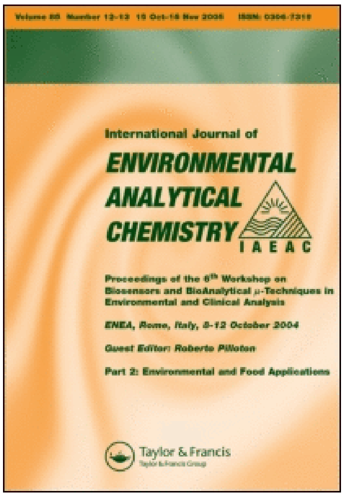

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article de Toledo, Renata Alves , Simões, Marcelo Luiz , Silva, Wilson Tadeu Lopes da , Martin-Neto, Ladislau and PedroVaz, Carlos Manoel(2009) 'Determination of metal ions extracted by DTPA in a soil treated with effluent using an Hg-electroplated-Pt microelectrode', International Journal of Environmental Analytical Chemistry, 89: 15, 1099 — 1111

To link to this Article: DOI: 10.1080/03067310903094487

URL: http://dx.doi.org/10.1080/03067310903094487

Intern. J. Environ. Anal. Chem.
Vol. 89, No. 15, 20 December 2009, 1099–1111

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(*Received 5 August 2008; final version received 26 May 2009*)

Determinations of Cu, Fe, Mn and Zn were performed in an oxisol soil treated with effluent originated from a biodigester septic cesspool. The extracts were obtained from a DTPA/TEA (pH 7.3) solution and analysed by electroanalytical methodologies using square wave anodic stripping voltammetry (SWASV) for the analysis of Cu, Mn and Zn and square wave voltammetry (SWV) for Fe analysis, both of them with a thin mercury film microelectrode (ME-Hg). DTPA (diethylenetriaminepentaacetic acid) complexes with Cu, Mn and Fe show electroactivity in the potentials range close to the ones for the free ions in solution, but the Zn complex did not show any electroactivity at the potential range analysed. SWASV/ME-Hg and SWV/ME-Hg results were in good agreement ($r^2 = 0.996$) with the flame atomic absorption spectroscopy (FAAS) analyses, demonstrating that electroanalytical methodologies can be used for micronutrient determinations in soil extracts without serious interferences of the matrix components. Some characteristics of the complex formed between DTPA-metals are explored by EPR experiments.

Keywords: treated effluent; soil; micronutrients; Hg-electroplated-Pt microelectrode; square wave anodic stripping voltammetry; square wave voltammetry

1. Introduction

The total analysis and the single step extraction procedure to identify the bioavailable fraction of metals in soil are usually performed by the flame atomic absorption spectroscopy (FAAS) and also by the inductively coupled plasma optical emission spectrometry (ICP-OES) [1–3]. These techniques are relatively time-consuming and expensive due to the reagents used in the analytical procedure. For this reason, several efforts have been made for the development of alternative analytical techniques to provide fast, inexpensive, reliable and even *in-field* estimation of soil micronutrients [4–6].

Electrochemical techniques represent a highly attractive alternative for soil micronutrients analysis due to their unique features such as high sensitivity, compact and portable instrumentation and low costs of maintenance and operation [7]. The development of microelectrodes and their use with square wave anodic stripping voltammetry (SWASV) have built up the area of electroanalysis by the high sensitivity reached in metal trace analysis [8,9].

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903094487
http://www.informaworld.com

Analyses of Cd, Pb and Cu trace concentrations in soil were done with SWASV and an Hg-electroplated-Ir microelectrode. Barium chloride ($0.1\,\mathrm{mol\,L^{-1}}$), sodium pyrophosphate ($0.1\,\mathrm{mol\,L^{-1}}$) and oxalic acid plus ammonium oxalate ($0.2\,\mathrm{mol\,L^{-1}}$) were used as bounding agents for the extraction of such metals [8]. The developed electroanalytical methodology provided accurate determination of these metal concentrations in the soil extracts.

Mobile forms of Zn, Cd, Pb and Cu were determined in a soil solution extracted with ammonium nitrate ($0.1\,\mathrm{mol\,L^{-1}}$) using an appropriate combination of anodic and cathodic stripping voltammetry with a hanging mercury drop electrode (HMDE). The voltammetric results were in good agreement with those obtained with FAAS [10].

In the recent literature, there is no study related to the development of electroanalytical methodologies for the analysis of extractable micronutrients (Cu, Fe, Mn and Zn) using the diethylenetriaminepentaacetic acid/triethanolamine (DTPA/TEA) solution in soil matrices, although this type of extracting procedure has been widely used to quantify micronutrients available to the plants [11].

DTPA can also be used for the evaluation of polluted elements (Cr, Co) and also methylmercury traces in biological and in environmental matrices using electroanalytical methodologies instead of the commonly spectrophotometry ones [12–14].

