

# Biogeochemical aspects of selected elemental content in *Ilex paraguayensis* S.H from Eastern Paraguay

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**Abstract**— *Yerba mate, Ilex Paraguayensis, is a plant of Paraguayan origin used in infusions/macerations by the ancient inhabitants of Paraguay as a “reviver”/energy beverage and mineral supplier which consumption is lasting up today; furthermore, it is extended almost worldwide. In regard to its mineral content very few studies are known; moreover, none has been published related to the occurrence of REE (rare earth) and other refractory elements in the leaves. In this work, minor and trace elements composition have been investigated by XRF techniques to determine their correlation as well as provenance. The analysis of complex spectra was performed by the AXIL software and the quantitative analysis by the QAES software. Analyzed trace elements were the refractory Rb, Sr, Y, Zr, Nb, Ba, La, Ce, Nd, 3d as Ti, Cr, Ni, Cu, Zn. Minor elements were Mn, Fe which are often related to the above refractory together with S and K.*

**Keywords**— *Ilex Paraguayensis, uptake of minerals, elementary content, refractory and rare earth elements.*

## I. INTRODUCTION

The role of mineral constituents and nutrients is of remarkable importance for *Illex paraguayensis*, according to its generally mentioned/accepted properties. *Ilex paraguayensis* or *Ilex paraguariensis* (Aquifoliaceae), is a small tree of Paraguayan origin called *yerba mate* in Spanish and *Ka'a* in Guaraní (*Paraguay is a bilingual country. Official languages are Spanish and Guaraní*), and is used as infusions in hot water (*mate*) or as a maceration in cold water (*tereré*) by the ancient inhabitants of Paraguay as a “reviver”/energy beverage (*According the Paraguayan Codex Alimentarius the minimum caffeine content shall be no less than 1%*) and mineral supplier whose consumption continues and is expanded to these days especially in Paraguay, Argentina, Brazil and Uruguay; furthermore, it is used almost worldwide as an infusion. When people, including those who are undernourished, drink in appropriate amounts any of these beverages, they gain/recover strength and their working yield improves. (*Usually workers are entitled to a “mate break” or “tereré break” of about 30 min*). In the *Codex Alimentarius* of Paraguay and elsewhere *yerba mate* is considered as a true food stuff [1-2].

Mate tea, as it is cited in [3] has, due to some of its components, important pharmacological properties. Chlorogenic acid and caffeoyl derivatives, among others polyphenols such as tannins, rutin etc, contribute prominently for its antioxidant capacity. Xanthines, as theophylline, theobromine and caffeine (the latter present at higher concentration), account for diuretic, CNS stimulant, hepatoprotective as well as other biological/ pharmacological properties. Saponins (so called matesaponins) in addition to their role in the flavor, have hypocholesterolemic and antiinflammatory properties; some of them have antiparasitic effects, *inter alia* anti-trypanosomal, due to its content in tri-terpenoids (IC<sub>50</sub> around 4µM for *Trypanosome brucei*). An excellent and comprehensive review in this regard is presented in [4& references therein].

The traditional way of manufacturing the *yerba mate* or *yerba* (*as the product is called*) is as follows: after raking leaves, twigs, and petioles, one proceeds to make bundles which are quickly passed over the flames of a bonfire, for achieving by fast evaporation a desiccation that prevents the decomposition of the *yerba*; the fire must be strong but without smoke; (a treatment called *sapecado*). Then comes the roasting, made over a framework of canes and sticks, done very carefully to

avoid the loss of the green of the leaves. Afterwards, the material is submitted to milling [5]. The product consists of a powder-like mixture of tiny crushed leaves and small sticks.

Despite its wide use in Paraguay few papers & reports were produced in the country, such as those from the middle of the 20th century [6-8]; more recently, two interesting and recommended papers can be cited [9,10] & references therein, mainly related to minerals content, as well as others more recent in similar line [11,12]. Also some more can be cited from the neighboring countries of Brazil and Argentina, *inter alia* at the State do Parana [13,14] in the former at the Provinces of Misiones and Corrientes in the later, [15,16] all of them bordering the East of Paraguay; just for expand the comparison, some other *data*[17] are also included.

They are mainly related to their multi-elemental contents; many of them, such as the essential microelements, are of utmost importance for living organisms.

An aspect very little considered is the geochemical approach, which represents a significant perspective on the ecology of plant nutrition [18-20], particularly in relation to their content of incompatible and refractory elements and their relationship in the *Ilex Paraguayensis*.

In this work, the composition of selected minor and trace elements have been investigated by EDXRF (Energy Dispersive X-Ray Fluorescence) techniques to determine their correlation as well as *provenance*. Analyzed elements were: K, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Y, Zr, Nb, Ba, La, Ce, Nd .

