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Microwave-assisted acid Digestion Procedure for Trace Elements Determination in Polychaete *Chaetopterus variopedatus*

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Procedimento de Digestão Assistido por Micro-ondas para Determinação de Elementos-traços no Poliqueta *Chaetopterus variopedatus*

Resumo: Este estudo avaliou e aplicou uma digestão, com ácido diluído, assistida por micro-ondas para determinar elementos-traço (As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni e Sb) em amostras de poliquetas (*Chaetopterus variopedatus*). Após a otimização do método, empregando-se material certificado de referência (1566b CRM, tecido Oyster), os analitos foram determinados por espectrometria de massas com plasma indutivamente acoplado (ICP-MS) e espectrometria de emissão atômica com plasma indutivamente acoplado (ICP OES). A repetibilidade foi adequada (<10%), os resultados obtidos com o CRM concordaram com os valores certificados e o teor de carbono residual valores (RCC) foi menor do que 15%. Além disso, até dez amostras podem ser simultaneamente decompostas em 34 min., resultando num elevado rendimento. O método desenvolvido foi aplicado em amostras de poliquetas coletados na Baía de Todos os Santos, Bahia, Brasil.

Palavras-chave: Elementos-traço; digestão assistida por micro-ondas; ácidos diluídos; tecido de poliquetas.

Abstract

This study evaluated and applied a microwave-assisted diluted acid digestion to determine trace elements (As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni and Sb) in polychaete (*Chaetopterus variopedatus*) samples. After the optimization of the method with certified reference material (CRM 1566b, Oyster tissue), the analytes were determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP OES). Repeatability was adequate (< 10 %), results for CRM agreed with the certified values and the residual carbon content (RCC) values were lower than 15 %. Moreover, up to ten samples can be simultaneously decomposed in 34 min., resulting in a high sample throughput. The developed method was applied to polychaete samples collected from Todos os Santos Bay, Bahia, Brazil.

Keywords: Trace element; microwave-assisted digestion; dilute acid mixture; polychaete tissue.

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Microwave-Assisted Acid Digestion Procedure for Trace Elements Determination in Polychaete *Chaetopterus variopedatus*

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1. Introduction

2. Experimental

2.1. Study area and sample collection

2.2. Instrumentation

2.3. Reagents and solutions

2.4. Optimization of sample digestion

2.5. Determination of the acidity and residual carbon content (RCC) of the digests

2.6. Quality assurance and quality control

3. Results and discussion

3.1. Techniques performance

3.2. Quality assurance and quality control

3.3. Optimization of the sample digestion procedure

3.4. Effect of the sample size on the efficiency of the microwave-assisted digestion process

3.5. Applications

4. Conclusions

1. Introduction

Many species of marine invertebrates, such as clams, mussels and polychaete worms are known to accumulate trace metals in their tissues and are therefore widely used

in environmental monitoring programs. Accumulated metal concentrations provide a relative measure of the total metal intake of an organism, including all routes, integrated over its life time.¹⁻³ Marine invertebrates, such as polychaete, have an important role in the transfer of metal from prey to predators

(e.g. crustaceans and fish).⁴ At the same time, bivalves and fish represent a major part of the human diet, especially for coastal populations, which are more susceptible to the toxic effects that elevated metal concentrations may pose to marine shellfish and fish.⁵

Several analytical techniques are available for the determination of trace elements in polychaetes, such as inductively coupled plasma optical emission spectrometry (ICP OES),^{6,7} inductively coupled plasma-mass spectrometry (ICP-MS),⁸ flame atomic absorption spectrometry (FAAS) and electrothermal-atomic absorption spectrometry (ETAAS).^{9,10} However, it is usually necessary to convert the solid sample to a solution which represents the original material. Several procedures have been developed in order to shorten analysis time and also to minimize the problems associated with solid sample pre-treatment, such as sample contamination and analyte loss. Microwave-assisted digestion procedures using closed vessels and various acid mixtures have been employed to destroy the organic matrix of the biological samples, rapidly at elevated temperatures and/or pressure, to accelerate sample digestion and to minimize contamination and losses of volatile elements.¹¹⁻¹³ Multiple combinations of acid mixtures (HNO₃, HNO₃-HCl, HNO₃-H₂O₂, HNO₃-HF, HNO₃-HCl-H₂O₂, HNO₃-H₂SO₄) have been reported in the literature for the digestion of environmental samples using microwave energy.¹³⁻¹⁸ For polychaetes tissues is required to do a total digestion to obtain reliable results.^{19,20} The use of diluted solutions is recommended, once it strongly minimize the generation of acid residues, which is in accordance to green chemistry recommendations.²⁰⁻²⁸

