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Sequential injection method for bi-parametric determination of iron and manganese in soil leachates

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Abstract

The aim of this work was to develop a sequential injection (SI) method for the determination of the micronutrients iron and manganese, in soil leachates, as a tool to assess potential groundwater contamination. The described sequential injection method was based on the reaction of iron with chelator MRB12, a greener alternative chromogenic reagent, and the reaction of manganese with zincon, within a single manifold. The developed SI method enabled the determination of iron in the range 0.10 - 1.00 mg/L, and manganese in the range 0.25 – 2.5 mg/L with a limit of detection of 0.09 mg/ for iron L and 0.19 mg/L for manganese. The determination of both parameters was made in 6 minutes, in triplicate. The application to monitor laboratory scale soil core columns (LSSCs), as a simulation of the soil leaching process, proved its efficiency to assess potential contamination of ground waters. Iron and manganese contents were effectively analysed in two different scenarios to mimic the leaching process with rainwater and fertilizer.

1. Introduction

The structure of soil has great impact in the storage and movement of water, gases and macro and micronutrients [1-3]. Organic matter content is another important characteristic in the quality and productivity of the soil and has impact in soil properties, namely water retention, soil porosity and stability. In this context, soil fertilization intends to improve soil productivity by increasing nutrients availability to plants [3-6].

The soil leaching process has a huge impact on the quality of surface and ground waters, therefore the monitoring of nutrients and contaminants is an essential procedure in environmental and agricultural studies [7]. The increase of soil fertilization, by means of the use of chemical fertilizers, has become a potential source of contamination. Additionally, that practice decreases organic matter and soil fertility and increases soil erosion and environmental degradation [8, 9].

Iron (Fe) is the fourth most abundant element in the Earth's crust [10] and it is relatively abundant in many soils (20 to 40 g/kg of soil) [7]. Nevertheless, the use of iron fertilizers is a common practice to address iron deficiency in plants and it may change soil properties, independently of the organic matter content [4, 8]. Manganese (Mn) is a relatively abundant element (0.085%) in the Earth's crust and it is broadly dispersed in soils, sediments, water and biological materials [11]. Manganese displays an essential role for organisms and plants, causing lesions when in excess but its deficiency results in reduced growth rate [12-15]. Therefore, manganese supplements are extensively used, making its potential accumulation in ground waters a key parameter to monitor. Consequently, the study of the soil leaching process requires the development of real-time analytical tools and laboratory models to mimic the natural process.

The traditional detection methods, like inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrophotometry (AAS), provide high sensitivity in the determination of trace metals, especially in water samples [16]. However, these techniques have significant acquisition and maintenance costs and may require laborious sample preparation. Thus, the development of new methods, specially those able to determine several parameters with high accuracy and precision, becomes crucial [17]. Flow-based methods coupled with spectrophotometric detection present an effective alternative allowing for an efficient miniaturization of chemical assays with low volume samples, low reagent consumption and waste generation and can be configured for fast analysis of many parameters (multi-parametric analysis) [17-19].

There are several reagents that can be used for iron quantification, but most of them present a relatively high toxicity. In this context, 3-hydroxy-4-pyridinone (3,4-HPO) chelators family have been considered as environmentally friendly reagents but presented the limitation of a low solubility in water, which could limit their efficiency [20-22]. The great potential of this family of ligands has been demonstrated with an especially designed, highly hydrophilic, 3,4-HPO chelator (MRB12) for iron determination in water samples [22]. More recently, other hydrophilic and structurally related ligands from the same class of molecules have also been used for iron determination in biological samples, using flow-based methodologies and revealing their potential for the desired purpose [23, 24]. Lately, the applicability of the same ligands has also been demonstrated in the development of a microfluidic paper-based analytical device for iron determination in natural waters [25].

Regarding manganese(II), several reagents have been used for its spectrophotometric determination [26]; however they usually require laborious pre-concentration techniques and sample preparation, thus being complicated, time consuming and expensive procedures [27]. In this work, zincon (2-carboxy-2’-hydroxy-5’-sulfoformazylbenzene) was chosen for the spectrophotometric determination of manganese as an alternative reagent due to its known sensitivity for the determination of metallic ions [28-35].

The aim of the described work was to develop a bi-parametric automatic integrated method for the quantification of two different micronutrients, iron and manganese, by using low toxicity chromogenic reagents. The idea was to set up laboratory scale soil core (LSSC) columns to understand the behaviour of metal ions in the soil leaching process, as previously described [36], namely mobility, transport and fate [37-39]. For this purpose, rain simulations with rainwater and a commercial fertilizer were performed in the established LSSCs.

2. Material and Methods

2.1. Reagents and solutions

All solutions were prepared with analytical grade chemicals and Milli-Q water (resistivity > 18 MΩ cm, Millipore, Bedford, MA, USA).

