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## Determination of Ni, Cr, Cu, Pb and Cd on the Mediterranean endemic plant *Posidonia oceanica* using the green extraction method “Microwave Assisted Micellar Extraction” and GFAAS

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An effort is currently being made to reduce the acid concentrations in traditional methodologies for the analysis of metals based on green chemistry. However, it becomes necessary to develop alternative extraction and analytical environmentally friendly methods that completely avoid the use of acids. In this sense, a Microwave Assisted Micellar Extraction (MAME) method was developed, which employs biodegradable surfactants as extractants. On the other hand, *Posidonia oceanica* has demonstrated its potential as a bioindicator for metal pollution in the marine environment. Therefore, a new green method has been tested for the extraction of heavy metals (Ni, Cr, Cu, Pb, Cd) from *Posidonia oceanica*, by using a mixture of biodegradable surfactants: Sodium Dodecyl Sulphate (SDS) and Triton X-100 as extractants and analysis by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The proposed method showed, in general, satisfactory recovery percentages, RSD below 7.29%, and LOD ranging from 0.04 to 0.22  $\mu\text{g g}^{-1}$ . On the other hand, the proposed method was applied to aged *Posidonia oceanica* as well as different macrophyte samples. The results obtained were compared to those from ISO 11047:1998.

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### Introduction

Despite the lack of consensus about a clear definition of “heavy metal”, the following elements are accepted as heavy metals: arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, tin and zinc, as suggested by Hübner *et al.*<sup>1</sup>

Heavy metals considered in this study (Ni, Cr, Cu, Pb and Cd) are also categorized as priority pollutants by the U.S. Environmental Protection Agency.<sup>2</sup> They have proven to be toxic and harmful to various marine organisms through the food chain<sup>3,4</sup> and present mutagenic and/or carcinogenic properties.<sup>5</sup> On the other hand, bioindicators are very interesting tools for the detection of the environmental pollution induced by the presence of metals due to the extremely low concentration of these elements in the marine environment.

In this sense, *Posidonia oceanica*, an endemic plant of the Mediterranean Sea, has been considered for some decades an important indicator of water quality, as ruled by the European Union through the Water Framework Directive 2000/64/EC, and particularly as a bioindicator for contamination by metals.<sup>3,6–11</sup>

*Posidonia oceanica* bioaccumulates metals according to their presence in the environment, offering likewise good resistance to metal pollution.<sup>12,13</sup> This plant accumulates metals mainly in its leaves.<sup>6–9,11,14–17</sup> It loses its leaves every 5–8 months,<sup>18,19</sup> which could act as a natural pathway for detoxification. Hence, the best parts of the plant for the extraction and analysis of metals are either the limbs of the leaves or the scales, depending on whether interested in recent or old pollution analyses, respectively (lepidochronological studies).<sup>11,17,20</sup>

On the other hand, the traditional methods employed for extraction and analysis of metals in solid matrices employ highly toxic and corrosive extractants such as strong acids. These methods are applied for long periods (12 h) and high temperatures (180 °C) to achieve complete removal of metals, a process known as acid digestion.<sup>21</sup>

This methodology can be combined with microwave energy, a process known as microwave assisted digestion,<sup>22,23</sup> for heating samples faster and hence shortening extraction times (5–10 min).<sup>24,25</sup>

Furthermore, this methodology allows simultaneous extraction of several samples in a single step, thus reducing the total time of extraction,<sup>26</sup> a smaller amount of both sample and extractant is required,<sup>21,27</sup> and ensures greater extraction efficiency as compared with other traditional digestion methods.<sup>28</sup> Nevertheless, strong acid mixtures are used when applying microwave digestion to the extraction of metals.

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In this sense, a variation of the microwave assisted digestion method employs diluted  $\text{HNO}_3$  solutions.<sup>29–31</sup> This alternative method generates less waste, leads to lower standard deviations and does not require high dilution factors before analyte measurements. Nevertheless, the use of diluted solutions of nitric acid involves the generation of  $\text{NO}$ ,  $\text{NO}_3^-$  and other types of organic residues.<sup>29–31</sup>

Surfactants are safer and environmentally friendly alternatives, as they are not toxic, not volatile, not easily flammable<sup>32</sup> and are also biodegradable.<sup>33,34</sup>

Using surfactants as extractants for metals avoids the use of toxic and corrosive extractants in a microwave assisted extraction technique that may be termed as Microwave Assisted Micellar Extraction (MAME).<sup>35</sup> Surfactants as extractants for metals has already been tested in aqueous samples with satisfactory results,<sup>36,37</sup> though we have not found any reference whether metals in solid matrices are either extracted or determined.

