POSSIBILITIES OF LOW-POWER X-RAY FLUORESCENCE SPECTROMETRY TECHNIQUES FOR RAPID MULTIELEMENTAL ANALYSIS AND IMAGING OF VEGETAL FOODSTUFFS

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Abstract

In the present contribution the possibilities and drawbacks of two analytical strategies based on the use of low power X-ray fluorescence systems (EDXRF and μ-XRF) have been explored for rapid multielemental analysis and chemical imaging of different edible vegetal species. The proposed methodologies allow analysis of vegetation material without complex sample treatments providing relevant advantages in terms of simplicity and costs compared to classical destructive methods.

As study cases, we have focused on the analysis of different parts of several vegetal species (*Daucus carota, Spinacia oleracea, Cynara scolymus, Raphanus staivus, Coriandrum sativum*) collected in agricultural soils irrigated with municipal treated wastewater samples. EDXRF and μ-EDXRF data revealed that content of some major elements such as P and S increased if crops are irrigated with municipal treated wastewater although the distribution of these elements is not depending of the type of water used for irrigation purposes. On the contrary, trace element content is not significantly higher in comparison to vegetables irrigated with fresh water, except for bromine.

The findings of this contribution can contribute to expand the knowledge about the impact of the use of reclaimed wastewaters for irrigation on vegetal composition but the use of the aforementioned XRF techniques could be also extended to other food safety and nutritional studies.

Keywords: Multielemental analysis, edible vegetable, carrot, spinach, artichoke, radish, coriander, EDXRF, μ-XRF, treated wastewater irrigation

1. Introduction

Human health and life quality is strongly dependent on the quality of food consumed daily. Increasing consumption of vegetables in recent years is associated with widespread consumer interest in a well-balanced and healthy lifestyle. Vegetables are an essential part of human diet and they are one of the main sources of major, minor and trace elements (Jolly et al., 2013). However, vegetation is the primary recipient of elements from the environment (soil, water, air) that are transferred via roots and foliage and thus, multielemental composition of vegetable foodstuff is important for both safety and nutritional purposes (Fraga, 2005).

Usually, atomic spectrometry techniques, including FAAS (Flame atomic absorption spectrometry), ETAAS (Electrothermal atomic absorption spectrometry), ICP-AES (Inductively coupled plasma emission spectrometry) and ICP-MS (Inductively coupled plasma mass spectrometry), are the techniques of choice for element determination in biological and environmental samples. At present, ICP-MS is usually preferred among the atomic spectroscopic techniques due to its multielemental capability, the extremely low limits of detection for most elements and high sample throughput (Chamberlain et al., 2000). However, the use of this technique usually involves sample preparation procedures for the total destruction of the solid matrix by means of a previous chemical treatment. Commonly, wet ashing (involving digestion with strong acids) is used to destroy the organic matter and dissolve the analytes in such kind of solid organic matrices (Jin et al.2001 and King et al., 2010). Sample dissolution is usually a tedious, expensive and time-consuming step in the analytical procedure that limits sometimes its application in both environmental studies and quality control processes. In view of these problems, the use of other methodologies for direct analysis of environmental solid samples has been increased over the last few years, including X-ray fluorescence spectrometry (XRF). A great advantage of XRF techniques compared to wet chemical procedures is that the multielemental analysis can be directly carried out on solid samples. This avoids the tedious and laborious wet digestion steps and the possible analyte losses and / or sample contamination as well as a considerable decrease in analysis time (Margui et al., 2009a and Maulvault et al., 2012). Moreover, recent developments and commercialization of benchtop and table-top XRF spectrometers, which offer extreme simplicity of operation in a low-cost compact design, have further promoted the approach of XRF in the environmental field for many analytical problems (Marguí et al., 2010 and Marguí et al., 2012). In recent years several scientific contributions have been published about the use of high power wavelength-dispersive XRF (WDXRF) and energy-dispersive XRF (EDXRF) systems to monitor metal content in vegetation samples grown in contaminated environments (Marguí et al., 2009) but the benefits and drawbacks of low-power XRF systems to determine multielement composition of edible plants is still scarce.

In addition to the determination of the total elemental composition of the vegetal foodstuffs, in some studies it is also of interest to get information about the element distribution within vegetal tissues (Choi et al., 2014 and Romarís-Horta et al., 2014). For that, imaging techniques such as laser-ablation ICP-MS (LA-ICP-MS) or μ-XRF are required. The use of the latter one is

increasing in importance year to year as it is highlighted through the annual reviews published by the Royal Society of Chemistry "Atomic spectrometry update: review of advances in the analysis of clinical and biological materials, foods and beverages" (Taylor et al., 2015). However, most of the published contributions dealing with the use of μ-XRF are combining high-brilliance synchrotron radiation and high-performance X-ray microfocusing optics that are not usually available to the general user's community (Gherase et al., 2013 and Meng et al., 2014).