A stock solution of pegylated 3,4-HPO ligand (MRB12), synthesized as previously described [21], was prepared by dissolution of 350 mg of MBR12 in 100 mL to a final concentration of 3.5 g/L. The MRB12 working solution of 600 mg/L was weekly prepared by dilution from the stock solution.

The carbonate buffer solution was prepared by dissolving 2.1 g of $NaHCO_3$ (Panreac, Spain) in 50 mL of water to achieve a final concentration of 0.5 M and the pH adjusted to 10.5 with NaOH.

The zincon stock solution was prepared by dilution of the 100 mg of the solid, zincon monosodium salt (Merck, Germany) in 100 mL of boric acid buffer to a final concentration of 2 mM. A working solution of 100 µM was monthly prepared by proper dilution of the stock solution in the boric acid buffer.

The boric acid buffer was prepared dissolving 15.5 g of boric acid (Aldrich, Germany) and 4.2 g of sodium hydroxide (Panreac, Spain) in 500 mL of water to a final concentration of 0.5 M H_3BO_3 in 0.2 M of NaOH at pH 9.

A 10 mg/L iron(III) stock solution was obtained by dilution of the atomic absorption standard of 1000 mg/L iron (III) (Fluka, Germany). The working standards, here named iron standards, were prepared from the stock solution in range 0.10 - 1.00 mg Fe /L in 0.01 M nitric acid.

A 10 mg/L iron(II) stock solution was obtained by dissolution of 7 mg of ammonium iron(II) sulfate hexahydrate, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, in 100 mL of water, previously purged with gaseous nitrogen for half an hour to remove the oxygen, and in 0.05 M sulphuric acid. The working standards were prepared, also with the nitrogen purged water, from the stock solution in the range 0.10 - 1.00 mg Fe /L in 0.05 M sulphuric acid and purged again prior to use.

A 100 mg/L manganese stock solution was obtained by dilution of the atomic absorption standard of 1000 mg/L Mn(II) (Supelco, Germany). The working standards were prepared from the stock solution in the range 0.25-3.0 mg Mn /L in 0.01 M of nitric acid.

A stock solution of 0.1 M nitric acid (Merck, Germany) was prepared by dilution from the commercial solution (d=1.39; 65%). A stock solution of 1 M sulphuric acid (Merck, Germany) was prepared by dilution from the commercial solution (d=1.83; 95-97%).

The commercial fertilizer solution (Universal fertilizer KB ®) was prepared according to its instructions, with 7 mL (mark in the flask cap) in 1 L of water to a final composition of: 6% total N (2.7% ammoniacal and 3.3% nitric); 3% soluble P_2O_5; 6% soluble K_2O; 0.002% total Cu chelated with EDTA; 0.03% total Fe chelated with DTPA; 0.01% Mn chelated with EDTA; 0.001% total Mo; 0.002% Zn chelated with EDTA.

To measure the soil pH, a 0.01 M calcium chloride (Merck, Germany) solution and a 1 M potassium chloride (Merck, Germany) solution were prepared by dissolving 0.74 g of $CaCl_2$ and 37 g of KCl in 500 mL of water, respectively.

2.2. Apparatus

Solutions were propelled with a Gilson Minipuls 3 peristaltic pump with a PVC pumping tube connected to the central channel of a ten-port electrically actuated selection valve (Valco VICI Cheminert C25-3180EUHB). All tubing connecting the different components of the flow system were made of PTFE (Omnifit) with 0.8 mm inner diameter (i.d.) including all the ports connections, the holding coil and the reaction coil.

An Ocean Optics HR 4000 charged coupled device detector (CCD), equipped with a pair of 400 nm fibre optic cable and a Micropack DH-2000 deuterium halogen light source, was used as the detection system. A Hellma 178.011-OS flow-cell with 10 mm light path and 30 µL inner volume was used as flow cell.

Data acquisition signal was obtained at 460 nm to iron determination and 490 nm to manganese determination and it was performed through the OceanOptics – Spectrasuite software running in a personal computer (HP L1706).

A computer (HP Pavilion zt3000) equipped with a National Instruments DAQcard-DI0 interface card, running a homemade software, was used to control the selection valve (SV) position and the peristaltic pump direction and speed.

2.3. Sequential injection manifold and procedure

The manifold for the bi-parametric determination of iron and manganese is depicted in Fig. 1.