Previous studies focused on extracting metals in this matrix, which are never explored obtaining an environmentally friendly methodology.

Moreover, surfactants generally used for metal extraction are anionic surfactants such as Sodium Dodecyl Sulphate (SDS) which has the ability to extract metals by ionic interactions.<sup>32,36,38</sup> Non-ionic surfactants are also used, such as Triton X-100 or Triton X-114, due to their hydrophobic interaction with metals.<sup>32,36,38</sup> Finally, combinations of these two types of surfactants are also used due to their improved synergistic action as shown in various studies.<sup>32,38,39</sup>

Therefore, our goal is the optimization of the green methodology MAME for the extraction of heavy metals (Ni, Cr, Cu, Pb and Cd) in *Posidonia oceanica* and its further determination by GFAAS.

## Materials and methods

### Reagents

All reagents are provided by PANREAC (Barcelona, Spain). Heavy metal standards (Ni, Cr, Cu, Pb and Cd) are of  $1000 \text{ g L}^{-1} \pm 0.002 \text{ g L}^{-1}$ . Stock solutions of each metal are prepared at  $30 \mu\text{g L}^{-1}$ , except Cd which is prepared at  $3 \mu\text{g L}^{-1}$ , with  $\text{HNO}_3$  Hyperpure (1%, v/v) and ultra-high quality water.

A standard solution of palladium (MERCK, Darmstadt, Germany) and a solution of ammonium phosphate,  $\text{NH}_4\text{H}_2\text{PO}_4$ , (PANREAC, Barcelona, Spain) are used as chemical matrix modifiers in metal determination by GFAAS. The palladium modifier is prepared at a concentration of 100 and 500 ppm for Cd and Pb, respectively, with  $\text{HNO}_3$  Hyperpure (1%, v/v) and ultra-high quality water. The ammonium phosphate modifier is prepared at 10% (v/v), with  $\text{HNO}_3$  Hyperpure (1%, v/v) and ultra-high quality water. The anionic (Sodium Dodecyl Sulphate, SDS) and the non-ionic (Triton X-100) surfactants are prepared in ultra-high quality water. The buffers employed in this study are the following: phosphoric acid/monopotassium phosphate, sodium acetate/acetic acid, monopotassium phosphate/dipotassium phosphate, ammonium chloride/ammonia and potassium chloride/sodium hydroxide. These buffers stabilize the

pH at values of 2, 4, 7, 9 and 12 respectively. These buffers are also prepared in ultra-high quality water.

### Apparatus

The microwave system used to perform the microwave assisted extraction process is a CEM® Xpress chamber (CEM Corporation, Matthews, NC, USA), with a rotor of 16 CEM® Xpress Teflon vessels (CEM Corporation, USA) and termstrips KAGER GmbH model C (Kager Industrietechnik, Germany) for temperature control.

The Atomic Absorption Spectrometer is provided by VARIAN® Model AA240Z (Agilent Technologies, USA), with a longitudinal Zeeman effect background correction system furnished with a graphite tube atomizer (GTA 120). Sample solutions are injected into the atomizer by using a program sampler dispenser (PSD 120).

An electrical accurate balance (Ohaus Model PA214C with a functioning range of 0.0001–210 g) is used to prepare all solutions.

Samples are lyophilized using a SENTRY VERTIS lyophilizer, and sieved using a Sieve Shaker CISA model RP-80.

### Procedure

**Sample preparation.** *Posidonia oceanica* leaves are frozen, lyophilized at  $-53 \text{ }^\circ\text{C}$  for 18 h, homogenized and crushed in a domestic breaker and sieved. A fraction of 250  $\mu\text{m}$  to 1 mm is selected.

