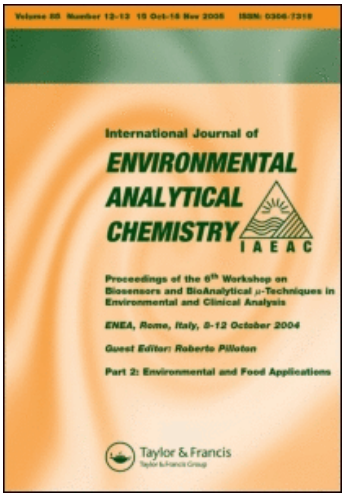

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Sequential voltammetric determination of mercury(II) and toxic metals in environmental bio-monitors: application to mussels and clams

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Sequential voltammetric determination of mercury(II) and toxic metals in environmental bio-monitors: application to mussels and clams

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This article reports the sequential voltammetric determination of Hg(II) and Cu(II) at gold electrode (GE), and of Cu(II), Pb(II), Cd(II), Zn(II) at hanging mercury drop electrode by square wave anodic stripping voltammetry in mussels and clams, possible environmental bio-monitors. The analytical procedure was verified by the analysis of standard reference materials: oyster tissue NBS-SRM 1566a, mussel tissue BCR-CRM 278 and cod muscle BCR-CRM 422. Precision and accuracy, expressed as relative standard deviation and relative error, respectively, were always less than 6%. Then, the analytical procedure was transferred and applied to mussels and clams sampled in two lagoon ecosystems connected with Adriatic Sea (Italy): the Goro Bay, located in the Po river mouth area and the lagoon ecosystem located in proximity to Ravenna. A critical comparison with spectroscopic measurements is also discussed.

Keywords: mercury; toxic metals; voltammetry; spectroscopy; mussel; clams

1. Introduction

One of the major problems in ecology is related to the path of heavy metals contained in wastewaters polluting the aquatic environment. Heavy metals may be accumulated in certain species of marine organisms, like algae, mussels, clams, shrimps or fish, which sequestrate and concentrate several elements from their aqueous environment [1]. For this reason they may be utilised as biological monitors for trace metal pollution in a limited ecosystem [2–6].

Together with numerous sample treatment procedures [7,8], several analytical methods have been described for trace metal determination [9]. Although spectroscopic [10,11] and voltammetric [12,13] techniques have been the most frequently employed, while among all the toxic metals, certainly Hg(II), Pb(II) and Cd(II) show to be, perhaps, the most dangerous for the man and wildlife, and consequently largely investigated [14–19]. In particular, as regards mercury(II), for its determination, cold vapour atomic absorption spectroscopy (CV-AAS) is prevalently used [20–30].

Voltammetric measurements are seldom employed even if voltammetry may be a good alternative to spectroscopy, since it allows the determination without the employment of

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too expensive equipment. Such a technique provide for the employment of gold [31–37], glassy carbon [38–40] and platinum electrodes [41], generally using acidic pH solutions and/or complexing agents as supporting electrolytes. Recently, also modified solid electrodes are successfully employed for the voltammetric determination of mercury(II) [42–48].

Then, considering the high toxicity of mercury(II), copper(II), lead(II), cadmium(II) and zinc(II), and their increasing presence and concentration in the environment, it seemed attractive to set up a new sensitive analytical procedure for sequentially determining in two steps, at ultratrace concentration level, these metals in mussels and clams by voltammetric techniques, employing two different working electrodes: at first Hg(II) and Cu(II) at gold electrode (GE) and successively Cu(II), Pb(II), Cd(II) and Zn(II) at hanging mercury drop electrode (HMDE).

The metals to determine the matrix kind were chosen considering important factors: (a) these metals, extremely toxic, are generally always and contemporaneously present in the environmental matrices; (b) the use of biological species to monitor qualitatively the environmental pollution load is continually increasing [49] and certainly mussels and clams can be employed to this purpose, and finally (c) the possibility of using well-known standard reference materials is certainly an important advantage in order to establish and verify the applicability of the analytical procedure, determining its accuracy and precision.

2. Experimental

2.1 *Apparatus*

A Multipolarograph AMEL (Milan, Italy) Mod. 433 was employed for all the voltammetric scans, assembling two conventional three electrode measuring cells, having in both cases an Ag | AgCl | KCl saturated reference electrode and a platinum wire auxiliary electrode, with a GE (surface area: 0.785 mm^2, AMEL, Milan, Italy), activated before and cleaned after the measurements following the procedure proposed by Bonfil *et al.* [32] and a HMDE as working electrodes, respectively.

The supporting electrolyte was $0.01\,mol\,L^{-1}$ EDTA-Na_2 + $0.06\,mol\,L^{-1}$ NaCl + $2.0\,mol\,L^{-1}$ $HClO_4$ and the experimental conditions for both electrodes are reported in Table 1. The choice of the supporting electrolyte was carried out following the procedure suggested by Sancho *et al.* [33], optimising either the instrumental parameters or the supporting electrolyte composition.

However, it is important to highlight that, when GE is employed, always it is present as a problem linked to the presence in solution of anions. In fact, anions are known to adsorb strongly on gold surfaces [50], and this adsorption is dependent on the potential of the electrode and on the nature of the anion. According to Salaun and van den Berg [36], to minimise the excessive adsorption effect, in order to have higher voltammetric peaks and flatter voltammetric baseline, a negative desorption potential of $-0.8\,V$ per Ag | AgCl | KCl saturated was applied for a short time (desorption time) between the deposition and the stripping steps. It has been shown that, at this potential Cl^-, Br^-, I^- and SO_4^{2-} do not adsorb on the GE [50,51].

Before the measurements, to avoid accidental contamination, the Teflon voltammetric cell was rinsed with suprapure concentrated 1 : 1 HNO_3 and then many times with Milli-Q water. The solutions were thermostated at $20 \pm 0.5°C$ and deaerated with Milli-Q water

Table 1. Instrumental parameters for the determination of Hg(II), Cu(II) at GE and Cu(II), Pb(II), Cd(II), Zn(II) at HMDE by SWASV. Supporting electrolyte: $0.01\,mol\,L^{-1}$ EDTA-$Na_2 + 0.06\,mol\,L^{-1}$ NaCl $+ 2.0\,mol\,L^{-1}$ $HClO_4$.