In the present contribution we explore the analytical capabilities of two benchtop and low-cost XRF systems (EDXRF and μ-XRF) for multielemental analysis and imaging of vegetal foodstuffs. As study case, we have focused on the analysis of different parts of several vegetal species (*Daucus carota* (Carrot), *Spinacia oleracea* (Spincach), *Cynara scolymus* (Artichoke), *Raphanus staivus* (Radish), *Coriandrum sativum* (Coriander)) collected in agricultural soils irrigated with municipal treated wastewater samples.

2. Materials and Methods

2.1 Vegetal samples

Several certified reference vegetal materials (CRMs) were used for quantification purposes: ZC73011 (Soy bean, NCS, China), ZC73012 (Cabbage, NCS, China), ZC73013 (Spinach, NCS, China), ZC73032 (Celery, NCS, China), ZC73033 (Scallion, NCS, China), ZC73036 (Green tea, NCS, China), DC73348 (Bush branches and leaves, NCS, China), DC73349 (Bush branches and leaves, NCS, China), DC73350 (Leaves of poplar, NCS, China), DC73351 (Tea, NCS, China), NMIJ7405a (Seaweed, NMIJ, Japan), TL-1 (Tea leaves, INCT, Poland), PVTL-6 (Polish Virginia Tobacco leaves, INCT, Poland) and OBTL-5 (Oriental Basma Tobacco leaves, INCT, Poland).These reference materials differ considerably both physically and chemically in order to assure the applicability of the EDXRF method developed to different vegetation matrices.

In order to test the possibilities of low-power EDXRF for multielement analysis of edible vegetal samples, different vegetal species were collected in cropped soils in a coastal agricultural area near Barcelona (Catalonia, NE Spain): *Cynara scolymus* (artichoke), *Daucus carota* (carrot), *Spinacia oleracea* (spinach), *Raphanus sativus* (radish), *Coriandrum sativum* (coriander). In all cases, crops were irrigated with municipal treated wastewater (TWW) for several years. Compound and representative vegetal samples were obtained by combining 5 up to 15 subsamples for each parcel (depending on the final wet vegetal mass).

μ-XRF analyses were performed in transversal and longitudinal sections of carrot specimens grown in experimental plots irrigated with fresh and TWW during its growth.

In both cases, vegetation specimens were uprooted from soils, stored in polyethylene bags and kept in a plastic container to avoid contamination during transportation to the laboratory.

2.2 Sample preparation

2.2.1 EDXRF analysis

Once at the laboratory, vegetal edible specimens were cut into different tissues (root, leaves, steam and fruit) and they were washed thoroughly with water and deionised water and oven-dried at around 40 ^{o}C until reaching a constant weight. To reduce particle size samples were ground in an agate ball mixer mill for 5-10 minutes. Once plant tissues were powdered and dried, they were preserved in a desiccator until analysis.

Considering the morphology of plant powder and the capacity to be compacted together, the preparation of pellets was performed without addition of a binder. Methodology used in the present study consist of weighing 5 g of powdered sample and pressing it at 10 Tm for 60 s to obtain a cylindrical pellet of 40 mm in diameter. This procedure was employed both for edible vegetal specimens and certified reference materials used for quantification purposes.

2.2.2 μ-XRF analysis

Once at the laboratory, carrot specimens were washed thoroughly with deionized water and cut into 0.2 mm tangential and longitudinal sections with a stainless steel surgical blade. Then they were ultra-frozen at -85 ^{o}C for a minimum of 24 h and lyophilized with a Cryodos Telstar® lyophilizer at -40 ^{o}C and a pressure lower than 0.1 mbar. After that, carrot sections were placed on a plastic support and directly measured by μ-XRF.

3.1 X-ray fluorescence instrumentation

For quantitative multielemental analysis of vegetal samples, a touch-control S2 RANGER EDXRF system (Bruker AXS, GmbH, Germany) with a Pd X-ray tube (Max. power 50 W) and a XFLASH™ Silicon Drift Detector (SDD) with a resolution <129 eV at Mn-K_α was used. The instrument is also equipped with nine primary filters that can be selected for improving measuring conditions for elements of interest. One of the most advantages of this spectrometer compared to other existing laboratory systems is that is equipped with an air-cooled low-power X-ray tube and a Peltier cooled SDD and thus, no cooling media and gas consumption are required. The evaluation of EDXRF spectra and calculation of the analyte net peak intensities were performed using the software (Spectra EDX, Bruker AXS) supplied with the equipment.