**Spiking of samples.** Fifty milligrams of sample are spiked with the metal mixture containing Ni, Cu, Cr, Pb at  $14.3 \text{ mg L}^{-1}$  and Cd at  $1.43 \text{ mg L}^{-1}$ ; later on, samples are homogenized and stored for 24 hours in order to obtain a dry and homogeneous sample.

**Microwave assisted micellar extraction.** Fifty milligrams of spiked *Posidonia oceanica* leaves are transferred to the Teflon digestion vessels. Fifty milligrams of non-spiked samples are also used to produce the blank samples. Then, 5 mL of acetic acid/sodium acetate buffer (pH 4) and 5 mL of the surfactant mixture composed of anionic surfactant SDS 1.25% (w/v) and non-ionic Triton X-100 0.1% (v/v) are added. The vessels are sealed and placed into the CEM® Xpress chamber for 10 min at 1600 W. The content of these vessels is then filtered with 10 mL sterile plastic syringes with cellulose acetate filters to avoid any possible contamination during the filtration process.

**Atomic absorption analysis.** The extracts obtained after filtration are diluted to 1 : 10 in  $\text{HNO}_3$  Hyperpure (1%, v/v) and analyzed using a VARIAN® Model AA240Z (Agilent Technologies, USA) Atomic Absorption Spectrometer, with a longitudinal Zeeman Effect background correction system furnished with a graphite tube atomizer (GTA 120). Graphite OMEGA Platform Tubes and GTA Tubes Partitioned Pyro-coated from Agilent Technologies Spain, S.L. were used in the GTA. Sample solutions are injected into the atomizer by using the program sampler dispenser (PSD 120). The standard solution of palladium is used as the chemical matrix modifier in the determination of metals Cd and Pb, at concentrations of 100 and

**Table 1** Spectroscopic conditions for the analysis of the heavy metals under study

Analyte	MAME-GFAAS $\lambda$ (nm)	Temperature ramp		Matrix modifier	Graphite tube
		Drying and pyrolysis step	Atomization step		
Ni	232.0	1100 °C/17 s	2500 °C/5 s	No	No platf.
Cr	357.9	1100 °C/12 s	2600 °C/5 s	No	No platf.
Cu	327.4	900 °C/8 s	2300 °C/5 s	No	No platf.
Pb	283.3	600 °C/17 s	2100 °C/3 s	Pd (500 ppm)	No platf.
Cd	228.8	700 °C/22 s	2100 °C/5 s	Pd (100 ppm)	Platform

500 ppm respectively. The heavy metals are analyzed under the optimized analytical conditions given in Table 1. Hollow cathode lamps operate with lamp current in the range of 5–10 mA. Argon is used as protective and purge gas.

**Method validation, ISO 11047:1998.** In order to validate the optimized method, it was compared with results obtained using an adaptation of the method established by the International Organization for Standardization (ISO 11047:1998) based on MAE for the extraction of metals and GFAAS as the determination technique. Samples, 0.5 g each, of spiked *Posidonia oceanica* leaves were transferred to the Teflon digestion vessels CEM® Xpress. Then, 6 mL of pure HCl (37%, v/v) and 2 mL of hyperpure HNO<sub>3</sub> (65%, v/v) were added. The vessels were sealed and placed into a CEM® Xpress chamber in a program based on control of time and temperature, for 30 min at 190 °C. The content of vessels was then filtered in a vacuum filtration system, and transferred to a flask of 100 mL, the concentration of HCl being 21%, v/v and HNO<sub>3</sub> 7%, v/v. 500  $\mu$ L of the extracts obtained are diluted in HNO<sub>3</sub> Hyperpure (1%) and taken to a final volume of 1500  $\mu$ L to be then analyzed using a VARIAN® Model AA240Z (Agilent Technologies, USA) Atomic Absorption Spectrometer, with a longitudinal Zeeman effect background correction system furnished with a Graphite Tube Atomizer GTA 120. Sample solutions are injected into the atomizer by using the program sampler dispenser. The metals are analysed under the optimized analytical conditions (Table 2). Hollow cathode lamps are operated with lamp current in the range of 5–10 mA. Argon is used as protective and purge gas.