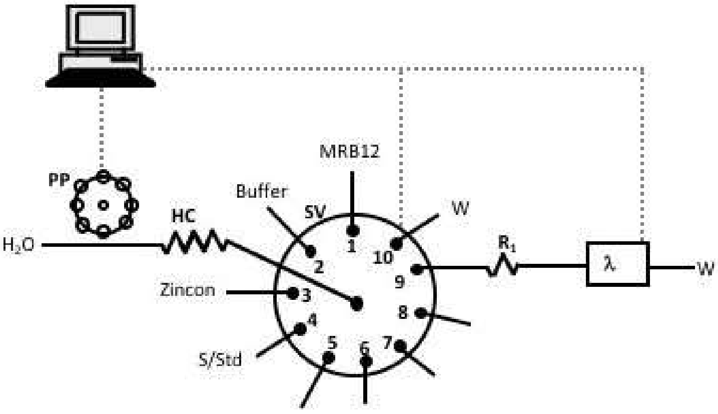

Figure 1. Sequential injection manifold for the bi-parametric determination of iron and manganese: SV, 10 port selection valve; HC, holding coil (3 meters length with 0.8 mm i.d.); PP, peristaltic pump; MRB12, iron ligand at 0.60 g/L; Buffer, 0.50 M hydrogen carbonate buffer; Zincon, manganese colour reagent (100 µM Zincon in 0.2 NaOH); S/Std, sample or standard; W, waste; R_1, reaction coil (65 cm length with 0.8 mm i.d.); λ, CCD UV/VIS spectrophotometer.

The sequence of the steps was described to combine the determination of iron with MRB12 at 460 nm and manganese determination with zincon at 490 nm and respective operation times are presented in Table 1.

Table 1. Protocol sequence for the bi-parametric determination of iron and manganese

Analyte	**Step**	**SV Position**	**Time (s)**	**Volume (µL)**	**Description**
Fe	A	1	3	250	Aspiration of colour reagent (MRB12)
	B	2	1	20	Aspiration of buffer
	C	4	8	650	Aspirate sample/standard
	D	9	40	3350	Propelling to detector for signal registration at 460 nm (iron determination) and washing up of the entire flow channel
Mn	E	3	4	150	Aspiration of colour reagent (zincon)
	F	4	6	400	Aspiration sample/standard
	G	9	30	2500	Propelling to detector for signal registration at 490 nm (manganese determination) and washing up of the entire flow channel

The iron determination started with the aspiration of the reagent, MRB12, and carbonate buffer (step A and B respectively). Then the sample/standard was aspirated (step C) and the stacked plugs sent to the detector (step D), promoting the mixture by flow reversal. To ensure that the flow channel is washed at each cycle, the volume propelled at this step corresponds to over 3.5 times the volume of the sum of the previously stacked plugs.

Following iron determination, the determination of manganese was initiated with the sequential aspiration of zincon reagent (step E) and the sample/standard (step F). Then, the mixture, promoted by the flow reversal, was sent to the detector (step G). Analogously to the determination of iron, the volume of this step is also quite higher than the sum of the aspirated volumes (over 4.5 times) to ensure the complete washing of the flow channel at each cycle.

2.4. Laboratory Scale Soil Column (LSSCs) setup

The soil samples were collected from two different agricultural locations, Maceiras (Vila do Conde, Portugal) and Lixa (Porto, Portugal) using acrylic cylinders (diameter = 75 mm; height = 66 cm) pushed into the ground to collect a superficial soil core (about 20 cm depth) [40]. This sampling process resulted in a highly compact soil core column which enabled to carry out the leaching process without soil loss. The LSSC columns were established in triplicate for each selected location (Figure 2A). To perform rain simulations, a strainer was place at the top and a plastic stopper (with a 5 mm hole) at the bottom (Figure 2B).