Information on element distribution within vegetal tissues was performed by a μ-XRF system (M4 Tornado, Bruker, Germany). The X-ray tube is a micro-focus side window Rh tube powered by a low-power HV generator and cooled by air. The generator is able to operate between 10-50 kV and 100-600 μA. A poly-capillary is used to obtain a spot size down to 25 μm for Mo K_α. Detection of fluorescence radiation is performed by an energy-dispersive SDD with 30 mm^2 sensitive area and energy resolution of 142 eV for Mn K_α. The system also allows to work under vacuum conditions. Evaluation of XRF spectra and calculation of the analyte net peak areas were performed using the software WinQXAS version 1.30 by the International Atomic Energy Agency (IAEA). In the present research, two types of measurement routines were

carried out using the μ-EDXRF system. On the one hand, imaging of samples was performed with scan resolution of 770x770 pixel, step size of 25 μm and a dwell time of 0.76 ms/pixel. In a second set of experiments single measurements of different parts of the carrot sections were performed.

Optimization of measurement conditions for both systems (EDXRF and μ-XRF) is displayed in sections 3.1.1and 3.2.1.

3. Results and Discussion

3.1 *Bulk multielemental analysis by EDXRF*

3.1.1 Optimization of measurement conditions

Different setting values were tested and properly selected to achieve better results in terms of detection limits and sensitivity for multielemental analysis. As it is shown in Table 1, two different excitation conditions were selected for elements with atomic number values up to 17 (Na-Cl) and for elements with higher atomic numbers (K-Pb). In the first case, a ratio between the X-ray tube voltage and current of 20 kV/0.171 mA was selected. This mode is most effective within the Kα emission energy up to 3 keV (light elements). For heavier elements, a combination of a primary filter made of Al (500 μm thickness) and a ratio between the X-ray tube voltage and current of 40 kV/0.349 mA was the best option. For both conditions, analyses were performed in vacuum atmosphere and using a measuring time of 200 s (total analysis time: 400 s). Measurement time was chosen as a compromise between counting statistics uncertainty (XRF system) and total analysis time. In Figure 1, as an example, obtained EDXRF spectra for the analysis of the OBTL-5 certified reference material is displayed.

3.1.2 Analytical performance

Once qualitative analysis was carried out using the analytical conditions displayed in Table 1, and taking into account the typical element contents in different vegetation specimens, a quantitative EDXRF method was developed based on empirical calibration mode.

Calibration curves were established using a set of 14 vegetal certified reference materials (see section 2.2 for details) in order to provide a suitable concentration range but also a good spread of calibration data points over the range of each element determined. A correction method for absorption effects based on the use of fixed alphas (referred to intensity correction) was used on the basis of the computerized routine program linked to the equipment. Table 2 presents the calibration data obtained for vegetation matrices. Results demonstrate the linearity over the whole concentration range studied (in most cases R^2>0.99) and also the suitability of the matrix correction method employed. Limits of detection (LOD) were calculated according to the 3σ criteria (Van Grieken & Markowicz, 2002) and are displayed in Table 2 as well. It is apparent that low-power EDXRF systems enable the detection of mineral elements in vegetation samples at mg kg^{-1} concentration levels. LOD for light elements (i.e., Na) are higher due to the inherent low fluorescent yield but they are low

enough if we consider that these elements are present at high concentrations in plant tissues. Despite that metal contents that can be detected by EDXRF are higher than those usually associated with other atomic techniques such as ICP-MS technique, the simplicity of sample preparation (acid digestion is avoided) and measurement makes the use of low-power EDXRF a promising alternative technique to be used in some applications where the analytical procedures for elemental determination in plant tissues should be fast and cheap.

At present, only two metals (Pb and Cd) are listed as contaminants in vegetable foodstuffs products in the Official Journal of the European Union CE 1881/2006. Unfortunately, the limits established for these elements (Pb: 0.1-0.3 mg kg^{-1} and Cd: 0.05-0.2 mg kg^{-1}) are too low to be detected using the developed EDXRF method (calculated detection limit for Pb is around 2.5 mg kg^{-1}). However, the developed analytical methodology can be useful to obtain quantitative information about other elements present at higher levels which are also included as contaminants in foods in other countries. For instance, in the Czech Republic, Fe, Zn and Cu concentrations are limited to 50 mg kg^{-1}, 25 mg kg^{-1} and 10 mg kg^{-1}, respectively (Krejcová et al., 2016). According to the detection limits of our methodology (see Table 2) the quantification of these elements is then feasible.

Finally, accuracy and precision of the developed EDXRF method were checked by analyzing five replicate samples of a certified reference material under the measurement conditions and the quantification mode previously described. Results obtained are presented in Figure 2. As it is shown, good agreement was obtained between determined and certified concentrations for all elements and no significant differences were found at 95% confidence level. Relative standard deviations estimated were in all cases lower than 10%.

3.1.3 Application to different edible plants tissues and species

The developed methodology was applied to multielemental analysis of different edible plant species and tissues. As it is shown in Figure 3, this simple and fast methodology allows the determination of a wide range of major (Na, Mg, Si, P, S, Cl, K, Ca), minor (Al, Fe, Br, Sr) and trace elements (Mn, Cu, Zn, Rb) in the edible part of each species as well as in other parts of the target vegetables. From the obtained results it is apparent that there is a common pattern with regards to the distribution of the elements within the plant tissues. This fact can be interesting to understand process of plant biology or for nutritional purposes. For instance, Al and P, are present at higher concentrations in the edible part of plants. On the contrary, Fe is greatly accumulated in leaves, especially in artichokes, where Fe concentration in leaves is one order of magnitude higher than in other parts of the plant. Regarding major elements (Ca, Mg Na and S) they are mainly concentrated in leaves and stems.

