Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 1415–1424
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Fractionation analysis of manganese and zinc in tea infusions by two-column solid phase extraction and flame atomic absorption spectrometry

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Received 28 June 2006; received in revised form 30 August 2006; accepted 12 September 2006

Abstract

The fractionation pattern of Mn and Zn in the infusions of black and green teas was studied using the Amberlite XAD7 and the Dowex 50Wx4 sorbents connected in a series, after the thorough examination of the sorption and the desorption characteristics of the resins applied. Under the optimized conditions, the one step two column approach developed enabled to distinguish three different operationally defined classes of the metal species, namely the polyphenolic, the cationic and the residual metal fractions. The results indicate that Mn and Zn in tea infusions are predominantly present in the form of the cationic species (up to 80% or more, especially in the green teas). Both metals are also attached to the polyphenolic compounds and possibly to other macromolecules.

Keywords: Mn; Zn; Fractionation; Tea infusions; Amberlite XAD7; Dowex 50Wx4; Flame atomic absorption spectrometry

1. Introduction

Tea is regularly drunk in many countries mostly for its taste and reputed health benefits. For the reason that it is included in everyday diet and largely consumed, the contributions concerning the determination of major (Al, Ca, Mg, or Mn) and trace (Ba, Co, Cr, Cu, Fe, Ni, Zn) mineral constituents in tea brews and leaves are of very high importance and interest regarding the estimation of the daily intake of these elements in diet or their possible negative effects and implications on health (Alberti, Biesuz, Profumo, & Pesavento, 2003; Cairns, Hill, & Ebdon, 1996; Fernandez, Pablos, Martin, & Gonzalez, 2002). Usually, for that purpose, the total metal contents in leaves as well as the totally dissolved metal concentrations in the infusions are measured using different methods of sample digestion and analysis (Fernandez et al., 2002; Gallaher, Gallaher, Marshall, & Marshall, 2006; Marcos, Fisher, Rea, & Hill, 1998).

The studies focused on the analysis of variety of the tea brands and their infusions on the metal composition are obviously valuable in terms of the determination of the geographical origin of the tea products, the assessment of their potential nutritional value, or finally, the estimation of the alimentary intake of the metals through the consumption of tea beverages. However, it is recognized that the identification and the quantification of the ultimate physicochemical forms of metals found in the tea infusions are determinant in the evaluation of their actual bioavailability or toxicity to the human organisms (Cairns et al., 1996; Flaten, 2002). Apparently, the trace and major metals can be present in the brews as simple ions but also as the complexes of different stability formed with the endogenous bioligands present in the tea. The most frequently, the metals are bound to the polyphenols, i.e., the theaflavins and the thearubigins, which are maintained to be the most abundant class of the organic compounds complexing the metals through the hydroxyl, carboxylate and phenolate oxygen-donor groups. Certainly, the comprehension of this metal complexation and the distribution among

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doi:10.1016/j.foodchem.2006.09.007

different classes of the compounds is crucial in order to predict the metal availability from the tea and the absorption by humans through the gastric intestine.

Direct determination of the specific forms of metals in the tea is extremely difficult due to a great number of the individual species that can exist in that kind of beverage. So far, only a small number of works have been dedicated to the speciation of metals in the tea infusions and usually the operationally defined fractionation approach was preferred and resulted in the discrimination of the metal species groupings having comparable physical or/and chemical properties. The overwhelming majority of the papers regarded the operational fractionation of aluminum undertaken by means of cation exchange, size-exclusion or ultrafiltration (Alberti et al., 2003; Erdemoglu, Pyrzynska, & Gucer, 2000; Flaten, 2002; Flaten & Lund, 1997; French, Gardner, & Gunn, 1989; Owen, Crews, & Massey, 1992; Ruszczynska, Pyrzynska, & Bulska, 2004; Zhou et al., 1996). The speciation of other metals, which are essential to life processes and which amounts in the teas and their infusions are considerable, i.e., Ca, Mg, Mn, or Zn, seems to be much lesser known (Alberti, Biesuz, D'Agostino, & Pesavento, 2004; Odegard & Lund, 1997; Ozdemir & Gucer, 1998; Pohl & Prusisz, 2006). In mentioned above contributions, normally the samples were treated with a one specified ion exchange resin in order to distinguish a one group of the metal species of the same charge (the cationic or the anionic metal fractions) or several groups of the metal species differing in the stability (the fractions of stable, weak, or very weak metal complexes).

In this work, the relevant information about the distribution of Mn and Zn in the infusions of black and green teas was obtained using an one-step two column assemblage containing the adsorbing resin Amberlite XAD7 and the cation exchanger Dowex 50Wx4 connected in a series. The concentrations in the tea leaves and the tea infusions were measured, and the extraction efficiency of the brewing process was evaluated. Three different classes of the metal species in the tea infusions were discriminated through the analytical protocol developed. The possible associations of the metals with distinct endogenous tea constituents in the separated metal fractions were discussed and compared with previously reported experimental findings.

2. Experimental

2.1. Apparatus

A Perkin Elmer AA-1100 B spectrometer was used to the determination of Mn and Zn concentrations in all the sample solutions applying a method of two standard additions. The apparatus was equipped with a single slot 10-cm titanium burner head for air/acetylene flame operation, a burner assembly comprising a plastic mixing chamber with a flow spoiler and a stainless steel nebulizer, as well as a drain safety siphon interlock. The gas flow rate settings corresponded to the manufacturer recommendations, i.e., $8.0\ \mathrm{l\ min^{-1}}$ for air and $2.5\ \mathrm{l\ min^{-1}}$ in case of acetylene. The hollow cathode manganese and zinc lamps were operated at 20 and 15 mA, respectively. For the absorbance measurements, the standard wavelengths and spectral band passes were selected, i.e., 279.5 and 0.2 nm for Mn, 213.7 and 0.7 nm for Zn. The time-shared background correction by means of a deuterium lamp was normally used. For the signal readout, a time-average integration (hold mode) was used with an integration time of 1 s and three replicates. The instrumental detection limits established were 0.003 and $0.004\ \mathrm{mg\ l^{-1}}$ with the upper linearity ranges of 3.0 and $1.0\ \mathrm{mg\ l^{-1}}$, respectively, for Mn and Zn.

For the solid phase extraction, Supelco (USA) liquid chromatographic columns (10 mm of ID) with coarse frits and Teflon stopcocks were used throughout.

