DO METAL TOLERANCE TRAITS EXPLAIN SPATIAL DISTRIBUTION PATTERNS IN METALLICOLOUS VASCULAR PLANT SPECIES?

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ATA DE DEFESA DE DISSERTAÇÃO DO CANDIDATO JUAN CARLOS ARIAS JIMENEZ DO PROGRAMA DE MESTRADO EM ECOLOGIA DE BIOMAS TROPICAIS

Aos catorze dias do mês de março do ano de dois mil e dezesseis, às 14h, na Sala Multimídia do ICEB - Campus/Morro do Cruzeiro/UFOP, em Ouro Preto/MG, foi instalada a sessão de defesa pública da dissertação **"Do Metal Tolerance Traits Determine Spatial Distribution Patterns in Metallicolous Vascular Plant Species?"**, do candidato ***Juan Carlos Arias Jimenez***, sendo a banca examinadora composta pela Professora Dra. Alessandra Rodrigues Kozovits- UFOP (presidente); Professor Dr. Fernando Augusto de Oliveira e Silveira – UFMG – Universidade Federal de Minas Gerais (membro), Professor Dr. Hildeberto Caldas de Sousa – UFOP (membro). Dando início aos trabalhos, a presidente com base no regulamento do curso e nas normas que regem as sessões de defesa de dissertação, concedeu ao candidato ***Juan Carlos Arias Jimenez***, 30 (trinta) minutos para apresentação do seu trabalho intitulado **"Do Metal Tolerance Traits Determine Spatial Distribution Patterns in Metallicolous Vascular Plant Species?"**. Terminada a exposição, a presidente da banca examinadora concedeu, a cada membro, um tempo máximo de 30 (trinta) minutos, para perguntas e respostas ao candidato sobre o conteúdo da dissertação, na seguinte ordem: 1º) Professor Dr. Fernando Augusto de Oliveira e Silveira, 2º) Professor Dr. Hildeberto Caldas de Sousa, tendo ela própria realizado sua arguição em último lugar. Dando continuidade, ainda de acordo com as normas que regem a sessão, a presidente solicitou aos presentes que se retirassem do recinto para que a banca examinadora procedesse à análise e decisão. A seguir foi anunciado publicamente que o candidato foi ...APROVADO...... por unanimidade, condicionando que a versão definitiva da dissertação deverá incorporar todas as exigências da banca, devendo o exemplar final ser entregue à Secretaria do Programa, em até 60 (sessenta) dias, juntamente com o comprovante de submissão de artigo em publicação com fator de impacto mínimo B2. Para constar foi lavrada a presente ata que, após aprovada, vai assinada pelo mestrando e pelos membros da banca examinadora.

Ouro Preto, 14 de março de 2016.

Mestrando: Juan Carlos Arias Jimenez

Presidente: Professora Dra. Alessandra Rodrigues Kozovits

Membro: Professor Dr. Fernando Augusto de Oliveira e Silveira

Membro: Professor Dr. Hildeberto Caldas de Sousa

"Through chances various, through all vicisitudes, we make our way..." Virgil, The Aeneid

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Table of Contents

List of figures

List of tables

Appendix list

Attachment list

Do metal tolerance traits explain spatial distribution patterns in metallicolous vascular plant species?

Abstract

Knowledge on natural spatial variation of metallicolous vascular plant species is fundamental both for theoretical (ecological, evolutionary) and applied (ecological restoration, bioremediation) purposes. However, the local variation between species and populations remains almost unexplored and it is still not known if there exists a dominant functional trait in metal tolerance or spatially related patterns among cohabiting metallicolous vascular plant species. For this reason, this study proposes to test, 1) if a dominant plant functional trait of metal tolerance can be found in ferruginous rocky ironstone outcrops, and 2) if there are local spatial patterns related to metal tolerance traits in these metallicolous plant functional groups. Through a systematic sampling design in a continuous grid of 115 plots (10x10m^2), data on plant density, distribution and foliar concentration of 25 chemical elements were collected for seventeen native vascular plant species in a rocky ironstone outcrop (also known in Brazil as *Canga*). Two different metal tolerance traits of foliar Al-accumulation and non-accumulation were identified among the studied vascular plant species. The most of the studied species were non-accumulators, while only 3 from the same family (Melastomataceae) were Al-accumulators (including both the most and the least frequent species), suggesting a phylogenetically constrained pattern. No clear evidence of a dominant metal tolerance trait was detected among functional groups; neither on relating plant spatial patterns to strategies of accumulation or exclusion of metals and other chemical elements. Thus, other factors may explain better the spatial distribution of metallicolous vascular plant species. Possibly, the local spatial variation of the studied species and functional groups is more related to other adaptive strategies (like resilience or competence) than to eco-physiological traits of metal tolerance or avoidance.

Keywords: plant functional groups, metal accumulators and excluders, spatial distribution, rocky ironstone outcrops.

Estratégias de tolerância a metais serão explicativas da distribuição espacial de plantas vasculares metalófitas?

Resumo

O conhecimento da variação espacial natural das plantas vasculares metalófitas é fundamental tanto para fins teóricos (ecológicos e evolutivos) quanto aplicados (restauração ecológica e biorremediação). A variação espacial local de espécies e populações metalófitas ainda permanece quase inexplorada, sendo preciso entender se existe alguma diferenciação funcional ou padrões espacialmente relacionados entre espécies de plantas vasculares metalófitas. Por esta razão, o presente estudo se propõe a testar, 1) se espécies metalófitas cohabitantes apresentam uma estratégia funcional dominante de tolerância a metais, e 2) Se a distribuição espacial de grupos funcionais de metalófitas está relacionada com estratégias de tolerância a metais. Por meio de um plano de amostragem sistematizado em uma grade contínua de 115 parcelas ($10x10m^2$), foram amostrados dados sobre densidade, distribuição de plantas e concentração foliar de 25 elementos químicos em 17 espécies de plantas vasculares nativas de um Campo Ferruginoso, localizado no Quadrilátero Ferrífero (QF), Minas Gerais, Brasil. Entre as espécies estudadas, duas estratégias de tolerância a metais foram reconhecidas: acumuladoras de Al e não acumuladoras. Não foi detectada evidência concluinte da existência de padrões espaciais relacionados ao acúmulo de metais em folhas; nem sobre o domínio de grupos funcionais de acumuladoras ou não acumuladoras de metais. Isto sugere outro tipo de relação entre os padrões funcionais e espaciais das espécies metalófitas de plantas vasculares. Possivelmente, as variações populacionais locais são expressões mais relacionadas às capacidades adaptativas das espécies estudadas (resiliência ou competência) do que a estratégias funcionais de acumulação e exclusão de metais.

Palavras-chave: grupos funcionais de plantas, acumuladoras e exclusoras de metais, distribuição espacial, Cangas.

Introduction

Processes that structure plant communities continue to be a primary focus of plant ecological research (Rayburn et al. 2011). Among these processes, plant adaptations to extreme environments are of key interest in evolutionary biology (Broadley et al. 2001) and for the ecological modeling and restoration of degraded areas (Whiting et al. 2004). Such is the case of metallicolous plant communities related to natural metal ore formations, which are highly prone to suffer severe disturbances due to mining industry operations. Negative consequences of mining activities, however, are not restricted to vegetation, but also can reach the local biota as a whole, spreading to larger areas through the pollution of soils, atmosphere and water bodies (Salomons 1995). These widely documented processes of environmental degradation derive into the need to restore soils, vegetation cover, and the ecological balances (biotic interactions, functional processes and biogeochemical cycles) that had been disturbed or even lost. The successful management of natural metal-rich ecosystems requires not only understanding the plant species specific potential of metal tolerance, but also their patterns of natural spatial variation in order to conserve or restore these ecological balances (Baker et al. 2010; Baumbach 2012).

Knowledge on plant processes of accumulation, translocation and environmental deposition of metals or trace elements had substantially grown on the recent years (Clemens et al. 2002; Ernst 2006; Baker et al. 2010; Krämer 2010; Kabata-Pendias 2011; Pilon-Smits & El Mehdawi 2012; Metali et al. 2015). However, natural spatial distribution of metal accumulators and non-accumulators functional groups remains almost unknown. It is known that small-scale variation of metal distribution in soils may influence recruitment and establishment of plants with different strategies of tolerance/sensitivity to metals (Ginocchio et al. 2004) and that metal tolerant individuals can reflect gradients of plant-available metal levels (Ernst 2006). Some authors suggest that metal excluder species dominate on metalliferous soils (Verbruggen et al. 2013), but this assertion still require further field research.

In Brazil, phytosociological field studies have expanded the knowledge on plant diversity patterns and vegetation structure in ecosystems covering naturally metal-rich soils, especially in ferruginous rocky outcrops locally known as *Canga* (Messias et al. 2013; Skirycz et al. 2014; Jacobi et al. 2015). These *Cangas* consist of fragments of iron-formation and hard hematite cemented by limonite (Vincent & Meguro 2008) covered by a complex mosaic of shrub-grassland vegetation (Fernandes et al. 2014) considered among the most threatened geo-ecosystems around the world due to their restricted distribution associated with some of the main iron ore deposits worldwide subjected to mining exploitation (Jacobi & Carmo 2008; Fernandes et al. 2014; Silveira et al. 2015).

In the context of ecological modeling for the conservation and recovery of metal-rich ecosystems, *Cangas* vascular plant species are a highly promising prospect for understanding how natural spatial variation occurs among plant functional groups aiming to parameterize the ecological requirements for the successful ecological management of ferruginous rocky ironstone outcrops. For this reason is critically important to find the answer to these questions: 1) if a dominant plant functional strategy of metal tolerance can be found in ferruginous rocky outcrops, and 2) If there are spatial patterns related to metal tolerance strategies among plant functional groups.

Hypothesis

Among metallicolous vascular plant species in ferruginous rocky outcrops could be expected that metal excluders dominate as a whole, and that metal accumulators evidence a relationship between spatial aggregation patterns and metal concentration in foliar tissues. In that sense, two specific hypotheses were tested in this work: 1) the dominance of a functional pattern of metal tolerance among vascular plants in ferruginous rocky outcrops, and 2) local spatial distribution patterns are related to strategies of metal tolerance among metallicolous plant functional groups.

Materials and Methods

Study Area

With an approximate extension of 1.20 ha, the study area is a *canga* spot located inside an environmental protection area (APA *Cachoeira das Andorinhas*) between an altitudinal range of 1420 and 1490 masl on the east slope of *Serra da Brigida*, eastern portion of the *Quadrilatero Ferrifero*, Brazil (lat. 20° 21’, long. 43° 30’; max. alt. of 1510 masl) (Figure I). In particular, *cangas* are superficial hematitic deposits from tropical Banded Iron Formations (BIFs) characterized by its heterogeneous rocky surface which leads place to shallow “soil pockets” and fissures occupied by vegetation islands (Simmons 1963; Jacobi et al. 2007; Vincent & Meguro 2008; Messias et al. 2013).