	GE [Hg(II)–Cu(II)]	HMDE [Cu(II)–Pb(II)–Cd(II)–Zn(II)]
E_i	−0.400	−1.150
E_d	−0.400	−1.150
E_{des}	−0.800	–
E_f	+0.900	+0.100
t_d	240	180
t_{des}	30	–
t_r	10	10
dE/dt	100	100
ΔE	50	50
τ	0.010	0.010
ν	0.100	0.100
η	10	10
r	600	600

Notes: E_i: initial potential (V/Ag | AgCl | KCl(sat)); E_d: deposition potential (V/Ag | AgCl | KCl(sat)); E_{des}: desorption potential (V/Ag | AgCl | KCl(sat)); E_f: final potential (V/Ag | AgCl | KCl(sat)); t_d: electrodeposition time (s); t_{des}: desorption time (s) t_r: delay time before the potential sweep (s); dE/dt: potential scan rate ($mV\,s^{-1}$); ΔE: step amplitude (mV); τ: sampling time (s); ν: wave period (s); η: wave increment (mV); r: stirring rate (r.p.m.).

saturated pure nitrogen for 5 min prior to analysis, while a nitrogen blanket was maintained above the solutions during the experiments. The solutions were stirred with a Teflon-coated magnetic stirring bar in the purge step.

For all the metals the atomic absorption spectrometry (AAS) measurements were performed using a Perkin–Elmer Mod. A-Analyst 100 Atomic Absorption Spectrometer, equipped with a deuterium background corrector, Autosampler AS-72 and with HGA 800 graphite furnace. Single-element Lumina (Perkin–Elmer) hollow-cathode lamps were used. All measurements were carried out after the relative ashing and atomisation curves had been studied for each element considered [52].

Using a Perkin–Elmer Mod. FIAS-100, mercury(II) CV-AAS measurements were carried out in the recirculation mode [52] employing stannous chloride as reducing agent. The absorption wavelength was fixed at 253.7 nm and the spectral band-width at 0.7 nm. The spectroscopic experimental conditions relevant to Cu(II), Pb(II), Cd(II), Zn(II) are reported in Table 2 [52,53].

2.2 *Reagents and reference solutions*

All acids and chemicals were of suprapure grade (Merck, Germany). Acidic stock metal solutions ($1000\,mg\,L^{-1}$, Merck, Darmstadt, Germany) were respectively employed in the preparation of reference solutions at varying concentrations for each element, using, for diluting, water demineralised through a Milli-Q system.

A special treatment was applied to potassium dichromate to render it virtually mercury-free: the salt was kept heated at 350°C for 4 days, then the temperature was raised to 410°C and the mass kept melted for 24 h. The solidified salt was granulated and

Table 2. Instrument settings for the determination of Cu(II), Pb(II), Cd(II), Zn(II) by AAS.

	Cu(II)	Pb(II)	Cd(II)	Zn(II)
Wavelength (nm)	324.8	283.3	228.8	213.9
Slit (nm)	0.7	0.7	0.5	0.5
Drying temperature (°C)	110	110	110	110
Charring temperature (°C)	1350	950	1000	850
Atomisation temperature (°C)	2500	1900	1750	1950
Matrix modifiers	0.015 mg Pd + 0.03 mg $Mg(NO_3)_2$	0.3 mg $NH_4H_2PO_4$ + 0.01 mg $Mg(NO_3)_2$	0.3 mg $NH_4H_2PO_4$ + 0.03 mg $Mg(NO_3)_2$	0.07 mg $Mg(NO_3)_2$

homogenised by corundum ball-milling. The reducing agent $SnCl_2$ $2H_2O$ was dissolved in 10% (w/w) to give a 25% (w/w) solution which was bubbled with N_2 for 20 min to strip away any residual Hg and O_2.

Oyster tissue NBS-SRM 1566a, mussel tissue BCR-CRM 278 and cod muscle BCR-CRM 422 were employed as standard reference materials for optimising and setting up the analytical procedure.

2.3 *Sample preparation*

Approximately 1.0 g of sample, accurately weighed, was placed in a Pyrex digestion tube calibrated at 25 mL and connected with a Vigreux column condenser together with 3 mL 69% (w/w) HNO_3 + 2 mL 37% (w/w) HCl + 5 mL 96% (w/w) H_2SO_4. The tube was inserted into the cold home-made block digester, raising gradually the temperature up to 150°C, and keeping this temperature for the whole time of mineralisation (2 h). After cooling, the digest was filtered through Whatman No. 541 filter paper, evaporated to dryness and the soluble salts dissolved in 50 mL of 0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$ supporting electrolyte.

For the spectroscopic determination of Hg(II), a different sample preparation procedure was followed [29,54]. Approximately 1.0 g of sample, accurately weighed, was placed in a digestion tube together with 1.2 g $K_2Cr_2O_7$ and 20 mL H_2O. A condenser was connected to the digestion tube and 20 mL H_2SO_4 were slowly added. The digestion tube was transferred to the hot block preheated at 180°C, and the digestion was allowed to proceed for 60 min to completion. After cooling at room temperature, the condenser was removed, rinsed with three 5 mL portions of H_2O and the washings were added to the digested matter. The open digestion tube, without the condenser, was replaced on the hot-block for a further 30 min boiling span. Finally, after cooling, the digested solution was diluted to 50 mL.

2.4 *Sampling and pretreatment of* Mytilus galloprovincialis *and* Tapes philippinarum

About 8 kg of *M. galloprovincialis* and of *T. philippinarum* were collected in the Goro Bay, located in the Po river mouth area (Italy) and in the lagoon ecosystem located in proximity to Ravenna (Italy). Mussels and clams were sampled, immediately taken to the laboratory

and prepared for analysis. They were open with a plastic appliance and the organisms were carefully extracted and placed in polyethylene containers, previously treated with suprapure HNO_3 diluted in 1 : 1 proportion with water for 48 h and followed by repeated rinsing with Milli-Q water in order to avoid any contamination. The samples were frozen and then liophilised for 30 h. Such a procedure, employing very low temperatures for drying the samples not only avoids the loss of volatile analytes like mercury, but also methylmercury.

