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Titolo della tesi

ASSESSMENT OF TRACE METAL BIOAVAILABILITY IN SOILS AND RIVER WATERS USING DIFFERENT ANALYTICAL TECHNIQUES TO PREDICT METALS UPTAKE BY BIOTA

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PREFACE

The experimental activity described in the present work has been conducted at the laboratories of the Italian Environmental Protection Agency (APAT). Some experiments relevant to soils and plants were performed in collaboration with CNR (Pisa) and were carried out at the CNR department of Soil Chemistry by CNR and APAT personnel.

Leaching tests were conducted in parallel at APAT and at the expert laboratory of the Energy research Centre of the Netherlands (ECN). Instrumental Neutron Activation Analyses on soils were performed at the Jožef Stefan Institute, Ljubljana, Slovenia.

In situ water sampling was performed in the Sitzerri river in Sardinia in cooperation with the Regional Environmental Protection Agency (ARPA). Some experiments concerning metals bioaccumulation in fish were performed at the laboratory of ARPA.

Introduction

The bioavailability and mobility of potentially toxic trace metals largely determine the environmental impact of metal-contaminated soils and aquatic systems [1]. Increasingly, it is being realized that the total metals content in soils and waters is a poor predictor of availability of metals to biological living organisms [2]. At present, although existing legislation or guidelines on heavy metals are primarily based on total concentrations, the scientific community well recognised that the total metal content in soils and waters embraces large fractions which are unavailable to plants, (micro)organisms, soil fauna and aquatic biota [3]. Metals speciation influences the fraction of metal that is really available for biota uptake and hence its toxicity in the environment. Many factors determine the chemical forms, or species, in which metals may be present is soils and waters. These include soil/water pH, the redox potential, organic matter, the temperature as well as the concentration of the metals and of potentially interacting agents such as organic chelators and inorganic anions [4].

The aim of the present work is the assessment of metal bioavailability in both soils and river waters through the comparison of the most widespread techniques used to predict metal biota uptake.

Firstly, the present study focused on the assessment of metal bioavailability in soils (some applications were performed also on a compost sample). The first objective was to attain an harmonized approach for the assessment of metal speciation and bioavailability in soils using traditional procedures as leaching and extraction tests and a recently developed technique known as Diffusive Gradients in Thin films (DGT), capable of *in situ* measurement of labile metal species in natural waters, sediments and in soils [5].

The leaching or extraction procedures enable to measure the environmentally-relevant sub-fractions of contaminants [6]. This approach differs from the purest form of metal speciation which consists on the isolation and measurement of specific metal compounds. The complexity of soil matrix currently precludes routine isolation of many of the forms of metals associated with the soil. The leaching and extraction

procedures focus on the mobile metals fractions, allowing for an interpretation of data in the light of key environmental parameters controlling metal mobility.

Leaching is the process by which contaminants are released from the solid phase into the waterphase under the influence of mineral dissolution, desorption, complexation processes as affected by pH, (micro)biological activity and organic matter [6]. Leaching can occur in the field by exposure of material to natural infiltration or precipitation (for example a natural soil exposed to rainwater infiltration). Leaching tests have been developed to mime these natural processes and to assess the fraction of contaminants potentially mobile and available for biota uptake.

The development and use of extraction schemes started at the end of 1970s [7] and aimed to evaluate the metal fractions available to plants and the environmentally accessible trace metals, *e.g.* the mobility of metals from a soil. These measurements, if supported by geochemical modelling with the measured parameters as input, allow to estimate metals speciation in soils and for a proper and long-term risk-assessment of the contaminated environments.

In the past 15 years, many scientific researches demonstrated that DGT represents a promising tool for inferring metals availability to biota. DGT has been developed by Davison and Zhang in 1994 and it is based on Fick's first law of diffusion [5, 8, 9]. DGT plastic sampler could be applied directly *in situ*. It uses a chelating resin separated from natural water (or soil surface) by an ion-permeable hydrogel membrane. Metals are concentrated onto the resin after diffusing through the gel layer, so that metal uptake by DGT is controlled by diffusion. The pore size of the diffusive gel of DGT permits free metal ions and inorganic and small organic metal complexes to diffuse through to the resin, which acts as a sink. The gel excludes particles and large colloids which will not be measured. DGT distinguishes between species not only by size (whether they can pass through the diffusive gel layer) but also kinetically (according to their lability). Therefore, only labile complexes that can dissociate on time scale less than minutes are measured. The ability of DGT to infer metals bioavailability depends on the evidence that the most bioavailable metal fraction is represented by labile metal species.

In 1994 DGT was firstly developed to be used in natural waters and then its use was extended to soils and sediments [10, 11]. When applied on soils, DGT is capable to evaluate the flux of available species from solid phase to solution [12]. This aspect is very interesting since mimics processes that truly occur in nature for biota metals uptake. Actually, if metals is removed from solution by biological uptake it may be rapidly resupplied from the solid phase. Thus, the assessment of the availability of metals in soils need also to consider the kinetics of exchange between solution and solid phase [13]. Traditionally, the assessment of the potential supply from the solid phase has been made by extraction techniques that attempt to quantify the size of available solid-phase pool. DGT provides a different cheering approach to quantify metal resupply from the solid phase.

As a consequence of the above considerations, selected leaching and extraction tests and DGT were applied to a range of metal contaminated Italian soils and their limits and field applicability were discussed. Leaching and extraction tests were conducted in collaboration with the Energy Research Centre of the Netherlands (ECN).

As a further step of this study, DGT capabilities of measuring flux of available metals from solid phase to solution has been investigated through a direct comparison with the uptake of different plant species.

DGT gave several encouraging results in the prediction of metal availability to plants in soils [3]. DGT measured concentration was described as the best indicator of metal phytoavailability by authors considering large ranges of soil contamination and different soil types [1, 13, 14, 15].

It is recognized that the supply of metals to plants is through the soil solution [13, 16, 17, 18]. When metal is supplied from the solid phase, it must be transferred to solution before it can be taken up by plant roots. It is well recognized that depletion of metal concentration in solution in the immediate vicinity of the roots of plants can allow a transfer from solid phase to solution. Like plants, DGT locally lowers metal concentrations in the soil solution through the metal accumulation in the resin. It also responds to metal resupplied from labile species in solution and the labile metal pool in the solid phase. DGT then measures the metals available from the complete soil system. The measured supply to DGT is controlled by a combination of the

concentration in soil solution, the size of the available labile pool in the solid phase and the kinetics of exchange between the two [13, 19].

Extraction of soil solution from soil isolates the aqueous phase to which plant roots and microorganisms are exposed but the measurements of metals in soil solution fail to account for the ability of the soil to sustain the solution concentration following a depletion by uptake [14].

Considering these encouraging presupposes, APAT and CNR (Pisa) activated a collaborative study to assess DGT, metal chemical extractions and soil solution capabilities in predicting metal uptake by plants.

In particular, trace metal concentrations measured in soils, soil solutions, and by chemical extraction and DGT were compared to the metals taken up by plants of lupine and wheat.

