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An eco-friendly cellular phase microextraction technique based on the use of green microalgal cells for trace thallium species determination in natural water samples

Leticia B. Escudero,*[ab] Carolina B. García,[ac] Stela M. da Silva[c] and Jorge H. Barón[c]

A simple and environmentally friendly technique named cellular phase microextraction (Cell-PME) was developed for thallium (Tl) species determination. Basically, Tl(III) species were mixed with a solution containing *Chlorella vulgaris* microalgal cells (480 mg L^{-1}) at pH 7 and the resulting system was stirred with a vortex for 8 min. After a centrifugation process, the sediment phase was directly injected into the graphite furnace of an electrothermal atomic absorption spectrometer. In contrast to Tl(III), Tl(I) species were not retained by the biomass. Optimization of variables influencing the biosorption process, including sample pH, microalgal mass and sample volume, was performed. A biosorption efficiency of 65% and an enrichment factor of 50 were obtained with only 5.00 mL of sample. The limit of detection (LOD) was 8.3 ng L^{-1} Tl, while the relative standard deviation (RSD) was 5.1% (at 1 μg L^{-1} Tl and $n = 10$), calculated from the peak height of absorbance signals (Gaussian-shape and reproducible peaks). The proposed method was successfully applied to determine Tl species in environmental samples, including drinking and natural water. To the best of our knowledge, a biosorption process is applied for the first time for Tl species separation and determination in the analytical field.

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1. Introduction

Thallium (Tl) has been identified as an environmentally significant element due to its high toxic effects.[1] Its toxicity is highly dependent not only on its concentration but also on its chemical form or species. In fact, Tl(III) species are approximately 50 000-fold more toxic than the monovalent state.[2] In order to obtain complete information about the bioavailability and toxicological effects of Tl, the continuous development of analytical methods for preconcentration and determination of the analyte and its species at trace levels is important.

To date, some methods have been reported for the preconcentration and separation of Tl species, including solid phase extraction (SPE),[3–5] single-drop microextraction (SDME)[6,7] and liquid–liquid microextraction (LLME).[8] Nevertheless, SDME has the inconvenience of the high instability of the drop hanging from the needle tip, limited drop surface and consequently slow kinetics.[9] More recently, the development of a simple and fast methodology involving the use of ionic liquids (ILs) for Tl species separation and determination by inductively coupled plasma mass spectrometry (ICP-MS) has been reported.[10] However, conventional solvents including carbon tetrachloride and ethanol were still used in that methodology, which is not totally friendly to the environment. Moreover, the separation and determination of Tl species by reversed phase HPLC and ion chromatography (IC) prior to ICP-MS has been reported.[11,12] Despite its high sensitivity, selectivity and sample throughput, the high costs of instrumentation and maintenance could be still high for routine analytical laboratories. In contrast, electrothermal atomic absorption spectrometry (ETAAS) has been successfully used for the determination of Tl species in drinking and natural water samples, due to its reliability, sensitivity and relatively low cost of instrumentation.[13] Additionally, as ETAAS allows the injection of a minimal volume of samples, microextraction procedures for elemental preconcentration can be developed successfully.[9,14]

In order to follow the concepts of "green chemistry", biological substrates have been applied in analytical chemistry for metal extraction, preconcentration and speciation. These kinds of substrates offer advantages such as high surface to volume ratios and several potentially active binding sites at their surface, as well as their production is very simple and rentable.[15] Thus, biosorbents utilizing agricultural-based waste materials such as coconut husk, seaweeds and tea waste have been used

[a]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. E-mail: letibelescudero@gmail.com; lescudero@mendoza-conicet.gob.ar; Tel: +54-261-4259738*

[b]*Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, 5500 Mendoza, Argentina*

[c]*Grupo a-Oil, Institutos Multidisciplinarios, Espacio de la Ciencia y la Tecnología, Universidad Nacional de Cuyo, Mendoza, Argentina*

for metal extraction.[16–18] Moreover, microorganisms such as algae,[19] yeast,[20] bacteria,[21] and organelles (erythrocytes)[22] have demonstrated to be successful biosorbents for metal preconcentration and speciation analysis. Among those alternatives, yeasts have been widely applied for heavy metal extraction from aquatic environments. Although several yeast strains have been assayed in analytical developments, *Saccharomyces cerevisiae* has been the most extensively chosen one by researchers, probably due to its ease of obtaining and low costs of production.[23] Likewise, algae are very interesting alternatives to be employed as biosorbents due to their relatively easy growth,[24] wide species diversity and natural abundance,[15] and low cost of production.[25] In fact, *Chlorella vulgaris* has been employed as a selective biosorbent for As(III) over As(V), while *Ecklonia maxima* has been used in SPE procedures for the determination of Cd(II), Cr(III), Cu(II), and Pb(II).[26] On-line systems have also been developed using some algal species immobilized onto typical solid supports, such as silica gel[24] and the resin Cellex-T.[27] Although some technological contributions have reported the use of biomass materials for Tl removal,[28,29] algal biomass has not been attempted so far for the separation and preconcentration of Tl species.

