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Determination of heavy metals in environmental bio-indicators by voltammetric and spectroscopic techniques

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Abstract The determination of copper, lead, cadmium and zinc in matrices involved in the food chain as algae, species *Ulva rigida*, and clams, species *Tapes philippinarum* by differential pulse anodic stripping voltammetry (DPASV) was carried out. For the mercury determination in these matrices, a new accurate and precise method was developed employing a mixture of concentrated acids H_2SO_4-$K_2Cr_2O_7$ for digestion and subsequent cold vapor atomic absorption spectrometry (CV-AAS) by reduction with $SnCl_2$. The analytical procedures were verified for four reference standard materials: *Ulva lactuca* BCR-CRM 279, *Lagarosiphon major* BCR-CRM 060, *Oyster tissue* NBS-SRM 1566, *Mussel tissue* BCR-CRM 278. For all the elements the precision, expressed as relative standard deviation (s_r), and the accuracy, expressed as relative error (e), were in the order of 3 to 5%, while the detection limits were in the range 0.010–0.100 μg/g. The standard addition technique improved the resolution of the voltammetric method even in the case of very high element concentration ratios. The analytical procedure was used for real matrices sampled in the Adriatic Sea south to Po river mouth, in the zone "Goro bay", and at open sea north to the Ravenna shore.

Introduction

One of the major problems in ecology is related to the path of heavy metals contained in wastes and waste waters polluting the aquatic environment. Heavy metals may accumulate in certain species of marine plants and thus enter the food chain and endanger living organisms, like algae, mussels, clams or shrimps, which sequestrate and concentrate heavy metals from their aqueous environment [1]. For this reason they may be utilized as bio-indicators of metal pollution in a limited ecosystem [2, 3].

Seaweeds were found to accumulate several metals [4, 5]. Interestingly the trace element concentration of eighteen different species of algae collected along the Mediterranean shore near Israel did not differ significantly from that found in unpolluted regions [6].

However, it is important to emphasize that large differences in metal levels as those of copper, lead, cadmium and zinc, have been found from species to species and from tissues to tissues in algae, molluscs, shrimps, etc. [3].

Copper and cadmium exerted their toxic action by reducing the growth of *Dunaliella minuta* populations [7] and affected the photosynthetic potential of this alga by reducing the relative volume of the chloroplast [8].

Cadmium was found to accumulate as dense deposit within the cell walls and nuclei of alga *Cystoseira barbata* [9].

Uptake and growth inhibition of the algae *Chlorella ellipsoidea* [10] and *Selenastrum copricornutum* [11] were found to increase with increasing concentration of cadmium and mercury, respectively.

Similarly, heavy metal levels were found to increase upon exposure in algae *Nitzschia closterium* and *Prorocentrum micans* present in the Adriatic Sea [12].

Molluscs are also largely influenced by heavy metals. They show the possibility to concentrate several elements and, for this reason, can be usefully employed as biological monitors for trace metals in the aquatic environment [13–15].

Additionally other problems as sampling, digestion technique and the analytical procedure may affect the results, e.g., in the case of cadmium and lead determination in seaweed nitric acid – perchloric acid digestion was found to give significantly different results than that after dry ashing or other digestion techniques [16].

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Therefore, reliable methods were developed for monitoring copper, lead, cadmium, zinc by differential pulse anodic stripping voltammetry (DPASV), and mercury by cold vapor atomic absorption spectroscopy (CV-AAS) in matrices applicable as bio-monitors and involved also in the food chain.

The marine organisms under study were *Ulva rigida* and *Tapes philippinarum*, both very common in the Adriatic Sea.

In the case of the proposed analytical procedure, the presence of H_2SO_4 in the supporting electrolyte, absolutely necessary for the organic matrix digestion, influences the voltammetric peak potentials of each metal, causing strong interferences and signal overlappings. To overcome such a problem [17, 18], even in the case of very high metal concentration ratios, the standard addition method is employed.

Experimental

Apparatus

DPASV curves for the simultaneous determination of copper, lead, cadmium and zinc were recorded with a Multipolarograph AMEL (Milan, Italy) Mod. 433, employing a conventional three electrode measuring cell. The experimental conditions are reported in Table 1.

