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Determination of the maximum bioaccumulation capacity of various metals in leaves of two *Tillandsia* species

Alexandre Gonzalez¹ · Zohra Benfodda¹ · David Béniméris¹ · Damien Bourgeois² · Damien Herfurth³ · Jean-Xavier Fontaine³ · Roland Molinié³ · Patrick Meffre¹

Abstract

Tillandsia species are plants from the Bromeliaceae family which display biomonitoring capacities in both active and passive modes. The bioaccumulation potential of *Tillandsia aeranthes* (Loisel.) Desf. and *Tillandsia bergeri* Mez acclimated to Southern/Mediterranean Europe has never been studied. More generally, few studies have detailed the maximum accumulation potential of *Tillandsia* leaves through controlled experiments. The aim of this study is to evaluate the maximum accumulation values of seven metals (Co, Cu, Mn, Ni, Pb, Pt, and Zn) in *T. aeranthes* and *T. bergeri* leaves. Plants were immersed in different mono elemental metallic solutions of Co (II), Cu (II), Mn (II), Ni (II), Pb (II), Pt (IV), and Zn (II) ions at different concentrations. In addition, cocktail solutions of these seven metals at different concentrations were prepared to study the main differences and the potential selectivity between metals. After exposure, the content of these metals in the leaves were measured by inductively coupled plasma-optical emission spectrometry. Data sets were evaluated by a fitted regression hyperbola model and principal component analysis, maximum metal loading capacity, and thermodynamic affinity constant were determined. The results showed important differences between the two species, with *T. bergeri* demonstrating higher capacity and affinity for metals than *T. aeranthes*. Furthermore, between the seven metals, Pb and Ni showed higher enrichment factors (EF). *T. bergeri* might be a better bioaccumulator than *T. aeranthes* with marked selectivity for Pb and Ni, metals of concern in air quality biomonitoring.

Keywords *Tillandsia* · Bioaccumulation · Metals · PCA analysis · Soaking experiment · Cocktail effect

Introduction

Indoor or outdoor air pollution, according to the World Health Organization (WHO), was responsible for the premature death of 6.7 million people worldwide in 2022, against 2 million in 2002 (World Health Organization 2002, 2022;

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Vianna et al. 2011). As pollution is a global health issue and a real concern all over the world, many techniques have been developed to get more information on air, soil, or water pollution (Han and Naeher 2006; Mateos et al. 2018). Most of these techniques present a very expensive development and cannot be conceivable for some countries (Gurgatz et al. 2016). A low-cost technique has been increasingly developed in the recent: biomonitoring. It consists in the continuous observation and monitoring of polluted matrices such as water, soil, or air using bioindicators (Gurgatz et al. 2016; Marié et al. 2018; Kovács et al. 2020; Swislawski et al. 2020; Subpiramanyam 2021; Respondek et al. 2022; Varela et al. 2023). Air biomonitoring has been first described in the 1980's (Schrimppf 1984; Mhatre and Chaphekar 1985; Dash et al. 1988). Some plants can be good bioindicators as there are able to accumulate various pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), or persistent organic pollutants (POPs) (de Souza Pereira et al. 2007b, a; Rodriguez et al. 2010, 2012, 2015; Wannaz et al. 2013; Derakhshan et al. 2016; Marié et al. 2018; Subpiramanyam et al. 2018; Niu et al. 2019; Reboredo et al. 2019; Swislawski et al. 2020; Subpiramanyam 2021). Plants biomonitoring can be both active (plants growing in their natural habitats are kept there and studied in this area) or passive (plants are transplanted from their "clean" habitat to a polluted area to study the bioaccumulation in their tissues) (Wegener et al. 1992; Schmidt et al. 1999; da Costa et al. 2016; Urošević et al. 2022; Silva et al. 2023). Prolonged studies in several sites simultaneously considering several effects of the environment are other advantages of plants' biomonitoring (Nangeelil et al. 2022). Metal pollution is one of the main concerns about air pollution: various metals present high toxicity and a great environmental resistance (Lasat 2002; Pescott et al. 2015; Wilk et al. 2017; Manisalidis et al. 2020). Consequently, their monitoring is of great interest due to the constant increase of emissions (Vianna et al. 2011).

Epiphytic plants such as *Tillandsia* from the Bromeliaceae family are rootless and grow by up taking nutrients from the air, the rainwater, and ambient humidity (Estrella-Parra et al. 2019; Eggli and Gouda 2020; Gouda 2020). *Tillandsia* natural habitat can be rainforest with high humidity and temperature, or highlands with wind, cold, and dryness (Bernal et al. 2005; Garth 2015; Sáyago et al. 2018; Miranda et al. 2020; Belmonte et al. 2022). Consequently, their tissue composition is widely representative of the atmosphere they live in and its air pollution status (de Oliveira et al. 2021; Belmonte et al. 2022; Contreras et al. 2022). *Tillandsia* have already been studied for their pollution bioindicator potential (Brighigna et al. 1997; Wannaz and Pignata 2006; Bermudez et al. 2009; Sánchez-Chardi 2016). It has been shown that in their natural habitats, *Tillandsia* species such as *Tillandsia recurvata* (L.) L. and *Tillandsia usneoides* (L.) L. are very

