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Distribution of Rare Earth Elements in Citrus Leaves and Reference Materials (NIST SRM 1515 and ERM CD281)

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Abstract: The present study aims to discuss the data on levels and distribution of rare earth elements, including Y, (REYs) in leaves of three different citrus species (lemon, orange, and tangerine) and provide additional information about the major, minor and trace elements in two biological certified reference materials (CRMs), Apple leave (NIST SRM 1515) and Rye grass (ERM CD281). In all samples, element concentrations were determined by High Resolution Inductively Coupled Plasma Mass Spectrometry. The obtained data display substantial variability in the distribution of REY elements, not only between different citrus species but also between different genera of plants indicating their different uptake and accumulation abilities. Measured concentrations of REYs in citrus leaves were substantially lower compared to the literature values, although the fractionation indices were comparable. The data for CRMs provide additional information for the 14 elements in NIST SRM 1515 and the 30 elements in ERM CD281, including rare earth elements.

Keywords: citrus leaves, rare earth elements, trace elements, Apple leave (NIST SRM 1515), Rye grass (ERM CD281)

INTRODUCTION

THE flowering trees and shrubs of the *Citrus* genus include some of the most important cultivated fruits around the world.[1,2] Belonging to the family *Rutaceae*, one of the largest families of the order *Sapindales*, this genus encompasses about 140 genera and 1300 species; of which the most economically important are the lemon (*C. ×lemon*), sweet orange (*C. ×sinensis),* sour orange (*C. ×aurantium*), tangerine (*C. reticulata*), lime (*C. ×aurantiifolia*), grapefruit (*C. ×paradise*), citron (*C. medica*) and shaddock (*C. maxima*). Their worldwide distribution can be attributed to their great nutritional and medicinal value and growing interest in plant-based medications.[3–5] Citrus leaves are usually strongly fragrant, while their extracts contain many useful flavonoids and other compounds that are effective insecticides, fungicides, and medicinal agents.[5,6]

Despite their prevalence and widespread use, the literature on the chemical properties of citrus plants is relatively limited, while studies related to the multi-elemental characterization are very rare.[7–9] However, increasing anthropogenic pressures, which inevitably condition the continuous input of various toxic elements into our environment, put this topic at the forefront and require additional studies.

Although plants do not rely on most of the transition (heavy) metals for normal plant growth and development, they can suffer serious damage if exposed to their excessive concentrations.[10,15–17] In that terms, chemical characterization of biological samples provides insight into the concentrations of individual chemical elements, which further allows the determination of mechanisms of their uptake and accumulation as well as the assessment of anthropogenic impact on the system.[18,19] Despite the fact that the increased use of rare earth elements for industrial and agricultural purposes portrays them as emerging pollutants,[20,21] research on biological and toxicological effects of elements of this group is still rather limited.[22]

As for other trace elements, the capacity of plants or crops to accumulate REEs depends on the species and the REEs content in the substrate.[23] The latter can be artificaly increased by the application of NPK or other phosphate fertilizers. Recent findings of Turra *et al.*[23] suggested that under such conditions, citruses readily accumulate REE, with leaf-soil ratio up to 1.09. Unfortunately, very limited research has been done so far regarding the distribution of rare earth elements in citrus systems.[23]

The study of the distribution of the REEs in plant systems, however, goes beyond the primary interest of the determination of their total concentrations in certain plant systems. Namely, in the early 1990s, Bennet and Breen[24] identified some notable similarities in the plant uptake mechanisms of trivalent REEs and Al^{3+}, suggesting REEs as potential ultrastructural tracers for Al toxicity in plants. Additionally, Wei and Zhou[25] found that free Nd^{3+} at low concentrations can specially bind to the high-affinity Ca^{2+} binding sites on the Ca-ATPase. Later, the physiological interaction of REEs with calcium (Ca), the effects of the REEs on structure and function of cytoplasm membranes, photosynthesis, hormone metabolism, and enzyme activity, and the water use efficiency have been more extensively discussed by Zhengyi *et al.*[26]

Although the soil analysis is still the most used tool for estimation of metal bioavailability,[15,17,27–29] the development of fast analytical techniques for multielement analysis enhances the role of plant studies in environmental and agricultural management. Among different techniques, HR-ICP-MS has been repeatedly confirmed as a highly specific technique for multielement analysis of a large number of elements, over a wide linear range (up to 8 orders of magnitude), and in different types of samples.[30]

Moreover, the existing trends of many national and international regulations on environmental and food safety are becoming more strict, imposing lower maximum permissible levels of toxic metals in food, animal feed, soil, water, etc., and require further development of analytical techniques for fast and reliable analysis. Thereat, reference materials are considered an important tool in ensuring the quality of measurements and validation of the analytical methods. Although the biological reference standards in the field of food control and environmental biochemistry are numerous and versatile, they are very often certified for a limited set of elements, and rarely include data for rare earth elements.