After that treatment the samples were homogenised thoroughly in an agate mortar. About 0.5–1.0 g sample were exactly weighed, digested and analysed as described above (see Section 2.3 and 2.5).

2.5 *Total voltammetric analytical procedure*

The total analytical procedure consists of carrying out two steps in succession: 10 mL sample aliquots of $0.01\,mol\,L^{-1}$ EDTA-$Na_2 + 0.06\,mol\,L^{-1}$ NaCl $+ 2.0\,mol\,L^{-1}$ $HClO_4$ aqueous reference solution or of solutions obtained in the mineralisation step of the standard reference material and of the real samples, were pipetted into the two voltammetric cells, assembled as previously described (Section 2.1), and deaerated for 5 min by bubbling water saturated pure nitrogen. The square wave anodic stripping determinations of Hg(II)–Cu(II) and of Cu(II)–Pb(II)–Cd(II)–Zn(II) were carried out using a GE and a HMDE as working electrodes, respectively (Table 1).

3. Results and discussion

3.1 *Aqueous reference solutions*

For the voltammetric determinations of Hg(II) and Cu(II) at GE, and of Cu(II), Pb(II), Cd(II), Zn(II) at HDME, a preliminary study was carried out employing the relevant aqueous reference solutions [the blank concentrations for all the elements were lower than the respective limits of detection (LOD), expressed according to IUPAC [55] ($K = 3$), and calculated on 10 blank signals].

Using instrumental experimental conditions of Table 1, the analytical calibration functions of each individual element were determined in the aqueous reference solutions. In the range of concentrations investigated, a linear i_p *versus* metal concentration relationship was found for each single element. In all cases the correlation coefficients were good ($r^2 > 0.9987$). The experimental peak potentials, for each supporting electrolyte, are reported in Table 3.

3.1.1 *Interference problems*

Many times, in the commonly used supporting electrolytes, the reduction peak potentials of each metal are very close, and thus the simultaneous voltammetric determination of neighbouring elements would be hindered.

However, as well known, the voltammetric interference problems are fundamentally linked to the concentration ratios between the two neighbouring elements [56]. In the case of Hg(II) voltammetric determination, the more important interferent, qualitatively investigated already by Hatle [31], seems to be Cu(II). Partially, we disagree on data reported by Hatle [31]; in fact, the author affirms that Cu(II) does not interfere for

Table 3. Experimental peak potentials ($E_{p,}$ V, Ag | AgCl | $KCl_{sat.}$) in the aqueous reference solutions and in the standard reference material solutions.

		Hg(II)	Cu(II)	Pb(II)	Cd(II)	Zn(II)
0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$	a	0.523 ± 0.015	0.361 ± 0.010	−0.449 ± 0.015	−0.669 ± 0.010	−0.996 ± 0.015
	b		−0.077 ± 0.015			
Oyster tissue NBS-SRM 1566	a	–*	–*	−0.423 ± 0.010	−0.658 ± 0.015	−0.967 ± 0.010
	b		−0.089 ± 0.015			
Mussel tissue BCR-CRM 278	a	0.543 ± 0.010	0.387 ± 0.015	−0.439 ± 0.015	−0.647 ± 0.010	−0.955 ± 0.015
	b		−0.101 ± 0.010			
Cod muscle BCR-CRM 422	a	0.577 ± 0.015	0.403 ± 0.015	−0.475 ± 0.015	−0.677 ± 0.010	−1.023 ± 0.015
	b		−0.123 ± 0.015			

Note: Number of independent determinations: 5. Working electrodes: GE (a); HMDE (b).
*Not determined owing to totally voltammetric peak overlapping Cu(II)–Hg(II) (Figure 1).

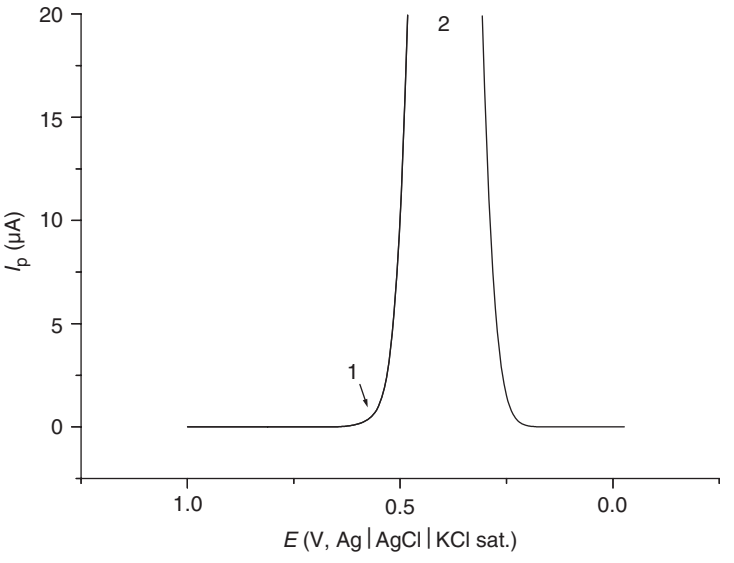

Figure 1. Square wave anodic stripping voltammogram at GE of Hg(II) (peak 1, overlapped) and Cu(II) (peak 2) in oyster tissue NBS-SRM 1566a standard reference material. Concentrations ($\mu g\,g^{-1}$): 0.0642 [Hg(II)]; 66.3 [Cu(II)]; $c_{Cu} : c_{Hg} = 1032.7$. Experimental conditions: see Table 1 and Section 2.1.

concentrations lower than $10\,mg\,L^{-1}$. Actually, the interference is strictly linked to the Hg(II)–Cu(II) concentration ratios.