good bioindicators for metal air pollution (Figueiredo et al. 2004; Cardoso-Gustavson et al. 2016; Salas-Luévano et al. 2017; Piazzetta et al. 2019; Beringui et al. 2021; Mendoza-Ramos et al. 2021; Nangeelil et al. 2022). Moreover, species such as *Tillandsia capillaris* Ruiz & Pav., taken from their natural habitats and placed in polluted areas revealed a good adaptation and bioindication of the transition metals air composition (De la Cruz et al. 2020). Finally, *Tillandsia* species cultivated in greenhouses and placed in polluted areas showed also a significant metal bioaccumulation (Zheng et al. 2020). The *Tillandsia* genus has proven its efficiency in bioaccumulating various metals, even though, the maximum ability of *Tillandsia* species to accumulate metals in their tissue has been poorly documented yet (Wannaz et al. 2011; Li et al. 2021; Sun et al. 2021).

Herein, in Southern/Mediterranean Europe, some *Tillandsia* species are well acclimated to the local climate: *Tillandsia aeranthos* (Loisel.) Desf. and *Tillandsia bergeri* Mez. Determining their maximum metal content is a necessary step to consider a possible biomonitoring of air pollution using these two *Tillandsia* species (Pandey and Sharma 2002; Wannaz et al. 2011). This allows to know the concentration of each studied metals that both species are able to accumulate before risking a decrease or an excretion of these metals from plant tissues. Such study could provide new information on physiological response and hormesis of these two species exposed to unusual high metal concentrations (Li et al. 2021). In the present, controlled laboratory experiments through soaking of *T. aeranthos* and *T. bergeri* plants into metal salt solutions were performed to determine the maximum accumulation levels in their leaves. A wide range of concentrations of the salt solutions was chosen, considering the leaf structure and that leaf surface is very important with dry deposition (Jeon et al. 2006; van Heerden et al. 2007). A cocktail effect experiment was also performed to study the potential selectivity of a metal from another under controlled conditions. Such experimental studies on *T. aeranthos* and *T. bergeri* have never been performed before to our knowledge.

Materials and methods

Plant material and chemicals

All the healthy *Tillandsia* species used for this study were provided by the *Tillandsia* PROD plant nursery located in Le Cailar (Occitanie, France, 43° 41' 31.98" N, 4° 14' 34.85" E) and were harvested in November 2022 with similar size from the same location in the plant nursery. Two species were used: *T. aeranthos*, *T. bergeri*. The chemicals used for the soaking experiment were ordered from Thermoscientific (Massachusetts, USA) companies: copper (II) chloride

hydrate (99.999%), nickel (II) chloride hydrate (99.995%), zinc (II) chloride hydrate (99.999%), lead (II) chloride (99.999%), manganese (II) chloride tetrahydrate (99.999%), platinum (IV) chloride (99.99 + %), and cobalt (II) nitrate hexahydrate (99.999%). The chemicals used for the plant digestion were ordered from the Carlo Erba Reagents company (Val de Reuil, France): nitric acid 67–69% (RPE quality), hydrochloric acid 35–37% (RPE quality), and hydrogen peroxide solution 30% (RPE quality). The chemicals used for ICP-OES and ICP-MS analysis were ordered from the Carlo Erba Reagents company (Val de Reuil, France): nitric acid 67–69% (RS quality) and Hydrochloric acid 34–37% (RS quality) both super pure for trace analysis. The standard solutions for quantification were ordered from SCP Sciences (Quebec, Canada) with mono element standards at 1000 mg. L⁻¹: Co (99.9%), Cu (99.3%), Mn (99.7%), Ni (100%), Pb (99.4%), Pt (99.4%), and Zn (99.9%).