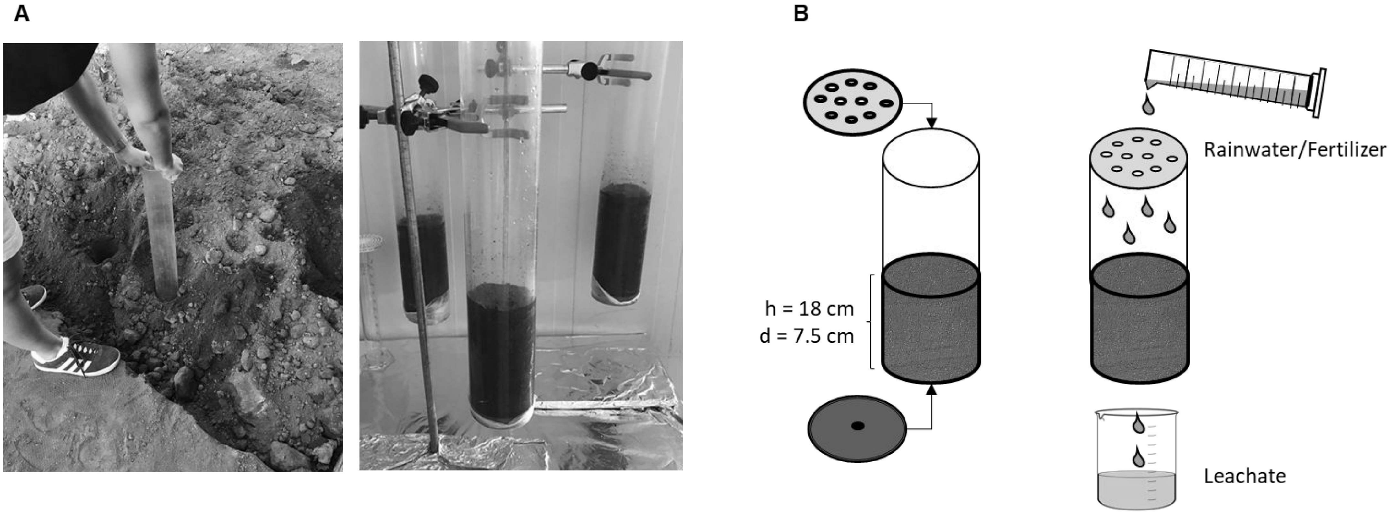

Figure 2. LSSCs assembly for leaching process simulation; **A**, soil core collection and setting up; **B**; schematic representation of process to perform rain simulations.

Some basic characterization, namely pH and granulometry, of the collected agricultural soil samples was made (ESI Table 1). The soil texture was determined based upon the results of the granulometry assessment, the pH values were assessed in water with a proportion of 1:2.5 (soil : water) [41].

The rain simulations were carried out with two different solutions, namely rainwater and the commercial fertilizer. The pH and the conductivity of the solutions used for rain simulations and of the produced leachates were measured. The pH measurements were made with a pH/mV meter (micropH 2002, Crison) and the conductivity measurements were made with a conductimeter (conductimeter basic 30, Crison).

2.5. Reference procedure

For the validation of the developed bi-parametric system, leachate samples were analysed with the reference method, atomic absorption spectrometry (AAS) [42], and the results compared to the developed system. For the AAS determination the leachate samples were acidified at collection to pH ≈ 2 according to the reference procedure [42].

3. Results and Discussion

3.1. Preliminary studies

As the chosen reagent for the spectrophotometric determination of iron was a recently synthesised iron ligand, some preliminary studies were carried out. As the targeted samples were leachates from surface soil core, the prevalent iron form should be iron(III). Nevertheless, throughout the leaching process, there could be some conversion of iron(III) into iron(II) so, being a recently synthesised iron ligand, it was important to assess that the ligand reacted with both iron forms. Therefore, calibration curves were established with individual iron(III) and iron(II) standards and with mixed standards of both iron forms (Figure 3).

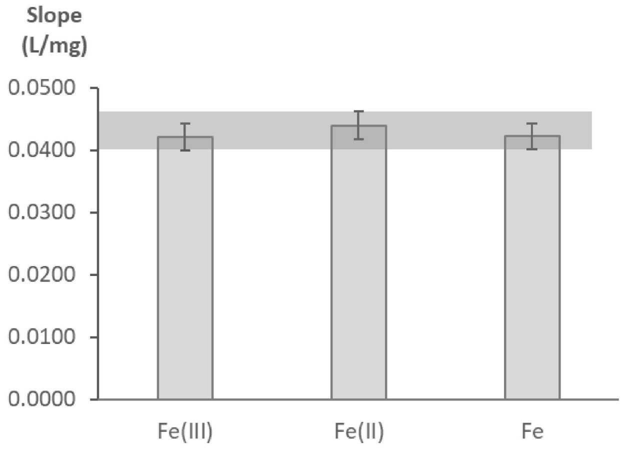

Figure 3. Comparison of the calibration curves slopes obtained with iron standards of iron(III), iron(II) and mixed standards of both iron forms (Fe); the error bars represent 5% variation of the slope; the shade illustrates the overlapping of the calibration curves slopes.

The results showed that there was no difference (relative deviation < 5%) between the calibration curve slope obtained from iron(III) standards and iron(II), or mixed standards. This clearly indicates that the reaction conditions with MRB12 ensure the quantification of both iron forms.

3.2. Study of colour reagent concentration and volume

The influence of the concentration and volume of each colour reagent was studied by establishing calibration curves and comparing the corresponding slopes and intercepts. For the iron determination, a higher slope would correspond to a higher sensitivity; as for the manganese determination, a higher sensitivity would correspond to the highest absolute value of the slope because the slope itself was negative. The increase in the calibration curve intercept is usually associated with an increase of the blank, so a lower intercept can potentially result in a lower limit of detection.