Only qualitative investigations show that Cu(II) concentrations higher than 200–250 times the Hg(II) concentration cause strong interference to the voltammetric signals, thus hindering evidently the correct determination of mercury(II). Then, considering that the Hg(II) concentrations in real matrices (environmental, food, etc) are generally very low, certainly it is not correct to fix a limit, since the Hg(II)–Cu(II) interference may be present also at Cu(II) concentrations decidedly lower than $10\,mg\,L^{-1}$, evidently depending on the Hg(II)–Cu(II) concentration ratios.

In fact, for example, in the case of oyster tissue NBS-SRM 1566a, where the concentration ratio Cu(II) : Hg(II) is greater than 1000, the respective voltammetric signals at GE show a strong overlapping, thus hindering their quantification (Figure 1), even if it is possible to determine Cu(II) employing HMDE where interference problems for Cu(II), Pb(II), Cd(II), Zn(II) are totally absent since the metal voltammetric peaks are separated enough.

It is important also to highlight that, in the case of Hg(II)–Cu(II) interference, the literature reports different approaches, for example electrochemical [57] or chemical [58] ones.

3.2 *Quality control and quality assessment*

3.2.1 *Standard reference materials*

The method set up in aqueous reference solutions was applied to standard reference materials oyster tissue NBS-SRM 1566a (from National Institute of Standards and

Table 4. Accuracy (e%) and precision (s_r%) of the analytical procedure (Section 3.2.1).

	Element		Certified value	Determined value	e (%)	s_r (%)
Oyster tissue NBS-SRM 1566a	Hg(II)		0.0642 ± 0.0036	–*	–	–
	Cu(II)	a	66.3 ± 2.8	–*	–	–
		b	66.3 ± 2.8	63.7 ± 3.5	−3.9	5.0
	Pb(II)		0.371 ± 0.023	0.387 ± 0.029	+4.3	4.3
	Cd (II)		4.15 ± 0.18	3.96 ± 0.21	−4.6	4.5
	Zn (II)		830 ± 27	796 ± 43	−4.1	5.1
Mussel tissue BCR-CRM 278	Hg(II)		0.188 ± 0.007	0.177 ± 0.012	−5.6	4.7
	Cu(II)	a	9.60 ± 0.16	9.96 ± 0.39	+3.8	5.1
		b	9.60 ± 0.16	9.25 ± 0.37	−3.6	4.7
	Pb(II)		1.91 ± 0.04	1.81 ± 0.15	−5.2	4.9
	Cd (II)		0.34 ± 0.02	0.36 ± 0.04	+5.9	4.3
	Zn (II)		76 ± 2	72 ± 5	−5.3	5.0
Cod muscle BCR-CRM 422	Hg(II)		0.559 ± 0.016	0.537 ± 0.025	−3.9	4.5
	Cu(II)	a	1.05 ± 0.07	1.10 ± 0.09	+4.8	4.9
		b	1.05 ± 0.07	0.99 ± 0.07	−5.7	4.8
	Pb(II)		0.085 ± 0.015	0.081 ± 0.010	−4.7	5.1
	Cd (II)		0.017 ± 0.002	0.016 ± 0.003	−5.9	4.7
	Zn(II)		19.6 ± 0.5	20.5 ± 1.1	+4.6	4.9

Notes: The determined values are the mean of five independent determinations ± confidence interval at 99% probability level. Concentrations: $\mu g\,g^{-1}$. Experimental conditions: see Table 1. Voltammetric measurements.
Supporting electrolyte: $0.01\,mol\,L^{-1}$ EDTA-$Na_2 + 0.06\,mol\,L^{-1}$ NaCl $+ 2.0\,mol\,L^{-1}$ $HClO_4$.
Working electrodes: GE (a); HMDE (b).
*Not determined owing to totally voltammetric peak overlapping Cu(II)–Hg(II) (Figure 1).

Technology, Gaithersburg, MD, USA), mussel tissue BCR-CRM 278 and cod muscle BCR-CRM 422 (from Institute for Reference Materials and Measurements, European Commission, Joint Research Centre, Belgium), in order to confirm and verify the applicability of the analytical procedure, determining its accuracy and precision (Table 4).

In the experimental conditions employed (Table 1), precision as repeatability [59] (expressed as relative standard deviation (s_r%) on five independent determinations) was satisfactory, being, in all cases lower than 5%, while accuracy expressed as relative error (e%) was generally of the order of 3–6%.

The accuracy and precision data relevant to spectroscopic measurements are reported in Table 5.

As example, Figures 2 and 3 show the square wave voltammograms at GE of Hg(II) and Cu(II) in mussel tissue BCR-CRM 278 and cod muscle BCR-CRM 422 standard reference materials, respectively.

3.2.2 *Limits of detection*

In the aqueous reference solution, in the solutions obtained by digestion of the standard reference materials, the LOD for both analytical procedures (Table 6) were obtained by the equation $LOD = K s_{y/x}/b$ [59], where $s_{y/x}$ and b are the regression estimated standard deviation and the slope of the analytical calibration function of each element, respectively, with a 98% ($K = 3$) confidence level [55].

Table 5. Accuracy (e%) and precision (s_r%) of the analytical procedure (Section 3.2.1).