**Table 2** Spectroscopic conditions for the analysis of heavy metals according to an ISO 11047:1998 method

Analyte	$\lambda$ (nm)	Temperature ramp		Matrix modifier	Graphite tube
		Drying and pyrolysis step	Atomization step		
Ni	232.0	900 °C/8 s	2650 °C/5 s	No	No platf.
Cu	327.4	800 °C/17 s	2300 °C/3 s	No	No platf.
Cr	357.9	1000 °C/13 s	2600 °C/5 s	No	No platf.
Pb	283.3	400 °C/20 s	2200 °C/3 s	Pd (500 ppm)	Platform
Cd	228.8	500 °C/19 s	1800 °C/5 s	Pd (500 ppm)	No platf.

## Results and discussion

### Optimization GFAAS analysis

The following variables are considered in optimizing the dependent variables for the analysis of metals: wavelength of maximum absorbance for each metal, furnace temperatures during drying, pyrolysis step (600 °C/110 °C) and during the atomization step (2100 °C/2600 °C), using or not using platform tubes and finally using or not using various matrix modifiers and their concentrations.

In the case of using platform tubes, some metals such as Pb and Cd are particularly sensitive to chemical interference when analysed by Atomic Absorption Spectroscopy. These interferences can be minimized by using platform atomization techniques and the appropriate matrix modifiers.

Therefore, analyses were performed for each metal with and without platform tubes. Results under optimized conditions are shown in Table 1.

In the case of Cd, the platform tubes gave the best results, providing a cleaner analytical signal. This is due to the effect of the platform, which allows the platform during atomization temperature to lag the furnace wall temperature by several hundred degrees. Under these conditions, the analytic compounds are not vaporized until the furnace wall and gaseous environment have approached steady-state temperature. This minimizes any chemical interferences.<sup>40,41</sup>

On the other hand, analyses were performed using two matrix modifiers: Pd modifier at two concentrations (100 ppm and 500 ppm) and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> modifier at 10% (v/v) in HNO<sub>3</sub> Hyperpure (1%, v/v). Optimized results are shown in Table 1. A cleaner signal was obtained when a Pd modifier was used in the case of Cd and Pb at 100 and 500 ppm concentrations, respectively. This is because the matrix modifier can decrease the volatility of the analyte or increase the volatility of a sample matrix component. The element could be prematurely lost in the graphite furnace temperature cycle, but with the addition of the matrix modifier, Cd and Pb can be stabilized at higher temperatures. This can greatly reduce matrix interferences.<sup>42–44</sup>

In the case of Cd, Pd matrix modifier concentration is lower than that of Pb, since at higher concentrations interferences occur. This is due to the high sensitivity of this analytical technique to determine Cd, which requires working with concentrations an order of magnitude lower than that of other metals, which results in a high probability of interferences.

### Optimization of Microwave Assisted Micellar Extraction

Since this work has been performed with *Posidonia oceanica* real matrices, blank samples are prepared under all conditions for the variables indicated in the next sections. We do so to eliminate the possible noise introduced by the matrix as well as the initial concentration of metal in the matrix.

**Effect of pH.** The effect of pH on the heavy metal recoveries is determined by the analysis of the spiked samples when using only SDS solution (1.25%, w/v) as the extractant at different pH values. Each case is achieved by adding 5 mL of a buffer solution with 2, 4, 7, 9 and 12 pH values and 5 mL of the surfactant

solution. The samples are irradiated in the microwave chamber at 400 W for 5 minutes and analyzed in GFAAS after their filtration.

Fig. 1 shows that the general trend in the recoveries increased until pH 4, whereas for higher pH values recovery either decreases (Cr, Pb) or shows a slight increase at pH 9 (Cu, Cd and Ni); in any case, the highest recoveries are obtained at pH 4.

This behaviour probably occurs because at higher acidities saturation occurs at anionic SDS micelles due to the high presence of  $H^+$  ions, making them catalytically ineffective as shown by some studies.<sup>45</sup>

On the other hand, many metals have reduced solubility at basic pH values. In the case of Cu, for example, this is probably due to the hydrolysis effect and osmotic changes in the cell walls of organic matrices.<sup>46</sup> Another example is the case of Ni, which at  $pH > 5$  precipitates as  $Ni(OH)_2$ , reducing the amount of free Ni ions.<sup>47</sup>

Therefore, pH 4 is taken as optimum for the extraction, corresponding to the buffer solution of acetic acid/sodium acetate.