3.2.1. Determination of iron

The concentration of MRB12 was evaluated and 0.60 mM was chosen from the tested values of 0.25, 0.40, 0.60 and 0.80 mM as the sensitivity increased up to that value (ESI Figure 1A).

Then, different volumes of MRB12 solution were tested in the range between 100 - 300 µL and as the sensitivity increased up to 250 µL, together with a decrease in the intercept (Figure 4A), so that was the volume chosen.

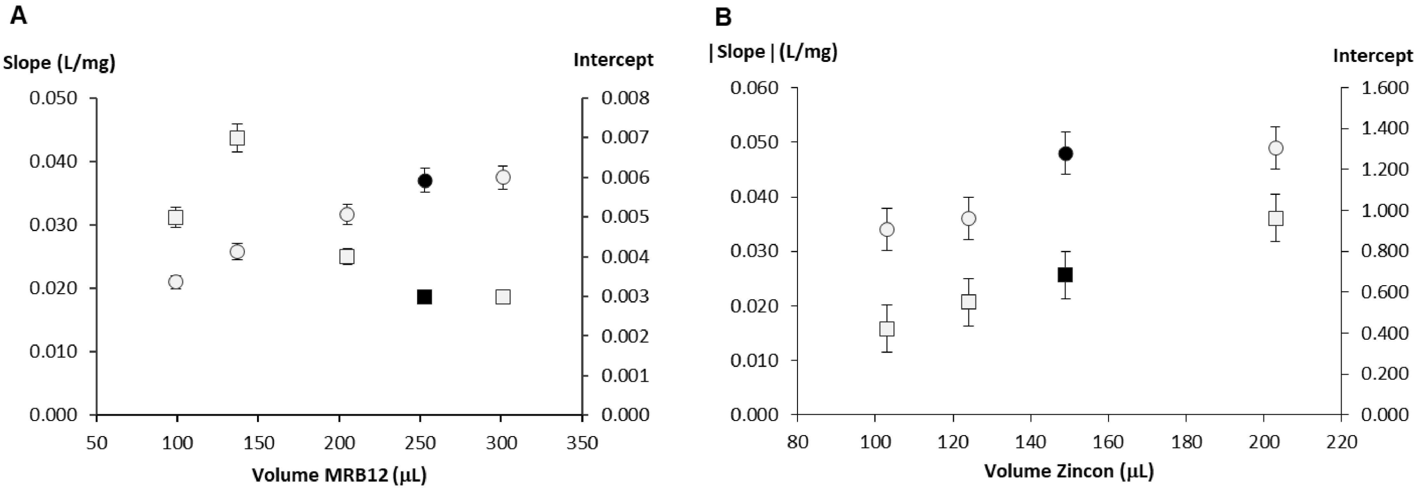

Figure 4. Study of the influence of the colour reagents volumes in the calibration curves slope (circles) and intercept (squares); **A,** Influence of MRB12 volume in the iron calibration curve; **B,** Influence of zincon solution volume in the manganese calibration curve; the values in black represent the slope and intercepts for the chosen volumes.

The stoichiometric formation of the iron complex of MRB12, ([FeMRB12)]$_3$, requires pH adjustment [20] so hydrogen carbonate buffer (at pH 10.5) was used [21] and the volume was set to 20 µL as the reported minimal reproducible amount [20].

3.2.2. Determination of manganese

The influence of the zincon concentration was also studied and reagent solutions with 50, 100, 150 and 200 µM were tested (ESI Figure 1B). Even though the highest sensitivity was observed for the highest concentration, the increase from 100 µM to 200 µM was only of 7% and as it also corresponded to the highest intercept (with an absorbance value > 1.5), 100 µM was the concentration chosen.

The volume of zincon solution was tested in the range 100 - 200 µL and the sensitivity increased with the increasing of the volume up to 150 µL (Figure 4B) therefore, that was the volume chosen.

3.3. Sample volume

The influence of the sample volume was studied for both iron and manganese determinations by tracing calibration curves for each tested volume (Figure 5).