## II. MATERIALS AND METHODS

### 2.1 Materials

The materials analyzed were from the north and the south of the Eastern Region of Paraguay main production areas of *Ilex*. In this way were analyzed:

#### 2.1.1 Specimens of different commercial brands of yerba mate

For the analysis, packages of samples were selected from major production areas, that is, in the north (Areas of San Pedro and of Pedro J Caballero and in the south, Itapúa,(Area of Bella Vista) in the Eastern Region of Paraguay. Thus, were analyzed samples from different brands taken at random in several shops four kg of 0,5 and 1kg packages , ea of the same brand. They are constituted by ground leaves and shoots mixed with small fragments of petioles and twigs, called usually sticks, whose presence in the product is admitted up to no more than 35% according to the art 1193 of the Código Alimentario Argentino. Moisture ranges from 8.3 to 12.5%.

#### 2.1.2 Specimens of leaves from fresh plants

Leaves, shoots, petioles and twigs were collected at localities of Azote'y in the North and of Capitán Miranda in the South (Itapua). Samples were taken at random from at least 12 plants in each cropping site. At the laboratory, materials were dried over night under a fan at room temperature, crushed, dry again and ground.

Composite samples from each pkg were prepared by quartering, being then dried at 105°C in an oven, ground and sieved. Samples from fresh plants were prepared in a similar way. For XRF measurements, the powdered samples were pressed into pellets of area weight of ~ 0.1 to 0.3 g.cm<sup>-2</sup>.Samples from commercial products were analyzed in Asunción while those from fresh materials in Ljubliana.

#### 2.1.3 Ashes samples

In order to check the results, aliquots of A and B materials were reduced to ashes at 550°C in an oven. Ashes content range from ~ 4.0 up to 6.5 %.

The distribution of sampling stations appears in **table 1**, including the soil typology.

**TABLE 1**  
**ILEX SAMPLING SITES**

<b>North: Departments of S. Pedro – Amambay</b>		
	<b>Coordinates</b>	<b>Soil tipology</b>
Nueva Germania (NG)	23° 54' 41.712" S, 56° 41' 56.734" W	Ultisol – Sandy from sandstones
Azotey (AZ)	23° 19' 7.855" S, 56° 29' 17.147" W	Ultisol – Sandy from sandstones
P.J.Caballero (PJC)	22° 32' 45.586" S, 55° 43' 55.622" W	Inseptisol– Sandy from sandstones
<b>South: Departments of Itapua</b>		
	<b>Coordinates</b>	<b>Soil tipology</b>
Cap. Miranda (CM)	27° 12' 53.592" S, 55° 47' 48.306" W	Oxisol– Sandy clay from basalt
Bella Vista (BV)	27° 3' 0.000" S, 55° 33' 0.000" W	Oxisol –Sandy clay from basal

## 2.2 XRF measurements and analysis

The XRF measurements and quantification were performed utilizing the facilities of the XRF laboratories at the Jožef Stefan Institute in Ljubljana and at the Atomic Energy Commission in Asunción. For the excitation of the fluorescence radiation the radioisotope sources of Cd109 (30 mCi) and Am-241 (100 mCi) were utilized as well as a X-Ray tube with the Mo anode & Mo secondary target. The energy dispersive X-ray spectrometer was based on Si (Li) semiconductor detectors. The analyses of complex spectra were performed by the AXIL software [21] which is based on iterative nonlinear least square fit of the spectra by the Gaussian shaped spectral lines. The resulting intensities of pure  $K_{\alpha}$  and  $L_{\alpha}$  lines of measured elements were then utilized in quantitative analysis, employing the quantification software of QAES (quantitative analysis of environmental samples) designed by P. Kump [22,23]. This software utilizes the i.e. transmission-emission (TE method) method for determination of the absorption in the sample and then iteratively finds the solution of the system of basic XRF equations (for each measured element there is one equation). The basic XRF equation namely relates the measured intensity to the respective concentration of the element in the sample. Since this relation is nonlinear and the intensities depend also on concentrations of all unmeasured elements, the information of the absorption in the total sample at single energy (Mo  $K_{\alpha}$  line) obtained by the mentioned TE method is crucial in solving such a system of equations. On the other hand the quantification would be possible only if a set of standards very much resembling the unknown samples would be at hand to perform the necessary calibrations. The absorption measurement on the sample was in principle equivalent to additional measurements on a set of standards.

The uncertainties of elemental concentrations obtained by the QAES software were assessed to be between 5 % and 15 % which has been confirmed by the analysis of some standard reference materials (RM Soil-7 and Sediments SL-1, SL-3, V8Flour rye from International Atomic Energy Agency, and Orchard leaves 1571 from NIST).