Therefore, the aim of the present study is twofold. The main aim is a detailed discussion of distribution of REY in leaves of three different citrus species, previously reported by Fiket *et al.*[31]. An additional goal is to report on the levels of 46 major, minor, and trace elements, including REYs, in two certified reference materials, Apple leave (NIST SRM 1515) and Rye grass (ERM CD281), that are often used in food, plant or pharmaceutical studies but are certified for only a limited set of elements.

METHODS

Sample Collection and Preparation

Discussed citrus leaf samples include three different citrus species: orange, lemon, and tangerine. Details of their sampling and sample preparation are described elsewhere.[31]

In addition to leaf samples, the following certified reference materials (CRMs) were also analysed: (i) Apple leave (NIST SRM 1515, The National Institute of Standards and Technology, USA) and (ii) Rye grass (ERM CD281, IRM, Belgium).

Subsamples (0.05 g) of CRM samples were subjected to total digestion in the microwave oven (Multiwave ECO, Anton Paar, Graz, Austria) in a one-step procedure consisting of digestion with a mixture of 6 mL nitric acid (HNO_3, 65 %, *traceSELECT*, Fluka) and 0.1 mL hydrofluoric acid (HF, 48 %, *traceSELECT*, Fluka).[32] Procedural blank solutions were prepared in the same way as sample solutions; equal volumes of an acid mixture, which have been used for sample digestion, were microwave-assisted heated in the same manner that was applied for the digestion of samples.

Prior to analysis, samples were acidified 2 % (*v* / *v*) HNO_3 (65 %, *supra pur*, Fluka, Steinheim, Switzerland), and indium (In, 1 μgL^{-1}) was added as the internal standard.

It should be noted that for both the citrus leaf samples[31] and CRMs, the same digestion and preparation protocol was used.

Sample Analysis

The multielement analysis of prepared samples was performed by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) using an Element 2 instrument (Thermo, Bremen, Germany). The typical instrument conditions and measurement parameters used throughout the work are reported elsewhere.[30] Mass calibration of the instrument (HR-ICP-MS) was performed using a multielement solution (Merck KGaA, Darmstadt, Germany) that contains the following elements: B, Ba, Co, Fe, Ga, In, K, Li, Lu, In, Rh, Sc, Tl, U, and Y.

Standards for trace element analysis were prepared by appropriate dilution of a multielement reference standard (100 ± 0.2 mg L^{-1}, Analytika, Prague, Czech Republic) containing Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Se, Sr, Ti, Tl, V, and Zn in which single element standard solutions of U (1.000 ± 0.002 g L^{-1}, Aldrich, Milwaukee, WI, USA), Rb (1.000 ± 0.002 g L^{-1}, Aldrich, Milwaukee, WI, USA), Sb (1.000 ± 0.002 g L^{-1}, Analytika, Prague, Czech Republic) and Sn (1.000 ± 0.002 g L^{-1}, Analytika, Prague, Czech Republic) were added. Zirconium (1000 mg L^{-1}, Alfa Aesar, Germany) was prepared as a separate single standard solution. For REEs determination, a multielement reference standard (Analytika, Prague, Czech Republic) containing Ce, La, Nd, and Pm (100 ± 0.2 mg L^{-1}) and Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y, and Yb (20 ± 0,4 mg L^{-1}) was used. For major elements determination, a multielement reference standard (Fluka, Germany) containing K, Mg, and Na was used.

All samples were analysed for the total concentration of 46 elements (Al, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Sr, Tb, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr).

RESULTS AND DISCUSSION

Method Validation

Method validation for analysis of listed 46 elements in CRMs included determination of limits of detection (LOD) and quantification (LOQ) (Table 1) as well as the demonstration of accuracy by presenting previously published data for Citrus leave CRM[31] obtained by the above-described method. The reason for the latter is that the Citrus leave CRM is certified for all elements encompassed by this study, with exception of Zr (Table 2).

Table 1. Measured element concentrations in procedural blank solutions (expressed in μg L^{-1}) and calculated limits of detection (LOD) and quantification (LOQ) (both expressed in mg kg^{-1}).

Element	Blank	LOD	LOQ	Element	Blank	LOD	LOQ
Al	5	6	18	Mo	0.005	0.005	0.015
As	0.005	0.003	0.01	Na	2	3	10
Ba	0.1	0.3	1.0	Nd	0.002	0.001	0.003
Be	0.002	0.002	0.006	Ni	0.05	0.03	0.1
Bi	0.002	0.002	0.006	Pb	0.05	0.03	0.1
Cd	0.002	0.002	0.006	Pr	0.002	0.001	0.003
Ce	0.002	0.001	0.003	Rb	0.01	0.03	0.1
Co	0.002	0.002	0.006	Sb	0.002	0.002	0.006
Cr	0.05	0.03	0.1	Sc	0.002	0.002	0.006
Cs	0.002	0.002	0.006	Se	0.04	0.02	0.06
Cu	0.05	0.03	0.1	Sm	0.002	0.001	0.003
Dy	0.002	0.001	0.003	Sn	0.005	0.003	0.01
Er	0.002	0.001	0.003	Sr	0.5	0.5	1.5
Eu	0.002	0.001	0.003	Tb	0.002	0.001	0.003
Fe	2	3	10	Ti	0.5	0.5	1.5
Gd	0.002	0.001	0.003	Tl	0.002	0.001	0.003
Ho	0.002	0.001	0.003	Tm	0.002	0.001	0.003
K	4	15	45	U	0.002	0.001	0.003
La	0.002	0.001	0.003	V	0.002	0.002	0.006
Li	0.015	0.005	0.015	Y	0.002	0.001	0.003
Lu	0.002	0.001	0.003	Yb	0.002	0.001	0.003
Mg	5	6	18	Zn	2.5	1.5	4.5
Mn	0.1	0.25	0.75	Zr	0.05	0.01	0.03