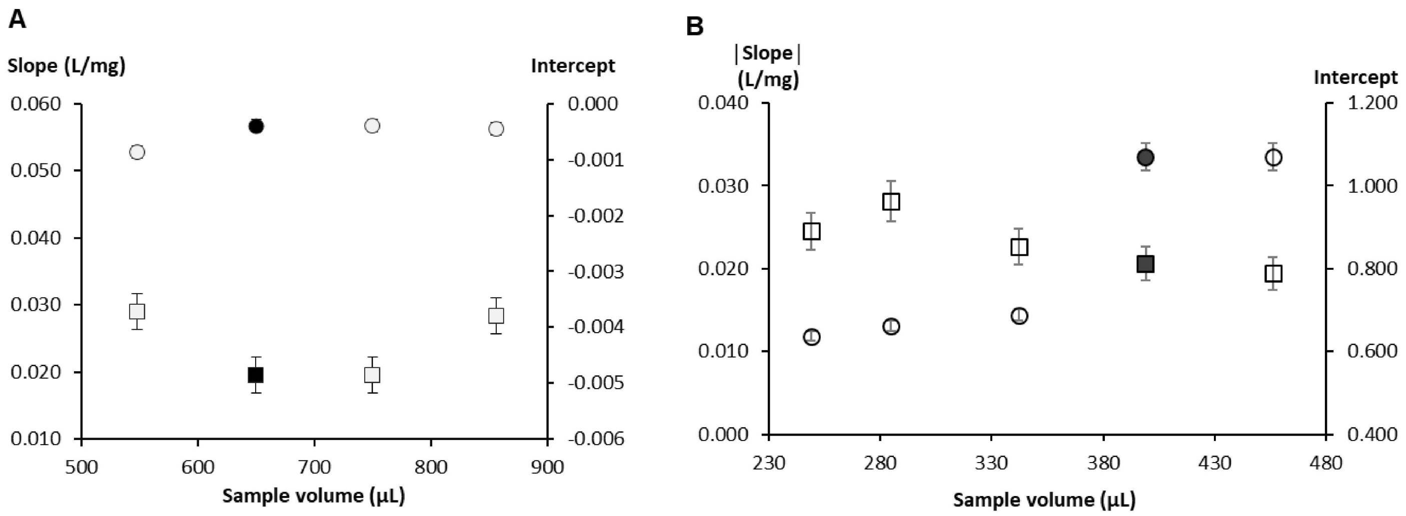

Figure 5. Study of the influence of the sample volume in the calibration curve slope (circles) and intercept (squares): A, the determination of iron; B, the determination of manganese; the points in black represent the slope and intercept for the chosen sample volumes.

For iron determination, values ranging from 550 to 850 µL were tested and sample volume of 650 µL was chosen corresponding to the highest sensitivity (calibration curve slope) and lowest intercept (Figure 5A). For the manganese determination, values between 250 and 450 µL were tested and, as the sensitivity increased up to the sample volume of 400 µL, this volume was chosen (Figure 5B).

3.4. Interferences study

As the LSSC leachates intend to simulate groundwater, the potential interference from ions likely to be present in natural waters was assessed.

For the iron determination with the ligand MRB12, a detailed study of potential interferences was performed testing metal ions and major anions expected to be found in natural waters. This study was carried out by comparing the absorbance signal of an iron standard (A_{Fe}) and the absorbance signal of a mixed standard with both iron and the potential interfering ion (A_{Mix}): % Interference = (A_{Mix} - A_{Fe})/ A_{Fe}. (Table 2).

Table 2. Study of potential interferences in the iron determination; percentage of interference calculated based on the signal of an iron standard with 0.5 mg/L and mixed standards also containing 0.5 mg/L iron and the potential interference; [Me], metal ion concentration; [Anion], major anion concentration.

Tested cation	**[Me] mg/L**	**% Interference**
Na^+	0.21	-6.0
	0.42	31
K^+	0.70	-3.0
	1.75	-13
Ca^{2+}	0.72	-5.8
	1.80	-13
Mg^{2+}	0.44	-6.7
	1.10	-13
Al^{3+}	0.24	0.1
	0.48	18
Cr^{3+}	0.46	0.2
	0.92	27
Mn^{2+}	0.49	0.1
	0.98	15
Co^{2+}	0.50	5.0
	1.00	68
Ni^{2+}	0.50	0.2
	1.00	18
Cu^{2+}	0.50	0.5
	1.00	33
Zn^{2+}	0.50	-2.5
	1.00	35
Cd^{2+}	2.00	-6.7
	5.00	22
Pb^{2+}	3.84	-6.3
	9.60	-11
Tested anion	**[Anion] mg/L**	**% Interference**
NO_2^-	610	2.4
	1220	-28
SO_4^{2-}	1250	1.7
	2500	31
PO_4^{3-}	735	-4.7
	1470	-44

Two concentrations are presented for each tested metal ion and major anion: the maximum amount not causing interference (% interference < 10%) and the minimal amount causing interference (% interference > 10%). The results were a clear evidence that for the concentration values expected in natural waters, there would be no significant interferences in iron determination with MRB12.

For the manganese determination, the potential interference of different metal ions was assessed by comparing the signal variation of different standards of the tested metal ion with the blank signal (ESI Figure 2). A signal variation above 5%, indicating interference in the determination, was observed for copper, nickel and zinc at a 1 mg/L concentration. As for iron and chromium the signal variation was negligible, 1.77% and 0.02% respectively, up to a concentration of 2 mg/L indicating no interference from these metal ions. As the tested amounts were considerably above the expected values in natural waters, like groundwater, it can be concluded that there is no significant interference from sample matrix.