The preparation of the samples, were carried out at the Laboratory of Hydroconsult in Asunción.

## III. RESULTS AND DISCUSSION

The primary uptake of metals from soils is through the root to the plant system [24]. In the rhizosphere the ability of the plant to absorb elements is often limited by their availability at the root surface; the concentration and oxidation state of them are significant in such an availability. In the dissolution of metallic compounds of the soil, pH and redox potentials, as well as the formation of complexes, play an important role in releasing the element, incorporating it into the soil pool [25,26]. Then, assisted by microorganisms activities as well as by the interactions of the root surface, which has the ability to release compounds that alter the solubility and bioavailability of nutrients, they can be absorbed and transported.

Rock minerals, silicates, oxides, sulfides, carbonates, sulfates, etc., due to weathering, undergo alterations, modifications, originating various compounds that precipitate or co-precipitate or become solubilized, being incorporated into the soil solution. In regard to 3d series, from Sc to Fe most of their minerals are oxides, while from Co to Zn, sulfides. In the soil solution they can be present as aquo-complexes according their most stable/important oxidation state  $Ti^{4+}$ ,  $VO^{2+}$ ,  $Cr^{3+}$ ,

$Mn^{2+}$ ,  $Fe^{2+}$ ,  $Co^{3+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$  [27], That compares, in broad sense, the relative stability in solution of 3d metals complexes, which order is  $Mn < Fe < Co < Ni < Cu > Zn$  according to Irving Williams Rule (IWR) [28].

Generally, the “3d” elements do not maintain the original geochemical composition of the precursor materials. The elements are considered mobile and this mobility is related to their oxidation states: is very low for states +3 and +4 and fairly high for the +2 state. As they usually do not precipitate directly (but Fe and Mn), their incorporation into sediments and eventually in the soil solution, are mainly due to co-precipitation processes. [29].

In general, their lower valence states are the most convenient, in this case  $M^{2+}$ , on the availability of the 3d series elements.

At  $pH < 5.5$ , which is the case for most of the soils at the *yerba* cropping area in Eastern Paraguay, ( $pH$  ranging from 4.50 to 5.50), in reducing ambience metals oxides are reduced to  $M^{2+}$ , increasing their availability. On the other hand these soils are low in organic matter (OM) with negative effect on such availability. Besides the humic complex that strongly affects the biogeochemical fate of micronutrients, a variety of other organic molecules can reduce and dissolve metallic oxides [30].

The humic complex are constituted basically by fulvic and humic acids (FA, HA) fractions. HA with various metals, forms much more stable complex than those formed with FA. Thus, the former are only partially soluble while FA metal complexes are more soluble: therefore more at hand to for the roots.

Recently, studies carried out in complexes of some transition metals, with ligands of carboxylic and phenol functional groups in humic substances (O-donor humic-like ligands) 1:1, indicate the following stability order  $Co (II) < Ni (II) < Cu (II) > Zn (II)$ , ie, in accord with IWR; however, if Pb (not transition) is included, its complexes with such ligands are comparatively more stable and the order results  $Pb >> Co < Ni < Cu > Zn$  [31]. On the other hand in a study of complexes of several metals with HA isolated from soils, the order of their stabilities results:  $Cu > Fe > Pb > Ni > Co > Ca > Cd > Zn > Mn > Mg$ . not following IWR [32]. This suggests that the complicated structure / composition of the humic substance and the concentration of the different functional groups present play an important role in the stability of the metal complexes. According, *inter alia*, another recent paper [33] the order of the binding capacity of these metals with HA is:  $Pb > Ni > Cr > Cu > Cd$  while with FA is  $Cr > Pb > Ni > Cu > Cd$ .

The average absolute values of K, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr occurrence in three different commercial brand materials from three Departments are shown in Table 2. The results for the fresh leaves analysis of Rb, Sr, Y, Zr, Nb, Ba, La, Ce, Pr, Nd are breaking down in Table 3.

