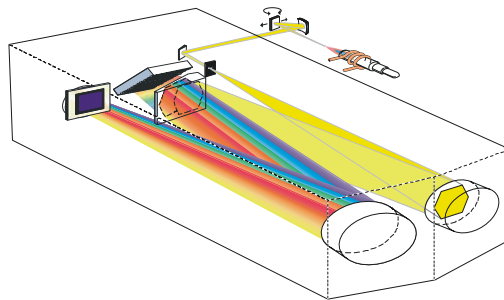**CNR-I.A.M.C.**

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Atomic emission spectrometry methods for determination of heavy metals (As, Cd, Co, total Cr, Cr(VI), Cu, Hg, Ni, Pb, Se, V, Zn) in marine sediments

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Contents

1. Introduction

Heavy metals play a relevant role in environmental processes since they are involved in natural geochemical and biochemical cycles; trace levels of several metals like Zn, Fe, Cu, Co are essential to marine organisms as constituents of enzymes ruling biological processes, but too high concentrations are cause of toxic effects on marine organisms.

Trace elements distribution in seawater and marine sediments results by a complex balance of both natural and anthropic inflow and outflow processes. Sediments are the last sink of heavy metals delivered by rivers, fallout processes and scavenging of particulate material (Nriagu, 1979) and could act as source of metals to seawater if a change in physical or chemical conditions at sediment-seawater interface occurs (Fergusson, 1990).

The assessment of trace metal distribution in marine sediments allows: i) the evaluation of the current status of environmental pollution, ii) the identification of the potential sources of pollution, iii) an adequate understanding of mechanisms ruling trace metals mobility and bioavailability in order to verify the existence of a real environmental risk.

Since a reliable dataset is essential to that relevant purpose, a correct procedure of sampling, storage, treatment and analysis of marine sediments plays a key role; the aim of this report is to describe in detail a protocol for preparation and analysis of sediment samples for determination of trace metals content using atomic emission spectrometry. All operating procedures were developed and checked for precision, reproducibility and accuracy in the geochemical laboratories of IAMC – CNR of Naples equipped with a VARIAN ICP-AES VISTA-MPX .

2. Inductively coupled plasma atomic emission spectrometry (ICP-AES)

Atomic emission spectrometry allows analysts to perform both qualitative and quantitative determinations of elements measuring their characteristic emission spectra by optical spectrometry. Due to its versatility and ease of use, ICP-AES is today widely used for determination of major and trace constituents of environmental matrices like soils, sludges, sediments, groundwater and industrial wastes (Becker, 2005).

Liquid samples are converted in aerosol in a concentric nebulizer and large droplets ($\Phi>5\mu m$) are removed passing through a spray chamber before introduction into the plasma; argon plasma is generated by an electrical spark and sustained by a magnetic field (RF= 40MHz); the finest fraction of sample aerosol is introduced into plasma where it is desolvated, atomized and excited so that single atoms could emit characteristic photons; in a polychromator all spectra lines are separated in single wavelengths trough a grating system and a Coupled Charged Detector measures simultaneously the intensities of each wavelength (Knowles, 2002).
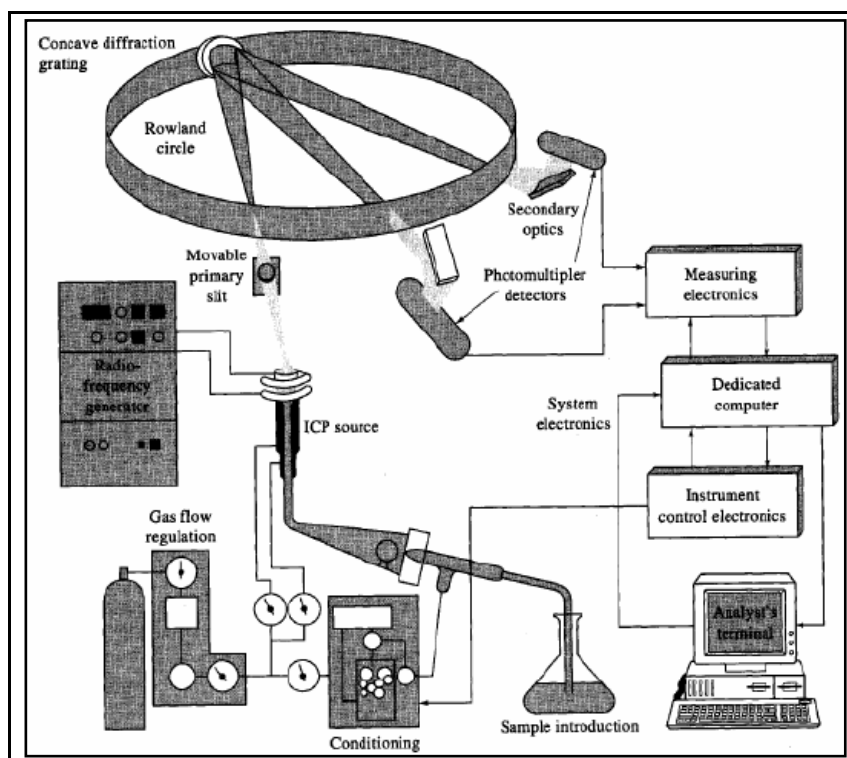
Fig. 1 Principal components of an ICP-AES system

3. Sampling and extraction procedures

Once the sediment core had been collected, samples had to be homogenized on site, placed in PVC bags and preserved at -20°C until preparation in laboratory. During sampling phases operators should use only non metallic materials and properly handle samples to prevent contamination.

Previous steps of sample preparation are: i) drying process, in a stove with forced air circulation, in three days; ii) fractionation of the finest grain-size through a Φ < 2mm microsieve; iii) reduction process to obtain a representative aliquot of the overall sample; that subsample is reduced to powder (< 200 mesh) in a planetary ball mill (Retsch PM100) or a mortar of agate to obtain a homogeneous sample.

4. Microwave assisted acid digestion

4.1 Instruments

Since atomic emission spectrometry allows determination of all elements of interest in liquid samples, it's essential a previous reduction of the sediment solid matrix in the solution form.

Three acid digestion procedures were developed and compared one another; the mineralization process was performed in the microwave oven Mars-5 of CEM Corporation (USA) (Fig. 2).

Digestion takes place in teflon vessels at high pressure and temperature thanks to an homogeneous microwave irradiation; Mars-5 equipment provides an automatic control system for temperature and pressure on vessel n. 1, so it is convenient realize a single mineralization cycle on samples resulting homogeneous in weigh, grain size, composition and acid mixture.

Before starting a digestion process is important: i) check safety membrane for each vessel both in position and level of stress;

ii) verify that all vessel surfaces are effectively dry; presence of liquid drops in joints may be cause of local overpressure and as a consequence of vessel deformation with a leak of sample.

A safety membrane break occurs if sediment sample is highly rich in carbonates or organic matter; in this case, reaction with acids provides evolution of a huge quantity of gaseous product so that is convenient let a "pre-digestion" under hood for 15-20 minutes in order to minimize a loss of analytes during digestion.

The choice of microwave assisted digestion was based on several advantages it provides in comparison with other extraction methods: i) rapidity; ii) prevention of sample contamination; iii) preservation of volatile elements like As, Hg, Sb, Se e Sn.

Fig. 2 Microwave assisted reaction acceleration system
MARS-5 (CEM Corporation)

4.2 Extraction methods

To develop adequate procedures of extraction we started studying the 3052 Method of US-EPA and following these fundamental steps:

1) Selection of an adequate acid mix to dissolve all elements of interest from sediment matrix;

2) Choice of the optimum value of sample quantity to gain both a concentration of dissolved metals >> instrument detection limits and a total attack of the sample;

3) Planning of microwave oven work (defining temperature, pressure, time and power parameters) to obtain an efficient acid attack keeping safety regulations.

Tests were performed on a standard reference material (PACS-2) from National Research Council of Canada (Ottawa), a sediment collected in the harbour of Esquimalt, B.C..

We made use of PACS-2 as reference material since it presents mineralogical composition and heavy metal content comparable to those of our samples. Certified values of PACS-2 are based on results of determinations performed at least using two independent analysis methods (surely atomic emission spectrometry) for heavy metals but Hg and Se, detected using other techniques of analysis like atomic fluorescence spectrometry, mass spectrometry and atomic absorption spectrometry.

An aliquot of PACS-2, weighed with a precision of 0.0001 g was subjected at three different acid attacks:

- aqua regia attack;

- acid attack (HNO_3 + HCl) + H_2O_2;

- total attack.

All dissolved samples were leached through whatman grade 1 filters (11 μm) to allow separation of residual sediment, reduced to a total volume of 100 ml using millQ water (Ω=18.2) in plastic flasks

(previously decontaminated using HNO_3 10%), transferred in 50 ml PP falcon and stored at 4°C until analysis.

Results of determinations performed in ICP-AES on PACS-2 samples subjected to different acid digestions are discussed above.

4.2.1 Aqua regia attack

Aqua regia attack is a "pseudo-total" attack, since it allows breaking of all mineralogical phases of samples but the silicates; it results a good solution in environmental studies because it enables scientists to extract from the solid matrix only bioavailable metals (non bonded to siliceous sediment structure).

Aqua regia results by mixing of three parts of HCl (reducing agent which allows to attack oxides, hydroxides, carbonates and sulfides at high temperature and pressure) and one part of HNO_3 (oxidant agent to digest organic matter). As aqua regia is a highly corrosive acid mixture, it had to be prepared using an adequate fume hood equipped with specific filters for acids. Mixing of HCl and HNO_3 is a highly exothermic process and produces a significant gas amount. It's advisable to conduct that mixing in a glass volumetric flask and avoid to completely close it to let gas output; in 5-10 minutes the acid mixture would change from a golden yellow color to an intense red one and become aqua regia usable to attack our samples.