The aim of the proposed work was the development of an environmentally friendly and simple microextraction technique based on the application of green microalgal cells of *C. vulgaris* for the determination of Tl species. Optimization of variables influencing the biosorption process, including sample pH, temperature, microalgal mass, stirring time, and sample volume, was performed. The proposed method was successfully applied to determine Tl species in drinking and natural water samples. To the best of our knowledge, a biosorption process based on the use of *C. vulgaris* is reported for the first time for Tl species separation and determination in the analytical field.

Table 1 Instrumental and experimental conditions for Tl species determination

ETAAS instrumental conditions	
Wavelength	276.8 nm
Spectral band width	0.7 nm
EDL lamp current	250 mA
Matrix modifier	5 μg Pd 2.5 μg Mg

Graphite furnace temperature program				
Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Injection	30	10	25	250
Drying 1	110	15	20	250
Drying 2	130	10	20	250
Pyrolysis	600	10	20	250
Atomization	1700	0	5	—
Cleaning	2400	1	2	250

Extraction conditions	
Sample volume	5 mL
Working pH	7
Biomass	*C. vulgaris*
Biomass amount	2.4 mg
Stirring type	Vortex
Stirring time	8 min
Centrifugation speed	3000 rpm
Centrifugation time	25 min
Injection volume in the graphite furnace	65 μL

2. Experimental

2.1. Instrumentation

Experiments were performed using a Perkin Elmer (Uberlingen, Germany) Model 5100ZL atomic absorption spectrometry equipped with a transversely heated graphite atomizer, a Tl Electrodeless Discharge Lamp (EDL) and a Zeeman-effect background correction system. Instrumental conditions used for Tl determination in the biomass-enriched phase are as shown in Table 1. A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used to accelerate the phase separation process. A vortex model Bio Vortex B1 (Boeco, Hamburg, Germany) was used for mixing the reagents. A Horiba F-51 pH meter (Kyoto, Japan) was utilized for pH determinations. A ZEISS Primo Star microscope (Göttingen, Germany) was used for microalgal identification.

2.2. Reagents

Stock standard solutions (1000 mg L^{-1}) of Tl(I) and Tl(III) were prepared by dissolving an accurate weight of either $TlNO_3$ (99.9%) (Aldrich, Milwaukee, USA) or $Tl(NO_3)_3 \cdot 3H_2O$ (98%) (Aldrich) in 1% (v/v) HNO_3. Working standard solutions were prepared daily by diluting the stock standard solution with ultrapure water. A stock TRIS buffer solution was prepared by dissolving 12.1 g of tris-(hydroxymethylamine)-methane (Merck, Darmstadt, Germany) in 100.0 mL of deionized water, and the pH adjusted to the required value with concentrated hydrochloric acid (37%) (Merck). For chemical modification, a 1000 mg L^{-1} Pd solution was prepared by dissolving 62.7 mg $Pd(NO_3)_2 \cdot 2H_2O$ (Fluka, Buchs, Switzerland) in 25 mL 0.1% (v/v) HNO_3. A 100 mg L^{-1} $Mg(NO_3)_2$ (Merck) stock solution was prepared in ultrapure water. Ultrapure water (18 MΩ cm) was obtained from a Millipore Continental Water System (Bedford, MA, USA). All glassware used in the experiments were cleaned with pure water, then soaked in 0.1 mol L^{-1} HNO_3 solution at least for 24 h and thoroughly rinsed 5 times with ultrapure water before use.

2.3. Biomass preparation

Green microalgae were obtained from natural water bodies of Mendoza. They were isolated and identified in successive cycles of indoor cultivation. The cells were grown under controlled conditions at 20 ± 1 °C. Illumination was supplied during a 8 h-photoperiod (16 h dark period) by a bank of fluorescent lights

during 13 days. The culture medium used was the modified Bold's basal medium (BBM).[30] The pH of the medium was adjusted to 7.0 with TRIS buffer. The green microalgal cells were cultured in a 1000 mL Erlenmeyer flask containing 100 mL of BBM and ultrapure water to make up to the final volume. Centrifugation of the culture at 3000 rpm (1360.8 × *g*) for 25 min was applied for preconcentration of microalgal cells. Before use for analysis, biomass was washed three times with ultrapure water in order to remove the nutrients from their surface.

2.4. Sample collection and conditioning

For collecting the tap water samples, domestic water was allowed to run for 20 min and approximately a volume of 1000 mL was poured into a beaker. Tap water samples were analyzed immediately after sampling. River water samples were taken from the Tunuyán River, and lake water samples were obtained from Llancanelo Lake, both located in Mendoza province, Argentina. River and lake water samples were collected in cleaned nitric acid-washed HDPE bottles rinsed three times with water sample prior to collection. A sample volume of 1000 mL was collected at a depth of 5 cm below the surface. These samples were filtered through 0.45 μm pore size PTFE membrane filters (Millipore Corporation, Bedford, MA, USA) immediately after sampling.