The local climate is mesothermic (*Cwb* according to Köppen), with a rainy summer during November-March and a dry winter (Álvares et al. 2013). A meteorological station (Watch Dog 2000) installed in the study area measured 1172mm of precipitation between February 2012 and January 2013, with an average annual temperature of 17ºC (Figueiredo et al. 2015).

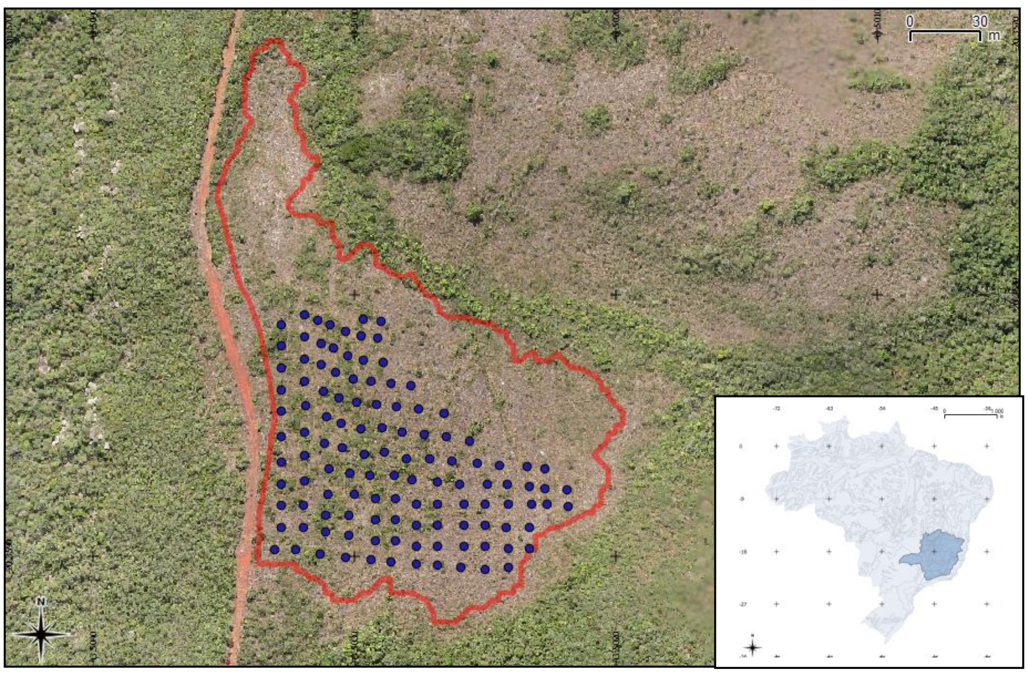

Figure I. Study area (the red line represents the perimeter and blue dots represent plot corners) located in Serra da Brigida, Minas Gerais, Brazil. On the inferior right corner, a scale map (1:1000.000) of Brazil, with the intense blue area representing the state of Minas Gerais, the points indicate a grid with distances of 1000 km.

Plant species and sampling method

A representative plant sample was taken to characterize the universe with respect to the measured species parameters (Taylor 1988). Due to the environmental heterogeneity of the study area, it was selected a systematic sampling design (Greig Smith 1952; Thompson 1958; Thompson & Seber 1995; Quinn & Keough 2002; Dungan et al. 2002). A grid of continuous plots (10x10 m^2) was established to study plant density, distribution and foliar accumulation patterns of metals and other chemical elements. From 115 plots, sixty were sampled to estimate frequency and 31 plots to estimate distribution data of seventeen native vascular plant species (all shrubs). These species were selected based on its frequency/rarity within the study area following the results on a previous phytosociological survey (Vale 2013), to satisfy the assumptions for hypothesis test. Ten frequent (I, II, III, IV, V, XI, XII, XIII, XIV, XV) and seven rare species (VI, VII, VIII, IX, X, XVI, XVII) were selected (See Table I for complete species names).

Table I. Species list by frequency categories.

Category[1]	**Code**[2]	**Species**[3]	**Family**[3]	**Voucher**[4]
Most frequent species	**I**	*Tibouchina heteromalla* (D. Don) Cogn.	Melastomataceae	46
	II	*Periandra mediterranea* (Vell.) Taub.	Leguminosae	47
	III	*Senna reniformis* (G.Don) H.S.Irwin & Barneby	Leguminosae	53
	IV	*Diplusodon microphyllus* Pohl	Lythraceae	51
	V	*Erythroxylum microphyllum* A.St.-Hil.	Erythroxylaceae	58
	XI	*Baccharis reticularia* DC.	Compositae	62
	XII	*Myrcia splendens* (Sw.) DC.	Myrtaceae	50
	XIII	*Matayba marginata* Radlk.	Sapindaceae	60
	XIV	*Heteropterys campestris* A.Juss.	Malpighiaceae	49
	XV	*Alibertia rotunda* (Cham.) K.Schum.	Rubiaceae	48
Least frequent (rare) species	**VI**	*Ouratea* sp.	Ochnaceae	55
	VII	*Eremanthus erythropappus* (DC.) MacLeish	Compositae	52
	VIII	*Byrsonima variabilis* A.Juss.	Malpighiaceae	61
	IX	*Miconia corallina* Spring	Melastomataceae	57
	X	*Trembleya laniflora* (D. Don) Cogn.	Melastomataceae	63
	XVI	*Myrsine* sp.	Primulaceae	54
	XVII	*Eremanthus incanus* (Less.) Less.	Compositae	56

[1]The order of the species names within categories does not necessarily reflect their abundance/frequency order. [2]Codes were assigned to each plant species for data ordination and simplification. [3]Taxonomically accepted names were obtained from The Plant List (2010). Version 1. Published on the Internet; http://www.theplantlist.org/. [4]Voucher specimens are deposited in the Herbarium "Professor José Badini" (OUPR); collection numbers correspond to B. V. Tavares.

As in Struik and Curtis (1962) the data was registered on a millimeter sheet for each sample unit (plot) with a representation scale of 1:100.

Geographic coordinates and altitude were collected using a Garmin *eTrex* GPS (Garmin Ltd. USA) device, both for the perimeter of the study area and for georeferencing all plots, with an error ≤ 3m for each coordinate (registered in DDD WGS 84).

Soil analysis

Samples for chemical and granulometric soil analyses were collected at 0-10 cm depth in 20 plots, by mixing five subsamples per plot. For granulometric analysis, soil samples of 2 Kg each were air-dried and the particles were separated by size fractions using sieves with different mesh sizes (4.0, 2.0, 1.0, 0.5, 0.25, 0.125, and 0.063 mm). After sieving, each size fraction mass was determined and the material was classified according to Wentworth (1922) nominal classes.

Concentrations of trace elements and macroelements (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, P, S, Ti, V, Y, Zn and Zr) in soils were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Agilent 725) after total acid digestion as described by Boss and Fredeen (1999). For this analysis, it was used the soil fraction <0.063mm. The total digestion method used concentrated chlorite, nitric and fluoride acids as extractors for subsamples of 0.25g. Certified reference material (GBW 07406 soil) was used in order to validate the extraction and quantification of chemical elements.

Sampling and foliar chemical analysis

Plant material was collected in the same 20 plots selected for soil analyses. In order to perform a representative sampling for foliar element concentration analysis, the procedure was standardized by collecting mature plant leaves from the same ontogenetic phase, at the same climatic period interval (Ernst 1995; Markert 1989, 1995; Markert & Klausmeyer 1990; Kovacheva et al. 2000) (Figures I-A, II-A, III-A & IV-A in the Appendix for an example of species foliar samples).

For each plant species, a sample consisted of $n \geq 30$ leaves from one or more plant individuals per plot. Among 4 and 9 samples per species were obtained across the 20 plots, resulting in a total of 121 samples.

Foliar concentrations of Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sr, Ti, V, Y and Zn were quantified by ICP-OES. Leaf samples were washed with deionized water, dried at 60 ◦C during 48 h and re-dried at 50 ◦C for 3 hours, prior to pre-digestion of approximately 0.3125 g of each sample on Teflon tubes with 8.750 mL HNO_3 (65%) and 1.250 mL H_2O_2 (23%) for 14 hours, and then digested in a microwave oven (MLS 1200: Milestone) to 150 ◦C for 10 minutes (with 60 min heat-up and 40 min cooldown time). The digests were checked to be clear and fully digested, then diluted to 20 ml with 18.2 mohm water (Milli-Q Plus, Millipore, Nepean, ON), and the diluted samples were analyzed by ICP-OES (Agilent 725, HP) (Zarcinas et al. 1987; Boss & Fredeen 1997).

A certified reference material (SRM 1515 Apple Leaves) from the National Institute of Standards and Technology NIST was also measured for quality assurance. The quantification limits (mg.Kg^{-1}) are provided in Table IV (Appendix).

Measures of spatial dispersion patterns

To analyze spatial dispersion patterns of those species and functional groups identified by multivariate (PCA) and non-metric ordination analysis, were calculated seven closely related indices: Index of Dispersion (ID), Index of Cluster Size (ICS), Green's Index (GI), Index of Cluster Frequency (ICF), Index of Mean Crowding (IMC), Index of Patchiness (IP), and Morisita's Index (IM). These indices, which primarily examine the deviation from a random (Poisson) distribution (Hurlbert 1990) were performed with PASSaGE software *v2* (Rosenberg & Anderson 2011).

Statistical analysis

Statistical analyses were performed using Minitab 17 for all variance analysis (chi-squared, ANOVA, t-tests). During model development, all data were explored for the normality of residuals and homogeneity of variances. A Principal Component Analysis (PCA) was performed to explore the underlying variance structure on plant foliar concentration data of different chemical elements. By simplifying the structure of the variables of study, this analysis accounts for all of the data variance including that found in the correlation coefficients and error variance (Brown 2001). Also, a multiple regression analysis was performed to identify significant relationships between response variables (density and spatial dispersion) and continuous predictors (foliar metal concentration) for the studied species (Zar 1999). For qualitative ordination was performed a Non Metric Dimensional Scaling by using PAST software *v3.10* (Hammer et al. 2001).

