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RESEARCH PAPER

Perspectives of bioremediation of heavy metals with native plants of the Fabaceae family present in Paraguay

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Abstract

Industrial pollution is a worldwide problem because many effluents do not receive proper treatment before being released into watercourses. In Paraguay, leather tanning is a common industrial activity and its main contaminant is Chromium. This heavy metal accumulates in both soil and water and can be harmful to human health in large quantities. This study presents the native Fabaceae found in Paraguay and highlights those plants found in the Sub-humid Flooded Forest of the Paraguay River which could be explored in future bioremediation assays. On the other hand, the phylogenetic study of the *rbcL* gene sequences present in the databases showed that *Glycine max,* a model species of the Fabaceae family, has the closest phylogenetic relationship with *Erythrina crista galli*, for which gene studies could be carried out to propose new strategies for pollution reduction.

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Introduction

Plants are exposed to different biotic and abiotic stimuli and in turn, plants have the ability to give a response that gives them tolerance or resistance to the stimulus. Pollutants can be considered a type of abiotic stress and plants use different types of mechanisms to survive. Pollutants found in the environment can be organic such as petroleum, hydrocarbons, organophosphate compounds, pesticides, herbicide (Borges *et al.*, 2021) or inorganic such as heavy metals (Masindi & Muedi, 2018; Mensah *et al.*, 2021).

There are various ways of bioremediation through plants which is known as phytoremediation (Yan *et al.*, 2020), among them is phytoextraction which uses the ability of plants to remove contaminants from either water or soil and subsequently harvest them in the form of biomass (Kanwal *et al.*, 2019), the plants that have high capacities to accumulate are known as hyperaccumulators. In phytostabilization, plants reduce the mobility of the contaminant and make it less bioavailable to other organisms among them animals (Zgorelec *et al.*, 2020).

Phytodegradation uses the degradation mechanism by plants, and is usually mostly related to the degradation of organic pollutants (Zazouli *et al.*, 2014). In phytostimulation, plants release substances like carbohydrates that allow interaction with microorganisms and thus enhance the degradation of contaminants (Zahoor *et al.*, 2017). Phytovolatilization is carried out through transpiration or evaporation, which allows the removal of contaminants from a matrix, whether soil or water (He *et al.*, 2015) and releases it into the air.

In Paraguay, there are dry and humid environments such as those found in the Chaco Dry Forest, or those in the Humid Forest of the Eastern Region, respectively. Intermediate environments between these two extremes are the sub-humid forests such as the so-called Humid Forest of the Cerrado and the Sub-humid Flooded Forest of the Paraguay River (BSHIRP) (MADES, 2015). In this work, we will focus on the study of native plants belonging to the Fabaceae family found in the BSHIRP. This forest is of special interest for having a wide range of native plants that can grow in soils derived from marine and alluvial sediments. BSHIRP soils are usually, be not completely drained and, due to its high moisture, can be considered similar to the soils in which industrial effluents are disposed. Thus, plants that grow in this type of environment could have a potential positive role in bioremediation.

Materials and methods

Search for native species

The search for native plants was carried out with the Families and genera of trees from Paraguay handbook (Perez de Molas, 2016) and with the National Forest Inventory of Paraguay of native forest strata (MADES, 2015).

Search for contaminants and data analysis

The search was performed by Chrome by entering the name of the native plant with an additional search term that could be “remediation”, “uptake metal” or “contamination”. To perform the data analysis, the R language and the Plantico (Quintana *et al.*, 2021) package were used.

Phylogenetic analysis

The Ribulose bisphosphate carboxylase large chain (*rbcL*) gene sequences were downloaded from the GenBank database from National Center for Biotechnology Information (NCBI). The alignment of the sequences was carried out with the MUSCLE algorithm and the evolutionary history used for the construction of the phylogenetic tree was by the Maximum Likelihood method and Kimura 2-parameter using the MEGA 11 software (Tamura *et al.*, 2021).