The second part of the present study focused on metal bioavailability in river waters. A collaboration with the Regional Environmental Protection Agency (ARPA) of Sardinia was instituted to study DGT applicability in a contaminated river in Sardinia. The main aim was to compare the bioavailable fraction of metal measured by DGT and by on site filtration [20, 21, 22]. In this way it was possible to investigate metal complexation and speciation focusing on the fraction of metal potentially available to aquatic organisms.

Metals bioaccumulation in fish was recognized to be an interesting topic of investigation in correlation with metals bioavailability in waters. A specific experiment was designed to study metals bioaccumulation in Cyprinus Carpio fish and a method to accurately determine metals accumulated in fish tissues was standardized.

Summary

PART 1: Metal Bioavailability in soils

SECTION 1

This section briefly describes the soils matrices (and a compost) under study in the present work. This section also reports some information concerning the sampling activity and samples pre-treatment.

SECTION 2

This section reports a comparison of different techniques for the determination of the total metal content in soil samples. Although the total metal content of soil is a poor predictor of metals bioavailability, the determination of the total metal concentrations still remains the first step in evaluating soils potential health or ecological hazard. In this section, the results for total trace metals content determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after acid digestion and those obtained by the non destructive Instrumental Neutron Activation Analysis (INAA) are discussed. The outcomes of this study were published in the Accreditation and Quality Assurance Journal and provided useful information to select the most appropriate digestion method to dissolve the samples under investigation (section 1) prior to ICP-MS analysis.

SECTION 3

This section reports the results obtained for the total metals content in the soils under study. The samples were digested and analysed using the information outcome from section 2.

SECTION 4-5

These sections describe the extraction and leaching procedures selected among the different leaching/extraction tests used across Europe for regulatory and research purpose, to be applied on the samples under investigation (section 1).

SECTION 6

This section describes the DGT theory of application and its application to evaluate metals resupply from the soils under study. Furthermore, the DGT capability in

predicting metal uptake by plants is compared with those of metal chemical extractions and soil solution.

PART 2: Metal Bioavailability in river waters

SECTION 7

The DGT results obtained in a contaminated river in Sardinia are reported and discussed in comparison to on site filtration. Metals uptake by Cyprinus Carpio in river waters is discussed.

SECTION 8

The validation of a method to accurately determine metals accumulated in fish tissues is also reported.

PART 1. METAL BIOAVAILABILITY IN SOILS

SECTION 1. Samples matrices under study

Metal speciation and bioavailability were investigated in 6 soils samples and in one compost sample.

Two agricultural soils were collected near the area of ACNA (Azienda Coloranti Nazionali e Affini), a chemical plant belonging to ENICHEM (Figure 1) located in the Cengio area, in the valley of the Bormida River (Savona, Italy). Since 1882, this plant discharged in the adjacent river its yellow, acid and smelly mud, providing the pollution and poisoning of the neighbouring waters and countries. In 1987, the Bormida valley was defined as an "highly risky area of environmental concern" and in 1999 the ACNA plant was finally closed. Afterwards, a land reclamation campaign was started on behalf of the Regional Environmental Protection Agency of Piemonte (ARPA- Piemonte). In the present study, one soil sample was collected in the agricultural area of Saliceto, in the Cuneo province, nearby to the ACNA discharge, and the second one was collected in the Millesimo area, in the same province but at higher distance from the ACNA plant. In the following sections, the samples codes of these soils will be *Saliceto* and *Millesimo.*

Figure 1. ACNA plant (Cengio).

One soil sample was collected in an agricultural area belonging to ERSA (Ente Regionale di Sviluppo Agricolo) in the centre of Italy (Abruzzo). In the following sections, the sample code of this soil will be *ERSA*.

The other two soil samples were collected from a contaminated area near Nuova Solmine S.p.A, a plant for arsenopyrite crushing and processing, located in

the zone of Scarlino, in the Grosseto province (centre of Italy). These soils originated from the residual mud of arsenopyrite manufacturing and from added soils carried from bordering zones (Figure 2). In the following sections, the sample codes for these two soils will be *Scarlino 1* and *Scarlino 2*.

One soil samples was collected by CNR (Pisa) in Bovisa (Lombardia) and was used, together with Saliceto soil, to perform experiments using plants (SECTION 6). Sample code: ***Bovisa.***

The compost sample (Figure 3) was collected in a plant for waste selection and composting of the centre of Italy. It derived from horticultural wastes collected from gardens and parks (bushes, leaves of trees, wood chippings, grass). It is a stabilized compost of high quality (sample code: *Compost*).

Figure 2. Pyrite fine ash (Scarlino).

Compost has traditionally been produced from plant materials and is not considered as waste but as an organic soil improver. Usually composts are used on agricultural land and it is important to evaluate the quality of the final product to protect the quality of the crops and of the environment.

Today, composts are also produced from other organic sources, including wastes from the food and agricultural industry, sewage sludge or household refuse [23]. Considerable remediation of contaminated soils can be accomplished by composting them with non-contaminated organic matter [23]. Trace metals are not degraded during composting but may be converted into organic species having less bioavailability than minerals combination of the metals. This substantial potentiality

for remediation of polluted soils makes compost as a matrix of great environmental concern [23].

Figure 3. Compost.

Sampling and samples pre-treatment

Soils samples were sampled by shovel after eradicating any surface vegetation. The soils were taken up to 20 cm depth. After sampling, the soils were weighed and stored in carton-board boxes and then dried into an oven fan at 36-40 °C until a constant weight was reached. Then they were dis-aggregated by using wood pestle and sieved at 2 mm. Compost was delivered in big tanks from the plant for waste selection and composting of the centre of Italy. It was also dried at 36-40 °C, until a constant weight was reached but sieved at 1 mm. Actually, the compost sample is a very complex matrix due to the presence of a lot of small wood residues. For this reason it was sieved at 1 mm, trying to remove the large part of them.

SECTION 2. The role of different soil sample digestion methods on trace metal analysis: a comparison of ICP-MS and INAA results

Abstract

The determination of trace elements in soil, sediment and waste, is generally a combination of a digestion procedure for dissolution of elements and a subsequent determination of the dissolved elements. “Partial” and “total” digestion methods can be used in environmental monitoring activities. To compare data coming from different methods, it is crucial to determine and to maintain under control the bias of the methods.

In this paper ICP-MS results obtained after matrix microwave digestion with modified aqua regia (HCl + HNO_3 + H_2O_2) method and two “total” digestion methods (aqua regia + HF and HNO_3 + HF) are compared with those obtained by Instrumental Neutron Activation Analysis, a non-destructive analytical method for the determination of the total content of inorganic components in environmental matrices.

The comparison was carried out on eight agricultural soil samples collected in one test area and analysed by INAA and ICP-MS to determine As, Co, Cr, Sb and Zn contents. The laboratory bias for As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn of the three digestion methods were assessed using selected reference materials. This paper highlights that the digestion procedure is an integral part of the measurement and can affect the measurement results in environmental analysis.