Results

Species inventory. *Tibouchina heteromalla* was the only species with 100% frequency across all sampled plots, followed by *Baccharis reticularia* (98%), *Diplusodon microphyllus* (96%), *Periandra mediterranea* (96%), *Erythroxylum microphyllum* (85%), *Alibertia rotunda* (78%), *Matayba marginata* (65%), *Senna reniformis* (65%), *Heteropterys campestris* (63%), *Myrcia splendens* (60%), *Eremanthus erythropappus* (45%), *Byrsonima variabilis* (41%), *Myrsine* sp. (41%), *Miconia corallina* (35%), *Ouratea* sp. (28%), *Eremanthus incanus* (33%) and *Trembleya laniflora* (23%). Along 31 sampled plots, 6807 individuals from the 17 studied species were recorded, with a density of 21958.06 individuals ha^{-1}; *T. heteromalla* was the most abundant species, with 3232 individuals, followed by *P. mediterranea* (1222 individuals), *D. microphyllus* (556), *E. microphyllum* (458), *B. reticularia* (241), *M. marginata* (216), *A. rotunda* (183), *H. campestris* (164), *S. reniformis* (127), *M. splendens* (106), *Myrsine* sp. (83), *M. corallina* (76), *B. variabilis* (51), *E. erythropappus* (31), *E. incanus* (30), *T. laniflora* (18) and *Ouratea* sp. (13). It was also observed a gradient in species richness (*R*) decreasing across the spatial matrix declivity (altitudinal range of ± 1490-1420 masl, Figure II).

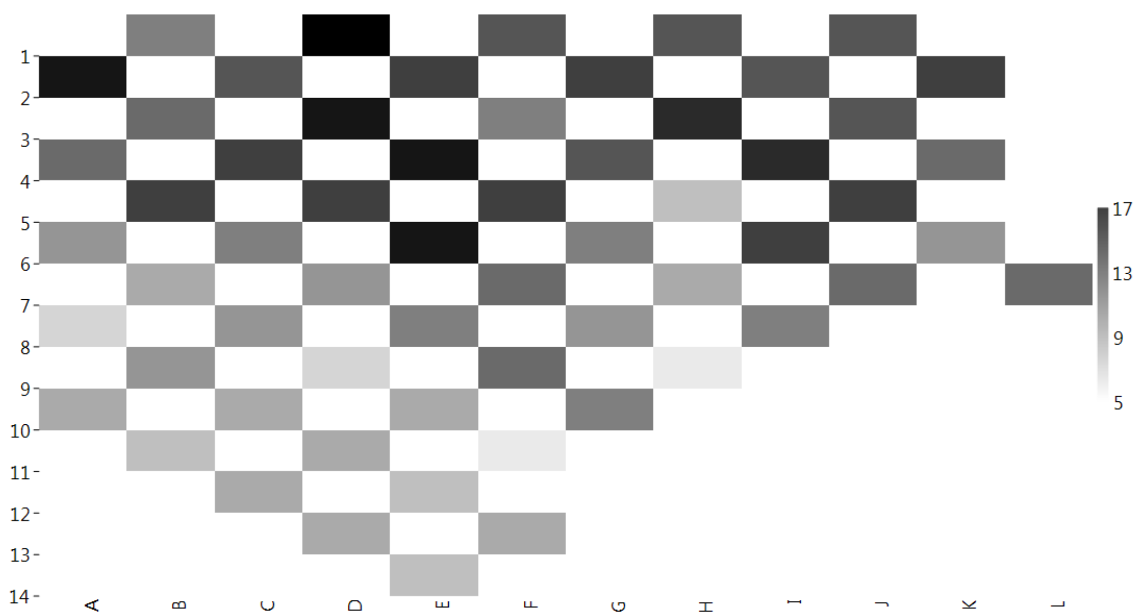

Figure II. Species richness per plot across the study area, increasing proportionally to dark intensity within plots (see grayscale at the right).

Soil particle-size distribution. The Figure III and Table I-A (Appendix) present the percentage distribution of each soil particle size class: 56.0% (>4mm), 18.2% (>2mm), 9.5% (>1mm), 4.9% (>0.5mm), 5.0% (>0.25mm), 3.4% (>0.125mm), 1.9% (>0.063mm) and 1.2% (<0.063mm).

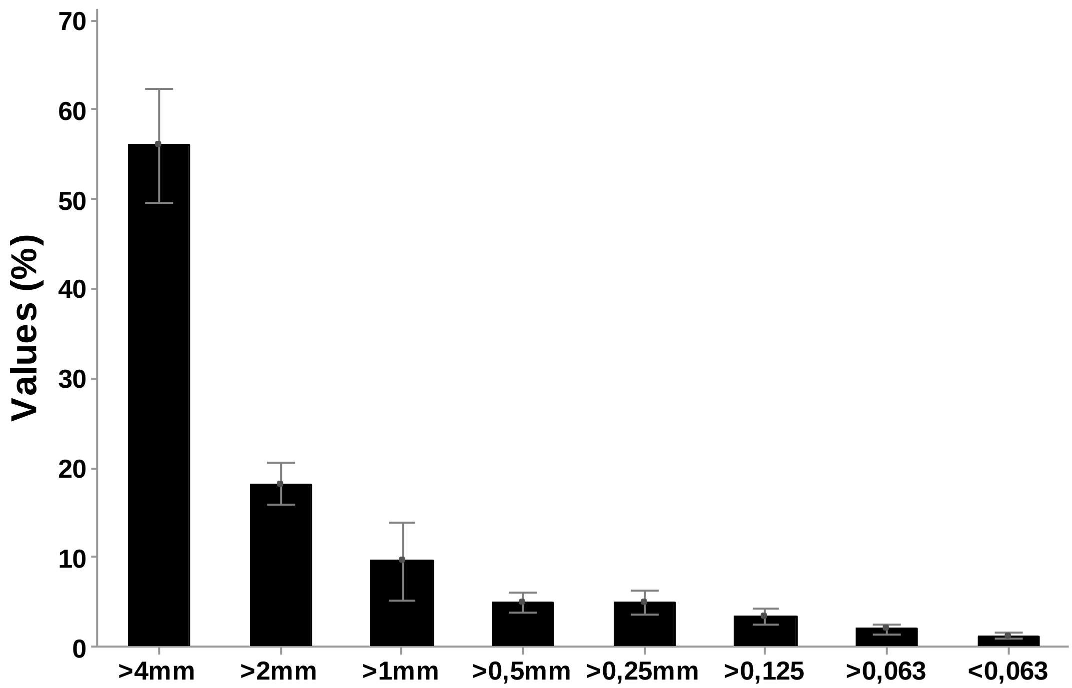

Figure III. Soil particle size distribution (values are expressed as percentages). 95% CI for the Mean. Individual standard deviations were used to calculate the intervals.

Total concentration of chemical elements in soils. Soil mean concentrations for the most of the analyzed chemical elements were under 1000 $mg.Kg^{-1}$ (see Table II). From a geochemical viewpoint those chemical elements are considered as "trace elements" (Kabata-Pendias 2011). Only Fe, Al, Ti and P presented concentrations over 1000 $mg.Kg^{-1}$. Fe represents 92.22% of soil mass, followed by Al (4.22%), and Ti (1.39%).

Table II. Soil mean concentration of the analyzed chemical elements. Standard deviations are shown as SE±.

Chemical element	Soil mean concentration (mg.Kg^{-1})	Standard Deviation
Al	10118.14	2055.63
Ba	34.32	9.30
Ca	915.28	291.08
Cu	86.99	71.79
Fe	231536.38	52420.51
K	662.60	158.45
Mg	411.96	70.21
Mn	502.87	83.61
Na	473.21	1253.87
P	1447.36	303.58
S	981.97	180.35
Ti	3371.29	544.20
Zn	44.06	6.32

Foliar concentration of chemical elements. Foliar mean concentrations of the analyzed chemical elements differed among plant species (Figure IV). Concentrations varied from 59.36 to 4195.29 (Al); 2.72-63.04 (Ba); 0.078-0.093 (Be); 2843.5-15315.46 (Ca); 3.08-17.41 (Cu); 103.82-453.81 (Fe); 1666.61-4893.01 (K); 1135.61-4164.41 (Mg); 75.95-2606.06 (Mn); 58.87-2055.86 (Na); 386.41-1212.51 (P); 967.12-2064.24 (S); 11.24-113.44 (Sr); 0.88-5.66 (Ti) and 13.62-76.08 (Zn) (Table II-A, Appendix). There were no significant differences among samples of the same species for each analyzed chemical element. In relative order of magnitude the foliar mean concentrations were Ca > K > Mg > S > Mn > P > Al > Na > Fe > Sr > Zn > Ba > Cu > Ti > Be.

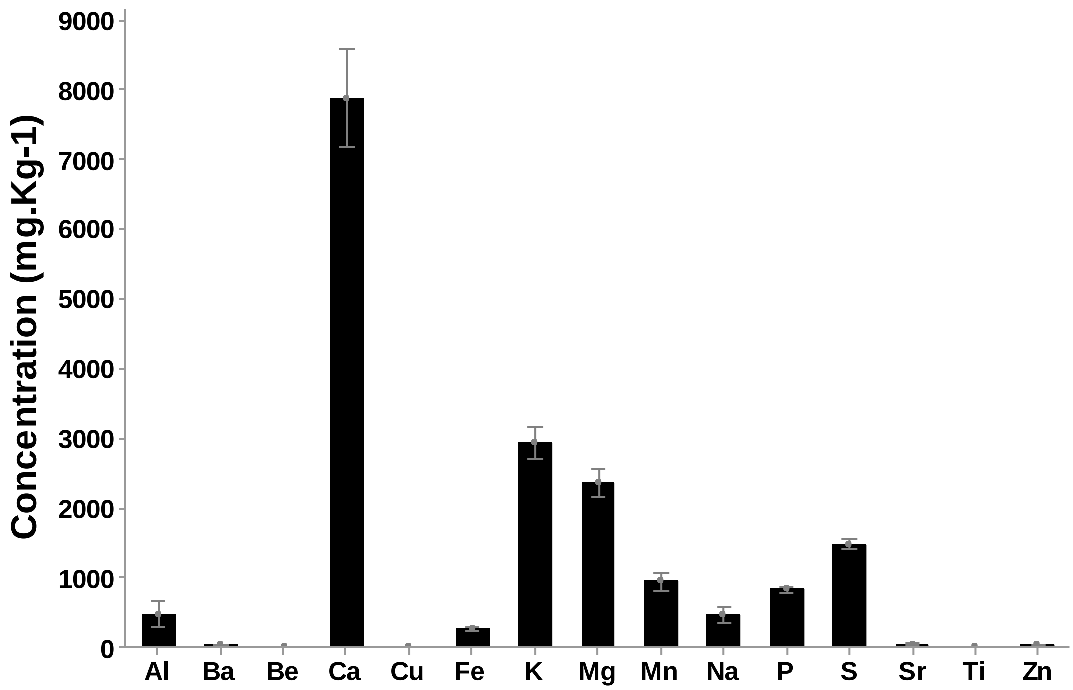

Figure IV. Average foliar concentration of 15 chemical elements. 95% Bonferroni CI for the Mean. Individual standard deviations were used to calculate the intervals.

