

Optimization of a microwave-pseudo-digestion procedure by experimental designs for the determination of trace elements in seafood products by atomic absorption spectrometry

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Abstract

A Plackett–Burman $2^7 \times 3/32$ design for seven factors (nitric acid concentration, hydrochloride acid concentration, hydrogen peroxide concentration, acid solution volume, particle size, microwave power, and exposure time to microwave energy) was carried out in order to find the significant variables affecting the metals acid leaching after a pseudo-digestion procedure by microwave energy from mussel. Nitric acid concentration, hydrochloride concentration or hydrogen peroxide, and exposure time to microwave energy were the most significant variables, and a 2^3 + star central composite design was used for their optimization. Nitric and hydrochloric acid concentrations between 4.1 and 5.3 M, and between 2.8 and 3.8 M, respectively, were found as optimum for many elements (Ca, Cd, Cr, Cu, Fe, Mg, Mn, Pb and Zn) yielding the acid leaching process for times in the 1.2–2.2 min range. However, As was quantitatively leached with hydrochloric acid concentrations between 4.8 and 5.3 M and an exposure time of 2.0 min, while Co and Se were extracted using nitric acid (1.0 and 5.0 M, respectively) and hydrogen peroxide (5.0 M) solution and an exposure time of 2.0 min. Finally, Hg was extracted using a hydrochloric acid/hydrogen peroxide solution at 3.5:2.0 M, and also for an optimum time of microwave radiation of 1.75 min. Trace metals were determined using flame atomic absorption spectrometry, electrothermal atomic absorption spectrometry and cold

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vapor — atomic absorption spectrometry. The methods were finally applied to several reference materials (DORM-1, DOLT-1 and TORT-1), achieving good accuracy. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Wet digestion with concentrated acids are the most common sample of pre-treatment for element determination of biological materials by atomic spectrometric techniques. The introduction of microwave energy has allowed the reduction of digestion time and has led to the development of convenient acid digestion procedures. These processes have been widely used as biological material pre-treatments, as can be seen in recent reviews [1]. These procedures offer a complete destruction of the solid sample matrix, so the liquid sample (digest) can be easily analyzed using conventional sample introduction systems in atomic spectrometry techniques. However, acid digestion involves the use of concentrated acids, so matrix interference is frequently present in the final determination step. In addition, an acid digestion using either high pressure or low pressure bombs involves the formation of nitrous vapors, highly carcinogenic, so the bombs must be cooled down before the digests are handled.

Acid leaching procedures could be an alternative sample pre-treatment for multi-element determination in solid biological samples. This type of process, using hydrochloric acid, has been applied since 1968 mainly to extract methylmercury from biological tissues (Westöö method) [2]. As was said by Lorentzen and Kingston [3], leaching is a term that has been applied to the extraction of metals from environmental samples and has become common terminology of the EPA and in the environmental analytical field. As acid leaching is not a total decomposition, recoveries can only achieve total values if an element is completely soluble in the acid leaching solution. Due to the fact that total sample decomposition is not needed, low acid concentrations can be used, which involves a low consumption of reagents and, in addition, the nitrous vapors are

also low. However, it must be said that partial dissolution of the solid sample is unavoidable when the solid sample and an acid solution are in contact, especially when oxidizing acids such as nitric acid are used. This fact is more important when the mixture is subjected to microwave energy. Therefore, the mechanism through which metals reach the solution is also matrix decomposition. By this way, the acid leaching procedures in which oxidizing acids are used must be called pseudo-digestion.

Some works have been developed using pseudo-digestion to extract elements, as in Puchyr and Shapiro [4] who used a HCl–HNO₃ leaching solution to extract several elements from foods. These authors conclude that elements, such as Al, Ca, Cd, Cr, Cu, Fe, Mg, Sn, Ni, Na, K, and Zn, are easily leached and none of the analytes determined by this acid leaching method could be found in the residues, thus attesting to the completeness of extraction. Other workers have recently developed procedures using either ultrasonic [5] or microwave energy [3,6–8]. El Azouzi et al. [5] use a HCl–HNO₃–H₂O₂ solution and room temperature sonication. This procedure is able to extract quantitatively, elements such as Ca, Cu, Fe, Mg and Zn from mussel samples, while low recoveries are reported for other elements, Co, Cr and Se. Lorentzen and Kingston [3,7,8] have reported a method based on microwave-assisted pseudo-digestion to extract several elements from sediments, sludge and soils. HNO₃ and HCl are the reagents used, added in two different steps, being the exposure time to microwave energy and the microwave power different for each stage. Finally, Szpunar et al. [6] have also used low power focused microwave technology for organotin speciation analysis.

Some works described above are time consuming, usually times for the pseudo-digestion

procedure between 30 and 180 min were used. In addition, some variables which can affect the process, such as acid volume or particle size of the solid biological samples are not considered in these studies, and other variables, which could be less significant, are widely investigated. Due to many variables being involved throughout the pseudo-digestion process, the optimization of each one separately can be a tedious labor. In addition, the interaction effects between variables on the optimum, which are not taken into account when each variable is optimized separately, can lead to false optimum values for each variable. So, studying all possible combinations of variables would only attain optimum operating conditions. From the experimental design theory and applying full factorial designs, a system with n factors (variables) at only two levels would be evaluated with 2^n experiments. In order to reduce the number of experiments, Plackett–Burman designs (PBDs) allow the evaluation of either system with fewer experiments. These are two-level designs for the study of $k = n - 1$ factors in n experiment runs where n is a multiple of 4 [9]. Therefore, different PBDs have been used in order to optimize several procedures [10–23].

The aim of the work has been the optimization of pseudo-digestion procedures for the extraction of metals (As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Pb, Se and Zn) from seafood products (mussel samples). Due to a great number of variables that can affect the extraction of metals from biological materials, Plackett–Burman designs were used to evaluate the most important factors (variables) affecting the process, and central composite designs were used to find the optimum conditions.

2. Experimental section

2.1. Instrumentation

A Perkin Elmer Model 3110 (Norwalk, CT, USA) flame atomic absorption spectrometer was used for Ca, Cu, Fe, Mg and Zn determinations using an air/acetylene flame. A Perkin Elmer Model 1100B (Norwalk, CT, USA) atomic absorp-

tion spectrometer equipped with a graphite furnace HGA-400, an autosampler AS-40, and deuterium lamp as a background correction system, was used for As, Cd, Co, Cr, Mn, Pb and Se measurements. A Perkin Elmer Model 4100ZL (Norwalk, CT, USA) atomic absorption spectrometer equipped with a Perkin Elmer FIAS 400 system with a five-port flow-injection valve was used for Hg determinations. The rotation speed of the two multichannel peristaltic pumps was programmed and automatically controlled by a separate PC. Hollow cathode lamps (Perkin Elmer) operating at recommended currents were used for all cases, except for Pb and Se, for which electrode discharged lamps, connected to a power supply (Perkin Elmer) were used. For As and Hg, electrode discharged lamps (system 2), connected to a power supply (system 2) from Perkin Elmer, were employed. A domestic microwave oven, Moulinex Model 700 (Barcelona, Spain) programmable for time, and with a microwave power between 70 and 900 W, was used to induce the acid leaching process. The acid leaching was carried out in laboratory-made poly(tetrafluorethylene) (PTFE) bombs which were hermetically sealed. A vibrating ball mill, Retsch (Haan, Germany), equipped with zircon cups (15 ml in size) and zircon balls (7 mm diameter) was used to pulverize and to reduce the particle size of the mussel samples. A Laser Diffraction Spectrometer, Coulter Series LS100, Fraunhofer optical model particle size (Coulter Electronics, Hialeah, FL, USA) was used to obtain the particle size of mussel samples. A Centrifuge Orto-Alresa (Barcelona, Spain) was also used in order to reach the separation between the solid mussel and the acid leaches liquid phase. A Panasonic domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for total digestion of the samples. The poly(tetrafluorethylene) (PTFE) bombs were laboratory-made and hermetically sealed and as a result they were adequate to work at low pressures.

2.2. Reagents

Chemicals were of ultrapure grade, using ultra-

pure water, resistance $18 \text{ M}\Omega \text{ cm}^{-1}$, which was obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). $\text{Cd}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_3$, $\text{Cu}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Pb}(\text{NO}_3)_2$, and $\text{Zn}(\text{NO}_3)_2$, and selenium stock standard solutions, 1.000 g l^{-1} , were supplied by Merck (Darmstadt, Germany). $\text{Mn}(\text{NO}_3)_2$ stock standard solution, 1.000 g l^{-1} , was obtained from Roimil (Cambridge, UK). AsCl_3 and $\text{Co}(\text{NO}_3)_2$ stock standard solutions, 1.000 g l^{-1} , were supplied by BDH (Poole, U.K.). $\text{Hg}(\text{NO}_3)_2$ stock standard solution, 1.000 g l^{-1} , was obtained from Panreac (Barcelona, Spain). $\text{Mg}(\text{NO}_3)_2$ stock standard solution, 1.000 g l^{-1} , was prepared from $\text{Mg}(\text{NO}_3)_2$ (BDH). $\text{Ca}(\text{CO}_3)_2$ stock standard solution, 0.500 g l^{-1} , was prepared from analytical reagent from Merck. LaCl_3 stock standard solution, 10.000 g l^{-1} , used as an ionization suppresser in Ca and Mg determinations, was prepared from LaCl_3 (Aldrich, Milwaukee, WI, USA). Pd stock standard solution, 3.000 g l^{-1} , used as a chemical modifier, was prepared from Pd 99.999% (Aldrich). $\text{Mg}(\text{NO}_3)_2$ stock standard solution, 3.000 g l^{-1} , used as a chemical modifier, was prepared from $\text{Mg}(\text{NO}_3)_2$ (BDH). Nitric acid (70.0%) was obtained from J.T. Baker B.V. (Deventer, Holland). Hydrogen peroxide (33%) was supplied by Panreac. Hydrochloric acid (37%) was obtained from J.T. Baker B.V. Sodium tetrahydroborate solution, 0.01% (m/v) was prepared from a sodium tetrahydroborate reagent (Aldrich) and it was used as a reducing agent in Hg CVAAS determinations. This solution was prepared daily by dissolution in a 0.5% (m/v) sodium hydroxide solution (prepared from sodium hydroxide supplied by Carlo-Erba, Milan, Italy). The National Research Council of Canada supplied the reference materials, DORM-1, DOLT-1 and TORT-1.