The voltammetric cell was rinsed every day with suprapure concentrated HNO_3 to avoid contamination.

The solutions were thermostated at 20 ± 0.5 °C and deaerated with pure nitrogen prior to analysis, while a nitrogen blanket was maintained above the solutions during the experiments. The solutions were stirred with a PTFE-coated magnetic stirring bar in the electrolysis step.

Mercury determination was performed using a Perkin-Elmer Model 50 mercury system (absorption wavelength 253.7 nm). The instrument settings were those recommended by the manufacturer [19].

Reagents and reference solutions

Nitric, sulfuric acids and all other chemicals were suprapure grade (Merk, Germany). Stock solutions of copper, lead, cadmium, zinc and mercury (1000 mg/L, BDH, England) were employed for the preparation of reference solutions at varying concentrations for each element, using demineralized water through a Milli-Q system for dilution.

A special treatment was applied to $K_2Cr_2O_7$ in order to render it virtually mercury-free. The salt was heated at 350 °C for 4 days, then the temperature was raised to 410 °C and the mass kept melted for 24 h. Successively, the solidified salt was completely homogenized by a corundum ball-mill.

The reducing agent $SnCl_2 \cdot 2H_2O$ was dissolved in 10% (v/v) H_2SO_4 to give a 25% (m/m) solution, which was bubbled with N_2 for 20 min to strip away any residual Hg and O_2.

Ulva lactuca BCR-CRM 279, *Lagarosiphon major* BCR-CRM 060, *Oyster tissue* NBS-SRM 1566 and *Mussel tissue* BCR-CRM 278 were employed as standard reference materials for optimizing and setting up the analytical procedure.

Table 1 Experimental conditions for the determination of copper(II), lead(II), cadmium(II) and zinc(II) by differential pulse anodic stripping voltammetry (DPASV)[a]

E_d	Electrolysis potential (mV/Ag, AgCl, KCl satd.)	–1100
E_f	Final potential (mV/Ag, AgCl, KCl satd.)	200
dE/dt	Potential scan rate (mV/s)	10
f	Pulse repetition (s)	0.1
ΔE	Pulse amplitude (mV)	50
ν	Pulse duration (ms)	40
t_s	Sampling time (ms)	8
t_d	Electrolysis time (s)	180
t_r	Rest time (s)	15
t	Purging time prior of the electrolysis (s)	300
u	Stirrer speed (r.p.m.)	600

[a] Supporting electrolyte: 5 mL HNO_3 + 5 mL H_2SO_4 diluted to 50 mL with deionized water (see text). Peak potentials (V/Ag, AgCl, KCl sat.): +0.010 [Cu(II)]; –0.470 [Pb(II)]; –0.535 [Cd(II)]; –0.890 [Zn(II)]

Sample preparation

Determination of Cu, Pb, Cd and Zn

For all the standard reference materials, approximately 0.5–1.0 g of the sample was exactly weighed and digested in a Pyrex tube with a mixture of 5 mL H_2SO_4 and 5 mL HNO_3. The tube was inserted into the cold home-made block digestor, raising gradually the temperature up to 150 °C, and keeping it for 4 h.

After digestion and cooling the solutions were filtered on Whatman No. 541, transferred into a volumetric flask and filled up to 50 mL with deionized water.

Voltammetric determinations were carried out after further dilution of this solution (1 : 20) with deionized water.

In the experimental conditions employed the blank concentrations for all the elements were lower than the respective limits of detection. The limits of detection (LOD), expressed according to IUPAC [20] (K = 3), correspond to a probability of 99% [21] and were calculated on 10 blank signals.

Determination of Hg

Several methods have been described for the mercury determination at low concentrations in environmental samples [22–28]. However, in all cases, the most important step of the analytical procedure is the sample preparation before the instrumental measurements due to the possible loss of mercury. A new sample digestion procedure, employing H_2SO_4 and $K_2Cr_2O_7$ as attack mixture, was carefully tested on standard reference materials of different origin [29–31].

The present work reports the first application of such a procedure to real biological environmental samples like algae and clams, which are more and more frequently employed as trace toxic metal biomonitors.