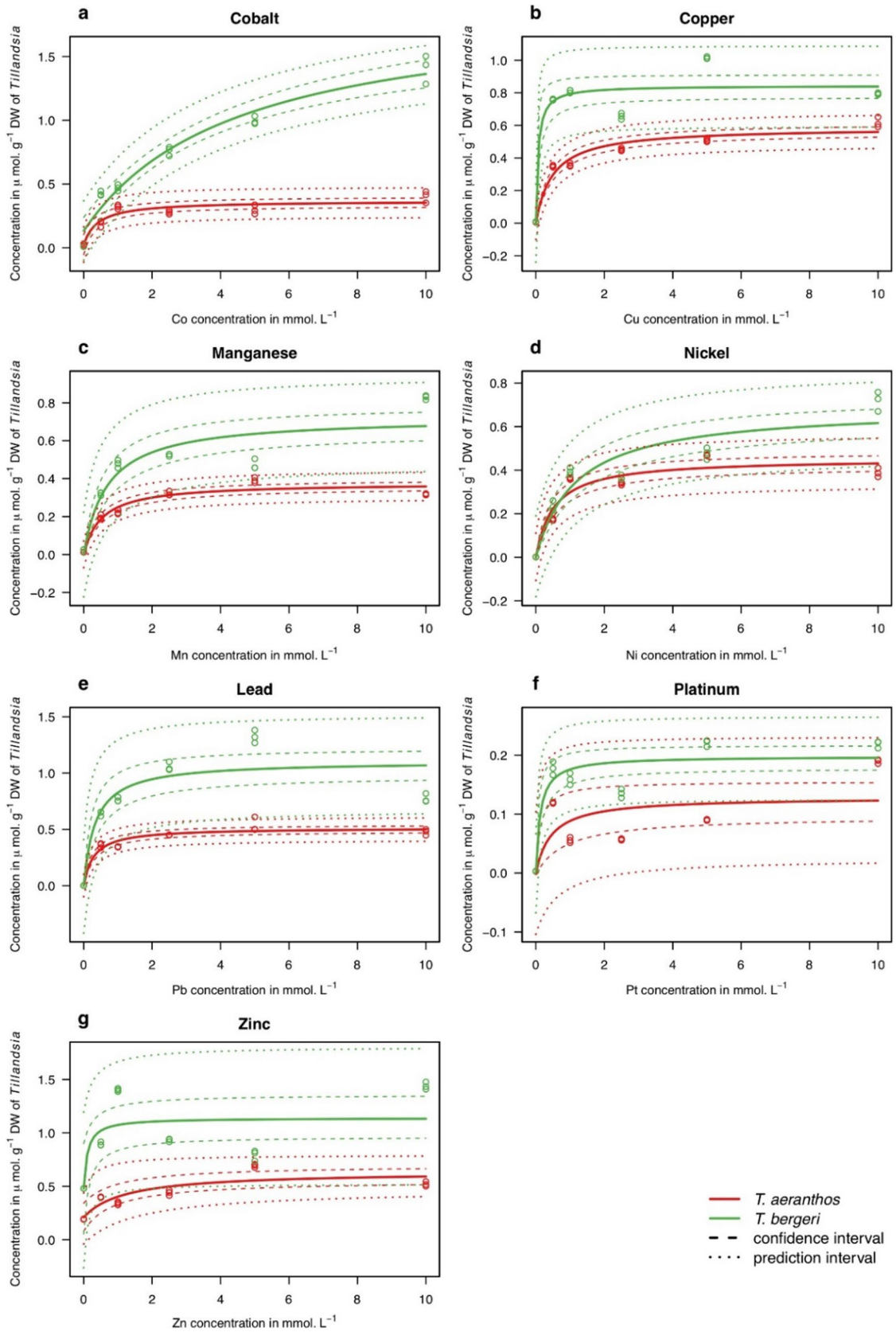
Sample preparation and treatment

The *T. aeranthis* and *T. bergeri* plants collected (around 5 g each plant) were soaked for 1 h in 250-mL metal salt solutions at the equilibrium at different concentrations and in 250-mL Milli-Q water (control treatment). The salt solutions were prepared from the salts: CuCl₂, NiCl₂, ZnCl₂, PbCl₂, MnCl₂, PtCl₄, and Co(NO₃)₂ at the following concentrations: 0.5 mM, 1 mM, 2.5 mM, 5 mM, and 10 mM. All solutions were at the equilibrium before soaking *Tillandsia* specimens. These concentrations were chosen according to Wannaz et al. (2011) to evaluate the maximal absorption values in *T. capillaris* leaves. The experiments were performed in the darkest possible conditions: due to their CAM photosynthesis process, *Tillandsia* species open their stomata at night, which favors the uptake of compounds such as metal cations. Carbon dioxide enters through the stomata at night and is stuck to malic acid. During the day, the plant survives with stock of carbon dioxide made at night and malic acid is decarboxylated (Freschi et al. 2010; Poczai and Hyvönen 2017; De La Harpe et al. 2020). The salt solutions have been prepared in a mono elemental way. Simultaneously, a cocktail effect experiment was carried out with salt solutions of Co, Cu, Mn, Ni, Pb, Pt, and Zn put together at the same concentration as before to evaluate the potential selectivity of *T. aeranthis* and *T. bergeri* for a metal or another. In the first part of the experiment, *T. aeranthis* and *T. bergeri* were immersed in mono elemental equilibrium solutions of seven different metallic ions (Co (II), Cu (II), Mn (II), Ni (II), Pb (II), Pt (IV), and Zn (II)) in various concentrations: 0.5 mM, 1 mM, 2.5 mM, 5 mM, and 10 mM. A control solution of pure milliQ water was used to determine the content in these seven metals in the two studied species. In the second part, *T. aeranthis* and *T. bergeri* plants were soaked in a cocktail equilibrium solution of the seven studied metals at

various concentrations, ranging from 0.5 to 10 mM. To help with the comprehension of the data obtained in the cocktail experiment, a principal component analysis (PCA) was used to analyze the quantity of the seven metals sorbed in the plants at the five different soaking concentrations in the two *Tillandsia* species. After the soaking time was over, *T. aeranthis* and *T. bergeri* plants were removed from the salt solutions and washed three times with Milli-Q water. Then, *Tillandsia* leaves were dried in an oven (Mettler UF55) at 50 °C until constant weight isolated from the environment. They were then grinded using a spex planetary ball mill apparatus, with a tungsten grinding bowl and three tungsten balls for 2 × 1 min. Then, dried and grinded *Tillandsia* samples were separated in three pools for digestion and next treatments.