2.2. Reagents and materials

The analytical grade reagents and doubly distilled water were used to prepare all the solutions. The solutions of Mn^{2+} and Zn^{2+} ($1000\ \mathrm{mg\ l^{-1}}$) were prepared from $MnCl_2$ and $ZnCl_2$ fixanals (Merck, Germany). Other chemicals were purchased from POCh (Poland), i.e., concentrated solutions of HNO_3 and HCl, 30% (m/v) solution of H_2O_2, solid NaOH, $C_8H_5O_4K$ (potassium hydrogen phthalate), $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (disodium salt of ethylenediaminetetraacetic acid, EDTA) and $C_{76}H_{52}O_{46}$ (tannic acid, TA). The 100-ml two-component working solutions of Zn^{2+} and Mn^{2+} ($0.50\ \mathrm{mg\ l^{-1}}$) were obtained by the dilution of the respective bulk solutions. For adjusting the solutions to the pH corresponded to 4.0, 4.5, 5.0, and 5.5, the $0.10\ \mathrm{mol\ l^{-1}}$ $C_8H_5O_4K$–NaOH standard buffer solutions, prepared according to the recommendations given by Bower and Bates (1995), were used. The 100-ml working solutions of the metal complexes with EDTA or TA were prepared by the addition of a $0.10\ \mathrm{mol\ l^{-1}}$ EDTA solution (10 ml) or a $1000\ \mathrm{mg\ l^{-1}}$ TA solution (10 ml), respectively, to the buffered metal solutions ($0.50\ \mathrm{mg\ l^{-1}}$).

The following resins were used for the solid phase extraction: the adsorbing, non-ionic, macroreticular acrylic ester polymer Amberlite XAD7 (particle size 20–60 mesh, $450\ \mathrm{m^2\ g^{-1}}$ of surface area) from Sigma–Aldrich (Germany), the strong anion exchanging, divinylbenzene 4-vinylpyridine copolymer Reillex™ 402 (particle size 100–200 mesh, pyridinyl functional groups) form Sigma–Aldrich (Germany) and the strong cation exchanging, gel type, styrene divinylbenzene copolymer Dowex 50Wx4 (particle size 200–400 mesh, sulfonic acid functional groups) from Supelco (USA).

2.3. Procedures

2.3.1. Resin pretreatment

The columns were packed with 2.0 g of the Amberlite XAD7, 2.0 g of the Reillex™ 402 and 1.0 g of the Dowex 50Wx4, respectively, using the water slurries of the resins as received. The resin beds were preconditioned before use. The preconditioning reagents and water used were passed at the flow rate of 1.0 ml min^{-1} controlled by means of a two-channel programmable peristaltic pump, type 306 (UniPan, Poland). In case of the Amberlite XAD7, at first, the resin beds were rinsed with 25 ml of a methanol-water solution (1 + 1). Washing of the resin beds with 10 ml of a 1.0 mol l^{-1} NaOH solution was followed and than, flushing with 50 ml of water was carried out. Next, 10 ml of a 2.0 mol l^{-1} HCl solution were passed through the resin beds. Finally, they were washed with water (about 50 ml) to obtain the neutral column effluents. The Reillex™ 402 resin beds were rinsed successively with about 50 ml of water. Then, 10 ml of 2.0 mol l^{-1} HCl solution were passed through the resin beds. The excess of HCl was removed by washing the resin beds with 100 ml of water. In case of the Dowex 50Wx4, the resin beds were flushed with 25 ml of water and then rinsed with 10 ml of 2.0 mol l^{-1} solution of HCl. Afterwards, washing with 25 ml of water was continued, followed by passing through the resin beds 10 ml of 1.0 mol l^{-1} solution of NaOH. The excess of NaOH was removed using about 25 ml of water.

2.3.2. Resin sorption and desorption conditions optimization

The flow rates of all the solutions passed through the columns was maintained using the two-channel programmable peristaltic pump. The metal recovery and retention efficiencies assessed were the mean values for three independent experiments. The relative standard deviations were normally within 3%. The column blanks for Mn and Zn were simultaneously undertaken; they were found below the detection limits.

The sorption of Mn^{2+} and Zn^{2+} as well as the metal complexes with EDTA and TA by the Amberlite XAD7 was studied at the pH changing from 4.0 to 5.5. For that aim, the columns were loaded with the respective 100-ml working solutions at the flow rate of 1.0 ml min^{-1}. The investigation concerning the sorption efficiency of the complexes of Mn and Zn with tannin versus the sample flow rate corresponded to 0.50, 1.0, and 2.0 ml min^{-1} was performed using the 100-ml working solutions of the pH equal to 4.5. The retention efficiencies for Mn and Zn were assessed comparing the metal concentrations in the working solutions to those determined in the column effluents collected (the volume about 10 ml). The effect of HCl and HNO_3 concentration in the solutions applied for the elution of the complexes of Mn and Zn with TA was examined at 0.50, 1.0, and 2.0 mol l^{-1}. After loading the columns with the 100-ml working solutions (the pH of 4.5) at the flow rate of 1.0 ml min^{-1}, 10 ml of the selected solvents were passed through the columns and the respective 10-ml eluate portions were collected. The recoveries of Mn and Zn were evaluated regarding the metal concentrations determined in the eluates and taking into consideration the amounts of the metals retained during the loading step.

The retrieval of Mn^{2+} and Zn^{2+} from the Dowex 50Wx4 was investigated using the HCl and HNO_3 solutions at the concentrations of 1.0, 2.0, and 4.0 mol l^{-1}. After loading the columns with the 100-ml working solutions (the pH of 4.5) at the flow rate of 1.0 ml min^{-1}, the metals were stripped with 10 ml of the respective eluents, passing them through the columns at the flow rate of 1.0 ml min^{-1}. The metal recoveries were calculated referring the concentrations of Mn and Zn measured in the eluates to the metal concentrations in the loaded working solutions. The influence of the eluent flow rate, related to 2.0 and 4.0 ml min^{-1}, on the recoveries of Mn and Zn was examined using 4.0 mol l^{-1} solution of HCl. The effect of the solution pH changed in the range from 4.0 to 5.5 (at the flow rate of 1.0 ml min^{-1}) and the sample flow rate varied from 1.0 to 4.0 ml min^{-1} (at the pH of 4.5) on the retention of Mn and Zn on the Dowex 50Wx4 resin was studied passing the respective 100-ml working solutions through the columns and then, stripping the metals retained applying 4.0 mol l^{-1} HCl solution (10 ml of the eluent were passed through the columns at the flow rate of 1.0 ml min^{-1}). To determine the retention efficiencies of Mn and Zn, the metal concentrations were measured in the collected 10-ml eluates and related to the metal amounts in the working solutions.