As stated in the experimental section, in all cases, target crops were irrigated with municipal treated wastewater (TWW) for several years. In Table 3, a comparison of mean concentrations for major, minor and trace elements in the edible part of the studied vegetal foodstuffs were compared with those reported in other scientific studies where vegetal samples were irrigated with TWW and fresh water. As it can be seen, in a general way, trace element content for

vegetables irrigated with TWWs is not significantly higher than those irrigated with conventional fresh water. The only exception is Br content in radish which is almost three times higher for those specimens irrigated with TWW. However, additional experimental tests are required to support this statement.

The effect of TWWs irrigation on major and minor element content in vegetal foodstuffs is difficult to be assessed due to the lack of available data in the scientific bibliography. Most of the studies related to wastewater irrigation are focused on the study of a limited group of elements (Arora et al., 2008) and most of them are dealing with the study of trace and potentially toxic elements (Holm et al. 2010 and Jaward, 2010) rather than an evaluation of the multielement composition of vegetables, including major and minor elements. However, from available data (see Table 3), it seems that there is an increase of major element content when vegetables are irrigated with TWW.

3.2 Imaging by *μ-XRF*

3.2.1 Optimization of measurement conditions

One of the main parameters to improve measuring conditions for elements of interest is the use of primary beam filters that are placed between the X-ray source and the sample. In the μ-XRF system used, five internal filters are available (Al 12.5 μm, Al 100 μm, Al 630 μm, Al/Ti_100/25 μm and Al/Ti/Cu_100/50/25 μm). The best strategy was to use a filter made of Al/Ti/Cu_100/50/25 μm to determine elements with an atomic number higher than 22. On the contrary, for light elements (from Na to Ti), better results in terms of sensitivity were assessed if no primary filter was used. X-ray tube current (mA) and voltage (kV) were automatically adjusted depending on the selection of the aforementioned primary filters. Regarding the measurement time, it was set at 300s since the signal to noise ratio was better than using 200 s and there was not a clear improvement using longer measurement times. A summary of the main instrumental conditions used to perform the μ-XRF analysis is presented in Table 4.

As detailed in Table 4, μ-XRF measurements on vegetal sections were performed under vacuum conditions. This fact enables the possibility to study light element distribution in the target samples which can be really useful to monitor the effect of TWWs irrigation of vegetal foodstuffs, as already pointed in section 3.1.3. Until recently, available commercial μ-XRF systems performed measurements under air conditions and therefore the determination of light elements in vegetation specimens was not possible. For this reason, most of published scientific contributions dealing with vegetal imaging by μ-XRF were focussed on the study of metal accumulation in contaminated areas rather than the study of major and minor elements distributions (Marguí et al., 2009b). An additional advantage of the μ-XRF system used is the reduced focal spot (25 μm for Mo-K_α) that allows a better mapping of the vegetation tissues.

3.2.2 Application to Daucus carota specimens irrigated with municipal TWW

As stated in section 2.1, μ-EDXRF analyses were performed in transversal and longitudinal sections of carrot specimens grown in experimental plots irrigated with fresh and TWWs

during its growth. Two-dimensional elemental mappings showed that for some elements a different distribution pattern within transversal and longitudinal sections exists. For instance, P, S and K were mainly concentrated in the central part and in the carrot peel (see Figure 4). However, for other studied elements (including other minor and trace elements) no distribution differences within sections were observed. It is also interesting to remark that these distribution tendencies were found both in carrot specimens irrigated with fresh and TWW waters during its growth. So, it seems that the type of water used for irrigation purposes did not affect the element distribution within vegetal tissues.

In order to study in more detail if there were differences with regards to element concentrations, single measurements of different parts of the carrot sections were performed both in carrot specimens irrigated with fresh and TWW and then a semiquantitative approach was used to estimate element concentrations. The relation between the fluorescence intensity of an element and its concentration is quite simple when a thin sample approximation is considered. When the mass per unit area is very small (thin sample) it is possible to consider that the total mass absorption coefficient of the sample is almost negligible and a linear relationship between the intensity of the fluorescent radiation and the concentration is observed (Marguí et al., 2009b). In the present contribution, calibration was performed using a set of nine commercially available certified reference materials of different vegetation species prepared as pressed pellets of similar mass (200 mg) to carrot samples. Then each certified reference material was measured in 12 points distributed along the pellet surface and the average peak area for each element was plotted versus known element concentrations and a straight line was fitted to measured points by the least-square method. In Table 5, information on the concentration range and regression coefficients obtained for 13 elements are displayed. As it is shown, regression coefficients for all elements were higher than 0.98, indicating an acceptable linearity taking into account the inherent heterogeneous nature of calibration samples at microscale level (Janssens et al., 2010). Using this quantitative approach it was found that P, S and Br content in the inner part of the carrot sections irrigated with TWW was approximately two times higher than those found in carrot specimens irrigated with fresh water (see Table 6 for details). Therefore, despite the fact that there were no significant differences with regards to the element distribution in carrot specimens irrigated with fresh and municipal treated wastewaters, a difference between some element contents exists depending of the type of water used for irrigation purposes.