**TABLE 2**  
**MINERAL CONTENT OF COMMERCIAL PRODUCTS**

	Ti	Cr*	Mn	Fe	Ni*
San Pedro (whole)	18.6±3.7	1.1± 0.13	510.25 ± 42.7	844.75± 57.02	1.7 ±0.5
San Pedro (leaves)	17.25±1.33	1.1±0.13	776.3± 22.2	1433.8±126.3	1.7±0.4
PJC (whole)	18±1.54	1.1±0.14	609.8± 60.3	620±110.12	1.6±0.7
PJC (leaves)	17.6 ± 2.1	1.16±0.11	624.95± 63.8	1034.48±111.74	1.74±0.98
Itapua (whole)	38.2±3.8	4.2±0.7	213.36± 29.2	213.36±29.171	1.248±0.524
ItapuaA (leaves)	37±4.0	4.4±0.7	1036± 150	263.2 ±19	1.6 ±0.38
	Cu*	Zn	K	Rb	Sr
San Pedro (whole)	6.57 ±1.76	38.17 ±6.02	16653± 1824.	20.36 ± 0.93	34.47 ±1.66
San Pedro (leaves)	7.34±1.26	43.92±3.65	19100.± 614.	29.82±1.95	45.12±0.88
PJC (whole)	9.2±1.5	36.7±6.4	16170.± 2398	23.4±2.4	25.72±2.51
PJC (leaves)	8.4±1.5	38.1±3.4	16507 ± 3295	27.8±3.1	38.4±2.67
Itapua (whole)	13.8±1.9	100.2±13.0	22132 ± 2445.	21.3±2.5	26.5±20.25
ItapuaA (leaves)	15.4±2.1	114.5 9.46	22107 ± 1080	21±2.5	36.5 ±3.2

\*recalculated from ashes

**TABLE 3**  
**ANALYSIS OF FRESH LEAVES**

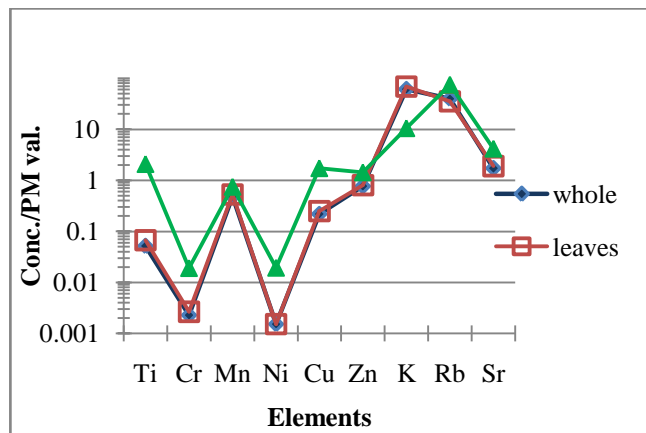
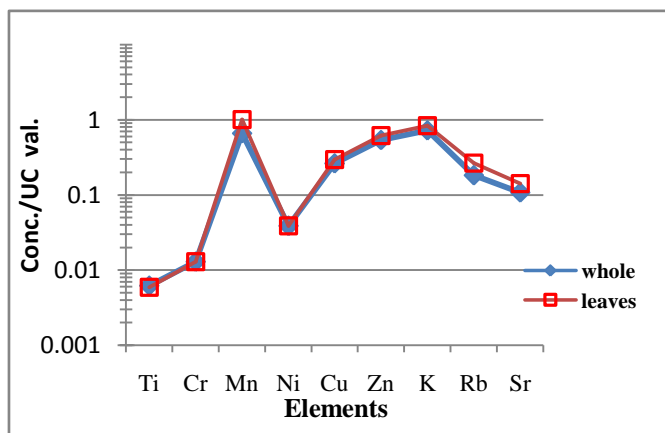
	K	Ti	Cr*	Mn	Fe	Ni*	Cu*	Zn
S1 (Azotey	7948 ± 916	21±2.6	2.0±3.1	625±64.9	295±24.9	5.4±0.8	11.9±1.2	19.7±1.8
S2 (Cap.Miranda)	25300 ± 2130	45.0±4.5	5.5±1.0	1680±142	318±26.9	9.8±21.5	18.1 ±1.9	188±24.1
	Rb	Sr	Zr*	Nb*	Ba	La*	Ce*	Nd*
S1 (Azotey	16.6±1.8	46.5±4.13	1.0±0.11	0.06±0,008	25.7±2.1	1.3±LOD	1.4± LOD	1.99±0.6
S2 (Cap.Miranda)	44.9±4.1	42.7±3.9	1.1±0.15	0.06±0.008	56.1±4.4	2.0±0.5	1.6±0,20	2.2±0,25
	Y*							
S1 (Azotey)	0.33±0.06							
S2(Cap.Miranda)	0.7±0.09							