The effect of the concentrations of nitric acid and hydrogen peroxide, largely used in biological tissues digestions, on closed vessel microwave-assisted systems, was previously described.²⁰ A closed vessel microwave-assisted procedure combined with diluted nitric acid solution (7 mol L⁻¹ of HNO₃) for digestion of soybeans grains, bovine blood,

bovine muscle and bovine viscera obtained residual carbon content (RCC) of 16% and good recoveries. The RCC and standard deviation were smaller than those obtained with digestions using concentrated HNO₃.²⁹ The efficiency of diluted nitric acid for the oxidation of organic matter can be explained by the regeneration of nitric acid promoted by the combination of the nitrogen oxide species with the oxygen present inside the reaction vessel.^{25,29} Bizzi and colleagues²⁶ reported the efficiency of diluted nitric acid solutions combined with oxygen gas for microwave-assisted digestion of bovine liver and non-fat milk powders samples.²⁷ The experimental data suggested that the regeneration of HNO₃ could be explained by the oxygen atmosphere that improved the effectiveness of digestion using diluted nitric acid solutions.^{28,30}

The main purpose of this study was to develop a microwave-assisted digestion procedure, using diluted acid for the determination of trace elements in polychaete (*Chaetopterus variopedatus*). There is little information on the content of trace elements in the *C. variopedatus* polychaete.³¹ This is a cosmopolitan polychaete specie occurring in several shallow coastal habitats, from temperate to tropical locations worldwide.^{32,33} In the Brazil, this specie occurs from the Northeast (Alagoas State) to the South (Rio Grande do Sul).³⁴ The procedure was chosen by selecting the best analytical conditions from several experiments to provide suitable recoveries of elements from certified reference material. The developed method was then applied to the trace elements measurement in *C. variopedatus*, collected at the Todos os Santos Bay, Bahia, Brazil.

2. Experimental

2.1. Study area and collection

The BTS is the second largest bay in Brazil, with an area of 1,223 km² and an average

depth of 9.8 m. The climate of this region is tropical humid, with average temperature of 25.3 °C and average annual rainfall of 1,002 mm.³⁵ Polychaete *Chaetopterus variopedatus* were collected at Maré (Praia Grande and Botelho) and Bimbarras islands and Itapagipe Bay, Todos os Santos Bay, Bahia, Brazil (Figure 1). The sampling points were selected based on the occurrence of this species in the

BTS, in areas subject to anthropogenic activities. The *C. variopedatus* are small organisms that live in an upright u-shaped tube, lined with mucous, that they construct in unconsolidated sediments. Although the tubes can be relatively large (40 cm), the organisms collected measured only about 15 cm (Figure 2).