Elemental analysis of *T. aeranthis* and *T. bergeri* leaves for maximum values

Dried samples were digested using a high-performance microwave digestion system (Ethos one, Milestone) with Teflon reactors under pressure. About exactly 60 mg of dried and grinded sample were weighed in a Teflon reactor, and 8.1 mL of HNO₃ 67–69% (RPE quality), 1.6 mL of H₂O₂ 30% (RPE quality), and 0.3 mL of HCl 35–37% (RPE quality) were added to the Teflon reactor which was then sealed. Reactors were placed in the microwave assisted device and temperature was brought to 200 °C in 15 min at 12 °C/min; then, the temperature was maintained at 200 °C for 15 min; and finally, the reactors were left until room temperature was reached. The digested samples were diluted in 100-mL volumetric flasks, then filtered using 0.2-µm cellulose acetate syringe filters. Then, 5 mL were poured in ICP tubes and kept at 4 °C until analysis using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The soaking solutions were filtered and diluted to be also analyzed using ICP-OES. There were also analyzed before the soaking to compare the evolution of metal content. The concentrations of the seven metals: Co, Cu, Mn, Ni, Pb, Pt, and Zn were calculated. All samples were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Thermoscientific, iCAP PRO series with a CETAC Technologies ASX-560 autosampler). Concerning the cocktail effect experiment, all samples were analyzed the same way as described before. Each batch of samples included a blank ultrapure water, a blank acid and replicates. Limits of detection (LOD) and limits of quantification (LOQ) were calculated in each analyzed phase for each metal from the dilution factor applied and the background equivalent concentration calculated by the spectrometer. The standard solutions of the targeted metals were diluted to make a seven-point calibration curve for each metal. Concentration of the seven metals was quantified applying a blank correction.



◀**Fig. 1** Concentrations of the seven metals in *T. aeranthis* and *T. bergeri* leaves soaked in different concentrations of mono elemental salt solutions at equilibrium. **a** Total Co concentration in $\text{Co}(\text{NO}_3)_2$ soaking solutions. **b** Total Cu concentration in CuCl_2 soaking solutions. **c** Total Mn concentration in MnCl_2 soaking solutions. **d** Total Ni concentration in NiCl_2 soaking solutions. **e** Total Pb concentration in PbCl_2 soaking solutions. **f** Total Pt concentration in PtCl_4 soaking solutions. **g** Total Zn concentration in ZnCl_2 soaking solutions

The given concentrations were calculated as the average of three replicates at different wavelengths for each replicate, for the seven metals. The selected spectral lines to assay metals were free from interferences.

Data analysis

Fitting of all experimental data was carried out using R software (version 4.2.3). The modeling of the accumulation of metals was carried out using a hyperbola according to the following function (Wannaz et al. 2011).

$$y = \frac{ax}{b+x} + c$$

where y represents the concentration of the metal in $\mu\text{mol.g}^{-1}$ in *T. aeranthis* and *T. bergeri*, x the concentration of the metal in mmol.L^{-1} in the soaking solutions, a and b are the constants of the equation, and c is the y -intersect. This equation is adapted from the Langmuir isotherm model, a y -intersect has been added since the plants contain some of these metals before the exposition to metals. The general equation of a Langmuir model is as follows:

$$y = \frac{Q_{\text{max}} * K * x}{1 + K * x}$$

The correlation between the equation used and the Langmuir isotherm are as follows:

$$Q_{\text{max}} = \frac{a}{b}$$

$$K = \frac{1}{b}$$

where Q_{max} represents the maximum capacity of a plant to absorb metals and K represents the thermodynamic affinity constant of plants for metals.

Determination of parameters a , b , and c of these function was achieved using the 'nls' function of the 'stats' package (version 4.2.3). Confidence and prediction intervals were determined using the 'propagate' package (version 1.0–6). The goodness-of-fit for nonlinear regression (pseudo R^2) was achieved using the 'aomisc' package (version 0.650). Principal component analysis (PCA) was carried out the 'FactoMineR' package (version 2.8).

Results

Various transition metal accumulation from mono elemental solutions

In this first part, the results obtained on the mono elemental experiment are presented. Figure 1 reveals the absorption of each metal by the two *Tillandsia* species in their leaves as a function of the concentration of the equilibrium salt solutions. Latter concentrations did not significantly change before and after the soaking of *Tillandsia* plants. The concentration of each metal in $\mu\text{mol.g}^{-1}$ (dry weight of *Tillandsia*) rose in *T. aeranthis* and *T. bergeri* leaves as the concentration of the soaking solutions increased to reach a saturation level. For both species, the maximum accumulation values of the seven metals into the leaves was reached at low concentrations, indicating a strong affinity of the plants for the metals. The curves equations are presented in the supplementary data (Table S1). This kind of curve equation has already been used in studies of the absorption of metal in plants through the Langmuir isotherm model (Benhammou et al. 2005; Argun et al. 2007).