Introduction

Determination of trace element contents in soils is the first step in evaluating their potential health or ecological hazard. Sample digestion is often a necessary step before determining “total” metal mass fractions in soils.

A standard, relatively safe, dissolution method that provides an analytical recovery of at least approximately 90% of soil bound metals is required in most laboratories working with trace metal in soil.

Various digestion methods are used to determine the content of trace elements in solid matrices, including different combinations of concentrated acids [24, 25, 26]. Open beakers heated on hot plates, digestion tubes in a block digester and digestion bombs placed in microwave ovens are the most commonly used equipments to digest solid sample matrices.

In particular, since the 1980s, the microwave-assisted sample digestion technique has become popular and at present it is widely used due to its safe, rapid and efficient performance [27, 28, 29].

Since different acid digestion methods applied to soil samples can release different amount of metals from this matrix, it is critical to compare different digestion methods used to determine elemental mass fractions in soils.

As reported by Chen and Lena [27], the amount of trace metals extracted by the commonly used digestion methods might depend on the element, their origin (anthropogenic or natural), soil properties and element mass fractions.

Aqua regia digestion method (USEPA 3050 [30] or ISO standard 11466 [31]) is considered effective for determining "total" trace elements in soils and is usually used to give an estimate of the maximum element availability to plants [27, 32]. This method consists of treating a soil sample with a 3:1 mixture of hydrochloric (HCl) and nitric (HNO_3) acids. The nitric acid destroys organic matter and oxidizes the sulphide material. In addition, it reacts with concentrated hydrochloric acid to generate aqua regia: $3HCl + HNO_3 \rightarrow 2\ H_2O + NOCl + Cl_2$. Aqua regia is considered adequate for dissolving most base metal sulphates, sulphides, oxides and carbonates but only provides a "partial" extraction for most rock forming elements and elements of a refractory nature. For example, aqua regia extraction might give complete recovery for metals such as Cd, Cu, Pb and Zn while it is known to provide partial recovery for metals like Cr, Ni and Ba. The latter elements can only be efficiently recovered by using hydrofluoric acid (HF). However, aqua regia digestion method is internationally accepted to determine the metal content in soil, considering as not available for biological uptake the fraction of elements not extracted by this method. The ISO standard on aqua regia digestion of soil includes only digestion by the use of hot plate heating, while the modified aqua regia digestion method, suggested by the Italian legislation, includes both hot-plate heating and microwave-oven heating

[33]. The aqua regia modified method adds, in the first step, hydrogen peroxide (H_2O_2) in order to enhance the destruction of the organic matter in the soil.

More vigorous HNO_3 + HCl + HF digestion methods (like EN 13656 applied to wastes [34]) provide satisfactory dissolution of silica matrices [35, 36, 37, 38]. These methods use microwave-assisted acid digestion for "total" sample decomposition and are applicable to up to 30 elements.

HNO_3 + HCl + HF and HNO_3 + HF mixtures dissolve silica matrices due to the presence of HF, via the following reaction: $HF + SiO_2 \rightarrow H_2SiF_6 + H_2O$.

In the present study, the three digestion methods reported above were compared for the analysis of eight agricultural soil samples collected at an Italian reference site, previously characterized within the framework of an APAT project focused on soil sampling uncertainty estimation [39, 40]. The target elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn) were determined in the solutions of digested samples using inductively coupled plasma mass spectrometer (ICP-MS). Furthermore, five elements (As, Co, Cr, Sb, Zn) were determined by the k_0-standardization method of Instrumental Neutron Activation Analysis (k_0-INAA). INAA is a non-destructive analytical method for the determination of the total content of inorganic components in solid matrices, because this method does not require any sample dissolution. INAA is a valuable technique particularly for elements that form or are in refractory phases that may be difficult to dissolve [41]. In this way, the effect of the dissolution step on the final analytical results for As, Co, Cr, Sb and Zn was investigated.

The difference between the reference values and the ICP-MS results on the solutions obtained in the laboratory with the three different digestion procedures was estimated by the laboratory bias. The modified microwave aqua regia method was applied to two different certified reference materials (LGC-6187 and BCR-141R) characterized for hot aqua regia total-recoverable trace metals. The microwave aqua regia + HF and HNO_3 + HF methods were applied to digest two IAEA reference materials (SL-1 and Soil-7) characterized for total metal content. IAEA Soil-7 was as well used as quality control material for INAA determinations.

Material and methods

Sample collection

Eight soil samples were collected at an agricultural site, located in the North East of Italy (Pozzuolo del Friuli, Udine), within the framework of an APAT project [39]. Sampling was performed in June 2001, using strictly controlled protocols. The details of sampling are fully described in Barbizzi et al. [40]. The agricultural area sampled reveals a quite balanced soil grain size distribution with a slight dominance of the silt fraction (47 %) and a low percentage of clay (below 16 %). In average, the fraction above 2 mm represents only the 13 % of the sampled soil. Relatively high pH values (about 7,7) and a low percentage of organic carbon content are observed. The Cation Exchange Capacity (CEC), along the area, reveals low values (in average below 16 $cmol_{(+)}$ kg^{-1}). These are compatible both with the slight contribution derived by low clay content and the poor level of organic carbon.

Sample preparation

Soil samples were weighed and stored in cardboard boxes and then dried in a oven fan at 36-40 °C until constant weight was reached. Then they were disaggregated using a wood pestle, sieved at 2 mm, the volume was reduced by quartering and riffling and at the end the laboratory samples were milled to 90 □m to obtain the test samples [42]. Barbizzi et al. [40] report in more detail the sample preparation steps. From each of the eight test samples, 9 test portions [42] were taken for trace metals analysis by ICP-MS and 1 test portion was taken for INAA analysis. The homogeneity of the test samples has been tested by INAA, analysing 10 test portions from three different test samples.

Digestion methods

Microwave digestions were performed in a CEM Mars 5 microwave oven (Matthews, NC).

For the three digestion methods, a test portion of about 0.1 g was weighed into a 120 mL Teflon-PFA microwave digestion vessel after manually shaking the bottles for at least 1 minute.

The HNO_3 + HF digestion (Method A) was performed by adding to the soil a mixture of 3 mL of HNO_3 and 2 mL of HF.

The aqua regia + HF digestion (Method B) used in the present work followed the EN 13656 method [34] developed for elemental determination in wastes. A freshly prepared mixture of 2 mL HNO_3 + 6 mL HCl + 2 mL HF was added to the sample.
The modified aqua regia digestion followed the method suggested by the Italian legislation (Method C) [33]. 1.5 mL H_2O_2, 4.5 mL HCl and 1.5 mL HNO_3 were added to the soil. Hydrogen peroxide was used to enhance the destruction of organic matter.
All the samples were microwave digested following the digestion cycles reported in Table 1. The time integrated energy was 5265 kJ and 3192 kJ respectively for method A and B. Every digestion cycle was performed using the maximum number of vessels available (12 vessels).