Measured element concentrations in procedural blank solutions and the calculated LOD and LOQ limits for all measured elements are presented in Table 1. Limits of detection and quantification were calculated, respectively, as three and ten times the standard deviation of ten consecutive measurements of the analyte concentration in the procedural blank. The LODs and LOQs range from 0.001 mg kg^{-1} to 1.5 mg kg^{-1} and from 0.003 mg kg^{-1} to 4.5 mg kg^{-1}, respectively, for the trace elements (including the group of REEs), and from 3 mg kg^{-1} to 15 mg kg^{-1} and from 10 mg kg^{-1} to 45 mg kg–1, respectively, for minor and major elements (Table 1).

To assess the precision of the measurements, for each element a relative standard deviation (RSD) from the mean of six replicates per CRM, both NIST SRM 1515 and ERM CD281, was calculated. The obtained RSDs amounted to 7 % and 9 %, respectively.

Major, Minor and Trace Elements in CRMs

The results of measurement of 46 elements (Al, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Sr, Tb, Ti, Tl, Tm, U, V, Y, Yb, Zn, and Zr) in studied CRMs, associated average recovery values and calculated REY fractionation indices are shown in Table 3 and Table 4.

In Figures 1 and 2, the measured concentrations are shown as recovery ranges calculated from the average concentrations of the elements and their standard deviations. Certified values were presented in the same manner, including their uncertainties. For simplicity, the data for major, minor, and trace elements were shown separately from the REEs data.

In NIST SRM 1515, certified values are listed for only 18 out of 46 elements encompassed by the present study, while an additional 14 elements are noted only with informative values; meanwhile, the concentrations of REEs are not listed or are only informative. For this reference material, the average recoveries range from 92 % and 108 % (Table 3), for all measured elements listed in the reference sheet both certified and informative, and show complete overlap with the range of certified values (Figure 1b and Figure 2b). Informative values are specified as mean value and 95 % confidence interval.

The elements present at the lowest concentrations (< 0.05 mg kg^{-1}), Co, Sb, Sc, and U, display the largest standard deviations. For these elements, only informative values are listed in the reference sheet; and on Figure 2a they are displayed as small points, instead of ranges.

Ivanova *et al.*[33] reported concentrations for 31 elements in NIST SRM 1515, of which 28 are included in the present study. For the certified elements, here presented data display higher accuracy and precision than those obtained by quadrupole ICP-MS.[33] For other elements (Be, Bi, Dy, Er, Ho, Lu, Pr, Tl, and Y), not included in the reference sheet, values reported by Ivanova *et al.*[33] were found comparable to those obtained by HR-ICP-MS in this study, although slightly lower.

In ERM CD281, only 16 elements are stated in the reference sheet and no certified or informative values are reported for the group of REEs. For this CRM, the average recoveries range from 91 % and 108 % (Table 4), for all measured elements listed in the reference sheet, both certified and informative and show partial or complete overlap with the range of certified values (Figure 1c). Informative values are specified as mean value and 95 % confidence interval. Despite the lack of data for this CRM in the literature, available data for Rye grass (BCR 261), obtained by

Table 2. Comparison of measured element concentrations (mean ± standard deviation; $n = 6$) and certified values (with associated uncertainties), expressed both in mg kg^{-1}, obtained recoveries (%),[31] and calculated REY fractionation indices in Citrus leave (NCS ZC73018).