3.4.1. Humic acid interference assessment

As the targeted samples were soil leachates, the potential interference from humic acid was evaluated. Standards with 0.5 mg/L of metal ion, iron and manganese, were prepared with and without humic acid at two concentrations, and the percentage of interference calculated (Table 3).

Table 3. Assessment of potential interference from humic acid in both determinations, iron and manganese; percentage of interference calculated based on the signal of a 0.5 mg/L iron or manganese standard and mixed standards containing the same amount of target ion and two quantities of humic acid.

Parameter	Humic acids (mg/L)	% Interference
Fe	75.0	9.8%
	150	18%
Mn	75.0	-2.4%
	150	-17%

It was observed that up to 75 mg/L there was no interference (interference < 10%).

3.5. Features of the developed SI method

The features of the developed method for the bi-parametric determination of iron and manganese in natural waters and soil leachates are summarized in Table 4.

Table 4. Features of the developed method for the bi-parametric determination of iron and manganese; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Parameter	Dynamic range (mg/L)	Calibration curve* A = S x mg/L + b	LOD (µg/L)	LOQ (µg/L)	RSD** (mg/L ± SD)	Injection rate (h^{-1})
Fe	0.100 – 1.00	$A = 5.22 \times 10^{-2}(\pm 1.5 \times 10^{-3})\ [Fe^{3+}] + 2 \times 10^{-3}(\pm 1 \times 10^{-3})$ $R^2 = 0.998\ (\pm 2\times 10^{-3})$	10	75	4.6% (0.273 ± 0.013)	48
Mn	0.250 – 3.00	$A = -3.96 \times 10^{-2}(\pm 5 \times 10^{-4})\ [Mn^{2+}] + 0.873\ (\pm 2 \times 10^{-3})$ $R^2 = 0.998\ (\pm 3\times 10^{-3})$	98	242	4.9% (0.615± 0.030)	68

* n = 5
** n = 10

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as the concentration corresponding to 3 and 10 times, respectively, the standard deviation of the blank signal (n=10) added to the blank average, following IUPAC guidelines [43, 44].

The repeatability was evaluated for each parameter determination through the calculation of the relative standard deviation (RSD) of 10 consecutive analysis of a sample and it was below 5% for both parameters.

The injection rate was calculated based on the time spent for each parameter analytical cycle. As for the determination rate of the bi-parametric determination, it was 32 determinations /hour, calculated using mixed standards with iron and manganese.

The consumption values were calculated per determination of each parameter: 150 µg of pegylated 3,4-HPO ligand, 0.84 mg of sodium hydrogen carbonate and 650 µL of sample were used for the iron determination; 7 µg of Zincon, 4.6 mg of boric acid, 1.3 mg of sodium hydroxide and 400 µL of sample were used for the manganese determination.

3.6. Application to natural waters and leachates

3.6.1. Validation of the procedure

To evaluate the accuracy, different samples were tested in the developed system (SI), and the results were compared to the obtained with the reference method, the atomic absorption spectroscopy (AAS) method (ESI Table 2). The relative deviation percentage (RD) calculated indicated that there were no significant differences between the two set of results (RD < 9%).

A linear relation between the two sets of results was established (ESI Fig.3): $[Fe]_{SI} = 1.01 \pm 0.08 \times [Fe]_{AAS} - 0.002 \pm 0.012$ with $R^2 = 0.994$; and $[Mn]_{SI} = 0.990 \pm 0.084 \times [Mn]_{AAS} - 0.002 \pm 0.078$ with $R^2 = 0.993$, where $[Me]_{SI}$ is the metal ion concentration obtained with the developed method and $[Me]_{AAS}$ the metal ion concentration obtained from the reference method. These values show that the slope is close to unit and the intercept is close to zero; the analysis of the results for a 95% confidence level shows that there is no statistical difference between the reference method and the developed method.

3.6.2. Application to the laboratory scale soil columns

Several rain simulation processes were performed using rain water and a commercial fertilizer (illustrated in Figure 2B). The determination of iron and manganese in all obtained leachates was attained using the developed SI method (Table 5). The physical-chemical parameters, pH and conductivity, were also assessed for all the analyzed samples (Table 5).

Table 5. Results for the determination of iron and manganese with the developed SI method applied to LSSC leachates obtained from rain simulation with different solutions; G, conductivity; SD, standard deviation; RSD, relative standard deviation.