2.3. Mussel samples

Fresh mussel samples were collected from the Galician coast (north-west Spain). The samples were triturated, homogenized and lyophilized. Then, they were transferred into polyethylene vials. The particle size of the lyophilized mussel was determined by laser diffraction, and it was approximately $200 \text{ }\mu\text{m}$. In order to study the

particle size effect on the acid leaching efficiency, the mussel samples were pulverized by means of a vibrating mill ball for 20 min, obtaining mean particle sizes of approximately $20 \text{ }\mu\text{m}$.

2.4. Microwave acid-digestion procedure

An optimized acid digestion assisted by microwave energy was used in order to know the total content of metals studied and also for comparative purposes. The optimized conditions for acid digestion have been described elsewhere [24], and a domestic microwave oven with laboratory-made low pressure PTFE bombs, and concentrated HNO_3 and H_2O_2 as reagents, were employed.

2.5. Microwave pseudo-digestion procedure

Mussel samples approximately 0.2 g (or also 0.3 g for some elements such as As, Ca, Cd, Cu, Fe, Hg, Mg, Mn, Pb and Zn) were directly weighted into PTFE bombs, then 5 ml of the oxidizing acid solution was added and, after homogenization the bombs (three to six bombs) were subjected to a microwave power of 64 W for a variable time (between 1.5 and 2.0 min). The bombs were left to cool down in an ice bath for 5 min , and the mixture (solid mussel and acid extract) was transferred to test tubes and was centrifuged at $3000 \text{ rev. min}^{-1}$ for 10 min , and the acid liquid phase was separated with a Pasteur pipette. In order to clean the solid residue, 2 ml of ultrapure water was added, the solid resuspended and centrifuged again at $3000 \text{ rev. min}^{-1}$ for 20 min . The liquid phase was also separated with a Pasteur pipette and combined with the acid extract. The solution was finally made up to 10 ml with ultrapure water and kept in polyethylene bottles at 4°C .

3. FAAS and ETAAS determinations

Calcium, Cu, Fe, Mg and Zn were determined by FAAS under optimum conditions (Table 1), and after appropriate dilution. For Ca and Mg determination adequate volumes of LaCl_3 solution (an ionization suppresser), to give a final

Table 1

FAAS conditions for the determination of Ca, Cu, Fe, Mg and Zn in acid extracts from mussel

	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Air flow rate (l min ⁻¹)	C ₂ H ₂ flow rate (l min ⁻¹)
Ca	422.8	0.7	15	23.5	2.0
Cu	324.8	0.7	15	23.5	2.0
Fe	248.3	0.2	30	23.5	2.0
Mg	285.2	0.7	15	23.5	2.0
Zn	213.9	0.7	15	23.5	2.0

concentration of 10.0% (m/v), were added. Aqueous calibration was used for Cu, Mg and Zn determination, while the standard addition technique was needed for Ca and Fe measurements.

As, Cd, Co, Cr, Mn, Pb and Se determinations were carried out by ETAAS using optimized graphite furnace temperature programs (Table 2). Pd(NO₃)₂, as a chemical modifier, at optimum concentrations of 10 and 20 mg l⁻¹, for Mn and Pb, respectively, was used. Mg(NO₃)₂, also as a chemical modifier, was used in Co and Cr determinations at a concentration of 20 mg l⁻¹. A solution containing Pd(NO₃)₂/Mg(NO₃)₂, at concentrations of 20 mg l⁻¹, was used as a chemical modifier for As measurement, while concentrations of 15 and 20 mg l⁻¹ for Pd(NO₃)₂ and Mg(NO₃)₂, respectively, was used for Cd. The Se determination was carried out using Pd(NO₃)₂/Mg(NO₃)₂ as a chemical modifier, at concentrations of 300 and 250 mg l⁻¹, respectively. For each case, portions of the acid extracts were mixed with appropriate volumes of the chemical modifier solution directly into the autosampler cups. Aqueous calibration was a real possibility for all elements, except for As and Se determination.

4. CVAAS determination

Hg determination was carried out by cold vapor generation — flow injection system on line atomic absorption spectrometry, with an electrically-heated quartz cell, and using sodium tetrahydroborate as a reducing agent, and hydrochloric acid as a carrier solution. Optimum conditions for the cold vapor generation and optimum flow injection parameters are given in Table 3.

5. Results and discussion

Seven variables which could potentially affect the metal extraction by a pseudo-digestion procedure (nitric acid concentration [HNO₃]; hydrochloric acid concentration [HCl]; hydrogen peroxide concentration [H₂O₂]; acid leaching solution volume, V; exposure time to microwave energy, *t*; microwave energy power, *P*; and mussel particle size, *φ*) were studied. Before starting with the experimental factor designs, some considerations must to be taken into account.

Firstly, the microwave oven is not a focused microwave oven, so in order to guarantee that all PTFE bombs are subject to microwave energy at the same conditions, we have used each microwave oven cycle only with three PTFE bombs. For some cases (certain experiments) we have placed one or two PTFE bombs with the same acid leaching solution volume, but without a sample, in order to develop all experiments with three bombs into the microwave oven. The numbers of PTFE bombs will be optimized after.

As a domestic microwave oven was used, the real microwave power, expressed in *W*, at each microwave power setting, was evaluated according to the equation [25]

$$P = \frac{KC_p m \Delta T}{t}$$

where *P* is the microwave power absorbed by a certain ultrapure water mass, *K* is a constant (4.184 J), *C_p* is the water calorific capacity, *ΔT* is the temperature variation of the water, and *t*, is the exposure time to the microwave energy.

The microwave power calculated was lower than

Table 2

ETAAS operating conditions and temperature programs for the determination of As, Cd, Co, Cr, Mn, Pb and Se in acid extracts from mussel^a

Operating conditions	As	Cd	Co	Cr	Mn	Pb	Se
Wavelength (nm)	193.7	228.8	242.5	357.9	279.5	283.3	196.0
Slit (nm)	0.7	0.7	0.2	0.7	0.2	0.7	2.0
Background correction	D ₂	D ₂	D ₂	–	D ₂	D ₂	D ₂
Injection volume (μl)	20	20	20	20	20	20	20
Pyrolytic-coated graphite tubes with L'vov platforms; integrated absorbance measurement							
<i>Graphite furnace temperature program</i>							
	Step	Temperature (°C)	Ramp heating (s)	Hold (s)	Ar flow rate (ml min ⁻¹)		
As	Drying	110	20	20	300		
	Pyrolysis	250	10	15	300		
	Atomization	2100	0	3	0 (READ)		
	Cleaning	2500	2	2	300		
Cd	Drying	150	15	20	300		
	Pyrolysis	600	10	15	300		
	Atomization	1600	0	2	0 (READ)		
	Cleaning	2200	2	3	300		
Co	Drying	150	15	20	300		
	Pyrolysis	1500	10	5	300		
	Atomization	2500	0	5	0 (READ)		
	Cleaning	2650	1	2	300		
Cr	Drying	150	15	20	300		
	Pyrolysis	1200	10	15	300		
	Atomization	2500	0	5	0 (READ)		
	Cleaning	2650	1	2	300		
Mn	Drying	150	15	20	300		
	Pyrolysis	1100	10	15	300		
	Atomization	2500	0	3	0 (READ)		
	Cleaning	2650	1	2	300		
Pb	Drying	150	15	20	300		
	Pyrolysis	800	15	10	300		
	Atomization	1600	0	3	0 (READ)		
	Cleaning	2200	2	3	300		
Se	Drying	150	15	20	300		
	Pyrolysis	1200	15	10	300		
	Atomization	2100	0	5	0 (READ)		
	Cleaning	2650	2	2	300		

^aMg(NO₃)₂ as a chemical modifier for Co (20 mg l⁻¹) and Cr (20 mg l⁻¹). Pd(NO₃)₂ as a chemical modifier for Mn (10 mg l⁻¹) and Pb (20 mg l⁻¹). Mg(NO₃)₂/Pd(NO₃)₂ as a chemical modifier for As (20/20 mg l⁻¹), Cd (15/20 mg l⁻¹) and Se (300/250 mg l⁻¹).

the given one by the supplier. So, we consider microwave powers of 64 and 209 W instead 70 and 250 W, respectively.