Multivariate analysis. A Principal Component Analysis (PCA) was performed to analyze the variance contribution on the foliar concentration of metals and other chemical elements (Figure V). To avoid statistical noise, those variables with low contribution weight to the most significant principal components ($\lambda \geq 1.0$) as well as the highly correlated ones were excluded after an exploratory PCA. The first two principal components explained over 64% of the cumulative total variance; with the first principal component accounting for about 45.3% and the second for 19.2% of the total variance. On the loadings, the first component reflects high values for Sr, Al, and the second component for Ti and Ba. In that sense, the first axis (PC1) was highly positively related to Sr and Al concentrations; and the second axis (PC2) was positively related to Ti and Ba concentrations. In PC1, the most positively related species were *M. corallina* (IX), *T. laniflora* (X) and *T. heteromalla* (I). On the other hand, *B. variabilis* (VIII) and *Myrsine* sp. (XVI) were negatively related to this axis. In PC2, the most positively related species were *E. erythropappus* (VII), *B. reticularia* (XI) and *M. marginata* (XIII).

By performing a t-test statistical analysis, no significant differences within (p=0.198; p=0.734) or between (p=0.185) score variances of the PCA's first and second principal components were detected. Thus, there was no statistical evidence of a major variance contribution related to these element concentration groups.

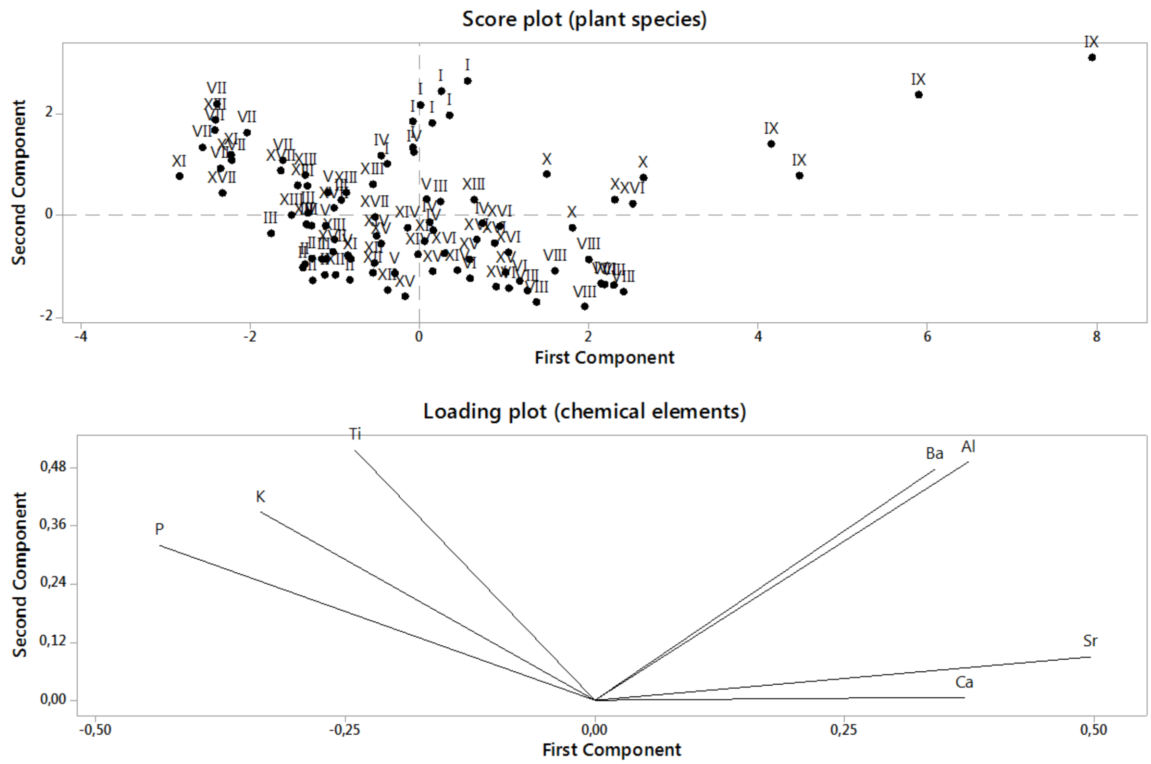

Figure V. Diagram of ordination showing the first two axes of a principal component analysis (PCA) of foliar concentration of different chemical elements in the 17 studied vascular plant species. Species codes are listed in Table I.

The foliar concentration of metals expressed a considerable variation between those species associated with the first, and the second principal components of the PCA. The Melastomataceae species showed a significant foliar Al accumulation pattern (over >1000 mg.Kg^{-1}) with Al concentrations varying between three and sixty folds over the other vascular plant species (*Myrsine* sp., a frequent neighbor of *M. corallina* presented the lowest average foliar Al concentration) (see Table II-A, Appendix). The foliar concentrations of Ba (65 mg.Kg^{-1}<), Sr (115 mg.Kg^{-1}<) and Ti (6 mg.Kg^{-1}<) were considerably inferior, showing higher values in Al accumulators than in non-accumulators (Figure VI).

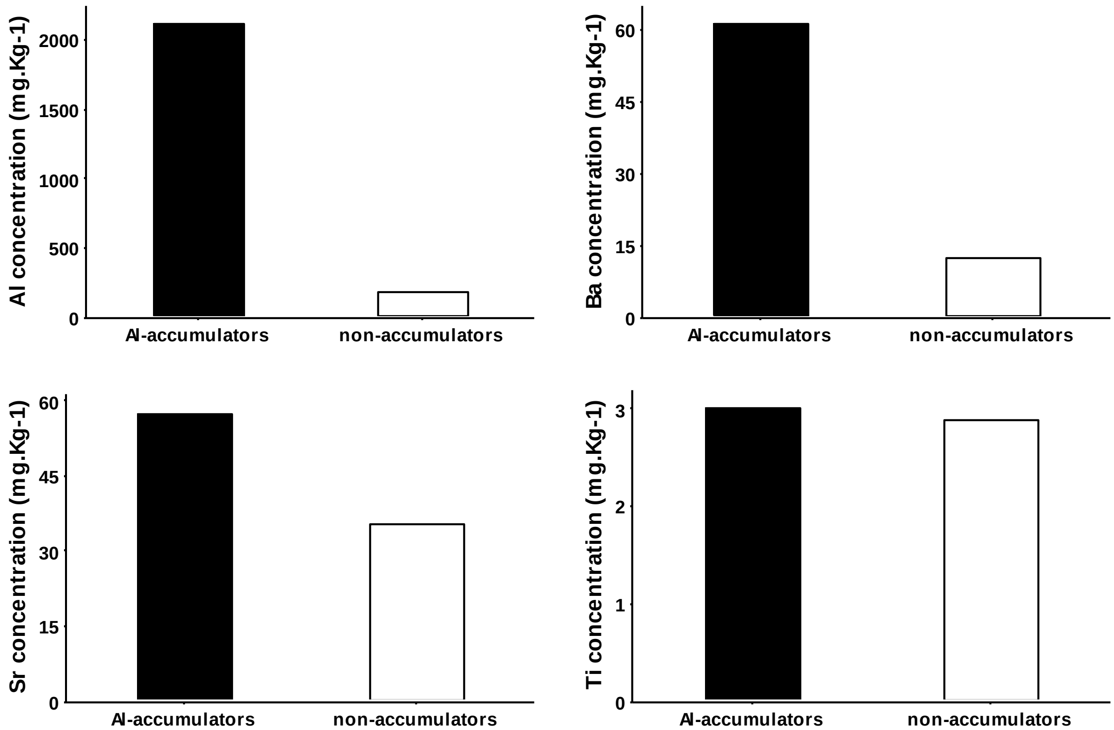

Figure VI. Average foliar concentration of Al, Ba, Sr and Ti between Al accumulators and non-accumulators.

The foliar concentration of the macronutrients Ca, K, and P varied as observed in Figure VII, with higher values in non-accumulators.

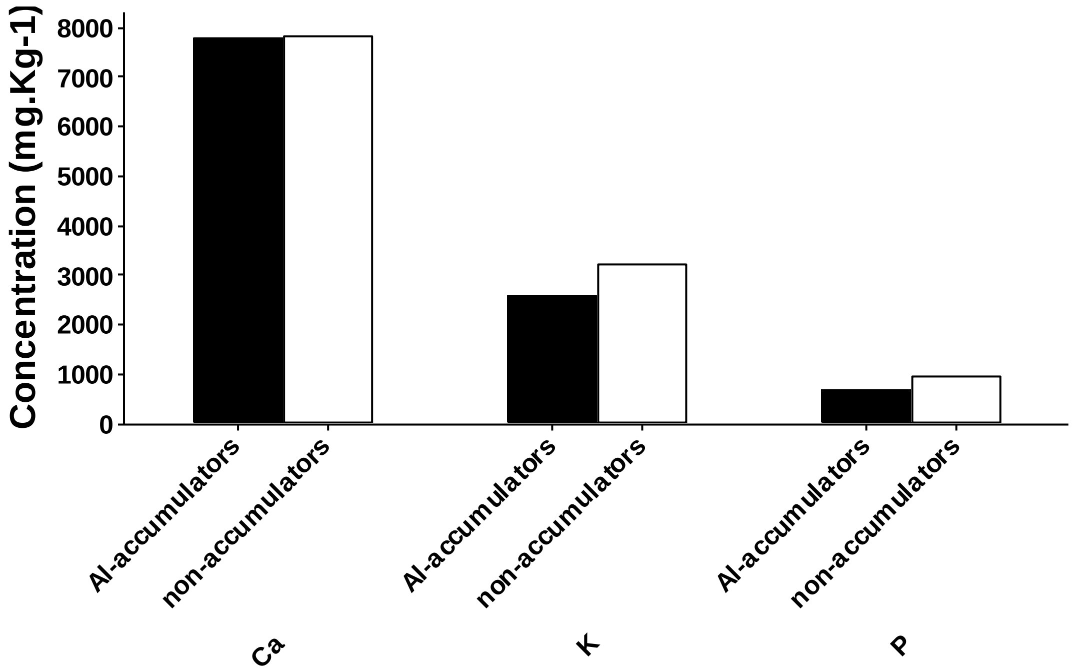

Figure VII. Foliar concentration of the macronutrients Ca, K and P ($mg.Kg^{-1}$) between Al accumulators and non-accumulators.

On the PCA multivariate ordination, the studied species were clearly identified with two functional groups of metal tolerance traits: Al accumulators (recognized on the first principal component) and non-accumulators (recognized on the second principal component) which were also confirmed by non-metric ordination (see Figure VIII). The pattern of Al accumulation in Melastomataceae included the most and the least frequent species across the studied area (*T. heteromalla* and *T. laniflora* respectively) which evidences a clear differentiation not only between accumulators and non-accumulating species, but also within Al accumulators.