Approximately 1.0 g of the standard reference materials, weighed accurately, was placed in a pyrex digestion tube, having a magnetic bar at its bottom, together with 1.2 g $K_2Cr_2O_7$ and 20 mL H_2O. A condenser, equipped with a lateral and removing funnel in the lower side, was connected to the digestion tube, placing this assembly on the magnetic stirrer. The stirring was started and 20 mL H_2SO_4 were slowly added drop by drop through a lateral funnel. The funnel was removed and the assembly was transferred to the hot-block preheated at 180 °C. The digestion was allowed to proceed for 60 min. The assembly was then removed from the block and kept for 5 min at room temperature. The condenser was removed, rinsed with three 5 mL H_2O portions and washings added to the digest; the open digestion tube, without the condenser, was replaced in the hot-block for a further 30 min boiling time. Finally, after cooling and extraction of the stirring bar, the digest was diluted to 100 mL.

Mercury analyses by CV-AAS were performed employing $SnCl_2$ as the reducing agent.

Results and discussion

Aqueous reference solutions

For the voltammetric determinations, a preliminary study was carried out employing an aqueous reference solution containing 5 mL H_2SO_4 + 5 mL HNO_3 diluted to 50 mL and successively again 1:20 with deionized water; the same mixture was used for the sample preparation before the voltammetric determinations (see "Sample preparation"). A linear i_p *vs.* metal concentration relationship was found for each element.

The simultaneous determination of copper, lead, cadmium and zinc was studied in a large range of concentration ratios, in order to verify the elemental concentration ratios, within which each single element could be determined without mutual interference. To a fixed, but very small concentration of one element, standards of the interfering element were added in such a way as to change their concentration ratios.

The peak current values obtained were then compared to those determined for the same concentration of the single element without interferent and the relative errors were calculated.

To be included in the non-interference concentration ratio intervals, the errors derived from such a comparison must not attain the limit of 5% at the confidence level of 95%. Figure 1 reports such errors as a function of the concentration ratio in the case of Pb and Cd and shows that the determination of Pb and Cd was possible within a maximum error of 5%, in the concentration ranges $13:1 > c_{Pb}:c_{Cd} > 1:15$, for concentrations expressed in mol/L.

Peak overlappings were found only in the case of Pb and Cd ($\Delta E_{p\ Pb\text{-}Cd}$ = 65 mV), while the determinations of Cu and Zn did not show problems; Cu was well separated from the Pb peak and Zn from the Cd peak ($\Delta E_{p\ Pb\text{-}Cu}$ = 480 mV; $\Delta E_{p\ Cd\text{-}Zn}$ = 355 mV).

However, this work concentrates on the determination of metals at the lowest concentration range together with an unfavorable concentration ratio of the interferent elements, outside the concentration ratios interval reported above.

By appropriately adding a standard solution of the metal with the lowest concentration, the concentration ratio can be shifted to the interval of non-interference. Due to the overlapping of the peaks, the i_p *vs.* concentration plots showed a non linear behavior. A linear section was obtained when the concentration ratios attain values within the validity of the bivariate analysis. An extrapolation of this linear section permitted the evaluation of the metal content in the mixture with acceptable accuracy. As example, Fig. 2 reports the fitting of the experimental data for the determination of cadmium in the presence of a high lead concentration in *Ulva lactuca* BCR-CRM 279 (see "Standard reference materials" section). The limit where linearity prevails was well defined and statistically evaluated according to the method of Liteanu et al. [32] using the t-test criteria.

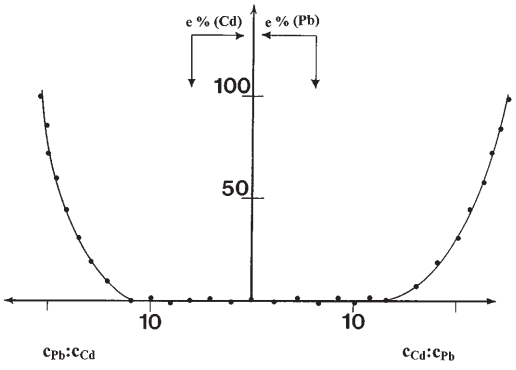

Fig. 1 Relationship between the metal concentration ratios and the relative errors in the determination of the element present at the lowest concentration. Supporting electrolyte: 5 mL HNO_3 + 5 mL H_2SO_4 diluted to 50 mL and successively again 1:20 with deionized water