In the present work, we developed an electroanalytical methodology for the determination of Cu, Fe, Mn and Zn in soil samples treated with an effluent produced by an anaerobic biodigester septic cesspool developed in Brazil by Embrapa Agricultural Instrumentation. The micronutrients were extracted with DTPA/TEA solution and analysed using SWASV (Cu, Mn and Zn) and SWV (Fe) with an Hg-electroplated-Pt microelectrode. The results were compared to the ones reached by the standard method of micronutrients analysis (FAAS) in order to verify the possibility of using the developed electroanalytical methodology in soil analyses. The complexes formed between DTPA-metals were studied by electronic paramagnetic resonance (EPR) technique.

2. Experimental

2.1 *Reagents and equipments*

All reagents were of analytical grade and used as received. $CaCl_2.2H_2O$, $Fe(NO_3)_3$, $Zn(NO_3)_2.6H_2O$, $CuSO_4.5H_2O$, $MnSO_4.1H_2O$ were purchased from Aldrich Co, HNO_3 and HCl were from Merck and DTPA and TEA were from Sigma. All the solutions were prepared with distilled water purified in a Milli-Q system (Millipore®).

Square wave anodic stripping voltammetric experiments were performed using a Potenciostat/Galvanostat AUTOLAB PGSTAT® under optimised conditions of square wave frequency (f), pulse amplitude (a), scan increment (ΔE_s) and pre-concentration step (time and potential) specific for each micronutrient (Cu^{2+}, Mn^{2+} and Zn^{2+}). The application of pre-concentration steps (time and potential accumulations) is not necessary for the Fe^{3+} analysis since the reaction Fe^{3+}/Fe^{2+} occurs in solution. In this case, square wave voltametry was used for Fe^{3+} determination and only f, a and ΔE_s were optimised.

The working electrode (Hg-electroplated-Pt microelectrode), the reference (saturated calomel electrode, SCE) and the auxiliary (platinum wire) ones were placed into a conventional Pyrex® glass cell with capacity of 10 mL through three holes in its Teflon® cap. All the experiments were carried out at room temperature. The Pt wire used to construct the working microelectrodes (Pt, $\phi = 25\,\mu m$) was from Goodfellow (Cambridge, UK).

FAAS analyses were carried out in a Varian SpectrAA-800 atomic absorption spectrometer (Varian, Mulgrave, Australia). The analytical wavelengths for the micronutrients were: Cu (324.8 nm), Fe (248.3 nm), Mn (279.5 nm) and Zn (213.9 nm). An air/acetylene flame (13.5:2 L min^{-1}) was used for all measurements.

Electron paramagnetic resonance (EPR) spectra were acquired in a Bruker EMX spectrometer operating in the X-band (~9 GHz) using a spherical cavity (high Q) at room temperature.

2.2 *Preparation of the Hg-electroplated-Pt microelectrode*

The disc shaped microelectrode of Pt ($\phi = 25\,\mu\text{m}$) was constructed by insulating Pt micro-wire into a glass capillary tube. For electrical contact, a copper fibre was sealed with the micro-wire by tin-lead solder. After construction, a surface treatment was carried out with sandpaper and by polishing in a wet cloth embedded with alumina emulsion of several decreasing granulations until 0.05 µm. The procedure was followed by optical microscopy to verify the presence of micro-crunch or micro-bubble in the borders of the exposed micro-disc.

The cronoamperometric technique was used for the electro-deposition of Hg on the Pt microelectrode surface using a $7.0 \times 10^{-2}\,\text{mol}\,\text{L}^{-1}$ of $Hg(NO_3)_2$ solution (pH ~1), which was prepared by dissolving high-purity Hg (m = 1.4 g) in some drops of concentrated HNO_3. The solution was then made up to volume (100 mL) with distilled water.

Prior to Hg deposition, the solution was purged with super dry N_2 (White Martins) during 10 min to remove oxygen. A stable mercury hemispheric deposit was obtained by applying a constant potential of –1.0 V *vs.* SCE during 90 s. The Hg-electroplated-Pt microelectrode was very stable during a full day analysis and the renewing of the hemispheric deposit was done by maintaining the electrode potential at 0 V during 15 s.

2.3 *Preparation of DTPA/TEA and the metals stock solutions*

The DTPA/TEA extracting solution (V = 1.0 L) was prepared from 1.9 g of DTPA ($5.0 \times 10^{-3}\,\text{mol}\,\text{L}^{-1}$), 13.3 mL of triethanolamine ($0.1\,\text{mol}\,\text{L}^{-1}$) and 1.4 g of $CaCl_2.2H_2O$ ($1.0 \times 10^{-2}\,\text{mol}\,\text{L}^{-1}$). The solution pH was adjusted to 7.3 by the addition of $1.0\,\text{mol}\,\text{L}^{-1}$ HCl solution. The stock solutions ($2.0 \times 10^{-3}\,\text{mol}\,\text{L}^{-1}$) of Fe^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} ions were daily prepared in acidified distilled water (pH 1.8).

TEA was selected as a buffer for its pKa (pKa 7.8) and because it burns cleanly during flames atomisation in atomic absorption spectroscopy [15].