2.3.3. Tea infusion preparation

The black and green teas available in Polish markets were taken for the analysis. The teas were brewed in the following way: 5-gram quantities of the tea leaves were placed in 1-liter beakers and filled with 400 ml of boiling doubly distilled water. The tea leaves were infused with swirling for about 6 min. After that, the resulted infusions were filtered through the narrow filter papers (Filtrak, Germany). The grinds were washed then with about 50 ml of water, the filtrates and the washings were combined and allowed to cool. Finally, the infusions were completed to the total volumes of 500 ml. These samples were used for the determination of the total metal amounts in the infusions as well as for the operationally defined metal fractionation analysis. The blank experiments were performed applying the same procedure and the equipment described above but without the tea leaves. The names of the tea brands as well as the pHs of the infusions prepared are given in Table 1.

2.3.4. Total metal content determination

The total concentrations of Mn and Zn in the tea infusions were measured directly by the FAAS using the method of two standard additions. The total contents of metals under discussion in the tea leaves were determined in the solutions obtained after the wet digestion of 0.5-gram leaf portions in the mixture of concentrated HNO_3 and 30% H_2O_2, carried out in glass beakers covered with watch glasses and placed on a hot plate. At first, the leaves were poured with 5 ml of the concentrated nitric acid and allowed to boil. After the decomposition of the leaves, 5 ml of the 30% hydrogen peroxide solution were added to the resulted aliquots and heating was continued.

Table 1
The total concentrations of Mn and Zn in the leaves (in mg g^{-1}) and in the infusions (in mg l^{-1}) of different black (B) and green (G) tea brands

Tea brand	pH[a]	Mn			Zn		
		Leaves	Infusion	In brew[b] (%)	Leaves	Infusion	In brew[b] (%)
B1, Earl Grey (Lipton, Poland)	5.0	0.286 ± 0.008	1.56 ± 0.01	53.7 ± 0.4	0.0262 ± 0.0011	0.121 ± 0.011	45.5 ± 4.4
B2, Saga (Unilever, Poland)	5.2	0.892 ± 0.008	2.77 ± 0.01	30.6 ± 0.1	0.0280 ± 0.0015	0.107 ± 0.006	37.7 ± 2.1
B3, Earl Grey (Bastek, Poland)	5.0	1.29 ± 0.01	6.02 ± 0.02	46.1 ± 0.2	0.0223 ± 0.0050	0.107 ± 0.004	47.3 ± 2.0
G1, Orient (Lipton, Poland)	4.8	0.833 ± 0.047	1.53 ± 0.04	18.2 ± 0.5	0.0340 ± 0.0038	0.121 ± 0.006	35.4 ± 1.8
G2, Loyd (Mokate, Poland)	4.8	1.44 ± 0.02	2.02 ± 0.04	14.0 ± 0.3	0.0346 ± 0.0020	0.153 ± 0.003	44.1 ± 0.9
G3, Original (Bastek, Poland)	4.6	0.618 ± 0.066	2.01 ± 0.12	33.0 ± 2.0	0.0232 ± 0.0070	0.150 ± 0.018	65.6 ± 7.9

The results are means ± standard deviations ($n = 3$).

[a] Measured in the infusion.

[b] The percentage of the metal amount extracted from leaves to the infusion.

Lastly, the solutions were evaporated to near dryness and then diluted with water to the volumes of 50 ml. Before the measurements, the digests were filtered through the narrow filter papers (Filtrak, Germany). The respective blank samples were also prepared and considered in the calculations.

2.3.5. Metal fractionation analysis

The procedure of the operational fractionation of Mn and Zn species in the tea infusions was as follows: the 50-ml infusion samples of different tea brands were passed at the flow rate of 1.0 ml min^{-1} through the Amberlite XAD7 adsorbent (1st column) and the Dowex 50Wx4 strong cation exchanger (2nd column) connected in a series (Fig. 1). Afterward, the columns were disconnected and the metal species retained were subsequently eluted by means of 10 ml of 2.0 mol l^{-1} HNO_3 (1st column) and 10 ml of 4.0 mol l^{-1} HCl (2nd column); the stripping solutions were passed through the columns at the flow rate of 1.0 ml min^{-1}. The resulted 10-ml eluates along with the effluents of the volume of about 10 ml, originated from loading the linked columns with the tea infusion samples, were subjected to the analysis in order to determine the concentrations of Mn and Zn in the separated fractions, i.e., the fraction of the hydrophobic metal species primarily attributed to the complexes of Mn and Zn with polyphenolic compounds (1st column), the fraction of the cationic metal species including mostly free cations and stable cationic complexes with organic acids (2nd column) and finally, the fraction of the residual metal species presumably referred to some anionic or neutral, hydrophilic complexes of the metals with low molecular mass ligands.

For comparison, another two column device was used for the partitioning of Mn and Zn metal species in the analyzed tea infusions (Fig. 1). Instead of the non-ionic sorbent Amberlite XAD7, the cross-linked polyvinylpyridine Reillex™ 402 resin, capable of forming very strong hydrogen bonds with the phenolic, hydroxyl and carboxyl groups of the dissolved organic matter, was applied. The tea infusions (50 ml) were passed through the columns connected in a series at the flow rate of 1.0 ml min^{-1}. Prior to the evaluation of the amounts of the Mn and Zn species retained