At each digestion vessel were added 5 ml of millQ water (in order to increase mineral solubility and avoid temperature spikes) and 10 ml of aqua regia; vessels were sealed and subjected to the mineralization cycle (Tab. 1) in Mars-5.

An efficient attack could be performed keeping samples at $180 \pm 5°C$ at least for 10 minutes (EPA method 3052). How to achieve

such a temperature is defined by the operator setting the plan of work of the microwave oven.

At the end of a digestion cycle it is necessary let vessels cooling at room temperature before proceeding in sample preparation to assure security rules in operating steps and avoid a leak of volatile substances like fluorides and chlorides.

Step	Power (W)	Time (min)	Pressure (PSI)	Temperature (°C)	Period (min)
1	1200	12:00	130	170	03:00
2	1200	05:00	220	200	45:00

Tab. 1 Mars-5 settings for a simultaneous digestion of 12 samples

4.2.2 Total attack

Total attack allows to dissolve completely a sediment sample prior to analysis. We tested a total attack by use of two different acid mixtures:

Mix (A) : 3 ml HF 48% V/V (silicate matrix dissolution to a SiF_6^{2-} acid solution), 2 ml MillQ water, 2 ml HCl 37% V/V (in order to stabilize Ag, Ba, Sn, Sb and high Al and Fe level solutions), 9 ml HNO_3 67% V/V.

Mix (B) : 5 ml MillQ water, 2 ml HF 48% V/V, 10 ml aqua regia.

Sample digestion was performed using the same program as for the aqua regia attack (Tab.1) but an important second step with addition of 30 ml of a 4% m/V boric acid solution at each vessel was performed in order to avoid corrosion phenomena (due to non complexed fluorides) on glass spare parts of the analysis instrument as torch, spray chamber and nebulizer. The presence of boric acid causes both a longer mineralization process and a major

solid content (% m/V) in sample solutions and hence a more evident matrix effect.

Alternatively, was used an instrument "inert kit" provided by Varian with torch, spray chamber and nebulizer HF resistant; it allows the instrument to work with a lighter matrix but results in a less reading sensitivity and a lesser stability of certain compounds like fluorides in sample solutions (Kamal et al., 2005).

These experiences let us to identify as the optimum process for total attack the one with boric acid and a further dilution of sample solutions to achieve a maximum value of 1 % m/V solid content .

Total attack by the acid mix (A) resulted more effective than the attack by the acid mixture (B), especially digesting marine sediments with a high organic matter content (as consequence of a greater oxidant power of HNO_3).

Step	Power (W)	Time (min)	Pressure (PSI)	Temperature (°C)	Period (min)
1	1200	12:00	130	170	03:00
2	1200	05:00	200	200	15:00

Tab. 2 Microwave working plan to achieve HF complexation by boric acid

4.2.3. (HNO_3+HCl) + H_2O_2 acid mix attack

A further attack was performed following the regional agency for environment ARPAC rules for acid digestion of sediment samples; each sample was treated in an open vessel under a laminar hood with 3 ml H_2O_2 for 20 minutes at room temperature; then were added 9 ml HCl and 3 ml HNO_3. The addition of HCl could provide a huge development of gaseous compounds causing a loss of sample; simply adding 2 ml millQ water prior to acid addition one could avoid such a problem.

Samples were mineralized following two different digestion programs: the first one, provided by ARPAC (Tab. 3) and the other one optimized in our laboratories (Tab. 1).

The digestion process conducted following ARPAC guidelines were quite difficult for continuous breaking of security membranes in vessels; it revealed a necessary modification of that program ruling microwave emissions by a precise temperature-time law and control on maximum pressure value (Tab. 4).

Metal determinations were found out quite similar suggesting that no significant difference exists between the two digestion programs processed (Tab.1, Tab.4).

Step	Power (W)	Time (min)
1	250	10:00
2	450	10:00
3	600	10:00
4	250	05:00

Tab. 3 Microwave digestion program provided by ARPAC guidelines

Step	Power (W)	Time (min)	Pressure (PSI)	Temperature (°C)	Period (min)
1	250	10:00	130	150	0:00
2	450	5:00	220	185	0:00
3	600	10:00	220	185	10:00
4	250	05:00	220	185	05:00

Tab. 4 Microwave digestion program optimized in the IAMC laboratory to perform an attack by the acid mix $(HNO_3+HCl)+ H_2O_2$.

5. Metal content determinations (As, Cd, Co, Cr, Cu, Ni, Pb, V, Zn) by ICP-AES technique

5.1 Instrument

Sample solutions were analysed by inductive coupled plasma emission spectrometry to determine metal content (As, Cd, Co, Cr, Cu, Ni, Pb, V, Zn); analytical procedures performed were based on EPA method 6010b directives. Analysis were performed by an assial VARIAN ICP-AES VISTA-MPX (Fig. 3), equipped with a peristaltic pump, a cyclonic spray chamber, a glass concentric nebulizer as sample introducing system and a high sensitivity torch of quartz. Such an equipment allows to analyse sample solutions with a total acid content (and HF free) smaller than 10 % V/V and a dissolved solid content smaller than 1 % m/V.

5.2 Instrument calibration and optimization of operative parameters

Prior to analysis is important perform a check of the whole system of sample introduction and of spare parts of the instrument; after that, next step to follow is to turn on the plasma and let the

instrument stabilization (about 20 minutes) before proceeding to the tuning phase; this one consists in pumping a 5 ppm Mn tuning solution and verify a reading sensitivity at 257.610 nm of about 1000000 c/s (Fig. 4); a smaller value in Mn reading identifies a loss in the sensitivity of the instrument probably due to one or more of the following causes:

a) Non optimum operative parameters setting;

b) Bad torch alignment;

c) Failure of instrument calibration parameters;

d) Bad working of the optic system.

If the bad working of the instrument is due to the cause (a) one should be able to increase instrument sensitivity simply adjusting operative parameters values for argon flows, RF power and rate pump; if such attempt fails, it is essential to realize a torch alignment to achieve the maximum level in signal reading. After a torch alignment one could only perform an instrument calibration pumping a multielement solution (5 ppm Al, As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Mo, Sr, Zn and 50 ppm K) in order to calibrate the hardware and the detector for all λ of interest. At last, if still a weak signal were read it would be due to a bad working of optics and would be resolved by Varian technicians. An important topic however must be considered: the natural decrease in instrument sensitivity due to hard daily working (of about 25%).
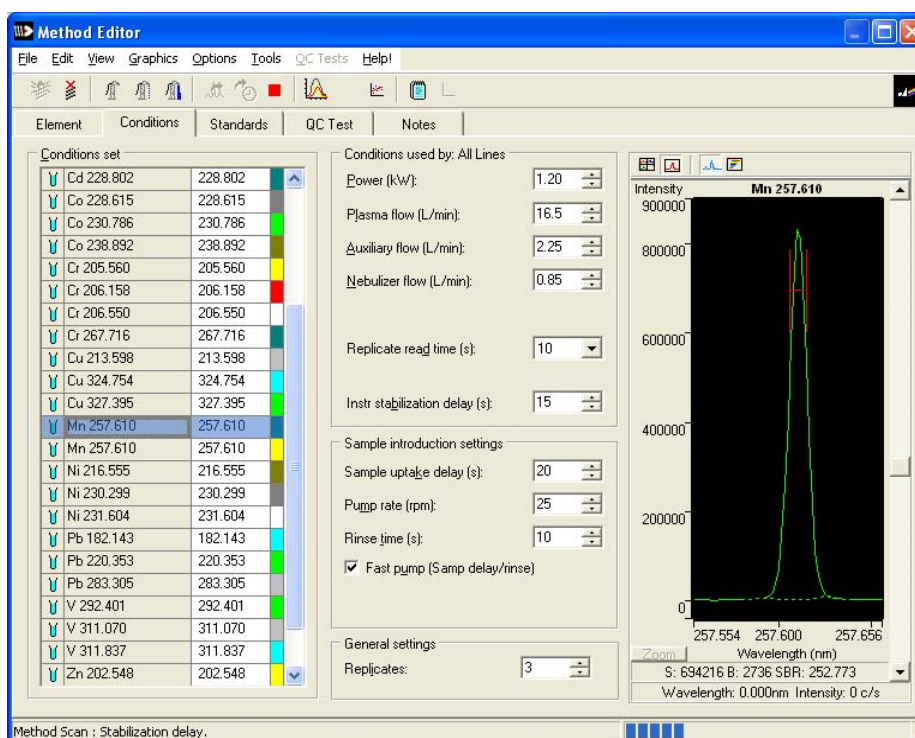
**Fig.4** Optimization of signal intensity for Mn 257.610

5.3 Analytical determinations

After the instrument stabilization and optimization steps was planned a method for quantitative analysis of all elements of interest basing on:

✓ **Operative parameters** (Fig. 5) values chosen in order to optimize the signal/noise ratio, running costs and the timetable of analysis (Tab. 5);

✓ **Reading wavelengths** (Tab. 6) chosen basing on characteristics of the interference windows, on the element emission intensity levels and on the presence of a possible background effect;

✓ **External calibration curve characteristics** (Fig. 6) obtained fitting at least five points correspondent to four multistandard solutions and a calibration blank readings with a maximum error of 10 % for each standard solution and a correlation coefficient greater than 0.95.