2.4 *Soil samples and extraction procedure*

Oxisol soil samples (containing 83% of sand, 9.5% of clay and 7.5% of silt, determined by granulometric analysis [16]) were collected at different depths (0–20, 20–40, and 40–60 cm) using a Dutch type auger, in Santa Candida Farm, located in the city of São Carlos, State of São Paulo, Brazil. The collected soil samples (100 g) were stored in plastic bags for transportation to the laboratory, dried at room temperature, grounded and passed through a 2 mm sieve.

The soil samples were collected in two areas, one treated exclusively with effluent (S) and other of reference or control that did not received any effluent or fertiliser application (C). Both areas have been cultivated with guava plants (*Psidium guajava* L.) during four years. Treated effluent was obtained from an anaerobic biodigester septic cesspool located in Santa Candida Farm. The effluent was monthly spread on the soil surface (100 L/plant) and, occasionally, this soil also receives supplements of K and P.

In order to verify chemical modifications in the studied oxisol soil, caused by the addition of treated effluent, some chemical parameters were evaluated, such as, pH in $CaCl_2$, organic matter quantity (OM), soil potential acidity (H + Al), exchangeable aluminium (Al) and cation exchangeable capacity (CEC). All these experimental chemical parameters were obtained in accordance with the *Manual of Soil Analysis Methods*, edited by EMBRAPA [17].

The soil micronutrients were extracted according to the procedure developed by Lindsay and Norvell [15]. Ten grams of air-dried soil were placed in a 125 mL conical flask and 20 mL of the DTPA extracting solution was added. Each flask was covered with parafilm and positioned upright on a horizontal shaker at 120 cycles min^{-1} in room temperature (25°C) during 2 hours. The suspensions were filtered in a filter paper Whatman 42 and the resulting solutions were analysed using SWASV/Hg-ME (Zn^{2+}, Mn^{2+} and Cu^{2+}), SWV/Hg-ME (Fe^{3+}) and FAAS methodologies. All laboratory glassware was cleaned with HNO_3 and rinsed with distilled water.

2.5 *Electroanalytical procedure*

The electroanalytical procedures for the determination of micronutrients contents were developed from SWASV (Cu^{2+}, Mn^{2+} and Zn^{2+}) and SWV (Fe^{3+}) optimised conditions. A 10 mL volume of acidified distilled water (pH 1.8) was introduced into an electrochemical cell and the analytical curves were obtained through standard addition procedure by spiking appropriate volumes of each micronutrients standard solution. Prior to the measurements, the electrolyte was purged with super dry N_2 (White Martins) during 10 min to remove dissolved oxygen. Analytical curves were constructed by plotting the peak current against the micronutrients concentration.

The sensitivity of the methodologies was checked through the calculation of the detection (DL) and the quantification (QL) limits. The DL was calculated from experimental parameters by the equation $DL = 3\sigma/\theta$ (σ is the standard mean deviation of ten voltammograms registered for the blank and θ is the slope of the analytical curve) [18]; QL is established as the lowest standard concentration level in the analytical curve range [19].

SWASV/ME-Hg and SWV/ME-Hg methodologies were applied in DTPA/TEA soil extracts in order to evaluate the extractable contents of Cu^{2+}, Mn^{2+}, Zn^{2+} and Fe^{3+}. After the extraction process (DTPA/TEA solution pH 7.3), the final extracts were acidified (pH 1.8). A 10 mL volume of the acidified extracts was introduced into an electrochemical cell and the voltammogram of each micronutrient was registered with the optimal SWASV (Cu^{2+}, Mn^{2+} and Zn^{2+}) and SWV (Fe^{3+}) conditions. In order to obtain the concentration of the extractable micronutrients, recovery experiments were carried out by adding different concentration of micronutrient standard solutions. The quantity of micronutrient recovered through the method was directly obtained through extrapolation, in the x-axis, from the linear regression of the peak current (I_p) *vs.* micronutrient concentration (C_{micro}).

2.6 *Electron paramagnetic resonance (EPR) spectroscopy analyses*

Aqueous solutions containing 14.4 mg L^{-1} of Mn^{2+} ions, in different pH values, were placed in micro capillary tubes (50 µL), and then placed inside EPR quartz tubes (2.5 mm I.D.) for analyses. The sample amount used for each spectrum was around 25 µL. This amount was chosen to be enough to fill all the extension of microwave resonant cavity in order to permit the comparison of the EPR spectra signal intensity. The experimental parameters were the following: 10 mW for the microwave power; 100 kHz for the modulation frequency; 5 Gauss for the modulation amplitude, and with at least 64 sweeps per determination.

3. Results and discussion

3.1 *Evaluation of soil chemical parameters after the addition of treated effluent*

The soil used in this study is very sandy (83% of sand), classified as oxisol, with low contents of organic matter, cation exchange capacity and pH (Table 1, see control sample).

The addition of treated effluent caused an improvement of some chemical parameters of the soil, such as increase of pH, amount of organic matter (OM), and cation exchangeable capacity (CEC); and decrease of potential acidity (H + Al) and exchangeable aluminium (Al), as it can be seen in Table 1.