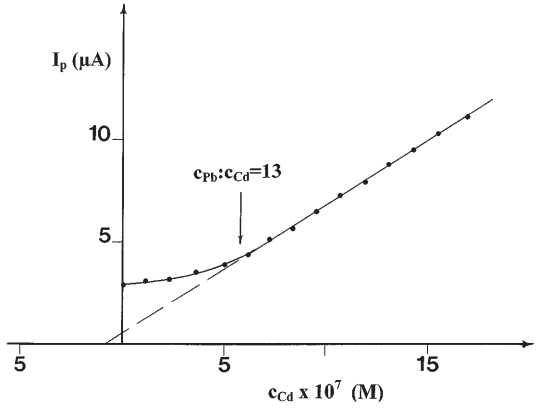

Fig. 2 Analytical calibration function of Cd in *Ulva lactuca* BCR-CRM 279 ($c_{Pb}:c_{Cd}$ = 26.7). Technique: differential pulse anodic stripping voltammetry. Experimental conditions: see Table 1

Standard reference materials

The method developed for aqueous reference solutions was applied to standard reference materials, in order to confirm and verify the applicability of the analytical procedure, determining its accuracy and precision.

The standard reference materials *Ulva lactuca* BCR-CRM 279, *Lagarosiphon major* BCR-CRM 060, *Oyster tissue* NBS-SRM 1566 and *Mussel tissue* BCR-CRM 278 have been employed for algae and clams, respectively.

Using the experimental conditions of Table 1, both statistical parameters, the former expressed as relative error e, and the latter as relative standard deviation s_r, are satisfactory, being in all cases less than 5% (Tables 2 and 3), and also for Pb and Cd in *Ulva lactuca* BCR-CRM 279 and *Lagarosiphon major* BCR-CRM 060, where the Pb-Cd concentration ratios are unfavorable (Pb:Cd = 26.7 and 15.7, respectively).

Table 2 Accuracy and precision of the analytical procedure in the algal matrix[a]

	Element	Certified value (μg/g)	Determined value (μg/g)	e (%)	s_r (%)	LOD[b] (μg/g)
Ulva lactuca BCR-CRM 279	Cu(II)	13.14 ± 0.37	12.7 ± 0.5	–3.4	2.8	0.011
	Pb(II)	13.48 ± 0.36	13.8 ± 0.3	+2.3	3.1	0.018
	Cd(II)	0.274 ± 0.022	0.29 ± 0.02	+4.4	2.5	0.009
	Zn(II)	51.3 ± 1.2	53.1 ± 2.1	+3.5	3.9	0.101
	Hg (II)	0.05[c]	0.047 ± 0.004	–6.0	3.7	0.027
Lagarosiphon major BCR-CRM 060	Cu(II)	51.2 ± 1.9	49.9 ± 1.9	–2.5	3.1	0.016
	Pb(II)	63.8 ± 3.2	62.0 ± 2.1	–2.8	3.6	0.025
	Cd(II)	2.20 ± 0.10	2.26 ± 0.09	+2.7	1.8	0.017
	Zn(II)	313 ± 8	305 ± 10	–2.6	4.3	0.096
	Hg(II)	0.34 ± 0.04	0.32 ± 0.03	–5.9	3.8	0.037

[a] Experimental conditions: see Table 1. Number of independent determinations: 5
[b] The limit of detection (LOD) corresponds to a probability of 99% [20, 21]. In both voltammetric and spectroscopic measurements, the blank concentrations for all the elements were less than the respective limit of detection
[c] Value not certified

Table 3 Accuracy and precision of the analytical procedure in the clam matrix[a]

	Element	Certified value (μg/g)	Determined value (μg/g)	e (%)	s_r (%)	LOD[b] (μg/g)
Oyster tissue NBS-SRM 1566	Cu(II)	63.0 ± 3.0	65.4 ± 2.8	+3.8	4.0	0.023
	Pb(II)	0.48 ± 0.02	0.46 ± 0.03	–4.2	2.3	0.048
	Cd(II)	3.5 ± 0.2	3.4 ± 0.3	–2.9	3.6	0.013
	Zn(II)	852 ± 23	869 ± 27	+2.0	3.9	0.077
	Hg(II)	0.057 ± 0.002	0.054 ± 0.004	–5.3	4.2	0.035
Mussel tissue BCR-CRM 278	Cu(II)	9.60 ± 0.16	9.77 ± 0.23	+1.8	2.9	0.015
	Pb(II)	1.91 ± 0.04	1.82 ± 0.07	–4.7	3.1	0.027
	Cd(II)	0.34 ± 0.02	0.35 ± 0.03	+2.9	1.9	0.007
	Zn(II)	76 ± 2	78 ± 3	+2.6	3.6	0.069
	Hg(II)	0.188 ± 0.007	0.181 ± 0.010	–3.7	2.3	0.021