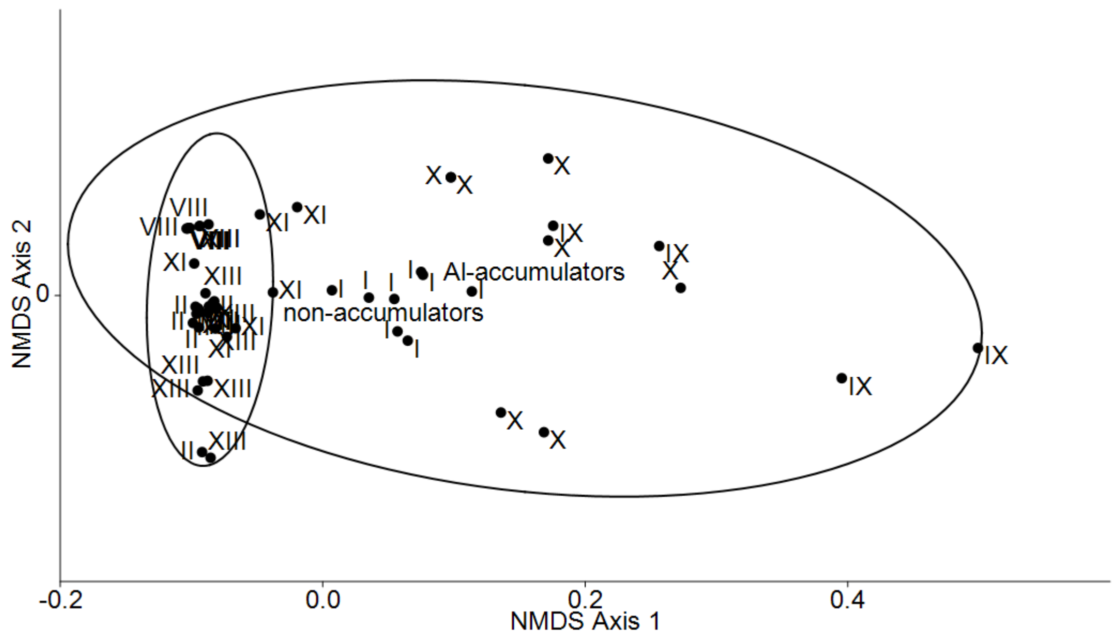

Figure VIII. Functional groups ordination (for metal tolerance traits) by Non-Metric Dimensional Scaling (NMDS). Species significantly related to the first and second principal components in the PCA were selected for this analysis, were the functional groups are Al-accumulators (I, IX, X) and non-accumulators (II, VIII, XI, XIII) (see Table I for species names). Euclidean distances were used for ordination. Accumulators: plants which accumulate more than 1000 $mg.Kg^{-1}$ of Al in their leaves (Jansen et al. 2002).

Plant species distribution

Dispersion indices. Comparing the populational variability of the sampled species, there were evident differences among all species in the terms of mean densities, and smoother differences in spatial dispersion patterns. As seen in Table III (Appendix), the most related species with the first and second principal components of the PCA (*M. corallina*, *M. marginata*, *B. reticularia*, *B. variabilis*) and also the most and the less abundant species (*T. heteromalla* and *T. laniflora* respectively), presented some degree of aggregation expressed by the coefficients of seven (different but related) dispersion indices (ID, ICS, GI, ICF, IMC, IP, IM). In other words, all the dispersion indices expressed a significant departure from randomness. These clustering patterns were present as follows: *T. laniflora* > *M. corallina* > *B. variabilis* > *M. marginata* > *P. mediterranea* > *B. reticularia* > *T. heteromalla* (for IP and IM). For the ICS and GI, the maximum aggregation value corresponded to *T. heteromalla* while the minimum to *B. reticularia*. For the ICF the maximum coefficient value corresponded to *T. heteromalla*, and the minimum to *T. laniflora*.

Spatial distribution and foliar concentration relationship.

Multiple regression analysis: This analysis was performed by computing the species and functional group (Al-accumulators/non-accumulators) values of density (m^2) and dispersion coefficients (from standardized IM) as responses in each case for the continuous predictors: foliar Al, foliar Ba, foliar Fe, foliar Sr, foliar Ti and versus species richness per plot. The idea on this analysis was to find if there exist any relationship between spatial aggregation and foliar concentrations of chemical elements (with species richness as control). Neither the foliar concentrations of Al, Sr, Ti or species richness were good predictors for functional group aggregation or for individual species aggregation. In the other hand, foliar Al was a suitable predictor for Al-accumulators density (m^2) showing a decrease in foliar Al when increasing Al-accumulators functional group density (see Figure IX).

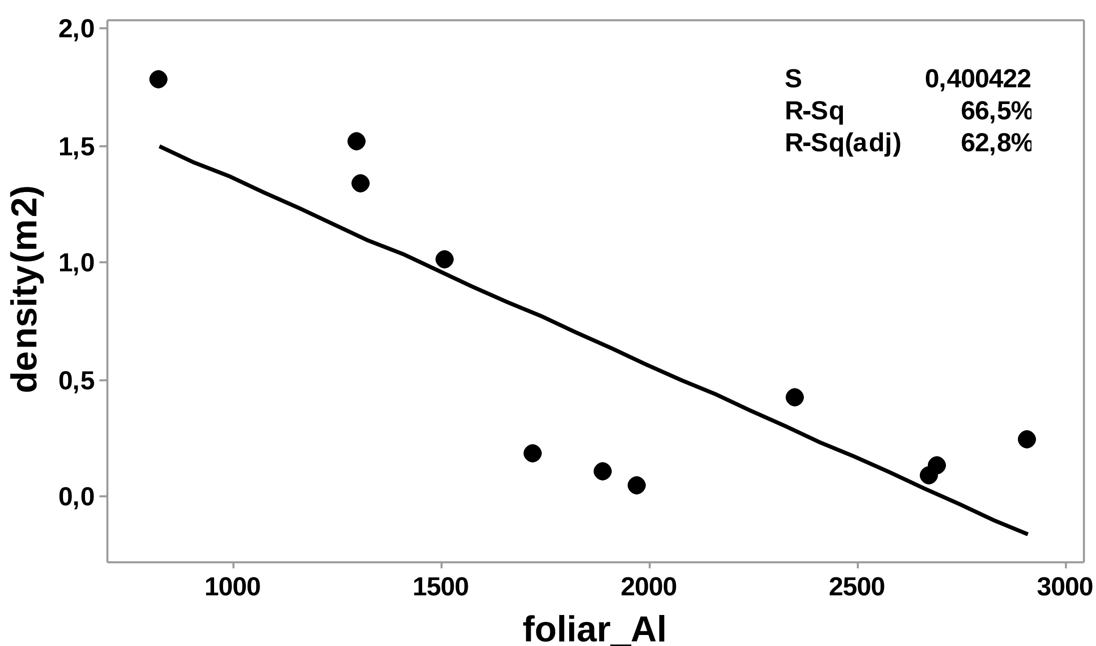

Figure IX. Density distribution of Al-accumulators functional group inversely related to foliar Al concentrations.

Also, species richness was a suitable predictor for density (m^2) of the dominant *T. heteromalla* with an *R-sq*(adj) of 59,2% (*S* = 0.256423) evidencing an inversely proportional relationship between density and species richness (see Figure X).

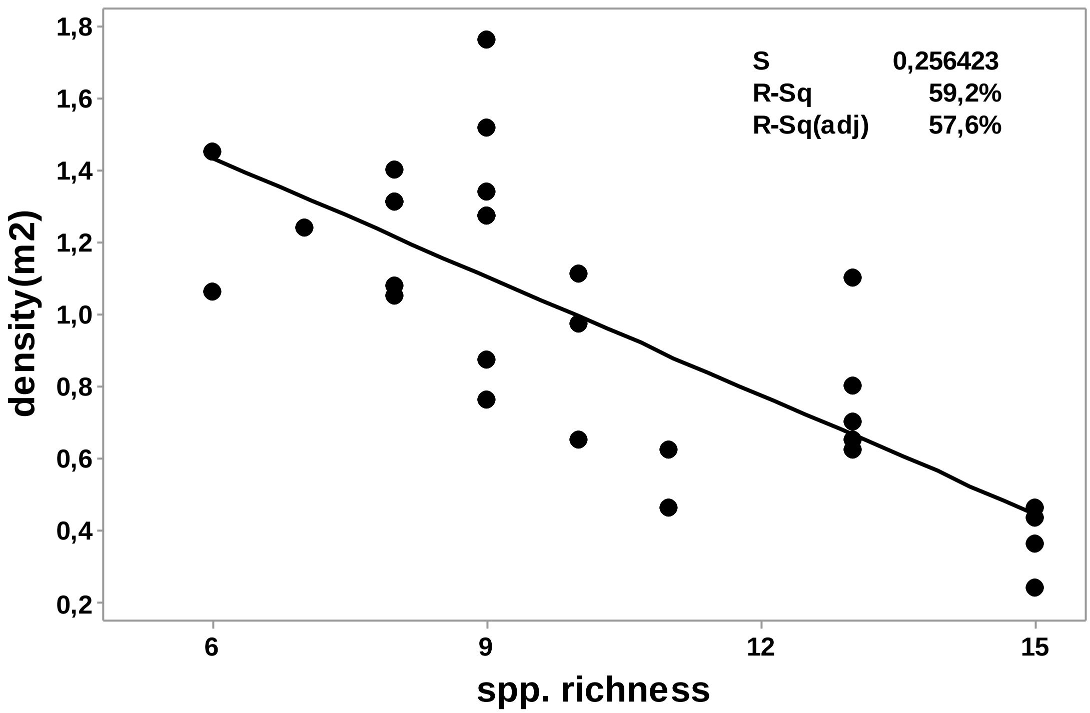

Figure X. Density distribution of *T. heteromalla* versus species richness.

When observing functional groups, only for Al-accumulators the density showed a linear relation with foliar Al accumulation, while for individual species there were no linear relationship between foliar concentration of any of the chemical elements and density per plots. Al, Ba, Fe, Sr and Ti foliar concentration were not suitable predictors for individual species spatial aggregation. For the dominant *T. heteromalla*, species richness explained better the density distribution than foliar concentration of Al, Ba, Fe, Sr or Ti.

The average densities of Al-accumulators and non-accumulators are shown in Figure XI, in which the slightly higher density (m^2) value corresponds to the non-accumulators functional group. When performing a test for equal variances (σ^2) no significant differences were detected among Al-accumulators and non-accumulators (p=0.275 for Multiple comparisons; p=0.248 for Levene's Test).