The particle size was determined by Laser Diffraction Spectrometry. Mussel samples, after the homogenization and lyophilization stages

Table 3
Hg cold vapor generation program for CVAAS^a

Flow rate (ml min ⁻¹)				Time (s)	Valve	Read	Function
Step	Pump 1	Pump 2					
		Carrier solution	Reducing solution				
1	10	9	5	30	Fill	*	Sampling step
2	0	9	5	15	Inject		Hg cold vapor generation

^aWavelength, 253.7 nm; slit, 0.7 nm; sample loop, 500 μ l; Argon flow rate, 100 ml min⁻¹; quartz cell temperature, 20°C; measurement mode, peak height; carrier solution HCl 0.1% (m/m); reducing solution NaBH₄, 0.01% (m/v).

offered a mean particle size (particle diameter) of approximately 200 μ m, while particle sizes for lyophilized mussel samples, after a pulverization stage in a vibrating ball mill for 20 min., was 20 μ m.

A dummy factor, *D*, was also considered. A dummy factor is an imaginary variable for which the change from one level to another is not supposed to cause any physical change. In other words, the value that can be adopted this variables would not affect the analytical response. The introduction of dummy factors is important in order to evaluate the possible systematic errors, which can be present when a certain procedure is being carried out. If this factor is not significant, the process under study is not subjected to systematic errors.

The effect of changing a factor from a low to a high level value was examined through the response, which in our study has been the metal recovery expressed as

$$\% \text{Recovery} = \frac{[\text{microwave pseudo-digestion}]}{[\text{acid digestion}]} \times 100$$

where $[\text{acid digestion}]$ and $[\text{microwave pseudo-digestion}]$ are the metal concentration obtained after the application of microwave-acid digestion and a microwave-pseudo-digestion procedure, respectively. High recoveries will be obtained for a certain eight-variable array, which will show us the most significant variables, while recoveries close to 100% will be obtained for the optimum conditions. The sample mass used to develop all experiments was 0.3 g.

Finally, it must be said that the recoveries

shown in the tables throughout this work are mean recoveries. Each experiment was replicated. Replicates must be included in order to make an estimation of the experimental error. This gives information about the average experimental error in itself, and because it enables us to compare response variation due to controlled causes with uncontrolled response variation.

6. Plackett–Burman designs

A Plackett–Burman $2^7 \times 3/32$ design for seven factors, resolution III and 4 error degree of freedom, involving 12 runs [26], was carried out. In Table 4 the experimental field definition for the Plackett–Burman design is presented, while the Plackett–Burman design matrix is shown in Table 5, together with the recoveries obtained for each metal and each experiment. The analysis of results lead the standardized ($P = 95\%$) Pareto charts of main effects shown in Fig. 1a,b, which are a combination of the individual Pareto charts for each metal. In the figures are shown a minimum *t* value at the 95% CI of 3.1, and we consider as significant variables those that offer a *t* value higher than ± 3.1 .

Variables such as [HNO₃], [HCl] and time of exposure to microwave energy resulted significantly in the acid extraction by a microwave pseudo-digestion of all metals, except for Hg, for which [HCl], [H₂O₂] and acid solution volume were the most significant; and for Co and Se, for which [HNO₃], [H₂O₂] and time are the most significant variables. Other variables resulted in less important, as well as the dummy factor, which

Table 4

Experimental field definition for the Plackett–Burman and central composite designs^a

Variable	Symbol	High level (+)	Low level (–)
HNO ₃ concentration (M)	[HNO ₃]	4.8	0
HCl concentration (M)	[HCl]	4.8	0
H ₂ O ₂ concentration (M)	[H ₂ O ₂]	2.4	0
Acid solution volume (ml)	<i>V</i>	7	3
Microwave power (W)	<i>P</i>	209	64
Time of exposure (min)	<i>t</i>	2.0	0.5
Mussel particle size (μm)	<i>φ</i>	200	20
Dummy factor	<i>D</i>	+1	–1

^aThe sample mass was 0.3 g for all experiments.

is not significant for any case, so errors during the procedure and atomic absorption spectrometric determination are not present. This result is important due to the fact that we have used a domestic microwave oven, and a bad repeatability for the microwave heating would be expected.

In addition, particle size is also not a significant variable. This result offers a practical advantage as mussel samples can be subjected to the acid leaching process without a previous pulverization stage.

The design shown in Table 5 does not allow interaction between variables. However, the interactions between variables are important, and normally, the effect of the least significant variables can lead to incorrect results on the importance of the most significant variables. Due to this fact, the effects of some variables (least significant) were discharged, and the effect of the significant variables and interactions between significant variables were again evaluated. After this new evaluation, shown as the combined Pareto chart in Fig. 2a–e, it was obtained that [HNO₃] and [HCl] together with the exposure time to microwave energy were the most significant variables ($P=95\%$), in the acid extraction of all metals except Cr and Cu (Fig. 2b), for which only [HNO₃] and [HCl] are significant. [HCl], [H₂O₂] and acid solution volume were the most significant variables for Hg (Fig. 2c), while [HNO₃], [H₂O₂] and exposure time to microwave energy were the variables which led the acid extraction of Co and Se (Fig. 2d). For Ca only [HCl], and exposure time were significant (Fig. 2e).

7. Optimization of the most significant variables for the microwave pseudo-digestion: central composite designs

Although the exposure time to microwave energy is not significant for Cr and Cu acid extraction (Fig. 2b), and [HNO₃] reduces the efficiency in the As acid leaching (Fig. 2a) or is not significant for Ca (Fig. 2e), the set of three variables, [HNO₃], [HCl] and exposure time to microwave energy, were subsequently optimized by using a central composite 2³ + star design with d.f. 6 and 16 runs [26], for the acid leaching of all metals except Co and Se, for which a set of variables of [HNO₃], [H₂O₂] and exposure time to microwave energy, were also optimized. The acid solution volume resulted in a significant variable for Hg (Figs. 1a and 2c). It was affected by a negative sign, which indicates that an increase in the acid solution volume decreases the efficiency of the acid leaching process. So, the acid solution volume was fixed at a lower value, 3 ml, for Hg, and [HCl], [H₂O₂] and exposure time were the variables under study. Table 4 also lists the upper and lower values given for each variable, while Tables 6 and 7 show the experimental design matrix and the recoveries obtained in each run. The analysis of the results in Tables 6 and 7 offers Pareto charts (not given) that are similar to the obtained for the two interaction effects Pareto charts (Fig. 2a,b and d,e), so we can say that the discharge of least significant factors and the new evaluation of the significant factors (main effects) and two

Table 5
Plackett–Burman design ($n = 12$) for the significant variables determination

Run	t	P	[HNO ₃]	[HCl]	[H ₂ O ₂]	V	ϕ	D	Recovery (%)												
									As	Ca	Cd	Co	Cr	Cu	Fe	Hg	Mg	Mn	Pb	Se	Zn
1	+	−	+	−	−	−	+	+	55.7	90.7	117.6	65.4	65.5	88.0	59.8	20.7	93.0	93.0	93.0	83.9	93.1
2	+	+	−	+	−	−	−	+	100.0	70.6	111.8	88.5	58.9	62.5	93.7	24.1	70.1	89.2	110.0	27.2	72.5
3	−	+	+	−	+	−	−	−	36.9	48.9	91.2	61.5	64.4	53.6	29.5	51.8	53.5	72.0	107.9	62.5	55.1
4	+	−	+	+	−	+	−	−	100.0	81.8	100.0	57.7	56.7	82.6	80.6	0.0	90.6	82.8	104.0	64.2	89.3
5	+	+	−	+	+	−	+	−	98.9	92.6	120.6	111.5	64.4	96.0	78.4	93.1	93.1	81.1	106.7	37.0	92.1
6	+	+	+	−	+	+	−	+	53.6	73.7	97.1	61.5	67.8	61.9	87.4	27.6	63.1	89.3	98.2	70.8	63.7
7	−	+	+	+	−	+	+	−	78.6	62.9	94.2	61.5	66.7	64.0	64.3	24.1	76.0	65.9	102.2	46.9	72.5
8	−	−	+	+	+	−	+	+	67.7	85.8	111.8	65.4	78.9	90.5	56.7	100.0	86.0	87.1	113.5	68.3	89.1
9	−	−	−	+	+	+	−	+	98.7	67.1	100.0	92.3	48.9	64.1	59.7	51.7	65.1	77.1	106.1	30.4	69.4
10	+	−	−	−	+	+	+	−	70.1	70.4	76.5	96.1	40.0	39.8	12.7	0.0	67.8	64.0	59.0	46.9	65.6
11	−	+	−	−	−	+	+	+	53.6	48.6	55.9	57.7	28.9	30.3	14.1	0.0	40.8	86.7	37.6	12.3	46.4
12	−	−	−	−	−	−	−	−	68.2	36.6	52.9	61.5	38.9	21.0	7.9	0.0	31.1	25.5	22.7	12.3	30.1

order interactions is a good practice. So, they confirm that the set of variables ($[\text{HCl}]$, $[\text{HNO}_3]$ and time; $[\text{HNO}_3]$, $[\text{H}_2\text{O}_2]$ and time; and $[\text{HCl}]$, $[\text{H}_2\text{O}_2]$) are statistically significant. Some quadratic terms were also significant for some cases, and response surfaces with curvature (optimum region) were obtained. One of these surfaces is given in Fig. 3a (Cr, $[\text{HCl}]/T$). For other

cases, a slight curvature was obtained as shown in Fig. 3b for Hg ($[\text{HCl}]/[\text{H}_2\text{O}_2]$). The decrease in the As and Co recoveries is also confirmed with the response surfaces obtained, such as the Fig. 3c for As ($[\text{HCl}]/[\text{HNO}_3]$). For other cases, quadratic terms were not significant, and curvature in the surface was not observed. An example, corresponding to Se ($[\text{H}_2\text{O}_2]/T$) can be seen in