Conclusions

In this contribution, two benchtop and low-cost EDXRF systems have been successfully applied for multielemental analysis and chemical imaging of vegetal foodstuffs.

On the one hand, it could be concluded that the EDXRF method for direct multi element determination in dry powdered plant materials described in this paper provides relevant advantages in terms of simplicity and costs compared to classical destructive methods. Usually

high power EDXRF systems have been used to monitor metal content in contaminated vegetation samples but this contribution highlight also the potential of low power EDXRF systems to determine multielement composition of edible plants. An additional advantage of this methodology is the possibility to quantify elements that are difficult to determine by other analytcial methods (P, S, Cl, Br…) which can also play an important role in understanding the processes of plant biology and for instance the effect of municipal wastewater irrigation onto crops.

The lateral resolution of the μ-EDXRF applied in this work (25 μm for Mo K_α) was small enough to study element distribution between parts of the target vegetation specimens providing relevant information not available from standard bulk analysis. Although the brilliance and the sensitivity from the low power X-ray tube used in the μ-EDXRF system is limited it is still possible to generate highly resolved elemental maps from such a device and thus, obtaining information of the spatial distributions of elements in plant tissues even for light elements.

The analysis of EDXRF and μ-EDXRF data on the studied vegetation samples revealed that, in a general way, trace element content for vegetables irrigated with TWWs is not significantly higher than those specimens irrigated with conventional fresh water. On the contrary, it seems that content of some major elements such as P and S is higher if crops are grown in TWW conditions although the distribution of these elements is not depending of the type of water used for irrigation purposes.

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Table 1

Instrument parameters and measurement conditions used for bulk multielemental analysis of edible vegetables by EDXRF

EDXRF benchtop system (S2 RANGER, Bruker AXS)	
Anode X-ray tube	Pd
Focal spot	26 mm
Detector	SDD-XFlash
Total analysis time	400s (200s/per analytical condition)
Measuring mode	Vacuum
Analytical Lines	
- K_α line	Na, Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Rb, Sr
- L_α line	Pb
Measurement conditions	
- Condition-1: 20kV, 0.171 mA, No primary filter	Na, Mg, Al, Si, P, S, Cl
- Condition-2: 40kV, 0.349 mA, 500µm Al filter	K, Ca, Mn, Fe, Cu, Zn, Br, Rb, Sr, Pb
Absorption effects corrections	Fixed alphas (intensity model)

Table 2

Analytical performance of the EDXRF method used for bulk multielemental analysis of edible vegetables

Element	LOD (mg Kg^{-1})	Concentration range (mg Kg^{-1})	Regression coefficient
Na	150	600-32400	0.995
Mg	40	200-10500	0.995
Al	30	200-3800	0.974
Si	35	500-12400	0.985
P	8	150-5400	0.992
S	5	300-16400	0.993
Cl	4	500-35400	0.998
K	55	1000-54400	0.993
Ca	35	400-48000	0.998
Mn	4	20-2135	0.999
Fe	15	50-1650	0.997
Cu	2	7-27	0.980
Zn	2	8-120	0.996
Br	1	4-485	0.999
Rb	0.8	4-89	0.996
Sr	1	30-1550	0.999
Pb	2.5	8-65	0.992

Table 3

Comparison of major, minor and trace element concentrations in different vegetation species determined in this work with other published values. Concentration values are expressed as mean values in mg Kg^{-1}.