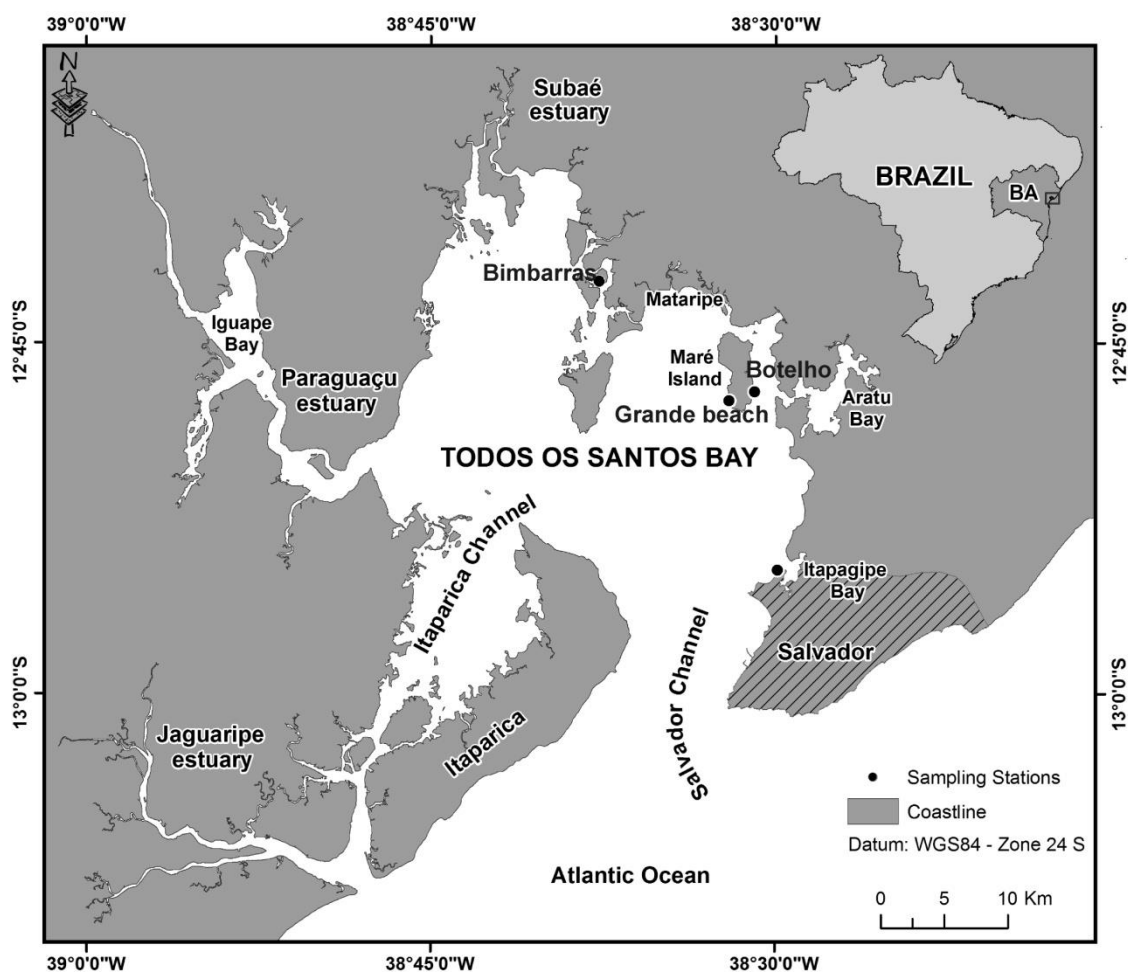


Figure 1. Study area showing the location (●) of polychaete sampling sites at the Todos os Santos Bay, Bahia

In each site, a minimum of 15 polychaetes were collected and combined in one composite sample. In the field, organisms were washed with seawater to remove encrustations and excess of sediment and placed in decontaminated plastic vessels with seawater to purge their intestines. The intestines were then removed using a plastic

spoon and the polychaetes were rinsed with ultrapure water. The biological tissues were freeze dried (Alfa 1-4 LD Plus, Christ, Germany). Dried samples were ground in a ball mill with a tungsten carbide vial set (Model 8000M, Spex Sample Prep, USA) and stored in clean polyethylene vials inside a desiccator at room temperature.



Figure 2. Sampling of (a) and (b) polychaete tube and (c) polychaete *Chaetopterus variopedatus* at the Todos os Santos Bay, Bahia

2.2. Instrumentation

Acid digestion of the samples was performed using a commercial high-pressure laboratory microwave oven (Milestone Ethos 1600 Microwave Labstation, Sorisole, Italy). This equipment was equipped with ten 100 mL tetrafluoromethoxy (TFM) tubes and operated at a frequency of 2,450 Hz, with an energy output of 1,000 W.

A quadrupole ICP-MS X series II (Thermo, Germany) equipped with a hexapole collision cell (CC) was used for As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni and Sb determinations. The elements Ge, Rh, Tl, In and Bi were used as internal standards in sample solutions to compensate for any matrix effects and instrument drift. The instrument software

allows for a rapid switch between standard mode (no gas, cell vented to mass analyzer chamber) to CC mode, whilst continuously aspirating the sample. The pre-mixed gases H₂ (7%) in He (H₂O and other impurities < 5 ppm) were admitted into the CC under flow control through stainless steel lines. The measurements were carried out with nickel sampler and skimmer cones (1.0 mm and 0.7 mm diameter orifices) and a standard concentric nebulizer. A glass impact bead spray chamber cooled to 4 °C using a Peltier cooler and a shielded Fassel torch was used to minimize plasma potential and thereby obtain low and narrow initial ion energy distribution. The detector was operated in dual mode and cross-calibration was performed. Table 1 presents the ICP-MS operational conditions.