\*recalculated from ashes

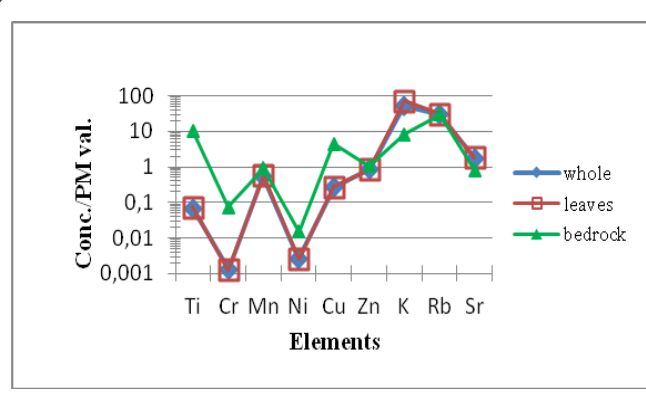
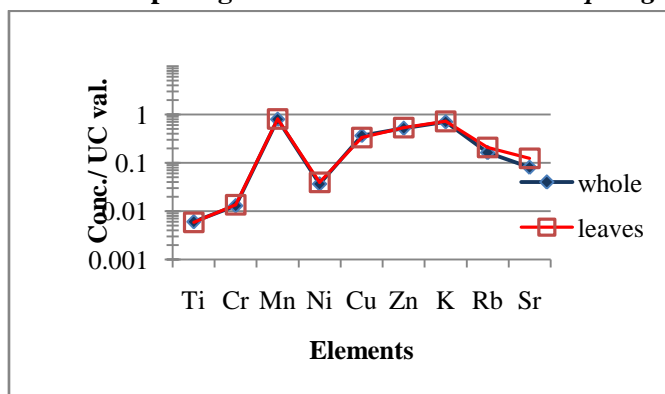
For comparison, correlation and *provenance* studies, as per the method widely used in geochemistry, the content of mineral components (specially at trace level) in soils and plants must be standardized in relation to their concentration / recommend values in primordial materials like, as chondrite, primordial mantle (PM), upper crust (UC), that is, by building the arachnograms or multi-element diagrams; the unit value obviously corresponds to those of the analyzed component layer [34,35].

Here we will refer to PM and to UC in relation to processes/products resulting from the *major* and the *minor* geochemical cycles [ 36,37].

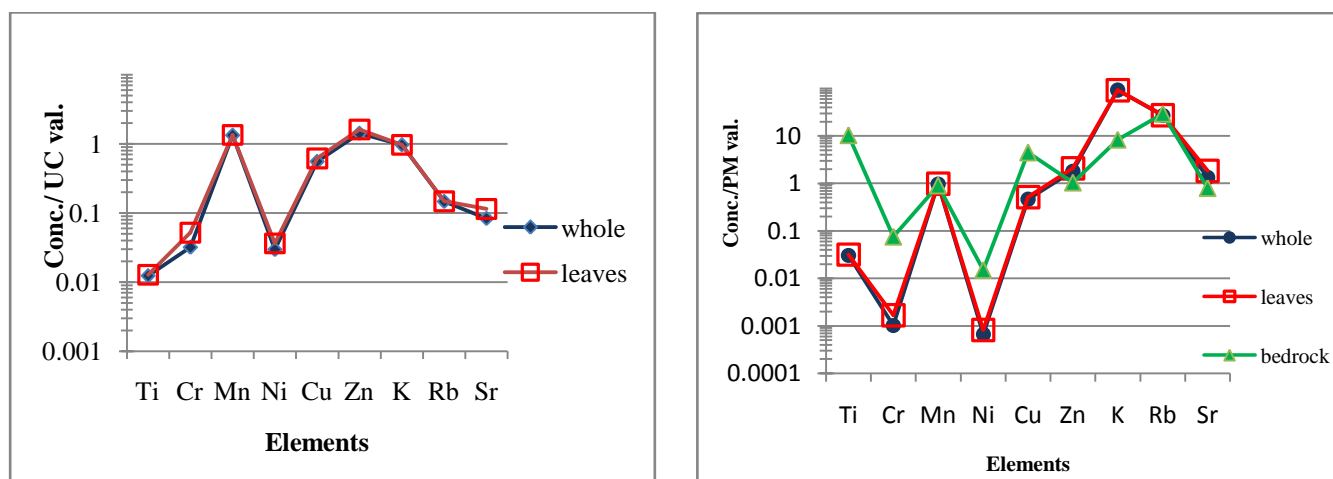
Thus, in figures 1a, 2a and 3a are presented the spidergrams of the results of the samples from San Pedro, PJ Caballero and Itapúa respectively, normalized to UC recommended values. The higher values indicate that the element is concentrated at the point of sampling.