2.5. Preconcentration and determination of Tl(III) species

For the development of the microextraction procedure, a volume of 5 mL of sample or a 1 μg L^{-1} Tl(III) standard solution (for optimization) was placed in a 10 mL graduated plastic centrifuge tube with 50 μL of stock buffer solution (pH 7) and 150 μL of a 16 g L^{-1} concentrated microalgal cell solution. The mixture was shaken for 8.0 min with a vortex stirrer. Finally, a centrifugation step at 3000 rpm for 25 min allowed the formation of two well-defined phases. The upper aqueous phase was manually discarded with a transfer pipette. The fully sedimented phase (65 μL) was directly injected into the graphite furnace of the ETAAS instrument for Tl determination (conditions as shown in Table 1). Calibration was performed against aqueous standards and blank solutions.

2.6. Determination of total Tl and Tl(I) species

Since Tl(III) species were selectively retained on the biomass, its determination was performed following the microextraction procedure described in Section 2.5. For the determination of total Tl, and before sample analysis, 5 mL-aliquots of samples were treated with 50 μL of bromine water (1%) in order to oxidise Tl(I) species. In fact, the use of bromine water has been previously reported by other authors.[31,32] Afterward, the preconcentration procedure described before was applied for total Tl determination. Finally, as Tl(I) was not retained on the biomass, its concentration was determined by the difference between the concentration of total Tl and Tl(III).

3. Results and discussion

3.1. Study of ETAAS operating conditions

A study on the analytical conditions for ETAAS determination was performed in this work. In order to reduce interference and increase the accuracy and precision for ETAAS measurements of Tl in the presence of the biomass, the use of a chemical modifier or a modifier mixture was evaluated. Thus, a preliminary experiment was developed by injecting a 200 μg L^{-1} Tl standard solution in the presence of 2.4 mg biomass. Individual amounts of $Pd(NO_3)_2$ and $Mg(NO_3)_2$, and their mixtures were assayed as chemical modifiers for Tl determination in ETAAS. Thus, 2.5, 5, and 7.5 μg of each chemical modifier were evaluated. Although signal improvements were observed when chemical modifiers were assayed individually, sharp and well-defined signals with a reduced background were obtained when a mixture of both modifiers was used. Therefore, a mixture of the chemical modifiers containing 5 μg of Pd $[Pd(NO_3)_2]$ and 2.5 μg of Mg $[Mg(NO_3)_2]$ was injected for Tl determination by ETAAS (Table 1). The volumes injected into the graphite furnace were 20 μL of a 250 mg L^{-1} $Pd(NO_3)_2$ solution and 25 μL of a 100 mg L^{-1} $Mg(NO_3)_2$ solution.

It was very important to select an appropriate pyrolysis temperature for removing possible organic matter resulting from the microextraction process in order to prevent Tl loss during the pyrolysis step. As mentioned earlier, Tl was determined in the presence of the biomass by direct injection of that phase into the graphite furnace of the ETAAS instrument. For this reason, the influence of pyrolysis temperature on the analytical absorbance was studied within a range of 400 to 800 °C. As can be seen in Fig. 1, optimal pyrolysis temperature was observed at 600 °C, with a holding time of 20 s. Therefore, 600 °C was selected as the working pyrolysis temperature. Subsequently, the effect of atomization temperature on Tl absorbance was evaluated within the interval of 1500–1900 °C. The highest signal was observed at 1700 °C, with a holding time

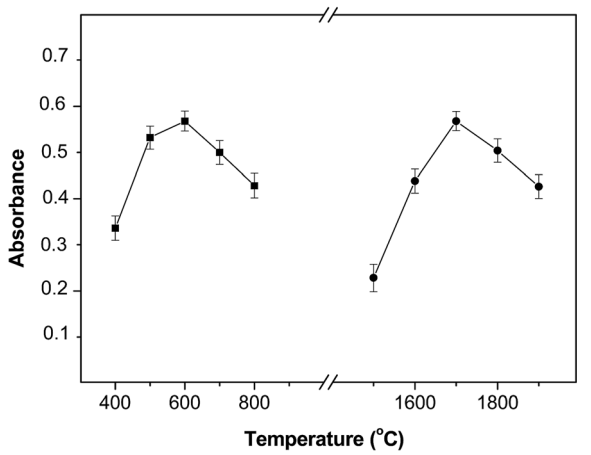

Fig. 1 Pyrolysis (■) and atomization (●) temperature curves for 200 μg L^{-1} Tl solution mixed with 2.4 mg biomass and using a mixture of 5 μg of Pd and 2.5 μg of Mg for chemical modification. Other conditions were as reported in Table 1.

of 5 s (Fig. 1). Moreover, a temperature of 2400 °C and a holding time of 2 s were chosen for the cleaning step. Thus, the sediment phase was successfully analyzed by ETAAS under the conditions shown in Table 1.