The pH is the soil property that interferes more significantly in metal availability [20]. As can be seen, the addition of treated effluent increases the pH of the soil by approximately one unit in all soil depths analysed. This is a favourable characteristic of the treated effluent, since its use provides the neutralisation of soil acidy, and consequently, the complexation of exchangeable Al^{3+} (toxic) by the soil OM [21].

The addition of treated effluent also promoted an increase of soil OM in all profile depths. The more significant increase (from 26 g dm^{-3} to 32 g dm^{-3}) was observed for superficial layer (0–20 cm), which is considerable when verified that the content of OM in soil sand texture is 15 g dm^{-3} [22].

Other benefits of treated effluent application are the reduction of the soil potential acidity (H + Al) and the increase of cation exchangeable capacity (CEC), indicating the rise of the bases capable to exchanging in soil, which contributed to retain essential plant

Table 1. Chemical analyses of soil samples treated with: (S) treated effluent and (C) control sample in different depths (0–20, 20–40, and 40–60 cm). Parameters analysed: pH $CaCl_2$, organic matter (OM), soil potential acidity (H + Al), exchangeable Al (Al) and cation exchangeable capacity (CEC).

Sample depth cm	pH $CaCl_2$	OM g dm^{-3}	mmol$_c$ dm^{-3} H + Al	mmol$_c$ dm^{-3} Al	mmol$_c$ dm^{-3} CEC
S* (0–20)	4.9	32	34	0	67
S (20–40)	4.9	27	34	0	65
S (40–60)	4.6	21	38	2	59
C* (0–20)	3.7	26	58	8	38
C (20–40)	3.7	23	52	10	34
C (40–60)	3.8	17	42	11	28

Notes: *S: soil with treated effluent addition; C: soil with no effluent addition (control).

nutrients within the rooting zone due to the additional cation binding sites [23]. According to Martin-Neto and co-workers, the increase of CEC is due to the increment of OM quantity, which is strongly responsible for CEC in tropical soils [24].

3.2 *Voltametric measurements*

The concentrations of Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} ions were measurement in the soil samples in order to evaluate the availability of these elements and also to verify if the addition of the treated effluent improves their abundance in the soil. Table 2 presents the optimised parameters for the four metal ions and the angular (slope) and linear (intercept) coefficients of the analytical curves (standard addition) used for determination of the detection (DL) and quantification limits (QL). The lowest detection limit was obtained for zinc ($0.1\,mg\,L^{-1}$) and the highest for iron ($0.7\,mg\,L^{-1}$).

All the developed electroanalytical methodologies have good correlation coefficients, intercepts and slopes. The precision of the methods has been determined from the standard deviation (SD) and the correlation coefficient (r^2) for three replicate analyses of sample solutions, and it provides values greater than $1.7 \times 10^{-2}\,nAmgL^{-1}$ and 0.99 for all micronutrients, respectively. It is important to mention that the aim of this study is not the detection of metal traces, thus the detection limit reached (DL) is adequate for the analysis of each micronutrient in the studied matrices.

The chelating agent DTPA/TEA solution was chosen among other solutions available for extract micronutrients because it offered the most favourable combination of stability constants for the simultaneous complexing of Cu^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+} and good correlation with the absorbed micronutrient contents by plants [15,25].

After the optimisation of SWASV/ME-Hg and SWV/ME-Hg methodologies, the electrochemical response of each micronutrient was evaluated in DTPA/TEA solution (pH 1.8) and also in spiked metal ions samples (acidified distilled water, pH 1.8) in order to check the electroactivity of the complex forming between the extract solution and the micronutrient (Figure 1). Measurements were also carried out at pH 7.3, but the voltammograms exhibited a poor profile probably associated to the loss of electroactivity of the studied complexes. The acidic condition (pH 1.8) was more favourable to the study since at this pH the DTPA is mostly protonated (pK_a: 1.9, 2.9, 4.4, 8.7 and 10.5) [26].

Table 3 presents the range of potentials scanned for each individual ions at the specific optimised SWASV and SWV parameters, the peak potentials of the DTPA/TEA/ions

Table 2. Analytical features of the SWASV/ME-Hg (Cu^{2+}, Mn^{2+}, Zn^{2+}) and SWV/ME-Hg (Fe^{3+}) methodologies, presenting the optimised parameters, the detection (DL) and quantification (QL) limits, the intercepts, the slopes and the linear correlation coefficients (r^2).