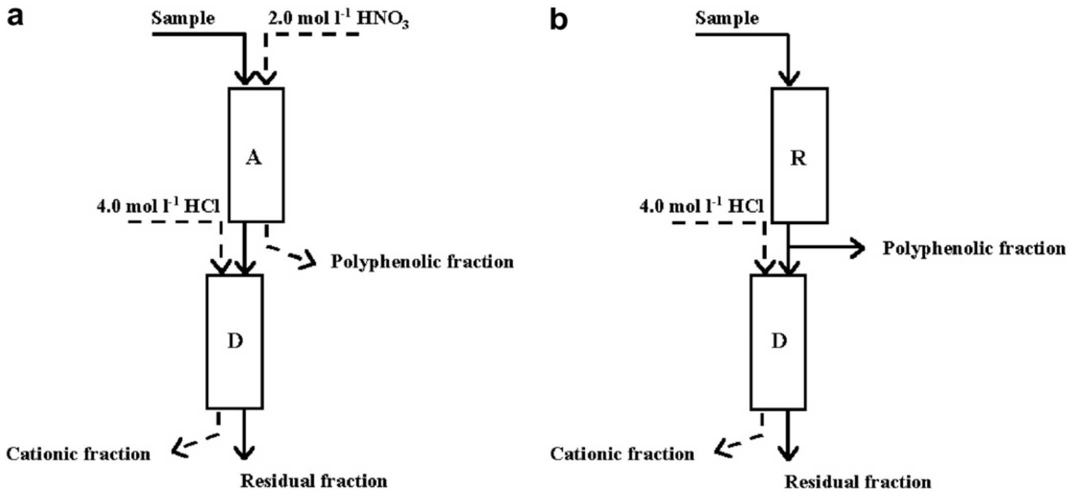

Fig. 1. The schemes of the Mn and Zn fractionation manifolds. (a) the Amberlite XAD7 (A) – Dowex 50Wx4 (D) system; (b) the Reillex™ 402 (R)–Dowex 50Wx4 (D) system.

by the Reillex™ 402, the 5-ml portions of the effluents incoming to the Dowex 50Wx4 were sampled. The effluents resulted from the passage of the infusions through the columns were also collected. Subsequently, the columns were split and the metal species were recovered from the Dowex 50Wx4 using 10 ml of 4.0 mol l^{-1} HCl solution. The analyses of the effluents and eluates were carried out for Mn and Zn contents.

All the experiment were undertaken in triplicate using the fresh infusion portions.

3. Results and discussion

3.1. Sorption and desorption conditions

3.1.1. Amberlite XAD7

The Amberlite XAD7 resin based on the polystyrene-divinylbenzene copolymer was previously used by Erdemoglu et al. (2000) for the operational fractionation of Al in the black tea infusions. The resin used enabled the separation of the broadly meant class of the complexes of Al with the polyphenols, however, a considerable uptake of simple Al^{3+} ions, probably due to different polar impurities present in that type of the polymer framework (Karadjova, Izgi, & Gucer, 2002; Terada, 1992), was also noted. Apparently, a special treatment in the mentioned work was required in order to inactivate the ion exchange sites by the initial treatment of the adsorbing resin with the solution containing In^{3+} ions.

In contrast to the cited paper, in the present contribution, the macroreticular, more hydrophobic methyl metacrylate-based copolymer Amberlite XAD7 resin was applied, and it was established that the resin did not retain the cations of Mn^{2+} and Zn^{2+} in the studied pH range, i.e., from 4.0 to 5.5. In addition, it was also ascertained that low molecular weight complexes of Mn^{2+} and Zn^{2+} with EDTA were either not adsorbed by the resin under the investigated pH sets.

The tannic acid was chosen as a model polyphenolic compound to study the sorption and the desorption properties of the metals bound to the polyphenols toward the Amberlite XAD7. It was found that Mn and Zn were retained from the solutions of the pH of 4.0–5.5 in the form of their complexes with TA with the average efficiencies equal to 53% and 38%, respectively. Unfortunately, there is a lack of the adequate literature in reference to the complexation of Mn^{2+} and Zn^{2+} by TA. Lately, Ucer, Uyanik, and Aygun (2006) determined that TA anchored on the activated carbon demonstrated a relatively low affinity to bond the cations of the metals under consideration. In view of this, it was assumed that the complexation efficiency of both metals by the tannic acid corresponded to the sorption efficiencies ascertained.

No effect on the sorption efficiency of the complexes of Mn and Zn with TA was found when the loading flow rate was lower than 1.0 ml min^{-1}. At higher loading flow rate used, i.e., 2.0 ml min^{-1}, the sorption efficiencies of the organically bound metals decreased. Hence, considering the particle size of the resin, the sample loading and further recovery by the solvent elution were performed at the flow rate of 1.0 ml min^{-1}.

Different concentrations of the solutions of HCl and HNO_3 were tested as the eluents and it was assessed that the exhaustive retrieval of the complexes of Mn and Zn with TA (100% and 101%, respectively, for Mn and Zn) was obtained using 10 ml of 2.0 mol l^{-1} HNO_3 solution. Other solutions examined resulted in the recoveries not higher than 90%.

3.1.2. Dowex 50Wx4

Characterized by a reasonably high cation exchange capacity, the Dowex strong acidic exchangers have been effectively applied in recent years to the operational fractionation analysis of different metals in various beverages and the discrimination of the broadly meant positively charged metal species class, including the simple metal cations, their stable cationic complexes with different inorganic and organic endogenous ligands, and the fraction of the labile metal species (Karadjova et al., 2002; Pohl & Prusisz, 2004, 2006; Ruszczynska et al., 2004; Yasar & Gucer, 2004).

Investigating the application of HCl and HNO_3 solutions of different concentrations for stripping the metals retained by the cation exchange resin beds, it was found that 4.0 mol l^{-1} solution of HCl (10 ml) was beneficial for the quantitative recovery of both metals in question. Its usage resulted in the retrieval of 99% of Mn and 100% of Zn from the columns. This was in the agreement with a general observation settled in the papers cited above wherein the moderately concentrated solutions of HCl were used for the efficient elution of metals from the strong cation exchangers. The effect of the flow rate of 4.0 mol l^{-1} HCl solution used for the elution was considerable, especially in case of the recovery of Mn. For this metal, the flow rates higher than 1.0 ml min^{-1} resulted in the recoveries lower than 90%.

The retention of Mn^{2+} and Zn^{2+} was found to be quantitative (close to 100%) regardless the solution pH in the range from 4.0 to 5.5 as well as the settled sample flow rate in the range from 1.0 to 4.0 ml min^{-1}. In addition, it was established that the complexes of Mn and Zn with EDTA were not sorbed by the resin under these conditions. Contradictory, both metals were completely retained from the analyte solutions containing TA, presumably due to the displacing of the complexation equilibrium between the metal cations and the tannin toward the dissociation of the complexes and the sorption of Mn^{2+} and Zn^{2+} ions by the sulfonic functional groups of the strong cation exchange resin. Formerly, comparatively high retention of the metal bound to tannic acid (almost 80%) on the Chelex 100 chelating resin was reported by Erdemoglu et al. (2000) in the study devoted to the operational fractionation of Al in the tea infusions.