**FIGURE 1 a: Spidergrams of whole & leaves of *I. paraguayensis* from San Pedro standardized to UC values.**  
**b: Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standardized to PM values**



**FIGURE 2 a: Spidergrams of whole & leaves of *I. paraguayensis* from P.J. Caballero standardized to UC values.**  
**b Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standardized to PM values**



**FIGURE 3 a: Spidergrams of whole & leaves of *I. paraguayensis* from Itapua standardized to UC values.**  
**b: Spidergrams of whole & leaves of *I. paraguayensis* and bedrock standardized to PM values**

The absorption of transition elements in yerba mate is little known [3]; efforts have been although made on the "complex paleo history" of the genus *Ilex* (~600 species) and besides, some species from Sud America such as *I. dumosa* and *I. guayusa* which infusions are also used as the *mate* [38-40]. In other plants it has been found the action of different NRAMPs in their absorption, transport and homeostasis. (NRAMP or natural resistance associated macrophage protein refers for an integrated membrane protein) [24].

### 3.1 Elements in the *Ilex* commercial samples

Uptake and distribution of  $K^+$  in plant cells is carried out by a variety of transporter proteins categorized into several families with varied structures and transport mechanisms that comprise the channel families *Shaker*-like voltage-dependent [41]. The  $K^+$  is strongly incompatible in the mantle melting and in basalt differentiation. During weathering and by hydrolysis, feldspar originated clay minerals rich in K that remains as a residue and thus the concentration of dissolved  $K^+$  in inland waters is low. [36]. It is an essential element for plant development and is considered as a macronutrient, taken in account the amounts absorbed by plants such as the yerba mate. The element promotes esteric changes in a great number of enzymes exposing reactive centres for chemical attack. It is essential for the synthesis and function of chlorophyll. It must be mentioned that potassium ( $K_2CO_3$ ) also neutralizes various anions and other compounds within the plant [25].

In the case of *yerba mate*, the plant concentrates K (See fig) and its recovery in dissolution is high [7-11]; its alkalinity (methyl orange) according to references 7 & 8 is around 22mg in  $K_2CO_3 \cdot L^{-1}$ . This supports the explanation given in [6,7] on the effect of .K of the neutralization into the lactic acid/lactate/  $[H^+]$  system, which promotes muscular fatigue. This should explain why people drinking the beverage, gain/recover strength and their working yield improves.

Essential microelements as well as of the other analytes found here, are also within the range of those from several studies on yerba mate and other vascular plants made elsewhere [12-17 & ref. therein].

It has been mentioned the existence of several large families of metal transporter genes. Members of the ZIP a metal transporter family first identified in plants, are capable of transporting cations, of the *transition d series*. [42]. Another important transporter of divalent metals are members of the Cation Diffusion Facilitator (CDF) super family and NRAMP homologues. In addition, members of the vacuolar cation proton exchange (CAX) and of the ABC transporter family are involved in metal homeostasis in plant cells [43].

Cations of the "d" elements like Cr, Cu, Fe, Mn have unpaired electrons that allow their participation in redox reactions. Several of the biological toxic effects of these elements can be explained by their capacity to catalyze the initiation of free radical reactions. On the other hand, Zn without unpaired electron however, functions as antioxidant when it replaces metals that are capable to catalyze free radical reactions; also, *inter allia*, Zn acts as a structural or regulatory co-factor for a certain number of enzymes.

Mn usually occurs in *Ilex paraguayensis* at relatively high concentration (see Table 2) perhaps also as a normal constituent of oxidizing enzymes. Small amounts of  $Mn^{+2}$  in the oxidases and peroxidases accelerate their oxygen carrying power. The major part of Mn in soils is in the higher oxidation states and only in very small quantities as  $Mn^{+2}$ . Fig 1a-3a show, in

comparison with the upper crust values, that the element is concentrated in the *yerba mate*; note in addition, that samples from the south present higher concentration [3].

Cu is a component of proteins found in enzymes that regulate the role of many reactions in plants, which would not grow without their specific presence; therefore, Cu plays essential roles in a wide range of physiological process (an example: chlorophyll formation) and promotes seed production and contributes to plant formation. A lower threshold (Cu deficiency) is not known but like several other plants, *Ilex* could be very sensitive to a low level concentration of Cu [20].

Ti is generally present in plants in low concentrations but is very dependent on the soils (6.12 to 85.1 mg.Kg<sup>-1</sup> in Chong Qing; with a wider range in different regions of China. [44] In Paraguay Ti has been studied in sandstones and its content ranges from 0.39 to 4.62mg/g in rocks from the Misiones and Eusebio Ayala Formations respectively [45]. The element is an inherent constituent of the ash from all plants and might participate in their metabolism as a redox catalyst, although its role in plant development is not well known. Some beneficial effects (and a few adverse though) of Ti application have been described. Recent efforts have been made to look for an explanation for the fact that a harmful substance at low doses can induce counter effects (“*hormesis*”) that neutralize those toxic [46].

