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ICP-EOS method development and validation for Cr(VI) determination in plants

Research article

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Abstract
The aim of this study was the development and validation of a new inductively coupled plasma optical emission spectrometry (ICP-EOS) method for determination of Cr(VI) from vegetable samples. The new method was completely validated through verification of performance parameters (linearity, inter-day and intra-day precision, limit of detection - LOD and limit of quantification - LOQ). The vegetable samples, mint and basil, were contaminated with a known amount of $K_2Cr_2O_7$. Cr(VI) was extracted from the vegetable samples by ultrasound assisted extraction technique, using 0.1 M Na_2CO_3 as extraction medium. The ICP-EOS method proved to be very sensitive, allowing to determine up to 3.0 μg/g Cr(VI) in vegetable matrices, value which was established as the method LOQ.

Keywords: Cr(VI), ICP-EOS, mint, basil, speciation

1. INTRODUCTION

The chemistry of chromium is very complex and depends on its solubility and mobility in soils, while its bioavailability strongly depends on different oxidation states of this metal (from 0 to 6), concentration, soil acidity, redox potential and salinity. Depending on oxidation state and concentration, chromium could act as a toxic metal or as an essential element for plants, animals and humans. The two most common species of chromium are Cr(III) and Cr(VI). Cr(III) is essential for animals and humans at low concentrations. It is very stable in soils, but it is usually found immobilized on iron and manganese oxides and hydroxides or complexed in organic matter. Cr(VI) toxicity strongly depends on its concentration, and the mechanism of absorption in soil is mainly based on passive diffusion [1,2].

The presence in the environmental waters of the two main oxidation states of chromium, Cr(III) and Cr(VI), is significantly influenced by biological, geochemical and toxicological factors. Hexavalent chromium is easily soluble in water and can accumulate in soil and plants, while Cr(III) probably exists in environmental waters in the form of many different species: hydrolysed, complexed and adsorbed on colloidal matter. Cr(III) may also undergo species change from the inorganic form to an organic complex, being thus transported as a carboxylate complex. Fortunately, not all chromium released from industrial processes is Cr(VI). In many cases, residual solutions are subject to reduction, and thereafter Cr(III) is released into the environment [3].

The interest for chromium speciation comes from the fact that the chemical properties and biochemical action of Cr(III) and Cr(VI) compounds differ a lot from each other. While Cr(III) belongs to the class of trace essential elements for the proper functioning of living organisms, Cr(VI) has toxic effects on biological systems and has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen in group 1. The toxic and carcinogenic properties of Cr(VI) compounds are due to the ability of the chromium anion (CrO_4^{-2}) to diffuse freely across the cell membranes and its action as an oxidizing

agent, as well as the formation of free radicals during the reduction of Cr(VI) to Cr(III) inside the cell [4-6].

Cr(VI) is ubiquitous in air, water and soil. It has been classified as a dangerous environmental pollutant by the Environmental Protection Agency (EPA, USA) (ATSDR 1998). The average global emissions of Cr from natural sources have been estimated at 43,000 tons/year. Most countries have set the value of 50 μg/L (ppb) Cr(VI) as the maximum admissible level of this contaminant in drinking water, while this level was set at 100 μg/L (ppb) by US drinking water legislation (ATSDR 1998). The amount of Cr in natural soils varies between 5 and 1000 mg/kg depending on the type of soil, but can reach up to 125 g/kg in serpentine soils (ATSDR 1998). In freshwater, its concentration can vary between 0.1 and 117 μg/L, while in seawater, it varies from 0.2 to 0.5 μg/L. Cr(VI) predominates in surface waters and aerobic soils, while Cr(III) is found in easily degradable environments such as sediments and wetlands. In remote and urban air samples, Cr can range from 5×10^{-6} to 1.2×10^{-3} μg/m^3 and 0.015 to 0.03 μg/m^3, respectively [7].

Mint commonly is known as an aromatic plant from *Lamiaceae* family, which includes *Satureja hortensis, Thymus serpyllum, Salvia officinalis, Ocimum basilicum*, etc. Although, not all mint species have therapeutic properties, *Mentha aquatica* (water mint), *Mentha viridis* or *Mentha spicata* (sweet mint) and *Mentha piperita* (peppermint) are recognized as mint species with medicinal properties. Mint is distributed especially in temperate and sub temperate regions and grows best in shady places. Cuttings or stolons with a very fast growth propagate mint [8, 9]. In Europe, mint was used into medicinal purpose in the mid-eighteenth century, the main symptoms treated being nausea, vomiting and gastrointestinal disorders. In addition to its therapeutic properties, mint also presents insecticidal properties [10].