Accordingly, it was substantiative that the Amberlite XAD7 adsorbent was placed before the Dowex 50Wx4 cation exchanger in the two column assembly proposed in the present work for the partitioning of Mn and Zn into the operationally defined metal fractions. The flow rate of 1.0 ml min^{-1} was chosen for the sample loading. For the elution of Mn and Zn from the resin beds of the adsorbent and the cation exchange resin, the solutions of 2.0 mol l^{-1} HNO_3 and 4.0 mol l^{-1} HCl, respectively, were applied. Ten-ml portions of the respective eluents were passed at the flow rate of 1.0 ml min^{-1}.

3.2. Fractionation of manganese and zinc in tea infusions

The analytical procedures based on ion exchange, size exclusion and adsorption liquid chromatography, attempted so far to speciate the trace and major metals in the tea infusions, usually provide the operational pattern of the existing distribution of the metals between different classes of the species. Furthermore, the studies concerning the metal fractionation analysis refer in a irresistible majority to aluminum (Alberti et al., 2003; Erdemoglu et al., 2000; Flaten & Lund, 1997; French et al., 1989; Owen et al., 1992; Ruszczynska et al., 2004). The investigations devoted to the classification in brewed teas of the metal groupings other than Al are less known and appear to be rarely reported in the literature hitherto (Odegard & Lund, 1997; Ozdemir & Gucer, 1998; Pohl & Prusisz, 2006).

In the present contribution, a simple and easily accessible instrumental methodology based on the one-step two-column (Amberlite XAD7-Dowex 50Wx4) solid phase extraction and the off-line FAAS detection was developed and used to partition Mn and Zn in black and green tea infusions into the hydrophobic, the cationic and the residual metal fractions.

3.2.1. Fractionation pattern of Mn

The amounts of Mn in the black and green teas analyzed in the study are given in Table 1. The total content of this metal was relatively high but very differentiated depending on the tea brand. The concentration of the totally dissolved metal extracted from the leaves to the infusion during typical 6-minute brewing process ranged from 1.0 to 6.0 mg l^{-1}. However, it was noticed that relatively higher amounts of Mn were transferred to the brews of the black teas, i.e., about 43% on average regarding the total metal content determined in leaves, than to the green tea infusions (22% on average). All these findings are very well comparable with the outcomes previously established by Odegard and Lund (1997) or Ozdemir and Gucer (1998). Taking into account the total content analysis of the tea brews under consideration, it was also considered that tea is a rich source of Mn in a daily diet since a single cup of tea served (about 250–300 ml) may contribute from 10% to 43% (medium 26%) of the average 4 mg safe and adequate daily dietary intake of this metal. Very close to that conclusion was formulated before by Powell, Burden, and Thompson (1998).

The distribution of Mn between the distinguished fractions is given in Table 2. Apparently, in reference to the total content of Mn in the infusions, the fraction of the metal bound to polyphenols contributed to 31–46% (39% on average) in case of the black teas and from 13% to 20% (16% on average) in case of the green teas. This difference between the tea species can be presumably attributed to the fact that in the manufacturing processes subjected to the black teas, the flavanoids present in the leaves polymerize into more complex and condensed polyphenols of a much higher molecular weight (Cairns et al., 1996). Such macromolecules can bind the metals more effectively as well as retain the metal ions inside the macromolecular structure. In the green teas, the flavanoids are usually simple polyphenols. Formerly, in Turkish tea infusions it was recognized that only about 5% of the total amount of Mn was in the form of the organically bound compounds (Ozdemir & Gucer, 1998).

The fraction of the broadly meant cationic species of Mn was the most abundant, i.e., contributed to 53–68% of the totally dissolved metal content in the black tea infusions and to 80–82% of the whole metal amount in the

Table 2
The concentrations of Mn (in mg l^{-1}) in the fractions separated using the Amberlite XAD7-Dowex 50Wx4 column system (in brackets the percentage contributions of the fractions to the total metal content are given)

Infusion	A	B	C	D[a] = A + B + C
B1	0.630 ± 0.030 (40.4 ± 1.9)	0.861 ± 0.010 (55.2 ± 0.6)	0.052 ± 0.006 (3.3 ± 0.4)	1.54 ± 0.03 (98.7 ± 1.9)
B2	1.26 ± 0.04 (45.5 ± 1.4)	1.47 ± 0.08 (53.1 ± 2.9)	0.013 ± 0.001 (0.48 ± 0.04)	2.74 ± 0.09 (98.9 ± 3.2)
B3	1.87 ± 0.06 (31.1 ± 1.0)	4.09 ± 0.01 (67.9 ± 0.2)	0.034 ± 0.006 (0.56 ± 0.10)	6.00 ± 0.06 (99.7 ± 1.0)
B3[b]	1.81 ± 0.03 (24.1 ± 0.4)	5.71 ± 0.11 (75.9 ± 1.5)	0.040 ± 0.001 (0.53 ± 0.01)	7.56 ± 0.12 (100 ± 2)
G1	0.228 ± 0.030 (14.9 ± 2.0)	1.22 ± 0.01 (79.7 ± 0.6)	0.023 ± 0.004 (1.5 ± 0.3)	1.47 ± 0.03 (96.1 ± 2.0)
G2	0.414 ± 0.075 (20.5 ± 3.7)	1.60 ± 0.03 (79.2 ± 1.5)	0.041 ± 0.012 (2.0 ± 0.6)	2.06 ± 0.08 (102 ± 4)
G3	0.258 ± 0.045 (12.8 ± 2.0)	1.66 ± 0.01 (82.5 ± 0.5)	0.043 ± 0.010 (2.1 ± 0.5)	1.96 ± 0.05 (97.5 ± 2.5)

A, the polyphenolic fraction; B, the cationic fraction; C, the residual fraction.
The results are means ± standard deviations ($n = 3$).

[a] The propagated standard deviations are given.