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Calibration standards were prepared by dilution of a certificated 100 $\pm$ 0.2 mg/l multistandard solution in volumetric flasks (class A) using acidified (1% HCl and HNO$_3$) millQ water. Calibration curves were constructed to cover a concentration detection range of 50-1000 ppb.
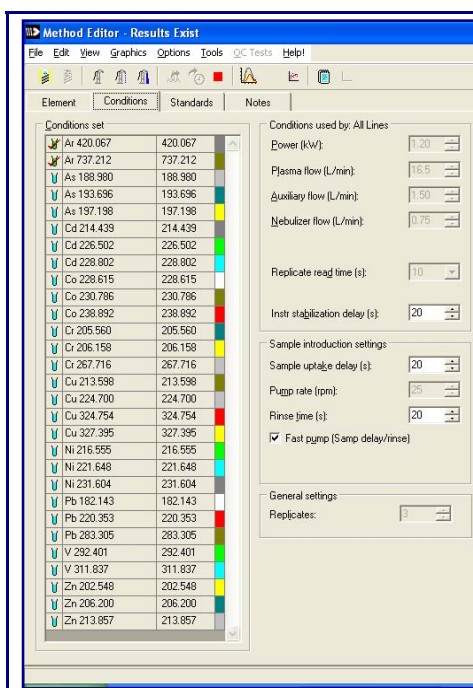

Fig.5 Table of operative parameters values optimized for metals analysis by ICP-AES

Sample source	time/sample (s)	argon/sample (L)	Medium cost/sample (€)
manual	90	30	
autosampler	120	40	

***Tab. 5** Time required and medium cost per sample for elements determination by ICP-AES*

Element	λ (nm)
As	188.980
Co	228.615
Cr	205.560
Cd	214.439
Cd	226.502
Cu	213.598
Hg	194.164
Hg	253.652
Ni	231.604
Pb	220.353
Se	203.985
Se	196.026
V	292.401
Zn	206.200

Tab. 6 List of reading wavelengths (λ)
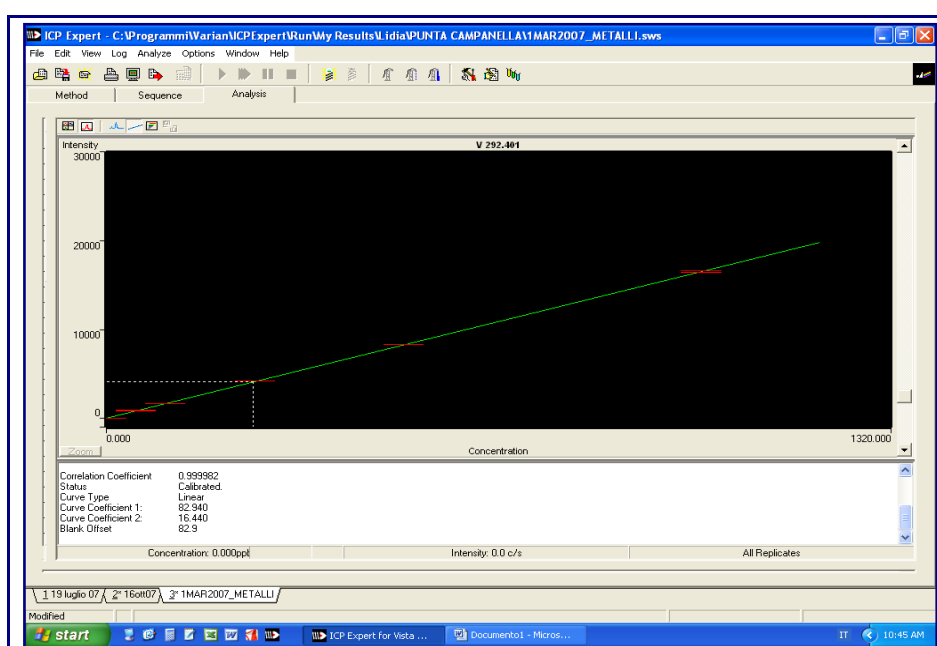
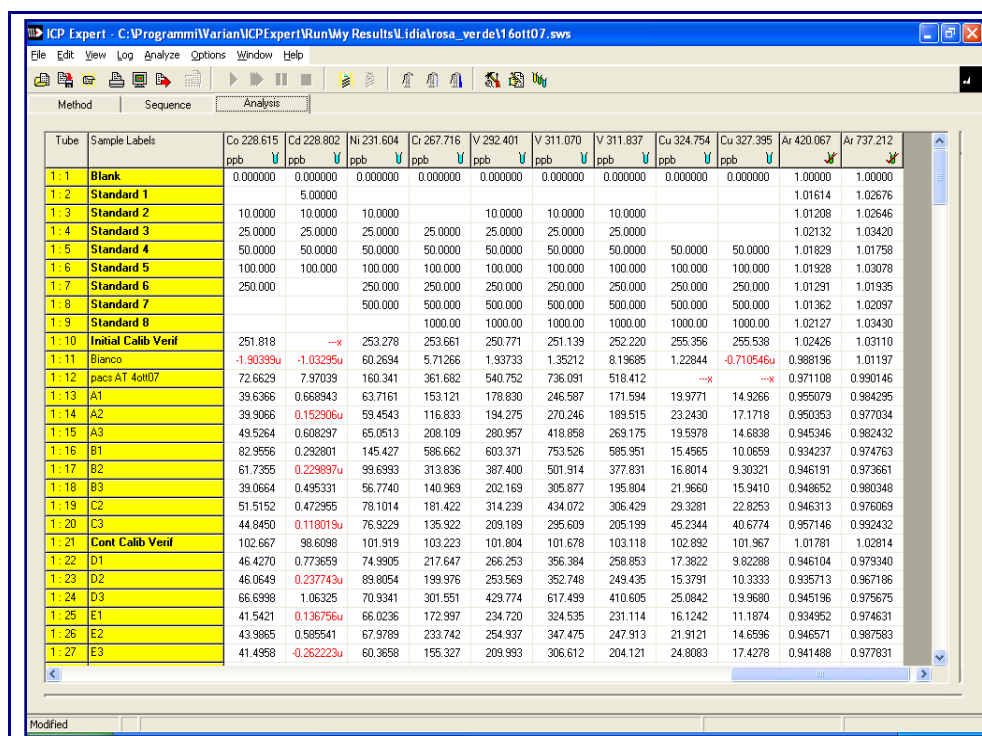**Fig. 6** Calibration curve constructed to carry out quantitative analysis by ICP-AES

5.4 Interferences

Single readings by atomic emission spectrometry suffer from two different kinds of interference classified as:

- *chemical-physical interferences* caused by the differences existing between matrices of the calibration standards and the real sample;

- *spectral interferences* due to the possible overlapping of emission wavelengths.

In general, when working at analytical determinations by atomic spectrometry, a good rule consists in the use of a calibration standard solution whose physical characteristics (like density and viscosity) and chemical setting are similar to those of the real sample matrix in order to avoid consistent interferences corresponding to the first item; otherwise, one has to follow the standard addition method for analysis or consider an internal standard (added both at standard solutions and samples) to perform a fair correction. Anyway, attention has to be paid at such method for the so called matrix effect weighs differently on the reading wavelengths in the range of interest, and could result of no effective use.

Despite its approximate role, an immediate and continuous monitoring activity was performed measuring the signal relative to Ar 420.067 and Ar 737.212 to test the presence of a matrix effect during analytical determinations of elements of interest. Most significant variations were relieved for samples digested by total attack, for their heavy matrix due to presence of boric acid at saturation level as indicated in fig.7 (Ar signal intensity corresponding to samples significantly low compared to those relative to calibration standards and blank solutions).

| Tube	Sample Labels	Co 228.615 ppb	Cd 228.802 ppb	Ni 231.604 ppb	Cr 267.716 ppb	V 292.401 ppb	V 311.070 ppb	V 311.837 ppb	Cu 324.754 ppb	Cu 327.395 ppb	Ar 420.067	Ar 737.212
1 : 1	Blank	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	1.00000	1.00000
1 : 2	Standard 1		5.00000								1.01614	1.02676
1 : 3	Standard 2	10.0000	10.0000	10.0000		10.0000	10.0000	10.0000			1.01208	1.02646
1 : 4	Standard 3	25.0000	25.0000	25.0000	25.0000	25.0000	25.0000	25.0000			1.02132	1.03420
1 : 5	Standard 4	50.0000	50.0000	50.0000	50.0000	50.0000	50.0000	50.0000	50.0000	50.0000	1.01829	1.01758
1 : 6	Standard 5	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	1.01928	1.03078
1 : 7	Standard 6	250.000		250.000	250.000	250.000	250.000	250.000	250.000	250.000	1.01291	1.01935
1 : 8	Standard 7			500.000	500.000	500.000	500.000	500.000	500.000	500.000	1.01362	1.02097
1 : 9	Standard 8			1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1.02127	1.03430
1 : 10	Initial Calib Verif	251.818	---x	253.278	253.661	250.771	251.139	252.220	255.356	255.538	1.02426	1.03110
1 : 11	Bianco	-1.90399u	-1.03295u	60.2694	5.71266	1.93733	1.35212	8.19685	1.22844	-0.710546u	0.988196	1.01197
1 : 12	pacs AT 4ott07	72.6629	7.97039	160.341	361.682	540.752	736.091	518.412	---x	---x	0.971108	0.990146
1 : 13	A1	39.6366	0.668943	63.7161	153.121	178.830	246.587	171.594	19.9771	14.9266	0.955079	0.984295
1 : 14	A2	39.9066	0.152906u	59.4543	116.833	194.275	270.246	189.515	23.2430	17.1718	0.950353	0.977034
1 : 15	A3	49.5264	0.608297	65.0513	208.109	280.957	418.858	269.175	19.5978	14.6838	0.945346	0.982432
1 : 16	B1	82.9556	0.292801	145.427	586.662	603.371	753.526	585.951	15.4565	10.0659	0.934237	0.974763
1 : 17	B2	61.7355	0.229897u	99.6993	313.836	387.400	501.914	377.831	16.8014	9.30321	0.946191	0.973661
1 : 18	B3	39.0664	0.495331	56.7740	140.969	202.169	305.877	195.804	21.9660	15.9410	0.948652	0.980348
1 : 19	C2	51.5152	0.472955	78.1014	181.422	314.239	434.072	306.429	29.3281	22.8253	0.946313	0.976069
1 : 20	C3	44.8450	0.118019u	76.9229	135.922	209.189	295.609	205.199	45.2344	40.6774	0.957146	0.992432
1 : 21	Cont Calib Verif	102.667	98.6098	101.919	103.223	101.804	101.678	103.118	102.892	101.967	1.01781	1.02814
1 : 22	D1	46.4270	0.773659	74.9905	217.647	266.253	356.384	258.853	17.3822	9.82288	0.946104	0.979340
1 : 23	D2	46.0649	0.237743u	89.8054	199.976	253.569	352.748	249.435	15.3791	10.3333	0.935713	0.967186
1 : 24	D3	66.6998	1.06325	70.9341	301.551	429.774	617.499	410.605	25.0842	19.9680	0.945196	0.975675
1 : 25	E1	41.5421	0.136756u	66.0236	172.997	234.720	324.535	231.114	16.1242	11.1874	0.934952	0.974631
1 : 26	E2	43.9865	0.585541	67.9789	233.742	254.937	347.475	247.913	21.9121	14.6596	0.946571	0.987583
1 : 27	E3	41.4958	-0.262223u	60.3658	155.327	209.993	306.612	204.121	24.8083	17.4278	0.941488	0.977831

Fig. 7 Multielement analysis worksheet settings for samples digested by total attack.