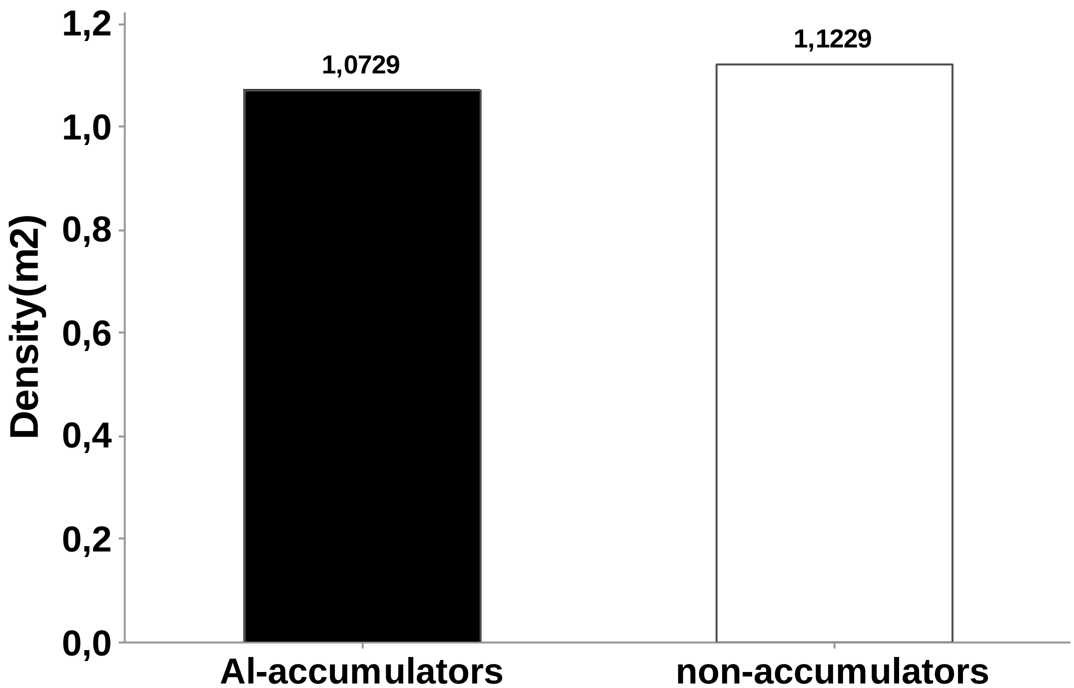

Figure XI. Density (m^2) compared in Al-accumulators and non-accumulators functional groups.

Discussion

Is there a dominant functional group between Al-accumulators and non-accumulators?

Although two functional strategies of metal tolerance (Al-accumulators and non-accumulators) were clearly identified in this study, there was no clear evidence of a dominant functional group among the studied vascular plant species, since the difference in relative abundances as in relative densities between Al-accumulators and non-accumulators was statistically not significant.

Considering the seventeen studied species only from a phylogenetic point of view, there is a dominant functional group of non-accumulators summarizing 14 species, and only 3 species (from the family Melastomataceae) being Al accumulators (including both the most and the least frequent species across the studied area). Foliar Al concentration is a phylogenetically constrained trait (Jansen et al. 2002), and Al accumulators are apparently more frequent among tropical woody plants than in plants of other life-forms and in different regions (Metali et al. 2012).

Is there a relationship between local spatial distribution and metal tolerance traits?

Both Al-accumulators and non-accumulators species and functional groups presented clustered spatial distribution patterns, evidencing a clear departure for randomness in metallicolous vascular plant species distribution in ferruginous rocky outcrops. The large majority of species tends to aggregate in species rich systems (Perry et al. 2009).

Despite of this, no evidence of a relationship between spatial aggregation patterns and foliar concentration of metals or other chemical elements was detected for any of the studied species and functional groups. Thus, there was no relationship between local spatial distribution and metal tolerance traits in the studied metallicolous vascular plant species.

A general feature in field studies of plant distribution is that even single species may display several scales of pattern, being common to find a combination of effects like clustering at large scales and regularity at small scales (Dale 1999; Dixon 2012). In the present study, different local patterns within Al-accumulators (*T. heteromalla*, *M. corallina*) and non-accumulators (*P. mediterranea*, *B. variabilis*) were observed with random distribution within plots and clustering being globally expressed in species and functional groups distribution patterns. This seems to indicate strong heterogeneity of exogenous (climate, topography, nutrient) and/or endogeneous (life history, competition, facilitation) factors occurring inside these plots (Pelissier & Goreaud 2001).

On the relationship of density patterns with metal tolerance traits, Al-accumulators showed a density response to foliar Al concentration, which surprisingly was inversely related; this relationship was not detected in non-accumulators. This could be an expression of a physiological constraint among Al-accumulators that will require further research and experimentation to know the extent and causes of this relationship. There is evidence supporting the positive influence of Al concentration on density and richness of herbaceous vegetation in Brazilian *Cerrado* soils (Ruggiero et al. 2002; Amorim & Batalha 2008) although this relationship was not observed in rocky outcrops from the Iron Quadrangle (Carmo & Jacobi 2015).

Very few studies have been conducted to test the relationship between metallicolous plant species distribution and metal concentration in soils. García-

Gonzalez & Clark (1989) detected differences between the distribution of *M. verna* and *T. alpestre* related to Ca, Cd, Pb and Zn in soils from the British Isles at a regional scale. Párraga-Aguado et al. (2013) founded that metal concentrations accounted for a minor role in the plant distribution of mine tailings from a Mediterranean semiarid area.

So far, two studies have tested the local spatial patterns of vascular plant species in metalliferous rocky outcrops. Ilunga et al. (2013) founded that Cu concentration was the most discriminant edaphic factor between different plant assemblages in a copper rocky outcrop from Upper Katanga, D.R.Congo; however, the same authors evidenced correlation for different edaphic factors (since they also found differences in soil properties due to horizon depth between habitats). In another study at the same geographic region, Séleck et al. (2013) identified a strong site effect in plant distribution.

Nunes et al. (2015) indicate soil properties as crucial in explaining the types and distributions of metallicolous vegetation associated with ferruginous rocky outcrops (*Canga*) in Carajás, eastern Brazilian Amazonia. Yates et al. (2011) similarly identified species habitat preferences associated with soil depth for banded iron plant communities in Western Australia.

This may suggest microsite constraints (restraining root development, accelerating water runoff) affecting functional group distributions (Carmo & Jacobi 2015; Carmo et al. 2016). This theory is supported by recent studies on plant traits focusing on the CSR strategy scheme (competitive, stress tolerant, ruderal) were fine-scale environmental differences explained better the distribution of distinct functional trait groups among highly diverse tropical grasslands (Negreiros et al. 2014) and were soil-related variables (depth, patch size) were the most important environmental filter influencing competitive vs. stress tolerant species proportions within an Atlantic rainforest inselberg (Paula et al. 2015).

The heterogeneous spatial and global pattern founded within functional groups evidences that the metal stresses (and consequently metal tolerance traits) are shaped by other adaptive strategies driven by major environmental filters which may explain better plant functional traits and species distributions and proportions in metalliferous rocky outcrops. Thus, not only geochemical but also geomorphologic heterogeneity would be a critically necessary condition to maintain the high natural biodiversity and the ecological functional processes in metalliferous rocky outcrops which may affect at regional scales the terrestrial biogeochemical balances.

The observed pattern in the present study fits into the theoretical context suggested by previous results in metalliferous rocky outcrops (Yates et al. 2011; Ilunga et al. 2013; Séleck et al. 2013; Serrano et al. 2014; Paula et al. 2015; Nunes et al. 2015; Carmo & Jacobi 2015). In addition, to the author's knowledge this seems to be the first study to test the relationship between plant species distribution and metal tolerance traits.

Considerations on some species as prospects for ecological modelling

In this study was found that *T. heteromalla* was clearly the dominant species across the studied environmental matrix, being mostly aggregated when species richness and abundance decreased. This may suggest a "dominant" resilience strategy among metallicolous vascular plant species, which decreases in relation to the availability of soil resources, were competitive strategies increase (see Negreiros et al. 2014). In the other hand *P. mediterranea*, the second species in dominance which is remarkably a "non-accumulator" seems to show a competitive strategy, suggested by its increase in density where the species richness increases.

A similar spatial and functional pattern (to that observed in *T. heteromalla*) was founded in the Mediterranean Al hyperaccumulator *Plantago almogravensis*, which was locally limited by shrub competition, also suggesting that the species has a poor capability to compete but has expressed the ability to find refuge in geochemical islands that are too harsh for most of the other species (Serrano et al. 2014).

When observing patterns of aggregation among different metallicolous vascular plant species *T. laniflora* was also the less co-occurring with the other studied taxa, which could be explained by a weak competitive ability in this environment. Baker & Brooks (1989) indicate that species confined to mineralized ground (referred as obligate metallophytes) are very sensitive to pathologies as fungal attacks (i.e. *Haumaniastrum robertii* and *H. katangense*) and it is probably this factor as well as their inability to withstand interspecific competition which restricts them to mineralized substrates in these environments. In that sense, resilience measurement rather than the metal tolerance trait of Al-accumulation could be a better predictor on this plant species distribution in ferruginous rocky ironstone outcrops.

Conclusions

Two different metal tolerance traits of foliar Al-accumulation and non-accumulation were identified among the studied vascular plant species. The most of the studied species were non-accumulators, but in the terms of relative abundances there was no clear dominant group between Al-accumulators and non-accumulators in this metallicolous plant community, since the Al-accumulators (Melastomataceae) represented half of the total abundance from all inventoried taxa.

For all studied species, there was a clustered spatial pattern and there was no relationship between spatial aggregation and foliar accumulation of metals or other chemical elements. Neither between density and foliar Al in the case of non-accumulators; but in the other hand, Al-accumulators showed a (inverse) density response to foliar Al concentration, which may reflect a phylogenetically functional trait among Al-accumulators, but further field and experimental research is required to identify the extent and causes of this pattern.

The relationship between density and species richness in *T. heteromalla* and the co-dominant *P. mediterranea* may suggest inversely related processes of environmental resilience and competition in this particularly stressed environment (heading to the CSR theory of Grime 2001), but experimental field research is required to prove that. The local spatial variation of the studied species seems to be more a product of adaptive strategies coping with major ecological filters than to eco-physiological traits of metal tolerance or avoidance.

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Appendix

Figure I-A. Plant material for foliar element concentration analysis: *T. heteromalla* (left, superior); *P. mediterranea* (right, superior); *S. reniformis* (left, inferior); *D. microphyllus* (right, inferior).

Figure II-A. Plant material for foliar element concentration analysis: *E. microphyllum* (left, superior); *Ouratea* sp. (right, superior); *E. erythropappus* (left, inferior); *B. variabilis* (right, inferior).

Figure III-A. Plant material for foliar element concentration analysis: *M. corallina* (left, superior); *T. laniflora* (right, superior); *B. reticularia* (left, inferior); *M. splendens* (right, inferior).

Figure IV-A. Plant material for foliar element concentration analysis: *M. marginata* (left, superior); *H. campestris* (right, superior); *A. rotunda* (left, inferior); *Myrsine* sp. (right, inferior).

Table I-A –Soil particle size distribution by percentage (%).