Element	Measured	Certified value	Recovery	Element	Measured	Certified value	Recovery
Al	1052 ± 30	1150 ± 100	91	Tl	0.057 ± 0.001	0.06 ± 0.008	95
As	0.995 ± 0.045	1.1 ± 0.2	90	U	0.046 ± 0.001	0.045 ± 0.01	102
Ba	97.7 ± 2.9	98 ± 6	100	V	1.11 ± 0.04	1.16 ± 0.13	96
Be	0.0265 ± 0.001	0.031 ± 0.007	85	Zn	18 ± 0.55	18 ± 2	100
Bi	0.229 ± 0.005	0.23 ± 0.025	100	Zr	1.74 ± 0.15	–(a)	–(a)
Cd	0.157 ± 0.004	0.17 ± 0.02	92	Y	0.412 ± 0.009	0.42 ± 0.04	98
Co	0.21 ± 0.012	0.23 ± 0.06	91	La	0.534 ± 0.017	0.57 ± 0.06	94
Cr	1.18 ± 0.05	1.25 ± 0.11	94	Ce	0.993 ± 0.043	1 ± 0.13	99
Cs	0.139 ± 0.006	0.14 ± 0.01	99	Pr	0.107 ± 0.004	0.108 ± 0.014	99
Cu	6.20 ± 0.3	6.6 ± 0.5	94	Nd	0.419 ± 0.021	0.42 ± 0.05	100
Fe	463 ± 15	480 ± 30	96	Sm	0.081 ± 0.006	0.08 ± 0.007	101
K	7189 ± 150	7700 ± 400	93	Eu	0.032 ± 0.002	0.033 ± 0.002	97
Li	1.02 ± 0.026	1 ± 0.1	102	Gd	0.080 ± 0.004	0.081 ± 0.01	99
Mg	2300 ± 50	2340 ± 70	98	Tb	0.010 ± 0.001	0.011 ± 0.001	92
Mn	29.6 ± 0.9	30.5 ± 1.5	97	Dy	0.057 ± 0.004	0.057 ± 0.05	100
Mo	0.193 ± 0.01	0.2 ± 0.01	97	Ho	0.011 ± 0.001	0.011 ± 0.001	98
Na	129 ± 4	130 ± 20	99	Er	0.026 ± 0.002	0.026 ± 0.006	101
Ni	1.11 ± 0.05	1.1 ± 0.055	101	Tm	0.004 ± 0.001	0.0038 ± 0.0009	102
Pb	9.74 ± 0.25	9.7 ± 0.9	100	Yb	0.026 ± 0.001	0.025 ± 0.005	103
Rb	2.86 ± 0.08	3 ± 0.2	95	Lu	0.004 ± 0.001	0.0037 ± 0.0009	106
Sb	0.176 ± 0.008	0.2 ± 0.06	88	ΣREY(b)	2.8		
Sc	0.137 ± 0.006	0.14 ± 0.02	98	Eu / Eu*(c)	2.02		
Se	0.17 ± 0.01	0.17 ± 0.03	100	Ce / Ce*(d)	0.80		
Sn	3.8 ± 0.1	3.8 ± 0.5	100	ΣLREE / ΣHREE(e)	14.4		
Sr	164 ± 3.5	170 ± 10	96	Ba / Eu(f)	3053		
Ti	37.8 ± 1.5	38 ± 10	99				

(a) - not specified.
(b) ΣREY – sum of all rare earth element concentrations.
(c) Eu / Eu* - europium anomaly (Eu / Eu*= EuNASC / (SmNASC × GdNASC)0.5).
(d) Ce / Ce* - cerium anomaly (Ce / Ce* = CeNASC / (LaNASC × PrNASC)0.5).
(e) ΣLREE / ΣHREE - ratio of sum of light rare earth and heavy rare earth element concentrations.
(f) Ba / Eu – ratio of Ba and Eu concentrations.

ICP-AES, show similar values for Eu, Ho, Lu, Nd, Tb, Y, and Yb, and higher values (up to 5 times) of other REEs.[34]

Rare Earth Elements in Citrus Leaf Samples

Concentrations of REEs + Y (REY) in all analysed samples ranged over three orders of magnitude, from 0.0003 mg kg^{-1} (Lu) to 0.646 mg kg^{-1} (La), with ΣREY ranging from 0.203 mg kg^{-1} to 1.71 mg kg^{-1} (Table 5). Among them, La was present at highest levels in lemon leaf samples, representing on average 38 % of the total REY, while Ce was present at the highest level in tangerine and orange leaf samples, accounting between 33 % and 38 % of the total REY. Thulium and lutetium, on the other hand, exhibited the lowest values. Highest concentrations of REYs were measured in lemon leaf samples where their average ΣREY value reached 1.54 ± 0.17 mg kg^{-1}, while orange and tangerine leaves exhibited lower REY concentrations, with average ΣREY values of 0.42 ± 0.11 mg kg^{-1} and 0.22 ± 0.02 mg kg^{-1}, respectively (Table 5). However, the measured REY concentrations in the leaf

Table 3. Comparison of measured element concentrations (mean ± standard deviation; *n* = 6) and certified values (with associated uncertainties), expressed both in mg kg^{-1}, obtained recoveries (%), and calculated REY fractionation indices in Apple leave (NIST SRM 1515).