Vegetal[a]	Irrigation[b]	Ref.	Na	Mg	Al	Si	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Br	Rb	Sr
C	TWW	This work	5279	2690	450	720	3970	2600	9810	32600	4232	6.9	44.8	8.8	25.6	44.5	5.9	17.6
	FW	Arora et al., 2008										15	17.5	7.5	42			
	TWW	Arora et al., 2008										14-20	200-235	12.5-21.6	40-51			
	FW	Krejcová et al., 2016	57-1100	49-213	nd-201		156-915	23-327		1400-7600	220-870	9.39	1.25-31	nd-5.4	nd-22			
	FW	Pandino et al., 2011	2500	800						20600	2800	7.2	33.2	3.6	10.8			
	FW	Kabata-Pendias, 2001			7.8								16-67	4.6-8.4	21-24			1.5-131
	FW	Azam et al., 2013		1700				7100		4600	1700	28	17		17			
S	TWW	This work	9600	9200	755	1340	5690	5120	12150	59300	15746	22	174	17	103	133	16	54
	FW	Arora et al., 2008										45	225	5	17			
	TWW	Arora et al., 2008										64-74	279-333	15-17	31-35			
	TWW	Khan et al., 2008												14	60			
	FW	Pandino et al., 2011								37300	9400	168	527	16.6	77.8			
	FW	Kabata-Pendias, 2001			104													45-70
A	TWW	This work	1840	2400	403	513	2967	2950	9008	23115	3529	13.7	24.7	8.0	29.2	16.8	1.8	6.7
	FW	Razic et al., 2008		3859						9031	11772	63.2	123.7	8	26.5			
	FW	Pandino et al., 2011	1500	1400						18800	4500	11.8	47	7.2	26.6			
R	TWW	This work	3300	2827	671	867	6340	5578	9681	59170	8301	12.8	50.3	8.3	47.3	84.3	12.5	28.3
	TWW	Khan et al., 2008												9	58			
	FW	Kabata-Pendias, 2001													27-708	24-26		
	FW	Azam et al., 2013	6900							35100		38			51			
C	TWW	This work	7189	3239	1094	2084	3758	5963	15836	52391	10359	31	394	13	51	41	10	39
	FW	Arora et al., 2008										30	275	7	20			
	TWW	Arora et al., 2008										41-48	292-326	10.9-13	29-33			

Information: nd (not detected), [a] In all cases the edible part of the vegetal is considered (C:carrot, S:Spinach, A:Artichoke, R:Radish, C:Coriander), [b] F: Fresh water, TWW: Treated wastewater.

460 **Table 4**

461 Instrument parameters and measurement conditions used for chemical imaging of vegetables

462 tissues by μ-XRF.

463

μ-XRF benchtop system (M4 Tornado, Bruker GmbH)	
Anode X-ray tube	Rh
Focal spot (Polycapillary)	25 μm (for Mo K_α)
Detector	SDD-XFlash
Total analysis time	600s (300s/per analytical condition)
Measuring mode	Vacuum
Analytical Lines (K_α lines)	Mg, Al, Si, P, S, Cl, K, Ca, Ti, Fe, Br, Rb, Sr
Measurement conditions	
- Condition-1: 10kV, 0.1 mA, No primary filter	Mg, Al, Si, P, S, Cl, K, Ca, Ti
- Condition-2: 50kV, 0.6 mA, 100/50/25 μm Al/Ti/Cu filter	Fe, Br, Rb, Sr

464 **Table 5**

465 Semiquantitative approach by external calibration for chemical imaging analysis by μ-XRF.

466

Element	Concentration range (mg Kg^{-1})	Regression coefficient
Mg	2200-8600	0.997
Si	170-2200	0.987
P	160-5800	0.996
S	800-4700	0.995
Cl	300-16400	0.997
K	1200-35400	0.986
Ca	3000-47500	0.986
Ti	400-39900	0.989
Mn	20-100	0.993
Fe	100-1470	0.989
Br	3-90	0.999
Rb	4-90	0.997
Sr	35-1470	0.998

467

468

469 **Table 6**

470 Comparison of element content in carrot specimens (inner part) irrigated with fresh water
471 (FW) and with municipal treated wastewater samples (TWW). Concentrations are expressed as
472 $mg \cdot kg^{-1}$ and were calculated using the μ-EDXRF semiquantiative approach described in section
473 3.2.2.
474

Irrigation	P	S	Cl	K	Ca	Fe	Br
FW	3380	745	7215	o.r.	2015	60	16
TWW	5660	1375	7960	28940	1770	55	9

475 o.r: out of calibration range

476

Figure 1

EDXRF spectra obtained for the analysis of the OBTL-5 certified reference material using the measurement conditions displayed in Table 1.