**Effect of SDS concentration.** In order to determine the effect of Sodium Dodecyl Sulphate (SDS) concentration on the recovery percentage, several samples containing different SDS concentrations are analyzed: 0.25, 1.25, 2 and 2.5% (w/v), in all cases over Critical Micellar Concentration (CMC = 0.1728%, w/v). The spiked samples are extracted with 10 mL of buffer with pH 4 and surfactant solution in the microwave system at 400 W for 5 minutes. The recoveries obtained for the metals under study can be seen in Fig. 2.

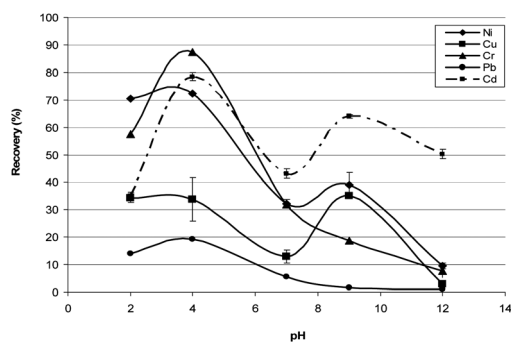


Fig. 1 Effect of the pH solution on the recovery of the heavy metals, when the SDS concentration was 1.25% (w/v) and the extractant volume was 10 mL ( $n = 3$ ).

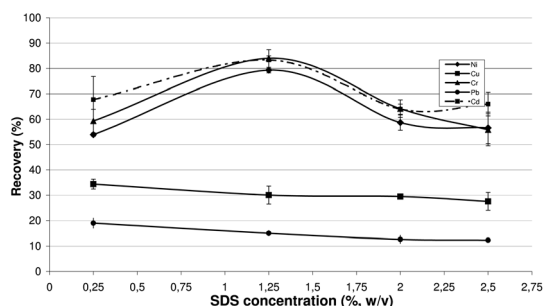


Fig. 2 Effect of the surfactant SDS concentration on the recovery of the heavy metals, when the pH was fixed at 4 and the extractant volume was 10 mL ( $n = 3$ ).

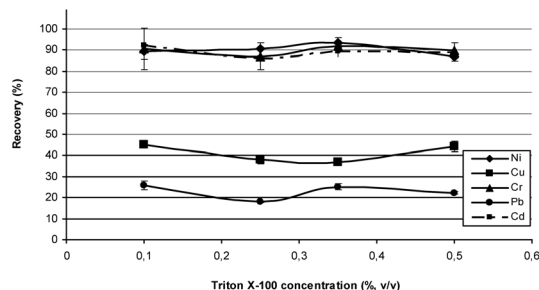


Fig. 3 Effect of the surfactant Triton X-100 concentration on the recovery of heavy metals, using 10 mL of buffer pH = 4 and SDS (1.25%, w/v) ( $n = 3$ ).

Fig. 2 shows that recovery percentages increase for Ni, Cr and Cd up to 79, 85 and 84%, respectively, when the SDS concentration increases until 1.25% (w/v); on the other hand, Cu and Pb recoveries remain constant or decrease slightly with increasing SDS concentration. Therefore, the optimal value taken for SDS concentration in the surfactant mixture is 1.25% (w/v).

**Effect of Triton X-100 concentration.** In order to determine the effect of Triton X-100 concentration on the recovery percentage, several samples containing SDS (1.25%, w/v) and different Triton X-100 concentrations are analyzed: 0.1, 0.25, 0.35 and 0.5% (v/v), in all cases over CMC (0.0155%, w/v). The metals from spiked samples are extracted with the buffer (pH = 4) and SDS:Triton X-100 solution in the microwave system at 400 W for 5 minutes. The recoveries obtained for the metals under study can be seen in Fig. 3.

Fig. 3 presents recovery percentages based on the concentration of Triton X-100 surfactant in the mixture. In general, recoveries are notably higher in the presence of this surfactant, though its concentration does not appear to affect the efficiency of extraction.

Thus, the percentage remains around 90% for Ni, Cr and Cd, increases slightly for Cu though below 50% recovery, and remains well below 40% for Pb.