Table 1. Operating conditions for ICP-MS

Parameter	Conditions
RF incident power	1300W
Plasma argon flow rate	13 L min ⁻¹
Auxiliary argon flow rate	0.7 L min ⁻¹
Nebulizer argon flow rate	0.87 L min ⁻¹
Scanning mode	Peak jump
Resolution	Standard
Dwell time	10 ms
Sweeps	100
Number of readings per replicate	3
Daily performance	¹⁴⁰ Ce ¹⁶ O ⁺ / ¹⁴⁰ Ce and ¹³⁷ Ba ⁺⁺ / ¹³⁷ Ba ⁺ < 2 %
CCT gas flow	6.5 mL min ⁻¹

An ICP OES with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with solid state detector

charge-coupled device (CCD), diffraction grating type echelle, cyclonic spray chamber, and concentric nebulizer was employed for C,

Mn and Cu determination. The operating conditions are summarized in Table 2.

Table 2. Operating conditions for ICP OES

Parameter	Conditions
Generator of radio frequency	40 MHz 1300W
RF incident power	15 L min ⁻¹
Plasma argon flow rate	0.7 L min ⁻¹
Nebulizer argon flow rate	1.5 L min ⁻¹
Auxiliary argon flow rate	V-Groove
Nebulizer type	Sturmann-Master
Spray chamber	Cu 327.395 (I = atomic line); Mn 257.610 (II = ionic line)
Emission lines (nm)	

2.3. Reagents and solutions

All labware was soaked in a HNO₃ acid bath (65% w/w, diluted 1/10 with high purity water) for 24 h and rinsed with high purity water. Subsequently, all material was dried in a clean bench at ambient temperature. All solvents and reagents were of the highest commercially available purity.

High purity deionised water (resistivity 18.2 MΩ cm; Millipore, Bedford, MA, USA) was employed to prepare all standards and sample solutions. Suprapur grade HNO₃ 65% (w/w) and H₂O₂ 30% (w/w) (Merck, Darmstadt, Germany) were used for sample digestion. Monoelemental, high-purity grade 1 g L⁻¹ stock solutions of As, Ba, Cd, Cr, Co, Cu, Hg, Mn, Ni and Sb and multielement solution 100 mg L⁻¹ of Bi, Ge, In, Tl, Rh and Sc were purchased from Merck (Darmstadt, Germany). Determination by ICP-MS was performed with external calibration using analytical solutions from 0.5 to 25 mg L⁻¹. For this technique, the reference analytical solutions and the samples were adjusted to acid concentration of 0.288 mol L⁻¹ HNO₃, as recommended by the manufacturer. Determination by ICP OES was performed with external calibration using analytical solutions ranging from 100-3,000 mg L⁻¹ for C and 0.01-15 mg L⁻¹ for Cu and Mn. The reference analytical solutions and the

samples were adjusted to acid concentration < 4 mol L⁻¹ HNO₃.

2.4. Optimization of sample digestion

The optimization of sample weight and volume of acid was performed in triplicate. There is no certified reference material (CRM) for polychaete tissue, so all experiments were realized with the CRM Oyster Tissue (Oyster tissue – NIST 1566b – National Institute of Standard and Technology, USA). In this study three experiments were performed in order to obtain the best conditions for the digestion of the samples.