[b] The 50-ml infusion was spiked with Zn (0.130 mg l^{-1}) and Mn (1.50 mg l^{-1}).

brews of the green teas. Considering the composition of tea infusions (Flaten, 2002), it can be presumed that this fraction is most probably associated with the presence of simple metal cations of Mn(II) as well as the cationic complexes of Mn^{2+} with the organic acids, i.e., oxalic, malic, citric and succinic, which content in the dry matter of the tea leaves reaches up to 2%. The size-exclusion chromatography experiments attempted by Odegard and Lund (1997) to relate the possible Mn species with the respective molecular mass ranges and established that Mn is associated with small organic molecules (<1 kDa) or not associated at all with any endogenous bio-ligands support this conclusion. The donation of the cationic fraction to the total Mn content found in the present study is, however, not so high as the abundance of that class of the metal species reported earlier (Odegard & Lund, 1997; Ozdemir & Gucer, 1998), i.e., 95–100%. Explaining this discrepancy, it should be indicated that different tea brands and the amounts of brews were taken for the fractionation analysis in the cited works. Furthermore, in both mentioned above papers, the contribution of the cationic fraction was ascertained using a single cation exchange column approach.

The concentration of Mn in the last distinguished fraction (the residual metal fraction), likely attributed to the anionic and/or neutral metal species associated with small ligands, was found to be the lowest, i.e., from 0.5% to 3% of the total metal content.

For comparison of the results referred to the differentiation of the metal species associated with the polyphenolics, in the two-column fractionation protocol developed in the present study, the non-ionic Amberlite XAD7 resin was replaced by the cross-linked polyvinylpyrolidone resin Reillex™ 402. The utilization of that last resin for the fractionation and the separation purposes of the organic matter is lesser-known but it has been recognized to form very strong hydrogen bonds with the phenolic groups of the dissolved organic matter (Peuravuori, Monteiro, Eglite, & Pihlaja, 2005). Here, due to the difficulties in the simultaneous exhaustive elution of Mn and Zn, a non-elution approach was used for the estimation of the metal amounts retained on the resin. The results obtained are given in Table 3. It was established that the contribution of the phenolic metal fraction distinguished using Reillex™ 402 resin was lower in comparison to that achieved using Amberlite XAD7 about 2 and 3 times, respectively for black and green tea infusions. Possibly, the fraction separated by the non-ionic Amberlite XAD7 adsorbent comprised not only the complexes of the metal with the polyphenols which were retained by the Reillex™ 402 resin but also some other species of the metal bound to high molecular weight compounds. These organo-metal species, not retained by the Reillex™ 402, are likely to be positively charged as they enhanced the donation of the cationic fraction separated by the Dowex 50Wx4 resin about 20–30%.

Certainly, the results obtained here evidence that the fractionation pattern of Mn in the tea infusions is much more complicated as it was supposed before and that a more comprehensive investigation is required for better understanding of the metal speciation in that kind of beverage.

3.2.2. Fractionation pattern of Zn

The total content of Zn determined in leaves was not so differentiated as that for Mn. Typically, it varied from 0.022 to 0.028 mg g^{-1} in case of the black teas and from 0.023 to 0.035 mg g^{-1} in case of the green teas. The extraction efficiency found for Zn, i.e., 44% and 48% on average, respectively, for the black and the green teas, resulted as well in comparable concentrations of the totally dissolved metal in the infusions, i.e., from 0.11 to 0.15 mg l^{-1}. All these results agree well with those reported in the contribution by Odegard and Lund (1997) and the papers cited therein.

The results of the fractionation experiments performed using the Amberlite XAD7-Dowex 50Wx4 two column assemblage are given in Table 4. They indicate that Zn is present mostly in the cationic forms which account for 59–71% and 84–86%, correspondingly in the black and the green teas. Similarly as for Mn, it was assumed that this fraction likely contains Zn(II) cations along with the simple cationic complexes of Zn^{2+} with some organic acids. The respective fraction of the metal groupings associated with the polyphenols (the polyphenolic metal fraction) contributed on average to 37% (the black tea infusions) and 14%

Table 3
The concentrations of Mn (in mg l^{-1}) in the fractions separated using the Reillex™ 402-Dowex 50Wx4 column system (in brackets the percentage contributions of the fractions to the total metal content are given)

Infusion	A	B	C	D[a] = A + B + C
B1	0.346 ± 0.047 (22.2 ± 3.0)	1.18 ± 0.03 (75.6 ± 1.9)	0.017 ± 0.001 (1.1 ± 0.1)	1.54 ± 0.08 (98.7 ± 5.1)
B2	0.367 ± 0.052 (13.2 ± 1.9)	2.28 ± 0.09 (82.3 ± 3.2)	0.023 ± 0.001 (0.83 ± 0.04)	2.67 ± 0.10 (96.4 ± 3.6)
B3	0.867 ± 0.136 (14.4 ± 2.2)	5.14 ± 0.06 (85.4 ± 1.0)	0.023 ± 0.003 (0.38 ± 0.05)	6.03 ± 0.15 (100 ± 2)
G1	0.040 ± 0.030 (2.6 ± 2.0)	1.53 ± 0.02 (100 ± 1)	0.011 ± 0.004 (0.72 ± 0.06)	1.58 ± 0.04 (103 ± 3)
G2	0.080 ± 0.040 (4.0 ± 2.0)	1.91 ± 0.05 (94.5 ± 2.5)	0.041 ± 0.005 (2.0 ± 0.2)	2.03 ± 0.06 (100 ± 3)
G3	0.081 ± 0.010 (12.8 ± 2.0)	1.92 ± 0.01 (82.5 ± 0.5)	0.034 ± 0.009 (1.7 ± 0.4)	2.04 ± 0.02 (101 ± 1)

A, the polyphenolic fraction; B, the cationic fraction; C, the residual fraction.
The results are means ± standard deviations ($n = 3$).

[a] The propagated standard deviations are given.

Table 4
The concentrations of Zn (in mg l^{-1}) in the fractions separated using the Amberlite XAD7-Dowex 50Wx4 column system (in brackets the percentage contributions of the fractions to the total metal content are given)

Infusion	A	B	C	D[a] = A + B + C
B1	0.047 ± 0.001 (38.8 ± 0.8)	0.071 ± 0.001 (58.7 ± 0.8)	<0.004 (<3.3)	<0.121 ± 0.002 (<100 ± 2)
B2	0.038 ± 0.002 (35.5 ± 1.9)	0.071 ± 0.002 (66.4 ± 1.9)	<0.004 (<3.7)	<0.114 ± 0.003 (<106 ± 3)
B3	0.028 ± 0.001 (26.2 ± 0.9)	0.076 ± 0.003 (71.0 ± 2.8)	<0.004 (<3.7)	<0.108 ± 0.003 (101 ± 3)
B3[b]	0.039 ± 0.001 (16.4 ± 0.4)	0.187 ± 0.001 (78.9 ± 0.4)	<0.004 (<1.7)	<0.230 ± 0.001 (<97.0 ± 0.4)
G1	0.022 ± 0.011 (18.2 ± 9.1)	0.102 ± 0.008 (84.3 ± 6.6)	<0.004 (<3.3)	<0.128 ± 0.014 (<106 ± 12)
G2	0.023 ± 0.010 (15.0 ± 6.7)	0.131 ± 0.006 (85.6 ± 3.9)	<0.004 (<2.6)	<0.158 ± 0.012 (103 ± 7.8)
G3	0.015 ± 0.002 (10.0 ± 1.3)	0.129 ± 0.006 (86.0 ± 4.0)	<0.004 (<2.7)	<0.148 ± 0.006 (98.7 ± 4.0)

A, the polyphenolic fraction; B, the cationic fraction; C, the residual fraction.
The results are means ± standard deviations ($n = 3$).