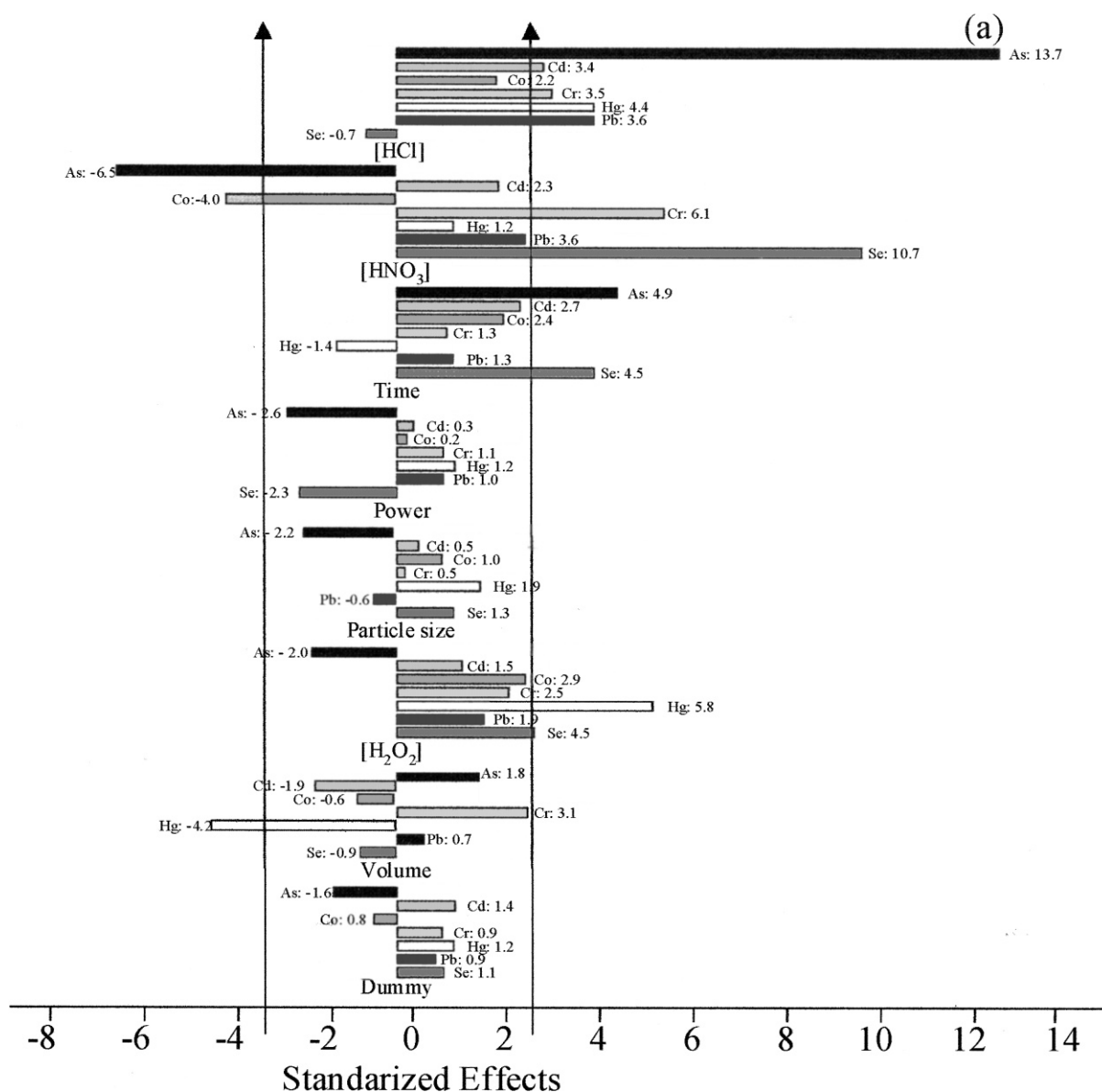


Fig. 1. Main effects combined Pareto chart in the Plackett–Burman design.

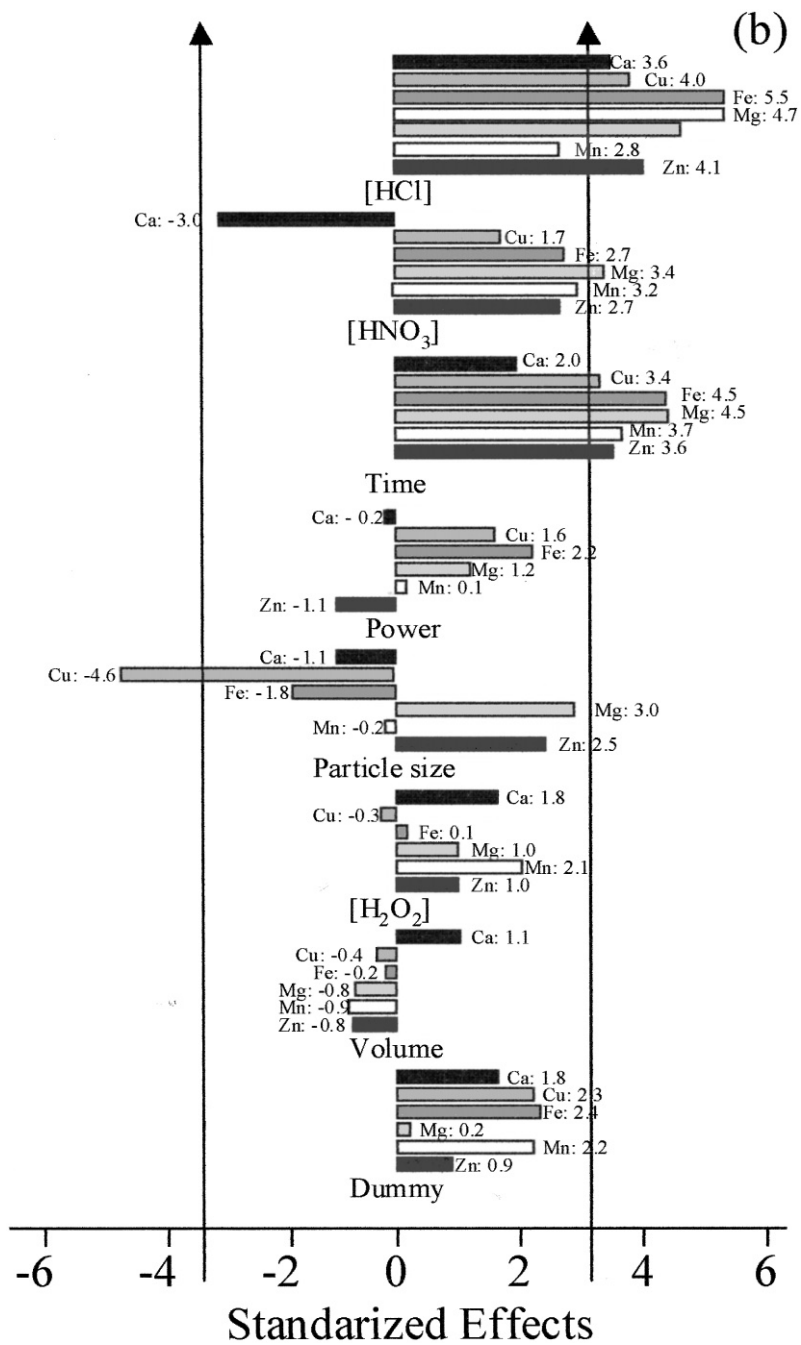


Fig. 1. (Continued).

Fig. 3d. In such cases, the maximum value for the variables were chosen as optimum, due to increases in acid concentration or exposure time

which would lead to acid digestion conditions. In addition, as it can be read in Tables 6 and 7, for some conditions (runs) quantitative recoveries

were reached. Finally, a response surface with two optima was also obtained (Fig. 3e). A response surface analysis with terms of the third order (not allowed by the software employed) would be desirable to find the real optimum. However, one of the optimums gives negative values for some variables, which is not a consis-

tent result. So, we have chosen the positive solution.