By looking at the curve equations and the pseudo R^2 in Table S1, it can be concluded that this model describes Pt and Zn absorption in *T. aeranthis* and *T. bergeri* leaves with moderate efficiency. However, for the sake of comparability between metals, it has been decided to include the data relative to these metals. In Fig. 1, the green curves represent the absorption of *T. bergeri* and the red curves the absorption of *T. aeranthis*. These curves are only a projection of all the measurements points. The dotted lines represent the prediction interval which means that no values are supposed to be out of that range and the dashed lines represent the 95% confidence interval. From these fittings, maximum metal loading capacity (Q_{max}) were obtained in $\mu\text{mol.g}^{-1}$ and $\mu\text{g.g}^{-1}$ and are presented in Table 1, along with thermodynamic affinity constants (K in L.mol^{-1}). For some metals such as Cu, Mn, and Co, the hypothesis that *T. bergeri* possesses higher maximum metal loading capacity (Q_{max}) than *T. aeranthis* could be made. However, the prediction intervals overlap for Cu, Mn, and Ni, which means there are no significant differences in the concentration of these metals accumulated in both *Tillandsia* species leaves. Consequently, the determined Q_{max} for these metals are not significantly different (Table 1). Some metals such as Cu, Pt, and Pb reach their maximum values into plant leaves in the lowest soaking concentration, with high K values for these metals, indicating strong affinity for these metals (Table 1). On the contrary, *T. aeranthis* and *T. bergeri* might have lower affinity for Mn and Ni, indicated by lower K values. Zn has low K value for *T. aeranthis* compared to *T. bergeri*.

Table 1 Maximum loading capacity (Q_{\max}) for the seven metals in *Tillandsia aeranthis* (Loisiel.) Desf. and *Tillandsia bergeri* Mez expressed in $\mu\text{mol.g}^{-1}$ and $\mu\text{g.g}^{-1}$ obtained from the mono elemental experiments

	<i>Tillandsia aeranthis</i>			<i>Tillandsia bergeri</i>		
	Q_{\max} ($\mu\text{mol.g}^{-1}$)	Q_{\max} ($\mu\text{g.g}^{-1}$)	K (L.mol^{-1})	Q_{\max} ($\mu\text{mol.g}^{-1}$)	Q_{\max} ($\mu\text{g.g}^{-1}$)	K (L.mol^{-1})
Co	0.40	23.9	2.39	1.41	83.0	0.22
Cu	0.62	39.2	2.05	1.02	64.5	15.63
Mn	0.39	21.5	1.96	0.83	45.5	1.46
Ni	0.49	27.5	1.92	0.72	48.2	0.86
Pb	0.54	111.2	3.44	1.32	274.2	2.98
Pt	0.19	37.0	2.20	0.22	43.1	7.94
Zn	0.69	45.3	0.82	1.44	94.1	9.09

Table 2 Enrichment factors of *Tillandsia aeranthis* (Loisiel.) Desf. and *Tillandsia bergeri* Mez for the seven studied metals in mono elemental solutions

	<i>Tillandsia aeranthis</i>	<i>Tillandsia bergeri</i>
Co	16	150
Cu	111	111
Mn	32	32
Ni	413	449
Pb	634	860
Pt	62	71
Zn	4	3

Maximum concentrations in the seven metals were obtained with *T. bergeri* rather than with *T. aeranthis*. However, that does not mean that *T. bergeri* accumulates more than *T. aeranthis*, because it depends on the initial metal content of the leaves regarding the studied metals. Therefore, to answer if *T. bergeri* is a better bioaccumulator than *T. aeranthis*, enrichment factors must be determined according to the following equation:

$$\text{EF} = \frac{C_{\max}[X]}{C_{\text{ref}}[X]}$$

where EF is the bioaccumulation factor, $C_{\max}[X]$ is the maximum metal content for each metal, and $C_{\text{ref}}[X]$ is the initial concentration of each metal in *Tillandsia* leaves in pure miliQ water after the soaking. The EF for each metal for *T. aeranthis* and *T. bergeri* are presented in the table below (Table 2). Analysis of the EF of the two *Tillandsia* species, leads that, except for Co, the six other metals are not more accumulated by *T. bergeri* than *T. aeranthis*. Concerning Co, there is a factor of 10 between the two species.

By calculating the EF (see Table 2) according to the control treatment values, it is possible to classify the seven metals for each *Tillandsia* species from the most tolerated metal to the less tolerated one: $\text{Pb} > \text{Ni} > \text{Cu} > \text{Pt} > \text{Mn} > \text{Co} > \text{Zn}$ for *T. aeranthis* and $\text{Pb} > \text{Ni} > \text{Co} > \text{Cu} > \text{Pt} > \text{Mn} > \text{Zn}$ for *T. bergeri*. As a comparison with another soaking experiment

performed on *T. capillaris* with four metals (Ni, Cu, Pb and Zn), the EF of Zn, Ni, and Cu are in the same range as the EF obtained in the present study whereas EF_{Pb} from the other study is about fourteen times lower than EF_{Pb} of the present study. However, the concentrations of metals in the *Tillandsia* leaves in $\mu\text{mol.g}^{-1}$ were higher in the *T. capillaris* study than in this study but these are different *Tillandsia* species and the conditions were not the same which makes it difficult to compare (Wannaz et al. 2011). Consequently, the EF showed the great resistance of *Tillandsia* species to toxic metal such as Pb tolerated at higher concentrations than the initial ones. However, other metals such as Cu or Co revealed high rates (high Q_{\max}) not especially higher than usual concentrations.