Element	Measured	Certified value	Recovery	Element	Measured	Certified value	Recovery
Al	270 ± 15	286 ± 9	94	Tl	0.012 ± 0.002	–(a)	–(a)
As	0.035 ± 0.002	0.038 ± 0.007	92	U	0.006 ± 0.0006	0.006*	100
Ba	47.5 ± 2	49 ± 2	97	V	0.261 ± 0.015	0.26 ± 0.03	100
Be	< 0.002	–(a)	–(a)	Zn	12.6 ± 0.8	12.5 ± 0.3	101
Bi	0.011 ± 0.002	–(a)	–(a)	Zr	0.728 ± 0.012	–(a)	–(a)
Cd	0.013 ± 0.002	0.013 ± 0.002	100	Y	9 ± 0.5	–(a)	–(a)
Co	0.085 ± 0.005	0.09*	94	La	20.4 ± 0.5	20*	102
Cr	0.3 ± 0.02	0.3*	100	Ce	3.05 ± 0.2	3*	102
Cs	0.005 ± 0.001	–(a)	–(a)	Pr	4.1 ± 0.2	–(a)	–(a)
Cu	5.42 ± 0.30	5.64 ± 0.24	96	Nd	17.3 ± 0.5	17*	102
Fe	84.1 ± 2.5	83 ± 5	101	Sm	3.1 ± 0.1	3*	103
K	15900 ± 250	16100 ± 20	99	Eu	0.195 ± 0.015	0.2*	98
Li	0.251 ± 0.026	–(a)	–(a)	Gd	3 ± 0.12	3*	100
Mg	2630 ± 150	2710 ± 8	97	Tb	0.39 ± 0.02	0.4*	98
Mn	51.5 ± 2.5	54 ± 3	95	Dy	1.85 ± 0.2	–(a)	–(a)
Mo	0.094 ± 0.006	0.094 ± 0.013	100	Ho	0.31 ± 0.02	–(a)	–(a)
Na	22.6 ± 1.1	24.4 ± 1.2	93	Er	0.64 ± 0.05	–(a)	–(a)
Ni	0.95 ± 0.05	0.91 ± 0.12	104	Tm	0.048 ± 0.003	–(a)	–(a)
Pb	0.45 ± 0.01	0.47 ± 0.024	92	Yb	0.28 ± 0.013	0.3*	93
Rb	9.6 ± 0.5	10.2 ± 1.5	94	Lu	0.032 ± 0.002	–(a)	–(a)
Sb	0.014 ± 0.001	0.013*	108	ΣREY(b)	63.7		
Sc	0.029 ± 0.003	0.03*	97	Eu / Eu*(c)	0.28		
Se	0.047 ± 0.003	0.05 ± 0.009	94	Ce / Ce*(d)	0.08		
Sn	0.164 ± 0.015	< 0.2*	–(a)	ΣLREE / ΣHREE(e)	14.4		
Sr	25.1 ± 0.5	25 ± 2	100	Ba / Eu(f)	243		
Ti	15.9 ± 0.6	–(a)	–(a)				

(a) - not specified.
(b) ΣREY – sum of all rare earth element concentrations.
(c) Eu / Eu* - europium anomaly (Eu / Eu*= EuNASC / (SmNASC × GdNASC)0.5).
(d) Ce / Ce* - cerium anomaly (Ce / Ce* = CeNASC / (LaNASC × PrNASC)0.5).
(e) ΣLREE / ΣHREE - ratio of sum of light rare earth and heavy rare earth element concentrations.
(f) Ba / Eu – ratio of Ba and Eu concentrations.

samples were up to two times lower compared to the Citrus leave CRM,[31] which is reflected in the substantially higher ΣREY in NCS ZC73018, amounting to 2.8 mg kg^{-1} (Table 2). Also, compared to the average REE concentrations in leaves from organic and conventional citrus systems reported by Turra *et al.,*[23] obtained concentrations for all studied leaf samples are substantially lower (up to an order of magnitude lower).

Compared to other plant species, the REY concentrations found in citrus leaves were lower than the ones reported for forest plants (*Betula*, *Pinus sylvestris*, *Vaccinium vitis-idaea*, *V. myrtillus*, *Deschampsia flexuosa*, *Polytrichum*, *Sphagnum)* from Germany,[34] comparable or higher than those reported for grass leaves (*Agrostis capillary*)[35] and higher than REY values reported for cabbage (*Brassica oleracea* var. *capitate*).[36] They were also found comparable to REY levels reported for olive leaves grown on reclaimed karst, rendzina, and calcocambisol soil, but lower compared to ones grown on cambisol soil characterised by lower pH (4.7–5.8) and elevated levels of

Table 4. Comparison of measured element concentrations (mean ± standard deviation; *n* = 6) and certified values (with associated uncertainties), expressed both in mg kg^{-1}, obtained recoveries (%), and calculated REY fractionation indices in Rye grass (ERM CD281).