Given these findings, the working conditions to lead the metal acid extraction from mussel samples by a microwave pseudo-digestion process are shown in Table 8. Copper, Fe, Mg and Zn are leached in the same acid leaching solution, how-

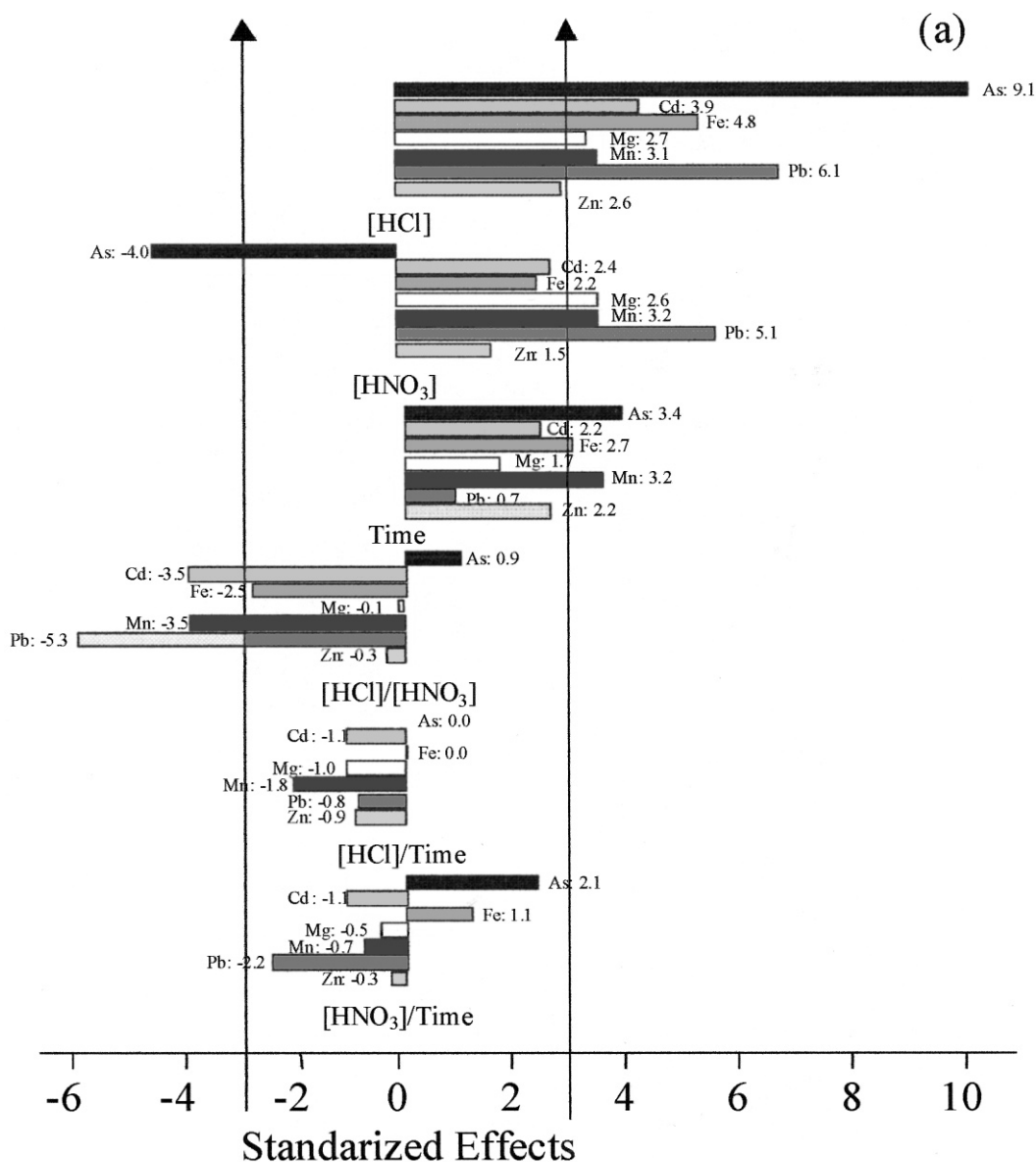


Fig. 2. Two order effect combined Pareto chart in the Plackett–Burman design for the $[\text{HNO}_3]/[\text{HCl}]/t$ set (a,b), the $[\text{H}_2\text{O}_2]/[\text{HCl}]/t$ set (c), and the $[\text{HNO}_3]/[\text{H}_2\text{O}_2]/t$ set (d).

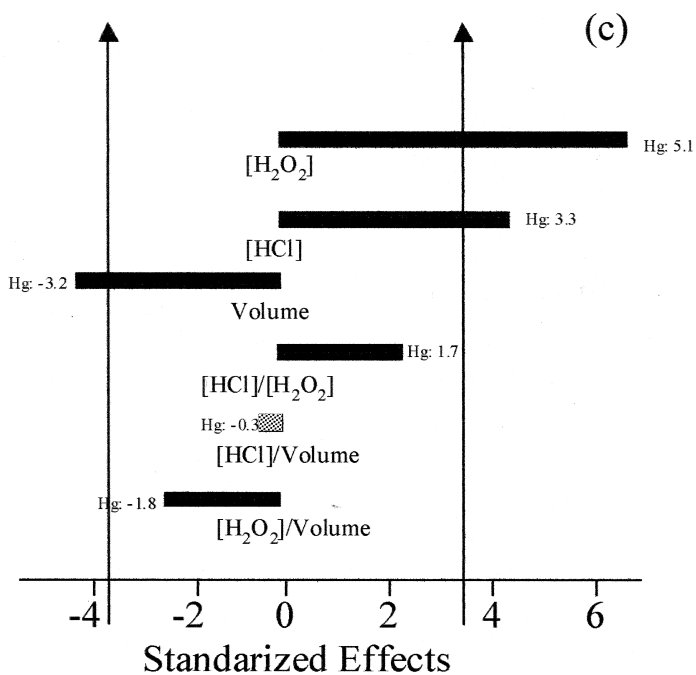
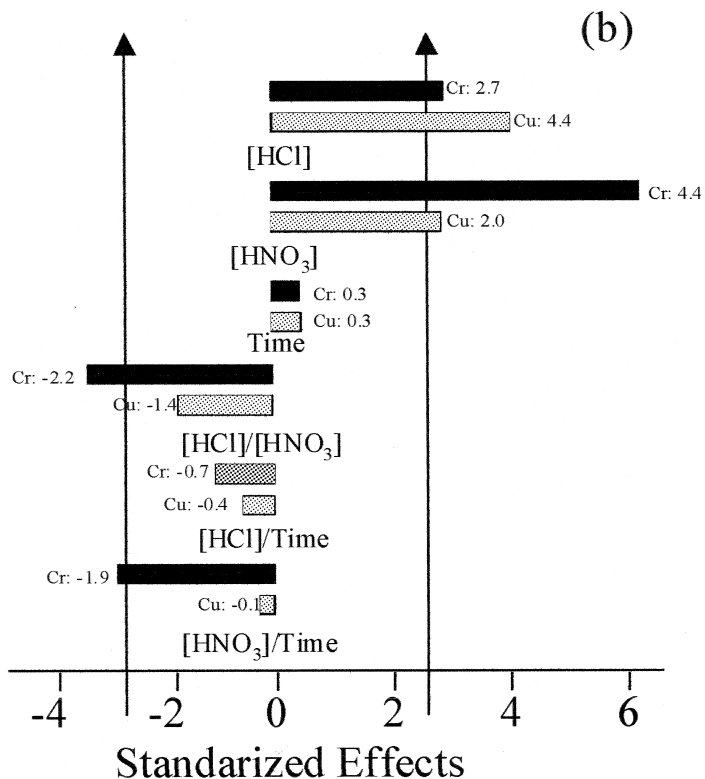


Fig. 2. (Continued).

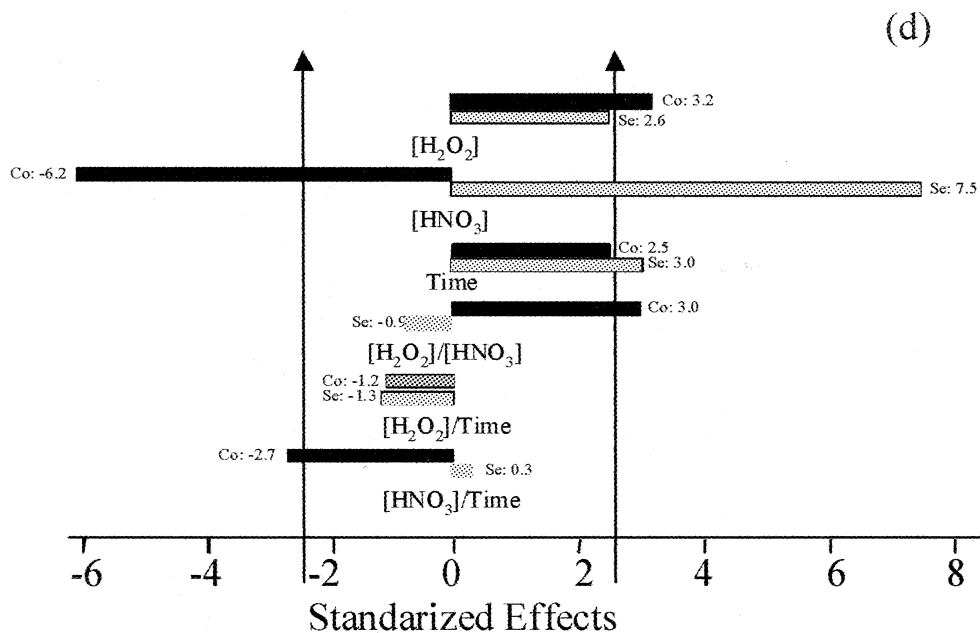


Fig. 2. (Continued).

ever, Ca, Cd, Cr, Mn and Pb require a more drastic conditions. Arsenic is extracted in hydrochloric acid medium, while the Co and Se acid

extraction needs hydrogen peroxide. A hydrochloric acid/hydrogen peroxide solution is chosen for Hg extraction.