Results and discussion

In Paraguay, the Fabaceae family has at least 55 species of native plants (Perez de Molas, 2016), of which 34 are found in the BSHIRP area. The study of the plants found in this region is of interest because could be considered that this type of soil is similar to the soils in which industrial effluents are disposed due to its high moisture content and a certain resemblance to the one that is found in areas of the riverbanks that sometimes present flooding of the coasts where the plants are found. However, should be noted that different variables can influence the growth of a plant, such as the difference in nutrients present on each soil.

In the sub-humid forest there are 13 native plants (Table 1) that have been used in other countries for bioremediation studies of heavy metals.

Table 1. Native plants of the Fabacea family in Paraguay.

Scientific name	Common name	Found in the sub-humid flooded forest of the Paraguay River	Used in bioremediation studies	Heavy metal
Enterolobium contortisiliquum	Timbo, oreja de negro	Yes	Yes	Cu, Zn, As (Rangel, *et al.*, 2014; Silva *et al.*, 2015; Silva *et al.*, 2016)
Parkinsonia aculeata.	Cina cina	Yes	Yes	Cd, Cr, Pb (Gonzalez-Villalobos *et al.*, 2021; Shahid, 1999)
Anadenanthera peregrine	Kurupa'y ita	Yes	Yes	As (Gomes *et al.* 2013; Gomes *et al.*, 2011; Gomes *et al.*, 2020; Gomes *et al.*, 2012)
Bauhinia sp.	Pata de buey	Yes	Yes	Pb , Mn, Cu, Cr, Mg, Zn, As, Cd Bhandarkar *et al.*, 2008; Kanwal *et al.*, 2019, Sharma *et al.*, 2017, Silva *et al.*, 2015)
Cassia sp	Canasita	Yes	Yes	Pb, Cd, Al, Hg, As, Zn, Cu (Annan *et al.*, 2013; Huang *et al.*, 2018)
Copaifera langsdorffii	kupa'y,	Yes	Yes	Cd, Cu, Pb, Zn (Asensio *et al.*, 2018; Meyer *et al.*, 2016)
Erythrina crista-galli.	Sui'yva, ceibo	Yes	Yes	Cr, Cu, Pb, Zn (Basilico & de Cabo 2018; Marco *et al.*, 2021; Scheid *et al.*, 2017; Scheid *et al.*, 2018)
Myroxylon peruiferum	incienso colorado	Yes	Yes	Cu (Marques *et al.*, 2018)
Parapiptadenia rigida	kurupa'y rã	Yes	Yes	Cu (Bicalho da Silva *et al.*, 2018; Silva *et al.* 2011)
Peltophorum dubium	Yvyra pytã	Yes	Yes	Cu (Marques *et al.*, 2018; Silva *et al.*, 2010)
Pterogyne nitens	Yvyra ro	Yes	Yes	Cu, Cr (Paiva *et al.*, 2014; Silva *et al.*, 2016)
Sesbania virgata	Unknown	Yes	Yes	As, Cu, Zn, Cr (Branzini *et al.*, 2012; Dias *et al.*, 2010)
Vachellia caven/Acacia caven	aromita; jukeri hovy; garabato negro	Yes	Yes	As, Cu, Pb (Jara-Medina, 2018; Pizarro *et al.*, 2015)
Albizia niopoides	Timbo moroti, yvyra ju	Yes	No	-

Scientific name	Common name	Found in the sub-humid flooded forest of the Paraguay River	Used in bioremediation studies	Heavy metal
Amburana cearensis	kumare, palo trébol, roble paraguayo, trébol, umburana	Yes	No	-
Acosmium subelegans	Unknown	Yes	No	-
Anadenanthera colubrina	kurupa'y kuru	Yes	No	-
Bergeronia sericea.	Yvyra itá	Yes	No	-
Caesalpinia paraguariensis.	guajakan, yvyra vera; sivipiruna	Yes	No	-
Cercidium praecox	Verde olivo, brea	Yes	No	-
Chloroleucon tenuiflorum	Tatare; guajakan arasa, pata de buey'i	Yes	No	-
Cynometra bauhiniifolia	Inga pytã	Yes	No	-
Geoffroea decorticans	chañar, mani de los indios. yvyra ajaka, manduvi guaikuru	Yes	No	-
Gleditsia amorphoides	Yvope, espina de corona	Yes	No	-
Holocalyx balansae	Yvyra pepe, alecrín	Yes	No	-
Inga uraguensis	Inga'i, inga guasu	Yes	No	-
Lonchocarpus fuvialis	yvyra ita; ka'a vusú, rabo de macaco, rabo ita; guatambú, palo de grasa, yvyra ñandy; yvyra moroti	Yes	No	-
Microlobius foetidus.	Yvyra ne	Yes	No	-
Mimosa detinens.	Araña niño	Yes	No	-
Platypodium elegans.	Unknown	Yes	No	-
Prosopis rubriflora	Algarrobo blanco	Yes	No	-