	Optimised SWASV parameters			Analytical curve				
Metal ion	*f* s^{-1}	t_{acc} s	E_{acc} V	Intercept nA	Slope nA mg^{-1} L	r^2	DL mgL^{-1}	QL mgL^{-1}
Cu^{2+}	20	300	–0.5	0.2	4.9	0.993	0.2	0.4
Fe^{3+}	40	–	–	0.1	1.3	0.999	0.7	2.0
Mn^{2+}	20	50	–1.7	0.5	2.6	0.992	0.2	1.4
Zn^{2+}	20	100	–1.2	4.5	1.8	0.998	0.1	0.8

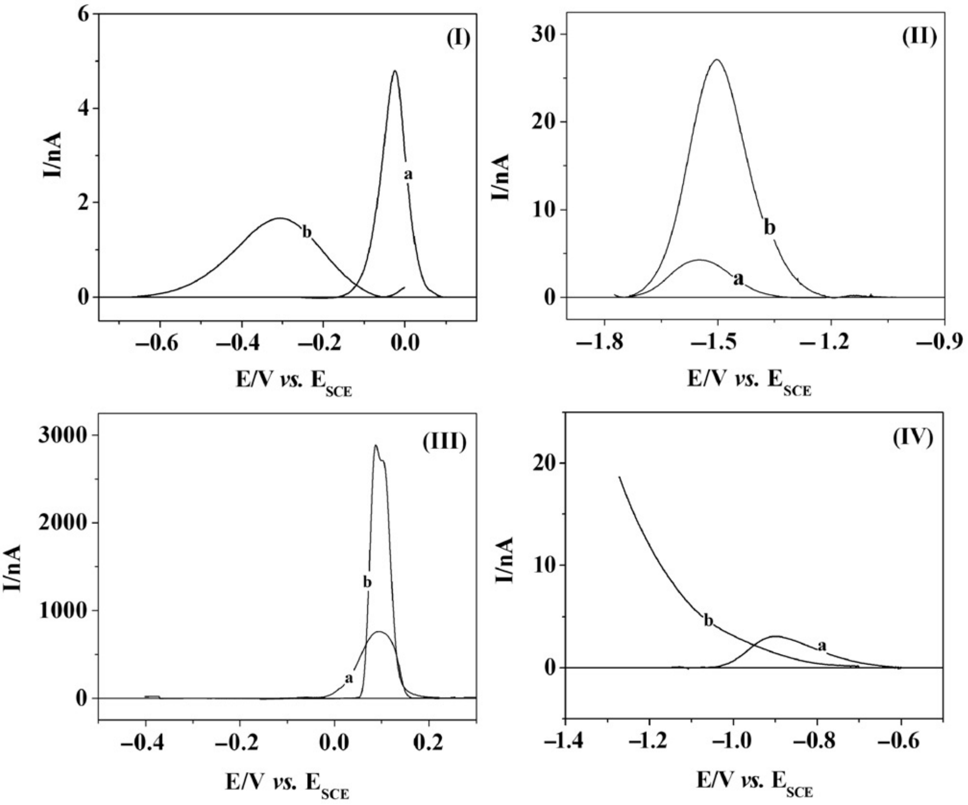

Figure 1. Voltammograms for: (I) Cu^{2+} (2.0 mg L^{-1}), (II) Mn^{2+} (2.6 mg L^{-1}), (III) Fe^{3+} (12.0 mg L^{-1}) and (IV) Zn^{2+} (4.6 mg L^{-1}). (a) Acidified distilled water (pH 1.8) and (b) DTPA/TEA solution (pH 1.8). Working electrode: ME-Hg. SWASV and SWV optimised parameters according to Table 2.

Table 3. Range of potentials scanned for each individual ions at the specific optimised SWASV and SWV parameters and the peak potentials of the DTPA/TEA/ions complexes and also of the free ions.

	Scan potentials (V)		Peak potentials (V)	
Metal ion	Initial	Final	Extractor/ion[a]	Free ion
Cu^{2+}	–0.75	0	–0.4	–0.03
Fe^{3+}	+0.2	–0.4	+0.12	+0.18
Mn^{2+}	–1.75	–1.1	–1.49	–1.55
Zn^{2+}	–1.30	–0.7	–	–0.9

Notes: [a]DTPA/TEA/ion complex.

complexes and also the peak potentials of free ions for comparison. It can be observed that the complex with Mn^{2+} shows electroactivity in the potentials range close to the ones for the free ions in solution, so it can be adequately detected by the SWASV/ME-Hg methodology. For Cu-DTPA complexes, there is a displacement of the peak potential for

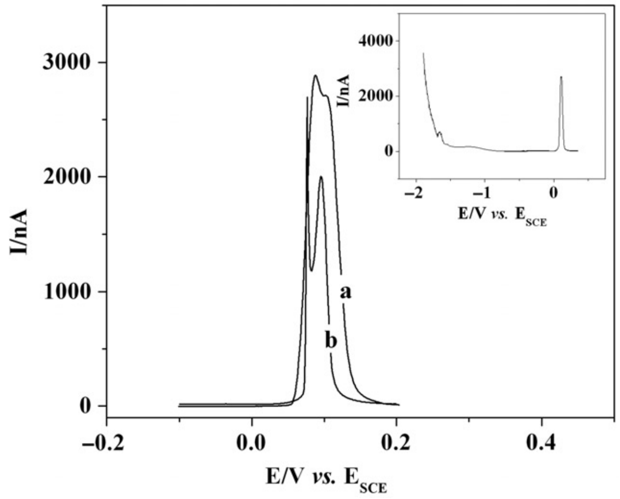

Figure 2. SWV voltammograms for Fe^{3+} (12.0 mg L^{-1}) in DTPA/TEA solution (pH 1.8): (a) $f = 40\,s^{-1}$ and (b) $f = 10\,s^{-1}$. Working electrode: ME-Hg. SWV optimised parameters according to Table 2. Insert: SWV voltammogram of DTPA/TEA solution (pH 1.8), $f = 40\,s^{-1}$, $a = 50$ mV and $\Delta E_s = 1$ mV.

more cathodic ones, which could be associated to the complexation mechanism of Cu^{2+} ions with DTPA molecule. This displacement does not affect its quantification.

