Biogeochemical Aspects of Manganese Content in *Ilex Paraguayensis* SH from Paraguay by EDXRF and INAA

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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. It has been recognized in Ilex paraguayensis, diuretic, CNS stimulant, hypocholesterolemic, hepatoprotective as well as other pharmacological properties. In regard to its elemental content few studies are known despite they play fundamental tasks in the structure and functioning of plants. One of them, Mn, usually occurs as a trace in Ilex paraguayensis, at relatively high concentration and in this work its concentration in plants from Paraguay have been investigated by EDXRF at Josef Stefan Institute at Ljubljana and at University of Asunción using radioactive isotopic sources and by INAA technique in the Faculty of Chemistry at Asunción with an Am-Be neutron source. These plants are grown mainly in two regions, north and south of Eastern Paraguay; results show that their manganese content can be used as a geochemical indicator to identify the region of origin. Besides, as in other plants, absorption, transport and homeostasis of Mn could be attributed to the action of different NRAMPs. In regard to its normal high content in healthy yerba plants, Mn in excess could be hidden in nodes and vacuoles being exported afterwards.*

***Keywords—* Ilex paraguayensis, Eastern Paraguay, yerba mate, Mn content, NRAMP, transporter.**

I. INTRODUCTION

Mineral constituents and nutrients play fundamental tasks in the structure and functioning of plants, sustaining them and supporting life on our planet. In such a perspective, their role is of remarkable importance for *Ilex paraguayensis* (Saint Hilaire)*, pursuant to its generally mentioned/accepted properties. *Ilex paraguayensis* (Aquifoliaceae), is a small tree of Paraguayan origin called *Ka'a* in Guaraní ** or yerba mate in Spanish and was used as infusion in hot water (mate & mate tea) or as a maceration in cold water (tereré) by the ancient/natives inhabitants of Paraguay as a "reviver"/energy beverage and mineral supplier whose consumption continues to these days especially in Paraguay, Argentina, Brazil and Uruguay; furthermore, it is used as the infusion almost worldwide. When people, including those who are undernourished, drink any of these beverages in appropriate amount, they gain/recover strength and their working yield improves. Thus, in the *Código Bromatológico del Paraguay* (1932) and elsewhere [1,2], yerba mate is considered as a true foodstuff.

The literature indicates that mate tea 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 theophilline, 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 antiinflamatory properties; some of them have antiparasitic effects, *inter allia* anti-trypanosomal, due to its content in tri-terpenoids (IC_{50} around 4μM for *Trypanosome brucei*). An excellent comprehensive review in this regard is presented in [3] and references therein.

In respect to minerals, it has been published papers referring to their concentration in *Ilex paraguayensis* from Paraguay [4-6] and in the neighbor countries of Brazil and Argentina, at the State do Parana [7,8] in the former; at the Provinces of Misiones and Corrientes in the later, [9-10] all of them bordering the east of Paraguay; just for expand the comparison, some other *data* [11] are also included.

They are mainly related to its multi-elemental contents that are prominent; many of them, such as the essential microelements are of utmost importance for living organisms. One of these essential elements is Mn that at trace levels usually occurs in *Ilex paraguayensis* at relatively high concentration on cropping lands from Paraguay as well as in Brazil, Argentina and elsewhere.

The element is essential for plant metabolism; participates actively in the photosynthesis process; also at the biosynthesis of several organic substances like some proteins and lipid acids, ATP, chlorophyl, flavonoids etc. Mn is a normal constituent of oxidizing enzymes; tiny amounts of Mn^{2+} in the oxidases and peroxidases accelerate their oxygen carrying power. It is a cofactor of superoxide dismutase (SOD) and in this regard, Mn SOD acts, *inter alia*, against plants oxidative stress. In excess Mn can be toxic; this can be explained (as well as for other 3d elements), by its capacity to catalyze the initiation of free radical reactions related to its impaired electrons. From human dietary aspects, the adequate daily intake (A.I) is 1.8-2.3 $mg.day^{-1}$ kg^{-1} b.w of Mn.

In Paraguay yerba mate is cultivated mainly in the north and in the south of the Eastern Region of the country on sedimentary and on basaltic *provenance* soils. In previous studies it has been shown that Mn content as well as other metals in Ilex, presents differences in concentration according to the harvesting zone and could be used as an origin indicator [9,12]. In this work has been investigated the content of the element in commercial as well as in fresh samples of yerba mate from several cropping areas of both, north and south zones of Eastern Paraguay, using EDXRF (Energy Dispersive X-.Ray Fluorescence) and INNA (Instrumental Neutron Activation Analysis), both non destructive techniques.