In the first experiment, 100 mg and 200 mg of CRM oyster tissue sample were used. The samples were weighed in microwave vessels, 7 mL of distilled nitric acid and 1.0 mL of H₂O₂ 30% v/v were also added to each vessel to test the mass size effect on the digestion. The digestion conditions were: power of 1,000 W, temperature of 90°C and 180°C in the first and last stages, respectively (Table 3). In the experiment 2, the mass of 200 mg of CRM was digested with acid mixtures of different concentrations: 14.1, 12.1, 10.1, 8.1, 6.1 and 4.1 mol L⁻¹. The power, time of each step and temperature were also modified. The objective of the

experiment 2 was to verify the possibility of using a more dilute solution, in order to reduce the volume of residues generated, while still obtaining satisfactory recovery for analytes in the CRM. The experiment 3 was performed to assess the possibility of

reducing digestion time under the digestion conditions using diluted acids. The time was reduced from 10 to 6 min in step 3 and the temperature was also reduced from 210°C (used in test 2) to 180°C in the last step of this test.

Table 3. Programs for digestion of CRM oyster (NIST 1566b) and polychaete tissues

	Step	Power (W)	Time (min)	Temperature (°C)
Experiment 1				
	1	1000	4	90
	2	1000	2	90
	3	1000	8	180
	4	1000	20	180
Experiment 2				
	1	500	5	120
	2	500	3	120
	3	1000	10	210
	4	1000	20	210
Experiment 3				
	1	500	5	120
	2	500	3	120
	3	1000	6	180
	4	1000	20	180

After digestion and cooling, the digests were transferred to LDPE flasks and made up to a final volume of 35.0 mL with ultrapure water. The digests were appropriately diluted, depending upon the concentration of the elements. Blanks were prepared in each batch of samples, using acid solution and H₂O₂ 30%. After ensuring the best conditions for oyster tissues digestion, the digestion was applied to polychaete tissues.

2.5. Determination of the acidity and residual carbon content (RCC) of the digests

To determine the final acidity, acid-base titrations of the digests were performed. The titration was carried out with a standard solution of sodium hydroxide (0.0997 mol L⁻¹, Merck, Germany) and phenolphthalein (1.0 % m/v in ethanol). The residual carbon was determined by ICP OES, using the line emission for C(I) at 193.025 nm. Carbon reference solution used for external

calibration RCC determination was prepared by dissolution of citric acid (Merck, Darmstadt, Germany) in water (25–500 mg L⁻¹ of C).²⁶

2.6. Quality assurance and quality control

All the samples were digested in triplicates. Element concentrations were calculated and expressed in µg g⁻¹ dry weight. Accuracy of analytical method was monitored by analyzing a CRM. At least one sample of the CRM and three blanks (reagents and digestion blanks) were included in each analytical batch. The precision of the method, defined as the closeness of agreement between mutually independent test results, was determined in terms of the percentage of the relative standard deviation (RSD %).

There are no certified reference materials for polychaete tissue samples. Therefore, it was used two different procedures in order

to validate the proposed method. Firstly, an oyster tissue certified reference material, containing most of the studied elements, was analyzed in triplicate using microwave-assisted digestion under selected optimum conditions. Secondly, the evaluation of the analytical performance of procedure was also made by a spike recovery study.

3. Results and discussion

3.1. Techniques performance

For ICP-MS, some of the studied elements suffer from polyatomic and isobaric interferences. This is the case for the Cr signal at m/z 52 by $^{40}\text{Ar}^{12}\text{C}^+$ and $^{36}\text{Ar}^{16}\text{O}^+$, the Cu signal at m/z 63 by $^{40}\text{Ar}^{23}\text{Na}^+$, the Mn signal at m/z 55 by $^{40}\text{Ar}^{15}\text{N}^+$, and the As signal at m/z 75 by $^{40}\text{Ar}^{35}\text{Cl}$. Collision/reaction cell was used to remove or reduce many polyatomic species. The daily optimization procedure involved the xyz alignment of the torch, determination of the optimum nebulizer gas flow rate and the ion lens voltage to maximize $^{115}\text{In}^+$ signal and to obtain low oxide ($^{140}\text{Ce}^{16}\text{O}^+ / ^{140}\text{Ce}^+$) and double charged ions ($^{137}\text{Ba}^{++} / ^{137}\text{Ba}^+$). The determination of As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni and Sb were monitored in the standard (STD) and cell collision technology (CCT) modes, in the presence of premixed 7% H_2 in He gas. Considering the agreement with the certified values, the following conditions were selected for analysis from the experimental uncertainties: CCT mode for the determination of ^{75}As , ^{59}Co , ^{52}Cr , ^{63}Cu , ^{55}Mn , and ^{60}Ni ; and STD mode for the