The complex formed with Zn^{2+} ions did not show any electroactivity in the entire potential window scanned (0 until –2.0 V).

For the Fe-DTPA complex, it was observed that its peak potential (+0.18 V, Figure 2a) is very close to the DTPA molecule peak (+0.1 V, insert in Figure 2) and the determination of Fe-DTPA complex is not possible. However, one additional study showed that if the SWV frequency is changed to $10\,s^{-1}$, the separation of both peaks can be obtained and the Fe-DTPA complex is detected, as shown in Figure 2b.

After establishing the electroanalytical methodologies, the concentration of each extractable micronutrient was obtained by recovery experiments (Figure 3), as mentioned in the experimental part above (Section 2.5).

Table 4 summarises the results obtained with SWASV + SWV/ME-Hg and FAAS methodologies for Cu, Fe and Mn analyses in DTPA/TEA ion extracts. The average relative standard deviation (RSD) of the samples measured in triplicate were 9.4% for SWASV + SWV and 7.8% for the FAAS, indicating the repeatability of both methods is very similar. A graphic correlation between SWASV + SWV and FAAS (Figure 4) shows a very good determination coefficient ($r^2 = 0.996$), showing that the electroanalytical methodology could be an alternative to the micronutrients measurements in soil extracts without serious interferences of the matrix components. Typical interferences fond are organic and mineral colloids and dissolved organic matter. The root mean square deviation (RMSD) obtained was 0.9 mg L^{-1}, that can be considered the average precision for the three metal together, if the FAAS is assumed as the standard method. It is interesting to verify that the RMSD obtained for Cu, Fe and Mn was of the same order of the DL and QL (Table 2).

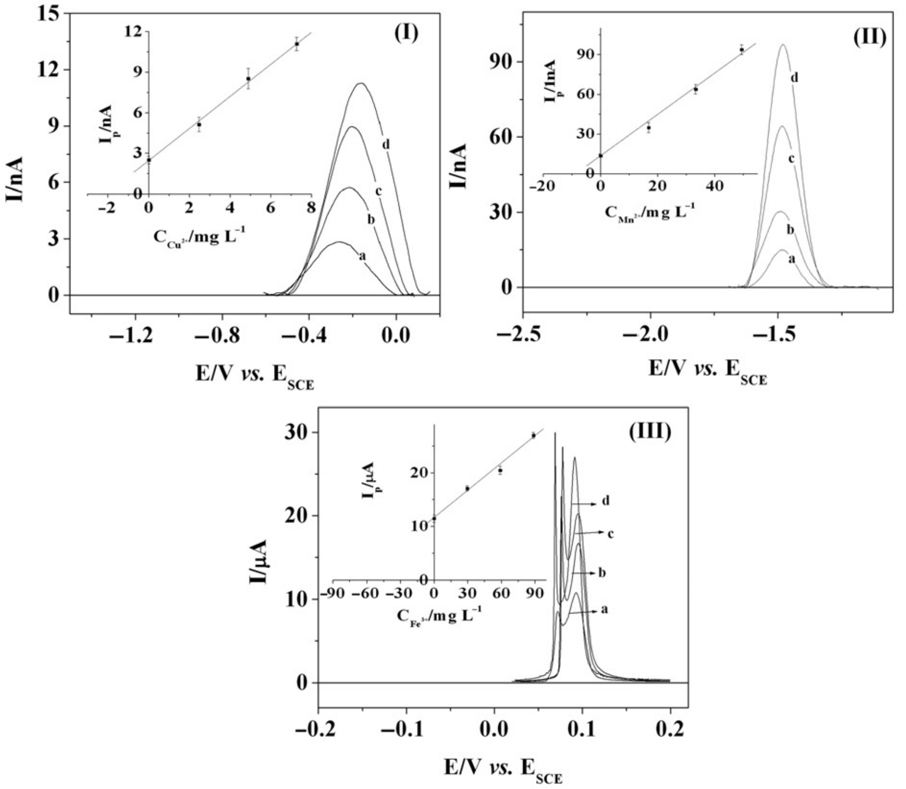

Figure 3. Voltammograms for the recovery experiments of the extractable micronutrients: (I) Cu-DTPA, (II) Mn-DTPA and (III) Fe-DTPA. (a) extracted soil sample and (b), (c), (d) standard addition of the metals solutions. Sample: soil treated with effluent addition. Working electrode: ME-Hg. SWASV and SWV optimised parameters according to Table 2 (except for the Fe^{3+} analysis, the *f* value used was $10\,s^{-1}$).