[a] Experimental conditions: see Table 1. Number of independent determinations: 5
[b] See footnote in Table 2

The detection limits for the metals are also reported in Tables 2 and 3.

Real samples

The method was applied to the determination of mercury, copper, lead, cadmium and zinc in the marine organisms under study, namely *Ulva rigida* and *Tapes philippinarum*.

Sampling and pretreatment of *Ulva rigida*

About 10 kg of *Ulva rigida* were collected employing a rubber boat near the mouth of the Po River, in the zone "Goro Bay" (salinity: 2.7%, temperature: 29 °C), and at open sea north to the Ravenna shore (salinity: 3.1%, temperature: 27 °C) with a small net and transferred into polyethylene bags and transported to the laboratory in a portable refrigerator in order to avoid alterations during transport.

The samples (water content > 89% in all cases) were dried in the laboratory for 48 h at 40–45 °C, then finely ground and homogenized.

Different aliquots (0.5–1.0 g) were taken from that batch, exactly weighed, digested and analyzed as described above.

Sampling and pretreatment of *Tapes philippinarum*

About 8 kg of *Tapes philippinarum* were collected near the mouth of the Po River in the zone "Goro Bay". The clams were sampled at the sea bottom and immediately taken to the laboratory and prepared for analysis. The clams were opened 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 lyophilized for 30 h. Such a procedure, employing very low temperatures for drying the samples, avoids the loss of volatile analytes like mercury.

Then the sample was homogenized thoroughly in an agate mortar. About 0.5–1.0 g sample were exactly weighed, digested and analyzed as described above.

The experimental results relevant to both matrices are listed in Table 4.

For *Ulva rigida*, from both sampling sites, even if Pb and Cd showed mutual interference and unfavorable concentration ratios ($c_{Pb} : c_{Cd} > 13$), both elements could be equally determined following the standard addition procedure previously described.

Table 4 Mean values of the metal concentrations (μg/g ± s.d., dry weight) relevant to the real matrices sampled in the Adriatic Sea[a]

Element	*Ulva rigida*		*Tapes philippinarum*
	Ravenna Shore	Po River Mouth	Po River Mouth
Cu(II)	23.0 ± 1.1	27.2 ± 0.9	11.7 ± 0.5
Pb(II)	6.96 ± 0.27	5.69 ± 0.23	7.69 ± 0.23
Cd(II)	0.09 ± 0.01	0.15 ± 0.01	1.96 ± 0.09
Zn(II)	87 ± 5	96 ± 4	77 ± 3
Hg(II)	0.123 ± 0.005	0.070 ± 0.003	0.169 ± 0.007

[a] The standard deviation (s.d.) is determined on 5 independent digestions

Conclusion

– The voltammetric technique has shown to be a sensitive and selective analytical procedure, suitable for the multicomponent element determination in complex real samples (in the present case environmental matrices like algae and clams).
– The standard addition method allows to simultaneously determine neighboring elements also in the case of strong interferences and signal overlappings.
– A final consideration: the results obtained for such matrices are substantially in the same order of magnitude and in agreement with those obtained in the same [33] and in other ecosystems [34–36].
– The set up of a correct analytical procedure for the trace metal determination in algae and clams allows to use such species as bio-indicators of toxic metals [2, 3], or also in biodetoxification procedures, and, last but not least, for checking high quality food [14].