A direct reading of the sample solution, in some cases, determined salt deposition at nebulizer nozzle and torch injection tube so to cause a strong drop in reading sensitivity and appearance of the memory effect; consequently, was decided to perform analytical determination on sample solutions diluted 1:2 or 1:3 when possible. Some elements present a too low concentration values (as Cd and Co in the reference material PACS-2) or a very low signal-noise ratio (As) and must be detected analysing non diluted sample solutions; in such cases is really important setting operative parameters with a longer rinse time between two consecutive readings. A correction was made on readings basing on data of the recovery percentage of measured reference spiked solutions (tab. 7).

Element	Recovery (%)
Cu	96,44
As	120,09
Cr	97,11
Ni	90,22
Pb	92,20
V	94,04
Zn	94,83
Cd	80,37
Co	74,31

Tab. 7 Medium values of recovery percentage determined
on certificated reference material spiked solutions

Spectral interferences cause an overestimation of the single reading we tried to avoid making a reasonable choice of element wavelength of emission based on those with relative overlapping and trying to correct the obtained data by an automated function provided by Varian software of management of the instrument: FACT subroutine which calculates automatically the correction factor to apply to the reading basing on: i) the form of the peak corresponding to the reading of the interfered analyte considered, ii) on the form of the peak corresponding to the non interfered analyte, and iii) on the form of the peak corresponding to the interferent element. FACT correction was performed for Cd concentration determinations at 226.522 nm; the Cd reading peak, in fact, presented a clear overlapping by Fe 226.505 due to the high content of iron in sediment matrix (fig.8, fig.9, fig.10).
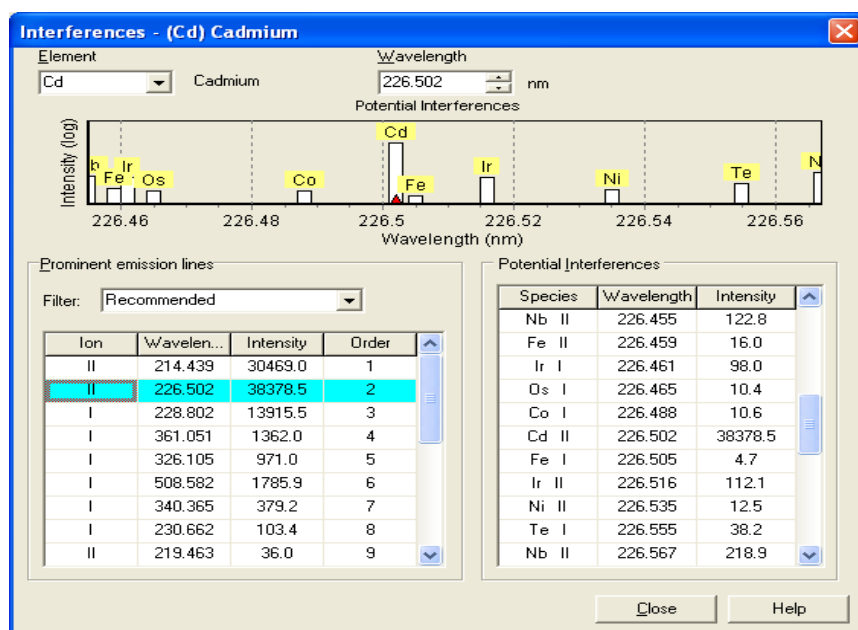
Fig 8 Window of spectral interferences corresponding to Cd 226.502
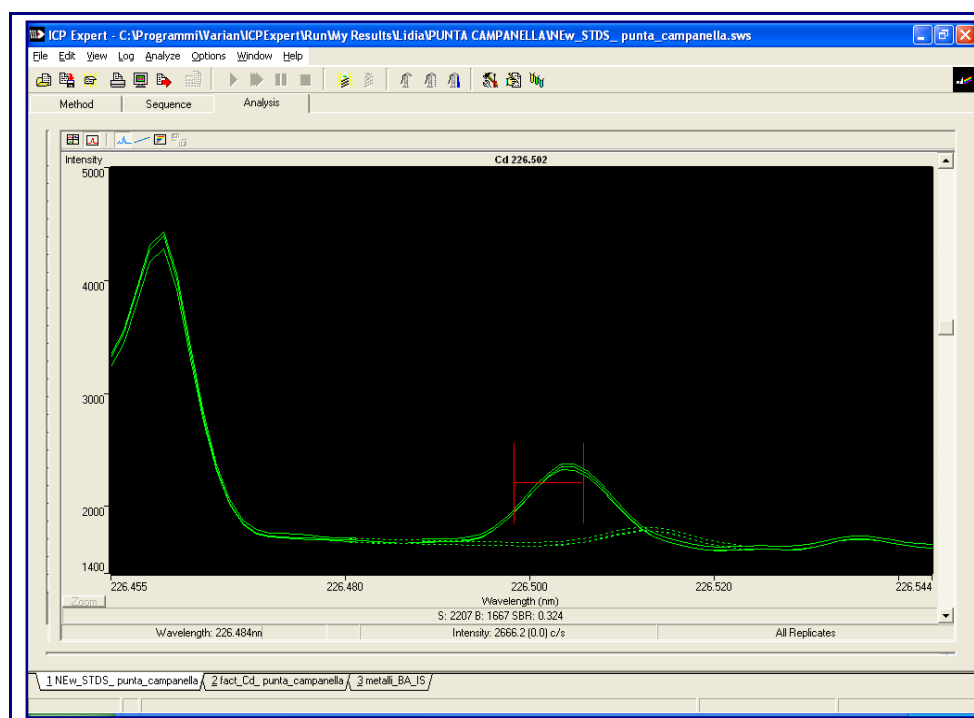
Fig. 9 *Marine sediment analysis spectrum for cadmium : Fe 226.505 interference on Cd 226.502*

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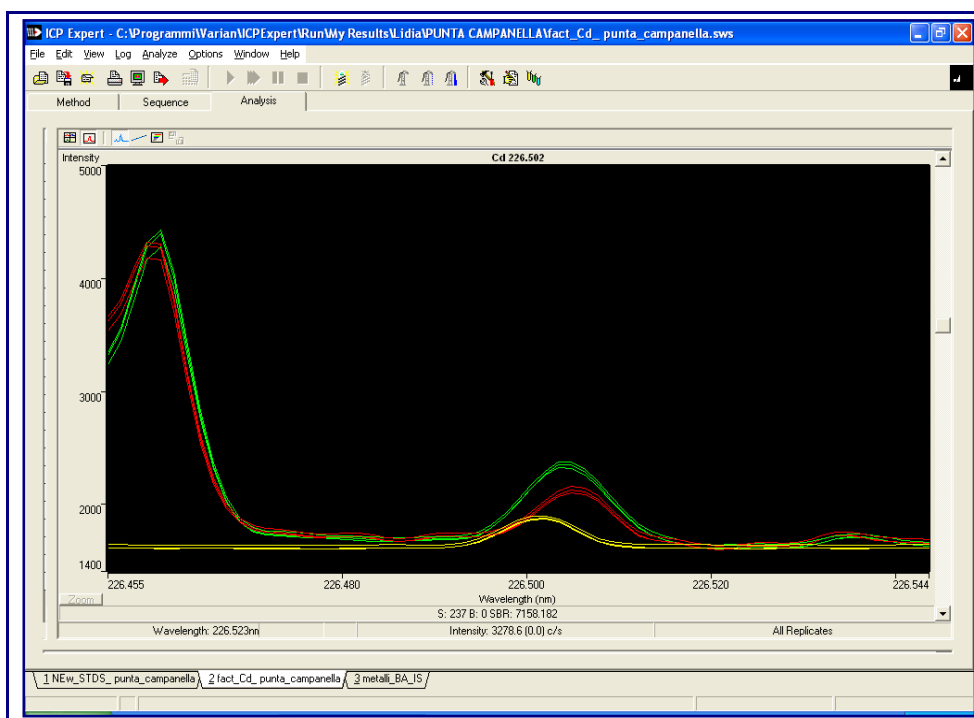
Fig.10 *Interference correction spectrum by FACT subroutine: Fe 226.505 (red line) and Cd 226.502 (yellow line) calculated basing on the sample interfered spectrum (green line)*

6. Determinations of Hg and Se in marine sediments by use of a hydride generation system

6.1 The hydride generation system VGA

Commonly, the low content of Hg and Se in marine sediments results in the impossibility to perform analysis directly by ICP-OES on the digested sample (analytes content in the solution under the instrument detection limits) and a coupling to VGA hydride generation system result essential in order to increase the signal/background ratio (Pohl, 2004).