3.2. Optimization of biosorption conditions with the Cell-PME method

Metal ion biosorption by algae is dependent on several variables, including pH, contact time, temperature and the presence of competing ions.[33,34] In this work, the influence of pH on biosorption efficiency was studied in the range of 2 to 10. As shown in Fig. 2, the highest biosorption efficiency of Tl(III) species was achieved between pH 6 and 8. Moreover, Fig. 2 shows that Tl(I) species were not retained on the biomass under the experimental conditions. Thus, complete separation of Tl species was feasible thanks to the selective retention of Tl(III) species by algal biomass.

It has been previously reported that the biosorption mechanisms between green algae and metals are extremely related to the chemical composition of the cell wall of the biomass.[35] Green algae mainly contain cellulose, polysaccharides like mannans, alginic acids, and xylans, and glycoproteins in their cell walls.[36] These chemical compounds present different functional groups, such as amine, carboxyl, sulphate, sulfhydryl, and thioether, which play an important role in the biosorption of metals.[35,37] Previous studies have proposed a complexation mechanism between functional groups present in the cell wall of algae and metals like Ni, Pb, Cu, Cd, and Zn.[38,39] In this work, considering that Tl(I) species do not form stable complexes and were not retained by the biomass,[40] it is proposed a hypothesis in which the selectivity for retention of Tl(III) species might be attributed to a selective complexation between functional groups of algal cell walls and the trivalent Tl species. As can be seen in Fig. 2, the low relative response observed under acidic conditions for Tl(III) species could be attributed to the high concentration of hydrogen ions which might change the protonation degree of ligands normally present in algal cell walls that could bind to Tl(III) species, thus leading to inhibition of their retention in the microalgal cells.[41] On the other hand, the marked decrease in the relative response at pH 9–10 could be due to the formation of a stable complex between the Tl(III) species and the hydroxyl group provided by the NaOH, which could avoid the subsequent complex formation between Tl(III) and ligands of the cell wall. However, further studies using Fourier Transform Infrared (FTIR) spectroscopy and X-ray Photoelectron Spectroscopy (XPS) techniques might be needed in order to confirm the involved mechanisms.

Previous studies support that a fixed amount of biomass could only offer a finite number of surface binding sites for the analyte, considering that some of which would be saturated by competing ions.[42] In this work, the influence of the mass of microalgal cells on biosorption efficiency was evaluated in the range of 0.3 to 4.0 mg. Fig. 3 shows how the recovery of Tl(III) increased when the amount of microalgae was increased up to 2.4 mg. Therefore, the higher amounts of algae lead to higher presence of polysaccharides, proteins or lipid on the cell wall structure, meaning that more functional groups are available to act as binding sites for Tl(III) species. Moreover, when the amount of microalgae was higher than 2.4 mg, the recovery of the analyte remained practically constant. Therefore, 2.4 mg of biomass was used for the following experiments.

Furthermore, the effect of the stirring time was studied in detail as it could be one of the most important variables affecting efficiency of the biosorption process. It could be observed that Tl(III) recovery was optimum from 8 min stirring time, while longer stirring time did not add further improvement to the analytical signal. On the other hand, shorter stirring time showed a decrease in biosorption efficiency, probably caused by an inefficient dispersion of the biomass particles in the sample. Thus, 8 min of stirring time was selected for the subsequent experiments.

The effect of temperature on analyte recovery was evaluated in the range of 20 to 60 °C. An optimum biosorption efficiency of the analyte within all the temperature ranges under study could be observed. Thus, *C. vulgaris* is able to retain Tl(III) without the need for having a control of the working

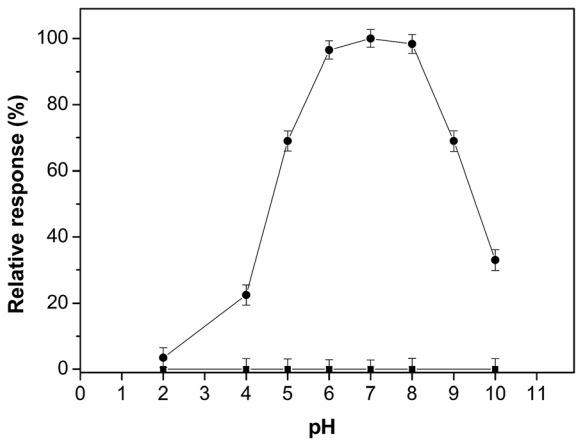

Fig. 2 Effect of pH on the retention of Tl(I) (■) and Tl(III) (●) species. Other conditions were as mentioned in Table 1.