Table 6

Central 2^3 + star orthogonal composite design ($n = 16$)^a

Run	[HNO ₃] (M)	[HCl] (M)	<i>t</i> (min)	Recovery (%)									
				As	Ca	Cd	Cr	Cu	Fe	Mg	Mn	Pb	Zn
1	K_o^*	K_o^*	K_o^{**}	90.7	74.3	81.3	58.6	92.1	81.6	79.7	85.0	96.0	97.0
2	—	—	—	69.9	21.3	39.1	30.4	30.8	51.1	38.7	17.3	21.5	56.2
3	+	—	—	53.1	64.2	82.8	51.0	92.1	61.7	77.7	65.4	82.3	86.8
4	—	+	—	105.2	56.6	79.0	50.6	76.3	51.4	88.1	77.5	120.3	86.8
5	+	+	—	85.3	53.6	47.6	42.6	108.1	92.8	78.0	57.7	72.4	100.1
6	—	—	+	79.8	31.7	60.3	30.9	15.3	10.1	46.1	23.3	57.0	56.5
7	+	—	+	54.9	53.1	93.1	60.1	76.9	66.3	86.3	79.3	76.2	97.2
8	—	+	+	110.4	105.8	91.0	36.3	77.9	81.4	89.7	74.7	92.8	89.4
9	+	+	+	69.8	74.5	94.0	45.0	92.3	97.2	99.3	78.4	103.5	101.0
10	$-\alpha^\dagger$	K_o^*	K_o^{**}	99.9	32.0	84.1	46.0	107.9	51.2	91.7	59.3	109.4	97.4
11	$+\alpha^\ddagger$	K_o^*	K_o^{**}	57.3	74.7	91.9	71.2	92.7	71.9	71.2	67.6	96.8	105.1
12	K_o^*	$-\alpha^\dagger$	K_o^{**}	40.3	121.3	80.1	52.9	61.8	25.7	82.8	39.2	64.9	120.1
13	K_o^*	$+\alpha^\ddagger$	K_o^{**}	48.7	74.6	62.5	33.1	77.0	51.2	71.0	39.1	63.3	89.9
14	K_o^*	K_o^*	$-\alpha^\S$	44.0	84.2	33.7	48.1	76.1	65.8	76.5	54.1	64.0	81.4
15	K_o^*	K_o^*	$+\alpha^\P$	65.7	115.3	82.5	39.6	92.5	102.5	77.5	67.8	96.4	82.5
16	K_o^*	K_o^*	K_o^{**}	49.0	85.6	78.7	56.8	92.5	87.5	84.3	85.8	78.0	71.5

^a K_o is the mean value for the variable: (*) $K_o = 2.4$ M and (**) $K_o = 1.25$ min; $\pm\alpha$ is the axial value (higher and low) for each variable: (†) $-\alpha = -0.689252$ M; (‡) $+\alpha = 5.489252$ M; and (§) $-\alpha = 0.284604$ min.; (¶) $+\alpha = 2.215391$ min.

Table 7

Central 2^3 + star orthogonal composite design ($n = 16$)^a

Run	[HNO ₃] (M)	[H ₂ O ₂] (M)	<i>t</i> (min)	Recovery (%)		[HCl] (M)	[H ₂ O ₂] (M)	<i>t</i> (min)	Recovery (%)
				Co	Se				Hg
1	K_o^*	K_o^*	K_o^*	5.0	53.3	K_o^*	K_o^*	K_o^{**}	77.9
2	—	—	—	7.8	24.5	—	—	—	0.9
3	+	—	—	33.1	25.5	+	—	—	30.8
4	—	+	—	60.5	58.0	—	+	—	0.9
5	+	+	—	37.0	51.3	+	+	—	95.1
6	—	—	+	52.0	24.5	—	—	+	1.8
7	+	—	+	40.7	45.2	+	—	+	81.5
8	—	+	+	71.4	78.1	—	+	+	11.9
9	+	+	+	66.9	95.7	+	+	+	80.3
10	$-\alpha^\dagger$	K_o^*	K_o^{**}	71.9	62.6	$-\alpha^\dagger$	K_o^*	K_o^{**}	0.9
11	$+\alpha^\ddagger$	K_o^*	K_o^{**}	15.5	63.2	$+\alpha^\ddagger$	K_o^*	K_o^{**}	52.7
12	K_o^*	$-\alpha^\dagger$	K_o^{**}	56.1	17.0	K_o^*	$-\alpha^\dagger$	K_o^{**}	10.9
13	K_o^*	$+\alpha^\ddagger$	K_o^{**}	49.0	54.2	K_o^*	$+\alpha^\ddagger$	K_o^{**}	93.6
14	K_o^*	K_o^*	$-\alpha^\S$	66.3	37.2	K_o^*	K_o^*	$-\alpha^\S$	51.2
15	K_o^*	K_o^*	$+\alpha^\P$	63.5	50.9	K_o^*	K_o^*	$+\alpha^\P$	81.7
16	K_o^*	K_o^*	K_o^{**}	36.5	45.6	K_o^*	K_o^*	K_o^{**}	61.9

^a K_o is the mean value for the variable: (*) $K_o = 2.4$ M and (**) $K_o = 1.25$ min; $\pm\alpha$ is the axial value (higher and low) for each variable: ($\dagger$) $-\alpha = -0.689252$ M; ($\ddagger$) $+\alpha = 5.489252$ M; ($\S$) $-\alpha = 0.284604$ min.; ($\P$) $+\alpha = 2.215391$ min.

8. Effect of the sample size on the efficiency of the microwave pseudo-digestion process

The effect of sample mass size on the efficiency of the acid extraction was studied in order to obtain a range of mussel mass suitable to carry out the procedure. A different mussel mass from the same mussel sample were taken within the 0.1–0.6 g range. Each sample mass was subject twice to the optimized process (different procedure for certain elements) and the metal concentration was determined, together with the metal content after a microwave acid digestion. Results are shown as a chart in Fig. 4a,b, and we can see that recoveries close to 100% are reached for metals such as Fe and Pb (Fig. 4a) or Ca and Mg (Fig. 4b) for all mass values studied. However, the acid extraction efficiency decreases moreover as the mussel mass is higher for elements such as Cd, Cu, Hg, and Mn (Fig. 4a), and As, Cr, Co, Se and Zn (Fig. 4b). For most of the metals (Cd, Cu, Hg, and Mn in Fig. 4a, and As and Zn in Fig. 4b) recoveries within the 90–110% range were reached for mass of 0.2 and 0.3 g, while, for Co, Cr and Se (Fig. 4b) high recoveries were obtained for mussel samples of 0.1 and 0.2 g. The decrease in the extraction efficiency when the sample mass

is increased could be explained through the microwave energy dissipation by the solid mussel mass into the PTFE bomb, and considering that the dissipation is proportional to the mussel mass. This effect is more important for some metals such as Cr, Co, and Se, and also As, Cd, Cu, Hg and Mn (Fig. 4a,b) than for other metals more easily extracted such as Ca, Mg or Fe.

It must be also noted that for Mn and Fe (Fig. 4a) recoveries higher than 110% were observed for a mussel mass of 0.1 g.

Therefore, and although 0.3 or 0.1 g can be a representative mass for many elements, a mass of 0.2 g was chosen due to the higher recovery for some elements (Cr, Co, or Se). This result is in agreement with the non-quantitative recoveries reached for some elements, mainly Cr and Se, in the Plackett–Burman and Central Composite designs (Tables 5–7), for which a sample mass of 0.3 g was used.