Our laboratory is equipped with a Varian VGA-77 hydride generation system (fig.11): sample solution, acid solution and a reducing solution are simultaneously introduced in a reaction cell through a peristaltic pump; a strong reaction produces some

hydrides like Hg and Se hydrides but also As, Sn and Te hydrides; a liquid-gas separator allows hydrides vapour transport to the ICP torch carried by an argon flow and the drainage of the liquid solution.
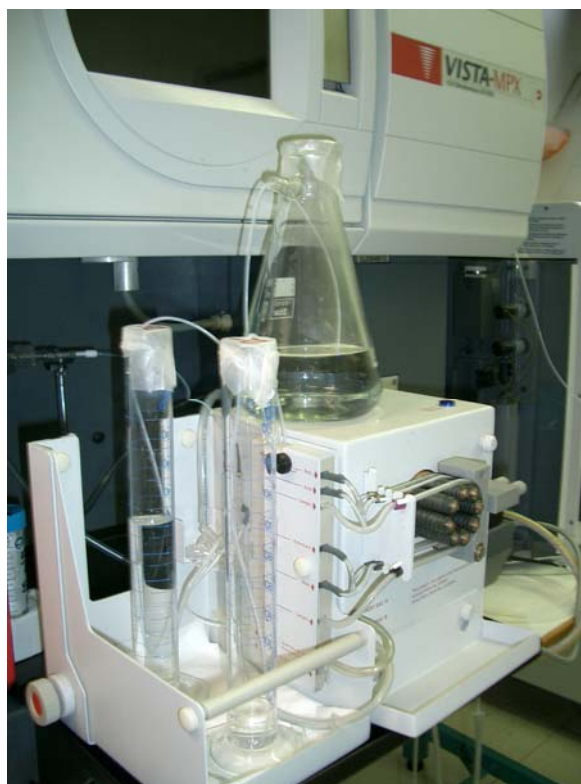
Fig.11 Varian hydride generation system VGA-77

Working at ICP-VGA a key role is played by regulation of both input and output flows at the liquid-gas separator in order to avoid the column overflow. The problem is strong in correspondence of the plasma lighting phase, when the drain pump stops for some seconds; a fine solution was provided according with the Varian technicians by splitting the drain flux in two parallel flows (fig. 12).
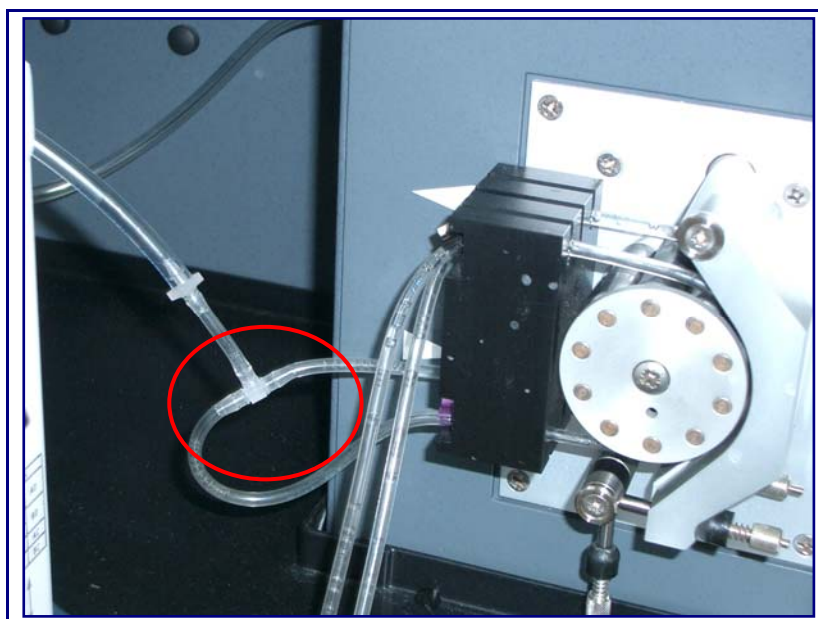
Fig.12 *Splitting of the drainage flux*

6.2 Sample preparation

Metal elements form hydrides more or less easily depending on their own oxidation state; Selenium in the reduced form Se(IV) shows a significantly higher attitude to form an hydride with respect to Se(VI) (10 times higher). Prior analysis is important subject sample solutions and calibration standards to a reducing process.

The following preparation method was optimized for Hg and Se determinations by VGA-ICP-OES:

a) transfer 15 ml of sample digested solution in a 50 ml centrifuge tube;

b) add 15 ml of 37% HCl;

c) mix the solution and keep it at 90 °C for 10-12 minutes;

d) Let the solution cool at room temperature prior to perform analysis.

6.3 Mercury and selenium determinations

Technical manuals generally report several procedures to perform analysis by VGA with respect to the specific element one must analyze.

We optimized a unique procedure for simultaneous determination of Hg and Se in order to minimize both costs and duration of single runs using a 3.7% (v/v) HCl solution as acid solution and a 0.3% (w/v) NBH_4 – 0.5% (w/v) NaOH solution as reducent agent.

The operative parameters of the instrument were set considering the better value of the signal/noise ratio in correspondence of the reading wavelengths; the rinse time and delay time determined to avoid a consistent memory effect. The RF power value was set at a lower value according to necessity (working at VGA) of limiting torch superheating caused by a dry plasma (fig. 13).

A calibration curve was constructed by interpolation of five measures (a blank and four standard solutions) in the concentration range of 0.5-10 ppb for Se determinations and of 2.5-20 ppb for Hg determinations; a maximum error of 10% on the single reading was imposed and a correlation coefficient >0.98 admitted.

The reliability of the obtained data was tested using the standard reference material PACS-2, results are reported in table 8.

At the end of the run a rinse of VGA system was performed as follows:

introducing 50 ml of a 10% (v/v) HNO_3 solution to decontaminate sample introduction tubes by adsorbed metals, introducing 200 ml of millQ water to rinse the system and attenuate corrosion of VGA metallic parts by acid residues and, finally, a drain step of all solutions by an argon flow for about 5 minutes.

PACS-2	Hg (mg/Kg)	Se (mg/Kg)
VGA-ICP-AES	3,12	1,02
certificated value	3,04 ± 0,2	0,92 ± 0,22

Tab.8 Comparison between Se and Hg readings at VGA-ICP-AES and certificated values for PACS-2.
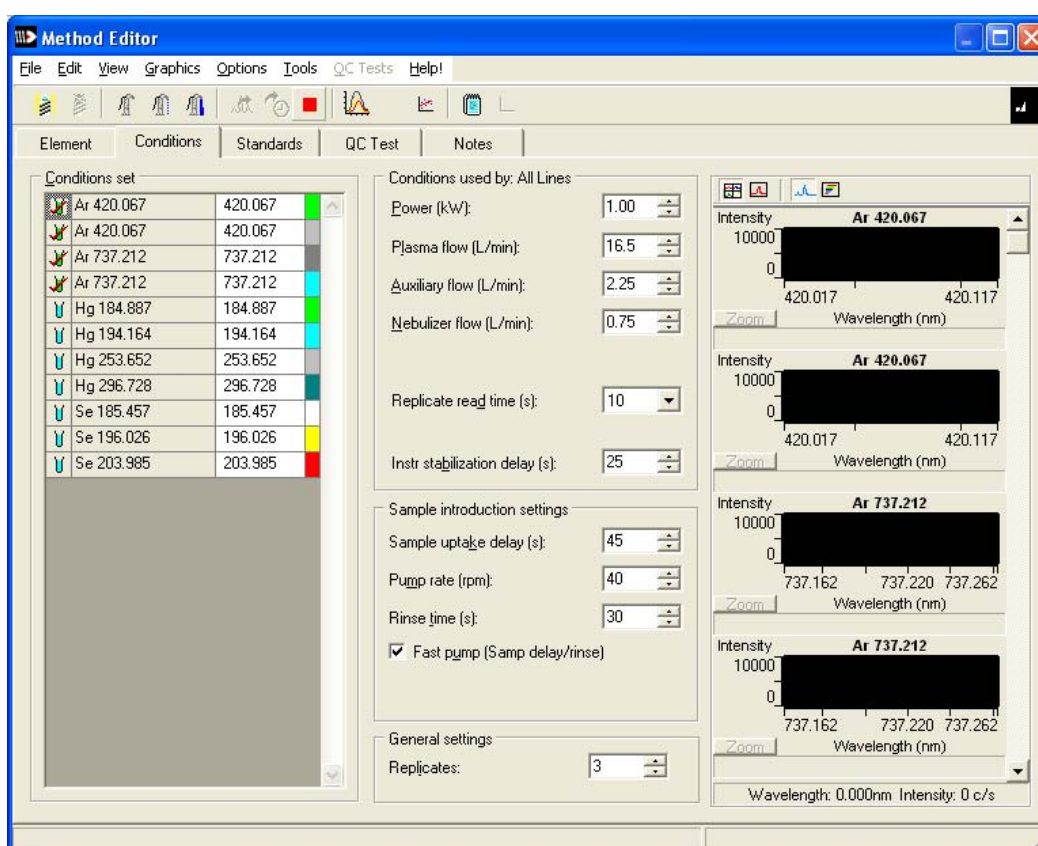
Fig. 13 Table of operative parameters setting for Hg and Se determinations by VGA-ICP-AES

7. Extraction and analysis of hexavalent chromium

Determination of hexavalent chromium concentrations in solids results very important in characterization studies for its human high toxicity and potential carcinogenicity (US EPA, 1998).

Prior to analysis determinations marine sediments must be adequately prepared in order to perform chromium extraction to solubilize Cr(VI) compounds from sediment matrix and keep the metal in the oxidated form (Giuriati, 2005).

Was used the alkaline extraction method 3060A provided by US EPA as reference procedure.