Accumulation of various metals from cocktail solutions

In this second part, the results obtained on the cocktail solutions experiment are presented. In Fig. 2, the first principal component (PC1) contributes to 74.39%, and the second principal component (PC2) contributes to 14.68% of the total variance in the metal concentration in the *Tillandsia* leaves data. PC1 allows the partition of metal concentrations of *T. bergeri* soaked in the 10 mM solution from the other individuals. PC1 also allows the global separation of *T. aeranthis* from *T. bergeri* concerning their metal content. Indeed, *T. aeranthis* leaves have lower concentrations for the seven metals than *T. bergeri* leaves. However, as in the previous part, that does not mean that *T. aeranthis* accumulates less than *T. bergeri* as it is necessary to compare the enrichment factors with the initial content of leaves. PC2 allows the partition of metal concentrations of *T. bergeri* soaked in the 0 mM solution from the other individuals. Moreover, for *T. aeranthis* soaked in solution from 0 to 5 mM, there is no increase of the metal content in leaves according to PC1 and therefore no separation between them. *T. aeranthis* soaked in the 10 mM solution is well separated in PC1 from the other concentrations. However, *T. aeranthis* from the 10 mM solution is similar to *T. bergeri* from 2.5 mM or

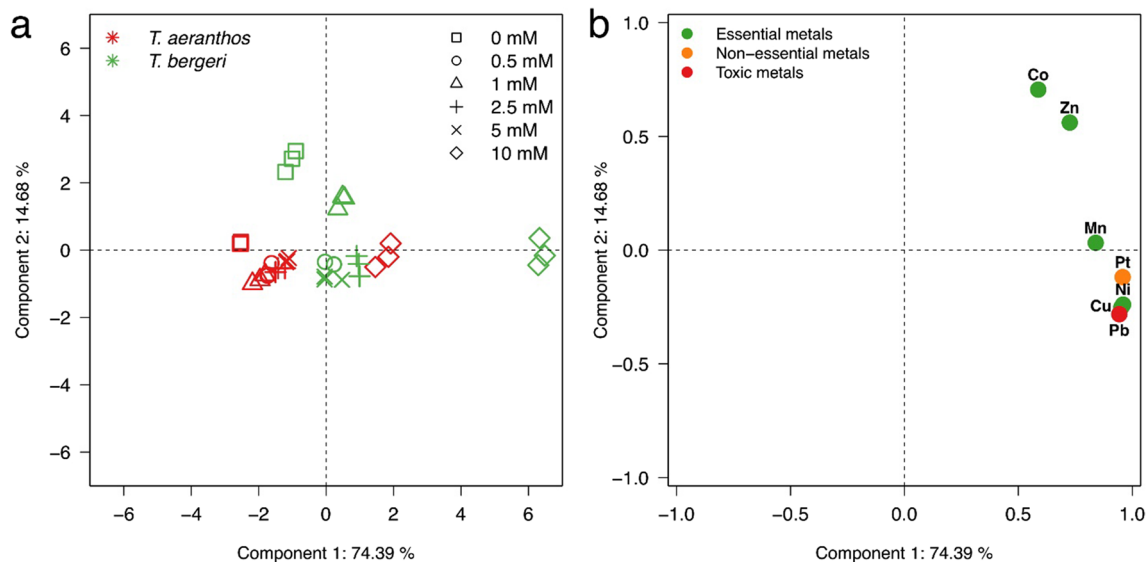


Fig. 2 PCA based on the seven metals accumulated by *Tillandsia aeranthis* (Loisiel.) Desf. and *Tillandsia bergeri* Mez. **a** Score plot of PC1 scores versus PC2 scores. **b** Loading plot of PC1 and PC2 contributing accumulation rates of metals in *Tillandsia*

5 mM solutions. That means that *T. bergeri* accumulates metals at lower soaking concentrations than *T. aeranthis* in the cocktail solutions of the seven metals. By studying the loading plot, it is possible to conclude that in both *Tillandsia* species, there is an increase of the metal content in leaves for the seven metals according to PC1 on the right side of the PCA. Furthermore, in *T. bergeri*, there is more Co and Zn than the rest of the metals as there are separated in PC2. These two metals are essentials for plants. The loading plot allows us to differentiate two groups: Co, Zn, and the rest of the metals. Concerning *T. aeranthis*, the differentiation between metals seems low whether there are essential, non-essential, or toxic for the plants.

The mono elemental experiment allows to anticipate the results obtained in the cocktail experiment since the ability of the two *Tillandsia* species to accumulate these metals is not supposed to change. However, a competition between the seven metals might occur once present together in the cocktail solutions.

Determination of the ratios of the EF (see Table 3) for each metal in the cocktail solution according to the control experiment values and the maximum accumulation values allows the classification of the seven metals for both *Tillandsia* species: for *T. aeranthis*, $Pb > Pt > Ni > Cu > Mn = Co > Zn$ and for *T. bergeri*, $Pb > Pt > Cu > Ni > Mn > Co = Zn$. The comparison between the two *Tillandsia* species leads to the conclusion that *T. bergeri* globally has better EF ratios than *T. aeranthis* for the seven studied metals. Finally, between the mono elemental soaking and the cocktail solution soaking, the EF and the maximum values are way higher in the mono elemental study.