Element	Measured	Certified value	Recovery	Element	Measured	Certified value	Recovery
Al	56 ± 3	–$^{(a)}$	–$^{(a)}$	Tl	0.006 ± 0.001	–$^{(a)}$	–$^{(a)}$
As	0.040 ± 0.005	0.042 ± 0.010	95	U	0.003 ± 0.001	–$^{(a)}$	–$^{(a)}$
Ba	12.5 ± 0.5	–$^{(a)}$	–$^{(a)}$	V	0.26 ± 0.02	–$^{(a)}$	–$^{(a)}$
Be	< 0.002	–$^{(a)}$	–$^{(a)}$	Zn	29.0 ± 1.3	30.5 ± 1.1	93
Bi	0.003 ± 0.001	–$^{(a)}$	–$^{(a)}$	Zr	0.75 ± 0.05	–$^{(a)}$	–$^{(a)}$
Cd	0.114 ± 0.006	0.12 ± 0.007	95	Y	0.034 ± 0.002	–$^{(a)}$	–$^{(a)}$
Co	0.24 ± 0.01	–$^{(a)}$	–$^{(a)}$	La	0.06 ± 0.005	–$^{(a)}$	–$^{(a)}$
Cr	24.2 ± 1.0	24.8 ± 1.3	98	Ce	0.11 ± 0.01	–$^{(a)}$	–$^{(a)}$
Cs	0.081 ± 0.002	–$^{(a)}$	–$^{(a)}$	Pr	0.014 ± 0.002	–$^{(a)}$	–$^{(a)}$
Cu	9.7 ± 0.5	10.2 ± 0.5	95	Nd	0.16 ± 0.01	–$^{(a)}$	–$^{(a)}$
Fe	172 ± 10	180*	96	Sm	0.009 ± 0.002	–$^{(a)}$	–$^{(a)}$
K	31000 ± 2000	34000*	96	Eu	0.021 ± 0.002	–$^{(a)}$	–$^{(a)}$
Li	0.054 ± 0.007	–$^{(a)}$	–$^{(a)}$	Gd	0.035 ± 0.002	–$^{(a)}$	–$^{(a)}$
Mg	1550 ± 100	1600*	97	Tb	0.014 ± 0.002	–$^{(a)}$	–$^{(a)}$
Mn	78.7 ± 3	82 ± 4	96	Dy	0.018 ± 0.002	–$^{(a)}$	–$^{(a)}$
Mo	2.19 ± 0.07	2.22 ± 0.12	99	Ho	0.011 ± 0.002	–$^{(a)}$	–$^{(a)}$
Na	3900 ± 250	4000*	98	Er	0.010 ± 0.002	–$^{(a)}$	–$^{(a)}$
Ni	14.7 ± 0.6	15.2 ± 0.6	97	Tm	0.016 ± 0.002	–$^{(a)}$	–$^{(a)}$
Pb	1.64 ± 0.05	1.67 ± 0.11	98	Yb	0.030 ± 0.002	–$^{(a)}$	–$^{(a)}$
Rb	41.1 ± 1.4	–$^{(a)}$	–$^{(a)}$	Lu	< 0.002	–$^{(a)}$	–$^{(a)}$
Sb	0.039 ± 0.002	0.042 ± 0.007	93	ΣREY$^{(b)}$	0.54		
Sc	0.014 ± 0.002	–$^{(a)}$	–$^{(a)}$	Eu / Eu*$^{(c)}$	5.25		
Se	0.021 ± 0.002	0.023 ± 0.004	91	Ce / Ce*$^{(d)}$	0.88		
Sn	0.067 ± 0.007	0.062 ± 0.011	108	ΣLREE / ΣHREE$^{(e)}$	4.1		
Sr	23.7 ± 0.8	–$^{(a)}$	–$^{(a)}$	Ba / Eu$^{(f)}$	595		
Ti	4.5 ± 0.3	–$^{(a)}$	–$^{(a)}$				

(a) - not specified.
(b) ΣREY – sum of all rare earth element concentrations.
(c) Eu / Eu* - europium anomaly (Eu / Eu*= EuNASC / (SmNASC × GdNASC)0.5).
(d) Ce / Ce* - cerium anomaly (Ce / Ce* = CeNASC / (LaNASC × PrNASC)0.5).
(e) ΣLREE / ΣHREE - ratio of sum of light rare earth and heavy rare earth element concentrations.
(f) Ba / Eu – ratio of Ba and Eu concentrations.