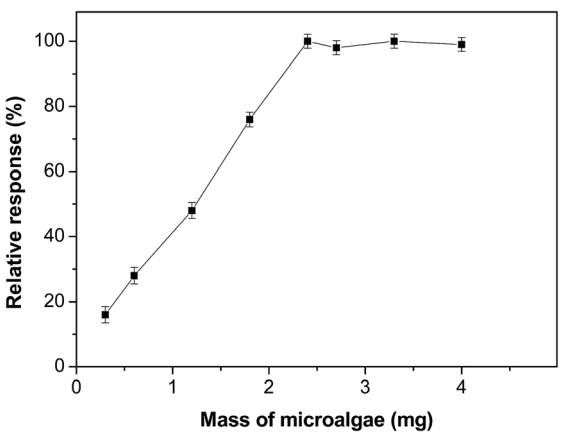

Fig. 3 Effect of the microalgal amount on Tl(III) retention with the proposed Cell-PME method. Other conditions were as indicated in Table 1.

temperature. For this reason, the following experiments were developed at room temperature.

3.3. Evaluation of centrifugation speed and time

The centrifugation speed and time of the sample solution were examined in order to achieve complete phase separation and the best frequency of analysis. Initially, the effect of centrifugation speed was evaluated in the range of 1500 to 3500 rpm for 30 min. The best phase separation was observed from centrifugation speeds of 3000 rpm (1509.3 × *g*). Then, the effect of centrifugation time was evaluated in the range of 5 to 40 min at 3000 rpm. It was observed that the separation of phases improved with the increase of centrifugation time and the highest analyte recovery was achieved from 25 min. Moreover, it was observed that very long centrifugation times did not improve the biosorption efficiency of the analyte. Therefore, a centrifugation time of 25 min was chosen to ensure the complete separation of phases.

3.4. Influence of the sample volume on Tl biosorption

Sample volume is one of the most important parameter to be studied when real samples are analyzed by a preconcentration technique, since it conditions the enrichment factor of the method. In this work, the effect of the sample volume on Tl recovery was studied. Thus, the Tl mass was kept constant while the sample volume was varied in the range of 2 to 8 mL. As can be observed in Fig. 4, Tl recovery remained constant up to 5 mL of sample, while higher sample volumes showed a decrease of analyte recovery, probably due to a major dilution effect. Therefore, a sample volume of 5 mL was selected for further experiments.

3.5. Influence of potential interfering species

Several ions including, Ca^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cl^{-}, NO_3^{-}, CO_3^{2-}, SO_4^{2-} and PO_4^{3-} are common concomitants regularly found in drinking and natural water samples. For this reason, the potential effect of concomitants on Tl biosorption and determination was evaluated. The study was performed by analyzing a 1 μg L^{-1} Tl standard solution containing concomitant ions in concentration levels at which these ions may occur in water samples, and applying the microextraction procedure proposed in this work. A concomitant ion was considered to interfere if it results in a ±5% variation of the analytical signal. Among the aforementioned ions, it can be observed that Ca^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Mg^{2+}, and Fe^{3+} could be tolerated up to at least 2500 μg L^{-1} and no interference was observed within the range under study (95% confidence level). Moreover, Mn^{2+} and Cu^{2+} did not cause interference up to 1800 μg L^{-1}. Finally, anions usually present in water samples did not produce any variation on quantitative Tl determination.

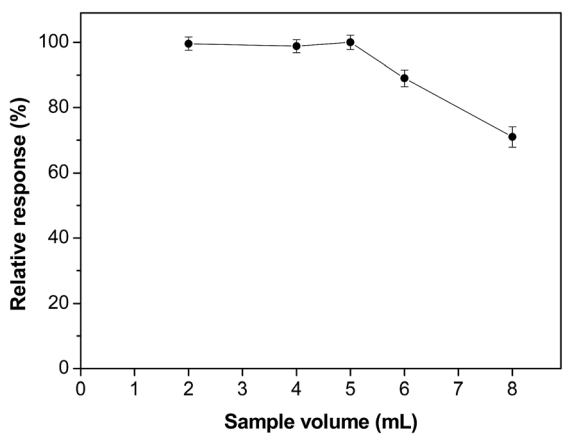

Fig. 4 Effect of the sample volume on Tl(III) retention with the proposed Cell-PME method. Other conditions were as reported in Table 1.

3.6. Adsorption capacity of the biosorbent

The adsorption capacity of *C. vulgaris* for Tl species was also studied. An amount of 100 mg of biomass was mixed with a solution containing 1 mg of Tl(III) species at pH 7. The mixture was shaken for 30 min and centrifuged. The supernatant was determined by ETAAS. The biosorption capacity was found as 4.61 mg g^{-1} for Tl(III) ions, which is comparable or even higher than the results obtained in similar studies.[24,43]

3.7. Analytical performance

In order to evaluate the performance of the proposed method, two main parameters were employed, namely: the biosorption efficiency and enrichment factor. The efficiency of biosorption was calculated using following equation:[44]

$$E = \frac{C_i - C_f}{C_i} \times 100$$

where C_i and C_f are the concentrations of the analyte in the sample phase and in the organic biomass phase, respectively. In this work, a biosorption efficiency of 65% was achieved when the procedure was developed under optimal experimental conditions (Table 1). Likewise, the enrichment factor (EF), calculated as the ratio of the slope of the calibration line derived from the preconcentration procedure using the algal cells to the slope obtained from direct measurements of aqueous standards solutions,[45] was 50.