Therefore, 0.1% (v/v) is taken as the optimal value for the concentration of Triton X-100, which is the lowest concentration used that allows acceptable performance in the extraction.

**Effect of extractant volume.** This section addresses if the volume of extractant would affect the extraction of the analytes due to possible evaporation losses or a non-complete

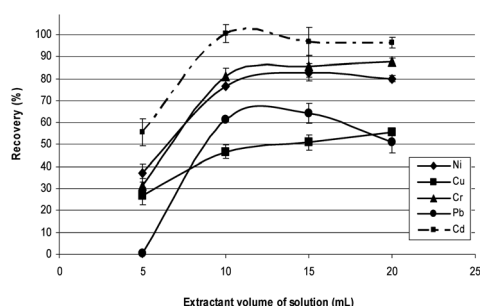


Fig. 4 Effect of the extractant volume on the recovery of the heavy metals using 10 mL of SDS:Triton X-100, 1.25% (w/v) and 0.1% (v/v) respectively ( $n = 3$ ).



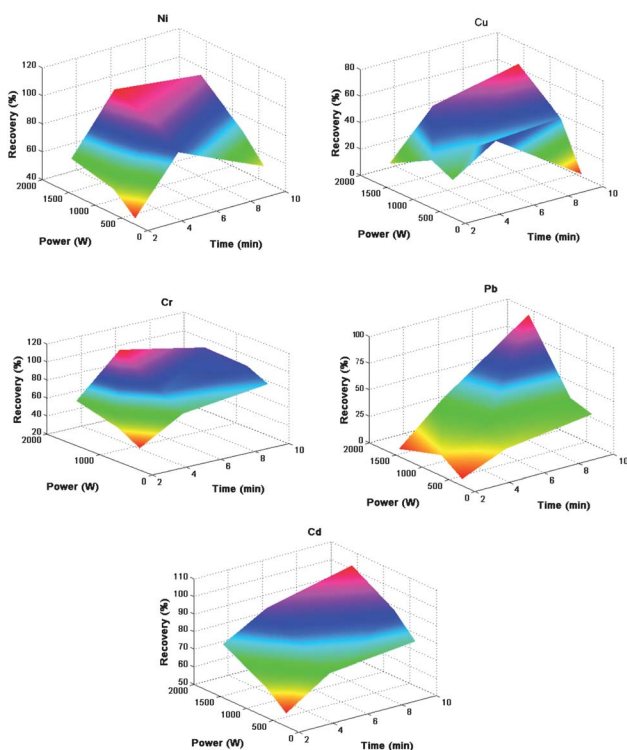
**Table 3** Radiation power and time conditions applied for each run

Run number	Time (min)	Power (W)
1	2.5	400
2	2.5	800
3	2.5	1600
4	5	400
5	5	800
6	5	800
7	5	800
8	5	1600
9	10	400
10	10	800
11	10	1600

interaction with the sample.<sup>35</sup> In this way, measurements of the analyte recoveries are performed using 5, 10, 15 and 20 mL of pH = 4 buffered solution of SDS:Triton X-100 (1.25%, w/v and 0.1%, v/v respectively), irradiated in the microwave system at 400 W for 5 minutes. The results are shown in Fig. 4.

Fig. 4 shows the results for recovery of metals according to the volume of extractant solution employed. There is a clear trend for the increasing recovery percentage with increasing volume extractant mixture up to 10 mL, where recoveries are stabilized for all metals.

Thus, neither a volume of 5 mL is sufficient to ensure good volatilization losses of analytes at high volumes as observed in other studies.<sup>35</sup>



**Fig. 5** Effect of the microwave radiation time and power on the recovery of the metal using 10 mL of buffer pH = 4 and SDS:Triton X-100 (1.25%, w/v and 0.1%, v/v) respectively.

Therefore, the optimum value of the extractant solution volume taken for the five metals is 10 mL.