The basil, or *Ocimum basilicum*, is one of the most popular herbs used as a spice, flavouring agent, or in traditional medicine, and along with its essential oil, the basil is well known for its pharmacological / therapeutic properties. The plant parts used for medicinal and culinary purposes are the stems, flowers, and leaves prepared as infusion, decoction, tincture, or different juices [10].

The ability of *Ocimum basilicum* to concentrate toxic metals (Cd, Cr, Pb, and As) and micronutrients (Fe, Zn, and Cu) in the plant tissues makes its use for the preparation of teas, spices, or raw consumption, a potential health concern [10].

Cr excess in soil, especially in the form of Cr(VI) can inhibit plant growth and pose a serious threat to presented organisms [11].

Cr(VI) detection in vegetable matrices using common spectrophotometric method is difficult due to the high LOQ limitation of this technique. For this reason, the aim of this study was to develop, validate and test a new ICP-EOS method for Cr (VI) determination in vegetable samples (mint and basil).

2. MATERIALS AND METHODS

2.1. Materials. Experiment description

Two different species of plants, mint (*Mentha Piperita*), and basil (*Ocimum Basilicum*), were acquired from the public market. The plant species were cultivated in 8 pots, 4 for each plant, the differences between the pots being the concentration of chromium in each of them. The description of experiments are presented in Tables 1 and 2.

2.2. Analysis methods

In this study an analytical method for the determination of Cr(VI) in plant matrix using inductively coupled plasma optical emission spectrometry (ICP-EOS Avio 500 Perkin Elmer) was developed and validated. The working conditions of the spectrometer were optimized in order to obtain the best results (Table 3).

The new method was applied to determine the amount of hexavalent chromium present in aqueous extracts of the plant matrix (plants, organs of plants). The method of extracting hexavalent chromium from vegetable matter consisted in treatment of samples with a solution of Na_2CO_3 in a ratio of 1: 100 (m/v) by boiling at 80^0C for 10 minutes.

Table 1. Experimental tests performed on mint plants.

Test	Number of tests	Number of plants/tests	Pot type/soil quantity	Images of mint plants
Blank Mint	1	4	hotchpotch 18 cm^3, 1 kg	
Test 4 mg/kg Cr(VI)	1	4	hotchpotch 18 cm^3, 1 kg	
Test 10 mg/kg Cr(VI)	1	4	hotchpotch 18 cm^3, 1 kg	
Test 30 mg/kg Cr_{total}	1	4	hotchpotch 18 cm^3, 1 kg	

Before extraction, after the soil residues were removed from the root, the plant samples were taken, washed with tap water and with ultrapure water and dried at 50°C. Over about 0.5 g of dry plant, 50 mL of 0.1 M Na_2CO_3 solution was added. The mixture was ultrasound treated for 20 minutes (10 minutes at 80°C). After the extraction time, the mixture was filtered through a 0.45 μm filter, and the aqueous phase was analysed using the new ICP-EOS method.

Table 2. Experimental tests performed on basil plants.

Test	Number of tests	Number of plants/tests	Pot type/soil quantity	Images of mint plants
Blank Basil	1	4	hotchpotch 18 cm^3, 1 kg	
Test 4 mg/kg Cr(VI)	1	4	hotchpotch 18 cm^3, 1 kg	
Test 10 mg/kg Cr(VI)	1	4	hotchpotch 18 cm^3, 1 kg	
Test 30 mg/kg Cr_{total}	1	4	hotchpotch 18 cm^3, 1 kg	

Table 3. Working conditions of the spectrometer ICP-EOS.

ICP-EOS Spectrometer parameters	
Plasma gas rate (argon)	15 L/min
Auxiliary gas rate	0.2 L/min
Nebuliser gas rate	0.75 L/min
Sample uptake rate (peristaltic pump)	1.9 mL/min
Power RF	1400 W
Number of replicates	4
Delay	60 s
Aria integration	10 points
λ (nm)	228,812
U5000AT+ UN	
Heater temperature	140°C
Cooler temperature	3°C

In the literature there are no data on Cr(VI) determinations by ICP-EOS, the detection being performed by other techniques (UV-Vis spectrometry, ion chromatography, atomic absorption spectrometry). The applied extraction method allows the preservation of the Cr(VI) species extracted from the plant without noticeable changes in the oxidation state for a period of 6 months [12,13].

The total Cr content was determined using the inductively coupled plasma optical emission spectrometry, using a method validated in the range 0.1-0.5 mg/L, the calibration solutions being prepared in 2.5% nitric acid solution.

3. RESULTS AND DISCUSSION

3.1. Method validation

To validate the new ICP-EOS method developed for Cr(VI) determination in vegetable matrices, the following parameters were tested: detection limit (LOD), quantitation limit (LOQ), linearity, dispersion homogeneity test, intra-day and inter-day precision method accuracy and measurement uncertainty. Four specific wavelengths for Cr (VI) detection were chosen: 267,716 nm, 205,560 nm, 357,869 nm and 206,158 nm. The results obtained from the validation tests are presented in Table 4.