The calibration graph for ETAAS determination of Tl was linear from levels near the limit of detection (LOD) and up to at least 7000 ng L^{-1}, with a correlation coefficient of 0.9986. The LOD, calculated based on the signal at the intercept and three times the standard deviation about regression of the calibration curve,[46] was 8.3 ng L^{-1} Tl. The relative standard deviation (RSD) for ten replicate measurements of 1 μg L^{-1} Tl was 5.1%. Furthermore, the consumptive index (CI) can be defined for practical purposes as the volume of the sample (in mL) consumed to achieve the EF value.[47] The CI obtained for the proposed method was 0.12 mL. Regarding the frequency of analysis, although the whole preconcentration procedure could take about 35 min, it is possible to simultaneously treat as many samples as can be placed in the centrifugation equipment. For

our work, the frequency of analysis was at least 8 samples per hour based on the time required for performing analyte determination in ETAAS.

Finally, a comparative study on analytical performance allows us to show the strengths of the proposed method with respect to others already reported in the literature. As can be seen in Table 2, our method shows comparable analytical performance with respect to previously reported methods. Thus, the proposed method presents a LOD that is comparable to, or better than other methodologies developed for Tl determination in water samples. Furthermore, our work avoids the use of volatile organic solvents that are not friendly to the environment. Moreover, the use of an inexpensive biomass, the minimal reagent consumption and waste generation plus an acceptable EF with a reduced sample volume, turns the proposed method into a valuable alternative for its application in routine analytical laboratories.

3.8. Validation study and determination of Tl in water samples

The accuracy of the proposed method was evaluated by analyzing a certified reference material (CRM) of natural water NIST SRM 1643e "Trace Elements in Water", with a Tl concentration of 7.445 ± 0.096 μg L^{-1}. Since the certified concentration value in the CRM was higher than the upper limit of the linear range achieved by this method, a dilution by a factor of 2 had to be implemented prior to the analysis. Using the method developed in this work, Tl concentration found in the CRM was 7.465 ± 0.150 μg L^{-1} (95% confidence interval; $n = 6$).

Moreover, a recovery study can be considered as a validation alternative in elemental speciation studies. The recovery of an amount of Tl(I) and Tl(III) ions added to water samples was examined by this procedure. The study shows recovery values for Tl(I) between 96.5 and 102, meanwhile the recoveries for Tl(III) were between 98.0 and 103% (Table 3). These results demonstrate the utility of green microalgal strains for the

Table 2 Analytical methods reported in the literature for preconcentration and determination of Tl in water samples (95% confidence interval; $n = 6$)[a]

Method	Speciation analysis	LOD (ng L^{-1})	RSD (%)	EF	Sample volume (mL)	Analysis frequency (h^{-1})	Ref.
SPE-ETAAS	Yes	9.0	3.9	20	2	18	7
IP-USAE-SFODME-ETAAS	No	4.7	4.8	443	20	[a]	50
AllylCA-GCE-SV	No	1000	~8.0	[a]	[a]	[a]	51
SDME-ETAAS	No	700	5.1	50	10	[a]	6
SPE-ETAAS	No	50	5.7	[a]	50	[a]	52
ETAAS	Yes	8.3	5.1	50	5	8	This work

[a] Not reported. IP-USAE-SFODME: ion pair formation-ultrasound assisted emulsification-solidified floating organic drop microextraction. AllylCA-GCE-SV: pallylcalix[4]arene LB film coated glassy carbon electrode-stripping voltammetry.

Table 3 Concentrations of Tl(I) and Tl(III) in river, lake and tap water samples (95% confidence interval; $n = 6$)

	Tl(III) species			Tl(I) species		
Sample	Added (μg L^{-1})	Found (μg L^{-1})	Recovery[a] (%)	Added (μg L^{-1})	Found (μg L^{-1})	Recovery[a] (%)
River water 1	0	[b]	—	0	0.12 ± 0.02	—
	2.0	1.97 ± 0.06	98.5	2.0	2.12 ± 0.08	100
River water 2	0	0.20 ± 0.03	—	0	0.25 ± 0.04	—
	2.0	2.22 ± 0.07	101	2.0	2.20 ± 0.10	97.5
River water 3	0	0.27 ± 0.04	—	0	0.31 ± 0.05	—
	2.0	2.33 ± 0.09	103	2.0	2.35 ± 0.11	102
Lake water 1	0	[b]	—	0	0.08 ± 0.02	—
	2.0	1.99 ± 0.08	99.5	2.0	2.06 ± 0.10	99.0
Lake water 2	0	0.11 ± 0.04	—	0	0.16 ± 0.06	—
	2.0	2.17 ± 0.08	103	2.0	2.16 ± 0.11	100
Lake water 3	0	0.14 ± 0.03	—	0	0.22 ± 0.05	—
	2.0	2.14 ± 0.10	100	2.0	2.15 ± 0.13	96.5
Tap water 1	0	0.08 ± 0.03	—	0	0.19 ± 0.07	—
	2.0	2.06 ± 0.08	99.0	2.0	2.16 ± 0.12	98.5
Tap water 2	0	0.09 ± 0.04	—	0	0.23 ± 0.05	—
	2.0	2.06 ± 0.09	98.5	2.0	2.27 ± 0.07	102
Tap water 3	0	0.12 ± 0.03	—	0	0.38 ± 0.06	—
	2.0	2.14 ± 0.11	101	2.0	2.36 ± 0.15	99.0

[a] [(Found-base)/added] × 100. [b] Not detected.

selective retention of Tl(III) in the samples under study, and its accurate determination with no matrix effect.