Table 3 Ratios of EF of metals in *Tillandsia aeranthis* (Loisiel.) Desf. and *Tillandsia bergeri* Mez for the seven studied metals in cocktail solutions considering the EF of Zn as reference

	<i>Tillandsia aeranthis</i>	<i>Tillandsia bergeri</i>
Co	2EF	1EF
Cu	6EF	20EF
Mn	2.5EF	1.7EF
Ni	9EF	12EF
Pb	55EF	157EF
Pt	13EF	39EF
Zn	1	1

For the seven metals together in the cocktail solution, their maximum loading were determined in mono elemental solutions and should not change. There are expressed in $\mu\text{mol.g}^{-1}$ and in $\mu\text{g.g}^{-1}$ and are presented in Table 4.

Discussion

A soaking experiment method was developed to determine, in laboratory conditions, the maximum accumulation potential of two *Tillandsia* species acclimated to Southern/Mediterranean Europe: *T. aeranthis* and *T. bergeri*. This experimental technique was used with diluted metal salt solutions that could represent rainwater deposited on *Tillandsia* leaves (Wannaz et al. 2011). This is a model of wet deposition to point out the accumulation of metals solubilized in rainwater that gives important information on

Table 4 Maximum values obtained with the 10 mM solution for the seven metals in *Tillandsia aeranthis* (Loisel.) Desf. and *Tillandsia bergeri* Mez expressed in $\mu\text{mol.g}^{-1}$ and $\mu\text{g.g}^{-1}$ in the cocktail experiment

	<i>Tillandsia aeranthis</i>		<i>Tillandsia bergeri</i>	
	$\mu\text{mol.g}^{-1}$	$\mu\text{g.g}^{-1}$	$\mu\text{mol.g}^{-1}$	$\mu\text{g.g}^{-1}$
Co	0.11	6.3	0.12	6.9
Cu	0.21	13.2	0.36	22.8
Mn	0.07	3.8	0.13	7.3
Ni	0.04	2.3	0.06	3.7
Pb	0.33	69.3	0.58	120.6
Pt	0.08	16.1	0.24	46.5
Zn	0.46	29.8	1.16	75.5

these two *Tillandsia* species abilities as bioaccumulators. Seven representative metal cations were chosen. There have been preferentially employed as chloride salts as this counter ion is naturally present in plants and can stabilize these elements in aqueous solutions (Inglezakis et al. 2005). In this study, the counter-ion effect was not investigated in detail, as all the cations studied are expected to be present in aqueous solutions as their free +2 cationic form (Co^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+}), except Pt(IV) which is readily hydrolyzed in water unless chloride ions are present, and then should be present as its chloride complexes, $(\text{PtCl}_4)_{\text{aq}}$ and $[\text{Pt}(\text{Cl})_6]^{2-}$ (Colombo et al. 2008). Five solutions at different concentrations and a control solution (miliQ water) were prepared at the equilibrium to soak *Tillandsia* plants during one hour under dark conditions. The first experiments set was conducted using mono elemental solutions for each of the seven metals and a second experiments set was conducted using cocktail solutions of the seven metals.

Between these two sets of experiments, the same trend has been observed: the bioaccumulation of the seven metals in both *Tillandsia* species increases while the soaking solutions concentrations increase themselves. Moreover, the maximum metal concentrations in leaves have been reached in the 5 mM or 10 mM soaking solutions for the seven metals whether in the mono elemental or cocktail experiment. The cocktail experiment trend could be anticipated by the mono elemental experiment. In cocktail solutions, the maximum accumulation capacity determined through the mono elemental solutions was not reached, indicating a competition between metals towards the sorption on the leaves. By regarding the prediction and confidence intervals, there are non-significant differences between the 1 mM or 2.5 mM and the 10 mM solutions for most of the studied metals. *K* values show that the two *Tillandsia* species have a high affinity for Pb, Pt, and Cu compared to other metals. Nevertheless, the accumulation starts at lower concentrations for *T. bergeri* compared to *T. aeranthis*. By summing the seven

Q_{max} of the cocktail experiment, $Q_{\text{max}_{\text{mono}}} < \sum Q_{\text{max}_{\text{cocktail}}}$ which means that these seven metals do not all have the same fixation sites in *Tillandsia* leaves. However, global accumulation values in the cocktail experiment are lower than in the mono elemental experiment which means there is a competition between metals in common absorption sites. In the mono elemental experiment, Pb and Ni are the two most accumulated metals by both *Tillandsia* species. The only major difference is the accumulation of cobalt which is ten times higher for *T. bergeri* than *T. aeranthis*. Concerning the cocktail effect experiment, the PCA analysis showed a lower bioaccumulation of the seven metals in *T. aeranthis* than in *T. bergeri*. Indeed, the metal content in *T. bergeri* leaves from the 2.5 mM or 5 mM solutions is almost the same as the one in *T. aeranthis* leaves from the 10 mM solution. Moreover, in comparison with the mono elemental experiment, the synergic effect of the cocktail solutions seems to induce a competition of selectivity between accumulation of the seven metals. In the cocktail solutions, Pb has more affinity in *Tillandsia* leaves than other metals such as Co, Mn, or Ni. The rates of the seven metals as well as the EF are way higher in the mono elemental experiment than in the cocktail experiment (see Tables 1, 2, 3, and 4). The EF ratios show a good suitability of *T. bergeri* species to biomonitor Pb, Ni, and Cu with a great difference between basal and final concentrations.