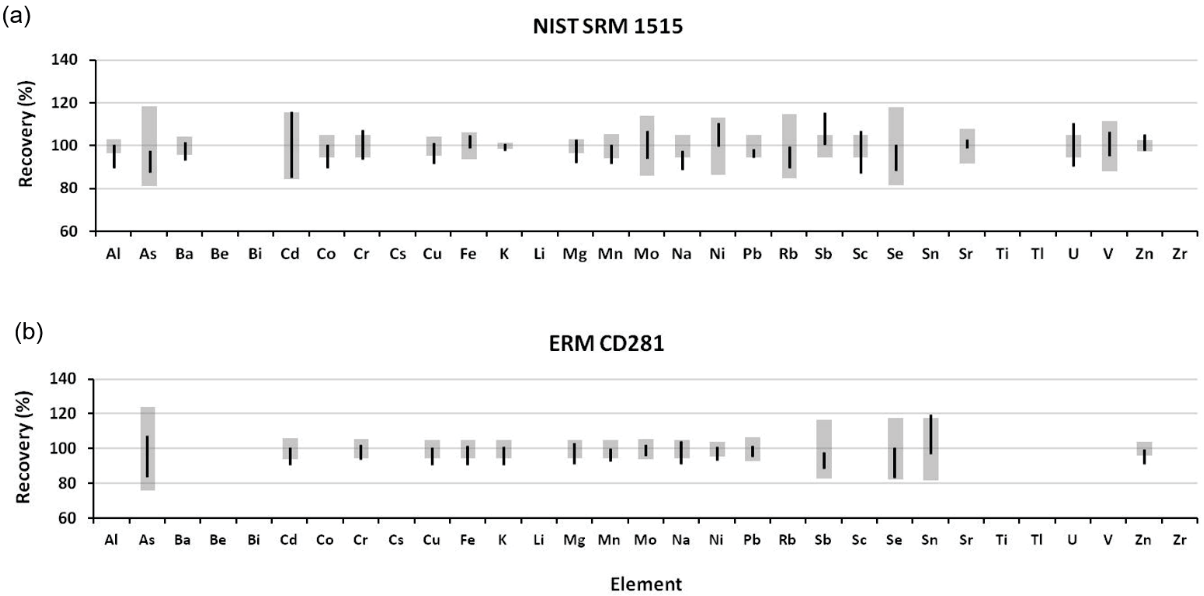

Figure 1. Comparison of measured (indicated by I) and certified (indicated by I) major, minor, and trace elements in (a) Apple leave (NIST SRM 1515) and (b) Rye grass (ERM CD281), presented as recovery ranges.

Al and P.[37] Even though the influence of soil characteristics on plant uptake of REY in a natural environment is still largely unknown, the study of Pošćić *et al.*[37] suggests that the specific local soil condition, such as pH and phosphorus availability, could explain most of the REY variability in plants, including also leaves.

Interestingly, the REY levels in studied citrus leaves were substantially lower or comparable to the lowest values reported by Miao *et al.*[38] for leaves of *Eucalyptus globulus Labill*, *Dicranopteris dichotoma*, and *Pinus massoniana*, although the concentrations of REY in soils reported by these authors are comparable to REY levels in soils from Croatia.[37,39,40] Thereby, it should be noted that the concentration levels of major and trace elements recorded in the ornamental plant substrate (pH 5.0–6.5) available on the Croatian market[41] are comparable or even lower than those in Croatian soils.[40]

Furthermore, the general order of REEs concentrations in different compartments of the citrus system was reported to decrease in the following order, soil > leaf > peel > pulp > seed > juice.[23]

In all investigated samples light rare earth elements, *i.e.* elements from La to Gd (LREE), were found to be more abundant compared to heavy rare earth elements, *i.e.* elements from Tb to Lu (HREE), with average ΣLREE / ΣHREE ratios of 11.6, 15.5 and 43.4 for tangerine, orange and lemon leaf samples, respectively (Table 5). Despite the differences in total REY, the ΣLREE / ΣHREE in NCS ZC73018 (15.1) is comparable to the average value obtained for orange leave (Table 1). Ratios similar to those obtained for tangerine and orange leaves were also reported for *Eucalyptus globulus Labill*, *Dicranopteris dichotoma*, and *Pinus massoniana* leaves from South China[38].

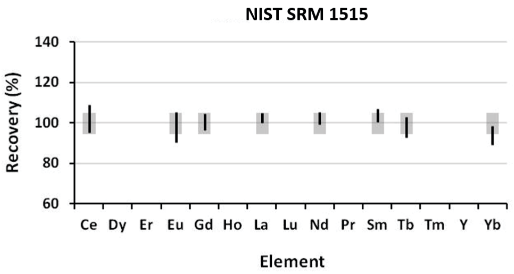

Figure 2. Comparison of measured (indicated by I) and certified (indicated by I) rare earth elements in Apple leave (NIST SRM 1515), presented as recovery ranges.

Citrus leaf samples exhibited a slightly positive to positive europium anomaly ($Eu / Eu^* = Eu_{NASC} / (Sm_{NASC} \times Gd_{NASC})^{0.5} = 1.05 – 2.82$), and a negative to slightly negative cerium anomaly ($Ce / Ce^* = Ce_{NASC} / (La_{NASC} \times Pr_{NASC})^{0.5} = 0.55 – 0.99$) (Table 5); both of which are in accordance with values obtained for NCS ZC73018 (Table 1). Interestingly, the lowest average values for both anomalies were observed for the lemon leaf samples (Eu / Eu* = 1.29 and Ce / Ce* = 0.56). The orange leaves, on the other hand, showed higher average values of both anomalies (Eu / Eu* = 2.35 and Ce / Ce* = 0.90), while the highest average values were observed for tangerine leaves (Eu / Eu* = 2.41 and Ce / Ce* = 0.95). The predominance of LREE in citrus leaves, accompanied by negative Ce anomaly, was also reported by Turra *et al.*[23].

Table 5. Measured element concentrations (average and standard deviation), expressed as mg kg^{-1}, in different citrus leaves,[31],and calculated REY fractionation indices.