METHOD A		METHOD B		METHOD C	
HNO_3 + HF		HCl + HNO_3 + HF		HCl + HNO_3 + H_2O_2	
Time (min)	Power (W)	Time (min)	Power (W)	Time (min)	Power (W)
10	250	2	500	10	250
10	400	2	0	10	450
10	650	5	500	10	600
5	400	5	800	5	250
10	250	5	1000		

Table 1. Microwave oven digestion cycles (method A-B-C).

The three digestion procedures were performed in triplicate for each test sample (CRMs, RMs and agricultural soil samples).
After digestion, each aliquot was quantitatively transferred to a volumetric flask (Brand) and diluted with MilliQ water to 100 mL. Before diluting, rhodium at a concentration of 10 □g/L was added as internal standard to minimize the instrumental signal fluctuation and matrix effects. The solutions were allowed to stand for 24h without removing the undissolved residue and then analysed by ICP-MS.

Nitric acid, hydrofluoric acid and hydrochloric acid of ultrapure grade were purchased from Merck.

ICP-MS determinations

Trace metal contents were determined on an Agilent technologies 7500c ICP-MS equipped with a collision cell to minimize polyatomic interferences and matrix effects. Babington nebulizer, standard spray chamber, Cetac ASX 500 auto sampler was used. The collision cell was pressurized with He gas (flow of 2.9 ml min^{-1}) to reduce interferences by dissociating interfering polyatomic species by collision and by charge transfer. The ICP-MS is optimized daily with a tuning solution at 10 □g L^{-1} of Li, Ce, Y, Tl. Optimization is performed using normal mode and collision cell mode. A typical analytical run after optimization of the ICP-MS consists of calibration standard solutions, procedure blanks, samples and CRM. Calibration standard solutions are daily prepared from a working standard solution containing 5 μg mL^{-1} of Cr and Zn, 0.5 μg mL^{-1} of Co, 1.5 μg mL^{-1} of Ni and Cu, 0.1 μg mL^{-1} of Cd and Sb and 2.5 μg mL^{-1} of Pb. The working standard solution has been prepared from 1000 μg mL^{-1} stock solutions of all elements by dilution with ultrapure water in a 100 mL volumetric flask. Calibration curve has been determined on five points for each element, in a range from 0 to 300 ng mL^{-1} for Cr and Zn, 0 to 30 ng mL^{-1} for Co, 0 to 90 ng mL^{-1} for Ni and Cu, 0 to 6 ng mL^{-1} for Cd and Sb and 0 to 150 ng mL^{-1} for Pb. The calibration solutions are traceable to standards issued by NIST.

^{75}As, ^{59}Co, ^{52}Cr, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{111}Cd, ^{121}Sb and ^{208}Pb isotopes were chosen for the analysis.

In the present work, He was demonstrated to effectively minimize the interferences of $^{40}Ar^{35}Cl$ on the isotope ^{75}As, $^{40}Ar^{12}C$ on ^{52}Cr, $^{40}Ar^{18}O$ on the isotope ^{59}Co and $^{44}Ca^{16}O$, $^{23}Na^{37}Cl$ on the isotope ^{60}Ni. For the agricultural soils the collision cell was used only for ^{59}Co and ^{75}As. Two procedure blanks and two RMs were analysed every eight samples.

k_0- INAA determinations

Determinations of As, Co, Cr, Sb and Zn by k_0-INAA were carried out at the Jožef Stefan Institute, Ljubljana, Slovenia. For details about k_0-INAA and the relevant nuclear data see Jaćimović et al. [41].

Test portions of about 0.2 g (one for each test sample) were sealed into suprapure plastic containers and irradiated for about 20 hours in the carousel facility of the TRIGA Mark II reactor, Ljubljana (thermal neutron flux $1.0*10^{12}$ n cm^{-2} s^{-1}). A 1.0 mm Al-0.1% Au alloy wire pressed into a disk (diameter of 6 mm, thickness 0.2 mm) was co-irradiated with the sample as a comparator. The irradiated samples were subsequently transferred to clean polyethylene vials and counted on calibrated coaxial HPGe detectors connected to a multichannel analyser (MCA). Each irradiated sample was measured three times: after 2-3, 8 and 30 days cooling time. k_0-INAA quality control was performed by using the reference material IAEA Soil-7. Results of quality control are reported in Table 2.

IAEA Soil-7	**Recommended Value (95% Confidence interval)**		I**NAA** (*n=14*)
		mg kg^{-1}	**mg kg^{-1}**
Cr	60	(49 - 74)	69.5 ± 5.2
Co	8.9	(8.4 - 10.1)	8.8 ± 0.6
Zn	104	(101 - 113)	103.1 ± 6.2
As	13.4	(12.5 - 14.2)	14.5 ± 0.6
Sb	1.7	(1.4 - 1.8)	1.8 ± 0.09

Table 2. Element contents determined by INAA in the RM IAEA Soil-7. Results are reported with their standard deviations at an approximate level of 95%. The table also reports the recommended values and the 95% confidence intervals for IAEA Soil-7 (n= number of independent replicates).

Laboratory bias determination

Reference materials (RMs) such as IAEA SL-1 (lake sediment) and IAEA Soil-7 from the International Atomic Energy Agency (IAEA), Austria, and certified reference materials (CRMs) BCR-141R (calcareous loam soil) from the Commission

of the European Communities, Belgium and LGC-6187 (river sediment) from the Laboratory of the Government Chemist (LGC), UK, were digested in triplicate following the procedures reported above. As previously stated, IAEA SL-1 and IAEA Soil-7 are characterized for total metals content and were processed by microwave digestion using aqua regia + HF and HNO_3 + HF. The certified reference materials BCR-141R and LGC-6187 are supplied with certified values for extractable metals using methods based on DIN 38414-S7 and ISO11466, respectively, and were digested in this study by using the modified aqua regia procedure. The BCR-141R is certificate for total mass fraction as well. Bias was judged by comparing the measured mass fraction with the certified/recommended values for the RMs [37].

Result presentation

In this paper the statistical terms (repeatability coefficient of variation, standard deviation, etc.) refer to ISO 3534-1 [43]. Repeatability coefficient of variation was defined as the ratio of the standard deviation to the average, obtained under repeatability condition expressed as CV%.

Standard deviations associated with the results of the measurements are multiplied by 2 at an approximate level of confidence 95%.

Analytical recovery is defined as the value observed divided by the value expected and multiplied by 100.

On the basis of the assumption that measurement results obtained on homogeneous material are distributed normally, the comparison between methods (A+B+C vs. INAA and A+B vs. C) was carried out using the grand mean, requiring data normally distributed. The assumption of normal distribution of the results is derived from inter-laboratory comparisons carried out by APAT, in which the normality of the distribution of the metal contents in sediment/compost RMs was verified on data from about 70 laboratories [44, 45].