II. MATERIALS AND METHODS

The XRF and NAA procedures were carried out in two different stages. At the first, the samples were analyzed by XRF; in the second, new materials were submitted to INAA.

2.1 Materials

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 and in the south of the Eastern Region of Paraguay. Thus, were analyzed samples from nine different brands taken at random in several shops of at least four of 0.5 kg packets, ea of the same brand. They are constituted by grinded 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 Nueva Germania, Azote'y and Katuete in the North and at Capitán Miranda in the South. 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 grinding; moisture ranges from ~ 46 to 50%.

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

TABLE 1
ILEX SAMPLING SITES

North: Departments of S. Pedro – Amambay –Kanindeyu		
	Coordinates	**Soil typology**
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
Caballero Alvarez (CA)	24° 4' 13.358" S, 54° 18' 17.863" W	Alfisol – Loamy sand from basalts
Katuete (K)	24° 14' 53.340" S, 54° 45' 27.655" W	Oxisol – Clayloam from basalt
Nueva Esperanza (NE)	24° 32' 25.865" S, 54° 49' 44.853" W	Oxisol
South: Department of Itapúa		
	Coordinates	**Soil typology**
Cap. Miranda (CM)	27° 12' 53.592" S, 55° 47' 48.306" W	Oxisol– Sandy clay from basalt
Obligado (Ob)	27° 3' 7.776" S, 55° 37' 8.434" W	Ultisol- Sandy clay
Bella Vista (BV)	27° 3' 0.000" S, 55° 33' 0.000" W	Oxisol –Sandy clay from basal

Samples from each of both A & B materials were sieved and prepared by quartering, being then dried at 105 ºC for 6 hs in an oven. For XRF measurements, powdered samples were pressed into pellets of area weight of ~ 0.1 to 0.3 $g.cm^{-2}$. For INAA, powdered samples in amounts of ~ 4-5 g were irradiated in polystyrene vials.

2.1.3 Ashes samples

In order to verify the results, aliquots of A and B materials were reduced to ashes at 600ºC in an oven. Ashes content ranges from ~ 4.3 up to 6.5 %.

2.2 X-ray Irradiation and Fluorescence 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 Paraguay.

For the excitation of fluorescence radiation the radioisotope source of Cd-109 (30 mCi) and the X-ray tube (at 40 kV and 20 mA) with the Mo anode and Mo secondary target were used. The energy dispersive X-ray spectrometer was based on a Si (Li) semiconductor detector coupled to a spectroscopic amplifier and a multichannel analyzer. The analysis of complex spectra was performed by the AXIL software [13] which is based on iterative nonlinear least square lines. The resulting intensities of pure K_α and L_α lines of measured elements were the utilized in quantitative analysis, employing the quantification software QAES (quantitative analysis of environmental samples) designed by Kump [14]. Details have been given elsewhere [15].

2.3 Neutron Irradiations and Radioactivity Analysis.

The samples were irradiated with neutrons of a 25 Ci ^{241}Am-Be annular source from Amersham, suitable for large samples. According to the maker the total flux is 5×10^7 $n.s^{-1}$ + 20% in good agreement with the known yield of 80 n/10^6 Bk; the neutron energy spectra are complex averaging 4-5 MeV with net spikes at 4.7, 6.5 and 8 MeV [16], suitable for (n p),(n α) as well as for (n γ) reactions [17].

By neutron irradiation of Mn, the radioactive isotope Mn-56 is formed trough ^{55}Mn (n, γ) ^{56}Mn reaction. This radioisotope has a $T_{1/2}$ of 2,54h, is a β- emitter that decays to exited states of ^{56}Fe that shows a prominent (98.85%) gamma emission of 0.847 MeV [18] which was used in this work for the analysis.

III. MEASUREMENTS

Irradiated samples were measured in a 3 x 3" $Bi_4Ge_3O_{12}$ (BGO) crystal, coupled to a MCA (Multi Channel Analyzer). The BGO has better counting efficiency (density of 7.1 g. cm^{-3}) than other solid scintillators.

The analysis of samples was performed in two steps; in the first, an aliquot of sample was irradiated and afterwards it was followed the half life through the 0,847 MeV photopeak, in order to check the absence of any significant tail; in the second step, samples were usually irradiated on about 2Θ, where $\Theta = t/T_{1/2}$ according to $A = A_s (1- e^{-\ln 2\Theta})$; A_s is the activity to saturation. For calculations, calibration curves were employed. In regard to ashes samples, they were irradiated ~ 0.52 Θ.