Plot sample	>4mm	>2mm	>1mm	>0,5mm	>0,25mm	>0,125	>0,063	<0,063
A2	49.0	31.5	7.7	2.9	2.9	3.0	1.6	1.3
A10	54.6	27.4	6.3	3.4	3.7	2.6	1.3	0.6
C2	49.6	21.0	8.8	7.0	5.6	4.1	2.7	1.2
C8	72.1	16.5	4.2	2.5	2.4	1.4	0.6	0.3
C10	65.4	16.2	5.2	3.2	4.3	3.2	1.6	0.8
C12	44.9	19.0	8.1	5.9	7.3	6.6	5.1	3.2
D1	48.4	19.8	8.6	6.6	7.1	4.8	2.7	2.0
E6	60.7	19.0	6.2	3.8	4.8	4.3	0.8	0.4
E8	72.6	14.3	4.4	3.0	2.6	1.6	1.0	0.6
G10	62.3	15.7	6.5	4.8	4.3	3.4	2.3	0.6
H3	64.6	16.1	5.7	4.1	3.4	2.6	2.1	1.4
H7	75.6	10.9	4.1	2.9	2.7	1.7	1.1	1.0
I4	29.1	17.0	47.1	2.4	1.8	1.1	0.8	0.6
I6	51.0	15.3	9.1	8.0	8.4	4.8	2.0	1.4
I8	49.3	22.0	11.4	6.1	5.1	3.2	1.7	1.2
J5	26.9	11.8	19.0	12.5	13.3	9.0	4.5	3.0
K4	76.4	11.1	3.1	2.9	2.8	1.9	1.2	0.5
K6	49.9	19.8	9.1	8.0	8.2	1.2	2.4	1.4
L7	57.6	20.2	6.9	4.3	5.2	3.3	1.5	0.8
E12	60.5	18.5	8.4	3.5	3.7	3.3	1.5	0.6

Table II-A - Foliar average concentration (mg.Kg^{-1}) of some chemical elements in 17 studied vascular plant species.

Species	Al	Ba	Be	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Sr	Ti	Zn
I	1243.33	63.04	0.081	6503.66	5.14	422.25	3361.04	2041.92	348.31	376.20	928.85	1504.64	30.82	3.90	16.62
II	135.11	2.72	0.081	4883.61	4.51	239.52	2449.14	2701.83	736.30	58.87	818.67	1352.80	11.24	2.39	47.05
III	160.48	16.57	0.081	6731.50	3.97	261.23	3108.87	2923.05	623.76	109.16	1133.99	1472.42	34.32	2.76	23.42
IV	223.03	31.83	0.088	10113.41	6.92	230.03	3318.33	2324.13	1483.51	404.65	760.21	1328.18	48.04	2.64	65.87
V	288.19	3.14	0.087	7200.41	5.58	391.92	1757.27	2262.20	415.05	145.43	823.55	1453.06	26.24	4.83	20.77
VI	59.85	24.78	0.079	8069.74	5.23	103.82	2120.17	1135.61	1639.86	115.89	595.11	1251.67	55.51	0.88	35.26
VIII	87.63	17.36	0.081	13071.75	3.99	160.91	1875.50	1468.27	777.99	64.52	544.27	967.12	68.63	1.38	18.82
IX	4195.29	59.08	0.084	15315.46	4.65	196.54	1757.07	3738.29	944.51	182.80	405.55	1456.83	113.44	1.91	49.32
X	1772.95	58.89	0.093	2843.50	3.08	222.59	1666.61	1424.23	313.58	1119.01	386.41	1001.56	55.81	2.29	25.51
XI	209.11	9.92	0.091	5520.26	17.41	287.69	3984.60	2385.41	2017.42	2055.86	1094.07	1989.91	21.93	3.47	67.11
XII	108.29	13.41	0.081	5140.45	7.49	140.22	2824.65	2172.79	1133.54	97.22	711.02	1645.61	26.47	1.50	24.38
XIII	155.56	20.26	0.083	8203.08	6.25	259.42	3819.47	4164.41	1472.39	206.86	1212.51	2064.24	38.64	2.43	30.51
XIV	166.68	9.45	0.088	12361.08	17.31	284.06	2563.71	1982.14	2606.06	177.94	822.94	1257.04	26.68	2.79	73.95
XV	125.95	8.07	0.082	8063.23	5.42	193.23	2264.83	2184.28	75.95	871.18	698.19	1744.00	49.07	2.04	13.62
XVI	59.36	47.81	0.078	8741.63	5.96	112.67	2828.09	2305.55	425.57	450.63	715.84	1391.26	53.83	1.04	15.57
XVII	162.35	10.79	0.086	6187.63	13.78	261.48	4893.01	2041.26	1023.48	391.62	899.97	1363.67	28.44	2.91	76.08
VII	325.52	8.23	0.085	5893.56	9.50	453.81	4592.41	2082.48	980.33	1609.79	1132.74	1651.15	27.67	5.66	71.82
QL*	**2.39**	**0.05**	**0.03**	**2.83**	**0.58**	**1.99**	**26.10**	**9.21**	**0.25**	**3.31**	**15.04**	**27.59**	**0.01**	**0.72**	**0.41**

*QL = quantification limit for each selected element.

Note: measurements on As, Cd, Co, Cr, Li, Mo, Ni, Pb, V and Y chemical elements were under the QL.

Table III-A. Dispersion indices.

Species	**Mean count**	**Variance**	**ID**	**ICS**	**GI**	**ICF**	**IMC**	**IP**	**IM**
B. reticularia DC.	7.77419	24.78065	3.18 (p = 0.00000)	2.18	0.07	3.55	9.96	1.28	1.27
B. variabilis A.Juss.	1.64	5.36	3.26 (p = 0.00000)	2.26	0.07	0.72	3.90	2.37	2.35
M. marginata Radlk.	6.96	62.36	8.95 (p = 0.00000)	7.95	0.26	0.87	14.91	2.14	2.10
M. corallina Spring	2.45	20.25	8.26 (p = 0.00000)	7.26	0.24	0.33	9.71	3.96	3.90
P. mediterranea (Vell.) Taub.	39.41	537.71	13.64097 (p = 0.00000)	12.64	0.42	3.11	52.06	1.32	1.31
T. heteromalla (D. Don) Cogn.	104.25	2513.46	24.10 (p=0.00000)	23.10	0.77	4.51	127.36	1.22	1.21
T. laniflora (D. Don) Cogn.	0.58	3.45	5.94 (p = 0.00000)	4.94	0.16	0.11	5.52	9.51	9.72

ID(Index of Dispersion) / ICS (Index of Cluster Size) / GI (Green's Index) / ICF (Index of Cluster Frequency) / IMC (Index of Mean Crowding) / IP (Index of Patchiness) / MI (Morisita's Index).

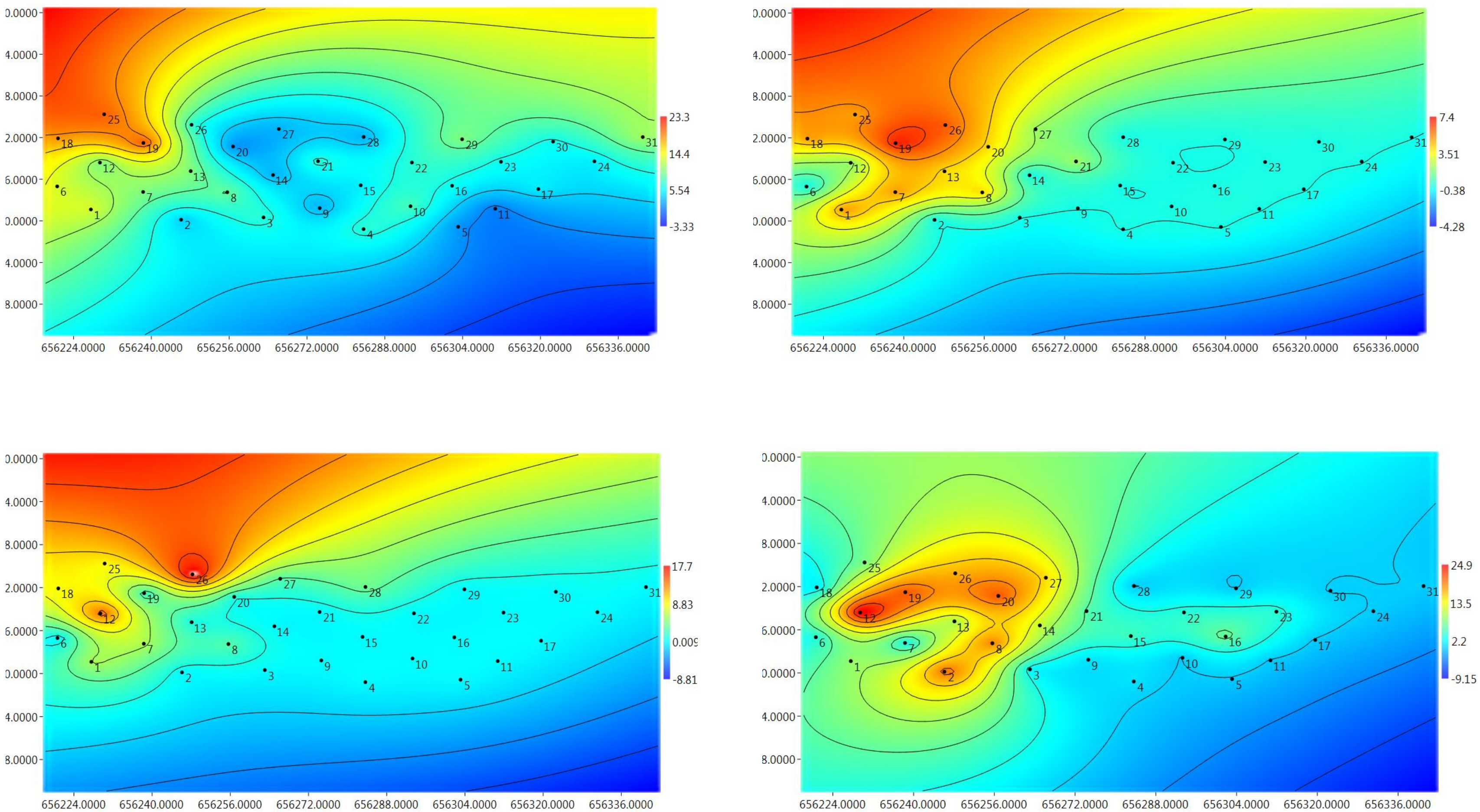

Figure V-A.Spatial distribution of *B. reticularia* (left, superior); *B. variabilis* (right, superior); *M. corallina* (left, inferior) and *M. marginata* (right, inferior) across the study area. Mapping method by ordinary kriging interpolation.