					Measured mass fractions			
					Method A HNO_3 + HF		Method B HCl + HNO_3 + HF	
	IAEA Soil-7		IAEA SL-1		IAEA Soil-7	IAEA SL-1	IAEA Soil-7	IAEA SL-1
	Recommended value (95% Confidence interval)		Recommended value (95% Confidence interval)		*(n=3)*	*(n=3)*	*(n=3)*	*(n=3)*
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Cr	60	(49 - 74)	104*	(95 – 113)	49 ± 10	109 ± 19	54 ± 16	106 ± 17
Co	8.9	(8.4 - 10.1)	19.8	(18.3 - 21.3)	9 ± 1	18 ± 3	9 ± 2	18 ± 4
Ni	26*	(21 - 37)	44.9*	(36.9 - 53.9)	26 ± 3	47 ± 4	25 ± 9	46 ± 7
Cu	11	(9 - 13)	30*	(24 - 36)	9.5 ± 2	30 ± 4	10 ± 3	30 ± 6
Zn	104	(101 - 113)	223	(213 - 233)	94 ± 13	189 ± 15	90 ± 22	193 ± 50
As	13.4	(12.5 - 14.2)	27.6	(24.7 - 30.5)	13.4 ± 1.4	28.2 ± 1.5	13 ± 3	28 ± 5
Sb	1.7	(1.4 - 1.8)	1.31*	(1.19 - 1.43)	1.7 ± 0.2	1.29 ± 0.09	1.6 ± 0.4	1.2 ± 0.2
Cd	1.3*	(1.1 - 2.7)	0.26*	(0.21 - 0.31)	1.20 ± 0.15	0.26 ± 0.03	1.2 ± 0.3	0.21 ± 0.09
Pb	60	(55 - 71)	37.7*	(30.3 - 45.1)	49 ± 7	32 ± 9	60 ± 14	37 ± 8

* = information value.

Table 3. Element contents for digestion methods A-B applied to IAEA Soil-7 and SL-1 RMs. Results are reported with their standard deviations at an approximate level of 95%. The table also reports the certified values and the 95% confidence intervals for IAEA Soil-7 and SL-1 (n= number of independent replicates).

	LGC6187	BCR 141R		Measured mass fractions			
	Certificate Aqua regia soluble mass fraction	Certificate Aqua regia soluble mass fraction	Certificate Total mass fraction	Method C $HCl + HNO_3 + H_2O_2$			
				LGC6187	(n=10)	BCR 141R	(n=4)
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	CV%	mg kg^{-1}	CV%
Cr	84 ± 9.4	138 ± 5	195 ± 7	101 ±14	6.8	155 ±7	2.4
Co		9.2 ± 0.5	10.5 ±0.4			10.1 ±0.1	0.5
Ni	34.7 ± 1.7	94 ± 5	103 ±3	41 ±8	9.3	93 ±8	4.3
Cu	83.6 ± 4.1	46.9 ± 1.8	46.4 ± 8	94 ±13	6.8	46.4 ±0.2	0.2
Zn	439 ± 26	270 ± 8	283 ±5	455 ±19	2.1	261±6	1.1
As	24 ± 3.2			29±2	3.1		
Cd	2.7±0.3	14 ± 0.4	14.6±0.5	2.9±0.4	6.1	13.6±0.2	0.6
Pb	77.2±4.5	51.3±2	57.2±1.2	82±4	2.2	50.7±0.6	0.6

Table 4. Element contents for digestion method C applied to LGC-6187 and BCR 141R CRMs. Results are reported with their standard deviations at an approximate level of 95%. The table also reports the certified values for extractable metals, total mass fraction and their uncertainties. The uncertainty represents the half-width of the 95% confidence interval (n= number of independent replicates).

RESULTS AND DISCUSSION

Laboratory bias and repeatability standard deviations in RMs and CRMs

In general, recoveries (% R) within 81% to 122% were obtained for all elements for the three digestion methods.

Table 3 reports the results and the associated standard deviations obtained with methods A and B compared with the certified values of the two RMs (IAEA Soil-7 and IAEA SL-1). Co, Ni, Cu, As, Sb and Cd contents are unbiased for both methods in the two RMs.

Low recoveries for Cr even using HF (method A and B) are described in the literature [46, 47] and are associated with the presence of insoluble refractory Cr minerals such as chromospinels and chromites ($FeCr_2O_7$). These minerals, frequently occurring in geological materials, are very difficult to dissolve; this behaviour can result in low recovery. In this investigation, all values of "total" Cr mass fraction determinations fall within the confidence intervals reported in the certificate of RMs. The lower efficiency of digestion methods in extracting Cr from soils can be detected aggregating the analytical data of the laboratories participating in the certification of IAEA Soil-7 and IAEA SL-1 [48, 49]. The mean values obtained for IAEA Soil-7 are 67 ± 6 mg kg^{-1}, in the case of determinations by direct INAA method, and 50 ± 11 mg kg^{-1}, in the case of digestion method associated with atomic absorption spectrometry. These values are respectively higher and lower than the recommended value. The same behaviour is shown for IAEA SL-1 where the aggregated values lead to 112 ± 19 mg kg^{-1}, for INAA, and 89 ± 29 mg kg^{-1}, for methods associated to atomic absorption spectrometry.

The Zn content determined with method A in IAEA SL-1 is significantly lower than the reference value. In the other cases, Zn mass fraction values overlap with the 95% confidence interval of the RMs, but all values are lower than the reference values. Zn is generally totally brought into solution, thus the ICP-MS procedure used in this study needs further investigation.

Pb content determined with method A in IAEA Soil-7 is significantly negatively biased. Low recoveries for Pb (81% and 85 %) using method A may be related to the internal standard used. Rh is not suitable to minimize the instrumental signal fluctuation and matrix effects. The comparison between the results obtained with methods A and B shows that there is no significant difference between the methods, with the exception of Pb in IAEA Soil-7. The results reported in Table 3 show that the standard deviations are generally lower for method A than for method B. This could be explained by the difference in total energy input used in the digestion steps. Method A uses a higher total energy input (5265 kJ) than method B (3192 kJ).

Table 4 reports the results obtained with method C in LGC-6187, certified for aqua regia method, and in BCR-141R certified for aqua regia extractable elements and for the total element contents. The uncertainties reported for the CRMs represent the

95% confidence limit. The standard deviations associated with the measured values are reported with an approximate level of confidence of 95%. Generally, the repeatability coefficient of variation for method C are lower for BCR-141R than for LGC-6187. This could be due to a higher homogeneity of BCR-141R in comparison with that of LGC-6187 certified reference material. The element mass fractions obtained on LGC-6187 were significantly positively biased for As, Cr and Ni, while on BCR-141R only Cr and Co are positively biased in comparison with element mass fractions certified using aqua regia. For Zn, Cu, Cd, and Pb the results with their standard deviations do not clearly overlap the 95% confidence interval of the recommended values. To check if the mass fraction values of these elements in LGC-6187 are biased, the criterion reported in ISO Guide 33 [50] has been used. On the basis of this criterion, that compare the bias with the uncertainty of the certified value combined with the standard deviation of the measurement process, Zn, Cu, Cd and Pb mass fractions are unbiased. The same ISO criterion was applied on the results obtained in BCR-141R. Zn content is negatively biased in comparison with the certified value for aqua regia soluble mass fraction.

The comparison between the certified total elements contents in BCR-141R and the values obtained with method C show that Cr and Co are negatively biased. The application of ISO criterion show that Zn content value is negatively biased.