IV. RESULTS AND DISCUSSION

Manganese is a relatively abundant element in universe: condrites carbonous show a value of 1920 ppm and for the earth it is estimated to be ~1680 ppm [19]. In the upper crust, concentration values mentioned are of 600 ppm [20] and 770ppm [21].

In Eastern Paraguay at the area of sampling stations of Nueva Germania, Azotey and PJ Caballero, sandstones of Aquidabán Group show Mn concentrations of 78ppm, but 600ppm where Aquidabán Group interfinger with sandstones of Misiones Formation [22] and ~ 500 ppm in sandstones/sediments of Acaray Formation[23]. All other sampling sites are located on the wide area of Alto Parana Formation with bearing stratum of basaltic rocks that show Mn0 concentration of about 0.19% [24]. Concerning to Mn and other *3d elements* concentrations, in soils of Paraguay very little is known except for Fe tenors. Punctual values of 6.5 and 22.2 mg kg^{-1} of Mn at sampling sites have been recorded though, according to Prof Alonso of the UNA [25]. In addition, in studies carried out in bottom sediments from several rivers and streams, the registered values of Mn are related to the geologic environment and the *provenance*.

In agreement with its half filled *3d* orbital's, Mn presents several oxidation states and forms a number of minerals. The highest occurrences are those of Mn^{2+} (silicates, carbonates) that through denudation/weathering are oxidised in the atmospheric environment. Thus, oxides/hydroxides are formed: $Mn(OH)_2$ which easily oxidises, varieties of MnO(OH) and of MnO_2 [26], which are prominent in soils, as well as other compounds.

For *yerba mate*, Tables 2a & b break down the results obtained in this work by EDXRF as well as by INAA. It must be note that Azotey and C. Miranda analyzed samples by XRF and INNA were from the same crop in each case; samples from Bella Vista are from the same sampling area, but do not belong to the same commercial brand. The closeness of the results obtained by both such techniques is remarkable. In addition it should be note that the samples "a" and "b" from Katueté come from native plants in the first case and cultured in the second.

TABLE 2a
MN CONCENTRATION IN ILEX – NORTH AREA
Departments of S. Pedro – Amambay –Kanindeyu

	FRX	INNA
Nueva Germania (NG)	510.25 ± 42.4	
Azotey (AZ)	625.0 ± 64.9*	595.0 ± 32.5*
P.J.Caballero(PJC)	609.8 ± 60.3	
Nueva Esperanza (NE)		1174.7 ± 117.1 1123.0 ± 46.6
Caballero Alvarez (CA)		1036.1 ± 109.4
Katuete (K)		2465.0 ± 30.0* 2005.0 ± 123.0*

**** fresh***

TABLE 2b
MN CONCENTRATION IN ILEX – SOUTH AREA
Department of Itapua

	FRX	INNA
Cap. Miranda (CM)	a)1680.0 ± 142*	a)1207.0 ± 169* b)1176.4 ± 111.2
Obligado (Ob)		824.0 ± 163.2
Bella Vista (BV)	a)903.5 ± 96.5 b)1036.0 ± 150	a) 993.1 ±113 b)1191.0 ±129

**** fresh***

On the dissolution of Mn-oxides, pH and redox potentials have a strong role [27]: in the soil solution, the most stable oxidation state is Mn^{2+} that occurs as $[Mn(OH_2)_6]^{2+}$ aquo-complex [28]. At pH < 5.5, which is the case for most of the soils at the *yerba* cropping area of Paraguay (pH ranging from 4.50 to 5.50), in reducing ambience manganese oxides are reduced to Mn^{2+}, increasing its availability. On the other hand these soils are low in organic matter (OM) with negative effect on Mn availability. Besides the humic complex that strongly affects the biogeochemical fate of micronutrients, a variety of other organic molecules can reduce and dissolve manganese oxides [29]. The humic complexes are constituted by fulvic and humic acids fractions (also humin).

Humic acid with Mn as well as with other metals, forms much more stable complex than those formed with fulvic acid. Thus, the former are only partially soluble while Mn-fulvic acid complex are more soluble: therefore more at hand to for the roots. Fulvic acid with $[Mn(OH)_6]^{4-}$ originate an outer sphere electrostatic structure complex with distorted octahedral configuration, and a low free energy [30].