9. Optimization of the number of simultaneous pseudo-digestions (effect of the number of PTFE bombs)

Due to microwave energy interactions with the

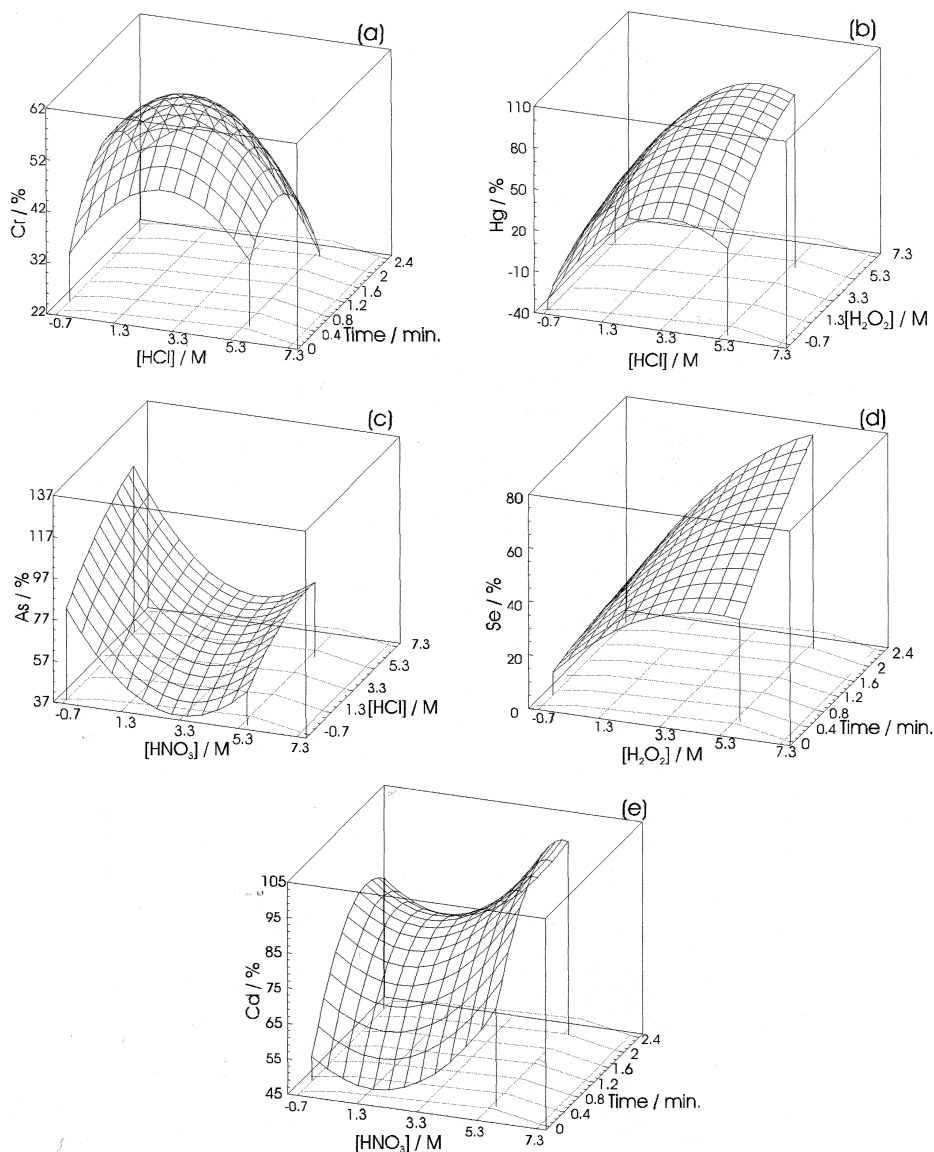


Fig. 3. (a–e) Some response surface estimated for the central composite designs (Tables 6 and 7).

solvent in the PTFE bombs and induction of the acid leaching from mussel, the amount of microwave energy that can interact with each bomb would vary in function with the number of bombs into the microwave oven (under fixed conditions of microwave power and exposure time). For this, the effect of the number of bombs on the efficiency of the procedure was studied. Several acid extractions were prepared from the same

mussel sample that was subjected to the optimized procedure three, four, five and six times (three, four, five and six bombs into the microwave oven). Results for some elements, expressed as recovery (%), are plotted in Fig. 5. We can see that the number of bombs does not affect element recoveries, so the procedure is suitable for the number of bombs ranging from three to six. This result offers a practical advantage due to

the fact that we can treat three different samples (twice each one) with a microwave oven cycle.

10. Analytical figures of merit

Four-point standard addition or aqueous calibration in suitable ranges according to sensitivity for each metal and each atomic absorption spectrometric technique, were performed daily. We used the standard addition technique in order to avoid possible differences due to the different acid composition of each experiment. The mean slopes of the standard addition graphs, expressed as $\bar{x} \pm \text{S.D.}$ ($1 \mu\text{g}^{-1}$), for $n = 9$, where $\bar{x}$ is the mean slope and S.D. is the standard deviation, were as follows: 0.0024 ± 0.0007 , for As; 0.070 ± 0.017 , for Cd; 0.0061 ± 0.0003 , for Co; 0.0086 ± 0.0015 , for Cr; 0.0064 ± 0.0001 , for Hg; 0.023 ± 0.005 , for Mn; 0.0046 ± 0.0014 , for Pb; and 0.0036 ± 0.0004 , for Se. For FAAS determinations, the

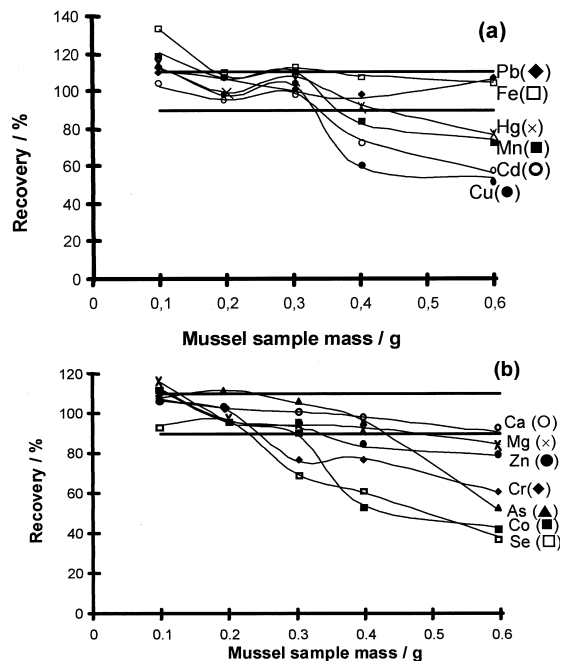


Fig. 4. Effect of the mussel sample mass on the acid leaching efficiency.

Table 8

Optimum conditions for the metals acid extraction from mussel samples by a microwave pseudo-digestion

	[HNO ₃] (M)	[HCl] (M)	Time (min)
Ca, Cd, Cr, Mn, Pb ^a	4.5–5.0	2.8–3.5	2.0
Cu, Fe, Mg, Zn	4.0–4.5	3.3–3.8	1.5
As ^b	–	4.5–5.0	2.0
	[HNO ₃] (M)	[H ₂ O ₂] (M)	Time (min)
Co ^c	1.0	4.5–5.0	2.0
Se	4.5–5.0	4.5–5.0	2.0
	[H ₂ O ₂] (M)	[HCl] (M)	Time (min)
Hg ^d	2.0	3.3–3.5	1.75

^a[H₂O₂], 0.5 M; acid solvent volume, 5 ml; microwave power, 64 W; particle size, 200 μm (mussel sample without pulverization); sample mass, 0.2 g.

^b[H₂O₂], 0.5 M; acid solvent volume, 5 ml; microwave power, 64 W; particle size, 200 μm (mussel sample without pulverization); sample mass, 0.2 g.

^c[HCl], 0.5 M; acid solvent volume, 5 ml; microwave power, 64 W; particle size, 200 μm (mussel sample without pulverization); sample mass, 0.2 g.

^d[H₂O₂], 0.5 M; [HNO₃], 0.5 M microwave power, 64 W; acid solvent volume, 5 ml; particle size, 200 μm (mussel sample without pulverization); sample mass, 0.2 g.

values expressed (1 mg^{-1}) were: 0.045 ± 0.02 , for Ca; 0.054 ± 0.015 , for Cu; 0.052 ± 0.017 , for Fe; 0.99 ± 0.12 , for Mg; and 0.42 ± 0.11 , for Zn. As it can be seen, good precision was obtained for all cases.

The sensitivity was studied by means of the detection (LOD) and quantification (LOQ) limits, defined as

$$\text{LOD} = 3 \frac{\text{S.D.}}{m}$$

and

$$\text{LOQ} = 10 \frac{\text{S.D.}}{m},$$

where S.D. is the standard deviation of 11 measurements of a blank, and m is the slope of the standard addition graph. In addition, the sensitivity, defined in FAAS as the analyte concentration which offers an absorbance signal of 0.0044 units

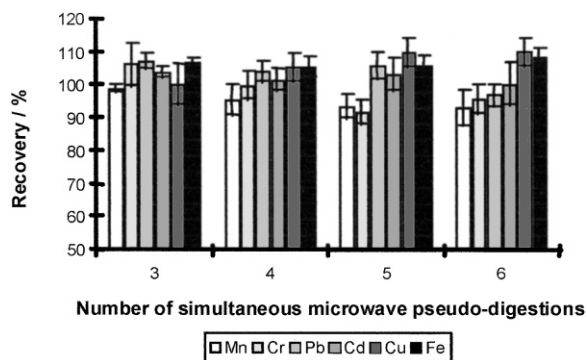


Fig. 5. Effect of the number of PTFE bombs on the extraction efficiency by microwave pseudo-digestion.

was also determined. The characteristic mass, defined for ETAAS as

$$m_0 = \frac{CV0.0044}{Q_{AC} - Q_{Ab}}$$

where C is the analyte concentration, V is the injection volume, and Q_{Ab} and Q_{AC} are the integrated absorbance for a blank and a concentration level, respectively, was also studied. Results are summarized in Table 9, and adequate sensitivity for our purposes was reached.

The repeatability of the procedure was studied by determining each element in a mussel sample that was subjected 11 times to the optimized

process also showed in Table 9 for each case. Results, expressed as R.S.D.s, varied between 12.9%, for Cd, and 5.4% for Pb.