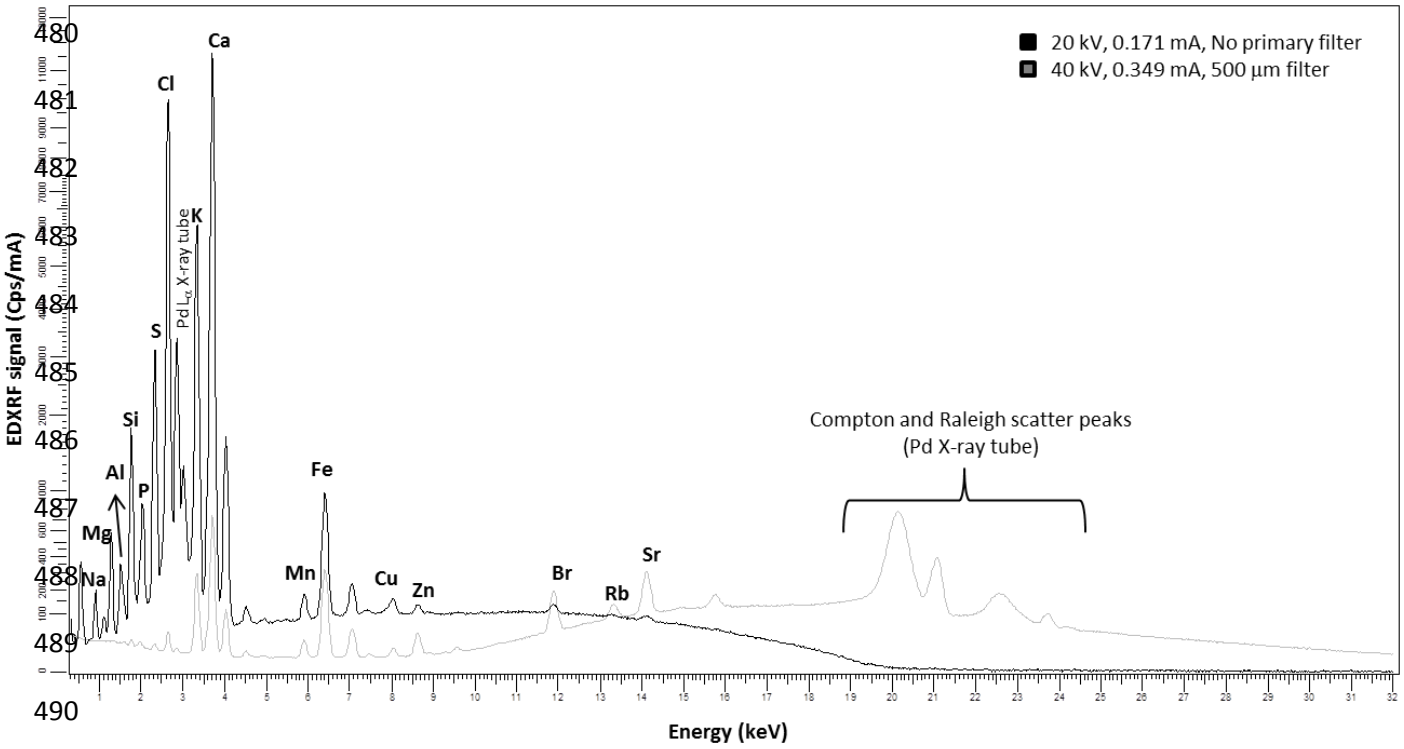


Figure 2

Concentrations obtained for the certified reference material CRM ZC73013 (Spinage) by using the developed EDXRF method. Error bars (EDXRF method) corresponds to the standard deviation of five replicate analysis.

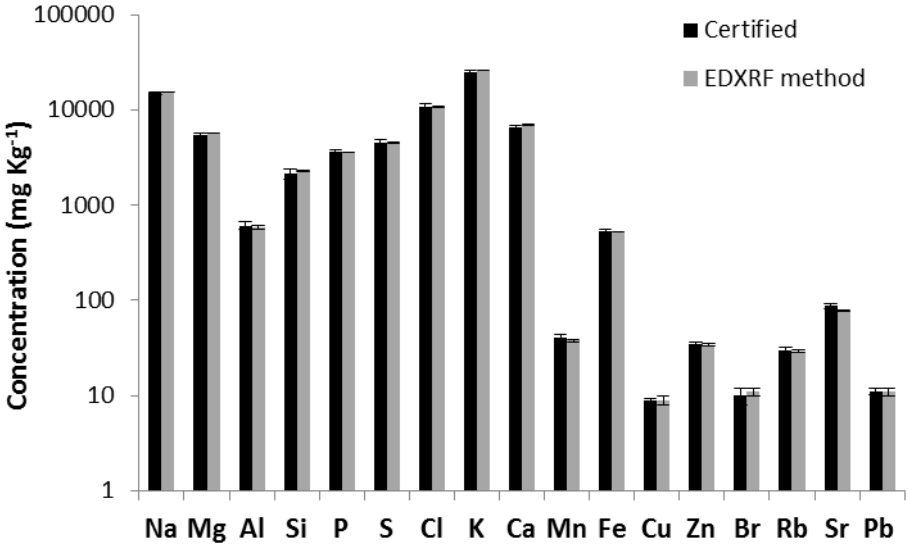


Figure 3

Multielemental concentrations in different edible vegetable tissues and species determined by the developed EDXRF method. Error bars corresponds to the standard deviation of duplicate analysis.