determination of ^{111}Cd , ^{121}Sb , ^{138}Ba and ^{201}Hg . For the quantification limit (LOQ), ten blanks were prepared and analysed. The result was multiplied for ten and divided for the angular coefficient of the analytical curve for each element. The LOQs obtained for ICP-MS were: As ($0.098 \mu\text{g g}^{-1}$), Ba ($0.907 \mu\text{g g}^{-1}$), Cd ($0.080 \mu\text{g g}^{-1}$), Cr ($0.695 \mu\text{g g}^{-1}$), Co ($0.233 \mu\text{g g}^{-1}$), Cu ($0.465 \mu\text{g g}^{-1}$), Hg ($0.819 \mu\text{g g}^{-1}$), Mn ($1.294 \mu\text{g g}^{-1}$), Ni ($0.520 \mu\text{g g}^{-1}$) and Sb ($0.109 \mu\text{g g}^{-1}$).

For ICP OES, since the signal intensities of each element depend on the sample matrix, it is important to optimize ICP OES parameters such as the radio-frequency (RF), incident power and nebulizer argon gas flow rate. The incident power and nebulizer gas flow-rate were optimized in the 1,000 – 1,400 W and 0.60 – 0.90 L min^{-1} ranges, respectively. The results showed that the greatest Mg(II)/Mg(I) ratio of 9.46 was obtained at 1,300 W incident power and 0.70 L min^{-1} nebulizer gas flow-rate. The ratio > 8 indicates that the conditions of plasma are robust.³⁶ These operational conditions were adopted throughout. The LOQ obtained for ICP OES were: $2.8 \mu\text{g g}^{-1}$ for Cu and $0.027 \mu\text{g g}^{-1}$ for Mn.

3.2. Quality assurance and quality control

The recoveries of spiked additions, made in polychaete samples prior digestions, ranged from of 85%, for Cu at 200 ng g^{-1} , to 110%, for Ba at 100 ng g^{-1} (Table 4), with standard deviations ($n = 3$) lower than 6% in all cases. The recoveries obtained confirmed that the proposed procedure showed good analytical performance.

Table 4. Recoveries (%) for spiked polychaetas samples under optimized digestion conditions

Element	Spike recovery (%)	
	100 ng g ⁻¹	200 ng g ⁻¹
¹³⁸ Ba	110	107
⁵² Cr	91	88
²⁰¹ Hg	104	94
⁶⁰ Ni	85	86
¹²¹ Sb	90	90

3.3. Optimization of the sample digestion procedure

Sample digestion is a critical step in most analytical procedures for the routine determination of chemical elements in environmental samples. The use of nitric acid for organic matrix digestion is the most usual approach for wet sample pre-treatment to facilitate the determination of trace elements for atomic spectrometric methods. Ideal digestions should lead to the complete decomposition of the organic material using minimal amounts of acid. Acid solutions should be as diluted as possible in order to decrease residual carbon and final acid concentration.¹¹ In the literature, there are various methods for the digestion of biological tissues using different reagents under specific conditions of pH, time and temperature. Compared to other traditional methods, an advantage of using microwave-assisted digestion is the short time required.^{37,38} In this study, samples of 100, 150 and 200 mg, were decomposed, in triplicate, in the experiment 1. A mass of 200 mg was analysed in experiments 2 and 3, using diluted acid, in only 34 min, which is a very important aspect for routine environmental analysis.

The efficiency of a microwave-assisted digestion is governed by three variables: acid concentration, digestion time and equipment

power. In order to optimize digestion, various programs involving different temperatures (180, 200 and 210 °C) and times (10 and 20 min in the fourth step) were evaluated and the best results obtained were considered to be the most appropriate and therefore were finally selected (Table 5).

The applied microwave-assisted digestion procedure with the acid mixture of 8.1 mol L⁻¹ in experiment 3, resulted in solutions with low residual acidity and carbon contents (9.6 ± 0.80%), which are important prerequisites for multielement determination using ICP-MS. The use of diluted acids has several advantages, such as cost reduction, minimization of residues, reduction of blank values, and prevention of damages to equipment.^{28,30,39-41} All digestion procedures were evaluated by comparing the residual carbon content, the residual acidity of digests and the trace element recoveries. The residual carbon contents ranged from 4.0 ± 0.32 % to 14.9 ± 0.90% (n = 3). According Nóbrega et al,⁴² the digestion efficiency increases at higher temperature and power, reducing the RCC. In this study, however, it was not possible to increase the temperature above 220°C due to the maximum limit permitted by equipment microwave manufacturer. The residual acidity obtained after the digestions, as expected, was highest for the most concentrated solutions. Residual acidity of the diluted digests was 1.6 to 3.8 mol L⁻¹ HNO₃.