11. Analysis of certified reference materials

Three different certified reference materials, DORM-1 (dogfish muscle), DOLT-1 (dogfish liver) and TORT-1 (lobster hepatopancreas), were used to assess the accuracy of the processes. These materials offer certified concentration (high, medium and low values) for all metals, except for Ca. Each reference material was subjected three times to each optimized procedure, which involves the use of a sample mass of 0.2 g in order to guarantee quantitative results. The metal was determined three times in each acid extract. Results are given in Table 10, where each found value is corresponding to nine replicates and it is expressed as $\bar{x} \pm \text{S.D.}$ In general, we can see that good accuracy is obtained for all metals in the reference materials under study. However, we cannot conclude that metals are quantitatively soluble in the optimized acid solution and under the optimized conditions at least all the elements have been transferred into the solution. In order to prove that the metal recovery was obtained for each metal and from each reference material. In

Table 9
Sensitivity and repeatability of the methods

	LOD ($\mu\text{g g}^{-1}$)	LOQ ($\mu\text{g g}^{-1}$)	Sensitivity (mg l^{-1})	m_0 (pg) ^a	R.S.D. (%) ^b
As	0.30	1.01	—	14.3 ± 0.3	8.3
Ca	0.7	2.4	0.10	—	5.2
Cd	0.03	0.11	—	0.34 ± 0.02	12.9
Co	0.05	0.16	—	5.8 ± 0.2	8.8
Cr	0.04	0.12	—	3.5 ± 0.2	7.5
Cu	0.04	0.10	0.08	—	6.3
Fe	0.3	1.3	0.08	—	6.8
Hg	0.05	0.17	—	—	6.1
Mg	0.1	0.3	0.04	—	6.6
Mn	0.43	1.44	—	2.3 ± 0.3	5.6
Pb	0.73	2.43	—	10.5 ± 0.4	5.4
Se	0.31	1.04	—	23.4 ± 0.6	7.0
Zn	0.07	0.2	0.01	—	5.9

^a $n = 4$.

^b Repeatability of the overall procedure (acid leaching and AAS measurement) for $n = 11$.

Table 10
Analysis of certified reference materials

	DORM-1		DOLT-1		TORT-1	
	Certified value ($\mu\text{g g}^{-1}$)	Found value ($\mu\text{g g}^{-1}$) ^a	Certified value ($\mu\text{g g}^{-1}$)	Found value ($\mu\text{g g}^{-1}$) ^a	Certified value ($\mu\text{g g}^{-1}$)	Found value ($\mu\text{g g}^{-1}$) ^a
As	17.7 ± 2.1	16.6 ± 0.5	10.1 ± 1.4	9.5 ± 0.3	24.6 ± 2.2	23.3 ± 1.1
Ca ^b	—	—	—	—	0.895 ± 0.058	0.876 ± 0.018
Cd	0.086 ± 0.012	0.079 ± 0.005	4.18 ± 0.28	3.98 ± 0.10	26.3 ± 2.1	25.4 ± 0.8
Co	0.049 ± 0.014	< 0.05	0.157 ± 0.037	0.170 ± 0.005	0.42 ± 0.05	0.45 ± 0.01
Cr	3.60 ± 0.40	3.52 ± 0.11	0.40 ± 0.07	0.41 ± 0.02	2.4 ± 0.6	2.3 ± 0.1
Cu	5.22 ± 0.33	5.06 ± 0.10	20.8 ± 1.2	20.0 ± 0.5	439.7 ± 22.0	435.3 ± 4.2
Fe	63.6 ± 5.3	61.6 ± 1.2	712 ± 48	697.3 ± 5.6	186 ± 11	179.8 ± 1.3
Hg	0.798 ± 0.074	0.765 ± 0.004	0.225 ± 0.037	0.208 ± 0.012	0.33 ± 0.06	0.32 ± 0.03
Mg ^b	0.121 ± 0.013	0.117 ± 0.006	0.110 ± 0.015	0.106 ± 0.004	0.255 ± 0.025	0.237 ± 0.011
Mn	1.32 ± 0.26	1.36 ± 0.09	8.72 ± 0.53	8.65 ± 0.12	23.4 ± 1.0	22.1 ± 0.4
Pb	0.40 ± 0.12	0.32 ± 0.04	1.36 ± 0.29	1.43 ± 0.06	10.4 ± 2.0	10.3 ± 0.8
Se	1.62 ± 0.12	1.55 ± 0.02	7.34 ± 0.42	7.37 ± 0.08	6.88 ± 0.47	6.95 ± 0.07
Zn	21.3 ± 1.0	20.8 ± 0.3	92.5 ± 2.3	91.8 ± 1.2	177 ± 10	171 ± 3

^a Expressed as $\bar{x} \pm \text{S.D.}$

^b Expressed in % (m/m).

Table 11 are listed the mean recovery, expressed as $\bar{x} \pm \text{S.D.}$ where as $\bar{x}$ and S.D. are the mean recovery and the standard deviation of nine determinations. The recovery was calculated according to the equation

$$\% \text{ Recovery} = \frac{[\text{microwave pseudo-digestion}]}{[\text{certified concentration}]} \times 100$$

where $[\text{certified concentration}]$ and $[\text{microwave pseudo-digestion}]$ are the mean certified metal concentration, and the mean metal concentration after the pseudo-digestion process, respectively. As it can be seen, most of the recoveries are within the 90–110% range, although recoveries lower than 95% were reached for metals such as As, Cd, Cr and Hg. A mean recovery of 80.0% was obtained for Pb from DORM-1 reference material, which is lower in comparison to the mean recoveries reached from the other reference materials (105.0 and 99.0% for DOLT-1 and TORT-1, respectively). This fact indicates that Pb is not quantitatively soluble in the acid solution, although the Pb concentration found is within the certified concentration range. This low recovery can be due to the low Pb concentration in DORM-1 ($0.40 \pm 0.12 \mu\text{g g}^{-1}$), in comparison to 1.36 ± 0.29 and $10.4 \pm$

$2.0 \mu\text{g g}^{-1}$ for DOLT-1 and TORT-1, respectively. That can lead to obtain low analyte recoveries from mussel samples with low analyte levels. This fact was also observed for Cd (91.9% from DORM-1), and it was less important for the other

Table 11
Mean recoveries ($n = 9$) from certified reference materials (DORM-1, DOLT-1 and TORT-1)

	Recovery (%) ^a		
	DORM-1	DOLT-1	TORT-1
As	93.8 ± 2.8	94.1 ± 3.0	94.7 ± 4.5
Ca	—	—	97.9 ± 2.0
Cd	91.9 ± 5.8	95.2 ± 2.4	96.6 ± 3.0
Co	—	108.3 ± 3.2	107.1 ± 2.4
Cr	97.8 ± 3.1	102.5 ± 5.0	95.8 ± 4.2
Cu	96.9 ± 1.9	96.1 ± 2.4	99.0 ± 0.9
Fe	96.8 ± 1.9	97.9 ± 0.8	96.7 ± 0.7
Hg	95.9 ± 0.5	92.4 ± 5.3	97.0 ± 9.1
Mg	96.7 ± 5.0	96.4 ± 3.6	92.9 ± 4.3
Mn	103.0 ± 6.8	99.2 ± 1.4	94.4 ± 1.7
Pb	80.0 ± 10.0	105.2 ± 4.4	99.0 ± 7.7
Se	95.7 ± 1.2	100.4 ± 1.1	101.0 ± 1.0
Zn	97.6 ± 1.4	99.2 ± 1.3	96.6 ± 1.7

^a The recovery expressed as $\% \text{ Recovery} = \frac{[\text{microwave pseudo-digestion}]}{[\text{certified concentration}]} \times 100$, where $[\text{certified concentration}]$ and $[\text{microwave pseudo-digestion}]$ are the mean certified metal concentration, and the mean metal concentration after the acid leaching process, respectively.

elements due to the relatively high concentration in the certified reference materials studied.

Therefore, we can see that although metal levels found in the certified reference materials agree with the certified concentrations, this is not a guarantee that all the metals are quantitatively extracted from the acid solution.

12. Conclusions

Microwave pseudo-digestion has been demonstrated to be a rapid sample pre-treatment procedure to multi-element analysis of mussel samples. At least six simultaneous pseudo-digestions (three different samples, twice each one) can be carried out in one run in less than 30 min (approx. 2 min. for the microwave pseudo-digestion process, 5 min for the cooling stage and dilution and 20 min for the centrifugation stages), as compared with 1 h for conventional microwave-acid digestion procedures.

It must be said that some quantitative recoveries obtained for certain elements have to be attributed to conditions close to a microwave total acid digestion. This is the explanation about the high recoveries reached for some elements, which are higher than the one found in some works dealing with pseudo-digestion procedures using microwave energy [3,7,8] or ultrasounds [5].

The sample mass was found as an important variable, and the extraction efficiency for most of the elements is reduced further when the sample mass is higher. A mass of 0.2 or 0.3 g can be used for the extraction of most of the elements, however, Cr and Se recoveries close to 90% are only reached for a sample mass of 0.2 g. Therefore, 0.2 g was chosen as representative mass to extract all the elements studied, however, 0.3 g can be also used for some elements such As, Cd, Mn, etc., and even 0.4 or 0.5 g can be used for Ca, Mg, Pb and Fe.

Experimental designs resulted in powerful techniques as a means of optimization for sample pre-treatments as an acid leaching procedure, for which many variables are involved. Variables such as particle size or microwave power, which were firstly taken into account as important variables,

resulted not significant, and other ones, such the exposure time to microwave energy, were optimized conveniently.

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