Cr<sup>+3</sup> and Ni<sup>+2</sup>, both with unpaired electrons, are essential elements, recently recognized as such. At low concentrations, it was reported that Cr<sup>+3</sup> promotes plant growth, stimulates chlorophyll synthesis and photosynthetic activity. In the human diet Cr deficiency causes lowered tolerance of glucose, which is the consequence of changes in insulin affinity of its receptors on the cells. In addition Cr also increases the functional activity of immune system of organism. Also, it has metabolic and cardiovascular benefits. When present in excess, Cr causes oxidative stress in cells. Ni has antioxidative effects on enzyme proteins; in addition, it is a unique component of urease, which is responsible for the hydrolysis of urea which nitrogen atoms are available for plants only if the compound is hydrolyzed. It has been called an “ubiquitous trace element” occurring in soil, water, air and the biosphere; however it has no known biological role in mammal metabolism. When present in excess Ni is genotoxic and causes oxidative stress. In this work, Cr could be quantified in the ashes; Ni also in some whole samples [20 & therein]. Other elements quantified by means of their ashes were Y, Zr, Nb, La, Ce, Nd. Their recalculated values are those included in Tables 2& 3.

An interesting aspect of the “*d*” elements in sediments/sedimentary rocks is that referring to the primordial mantle values [36], they present a W distribution (Allegre “*in W*” distribution); resulting from the crystal field stabilization energy (CFSE) and the electronic distribution. During the crystallization processes of the magma, those elements with high stabilization energy stay more stabilized in the solid but in the magma they become depleted. One can see this in the normalized multi-element diagrams of Fig 1b-3b of *Ilex* and bedrocks, with chromium and nickel at the bottoms of the troughs, whereas manganese and zinc are at the higher spikes; this could be an indicator that *Ilex* follows in some way the cations content/distribution of the sedimentary environment. In addition it should noted that it is apparent the enrichment of the *Ilex* fractions in Mn, K and perhaps Zn in relation with the UC values (Fig 1a-3a); enrichment that is higher in the southern samples in agreement with the nature of latheritic soils.

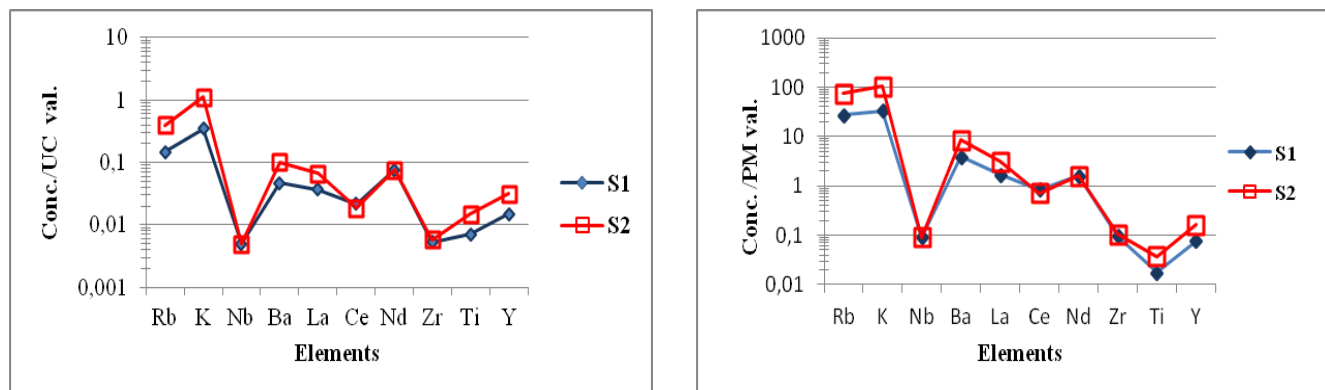
In those spidergrams were also included rubidium and strontium. Rb in soils is closely connected to K. Its occurrence in soil is strongly related to the bed rocks. This element is taken up easily by plants; it substitutes K sites in them but does not substitute K metabolic function. At high concentration is toxic to the plants. Rb uptake and transportation in plants seems too different from that of K. Most of higher plants as the *Ilex* show concentrations of rubidium ~ 20-70ppm.

Geochemical and biochemical properties of Sr are similar to those of Ca and very often are associated with it in the terrestrial environment. Interactions between both are complex; Sr usually cannot replace Ca in biochemical function. Its content in soils is mainly controlled by bedrock and climate: its concentration ranges from 750 to 1000ppm; in Paraguayan sandstones from ~15 to ~130ppm [45].