**Effect of the microwave radiation power and time.** Power and radiation time are key variables in the extraction efficiency for the microwave sample preparation.<sup>48,49</sup>

The temperature obtained inside the vessels is the variable that determines the efficiency of extraction. Since temperature depends on the radiation time and power applied, both variables affect directly the efficiency of the extraction<sup>24</sup> and are analyzed at the same time. A central composite design is followed in order to study the effect on the recovery. A two-level full factorial design, 2<sup>2</sup>, with a star orthogonal composite design and three central points (11 runs in total) allowed the direct evaluation of the considered variables.<sup>50</sup>

Therefore, extracts of *Posidonia oceanica* leaf samples are obtained using the previously optimized conditions, at different microwave powers (from 400 to 1600 W), and radiation times (2.5 to 10 minutes), to be then analyzed. Table 3 shows the different radiation conditions employed for each run.

Fig. 5 shows the recovery percentage of each metal as a function of time and extraction power. Recovery percentage increases with increasing irradiation time and power for all metals, with values between 90 and 100% for Ni, Cr, Pb and Cd, and around 63% for Cu. Therefore, 10 minutes and 1600 W are taken as optimum values, which reached a temperature of 160 °C.

### Analytical parameters

The corresponding calibration curves are obtained by the atomic absorption spectrometer system after analysing standard solutions containing a known concentration of the metals. The results reveal a linear relationship in the interval 5.00–30.00  $\mu\text{g g}^{-1}$  for all the metals except Cd, whose linear interval is 0.50–3.00  $\mu\text{g g}^{-1}$ , with high correlation coefficients (0.999) in all cases, as shown in Table 4.

In order to assess the reproducibility of the optimized method, it is also applied to the analysis of six samples containing the mixture of metals which are analyzed under the established conditions. The results obtained show RSD below 7.29% as shown in Table 4.

The limits of detection (LOD) are also calculated, once the MAME method is fully applied, for each analyte using the expression  $\text{LOD} = X_b + 3\sigma$ .<sup>51</sup> The results obtained, also listed in Table 4, show LOD ranging from 0.04 to 0.22  $\mu\text{g g}^{-1}$ . Finally, in order to validate the optimized method, the results obtained are

**Table 4** Analytical parameters for the optimized methodology

Metal	RSD <sup>a</sup> (%)	LOD <sup>b</sup> ( $\mu\text{g g}^{-1}$ )	LOQ <sup>b</sup> ( $\mu\text{g g}^{-1}$ )	$\gamma^c$	Linear Range ( $\mu\text{g g}^{-1}$ )	Slope <sup>d</sup>	R <sup>2</sup>
Ni	4.49	0.08	0.17	0.51	5–30	2.03	0.9998
Cr	7.29	0.22	0.18	1.37	5–30	8.63	0.9998
Cu	4.17	0.17	0.37	0.99	5–30	2.03	0.9999
Pb	2.07	0.06	0.18	0.66	5–30	1.28	0.9997
Cd	3.36	0.04	0.07	18.54	0.5–3	57.28	0.9995

<sup>a</sup> ( $n = 6$ ). <sup>b</sup> ( $n = 10$ ). <sup>c</sup> Analytical sensitivity ( $\times 10^{-3}$ ). <sup>d</sup> Slope ( $\times 10^{-3}$ ).

**Table 5** Comparison between the optimized MAME method and ISO 11047:1998 method for each metal. ( $n = 3$ )

Analyte	Added ( $\mu\text{g g}^{-1}$ )	Found by MAME-GFAAS ( $\mu\text{g g}^{-1}$ )	Found by ISO 11047:1998 method ( $\mu\text{g g}^{-1}$ )
Ni	30.03	29.14 $\pm$ 2.40	28.36 $\pm$ 0.37
Cu	30.03	19.05 $\pm$ 1.56	27.16 $\pm$ 1.56
Cr	30.03	27.49 $\pm$ 2.07	31.85 $\pm$ 1.10
Pb	30.03	28.68 $\pm$ 0.93	29.83 $\pm$ 1.05
Cd	3.00	3.06 $\pm$ 2.75	3.02 $\pm$ 0.14

**Table 6** Comparison between metal recovery percentages for aged and recent *P. oceanica* samples. ( $n = 3$ )

Analyte	Recovery (%) <i>P. oceanica</i>	Recovery (%) aged <i>P. oceanica</i>
Ni	97.03 $\pm$ 2.40	95.57 $\pm$ 1.65
Cu	63.43 $\pm$ 1.56	60.54 $\pm$ 0.57
Cr	91.54 $\pm$ 2.07	89.95 $\pm$ 5.12
Pb	95.49 $\pm$ 0.93	94.24 $\pm$ 3.44
Cd	102.00 $\pm$ 2.75	100.33 $\pm$ 1.41

compared with those obtained using an adaptation of the method established by the International Organization for Standardization (ISO 11047:1998) which consists of MAE as the extraction method and GFAAS as the determination technique. The recoveries obtained with both methods are very similar (Table 5), whereby the method is validated.