The results above reported could be explained by the different procedures used in this study in comparison with those used for CRM certification. The degree of dissolution of solid sample with aqua regia depends on the input of energy. Method C uses different power inputs and different reaction times during the digestion step than those used in the certification of LGC-6187 (conventional heating under open reflux condition) and BCR-141R. H_2O_2 used in method C enhances the dissolution of the organic mass fraction. To investigate the effects induced by the procedure used in this work, LGC-6187 was digested using aqua regia microwave assisted, without the addition of H_2O_2 (Table 5). In this case the contents of Cr, Ni, Cu and As are unbiased, while the content of Zn is significantly negatively biased. This confirms the need of further investigations on Zn determination by ICP-MS.

	Method aqua regia (ISO11466*)	
	LGC6187	***(n=3)***
	mg kg^{-1}	**Recovery %**
Cr	92±3	110
Ni	36±2	104
Cu	86±2	103
Zn	390±56	89
As	25.1±0.5	105

* = microwave assisted digestion method

Table 5. Element concentrations using aqua regia digestion method applied to LGC-6187 CRM. Results are reported with their standard deviations at an approximate level of 95%. (n= number of independent replicates).

Comparison of the three digestion methods in determining elements in agricultural soil samples

The homogeneity of the soil test samples was verified measuring by INAA 10 test portions from 3 different test samples. The coefficient of variation for all elements (As, Co, Cr, Sb and Zn) was less than 4%. These results indicate that the differences among methods can be detected when their differences are higher than the residual heterogeneity in the test samples.

In the soil samples the higher precision of method A compared with method B is not confirmed. As an example, CVs for As range from 1 to 14% (method A) and from 1 to 6% (method B), while for Cr CVs range from 1 to 13% (method A) and from 2 to 16% (method B). This different behaviour between the agricultural soils and the RMs (IAEA Soil 7 and IAEA SL1) could be due to the different matrices and to the levels of homogeneity in RMs and in the test samples used.

The results for As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn with methods A and B on the eight agricultural soils are pooled on the basis of studies on RMs. The results obtained with method A and B are compared with those measured after sample

extraction with method C (Table 6). All the results are reported with their standard deviations with an approximate level of confidence of 95%.

Analysis of variance using the ANOVA test at a confidence level of $\alpha = 0.05$ [51] was performed to assess the significance of differences among the three methods. ANOVA shows no significant differences for As, Cd, Co, Cu, Ni, Pb and Zn. These results suggest that for the soil analysed the partial digestion method aqua regia leads to results equivalent to those of the total digestion methods.

For Cr, the ANOVA test shows a statistically significant difference among the methods A-B-C, attributable to method C. As can be easily recognized from Figure 4 and Table 6, Cr mass fractions determined with method C are lower by a factor of two than methods A and B. This could be due to the presence of insoluble refractory Cr minerals which cannot be dissolved without using HF. Similar low recoveries (23%-74%) for Cr using the aqua regia digestion procedure are described in the literature for river sediments and soils [52, 53].

The ICP-MS results on sample digested with method C for Sb (Figure 5 and Table 6) show mass fraction values about 50% lower than method A and B. These results could be due to a not efficient extraction of Sb normally bound to silicates.

A t-test at a confidence level of $\alpha = 0.05$ between methods A and B, showed no significant difference for Cr and Sb.

Soil sample	Cr		Co		Ni		Cu		Zn		As		Cd		Sb		Pb	
	A + B* (n = 6) mg kg^{-1}	C** (n = 3) mg kg^{-1}	A + B (n= 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C** (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}	A + B (n = 6) mg kg^{-1}	C (n = 3) mg kg^{-1}
1	119 ± 9	60 ± 2	13 ± 1	13.3 ± 0.2	47 ± 4	50 ± 6	42 ± 5	43 ± 1	95 ± 6	95 ± 5	10.8 ± 0.7	12.0 ± 0.1	0.66 ± 0.05	0.66 ± 0.03	1.26 ± 0.13	0.660 ± 0.003	36 ± 3	38 ± 2
2	122 ± 27	57 ± 2	13 ± 2	12.3 ± 0.5	49 ± 4	46 ± 3	35 ± 4	34 ± 1	94 ± 11	85 ± 3	11 ± 2	11.2 ± 0.3	0.68 ± 0.12	0.66 ± 0.01	1.36 ± 0.24	0.7 ± 0.1	38 ± 7	31.4 ± 0.7
3	123 ± 24	59 ± 1	13 ± 1	12.9 ± 0.2	48 ± 7	47 ± 2	46 ± 5	47.2 ± 0.5	91 ± 10	88 ± 2	11.1 ± 0.9	11.9 ± 0.3	0.62 ± 0.08	0.64 ± 0.01	1.24 ± 0.10	0.66 ± 0.05	34 ± 2	31 ± 1
4	116 ± 21	62 ± 7	12 ± 1	12.3 ± 0.3	45 ± 4	48 ± 3	44 ± 4	46 ± 1	87 ± 12	88 ± 4	11.2 ± 1.5	12.1 ± 0.2	0.60 ± 0.15	0.6 ± 0.1	1.27 ± 0.23	0.67 ± 0.02	32 ± 3	29.7 ± 0.6
5	114 ± 22	62 ± 4	13 ± 1	12.9 ± 0.2	49 ± 9	48 ± 2	38 ± 4	39.2 ± 0.9	88 ± 10	90 ± 6	10.6 ± 1.2	11.3 ± 0.4	0.58 ± 0.12	0.58 ± 0.03	1.23 ± 0.23	0.61 ± 0.03	30 ± 3	29 ± 1
6	109 ± 39	50 ± 3	11 ± 1	10.6 ± 0.6	42 ± 6	39 ± 2	31 ± 4	31 ± 1	79 ± 9	81 ± 10	9.6 ± 0.3	9.9 ± 0.6	0.57 ± 0.08	0.55 ± 0.02	1.13 ± 0.13	0.57 ± 0.04	28 ± 1	24 ± 1
7	104 ± 20	49 ± 4	11 ± 1	10.5 ± 0.7	42 ± 12	39 ± 2	37 ± 8	36 ± 2	78 ± 11	77 ± 2	9.5 ± 0.4	9.9 ± 0.5	0.54 ± 0.06	0.55 ± 0.02	1.17 ± 0.11	0.55 ± 0.05	29 ± 2	27 ± 1
8	84 ± 15	42 ± 3	9 ± 1	9.0 ± 0.5	35 ± 12	31.0 ± 0.2	29 ± 6	28.8 ± 0.2	69 ± 6	67 ± 3	8.3 ± 0.9	8.7 ± 0.6	0.54 ± 0.10	0.51 ± 0.02	0.99 ± 0.09	0.49 ± 0.05	26 ± 1	23 ± 1

* A + B = results from method A (HNO_3 + HF) pooled with results from method B (HCl + HNO_3 + HF)

** C = results from method C (HCl + HNO_3 + H_2O_2)

Table 6. ICP-MS results for 8 Italian agricultural soils. Results obtained after digestion with method A and B are pooled and reported as A+B. Column C reports results obtained after digestion with method C. Results are reported with their standard deviations at an approximate level of 95%. (n= number of independent replicates).