	Element	Certified value	Determined value	e(%)	s_r(%)
Oyster tissue NBS-SRM 1566a	Hg(II)	0.0642 ± 0.0036	0.0613 ± 0.0041	−4.5	5.1
	Cu(II)	66.3 ± 2.8	69.6 ± 3.5	+5.0	4.8
	Pb(II)	0.371 ± 0.023	0.390 ± 0.025	+5.1	4.9
	Cd (II)	4.15 ± 0.18	3.93 ± 0.27	−5.3	4.3
	Zn (II)	830 ± 27	869 ± 43	+4.7	5.0
Mussel tissue BCR-CRM 278	Hg(II)	0.188 ± 0.007	0.196 ± 0.015	+4.3	5.3
	Cu(II)	9.60 ± 0.16	9.23 ± 0.43	−3.9	4.1
	Pb(II)	1.91 ± 0.04	2.00 ± 0.11	+4.7	4.5
	Cd (II)	0.34 ± 0.02	0.36 ± 0.03	+5.9	4.7
	Zn (II)	76 ± 2	79 ± 4	+3.9	4.9
Cod muscle BCR-CRM 422	Hg(II)	0.559 ± 0.016	0.531 ± 0.030	−5.0	5.0
	Cu(II)	1.05 ± 0.07	1.11 ± 0.09	+5.7	4.5
	Pb(II)	0.085 ± 0.015	0.090 ± 0.07	+5.9	4.3
	Cd (II)	0.017 ± 0.002	0.016 ± 0.003	−5.9	4.1
	Zn(II)	19.6 ± 0.5	18.8 ± 1.0	−4.1	4.8

Notes: The determined values are the mean of five independent determinations ± confidence interval at 99% probability level.
Concentrations: $\mu g\, g^{-1}$. Experimental conditions: see Table 2. Spectroscopic measurements.

In the case of voltammetric technique, since the analytical calibration functions were determined by standard addition method, it was possible to obtain the LODs directly also in the real matrices (Table 6).

3.2.3 *Comparison between voltammetric and spectroscopic measurements*

To better validate the voltammetric analytical procedure proposed, the metal concentrations have also been determined by atomic absorption spectroscopy.

The voltammetric and spectroscopic results reported in Tables 4, 5 and 7 relevant either to standard reference materials or to mussels and clams sampled in two lagoon ecosystems connected with Adriatic Sea (Italy), show a very good agreement (differences generally lower than 7% in all cases).

4. Practical application

The method was applied to the determination of mercury(II), copper(II), lead(II), cadmium(II) and zinc(II) in the marine organisms under study, namely *M. galloprovincialis* and *T. philippinarum*, sampled in the Goro Bay, located in the Po river mouth area (Italy) and in the lagoon ecosystem located in proximity to Ravenna (Italy) (Section 2.4).

The experimental results relevant to both matrices are listed in Table 7, while, as example, Figures 4 and 5 show the anodic stripping voltammograms of Hg(II) and Cu(II) and Cu(II), Pb(II), Cd(II), Zn(II) at GE and HMDE, respectively, in *M. galloprovincialis* sampled in the Goro Bay.

It is evident that the metal concentration levels in mussels and clams sampled in the lagoon ecosystem located in proximity to Ravenna show to be undoubtedly higher. This is certainly due to the fact that, in the Lagoon of Ravenna (today a peculiar and protected

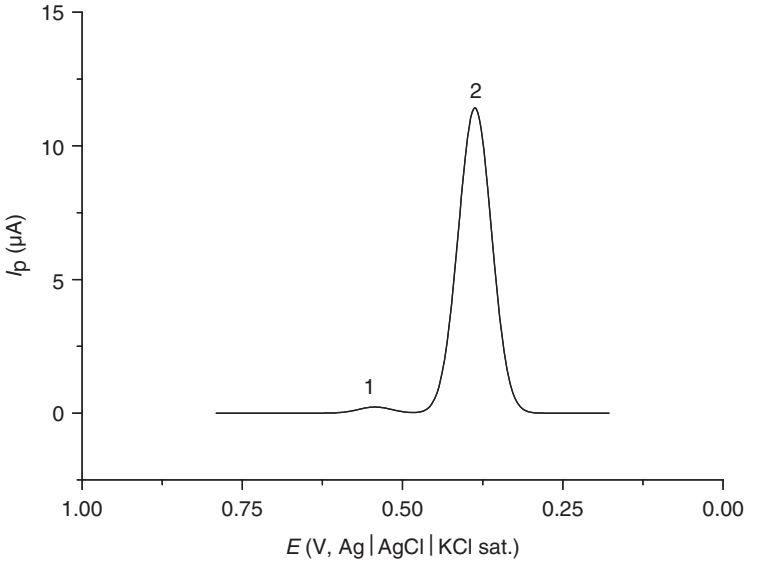

Figure 2. Square wave anodic stripping voltammogram at GE of Hg(II) (peak 1) and Cu(II) (peak 2) in mussel tissue BCR-CRM 278 standard reference material. Concentrations ($\mu g\,g^{-1}$): 0.188 [Hg(II)]; 9.60 [Cu(II)]; $c_{Cu} : c_{Hg} = 51.1$. Experimental conditions: see Table 1 and Section 2.1.

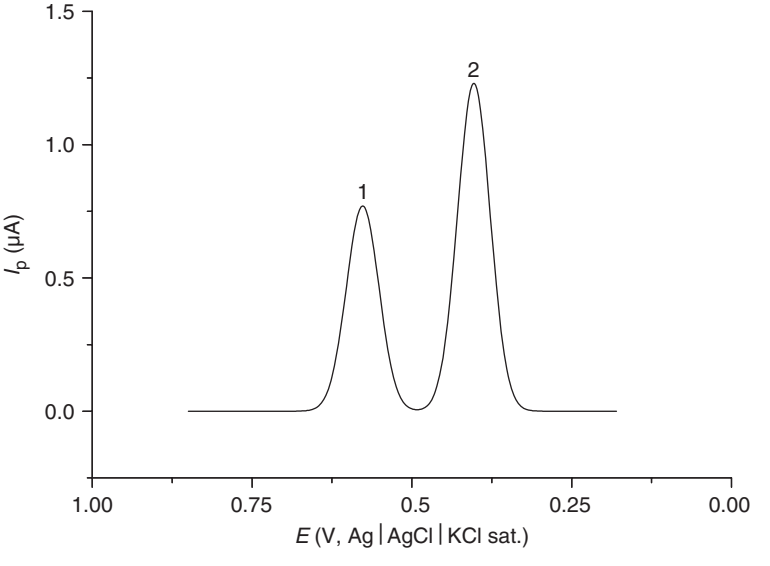

Figure 3. Square wave anodic stripping voltammogram at GE of Hg(II) (peak 1) and Cu(II) (peak 2) in cod muscle BCR-CRM 422 standard reference material. Concentrations ($\mu g\,g^{-1}$): 0.559 [Hg(II)]; 1.05 [Cu(II)]; $c_{Cu} : c_{Hg} = 1.9$. Experimental conditions: see Table 1 and Section 2.1.

ecosystem of naturalistic and tourist importance, but also an area of intense industrial and agricultural activity), during the 1950s, a very important industrial area was built in the southern border of the wetland. Unfortunately before 1973, because of the lack of environmental legislation, industrial wastes were released directly into the Lagoon without

Table 6. LOD, calculated in micrograms per litre in the aqueous reference solutions, and expressed in micrograms per gram in the standard reference materials.