Table 4 also shows an increment of the metal availability for the Cu^{2+} and Mn^{2+} and a decrement of the availability for Fe^{3+} with the addition of the treated effluent (comparing the control with the treated effluent treatments). Increment in the whole soil profile was about 100% for Cu^{2+} and Mn^{2+}. For the Fe^{3+}, there was a decrement of about 60% in the soil profile. The decreasing of Fe^{3+} ions extracted from the samples treated with effluent can be related to the increasing of the pH. According to Hooda and Alloway, a reduction of 0.5 pH unit can be significant to the metals availability in soil [27].

Through the results obtained above, it was possible to observe that the application of the treated effluent from the developed anaerobic biodigester cesspool improves some chemical properties of the studied soil.

3.3 *Evaluation of DTPA-metals complexation behaviour*

The EPR experiments were useful in order to observe the formation of inner and outer DTPA complexes with the studied metals and to associate these complexes nature with

Table 4. Comparison of the results obtained with SWASV + SWV/ME-Hg with FAAS methodologies for Cu^{2+}, Fe^{3+} and Mn^{2+} analyses in DTPA/TEA extracts. S (soil treated with effluent); C (control sample) in different depths (0–20, 20–40 and 40–60 cm).

		DTPA/TEA soil extracts					
		SWASV			FAAS		
Metal ion	Treatment	[Ion] $\mathrm{mg\,L^{-1}}$	SD	RSD %	[Ion] $\mathrm{mg\,L^{-1}}$	SD	RSD %
Cu	S (0–20 cm)	2.0	0.1	5.0	2.1	0.2	9.5
	S (20–40 cm)	1.6	0.2	12.5	1.0	0.0	–
	S (40–60 cm)	1.0	0.1	10.0	0.8	0.1	12.5
	C (0–20 cm)	0.8	0.1	12.5	0.8	0.0	–
	C (20–40 cm)	0.8	0.2	25.0	0.6	0.0	–
	C (40–60 cm)	0.4	0.1	25.0	0.6	0.07	11.6
Fe	S (0–20 cm)	68.9	3.9	5.6	73	8.5	11.6
	S (20–40 cm)	67.1	4.4	6.5	63	5.6	8.8
	S (40–60 cm)	49.3	8.1	16.4	47.5	2.1	4.4
	C (0–20 cm)	146.5	14.5	9.8	151.5	17.7	11.7
	C (20–40 cm)	123.0	4.1	3.3	136.5	4.9	3.6
	C (40–60 cm)	62.2	2.6	4.2	61.5	4.9	7.9
Mn	S (0–20 cm)	14.4	1.1	7.6	15.2	1.5	9.8
	S (20–40 cm)	9.9	0.7	7.0	11.5	0.9	7.8
	S (40–60 cm)	8.7	0.3	3.4	7.4	0.2	2.7
	C (0–20 cm)	13.1	0.6	4.5	12.6	0.6	4.8
	C (20–40 cm)	6.9	0.3	4.3	5.6	0.1	1.8
	C (40–60 cm)	3.2	0.2	6.2	2.5	0.2	8.0

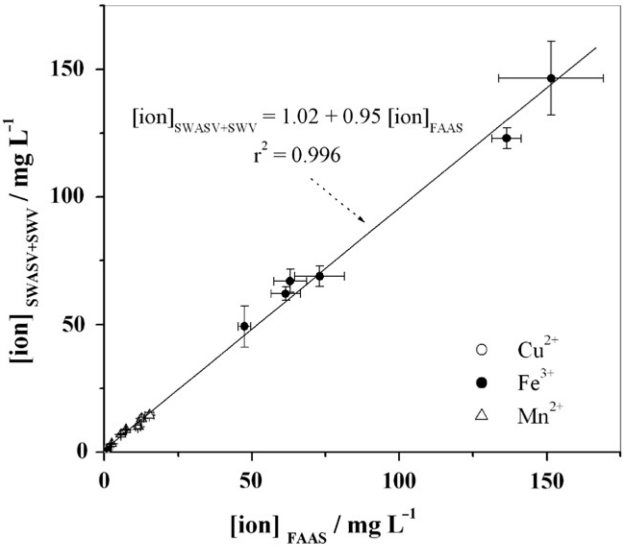

Figure 4. Correlation between SWASV + SWV and FAAS methodologies ($r^2 = 0.996$).