Table 5. Recoveries (%) of analytes obtained from the digestion procedures of CRM oyster tissue (1566b) for ICP-MS and ICP OES*, using various acid solution mixtures

Mass (mg)	Acid mixture [] mol L ⁻¹	As	Cd	Co	Cu	Mn
Experiment 1						
100	14.1	114	87	85	84	169
200	14.1	97	112	102	93	89
Experiment 2						
200	14.1	91	87	82	85*	74*
200	12.1	91	86	88	92*	90*
200	10.1	91	86	84	92*	91*
200	8.1	93	89	86	87*	86*
200	6.1	100	95	94	83*	70*
200	4.1	95	90	89	103*	64*
Experiment 3						
200	10.1	95	91	86	87*	92*
200	8.1	100	95	93	89*	102*

3.4. Effect of the sample size on the efficiency of the microwave-assisted digestion process

After the removal of the polychaete intestines, the dry weight obtained for the biological tissue of each organism was, in general, below 200 mg. In order to have enough biological tissue mass for the experiments realized in this study, it was necessary to combine 15 individuals from each sampling site. Considering the size of the polychaetes, the effect of a sample mass on the digestion is critical, and it was evaluated. This means that attempts to work with only one individual polychaete to characterize and/or to monitor the contamination of a site will require the selection of only the largest individuals. The size of the polychaete, however, cannot be estimated by observing the extremities of the tube, above the sediments surface, as a result it is necessary to collect a number of tubes to choose organisms with adequate size, which is not very practical.

Many species of polychaetes are small. However, *C. variopedatus* have mass and size comparable to some marine invertebrates frequently used as biomonitors, such as clams and mussels. The sample mass for the

digestion of polychaetes may also vary according to the species to be analyzed.^{43,44}

The digestion conditions applied in experiment 1, with acid mixture of 14.1 mol L⁻¹, showed good results and high precision for the majority of the studied elements in sample mass of 150 and 200 mg. However, triplicate samples of 100 mg produced very variable and discrepant results, suggesting that it is inappropriate to use such a small mass. Moreover, the As, Cu, Cd, Mn and Ni concentrations obtained for 200 mg, were closest to the certified values that values obtained for 100 mg, possibly due to the higher sample homogeneity in 200 mg samples. It is suggested that, for the polychaete species tested in this study, a minimum mass of 200 mg should be used to obtain more representative and accurate results.

3.5. Applications

The proposed method has been applied for the determination of trace elements in *C. variopedatus* collected in five sites at Todos os Santos Bay, Bahia (Table 5). Although this is only a preliminary evaluation, since only a reduced set of sites were assessed, it is clear

that concentrations were very variable both among elements and studied sites. The concentrations of Hg and Sb were < LOQ in all samples, except for Hg that was found in Itapagipe ($1.18 \pm 0.17 \mu\text{g g}^{-1}$). This region is contaminated by Hg due to the production of chlorine and caustic soda, during 12 years on the shores of Itapagipe bay.⁴⁵

The highest As and Ni concentrations were observed at Praia Grande and Bimarras. An important petrochemical complex and harbor is located in front of Maré Island, where Praia Grande and Botelho are found. Both the harbor and the petrochemical complex among other industries in the region are well documented sources of several metals.^{5,41,46} High values of Cd in Bimarras may be associated to intense port activity (mainly petrochemical products) at Madre de Deus Island and also the influence of the Subaé estuary, which is the main source of contaminated particulate material to BTS.⁴⁷

Arsenic levels in some polychaetes are generally high, and appear to be concentrated in lipids.⁴⁸ *Chaetopterus variopedatus* can store up to $46,000 \mu\text{g g}^{-1}$ of As in their tissue without being intoxicated.⁴⁹ Arsenic concentrations in polychaete tissue are determined by a variety of factors including exposure, diet and the physiology of the organisms.⁵⁰ In this work, the As

concentrations in the polychaete tissues were higher than reported results for others polychaete species⁵¹ and bivalves.^{5,52,53}