		Hg(II)	Cu(II)	Pb(II)	Cd(II)	Zn(II)
0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$	a	0.15	0.23	0.21	0.27	0.69
	b		0.29			
Oyster tissue NBS-SRM 1566a	a	–**	–**	0.047	0.029	0.077
	b		0.053			
Mussel tissue BCR-CRM 278	a	0.029	0.051	0.050	0.035	0.081
	b		0.057			
Cod muscle BCR-CRM 422	a	0.027	0.055	0.049	0.032	0.075
	b		0.054			

Notes: Number of independent determinations: 5.
The LoD were obtained by the analytical calibration functions [59] of each element ($K = 3$, 98% confidence level [55]) (Section 3.2.2).
*Working electrodes: GE (a); HMDE (b).
*In the case of spectroscopic measurements, the LoD (concentration: μg L^{-1}) calculated for the aqueous reference solutions were: 0.77 [Hg(II)]; 0.96 [Cu(II)], 0.69 [Pb(II)], 0.49 [Cd(II)], 1.09 [Zn(II)].
**Not determined owing to totally voltammetric peak overlapping Cu(II)–Hg(II) (Figure 1).

Table 7. Mean values of the Hg(II), Cu(II), Pb(II), Cd(II), Zn(II) concentration (μg g^{-1}) relevant to mussels and clams sampled in the Goro Bay (*) and in the Lagoon ecosystem of Ravenna (**).

		Hg(II)	Cu(II)	Pb(II)	Cd(II)	Zn(II)
Voltammetric measurements						
M. galloprovincialis	*	0.35 ± 0.02	23.5 ± 1.9[a] 24.0 ± 1.5[b]	5.7 ± 0.4	0.29 ± 0.03	149 ± 7
	**	1.69 ± 0.11	30.7 ± 1.2[a] 30.0 ± 1.8[b]	7.5 ± 0.5	0.96 ± 0.05	177 ± 10
T. philippinarum	*	0.43 ± 0.03	45.7 ± 2.5[a] 44.5 ± 2.3[b]	4.3 ± 0.3	0.77 ± 0.05	105 ± 8
	**	1.95 ± 0.15	67.2 ± 3.1[a] 69.0 ± 3.5[b]	9.6 ± 0.6	1.03 ± 0.06	123 ± 6
Spectroscopic measurements						
M. galloprovincialis	*	0.37 ± 0.03	24.3 ± 1.5	5.4 ± 0.5	0.31 ± 0.04	155 ± 9
	**	1.81 ± 0.15	31.0 ± 1.3	7.7 ± 0.7	0.99 ± 0.07	169 ± 13
T. philippinarum	*	0.41 ± 0.05	46.9 ± 2.3	4.5 ± 0.4	0.74 ± 0.05	96 ± 8
	**	2.09 ± 0.19	66.9 ± 2.9	9.3 ± 0.8	1.07 ± 0.07	129 ± 7

Note: Working electrodes: GE (a); HMDE (b).

any treatment. For example, it has been estimated that during the 1958–1973 period, tens of tons of mercury, coming from chemical plants which produced acetaldehyde and vinyl chloride from acetylene using mercury salts as catalysts, contaminated the Lagoon of Ravenna [60,61].

In the case of the Lagoon of Ravenna, the literature does not report previous data for a critical comparison. On the contrary, in the case of the Goro Bay ecosystem, it is important to highlight that the data reported in this work are in general agreement with the metal concentration data obtained in the same matrices in previous surveys [62,63].

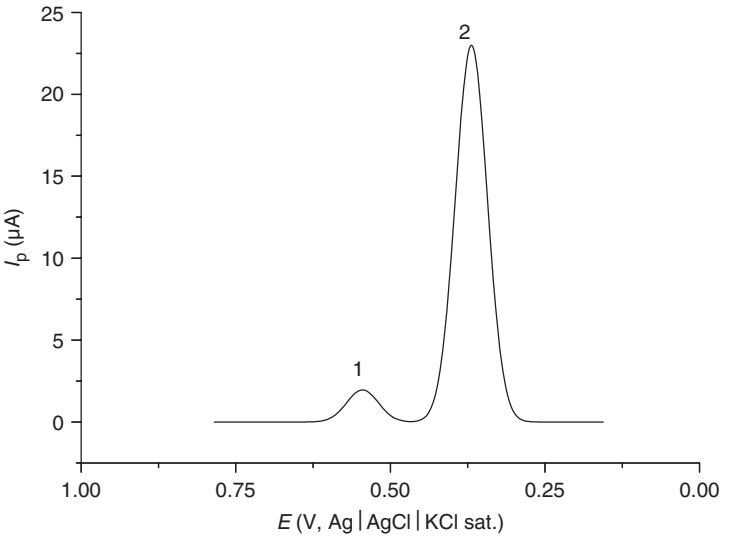

Figure 4. Square wave anodic stripping voltammogram at GE of Hg(II) (peak 1) and Cu(II) (peak 2) in *M. galloprovincialis* sampled in Goro Bay (Italy) (see Section 4.1). Concentrations: see Table 7. Experimental conditions: see Table 1 and Section 2.1.