[a] The propagated standard deviations are given.

[b] The 50-ml infusion was spiked with Zn (0.130 mg l^{-1}) and Mn (1.50 mg l^{-1}).

(the green tea infusions) of the total dissolved metal content. These findings reasonably well correspond to the observations of Odegard and Lund (1997) who determined that Zn is bound in the tea infusions to the species with the molecular weight in the range of 4.0–6.0 kDa, referring to the polyphenolic compounds. The content of the residual fraction was marginal as the concentrations of Zn measured in the effluents obtained after passing the brews through the columns connected in a series were below the detection limit.

For comparison of the fractionation pattern obtained for Zn using the Amberlite XAD7-Dowex 50Wx4 system, the distribution of the metal among the fractions distinguished by means of the Reillex™ 402 and the Dowex 50Wx4 columns connected in a series was also assessed. A substantial difference between the metal shares in the polyphenolic and the cationic fractions separated by both protocols was observed only for two black teas, i.e., B1 and B2 (Table 5). Apparently, a decrease of the Zn content in the fraction of the metal bound to the polyphenols was noted along with a relevant enhancement of the cationic fraction donation. Probably, in both specified teas, Zn was present not only in the forms bound to the polyphenolic species, presumed to be neutral or negatively charged (French et al., 1989), but also as the cationic complexes with other macromolecules retained on the Amberlite XAD7 adsorbent, and the Dowex 50Wx4 cation exchanger after passing the Reillex™ 402 resin. In case of other teas analyzed, the differences between the fraction amounts achieved using both analytical schemes were insignificant. It illustrates that the respective Zn hydrophobic fraction separated through the proposed procedure in the infusions analyzed is mainly attributed to the forms of the metal associated with polyphenols.

3.3. Spiking experiment

The volume of the infusions used in this study for the operational fractionation analysis of Mn and Zn was about 5–50 times higher than those reported in previous works (Erdemoglu et al., 2000; Odegard & Lund, 1997; Ozdemir & Gucer, 1998; Ruszczynska et al., 2004). Considering the amounts of the resin used in the present study it was assumed that such sample volume would not saturate the columns. To verify this assumption and that the fractions distinguished were not overestimated, the respective recovery test was performed. To the 50-ml infusions of Bastek Earl Grey tea (B3), Mn^{2+} ions (at final concentration of

Table 5
The concentrations of Zn (in mg l^{-1}) in the fractions separated using the Reillex™ 402-Dowex 50Wx4 column system (in brackets the percentage contributions of the fractions to the total metal content are given)

Infusion	A	B	C	D[a] = A + B + C
B1	0.014 ± 0.005 (11.6 ± 4.1)	0.092 ± 0.001 (76.0 ± 0.8)	<0.004 (<3.3)	<0.110 ± 0.005 (<90.9 ± 4.1)
B2	0.015 ± 0.006 (14.0 ± 5.6)	0.084 ± 0.001 (78.5 ± 0.9)	<0.004 (<3.7)	<0.103 ± 0.006 (<96.3 ± 5.6)
B3	0.026 ± 0.009 (24.3 ± 8.4)	0.078 ± 0.001 (72.9 ± 0.9)	<0.004 (<3.7)	<0.108 ± 0.009 (101 ± 8)
G1	0.012 ± 0.009 (9.9 ± 7.4)	0.104 ± 0.007 (86.0 ± 5.8)	<0.004 (<3.3)	<0.120 ± 0.011 (<99.2 ± 9.1)
G2	0.025 ± 0.021 (16.3 ± 13.7)	0.129 ± 0.001 (84.3 ± 0.6)	<0.004 (<2.6)	<0.158 ± 0.021 (103 ± 14)
G3	0.017 ± 0.016 (11.3 ± 10.7)	0.130 ± 0.008 (86.7 ± 5.3)	<0.004 (<2.7)	<0.151 ± 0.018 (101 ± 12)

A, the polyphenolic fraction; B, the cationic fraction; C, the residual fraction.
The results are means ± standard deviations ($n = 3$).

[a] The propagated standard deviations are given.

1.50 mg l^{-1}) and Zn^{2+} ions (at final concentration of 0.130 mg l^{-1}) were added and the samples were let to equilibrate for overnight. Afterwards, the spiked infusion samples were treated with the Amberlite XAD7-Dowex 50Wx4 assemblage and the metal concentrations in the distinct fractions were determined (Tables 2 and 4).

It was found that added Mn^{2+} ions was entirely found in the cationic metal fraction as the metal recovery was equal to 108%. The differences between the contents of the polyphenolic and the residual fractions assessed in the original and the spiked samples of the tea infusion B3 were irrelevant taking into consideration the uncertainty of the results obtained. The total recovery of Mn was estimated to be 101%. In case of Zn, it was established that added Zn^{2+} ions predominantly contributed to the cationic metal fraction since 85% of its amount was recovered from that class of the metal species. Almost 9% of Zn^{2+} ions added to the infusion was found in the fraction of the metal bound to the polyphenols. The total recovery of Zn from the spiked sample of the tea infusion B3 was established to be 97%.

The recoveries assessed prove the dependability of the whole operationally defined fractionation procedure developed in this study for the partitioning of Mn and Zn in the tea infusions. The correctness of the results assessed was confirmed as well by the sums of the metal concentrations determined in the fractions distinguished through the protocol devised. In relation to the respective metal concentrations measured in the infusions, the differences between these sums and the concentrations of the totally dissolved metals varied from −2% to +4% in case of Mn and from −3% to +6% for Zn.