A sample aliquot of 2.5 ± 0.1 g of wet sediment was transferred in a 250 ml borosilicate glass beaker with 50 ml of extraction solution at pH 13 ± 0.5, covered with watch-glasses, stirred in order to create a sediment suspension, heated at 90 ± 5 °C and kept at that temperature for 60 minutes. Then sample suspension was cooled at room temperature, filtered by a whatman paper filter (cut-off 11 μm) to allow separation of sediment matrix from the extract solution and acidified at pH 9 ± 0.5 using a 5M HNO_3 solution. Practically in all extracted solutions, once acidified, were formed flocculant precipitates so that was decided to perform a second filtration step through a 0.45 μm polycarbonate membrane.

According to US EPA method, all extraction procedures were performed in a month from sampling and analytical determinations performed in a week.

Chromium analysis were run at ICP-AES equipped with spray chamber, nebulizer and torch for high solids matrices.

Operative parameters were set to generate a powerful plasma in order to destroy the so heavy matrix of extracted samples (fig. 14).

Working sample solutions with a high salt content as those obtained by alkaline extraction method, the torch injection tube and the nebulizer nozzle are easily subjected to formation of saline deposits resulting in a loss both in stability and sensibility of the detection instrument; in order to pull down such a disadvantage in our laboratory was adopted as solution a consistent rinsing of all the sample introduction system and of the torch any 30 readings.

The accuracy of the extraction procedure was evaluated by mean of a series of spikes (adopted as alternative for the lack of a standard

reference material) performed using the soluble Cr(VI) salt $K_2Cr_2O_7$ both on blank solutions and on a selection of sediment samples; accuracy was calculated as the percent Cr(VI) spike recovery value. Recovery percentages resulted quite good (80-120%) for blanks while really low values were obtained for sediment spikes (10-40%). A check of all extraction and analysis procedures performed, of the right spiking process was performed to be sure that a new hypothesis could be considered: physical and chemical parameters of sediments could be responsible of the Cr(VI) to Cr(III) reduction process. The hypothesis was supported by pH and Eh data on sediment samples: they were clearly located in the chromium "reduction field" like showed in fig. 15.
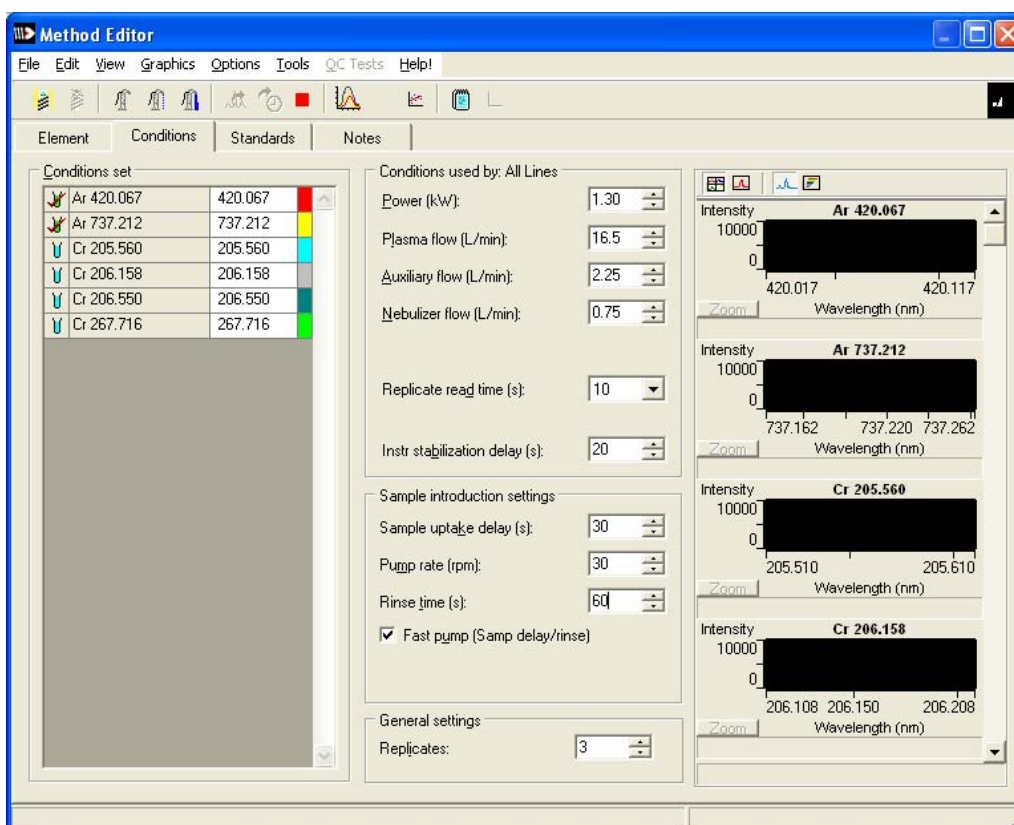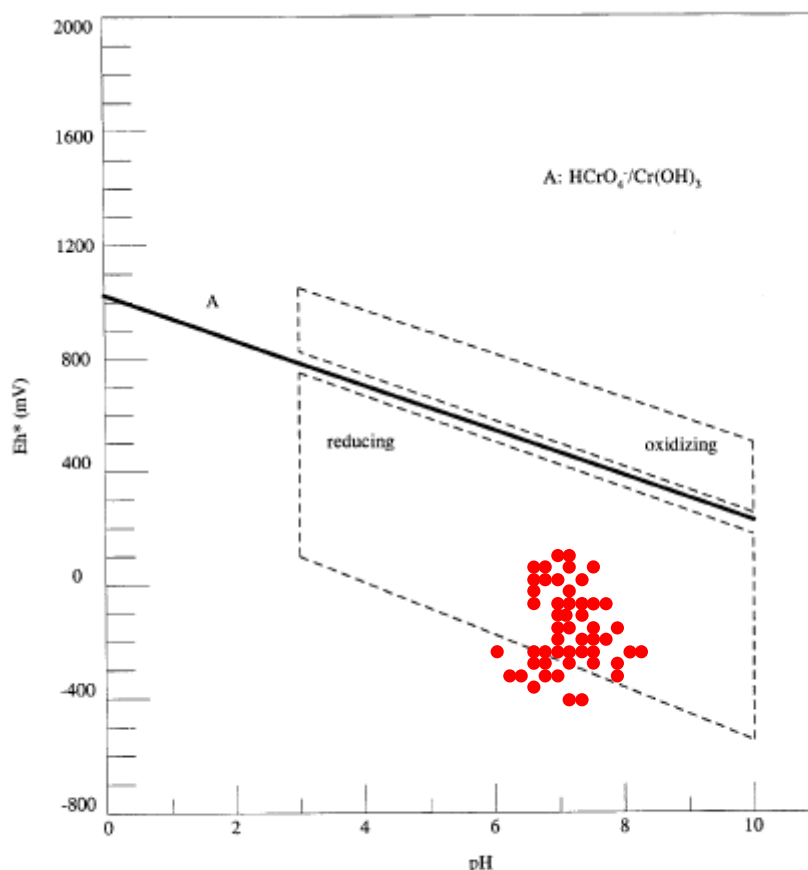

Fig. 14 Operative parameters setting for Cr(VI) analysis by ICP-AES

Fig. 15 Eh-pH phase diagram for chromium: red circles show values of Eh-pH corresponding to the investigated samples.

8. Quality control

Trace element determinations were performed according to quality control guidelines provided in the 6010b method of US EPA; a systematic test was performed on ICP-AES readings to evaluate the consistency of the obtained data. The validation was effectuated through a continuous check on: i) instrument performances (detection limits determinations, instrument stability), ii) efficacy of the sample preparation procedure (analysis of preparation blank solutions, of duplicate samples and of spiked samples) and iii) reliability of the obtained analytical date (precision, accuracy).

## 8.1 Instrument detection limit (IDL)

The detection limit of the instrument was estimated for any element calculating three times the standard deviation of a set of seven consecutive readings of a preparation blank solution performed in three non consecutive days (tab. 9).

Thus the detection limit corresponds to the lowest content of analyte the instrument can read, it could not be the "true" value of that concentration level (determination limit).

An example is given by the detection limit calculated for lead (3.6 μg/l for total attack, see tab. 9) an order inferior to the determination level of 40 μg/l evaluated studying the shape of reading peaks of Pb and the background characteristics in the reading range at λ= 220.353nm (fig.16, fig.17).

	Total Attack	*Aqua* Regia Attack	Acids + H$_2$O$_2$ Attack
	µg/L	µg/L	µg/L
Cu 324.754	0,6	0,5	0,2
As 188.980	7,8	11,1	6,1
Cd 226.502	0,1	0,2	0,01
Co 228.615	0,6	0,4	0,1
Cr 205.560	0,6	0,4	0,6
Ni 231.604	1,3	1,3	2,0
Pb 220.353	3,6	1,0	0,6
V 292.401	1,3	0,6	0,2
Zn 202.548	0,5	1,4	0,5