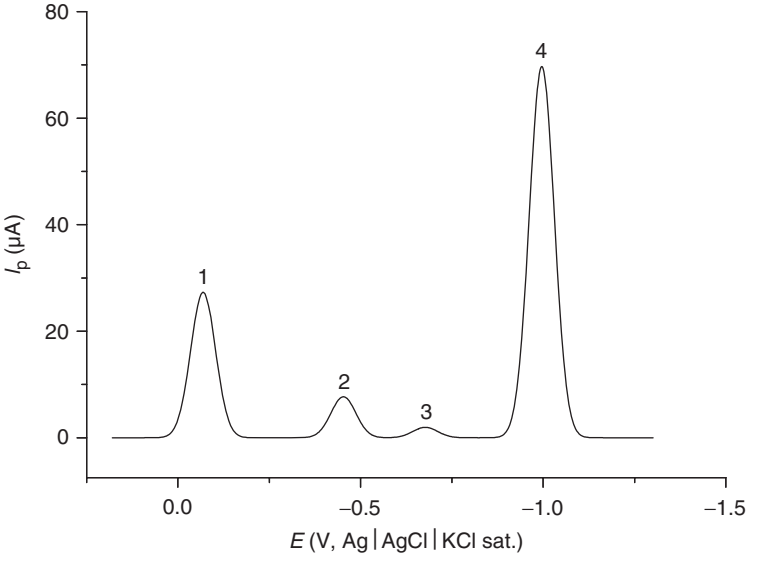

Figure 5. Square wave anodic stripping voltammogram at HMDE of Cu(II) (peak 1), Pb(II) (peak 2), Cd(II) (peak 3) and Zn(II) (peak 4) in *M. galloprovincialis* sampled in Goro Bay (Italy) (Section 4.1). Concentrations: see Table 7. Experimental conditions: see Table 1 and Section 2.1.

5. Conclusions

Voltammetry is certainly a valid analytical technique (good selectivity and especially sensitivity) for simultaneous metal determinations. Hence, the proposed procedure is certainly suitable for metal determinations in multicomponent complex matrices, also

Table 8. Voltammetry and spectroscopy: the comparison.

	Voltammetry	Spectroscopy
Sample mineralisation	Yes	Yes
Time of sample preparation (*h*)	2.5–3.0	2.5–3.0
Matrix modifier	No	Yes
Simultaneous determination	Yes	No
Signal interference	Possible	No
Accuracy (*e*%)	Generally <6	Generally <6
Precision (s_r%)	Generally <6	Generally <6
Equipment cost	Lower	Higher
r^2, Signal *vs.* metal concentration function	>0.9990	>0.9990

considering that it does not need enrichment steps, for example solvent extraction procedures, and particular sample treatments.

- A consideration about the comparison between the two techniques employed can be done, considering both the analytical and instrumental parameters (Table 8). As to precision, accuracy and limits of detection, good and comparable results can be observed in all cases with the two techniques. The two techniques are then equivalent, although voltammetry is better if compared with atomic absorption spectroscopy, allowing simultaneous metal determinations, even if in some cases, when the concentration ratios of two neighbouring metals is unfavourable, i.e. too high (see the case of oyster tissue NBS-SRM 1566a standard reference material, Table 4 and Figure 1), this is not possible. However, it is important to highlight that also inductively coupled plasma (ICP) and ICP/Mass Spectrometry (MS) allow a mult-element determination; but, in our opinion, the great advantage in using voltammetry is certainly the equipment, and also the running cost: very low in the case of voltammetry, extremely high in the case of ICP and ICP/MS, also 25–30 times higher in the case of ICP/MS).
- A final comment about the aim of the present work: the set up of a correct analytical procedure for the trace metal determination in mussels and clams allow to use such species as bio-indicators of toxic metals or in biodetoxification procedures [2,3], and, last but not least, also for checking high-quality food [5].

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References

[1] Y.P. Ting, F. Lawson, and I.G. Prince, Biotechnol. Bioeng. **37**, 445 (1991).
[2] D.J.H. Phillips, in *Biomonitoring of Coastal Waters and Estuaries*, edited by K.J.M. Kramer (CRC Press Inc., Boca Raton, FL, 1994), pp. 85–103.