Finally, the concentrations found in river water samples were in the range of 0.12–0.31 μg L^{-1} for Tl(I), and not detected-0.27 μg L^{-1} for Tl(III). The concentrations found in lake water samples were in the range of 0.08–0.22 μg L^{-1} for Tl(I), and not detected-0.14 μg L^{-1} for Tl(III). On the other hand, concentrations of Tl species in tap water were in the range of 0.19–0.38 μg L^{-1} for Tl(I) and 0.08–0.12 μg L^{-1} for Tl(III). These results are in good agreement with previous studies, where similar concentrations of Tl species in water samples were reported.[7,48,49]

4. Conclusions

The first ever reported application of microalgae for highly sensitive determination and speciation of Tl is presented in this work. A novel eco-friendly biosorption method, named Cell-PME, was developed based on the application of algal biomass for the efficient separation and preconcentration of Tl. The selection of algal cells as a biosorbent was really convenient because they are cheap, biodegradable and very friendly to the environment. The capability of *C. vulgaris* for selective retention of Tl(III) species under the experimental conditions defined in this work has been demonstrated. Sensitive and accurate determination of Tl by ETAAS has been feasible even in the presence of a complex organic matrix, such as microalgal biomass. The proposed method shows a detection limit that is comparable to, or better than, other methodologies developed for Tl species determination, and has a good calibration range with a reduced amount of sample. Moreover, our method fulfils the requirements of analytical selectivity and sensitivity and can be easily implemented in routine laboratories for reliable Tl species determination at trace levels in various water samples with good accuracy and reproducibility.

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References

1 A. K. Das, M. Dutta, M. L. Cervera and M. de la Guardia, *Microchem. J.*, 2007, **86**, 2–8.
2 C. H. Lan and T. S. Lin, *Ecotoxicol. Environ. Saf.*, 2005, **61**, 432–435.
3 Y. Li, Y. Peng, Y. Ma, Q. Hu and G. Yang, *Asian J. Chem.*, 2009, **21**, 4593–4598.
4 H. Altundag and M. S. Dundar, *Fresenius Environ. Bull.*, 2009, **18**, 2102–2107.
5 P. H. Pacheco, R. A. Gil, P. Smichowski, G. Polla and L. D. Martinez, *Anal. Chim. Acta*, 2009, **656**, 36–41.
6 M. Chamsaz, M. H. Arbab-Zavar, A. Darroudi and T. Salehi, *J. Hazard. Mater.*, 2009, **167**, 597–601.
7 R. A. Gil, P. H. Pacheco, P. Smichowski, R. A. Olsina and L. D. Martinez, *Microchim. Acta*, 2009, **167**, 187–193.
8 L. B. Escudero, P. Berton, E. M. Martinis, R. A. Olsina and R. G. Wuilloud, *Talanta*, 2012, **88**, 277–283.
9 S. Dadfarnia and A. M. H. Shabani, *Anal. Chim. Acta*, 2010, **658**, 107–119.
10 L. B. Escudero, R. G. Wuilloud and R. A. Olsina, *J. Hazard. Mater.*, 2013, **244–245**, 380–386.
11 P. P. Coetzee, J. L. Fischer and M. Hu, *Water SA*, 2003, **29**, 17–22.
12 Y. Chu, R. Wang and S. Jiang, *J. Chin. Chem. Soc.*, 2011, **58**, 219–225.
13 P. Berton and R. G. Wuilloud, *Anal. Chim. Acta*, 2010, **662**, 155–162.
14 E. M. Martinis, P. Berton, R. P. Monasterio and R. G. Wuilloud, *TrAC, Trends Anal. Chem.*, 2010, **29**, 1184–1201.
15 S. Marcellino, H. Attar, D. Lièvremont, M. Lett, F. Barbier and F. Lagarde, *Anal. Chim. Acta*, 2008, **629**, 73–83.
16 O. Amuda, A. Giwa and I. Bello, *Biochem. Eng. J.*, 2007, **36**, 174–181.
17 B. Amarasinghe and R. Williams, *Biochem. Eng. J.*, 2007, **132**, 299–309.
18 R. Senthilkumar, K. Vijayaraghavan, M. Thilakavathi, P. Iyer and M. Velan, *Biochem. Eng. J.*, 2007, **33**, 211–216.
19 A. Zou, X. Tang, M. Chen and J. Wang, *Spectrochim. Acta, Part B*, 2008, **63**, 607–611.
20 J. Koh, Y. Kwon and Y. Pak, *Microchem. J.*, 2005, **80**, 195–199.
21 M. Tuzen, O. Uluozlu, I. Karaman and M. Soylak, *J. Hazard. Mater.*, 2009, **169**, 345–350.
22 O. Rohling and B. Neidhart, *Anal. Chem.*, 1999, **71**, 1077–1082.
23 P. Pacheco, R. Gil, S. Cerutti, P. Smichowski and L. Martinez, *Talanta*, 2011, **85**, 2290–2300.
24 P. Tajes-Martínez, E. Beceiro-González, S. Muniategui-Lorenzo and D. Prada-Rodríguez, *Talanta*, 2006, **68**, 1489–1496.
25 A. Taboada-de la Calzada, M. Villa-Lojo, E. Beceiro-González, E. Alonso-Rodriguez and D. Prada-Rodríguez, *TrAC, Trends Anal. Chem.*, 1998, **17**, 167–175.
26 M. Romero-González, C. Williams and P. Gardiner, *J. Anal. At. Spectrom.*, 2000, **15**, 1009–1013.
27 B. Godlewska-Zyłkiewicz, J. Malejko, P. Hałaburda, B. Lesniewska and A. Kojło, *Microchem. J.*, 2007, **85**, 314–320.
28 Z. Y. Yin, D. Y. Zhang and X. L. Pan, *Int. J. Environ. Pollut.*, 2009, **37**, 349–356.
29 A. L. John Peter and T. Viraraghavan, *Bioresour. Technol.*, 2008, **99**, 618–625.
30 C. Sorokin, *Handbook of Phycological Methods: Culture Methods and Growth Measurements*, London, 1973.
31 P. Nagaraja, N. G. S. Ai-Tayar, A. Shivakumar, A. Shresta and A. Gowda, *Electron. J. Chem.*, 2009, **6**, 1153–1163.
32 A. Amin, A. El-Sharjawy and M. Kassem, *Spectrochim. Acta, Part A*, 2013, **110**, 262–268.
33 L. Shunxin, Q. Shahua, H. Ganquan and H. Fei, *Fresenius. J. Anal. Chem.*, 1999, **365**, 469–471.
34 G. Donmez and Z. Aksu, *Process Biochem.*, 2002, **38**, 751–762.