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References

1. Ting Y P, Lawson F, Prince I G (1991) Biotechnol Bioeng 37: 445–455
2. Phillips D J H (1994) In: Kramer K J M (ed) Biomonitoring of Coastal Waters and Estuaries. CRC Press, Boca Raton, FL, p 85–103
3. Johansen P, Hansen M M, Asmund G, Nielsen P B (1991) Papers from the Eighth International Ocean Symposium, 9–13th October, 1989, Dubrovnik, Yugoslavia, Part Two, Duedall IW (ed) 5: 35–55
4. Muse J O, Tudion M B, D'Huicque L, Troccoli O E, Carducci C N (1995) Environ Pollut 87: 249–253
5. Garcia E M (1993) Toxicol Environ Chem 39: 29–35
6. Homung H, Kress N, Friedlander M (1992) Fresenius Environ Bull 1: 84–89
7. Visviki I, Rachlin J W (1992) Arch Environ Contam Toxicol 20: 271–275
8. Visviki I, Rachlin J W (1992) Arch Environ Contam Toxicol 23: 420–425
9. Pellegrini L, Pellegrini M, Delivopoulos S, Berail G (1991) Br Physiol J 26: 1–8
10. Aoyama I, Okamura H (1993) Environ Toxicol Water Qual 8: 255–269
11. Chen C Y (1994) Water Res 28: 931–937
12. Carpenè E, Boni L (1992) Science of the Total Environment, Supplement 1992: 921–928
13. Tessier L, Vaillancourt G, Pazdernik L (1996) Water, Air Soil Pollut 86: 347–357
14. Locatelli C, Fagioli F (1997) Ann Chim (Rome) 87: 535–538
15. Schuhmacher M, Domingo J L, Llobet J M, Corbella J (1993) In: Sloof W, Kruijf H de (eds) Proceedings of the second European Conference on Ecotoxicology, Suppl Pts 1–2: 117–125
16. Fleurence J, Le Coeur C (1994) Bot Mar 37: 555–559
17. Locatelli C, Torsi G (1996) Electrochim Acta 41: 2011–2017
18. Huang W, Henderson T L E, Bond A M, Oldham K B (1995) Anal Chim Acta 30: 1–15
19. Perkin-Elmer (1982) Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer Corp., Norwalk
20. International Union of Pure and Applied Chemistry – Analytical Chemistry Division (1978) Spectrochim Acta 33B: 241–245
21. Miller J C, Miller J N (1984) Statistics for Analytical Chemistry, Ellis Horwood, Chichester
22. Vargas M C de, Romero R A (1992) Analyst 117: 645–647
23. Bulska E, Baxter D C, Frech W (1991) Anal Chim Acta 249: 545–554
24. Navarro M, Lopez M C, Lopez H, Sanchez M (1992) Anal Chim Acta 257: 155–158
25. Minganti V, Fiorentino F, De Pellegrini R, Capelli R (1994) Int J Environ Anal Chem 55: 197–202
26. Horvat M, Liang L, Azemard S, Mandic V, Villeneuve J P, Coquery M (1997) Fresenius J Anal Chem 358: 411–418
27. Donais M K, Saraswati R, Mackey E, Demiralp R, Porter B, Vangel M, Levenson M, Mandic V, Azemard S, Horvat M, May K, Emons H, Wise S (1997) Fresenius J Anal Chem 358: 424–430
28. Schantz M M, Demiralp R, Greenberg R R, Hays M J, Parris R M, Porter B J, Poster D L, Sander L C, Sharpless K S, Wise S A, Schiller S B (1997) Fresenius J Anal Chem 358: 431–440
29. Landi S, Fagioli F, Locatelli C, Vecchietti R (1990) Analyst 115: 173–177
30. Landi S, Fagioli F, Locatelli C (1992) J Assoc Off Anal Chem Int 75: 1023–1028
31. Locatelli C, Bighi C, Fagioli F (1996) Fresenius Environ Bull 5: 386–391
32. Liteanu C, Popescu I C, Hopirtean E (1976) Anal Chem 48: 2010–2013
33. Fagioli F, Locatelli C, Landi S (1994) Ann Chim (Rome) 84: 129–140
34. Forstner U, Wittman G T W (1983) Metal Pollution in the Aquatic Environment, Springer, Berlin Heidelberg New York
35. Karez C S, Magalhaes V F, Pfeiffer W C, Filho G M A (1994) Environ Pollut 83: 351–356
36. Seferlis M, Haritonidis S (1995) Fresenius Environ Bull 4: 309–314