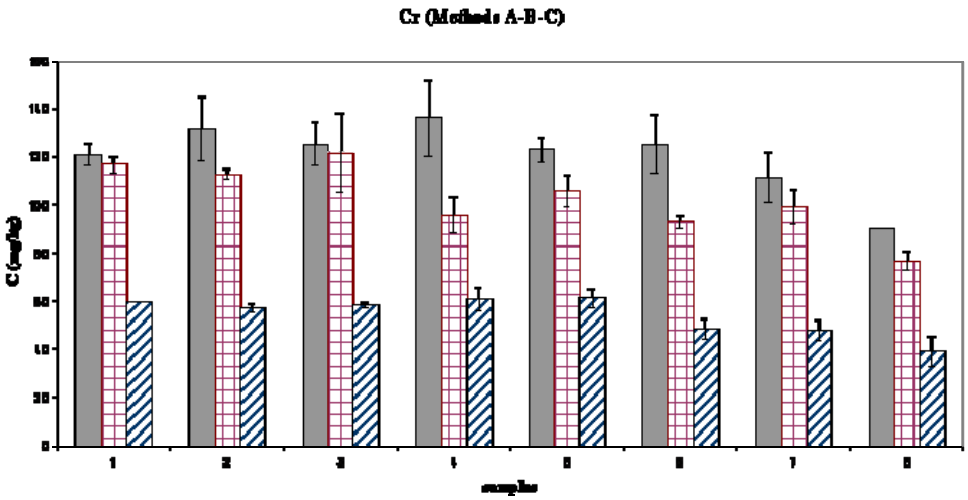


Figure 4. Cr results for eight agricultural soils determined by ICP-MS after acid digestion by methods A, B and C (grey = method A; squared = method B; striped = method C). The error bars represent the standard deviation of three replicates.

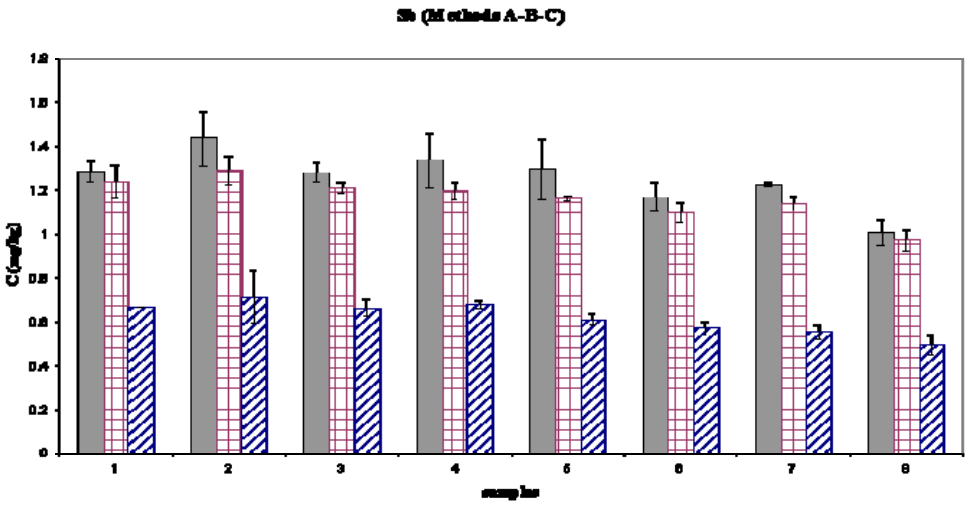


Figure 5. Sb results for eight agricultural soils determined by ICP-MS after acid digestion by methods A, B and C (grey = method A; squared = method B; striped = method C). The error bars represent the standard deviation of three replicates.

Comparison of ICP-MS measurements with INAA in determining elements in agricultural soils

Among the trace elements selected in this investigation, As, Zn, Co, Cr and Sb were determined by INAA as well. Table 7 reports the grand mean of As, Zn, Co mass fraction values obtained by

ICP-MS, after the soil digestion with methods A, B and C, and the INAA analytical results for the eight agricultural soils investigated. INAA data are reported with the standard deviation from counting statistics in gamma spectrometry. The comparison of k_0-INAA results with those obtained by ICP-MS measurements are in good agreement for all elements except for Zn that is slightly underestimated. This result confirms the needs of future studies on Zn determination by ICP-MS. Looking at the results obtained after the digestion with method A, Cr mass fractions in soil are lower than k_0-INAA values (Table 8). These results are attributable to an incomplete digestion of Cr, bound to residual fraction as refractory mineral, even using HF. Yang et al. [47] reported low recoveries for Cr in a certified reference material, HISS (sediment), using ICP-MS and closed vessel digestion with HF and HNO_3. The authors obtained mass fraction values which were almost three times lower than the value found by INAA. Sb mass fraction values in ICP-MS determinations are slightly higher than INAA determinations. The presence of HF in the digestion mixture, in this case, is sufficient for the complete digestion of silicate bound Sb.

Soil Sample	**As (mg kg^{-1})**		**Zn (mg kg^{-1})**		**Co (mg kg^{-1})**	
	ICP-MS Grand Mean methods A, B, C* (*n=3*)	**INAA (*n=1*)**	**ICP-MS Grand Mean methods A, B, C (*n=3*)**	**INAA (*n=1*)**	**ICP-MS Grand Mean methods A, B, C (*n=3*)**	**INAA (*n=1*)**
1	11 ±1	11 ±1	95 ±4	101 ±8	13.0 ±0.9	13 ±1
2	11 ±1	11 ±1	91 ±12	97 ±8	12.5 ±0.4	13 ±1
3	11.4 ±1.1	11±1	90 ±5	99 ±8	12.9 ±0.2	13 ±1
4	11.5 ±1.4	12 ±1	88 ±3	90 ±7	12.1 ±0.3	12 ±1
5	10.8 ±0.9	10.7 ±0.8	89 ±3	89 ±7	12.7±0.5	12 ±1
6	9.7 ±0.5	9.0 ± 0.7	80±5	83 ±7	10.9 ±0.5	11 ±1
7	9.6±0.5	9.5 ± 0.8	77 ±9	85 ++ 7	10.7 ±0.5	11 ±1
8	8.4 ±0.5	8.7 ± 0.7	68 ±2	73 ±6	9.1 ±0.4	9.5 ± 0.8

*methods A, B, C= grand mean of the ICP-MS results after digestion with method A (HNO_3 + HF), B (HCl + HNO_3 + HF) and C (HCl + HNO_3 + H_2O_2)

Table7. ICP-MS and INAA results on Italian agricultural soil. Grand means of data obtained after digestion with method A, B and C. Results are reported with their standard deviations at an approximate level of 95%. (n= number of independent replicates).