35 J. He and J. P. Chen, *Bioresour. Technol.*, 2014, **160**, 67–78.
36 M. E. Wurdack, *Ohio J. Sci.*, 1923, **23**, 181–191.
37 P. Kotrba, M. Mackova and T. Macek, *Microbial Biosorption of Metals*, New York, 2011.
38 P. X. Sheng, Y.-P. Ting, J. P. Chen and L. Hong, *J. Colloid Interface Sci.*, 2004, **275**, 131–141.
39 F. Yang, H. Liu, J. Qu and J. P. Chen, *Bioresour. Technol.*, 2014, **102**, 2821–2828.
40 F. Burriel Martí, F. Lucena Conde, S. Arribas Jimeno and J. Hernández Méndez, *Química Analítica Cualitativa*, Madrid, 1989.
41 M. Mahmoud, I. Kenawy, M. Hafez and R. Lashein, *Desalination*, 2010, **250**, 62–70.
42 Q. Li, S. Wu, G. Liu, X. Liao, X. Deng and D. Sun, *Sep. Purif. Technol.*, 2004, **34**, 135–142.
43 B. Godlewska-Zylkiewicz, *Spectrochim. Acta, Part B*, 2003, **58**, 1531–1540.
44 A. Shoaib, A. Naureen, F. Tanveerand and N. Aslam, *Int. J. Agric. Biol.*, 2012, **14**, 831–833.
45 E. M. Martinis, P. Berton, R. A. Olsina, J. C. Altamirano and R. G. Wuilloud, *J. Hazard. Mater.*, 2009, **167**, 475–481.
46 P. G. Rickert, D. C. Stepinski, D. J. Rausch, R. M. Bergeron, S. Jakab and M. L. Dietz, *Talanta*, 2007, **72**, 315–320.
47 Z. Fang, *Flow Injection Separation and Preconcentration*, VCH, New York, 1993.
48 R. Horiguchi, I. Nukatsuka, Y. Shimizu, S. Sekikawa and K. Ohzeki, *Bunseki Kagaku*, 2002, **51**, 675–679.
49 S. Asadpour, M. Chamsaz, M. Entezari, M. Haron and N. Ghows, *Arabian J. Chem.*, 2012, **1**, 1–6.
50 H. Fazelirad and M. A. Taher, *Talanta*, 2013, **103**, 375–383.
51 H. Dong, H. Zheng, L. Lin and B. Ye, *Sens. Actuators, B*, 2006, **115**, 303–308.
52 T. Asadoulahi, S. Dadfarnia and A. M. H. Shabani, *J. Braz. Chem. Soc.*, 2007, **18**, 1353–1359.