Thus the trace elements released by redox and hydrolysis reactions enriched the soil solution that, assisted by the root surface and micro organisms activities, constitute a pool that can interact with the root surface, get absorbed and transported.

Concentration of Mn in the pool can fluctuated [31] from very low up to few hundred micromoles according to the soil behaviour and as has been shown in rice, the *yerba* and other plants, should deal with this fluctuation. In these processes and not seldom, antagonism of metals can exist, for example it is believed the existence of antagonism in *Ilex paraguayensis* of the pair Mn- Fe according the differences found in their concentration despite their similar chemical pathway in dissolution and in condensed phase.

Very little is known about Mn absorption in *yerba mate*; but in other plants it has been found the action of different NRAMPs in its absorption, transport and homeostasis. NRAMP or Natural Resistance- Associated Macrophage Protein refers to an integral membrane protein (perhaps also plasmatic) with unique expression in macrophages [32]. The plants NRAMP proteins in many cases maintain similar amino acid sequences as the NRAMP of animals; for instance, in the widely studied *Arabidopsis thaliana*, At-NRAM proteins are close to mouse NRAMP s [33].

It has been pointed out [27] that a concentration of Mn above 500ppm usually is of toxicity for plants; however this is not the case for yerba mate. In this work as well as in others, concentrations of more than 2000 ppm have been recorded in healthy plants. In other plants with the same characteristics, Mn in excess is hidden in nodes and vacuoles, being exported afterwards according necessity for the homeostasis [32-34]; this could worth for an explanation in yerba mate plants.

The results are consistent with those registered in Paraguay (previously), Brazil and Argentine, as can be seen in table 3.

TABLE 3
MN TENORS (PPM) IN *ILEX PARAGUAYENSIS- PARAGUAY AND ELSEWHERE**

Sampling sites			
Department			
Itapua (Bella Vista)	**S1**	**S2**	**S3**
	651±128	880±220	720±282
	S4	**S5****	**S6****
	858±126	904±209	1030±113
Argentina Provinces			
Misiones	**M1**	**M 2**	
	2277±250	1776±106.6	
Corrientes	**C1**		
	2320±154.4		
Brasil			
Parana	**Prudentopolis**	**Laranj. do Sul**	**Palmerinha**
	521.5±3.13	209.6±0.63	872.0±6.98
	Pinhao	**Faxinal do Ceu**	**Cachoeira dos Turcos**
	894.5±17.88	971.15±97.1	1043.5±157

***Values registered in previous works adapted from references 7-14; commercial products.**

Besides, values for whole sample recalculated from the results obtained when ashes were irradiated (see table 4) validate, in some way, those found in the whole samples.

TABLE 4
VALUES RECALCULATED FROM IRRADIATED ASHES

	Concentration
North	
Azotey	593.9±44.5
Nva. Esperanza	1385.7±27.7 1233.3±24.7
Caballero Alvarez	854.7±53.0
Katuete	1687.2±43.9 2320±53.4
South	
Cap. Miranda	1396.2±140 936±21.5
Obligado	904 ±166
Bella Vista	1255.3±27.6 1228.3±35.0

It has been pointed out that more than regarding to soil typology, Mn concentration in surface soil is related to particles size, i.e, clay and silt content, smaller/fine grade components of soils which are basic for sorption of trace elements pursuant to equilibrium conditions. In this regard, results corresponding to NG, Az. PJC materials from sedimentary sampling areas have consistently much lower Mn content than those from basaltic; NG, Az, and PJC present sandy deep soils with low content in clays (sandy loam). On basalts *provenance* soils that are more clayed, tenors of Mn increased sharply. At Katueté with typically basaltic soils, the yield of the element is higher than on the other sampling stations of the area. Also, at this very point, concentration of Mn show to be higher in native plants than in the cultivated ones, in line with results of other works.

In a few experiments were irradiated also samples of sticks of *yerba* (fresh) from Azotey and Capitan Miranda. In the first case Mn yield was 236 ± 28 ppm and in the second 727±70 ppm in comparison of leafy materials (see Table 2). The much higher values registered in leaves than in sticks are in line with the role of Mn in the photosynthesis [34].

V. CONCLUSION

Yerba mate is good supplier of Mn and its content of this element can be an indicator of provenance. As in other plants, absorption, transport and homeostasis of Mn could be attributed to the action of different NRAMPs. Besides, high concentration of Mn in normal healthy plants suggests that Mn in excess is hidden in nodes and vacuoles being distributed afterwards.

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