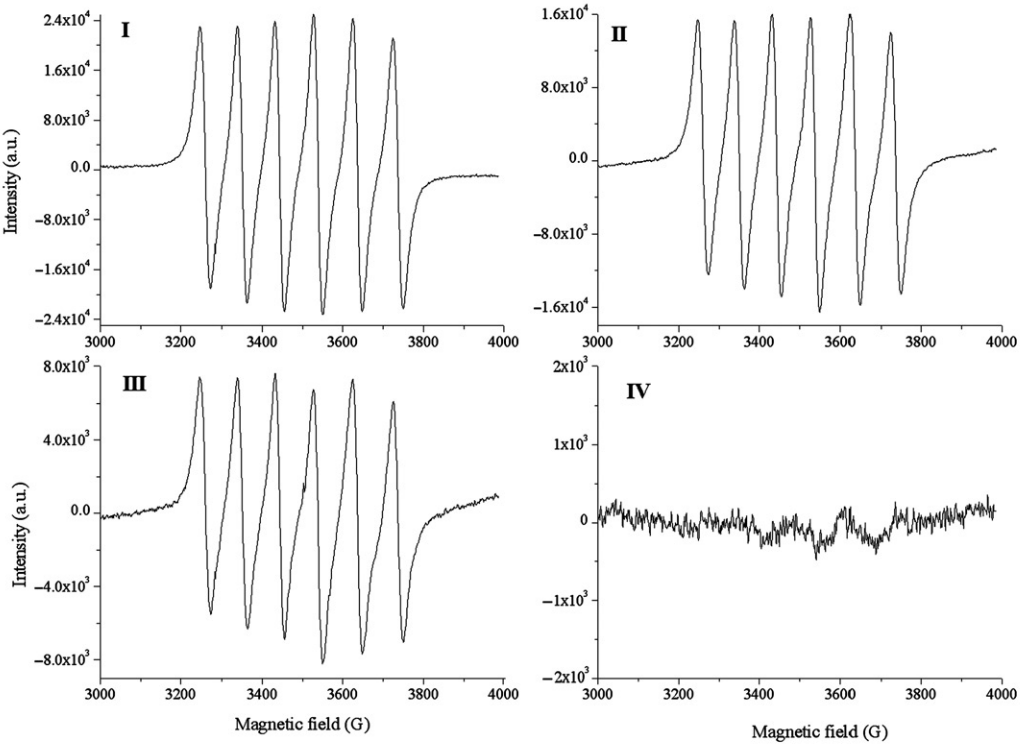

Figure 5. Mn^{2+} EPR spectra in the g ~ 2 region for a DTPA/TEA soil extract solution in different pH values: (I) 1.8, (II) 2.8, (III) 3.2 and (IV) 7.3. DTPA extract from soil treated with effluent (0–20 cm).

their electroactivity in different pH values. It was not possible to detect Fe^{3+} and Cu^{2+} ions, considering the experimental conditions used, and as well as the Zn^{2+} ions due to its diamagnetic nature.

In Figure 5, EPR spectra registered for a DTPA soil extracting solution in different pH values (1.8, 2.8, 3.2 and 7.3) are presented for Mn^{2+} ions. It could be seen that EPR Mn^{2+} signals decrease with the increase of pH and totally disappear at pH 7.3.

According to the literature, metal ions can be associated to humic substances, forming inner and/or outer sphere complexes [28]. These types of interactions explain the occurrence of EPR Mn^{2+} signals only in acid medium (pH 1.8–2.8), since in this pH interval Mn^{2+} ion is presented as $[Mn(H_2O)_6]^{2+}$ and the nature of the interaction between DTPA and $[Mn(H_2O)_6]^{2+}$ is basically electrostatic, i.e. they are more labile.

This behaviour could explain the electroactivity of the Mn-DTPA complexes only in acid medium, since in pH 7.3 the voltammograms exhibited a poor profile probably associated to the loss of electroactivity of the complex.

The loss of the EPR signal can be attributed to the exchange of water molecules by COO^- groups of the DTPA molecule, forming inner sphere complexes with high complexes stability (Fe^{3+} (log K = 28.6), Mn^{2+} (log K = 15.6), Cu^{2+} (log K = 21.5) and Zn^{2+} (log K = 18.6)) [29,30]. The nature of the DTPA complexes as inner or outer spheres were directed influenced by the pH solution and this behaviour could clearly explain the complexes electroactivity of the extractable metals.

4. Conclusion

The addition of treated effluent, produced by the anaerobic biodigester septic cesspool, caused an improvement in some chemical parameters of the studied soil, such as the pH, amount of organic matter, soil potential acidity ($H + Al$), exchangeable Al, and cation exchangeable capacity (CEC). These results suggest that this kind of effluent is an interesting alternative for the use in agriculture as a good source of plant nutrients.

The short analysis time in SWASV/ME-Hg and SWV/ME-Hg makes the electroanalytical methodologies very attractive for routine determination of the described elements in soil. Other favourable characteristics of the electroanalytical methodology, such as no-necessity of enrichment steps or particular sample treatments, compact and portable instrumentations, make possible the use of this kind of methodologies as an alternative to the standard FAAS methods.

There is a need for additional research in order to study the best experimental conditions to evaluate other extract solutions to be used in Zn electroanalytical determination using the SWASV/Hg-ME methodology and also to analyse Fe-DTPA and Cu-DTPA complexes by EPR.

Acknowledgements

The authors wish to thank CNPq (Processes: 151810/2005-2 and 310750/2006-7) and Embrapa Instrumentação Agropecuária (CNPDIA).

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