Scientific name	Common name	Found in the sub-humid flooded forest of the Paraguay River	Used in bioremediation studies	Heavy metal
Pterocarpus santalinoides	Pajaguá manduví, pajaguá manduvi mi, jatayva rã, yva rã	Yes	No	-
Samanea tubulosa	Manduvirã	Yes	No	
Zygia inaequalis	Guara pepe	Yes	No	-
Andira sp.	Unknown	No	No	-
Apuleia leiocarpa	yvyra pere, grapia	No	No	-
Ateleia glazioveana.	Timbo blanco, timbo raposã	No	No	-
Bowdichia virgilioides	Unknown	No	No	-
Calliandra foliolosa.	niño azote	No	No	-
Cyclolobium brasiliense	Quebracho	No	No	-
Dalbergia frutescens	Ysypo kopi	No	No	-
Dimorphandra mollis.	Unknown	No	No	-
Dipterys alata	Unknown	No	No	-
Guibourtia chodatiana.	kupa'y, kuruñai	No	No	-
Hymenaea stigonocarpa.	Jata uva	No	No	-
Machaerium acutifolium.	Jukeri vusu guasu, tanimbu yva, yvyra tanimbú; guajakan moroti, sapy'hu, ysapy'y hu	No	No	-
Mimozyganthus carinatus.	Lata	No	No	-
Myrocarpus frondosus	aju'y ñandú, cabriuva, kavure'y, yvyra paje, incienso	No	No	-
Piptadenia peregrina	Kurupa'y, kurupa'y ita	No	No	-
Piptadeniopsis lomentifera.	Yvyra hoví	No	No	-

Scientific name	Common name	Found in the sub-humid flooded forest of the Paraguay River	Used in bioremediation studies	Heavy metal
Plathymenia foliolosa	Morosyvo sa'y ju	No	No	-
Poecilanthe parviflora	Yvyra ita	No	No	-
Stryphnodendron rotundifolium	kurupa'y, timbo uva	No	No	-
Sweetia fruticosa	Taperyva guasu	No	No	-
Tachigali aurea	Sucupira	No	No	-

Having plants capable of carrying out bioremediation of heavy metals is of vital importance due to the adverse effects that heavy metals can have on human health. Heavy metals can affect cellular organelles and cell wall components and metal ions can cause DNA damage due to its interaction with DNA and nuclear proteins, which leads to apoptosis or carcinogenesis (Briffa *et al.*, 2020). Copper (Cu) is the most studied metal in native fabaceas that can be found in the BSHIRP as shown in Fig. 1; of 13 plants, 11 plants involve this metal in their studies (85%) followed by Arsenic (As), Lead (Pb), Zinc (Zn), Chromium (Cr), Cadmium (Cd), Aluminum (Al), Mercury (Hg) and Manganese (Mn) with 46%, 46%, 46%, 38%, 31%, 15%, 8%, 8% of the total studies, respectively. Most of the studies involve As and Cu because they are the most common pollutants derived from mining activities (García-Salgado *et al.*, 2012) and are also reported in agricultural soils (Hou *et al.*, 2020; Nuralykyzy *et al.*, 2021). Mining is not a highly exploited activity in Paraguay because this country does not have large deposits where the mining activity can be carried out.

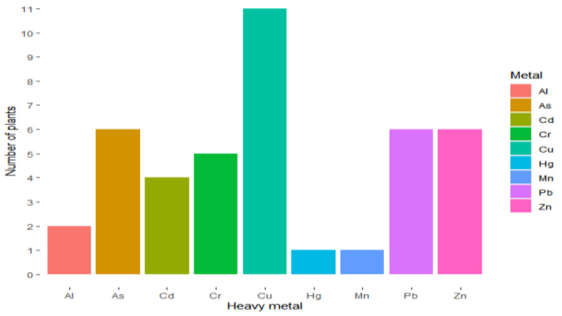

Fig. 1. Native plants in BSHIRP area involved in metal studies.