Table 4. Performance parameters of the Cr(VI) determination method using the ICP-EOS technique.

Wavelength, nm	LOD (µg/L)	LOQ (µg/L)	RSD_r %	RSD_R %	Uex %
267.716	3,2	11,0	4,54	7,88	18.5
205.560	1,8	6,0	6,28	5,73	19.5
357.869	**0,6**	**3,0**	**4,23**	**4,93**	**17.8**
206.158	2,6	9,0	2,50	7,14	14.1

To determine the Cr(VI) content using the inductively coupled plasma optical emission spectrometry technique, the wavelength of 357,869 nm was chosen as the most suitable, as it showed the lowest quantification limit values (LOQ), repeatability (RSD_r) and intermediate accuracy (RSD_r).

A calibration curve was plotted in the range of 20 µg/L ÷ 100 µg/L Cr(VI), using a $K_2Cr_2O_7$ standard (Supelco, Merck KGaA, Germany) in 0.1 M Na_2CO_3 solution (Supelco, Merck KGaA, Germany) (Figure 1). The detector response was linear through the entire concentration range, the correlation coefficient being higher than 0.999.

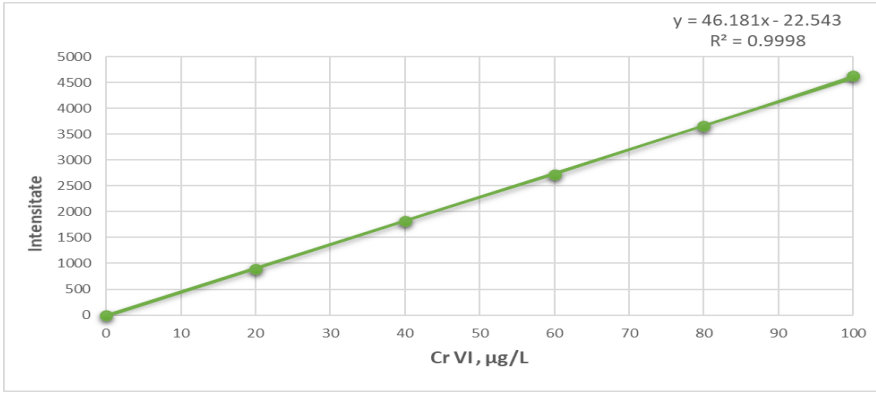

Figure 1. Linear regression plot for Cr(VI) determination by ICP-EOS (λ = 357,869 nm)

3.2. Tests on real samples of vegetable matter (mint and basil)

Two different plant species (basil and mint) were contaminated with Cr(VI) species, namely potassium dichromate, the concentration of Cr(VI) added to the soil being 4 mg/kg (concentration chosen in order to reach the alert threshold for soils with sensitive use according to Romanian Order no.756 / 1997), respectively of 10 mg/kg (intervention threshold for sensitive use). At the same time, one specimen from each of the two selected plants was contaminated with Cr(III) at a concentration of 30 mg/kg, representing the normal concentration in soil.

After 7 days from the contamination, plant samples were collected and the Cr(VI), respectively the total Cr content was determined, the results being presented in Figure 2.

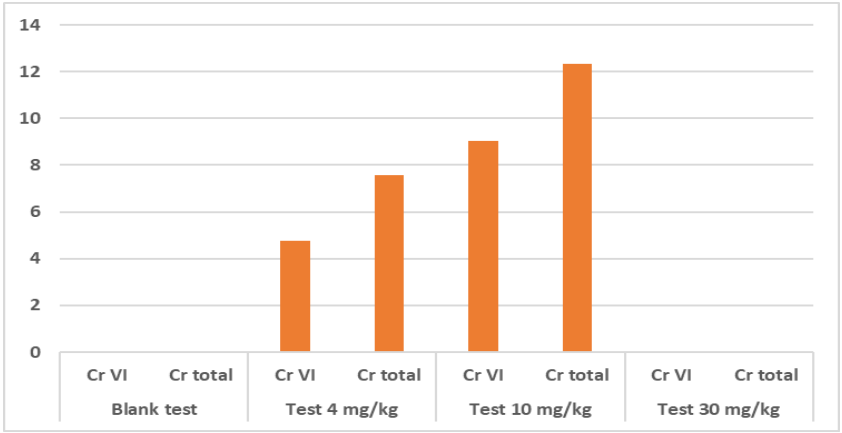

Figure 2. *Cr(VI) and total Cr in basil plants after 7 days, mg/kg*

It can be seen that after 7 days of plant poisoning with Cr(VI), for the test using 4 mg/kg and 10 mg/kg, both Cr(VI) and total Cr accumulated only in the basil. In the case of mint plants, after 7 days of contamination with both Cr(VI) and Cr(III), they did not bioaccumulate at all.