### 3.2 Elements in the fresh *Ilex* samples

Several of the elements analyzed K, Rb, Nb, Ba, La, Ce, Nd, Zr, Ti, Y like, are often used as provenance indicators in geochemistry. They are refractory as well as their compounds. Very little is known about their role in plants despite recent efforts made to its understanding [18,19] especially in relation to the REE ( Rare Earth Elements). Table 3 brakes down the average of their absolute values which show to be very low; in fact some of these values were recalculated from measurements of the ashes.

Comparison among light rare earth elements and other refractory is doing by normalizing the analyses results to primordial mantle (PM) [36] as well as upper crust (UC) reference values [37], the latter in order to look for enrichment/contamination of the upper crust materials from other sources.



**FIGURE 4 a: Spidergrams of fresh samples leaves of *I. paraguayensis* from Azotey (S1) & Cap. Miranda (S2) standardized to UC values.**

**b: Spidergrams of fresh samples leaves of *I. paraguayensis* from Azotey (S1) & Cap. Miranda (S2) standardized to PM values.**

The spidergrams of incompatible elements results from yerba mate, are assembled and compare in Figures 4a & 4b normalized to UC and PM values respectively; the first does not show any enrichment/contamination of the upper crust materials from other sources; they follow closely the uptake referred to PM of the second one. In both cases, results of these refractory are low, and except by monovalent cations (K & Rb), well below the line of reference when normalized to UC (fig 4a).

Their availability to the plant, both, below and above the ground, that is, roots and aerial parts are the key factor; in regard to the soil, it has been shown that the system Mn / Fe oxyhydroxides/pH in soil fractions are the major contributors to the release and availability of REE compounds [47,29].

Although REEs have not known biological role in plants, some toxic effects have been mentioned, although there is not much data [25]. They can compete with other cations and interfere their bioprocesses. Such is the important case of  $\text{Ca}^{2+}$ , which ionic radius ( $0,99 \text{ \AA}$ ) is close to those of REEs, whose their higher oxidation state motivates besides, a higher density of charges compared to Ca bivalent with a lower density. Thus it can be supplanted in its bonding site, generating therefore undesirable effects on the plant, especially when referring to the shoots/leaves, taken into account inter alia, the role of Ca channels on photosystem II [48 & ref therein].

It has been found some negative effects of REE on biomass development and also in the biogermination; among REE, Ce is of the more concern due its radius ( $1,0 \text{ \AA}$  &  $1,1 \text{ \AA}$  for IV & III valences) and its capacity to change its oxidation state. [48]. Lanthanides are toxic to cell metabolism, although there is not much data about. On the other hand, it was reported that La, Pr, Nd to, specifically and competitively, inhibit calcium uptake / accumulation by the mitochondria of microorganism cells [25].

Although the uptake of LREE is low in *Ilex* and other plants, the sustained increase in their use in modern / contemporary technology has turned them into “emerging” pollutants / toxics, which should be under permanent consideration as indicated by several articles among which can be cited [49 & ref herein].

As above mentioned, the multi-element diagrams show that aerial materials of both, S1 & S2 have almost the same behavior: a deep trough in Nb, Ce, Zr, Ti and bumps at K, Ba and Nd; it is apparent a negative anomaly for Ce as well as a *W distribution* of the LREE first tetrad in the spidergrams normalized either to UC as to PM values. According to the well documented study on *Vitis vinifera* [50 & ref herein], these *W distribution* of the first tetrad associated with the negative Ce anomaly in the aerial parts of the plants, found in this effort also, suggest the oxidation of  $\text{Ce}^{3+}$  to  $\text{Ce}^{4+}$  insoluble as  $\text{CeO}_2$  which, comes out of the solution and therefore originates the negative Ce anomaly. On the other hand, a *M distribution* was observed in the root of grapes associated with positive Ce anomaly being apparent the redox processes  $\text{Ce}^{3+} \leftrightarrow \text{Ce}^{4+}$  in the rhizosphere and its uptake as organic complexes of  $\text{Ce}^{4+}$  but changes to  $\text{Ce}^{3+}$  in the plant metabolic processes [50,51].



#### IV. CONCLUSION

The samples indicate that *Ilex paraguayensis* present in the aerial parts an important content of 3d elements, several of them considered essentials; besides K plays a prominent rol in the “reviver” property of mate beverages. On the other hand, results of the refractory are low, and except monovalent cations (K & Rb), well below the line of reference when normalized to UC. When compared to bed rocks PM normalized values, *Ilex* follows in some way the captions content/distribution of the sedimentary environment. In addition, spidergrams show the enrichment of the *Ilex* fractions in Mn, K and perhaps Zn in relation with the UC values, enrichment that is higher in the southern samples: this can be used as provenance indicator of *yerba mate* in Eastern Paraguay.

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