Although the contents of some elements (i.e. Cd and Ba) presented a large variability between sites, the concentrations of Ba, Ni, and Cr obtained in this study were, generally, smaller than the values reported in the literature for several species.⁴⁹ However, Cd and Cu values were similar to the reported values in other polychaetes species.^{9,54}

Between sampling points, the levels of Co were highest in Praia Grande. The Ba, Co, Cd and Mn concentrations obtained in this polychaete species were similar to the concentrations measured in shellfish of the Todos os Santos Bay.⁵ The highest Mn concentrations occurred at Botelho, which is possibly related to the anthropogenic introduction of this element by the industries and port activities at Aratu Bay.⁴¹ However natural background levels of Mn are relatively high at Todos os Santos Bay sediments ($344 \pm 105 \text{ mg kg}^{-1}$),⁴⁵ and may explain the concentrations observed at all other sites. Copper presented moderately high concentrations at all studied sites, with the exception of Bimarras. To mention just a few, there are inputs of untreated sewage all around the bay and several industries and intense port activities that contribute to the Cu burden in the sediments and biota.

Table 6. Mean \pm standard deviation element concentrations ($\mu\text{g g}^{-1}$, dry weight) in *Chaetopterus variopedatus* collected along Todos os Santos Bay, Bahia, Brazil

	Praia Grande ^a	Praia Grande ^b	Botelho	Itapagipe Bay	Bimarras
As	145 ± 4.78	138 ± 2.33	89.2 ± 2.48	81.4 ± 2.44	133 ± 2.48
Ba	2.30 ± 0.16	2.25 ± 0.09	2.93 ± 0.21	1.00 ± 0.02	1.64 ± 0.03
Cd	0.40 ± 0.02	0.58 ± 0.02	0.59 ± 0.03	0.18 ± 0.004	2.04 ± 0.028
Cr	1.02 ± 0.03	1.07 ± 0.05	1.15 ± 0.02	< LOQ	0.44 ± 0.03
Co	9.23 ± 0.36	10.6 ± 0.38	3.53 ± 0.27	4.35 ± 0.44	2.52 ± 0.03
Cu	7.76 ± 0.38	8.49 ± 0.27	8.35 ± 0.35	6.49 ± 0.54	2.38 ± 0.05
Hg	< LOQ	< LOQ	< LOQ	1.18 ± 0.17	< LOQ
Mn	27.9 ± 0.93	35.5 ± 2.25	50.6 ± 1.67	33.9 ± 1.01	30.8 ± 0.49
Ni	3.50 ± 0.13	3.50 ± 0.06	1.74 ± 0.08	1.13 ± 0.10	3.18 ± 0.07
Sb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

^a March 2010; ^b October 2010

Evaluating the scientific literature, the use of polychaetes, as a biomonitor, is much less than oysters, mussels and clams, suggesting that the potential use of these organisms is yet to be explored. Moreover, once this polychaete lives in physical and chemical conditions (e.g. substrate type, grain size of sediments, dissolved oxygen and organic matter concentrations) different from those observed for oyster and/or mussels, the data obtained for this organism complement the results of other biomonitor organisms in monitoring programs. The use of various organisms in monitoring programs provides information of the relative importance of bioavailable metal sources.⁵⁵

4. Conclusions

The proposed procedure is suitable for decomposition of polychaete samples *C. variopedatus* for subsequent trace elements (As, Ba, Cd, Cr, Co, Cu, Hg, Mn, Ni and Sb) determination by ICP-MS or ICP OES. Repeatability was satisfactory (< 10 %) and results for CRM agreed well with the certified values. The obtained RCC values were lower than 15 %, which is important for minimizing the generation of residues and improving detection limits. Moreover, up to ten samples can be simultaneously decomposed in 34 min, resulting in a high sample throughput that is an essential aspect for routine environmental analysis. The polychaete species studied showed sufficient mass for chemical analysis and accumulation of trace metals, especially As. The use of this species should be better explored as a biomonitor species for monitoring programs.

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