Plant samples were also taken 2 months after contamination and the Cr(VI) and total Cr content was determined, respectively. The obtained results are presented in Figure 3.

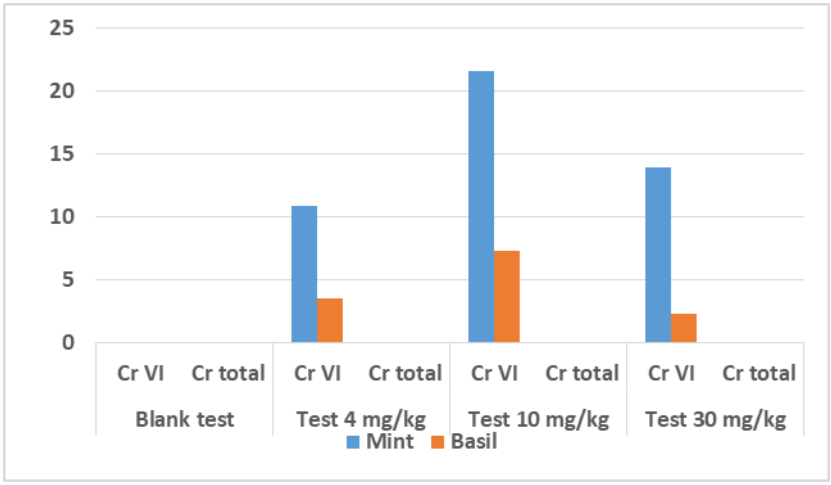

Figure 3. *Cr(VI) and total Cr in mint and basil plants after 2 months, mg/kg*

It can be seen that after 2 months of experiment, Cr(VI) accumulated both in mint and basil plants. In the mint plant, the accumulated Cr(VI) value was about 11 mg/kg in the experiment with 4 mg/kg Cr(VI) contamination, about 22 mg/kg in the experiment with 10

mg/kg Cr(VI) contamination, and 14 mg/kg in the experiment with Cr(III) contamination (30 mg/kg). Regarding basil, it can be seen that Cr(VI) has accumulated much less compared to mint plants but the bioaccumulation was faster. Mint plant needs more time to bioaccumulate the Cr(VI). As the Cr total was not detected in any plant sample we can conclude that the amount of bioaccumulated chromium is only in the form of Cr (VI).

Cr(VI) extracts in sodium carbonate were no longer appropriate for UV-Vis determination due to the low colour intensity of the obtained solutions (Figure 4), which presented different colour depending on the used plant. Determination by UV-Vis requires the formation of a pink-violet coloured complex (colour of the Cr(III) complex with diphenyl carbazide in phosphoric acid medium). Under the given conditions, no change was observed due to the low concentrations of Cr(VI) in tested solutions.

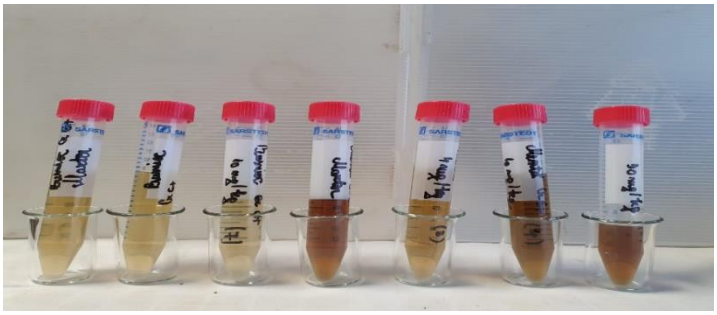

Figure 4. *Aqueous Cr(VI) extracts*

4. CONCLUSION

Cr(VI) was extracted from vegetable samples (basil and mint contaminated with $K_2Cr_2O_7$) in a solution of 0.1 M Na_2CO_3 (ratio m / v = 1: 100) by ultrasound treatment at 80°C for 10 minutes. The Cr(VI) species was subsequently determined by ICP-EOS using a calibration curve over 20-100 μg/L concentration range. Due to the low colour intensity of the extracts and the low chromium concentration levels in tested plants, the UV-Vis spectrometry method could not be applied for Cr(VI) determination.

It has been shown that mint accumulated Cr(VI) in a longer time period than basil, and Cr(VI) proved to accumulate in mint in greater amounts, only after two months from the plant contamination.

Further speciation studies will be done by using the ion-chromatographic method in order to separate and identify the Cr(III) and Cr(VI) species from the contaminated vegetable samples.

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