Rain simulation	Sample ID	pH	G (mS/cm)	[Fe]		RSD (%)	[Mn]		RSD (%)
				(mg/L)	SD		(mg/L)	SD	
-	Rainwater	6.58	3.42×10^{-2}	< LOD		-	< LOD		-
-	Fertilizer	5.28	4.21	0.561	0.044	7.8	1.87	0.05	2.6
RainWater	M_I	8.32	2.80	0.289	0.011	3.8	0.579	0.025	4.3
	M_II	8.39	2.13	0.452	0.030	6.6	0.500	0.031	6.2
	M_III	8.50	1.81	0.319	0.006	1.8	0.467	0.015	3.2
Fertilizer	M_I	8.04	2.48	0.276	0.011	4.0	0.485	0.033	6.8
	M_II	8.24	1.97	0.394	0.011	2.8	0.467	0.015	3.2
	M_III	8.07	1.51	0.302	0.020	6.6	0.514	0.025	4.9
RainWater	M_I	8.52	2.18	0.305	0.004	1.3	0.408	0.025	6.0

	M_II	8.46	1.85	0.309	0.011	3.6	0.565	0.038	6.7
	M_III	8.40	1.55	0.244	0.020	8.2	0.494	0.039	7.8
RainWater	M_I	8.34	1.59	0.326	0.008	2.5	0.598	0.015	2.5
	M_II	8.35	1.44	0.195	0.014	7.2	0.550	0.033	6.0
	M_III	8.09	1.76	0.181	0.008	4.4	0.464	0.039	8.4
Fertilizer	M_I	8.52	1.65	0.348	0.011	3.2	0.542	0.002	0.4
	M_II	8.56	1.33	0.259	0.005	1.9	0.554	0.046	8.3
	M_III	8.25	1.95	0.201	0.005	2.5	0.623	0.045	7.2

The values were all within the established dynamic ranges for both parameters and the calculated relative standard deviation (RSD, %) proved the robustness of the developed methodology as it was always below 10%. In fact, only the rainwater presented a value below the limit of detection making it impossible to access.

4. Conclusions

The methodology of a bi-parametric determination of iron and manganese in soil leachates proved to be an effective alternative to conventional procedures enabling a fast quantification analysis. The comprehensive understanding of dynamic environmental processes, namely the soil leaching to ground waters, requires innovative analytical tools that address the challenges of matrix complexity so that the watering water and the leachate can be analysed with the same method. This approach will enable to compare the results in a consistent and reliable manner.

The use of sequential injection analysis as flow technique provided robustness and versatility in attaining multiparametric assessment. The bi-parametric determination can be accomplished with minimal operator influence (high degree of automation), with 32 determination per hour and a minimal sample consumption (≈ 1 mL).

In the end, as the developed method was applied to leachates from laboratory scale soil core columns (LSSCs), a real-time assessment was attained to study the impact of micronutrients supplementation in soils to ground water contamination, through the soil leaching process. Two different sources of soil samples were tested and rain simulations performed with rainwater and the commercial fertilizer solution. The bi-parametric determination of iron-and manganese was carried out with the developed SI method to both the initial solutions and the obtained leachates.

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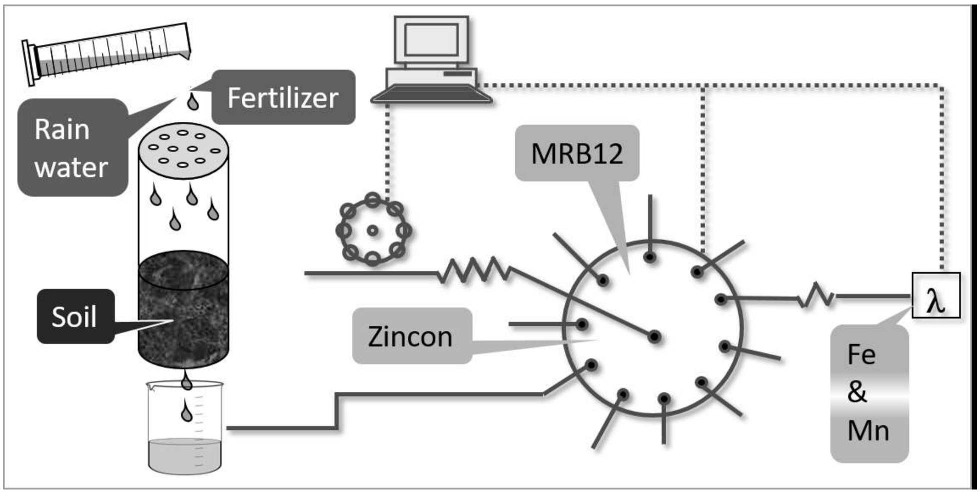

Sequential injection method for bi-parametric determination of Fe and Mn in soil leachates to access the impact of micronutrients supplementation.

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