[3] P. Johansen, M.M. Hansen, G. Asmund, and P.B. Nielsen, Papers from the Eighth International Ocean Symposium, 9–13th October, 1989, Dubrovnik, Yugoslavia, Part Two, I.W. Duedall Editor, **5**, 35 (1991).
[4] L. Tessier, G. Vaillancourt, and L. Pazdernik, Water, Air, Soil Pollut. **86**, 347 (1996).
[5] C. Locatelli and F. Fagioli, Ann. Chim. (Rome) **87**, 535 (1997).
[6] M. Schuhmacher, J.L. Domingo, J.M. Llobet, and J. Corbella, in *Proceedings of the Second European Conference on Ecotoxicology*, editers by W. Sloof and H. de Kruijf, **Suppl. Pts. 1–2**, 117 (1993).
[7] J.C. Van Loon, *Selected Methods in Trace Metal Analysis: Biological and Environmental Samples* (John Wiley and Sons Inc., New York, 1985).
[8] M. Stoeppler, *Sampling and Sample Preparation* (Springer-Verlag Publications, Berlin, Heidelberg, 1997).
[9] C. Sarzanini and E. Mentasti, Ann. Chim. (Rome) **81**, 185 (1991).
[10] W. Slavin, *Graphite Fornace AAS, A Source Book* (Perkin–Elmer Corporation, Norwalk, 1984).
[11] B. Welz, *Atomic Absorption Spectrometry* (VCH Publishers, Weinheim, 1985).
[12] A.M. Bond, *Modern Polarographic Methods in Analytical Chemistry* (Marcel Dekker Publications, New York, 1980).
[13] J. Wang, *Stripping Analysis – Principles, Instrumentation and Applications* (VCH Publishers, Deerfield Beach, FL, 1985).
[14] K.J. Irgolic and A.E. Martell, Editors, *Environmental Inorganic Chemistry*, (VCH Publishers, Deerfield Beach FL, 1985).
[15] D. Purves, *Trace Element Contamination of the Environment* (Elsevier, Amsterdam, 1985).
[16] R.D. Wilken and H. Hintelmann, in *Metal Speciation in the Environment*, edited by J.A.C. Broekaert, S. Gucer, and F. Adams (Springer-Verlag, Berlin, 1990).
[17] M. Sadiq, *Toxic Metal Chemistry in Marine Environments* (Marcel Dekker Inc., New York, 1992).
[18] S. Caroli, Editor, *Element Speciation in Bioinorganic Chemistry*, (John Wiley and Sons Inc., New York, 1996).
[19] T.R. Crompton, *Analysis of Sea Water* (Springer-Verlag, Berlin, 2006).
[20] H. Emteborg, E. Bulska, W. Frech, and D.C. Baxter, J. Anal. At. Spectrom. **7**, 405 (1992).
[21] M.C. de Vargas and R.A. Romero, Analyst **117**, 645 (1992).
[22] M. Navarro, M.C. Lopez, H. Lopez, and M. Sanchez, Anal. Chim. Acta **257**, 155 (1992).
[23] D.C. Baxter and W. Frech, Anal. Chim. Acta **236**, 377 (1990).
[24] G.A. Zachariadis and J.A. Stratis, J. Anal. At. Spectrom. **6**, 239 (1991).
[25] S.Y. Lamleung, V.K.W. Cheng, and Y.W. Lam, Analyst **116**, 957 (1991).
[26] Official Methods of Analysis, 15th Ed. AOAC, Arlington, VA, Secs. 971.21, 977.15, 952.14, 1990.
[27] M.F. Silva, I.V. Toth, and A.O. Rangel, Anal. Sci. **22**, 861 (2006).
[28] R.B. Voegborlo and H. Akagi, Food Chem. **100**, 853 (2007).
[29] S. Landi, F. Fagioli, and C. Locatelli, J. Assoc. Off. Anal. Chem. Int. **75**, 1023 (1992).
[30] N.S. Bloom, Fresenius J. Anal. Chem. **366**, 438 (2000).
[31] M. Hatle, Talanta **34**, 1001 (1987).
[32] Y. Bonfil, M. Brand, and E. Kirowa-Eisner, Anal. Chim. Acta **424**, 65 (2000).
[33] D. Sancho, L. Deban, F. Barbosa, R. Pardo, and M. Vega, Food Chem. **74**, 527 (2001).
[34] P. Ugo, S. Zampieri, L.M. Moretto, and D. Paolucci, Anal. Chim. Acta **434**, 291 (2001).
[35] G.A. East and E.P. Marinho, Biol. Trace Elem. Res. **103**, 261 (2005).
[36] P. Salaun and C.M. van den Berg, Anal. Chem. **78**, 5052 (2006).
[37] R.A.A. Munoz, F.S. Felix, M.A. Augelli, T. Pavesi, and L. Angnes, Anal. Chim. Acta **571**, 93 (2006).
[38] S. Meyer, F. Scholz, and R. Trittler, Fresenius J. Anal. Chem. **356**, 247 (1996).
[39] J. Lu, X. He, X. Zeng, Q. Wan, and Z. Zhang, Talanta **59**, 553 (2003).
[40] H. Yi, Anal. Bioanal. Chem. **377**, 770 (2003).

[41] J.M. Pinilla, L. Hernandez, and A.J. Conesa, Anal. Chim. Acta **319**, 25 (1996).
[42] C. Faller, N.Yu. Stojko, G. Henze, and Kh.Z. Brainina, Anal. Chim. Acta **396**, 195 (1999).
[43] W. Huang and S. Zhang, Anal. Sci. **18**, 187 (2002).
[44] K. Wu, S. Hu, J. Fei, and W. Bai, Anal. Chim. Acta **489**, 215 (2003).
[45] S.V. Romanenko and L.N. Larina, J. Electroanal. Chem. **583**, 155 (2005).
[46] H. Zejli, P. Sharrock, J.L. Hidalgo-Hidalgo de Cisneros, I. Naranjo-Rodriguez, and K.R. Temsamani, Talanta **68**, 79 (2005).
[47] I.K. Tonle, E. Ngameni, and A. Walcarius, Sensor. Actuat. B-Chem. **110**, 195 (2005).
[48] N. Yang, Q. Wan, and J. Yu, Sensor. Actuat. B-Chem. **110**, 246 (2005).
[49] H. Huang, J.Y. Wu, and J.H. Wu, Environ. Monit. Assess. **129**, 315 (2007).
[50] Z. Shi and J. Lipkowski, J. Electroanal. Chem. **403**, 225 (1996).
[51] Z. Shi, S. Wu, and J. Lipkowski, J. Electroanal. Chem. **384**, 171 (1995).
[52] B. Welz and M. Sperling, *Atomic Absorption Spectrometry*. 3rd ed (Wiley VCH, Weinheim, 1999).
[53] Perkin-Elmer, *Analytical Methods for Atomic Absorption Spectrometry*. (Norwalk, 1982).
[54] S. Landi, F. Fagioli, C. Locatelli, and R. Vecchietti, Analyst **115**, 173 (1990).
[55] International Union of Pure and Applied Chemistry – Analytical Chemistry Division, Spectrochim. Acta **33B**, 241 (1978).
[56] C. Locatelli and G. Torsi, Microchem. J. **75**, 233 (2003).
[57] J. Golimowski, A. Orzechowska, and A. Tykarska, Fresenius J. Anal. Chem. **351**, 656 (1995).
[58] K.S. Yoo, S.B. Woo, and J.Y. Jyoung, Bull. Korean Chem. Soc. **24**, 27 (2003).
[59] J.C. Miller and J.N. Miller, *Statistics for Analytical Chemistry* (Ellis Horwood Limited Publisher., Chichester, 1984).
[60] M. Miserocchi, L. Langone, and S. Guerzoni, Water Sci. Technol. **28**, 349 (1993).
[61] D. Fabbri, O. Felisatti, M. Lombardo, C. Trombini, and I. Vassura, Sci. Total Environ. **213**, 121 (1998).
[62] C. Locatelli and F. Fagioli, Ann. Chim. (Rome) **87**, 535 (1997).
[63] C. Locatelli, G. Torsi, and T. Garai, Fresenius J. Anal. Chem. **363**, 662 (1999).