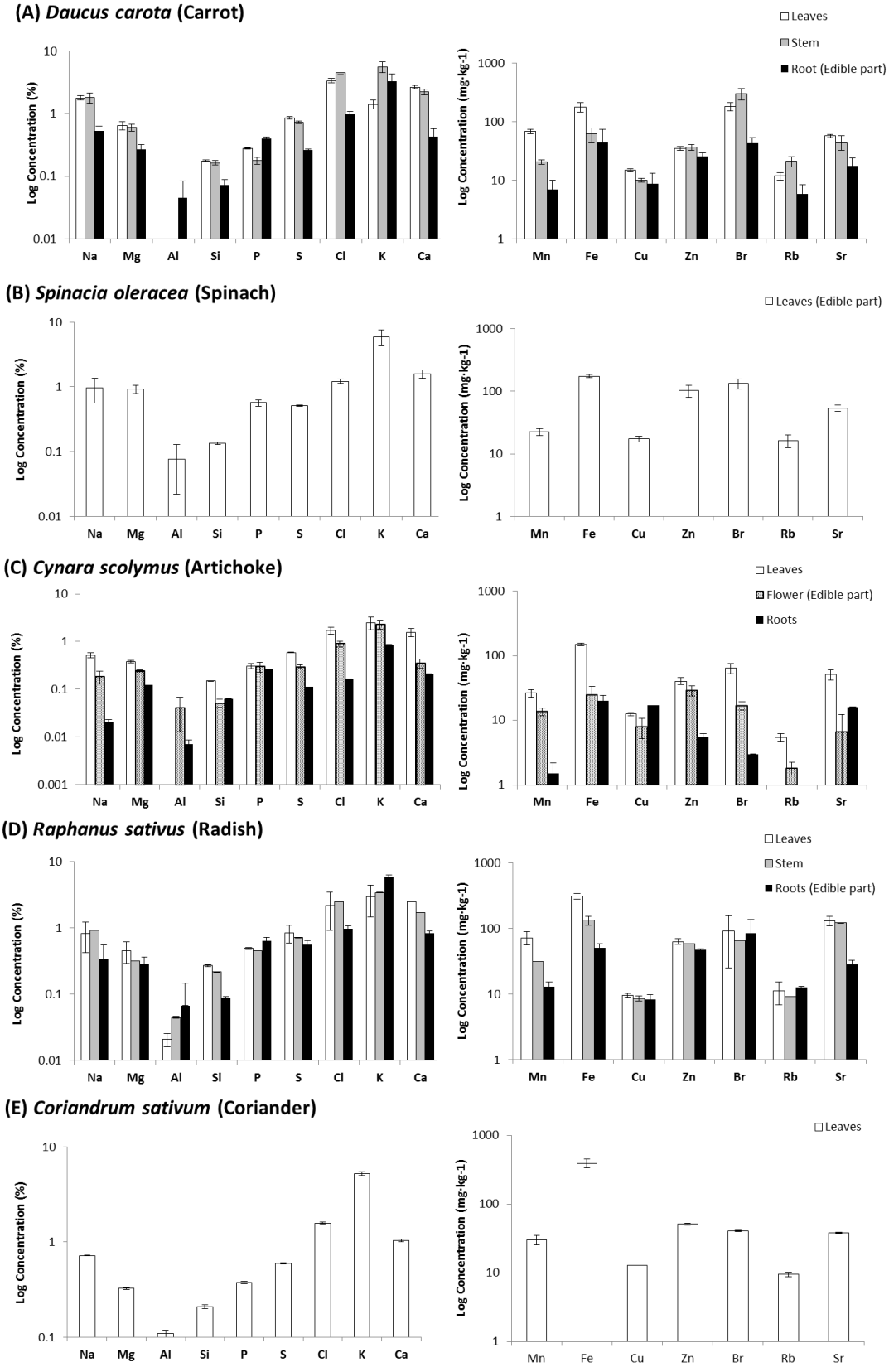
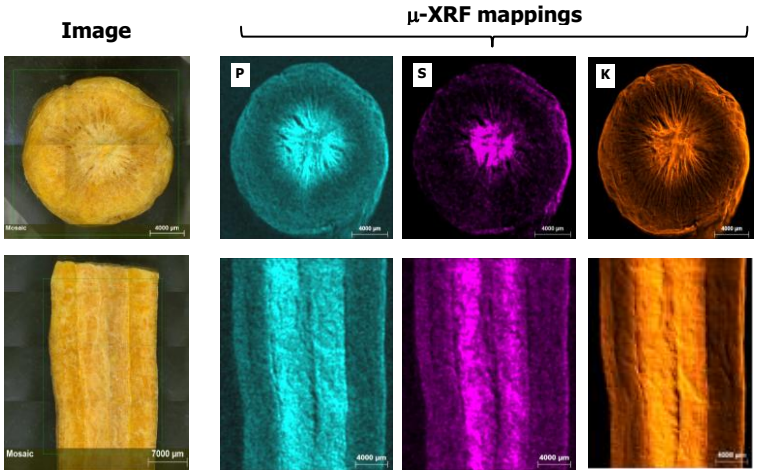


Figure 4

Mappings of tranversal and longitudinal sections of *Daucus carota* (carrot)



Highlights

- Rapid multielemental analysis and chemical imaging of vegetal foodstuffs
- Advantatges in terms of simplicity and costs to classical destructive methods
- Element distribution within vegetal is not depending on the irrigation water type
- P, S and Br increased if crops are irrigated with municipal treated waste waters