These two *Tillandsia* species have never been studied before concerning their bioaccumulation potential of metals whether in active, passive or laboratory biomonitoring. Other studies on *T. capillaris* or *T. usneoides* have shown a high accumulation of nickel into the leaves as the best accumulated metal (Pyatt et al. 1999; Wannaz et al. 2011). In *T. aeranthis* and *T. bergeri*, Ni is highly accumulated (2nd) but Pb is the most accumulated metal in these two species (see Table 3 and 4). This might lead to the hypothesis that there is a selectivity in the bioaccumulation of metals in these two *Tillandsia* species. Due to the high concentrations of the soaking solutions, the metal content into the leaves is way higher than in past studies of active or passive biomonitoring which makes sense because such metal loadings cannot be found in the ambient air (Pignata et al. 2002; Bermudez et al. 2009; Rodriguez et al. 2011; Cardoso-Gustavson et al. 2016; De La Cruz et al. 2021). These high concentrations level out K effect in this study. Consequently, *T. aeranthis* and *T. bergeri* could be used in active or passive biomonitoring future studies due to their ability to accumulate more metal than what is present in the atmosphere and their proven affinity with some metals.

Concerning the common metals of our study and the study of Wannaz et al., the rates are lower in our study for Cu, Ni, Pb, and Zn while the EF are higher in our study except for Zn (Wannaz et al. 2011). However, it is difficult to compare the results obtained in these two studies since there are three

different *Tillandsia* species and the laboratory experiments did not take place in the same location under the same exact conditions. Finally, the potential selectivity of a metal from another could be explained by their role in plants' metabolisms. Indeed, in addition to being a non-essential metal, Pb is toxic for plants. It is the second most toxic metal after Arsenic. Moreover, it can inhibit growth and germination even at micromolar concentrations (Pourrut et al. 2011; Zulfiqar et al. 2019). *T. aeranthos* and *T. bergeri* seem to be good bioaccumulators of Pb. On the contrary, Ni is an essential micronutrient for plants as it maintains the activity of metallo-enzymes. Ni is present in many biomolecules as it helps to keep their structure and activity. Ni is required by urease for hydrolyzing urea. However, when the Ni concentration is too high in plant cells it can reduce growth and inhibit cell division (Ahmad and Ashraf 2012; Sreekanth et al. 2013; Bhalerao et al. 2015). Concerning Co, few studies investigated its toxicity in plants. Co participates in the formation of chlorophyll b and is present in B12 vitamin. However, at high concentrations, Co can be toxic for plants (Palit et al. 1994; Jayakumar and Jaleel 2009; Nakos et al. 2017).

Conclusion

This study allowed the evaluation of the potential and the efficiency of two *Tillandsia* species (*T. aeranthos* and *T. bergeri*) to accumulate seven metals (Co, Cu, Mn, Ni, Pb, Pt, and Zn) by soaking them into various metal solutions at different concentrations. This study allowed us to conclude that these two *Tillandsia* species were able to accumulate metals through their leaves and that a potential selectivity of the metal accumulated occurred. The EF of the seven metals in the leaves followed Pb > Ni > Cu > Pt > Mn > Co > Zn for *T. aeranthos* and Pb > Ni > Co > Cu > Pt > Mn > Zn for *T. bergeri*. Pb seems to have the strongest affinity with *T. aeranthos* and *T. bergeri*. In a cocktail experiment, a PCA analysis was performed to compare the differences between *T. aeranthos* and *T. bergeri* and it showed that *T. bergeri* accumulates more and has a stronger affinity for metals than *T. aeranthos*. This might be explained by the leaf surface/volume ratio of the two *Tillandsia* species. Indeed, *T. bergeri* may have a greater surface per gram compared to *T. aeranthos* which might explain the higher metal concentration in its leaves. Moreover, the density of trichomes on leaves could also induce a difference between both species in metal accumulation. The mono-elemental experiment allowed us to determine the maximum metal loading capacity and the affinity of the two *Tillandsia* species for these metals. The cocktail experiment revealed the selectivity between metals with both proper accumulation sites for metals and competition in a common site between metals. These two *Tillandsia* species were never studied before concerning their bioaccumulator

potential and this study allows us to conclude that *T. aeranthos* and *T. bergeri* acclimated to Southern/Mediterranean Europe could be used as biomonitors for airborne metal pollution. Their marked selectivity for Pb suggests that they could be used as bioindicators for this metal. Finally, it could also be interesting to evaluate the potential for depollution of the air through selective sequestration of Pb given the high metal loading capacity of both *Tillandsia* species for Pb.

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Author contribution Alexandre Gonzalez: methodology, software, validation, formal analysis, investigation, writing—original draft preparation, and visualization. Zohra Benfodda: supervision and writing—review and editing. David Béniméris: resources. Damien Bourgeois: supervision and writing—review and editing. Damien Herfurth: software and formal analysis. Jean-Xavier Fontaine: software, formal analysis, and visualization. Roland Molinié: methodology, software, validation, formal analysis, writing—review and editing, visualization, and supervision. Patrick Meffre: conceptualization, writing—review and editing, supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Declarations

Ethics approval Not applicable.

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