Besides, Cr has acquired greater relevance because is obtained as a residue from different tanneries that often send their effluents without prior treatment directly to the riverbeds hydric.

The plants that are involved in studies with Cr and could be interesting to evaluate in in Paraguay are *Bauhinia* sp., *Erythrina crista-galli Parkinsonia aculeata*, *Pterogyne nitens*, and *Sesbania virgata*.

We must keep in mind that even though these plants could extract the metal from the soil, a limitation of the process is the amount of metal that can be recovered; however, if the metal is not absorbed plants may still have the ability to stabilize pollutants (Basílico & de Cabo, 2018; Huang *et al.*, 2018; Zgorelec *et al.*, 2020).

It should be noted that phytoremediation strategies can be enhanced if chemical studies are combined with molecular biology studies of those plants. Taking into account the progress of molecular techniques and new sequencing technologies, could be expected that there is a lot of gene information in all plant species, including the native ones, however this is not always the case.

In most of the native species of the BSHIRP, the databases only have sequences for the identification of plants, as observed in Table 2, where the access code to the *rbcL* sequence can be visualized, which is a common marker for the identification of plants.

Table 2. Access code and size of rbcL sequences from the GenBank database.

Scientific name	GenBank accession number	Size bp
Enterolobium contortisiliquum	MG833534.1	585
Parkinsonia aculeata	OL537716.1	553
Anadenanthera peregrina	MG833517.1	523
Bauhinia variegata	AF387981.1	510
Bauhinia purpurea	AF387980.1	510
Bauhinia forficata	MG833519.1	579
Cassia alata/Senna alata	JQ301848.1	607
Copaifera langsdorffii	MT304243.1	700
Erythrina crista-galli.	MK238893.1	551
Myroxylon peruiferum	MG833552.1	527
Parapiptadenia rigida	MG833554.1	572
Peltophorum dubium	MG833555.1	588
Pterogyne nitens	MG833561.1	530
Sesbania virgata	MG833567.1	558
Vachellia caven/Acacia caven	Z70145.1	1368
Glycine max	Z95552	1420
Oryza sativa	MK932669.1	850

Although the information of native plants is limited to these identification markers, gene studies could be started based on the previous information that exists in other model plants that do have a lot of information, such as *Arabidopsis thaliana*, *Oryza Sativa*, *Zea mays* and *Glycine max*, the latter is of special interest because belongs to the Fabaceae family.

After searching for the *rbcL* gene, sequence alignment and phylogenetic tree construction were performed to see which of the species could be more closely related to *G. max*. As can be seen in Fig. 2, the phylogenetic tree indicates that *E. crista galli* would be the one with the greatest relationship to the model plant *G. max*. In addition, was observed that *O. sativa* functioned correctly as an outgroup as it is a plant of the Poaceae family.

Fig. 2. Plant phylogenetic tree based on the *rbcL* gene.

These results could indicate that a particular sequence, for example the glyoxalase I (*GLYI*) gene sequence present in *G. max* which overexpression provides tolerance to stress by heavy metals (Ghosh & Islan, 2016), could also be found in *E. crista galli*. This type of gene similarity search can complement existing chemically focused information on native plants and improve phytoremediation processes.

Conclusions

The studies reported to this date indicate that there is a wide range of native plants in the sub-humid forest of Paraguay that are already explored in other countries as potential phytoremediators, it will also be shown that *E. crista galli* is a plant that has an interesting phylogenetic relationship with *G. max*, which makes it a candidate for molecular biology studies. Thus, these plants would become a practical strategy for future events of contamination from anthropic activity. In Paraguay, the main contaminants that could be removed with this strategy are heavy metals such as chromium, whose importance lies in the fact that it is one of the main contaminants of industrial effluents, in this way future tests can be proposed with some fabaceas that already have background uses for this kind of contaminant.

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