Tab. 9 *Detection limit* values determined for three different acid attacks
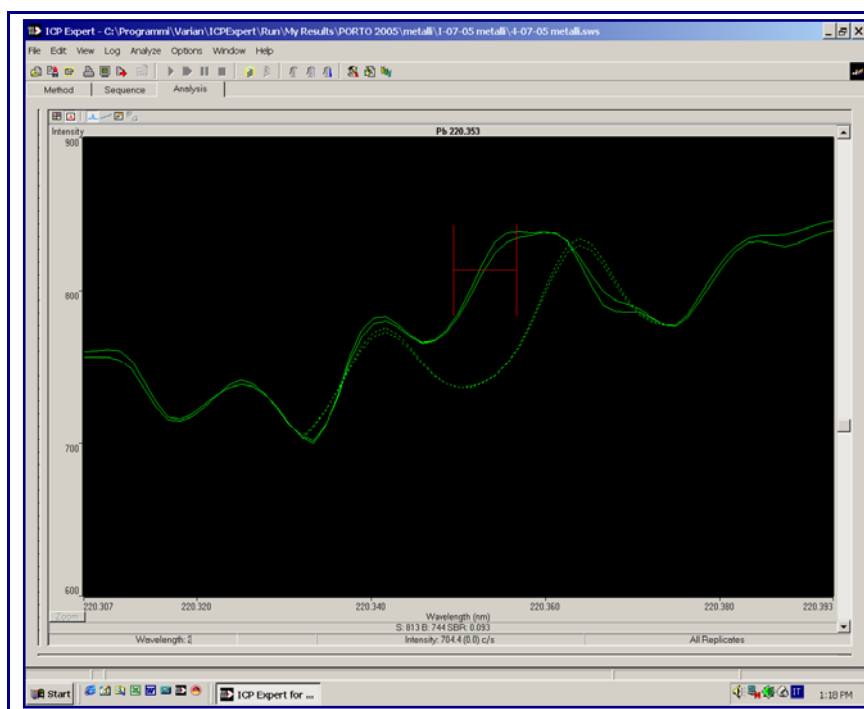
Fig.16 Reading peak of lead at 220.353 nm corresponding to a detected concentration < 40 µg/L
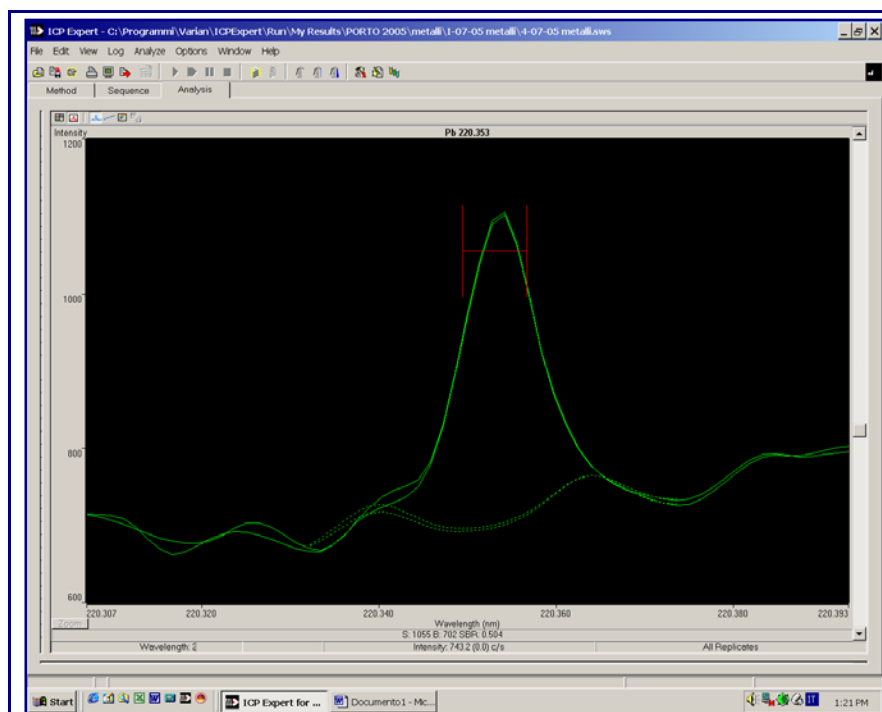
Fig.17 Reading peak of Pb 220.353 corresponding to a detected concentration > 40 µg/L.

8.2 Calibration verification

Analytical methods were optimized introducing quality control tests during a run in order to monitor in continuous the quality of the analytical data: an initial verification (ICV) was performed reading a standard solution immediately after the first calibration, and a continuous verification (CCV) was performed reading a calibration standard solution any 10-15 sample solutions. In case of difference in readings major than 10% of the true standard value, a calibration reslope or a new calibration must be performed.

## 8.3 Method precision and repeatability

To estimate the repeatability of the procedures adopted in the laboratory was prepared a duplicate every 20 samples.

The analysis performed on reference material PACS-2 and on its duplicate revealed a percent difference in readings inferior to 10% for all trace metals but As both for samples digested by aqua regia attack and those samples digested by acid mix + H_2O_2. The lower repeatability for As readings is probably due to the high reactivity of As with chloride to form volatile compounds (tab. 10).

Analytical precision was calculated as percent relative standard deviation of a set of seven different readings of the same sample of PACS-2 performed in a single run; results indicated very high values for precision (fig.18).

	Total Attack	*Aqua* Regia Attack	Acids + H_2O_2 Attack
Co	2,6	2,4	3,1
Cd	5,2	1,7	2,1
Cu	0,9	0,8	2,5
As	1,2	11,9	12,1
Cr	4,3	0,7	4,6
Ni	4,7	5,2	0,8
Pb	1,7	3,2	2,9
V	2,4	1,2	0,1
Zn	0,6	1,6	1,1

Tab. 10 *Percentage differences of Pacs2 duplicates for each typology of attack*
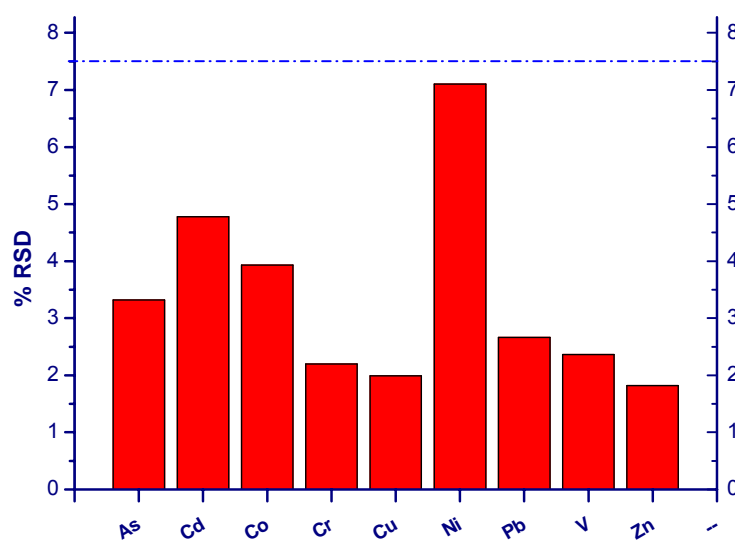
Fig. 18 Precision of the analytical method optimized for trace elements determination

8.4 Method accuracy

The accuracy of the method was estimated for three different kind of acid attack (see chap. 4): total attack, aqua regia attack and acid mix + H_2O_2 attack; analysis were performed on three different aliquots of PACS-2.

Analytical results revealed the greatest accuracy for total attack performed on a sample aliquot of 250 mg (tab.11); in particular, highest values were individuated for elements like Cr, Cu, Ni and Pb and V which generally are present in sediments as constituents of the most refractory minerals (oxides and silicates) and only partly destroyed by a weak attack (tab.2, fig.19) whilst Co and Se showed a very low accuracy level. Despite Se reading matched the reference material certified value (inner to confidence range) it shows a lack of accuracy (-25%) one could explain by a loss of the element during preparation procedures as Se is high volatile in acid solutions.

Cobalt inaccuracy is probably due to an additive effect by spectral interference on ICP-AES readings as suggested by non linear characteristics of background corresponding to Co 228.615 reading range (fig. 20). This hypothesis is supported by characteristics of the Co emission spectrum corresponding to the reading of a total attack preparation blank solution: a peak could be identified in correspondence of λ = 228.595 (fig.21). Probably a polyatomic specie forms during total attack digestion process and results high resistant to plasma. For weak attacks was not relieved this problem, background appears quite linear (fig.22) and a good accuracy was measured (tab.11, fig.19).
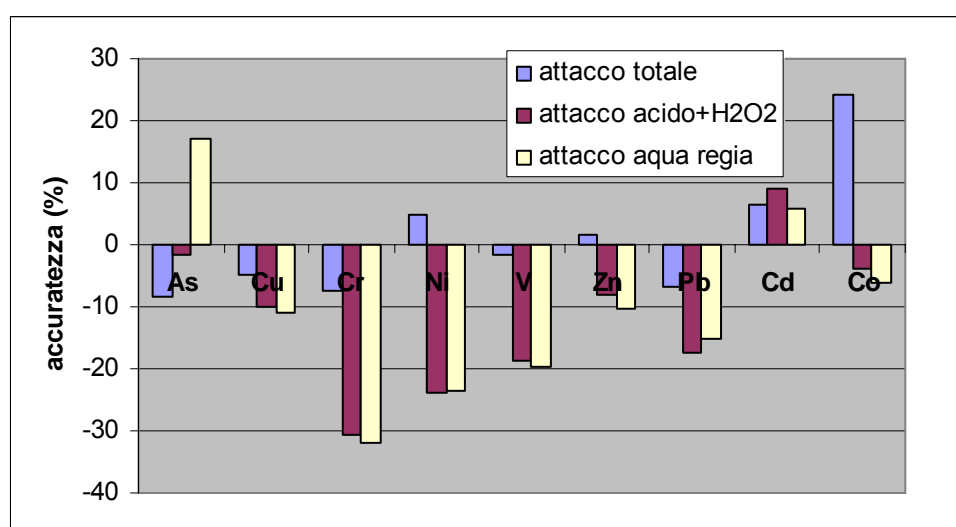
	PACS-2 reading	Pacs-2 certificated	Accuracy
	µg/g	µg/g	%
As	24,0	26,2 ± 1,5	-8,5
Cu	295,1	310 ± 12	-4,8
Cr	84,1	90,7 ± 4	-7,3
Ni	41,4	39,5 ± 2,3	4,7
V	130,9	133 ± 5	-1,5
Zn	370,2	364 ± 23	1,7
Pb	170,6	183 ± 8	-6,8
Cd	2,25	2,11 ± 0,15	6,6
Co	14,3	11,5 ± 0,3	24,2
Hg	3,02	3,04 ± 0,2	-0,7
Se	0,70	0,92 ± 0,22	-25,0