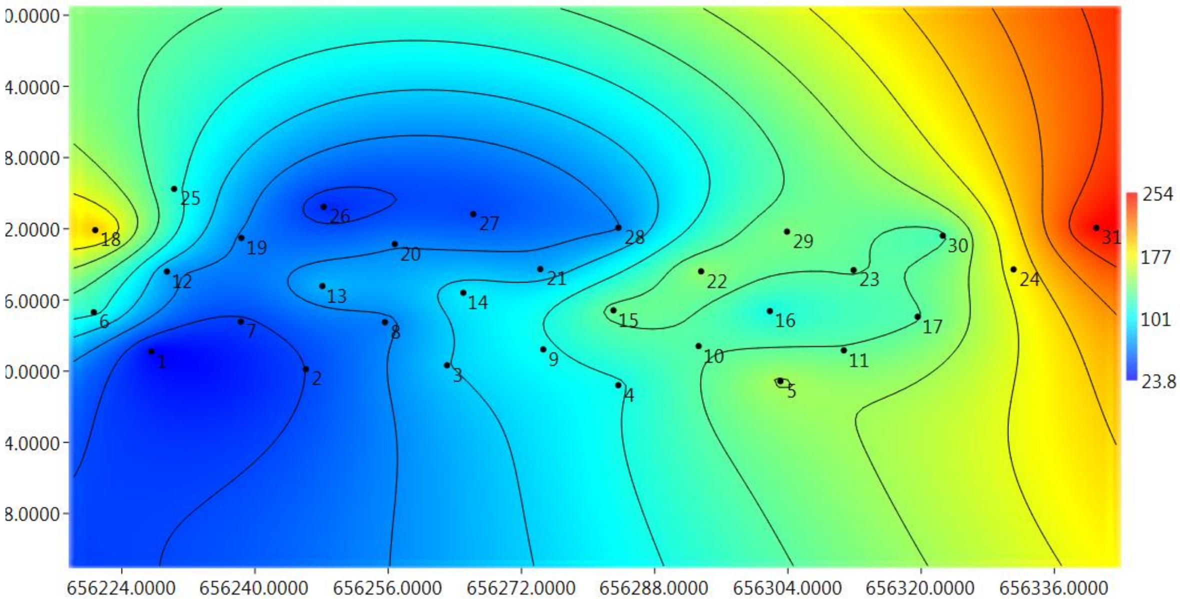

Figure VI-A. Spatial distribution of *T. heteromalla* across the study area. Mapping method by ordinary kriging interpolation.

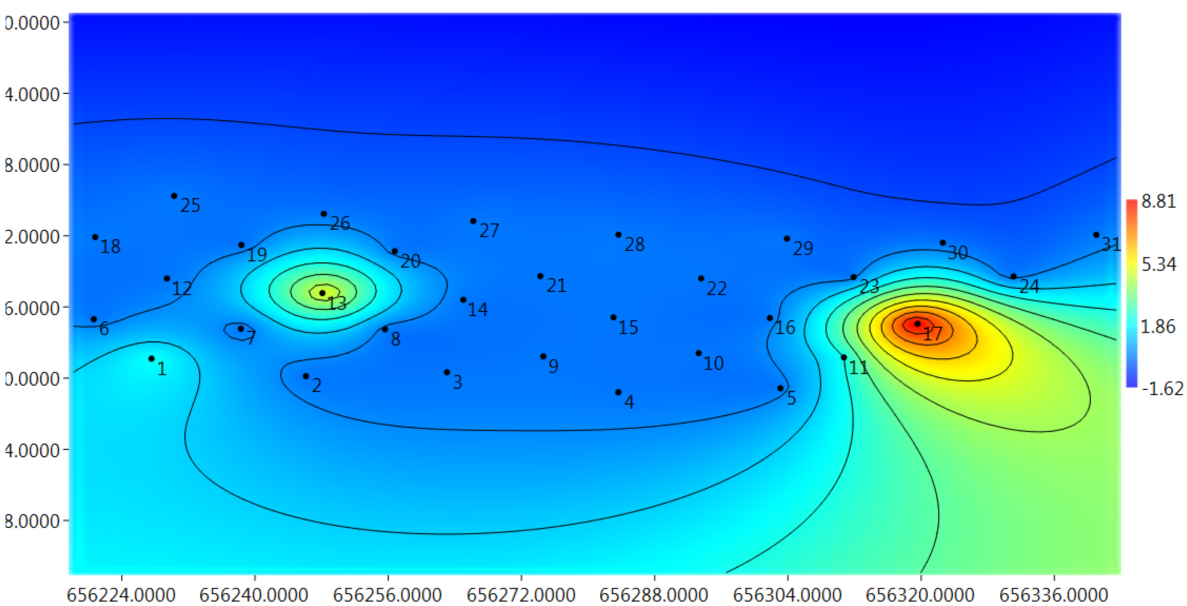

Figure VII-A. Spatial distribution of *T. laniflora* across the study area. Mapping method by ordinary kriging interpolation.

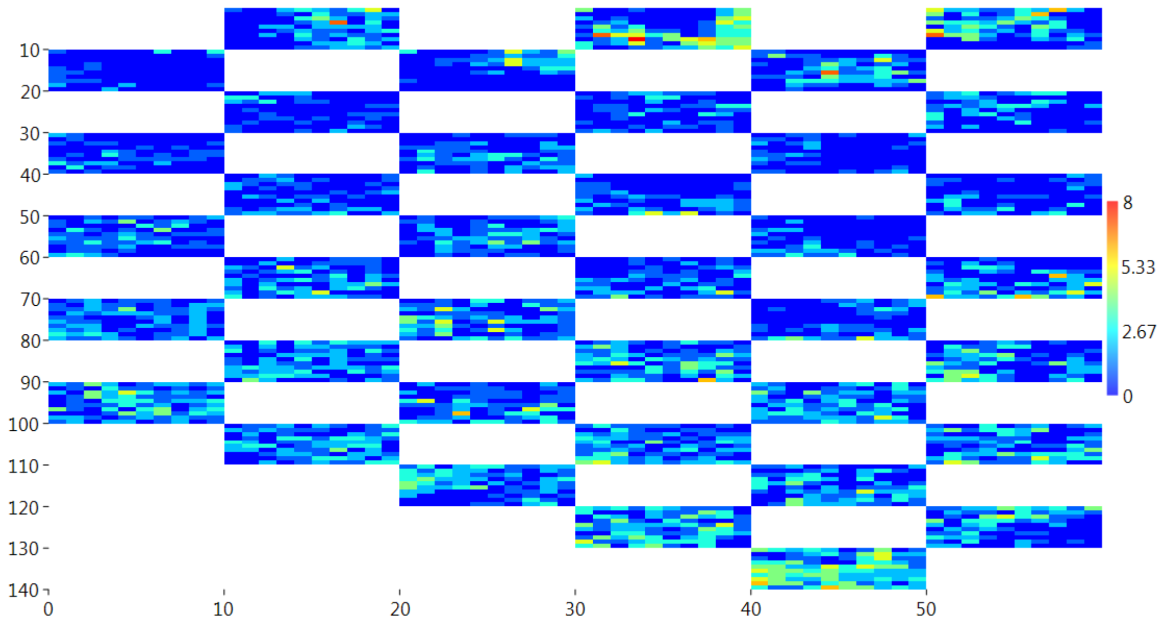

Figure VIII-A. Surface matrix plot of T. *heteromalla* across the studied area. The diagram shows a diagonal spatial pattern, also known as “bishop’s move” neighborhood (Dale 1999).

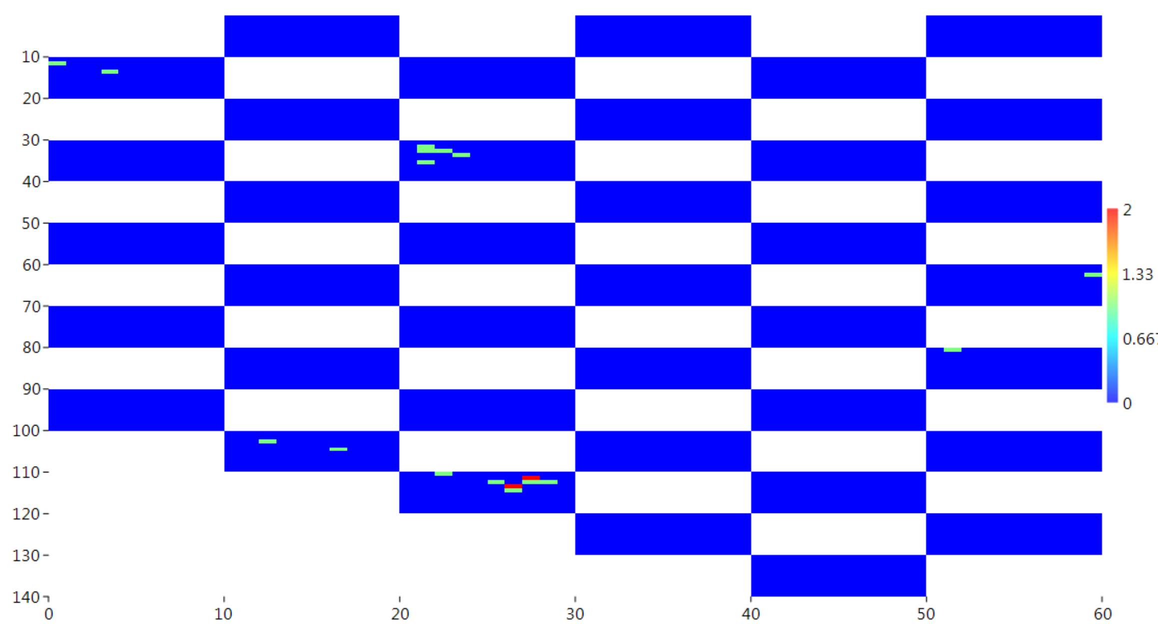

Figure IX-A. Surface matrix plot of *T. laniflora* across the studied area.

Attachment

Table I-aT. Comparative foliar concentrations of some metal elements in two metalliferous species from ferruginous rocky outcrops in Brazil.

Species			Chemical element concentration (mg/Kg^{-1})							Geographic region	Authors
	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn		
E. erythropappus*	0.53	nq	22.93	228.95	420.31	0.12	7.86	2.450	nq	Quadrilatero ferrifero - MG	Porto & Silva (1989)
	0.41	nq	26.58	258.33	443.75	0.32	4.64	4.070	nq		
	0.182	bql	11.6	395	454	nq	7.87	0.9	34.8	Quadrilatero ferrifero – MG	Teixeira & Lemos-Filho (1998)
	0,49	0,87	9,49	453,80	980,33	bql	5,21	bql	71,81	Quadrilatero ferrifero – MG	Present study
T. laniflora	bql	9.8	1.2	506	169		1.26	bql	14.9	Quadrilatero ferrifero – MG	Teixeira & Lemos-Filho, (1998)
	bql	0,62	2,91	212,73	334,82	bql	bql	bql	25,48	Quadrilatero ferrifero - MG	Present study

*Referred as *Vanillosmopsis erythropappa* (DC.) Sch.Bip on Porto & Silva (1989) and Teixeira & Lemos-Filho (1998)
nq = not quantified.
bql = below quantification level.
All average based values.