### Analytical applications

**Application of the optimized method to *P. oceanica* aged samples.** Since the chemical bonds between analytes and matrix change with time, recovery percentages are also affected by the aging effect of the samples.<sup>35</sup> This issue is addressed by enriching the matrix and leaving it in contact with the analytes for 6 months. Later on, extraction and analysis are performed under the optimized conditions. The results are shown in Table 6. As it can be seen, the recovery percentages obtained for the aged samples are very similar to those obtained with the optimized method applied to recently spiked samples. Therefore, the aging effect of the samples does not affect significantly the recovery percentages.

In this sense, the optimized procedure can be an efficient tool for extraction of heavy metals from *P. oceanica* leaves in their natural ecosystem, because this plant changes its leaves every 5 or 8 months as a natural detoxification pathway.<sup>18,19</sup>

Therefore, since the method is effective for samples in contact with the analytes for a period up to 6 months, the optimized method is applicable to natural *P. oceanica* samples regardless of time of exposure to pollution by metals.

**Application to other matrices.** The optimized method is further applied to different aquatic vegetal samples, such as the macrophytes *Ulva lactuca* and *Enteromorpha* sp. The samples are spiked with a mixture of metals under study with

**Table 7** Application of the optimized procedure to several aquatic vegetal samples. ( $n = 3$ )

Analyte	Rec. (%) <i>P. oceanica</i>	Rec. (%) <i>U. lactuca</i>	Rec. (%) <i>Enteromorpha</i> sp.
Ni	97.03 $\pm$ 2.40	83.22 $\pm$ 1.93	83.80 $\pm$ 5.28
Cu	63.43 $\pm$ 1.56	48.51 $\pm$ 0.23	60.70 $\pm$ 7.03
Cr	91.54 $\pm$ 2.07	89.44 $\pm$ 1.88	95.30 $\pm$ 3.92
Pb	95.49 $\pm$ 0.93	74.78 $\pm$ 1.36	71.62 $\pm$ 3.17
Cd	102.00 $\pm$ 2.75	105.56 $\pm$ 1.54	102.67 $\pm$ 1.34

the following concentrations: 30.03  $\mu\text{g g}^{-1}$  for Ni, Cr, Cu and Pb, and 3.00  $\mu\text{g g}^{-1}$  for Cd.

Later on, metals are extracted under optimized method conditions. The results in Table 7 show that recovery percentages for *U. lactuca* and *Enteromorpha* sp. are generally satisfactory except for Cu and Pb, though recoveries were slightly lower than those obtained in the optimization of the method. Therefore, this method is applicable to this type of marine macrophytes. In the case of Cu and Pb further studies are needed to optimize their application to this type of matrices.

### Conclusions

This study proves the suitability of the mixtures of anionic and non-ionic surfactants as extractants for heavy metals under study from *P. oceanica* samples, except for Cu.

Combining surfactants as extractants with the microwave assisted extraction enhances method skills because extraction is faster, and less extractant is needed, thus dropping off the costs dramatically. Further, this method is relatively straightforward because it does not require deep handling, and the extractant can be directly analyzed. Moreover, it can be applied to the extraction of several samples at the same time without toxic effects. Therefore, this promising method could be an alternative to extract Ni, Cr, Pb and Cd from this kind of marine samples and from other marine macrophytes.

In the case of Cu further studies are needed to improve their results.

In addition, the results obtained for the extraction of these heavy metals from aged samples are particularly relevant, because the optimized procedure could be applied for the evaluation of the heavy metal pollution in the marine environment by using *P. oceanica* leaves regardless of the time spent in contact with pollutants.

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