Tab. 11 Accuracy of the analytical procedure as % difference between PACS-2 solutions readings and PACS-2 certificated values

Analytical tests were performed on different aliquots of a sediment sample, respectively 0.100 g, 0.250 g and 0.500 g and for each one three different acid attacks were tested; in any case better results were obtained for a sediment aliquot of 0.250 g. That is because in correspondence of an increasing in instrument detection (higher concentration in solution) a major cost is needed for 500 mg of sample due to a greater quantity of acids needed to attack the sample whilst a non significant increase in accuracy and precision was registered; analysis performed on 0.100 g aliquot of sample relieved a difficult detectability for all elements characterised by a high detection limit or present in a very low content in the sediment (As, Co, Cd, Se).

|	PACS-2 *acids+H₂O₂ attack* µg/g	Accuracy *acids+H₂O₂ attack* %	PACS-2 *aqua regia* attack µg/g	Accuracy *aqua regia* attack %	PACS-2 Certificated µg/g
As	25,8	-1,5	30,7	17,2	26,2 ± 1,5
Cu	279	-10,0	276	-11,0	310 ± 12
Cr	62,8	-30,8	61,7	-32,0	90,7 ± 4
Ni	30,1	-23,8	30,2	-23,5	39,5 ± 2,3
V	108	-18,8	107	-19,5	133 ± 5
Zn	335	-8,0	327	-10,2	364 ± 23
Pb	151	-17,5	155	-15,3	183 ± 8
Cd	2,30	9,0	2,23	5,7	2,11 ± 0,15
Co	11,05	-3,9	10,8	-6,1	11,5 ± 0,3

Tab. 12 Method accuracy for a PACS-2 aliquot of 0.250 gr: comparison between *aqua regia* attack and acids+H₂O₂ attack


Fig. 19 Accuracy of the analytical procedure as % difference between PACS-2 solutions readings and PACS-2 certificated values for each kind of acid attack
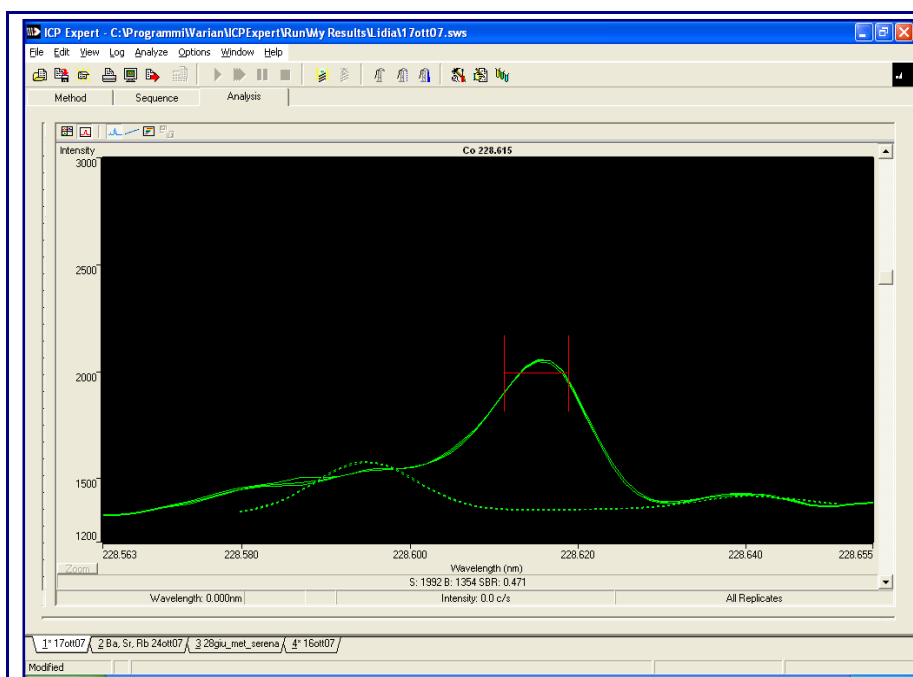
Fig 20 Reading peak of Co 228.615 for a PACS-2 solution digested by total attack (continuous line represents Co peak; the background is in dashed line
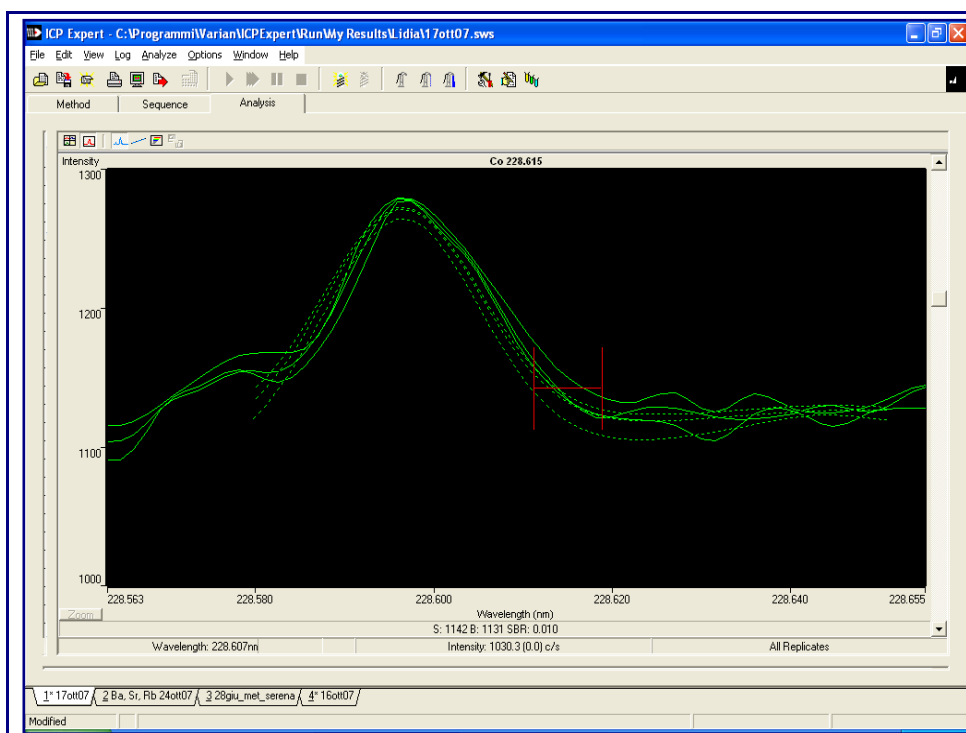
Fig 21 Reading window of Co 228.615 for a preparation blank by total attack (Co peak in continuous line; background in dashed line)
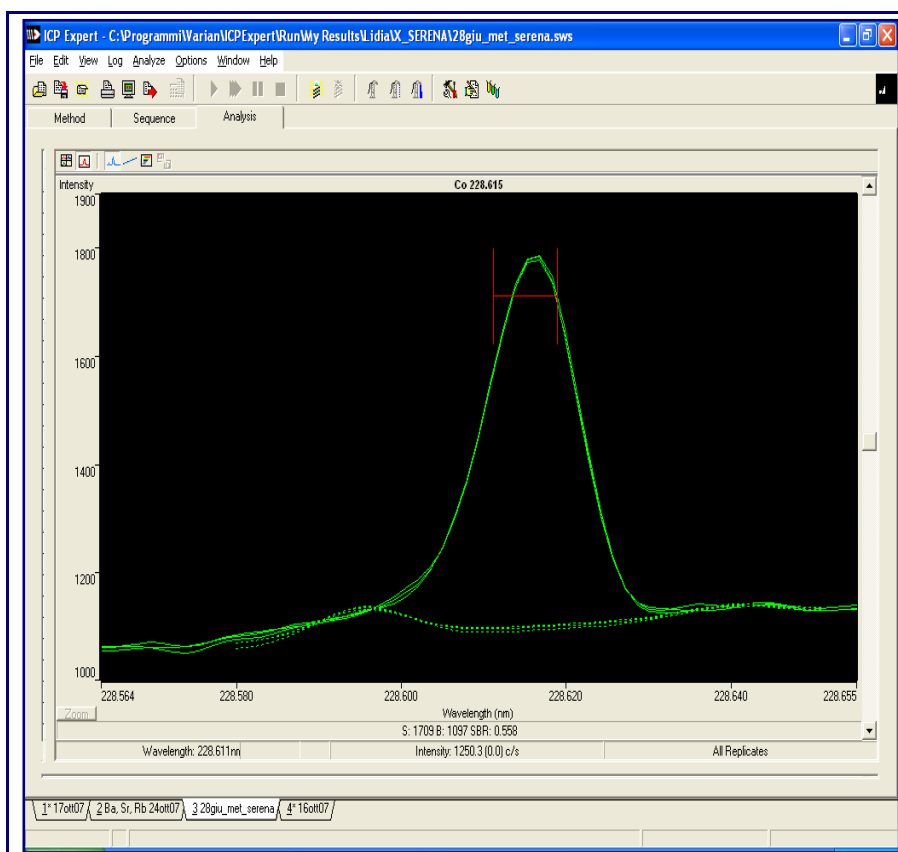
Fig. 22 Reading window of Co 228.615 for a PACS-2 solution digested by aqua regia attack

9. Applicability of the analytical method

The estimate of quality control parameters data obtained (in terms of precision, accuracy and repeatability) coupled to the evaluation of related costs (reagent acids, carrier gas, waste disposal, working hours) allows to consider all analytical method described in this technical report suitable to determination of metals in marine sediments both for a geochemical characterization target and a more general environmental survey. In particular, authors tested preparation procedures and analysis method for detection of As, Cd, Co, Cr totale, Cr(VI), Cu, Hg, Ni, Pb, Se, V, Zn, but the same analysis protocol could be successfully used for determination of other metals like Al, Fe, Mn, Mg, Sb, Sn, Ag in sediments as in some different matrices like soils, rocks, waste sludges.

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