4. Conclusions

The present study was attained to develop a simple and easily accessible analytical methodology of the fractionation of Mn and Zn in the tea infusions. The relevant data about the metal species partitioning was obtained using the extraction to solid phase of the Amberlite XAD7 (the adsorbing resin) and the Dowex 50Wx4 (the strong cation exchanger) linked in a series. The proposed one step assemblage enables the separation of the hydrophobic fraction of the metals bound to the polyphenolic compounds, the cationic metal species fraction and the residual metal fraction, presumably attributed to the neutral or/and the anionic complexes with low molecular mass ligands. The metal classes distinguished are operationally defined and relate to the sorption behavior of Mn and Zn species toward the resins applied.

The fractionation pattern assessed for Mn indicates that the complexation of this metal in the tea infusions is not so simple as it was supposed earlier. It appears that manganese can be present in brews not only in the form of the predominant simple cations or other small cationic species but also may in bound forms with the polyphenolic species or other macromolecular compounds. Zinc is likely to be present in the form of the cationic species (the most abundant fraction) as well as stable complexes of the metal with the polyphenols.

Acknowledgement

P. Pohl acknowledges receiving the fellowship of the Alexander von Humboldt Foundation in 2006.

References

Alberti, G., Biesuz, R., D'Agostino, G., & Pesavento, M. (2004). Speciation of Mn(II), Zn(II) and Cu(II) in tea infusion. In *Book of abstracts of XVIII National Congress of Analytical Chemistry*, 19–23 September, Parma, Italy.

Alberti, G., Biesuz, R., Profumo, A., & Pesavento, M. (2003). Determination of the total concentration and speciation of Al(III) in tea infusions. *Journal of Inorganic Biochemistry, 97*, 79–88.

Bower, V. E., & Bates, R. G. (1995). Standard buffer solutions. *Journal of Research of the National Bureau of Standards, 55*, 197–200.

Cairns, W. R. L., Hill, S. J., & Ebdon, L. (1996). Directly coupled high performance liquid chromatography-inductively coupled plasma-mass spectrometry for the determination of organometallic species in tea. *Microchemical Journal, 54*, 88–110.

Erdemoglu, S. B., Pyrzynska, K., & Gucer, S. (2000). Speciation of aluminum in tea infusion by ion-exchange resins and flame AAS detection. *Analytica Chimica Acta, 411*, 81–89.

Fernandez, P. L., Pablos, F., Martin, M. J., & Gonzalez, A. G. (2002). Multi-element analysis of tea beverages by inductively coupled plasma atomic emission spectrometry. *Food Chemistry, 76*, 483–489.

Flaten, T. P. (2002). Aluminum in tea – concentrations, speciation and bioavailability. *Coordination Chemistry Reviews, 228*, 385–395.

Flaten, A. K., & Lund, W. (1997). Speciation of aluminum in tea infusions studied by size exclusion chromatography with detection by post-column reaction. *Science of the Total Environment, 207*, 21–28.

French, P., Gardner, M. J., & Gunn, A. M. (1989). Dietary aluminium and Alzheimer's disease. *Food Chemistry Toxicology, 27*, 495–496.

Gallaher, R. N., Gallaher, K., Marshall, A. J., & Marshall, A. C. (2006). Mineral analysis of 10 types of commercially available tea. *Journal of Food Composition and Analysis, 19*(Suppl.), S53–S57.

Karadjova, I., Izgi, B., & Gucer, S. (2002). Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry. *Spectrochimica Acta Part B, 57*, 581–590.

Marcos, A., Fisher, A., Rea, G., & Hill, S. J. (1998). Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *Journal of Analytical Atomic Spectrometry, 13*, 521–525.

Odegard, K. E., & Lund, W. (1997). Multi-element speciation of tea infusion using cation-exchange separation and size-exclusion chromatography in combination with inductively coupled mass spectrometry. *Journal of Analytical Atomic Spectrometry, 12*, 403–408.

Owen, L. M., Crews, H. M., & Massey, R. C. (1992). Aluminum in tea – SEC-ICP-MS speciation studies of infutions and simulated gastrointestinal digests. *Chemical Speciation and Bioavailability, 4*, 89–96.

Ozdemir, Y., & Gucer, S. (1998). Speciation of manganese in tea leaves and tea infusions. *Food Chemistry, 61*, 313–317.

Peuravuori, J., Monteiro, A., Eglite, L., & Pihlaja, K. (2005). Comparative study for separation of aquatic humic-type organic constituents by DAX-8, PVP and DEAE sorbing solids and tangential utrafiltration: elemental composition, size exclusion chromatography, UV–vis and FT-IR. *Talanta, 65*, 408–422.

Pohl, P., & Prusisz, B. (2004). Pre-concentration of Cd, Co, Cu, Ni and Zn using different off-line ion exchange procedures followed by the inductively coupled plasma atomic emission spectrometric detection. *Analytica Chimica Acta, 502*, 83–90.

Pohl, P., & Prusisz, B. (2006). Fractionation of calcium and magnesium in honeys, juices and tea infusions by ion exchange and flame atomic absorption spectrometry. *Talanta, 69*, 1227–1233.

Powell, J. J., Burden, T. J., & Thompson, R. P. H. (1998). In vitro mineral availability from digested tea: a rich dietary source of manganese. *Analyst, 123*, 1721–1724.

Ruszczynska, A., Pyrzynska, K., & Bulska, E. (2004). On the use of solid-phase extraction for the fractionation of aluminum species. *Chemical Analysis (Warsaw), 49*, 19–28.

Terada, K. (1992). Preconcentration by sorption. In Z. B. Alfassi & C. M. Wai (Eds.), *Preconcentration techniques for trace elements* (pp. 211–241). Boca Raton, FL: CRC Press.

Ucer, A., Uyanik, A., & Aygun, S. F. (2006). Adsorption of Cu(II), Cd(II), Zn(II), Mn(II) and Fe(III) ions by tannic acid immobilised activated carbon. *Separation and Purification Technology, 47*, 113–118.

Yasar, S. B., & Gucer, S. (2004). Fractionation analysis of magnesium in olive products by atomic absorption spectrometry. *Analytica Chimica Acta, 505*, 43–49.

Zhou, C. Y., Wu, J., Chi, H., Wong, M. K., Koh, L. L., & Wee, Y. C. (1996). The behaviour of leachedaluminium in tea infusions. *Science of the Total Environment, 177*, 9–16.