Element	Orange leave	Lemon leave	Tangerine leave
Y	0.058 ± 0.015	0.108 ± 0.013	0.026 ± 0.001
La	0.082 ± 0.020	0.583 ± 0.070	0.038 ± 0.004
Ce	0.145 ± 0.038	0.461 ± 0.051	0.074 ± 0.008
Pr	0.017 ± 0.005	0.063 ± 0.006	0.009 ± 0.001
Nd	0.067 ± 0.020	0.225 ± 0.031	0.038 ± 0.002
Sm	0.013 ± 0.004	0.034 ± 0.003	0.008 ± 0.001
Eu	0.006 ± 0.002	0.009 ± 0.001	0.004 ± 0.001
Gd	0.015 ± 0.001	0.019 ± 0.001	0.009 ± 0.001
Tb	0.002 ± 0.001	0.003 ± 0.001	0.001 ± 0.0003
Dy	0.008 ± 0.001	0.014 ± 0.002	0.006 ± 0.001
Ho	0.002 ± 0.001	0.002 ± 0.001	0.001 ± 0.0003
Er	0.005 ± 0.001	0.006 ± 0.001	0.004 ± 0.001
Tm	0.001 ± 0.0003		
Yb	0.005 ± 0.002	0.005 ± 0.001	0.003 ± 0.001
Lu	0.001 ± 0.0003	0.001 ± 0.0003	0.001 ± 0.0003
ΣREY[(a)]	0.421 ± 0.111	1.54 ± 0.174	0.220 ± 0.018
Eu / Eu*[(b)]	2.35 ± 0.43	1.29 ± 0.25	2.41 ± 0.64
Ce / Ce*[(c)]	0.90 ± 0.02	0.56 ± 0.002	0.95 ± 0.24
ΣLREE / ΣHREE[(d)]	15.5	43.4	11.6
Ba / Eu[(e)]	2483	2044	2550

[(a)] ΣREY – sum of all rare earth element concentrations.

[(b)] Eu / Eu* - europium anomaly (Eu / Eu*= EuNASC / (SmNASC × GdNASC)0.5).

[(c)] Ce / Ce* - cerium anomaly (Ce / Ce* = CeNASC / (LaNASC × PrNASC)0.5).

[(d)] ΣLREE / ΣHREE - ratio of sum of light rare earth and heavy rare earth element concentrations.

[(e)] Ba / Eu – ratio of Ba and Eu concentrations.

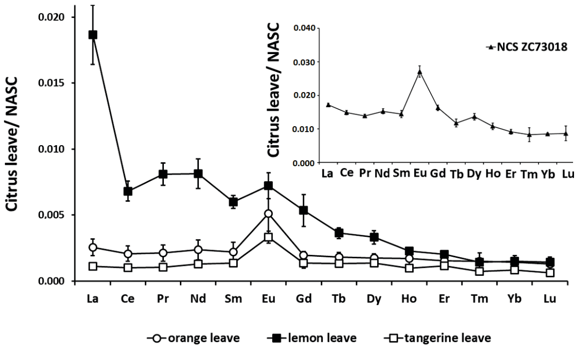

Figure 3. NASC-normalized REE patterns of citrus leaf samples, including the CRM NCS ZC73018.

Prevalence of LREE, along with positive Eu and negative Ce anomaly, is reflected in the average REE patterns of studied citrus leaves (Figure 3), normalized to the average composition of North American Shale Composite (NASC).[42]

The normalized curves of the orange and tangerine leaves are similar to that of CRM NCS ZC73018 (Figure 3), except for the less pronounced Eu anomaly, while the normalized curve of the lemon leaves in more inclined, showing a decrease in normalized values from LREE to HREE. Compared to the normalized curves of citrus leaves reported by Turra *et al.,*[23] all curves obtained in this study are less inclined and have significantly stronger Eu anomalies. The positive bias for the Eu anomaly could be, at least partly, attributed to the interferences from oxide species, specifically BaO. Namely, in case of high Ba concentrations and an abundance of Ba / Eu > 1000, notable BaO interference on both Eu isotopes (^{151}Eu and ^{153}Eu) and occurrence of false-positive Eu anomaly was observed in the samples.[43] In this study, Ba / Eu > 2000 was observed for all citrus leaf samples, including the NCS ZC73018 (Table 1 and Table 5). Usually switching to high-resolution (HR) mode improves the Eu determination and elimination of BaO interference;[43] however, in this case, low levels of Eu in all studied samples prevented the use of HR mode. Therefore, caution is advised when applying low and medium-resolution mode for quantification of Eu in biota samples containing high levels of barium.

Although the remaining two reference materials (NIST SRM 1515 and ERM CD281) showed much lower Ba/Eu ratios, the other calculated parameters indicate high variability between plant species not only in terms of REY fractionation but also in their total accumulation (Table 3 and Table 4). Thereat, Apple leave (NIST SRM 1515) displayed up to two orders of magnitude higher REY values (ΣREY = 63.7, Table 3) compared to citrus leaves. Given that the species in the genus *Malus*, along with species in the genus *Citrus*, are among the most commonly grown tree species, further research is needed on this topic.

CONCLUSION

Investigated citrus leaves displayed substantially lower REY levels compared to the literature available for the citrus system. However, they displayed similar fractionation, *i.e.* the prevalence of LREE, positive Eu and negative Ce anomaly. Compared to the certified reference material for citrus leaves (NCS ZC73018),[31] studied citrus leaves displayed overall lower REY concentrations.

The presented data also include concentrations of 46 elements in two biological reference materials, Apple leave (NIST SRM 1515), and Rye grass (ERM CD281), determined by HR-ICP-MS. In comparison with the certified and/or informative values available for these reference materials, additional information for 14 elements in NIST SRM 1515, and 30 elements for ERM CD281, including the REYs, was provided, extending their application in environmental and food studies.

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