Soil Sample	Cr (mg kg^{-1})		Sb (mg kg^{-1})	
	ICP-MS Method A* ***(n=3)***	**INAA** ***(n=1)***	**ICP-MS Method A** ***(n=3)***	**INAA** ***(n=1)***
1	121±10	223 ± 19	1.3 ± 0.1	1.2 ± 0.1
2	132 ± 27	260 ± 21	1.4 ± 0.3	1.2 ± 0.1
3	125 ± 18	215 ± 19	1.28 ± 0.09	1.3 ± 0.1
4	136 ± 31	215 ± 19	1.3 ± 0.3	1.3 ± 0.1
5	123 ± 10	219 ± 19	1.3 ± 0.3	1.2 ± 0.1
6	125 ± 24	234 ± 21	1.2±0.1	1.1 ± 0.1
7	111±21	250 ± 21	1.23 ± 0.02	1.1 ± 0.1
8	90 ± 2	224 ± 19	1.01±0.08	1.0 ± 0.1

*method A= HNO_3 + HF

Table 8. Cr and Sb contents in Italian agricultural soil. Comparison between the results obtained with method A and ICP-MS with INAA results. Results are reported with their standard deviations at an approximate level of 95%. (n= number of independent replicates).

CONCLUSIONS

For methods A and B, Co, Ni, Cu, As, Sb and Cd are unbiased, while for method C, Cu, Cd and Pb are unbiased in comparison with the certified values of the selected RMs.

Both methods A and B generally showed a good repeatability standard deviation. The higher precision of method A is found only in the case of the RMs and not for the real soil samples analysed. Concerning method C, the results on LGC-6187 show higher repeatability than those on BCR141R.

Modified digestion conditions, occurring in method C, in terms of energy input of microwave, determined positively biased values for Cr element for both CRMs certified for aqua regia soluble mass fraction (LGC-6187 and BCR141R). Cd, Pb and Cu mass fraction values are in agreement with the certified values in both CRMs. Zn is generally negatively biased for all methods. Zn is generally totally brought into solution, thus the ICP-MS procedure used in this study needs further investigation.

ANOVA was performed among the ICP-MS results obtained after the application of the three digestion methods on eight agricultural soil samples, collected in one test area. The only significant differences are related to Cr and Sb results obtained after extraction with method C (modified aqua regia). This could be due to the presence in the soil of insoluble Cr minerals and Sb bound to silicates.

k_0-INAA analytical results of the soil samples were compared with ICP-MS measurements on the same test samples. The results of As and Co are in good agreement regardless which of the three digestion methods is used. In this case, the procedures used do not affect the final analytical results.

Cr results, even if obtained with "total" digestion method with the best recovery (method A), are lower by a factor of 2 than the INAA results. Sb after digestion with HNO_3 + HF is in good agreement with INAA results.

In conclusion, the results reported in this paper highlight that the digestion procedure is an essential part of the definition of the measurement procedure in environmental analysis.

SECTION 3. Total trace metal concentrations

The acid digestion of solid matrices is a necessary step prior to the determination of total trace metal content by Inductively Coupled Plasma mass Spectrometry (ICP-MS).

The five soil samples (Saliceto, Millesimo, ERSA, Scarlino 1-2) and compost were digested and analysed using the information outcome from section 2. This section reports the results obtained using three different microwave acid digestion procedures (HF + HNO_3, aqua regia + HF and modified aqua regia) for the dissolution of eight agricultural soil samples and on four certified reference materials.

The target elements were determined in the digested solutions using an inductively coupled plasma mass spectrometer (ICP-MS).

As expected, the digestion method efficiency appears to be strongly dependent on element and soil matrices. As reported in more details in section 2, the three digestion procedures can be considered equivalent for the determination of almost all elements under study.

In addition, the digestion method using HF + HNO_3 (method A) provided more precise results for RMs with respect to aqua regia + HF procedure (method B). In addition, the use of HF for methods A and B provides higher recovery for Cr and Sb with respect to the aqua regia procedure (method C).

In this context, for the digestion of the 5 soils and compost, it was selected the digestion method A, following the procedure reported in details in section 2. For the compost sample, the procedure was slightly modified, using 0.5 ml of H_2O_2 instead of 0.5 ml of MilliQ water, in order to enhance the disruption of organic fractions, highly present in compost. The results are reported in Appendix 1, Table 9.

Appendix 1

Sample	Cr	RSD	Mn	RSD	Fe	RSD	Co	RSD	Ni	RSD	Cu	RSD	Zn	RSD	As	RSD	Cd	RSD	Pb	RSD
	mg/kg	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*
ERSA	93.32	0.7	1188.91	0.9	26983	1.1	11.47	1.0	46.42	1.1	39.07	1.1	92.11	0.8	11.02	1.4	0.42	5.8	33.20	0.7
Compost	19.18	0.5	323.08	0.6	15984	1.3	3.42	1.0	8.58	1.0	63.37	1.0	152.96	0.9	4.58	4.0	0.50	3.4	98.50	1.0
Scarlino 1	10.87	2.8	784.91	1.6	358460	1.1	257.19	0.5	11.10	1.6	272.96	0.6	1058.23	0.9	397.26	0.7	3.28	3.5	910.29	1.2
Scarlino 2	11.24	1.5	263.74	0.7	278599	1.2	191.82	0.6	6.93	1.6	208.42	0.6	403.96	0.7	385.27	0.6	0.96	5.5	1781.45	0.7
Millesimo	61.36	0.6	419.36	0.5	27772	1.2	7.79	0.6	30.20	0.7	14.01	0.9	107.78	1.0	25.80	0.8	0.42	2.7	31.61	0.3
Saliceto	145.96	0.3	1054.24	0.2	31962	0.7	23.21	0.6	101.08	0.6	24.08	0.7	99.11	0.9	32.37	1.3	0.48	4.8	49.35	0.7

Table 9. Total metal concentrations as determined in the five soils sample and compost by using microwave acid digestion method A.

SECTION 4. Single extraction procedures

Introduction

The development and use of extraction schemes started at the end of 1970s and aimed to evaluate the metal fractions available to plants and the environmentally accessible trace metals, *e.g.* the mobility of metals from a soil. These methods are still widely used for soils and sediments as reflected by the number of recently published papers on their applications to environmental studies.

Complexing agents solubilize not only the exchangeable element fraction but also the element fraction forming organic matter complexes and the element fraction fixed on the soil hydroxides.

Once the metal is adsorbed onto these fractions it is taken out of the environmental processes. To be de-sorbed and return into the soil solution, a large amount of energy is required. In order to quantify the concentration of heavy metals in the soils in the adsorbed and/or complexed forms it is necessary to use an extractant able to form particularly stable complexes with these elements. These procedures represent a useful approach for environmental studies for soils and sediments, providing practical information on the fractionation of metals. However, it has to be renowned that extraction procedures provide a simple classification of soil metal fractions, but these are based on arbitrary responses to chemical reagents rather than on a true reflection of metal lability. In other words, the extracted "forms" should be only related to the extractant used, for example EDTA-extractable element and not as "bioavailable" or "mobile" forms which are interpretations of data rather than results of actual measurements.

Material and Methods

The methods used in the present work use as extractant a solution of diethylen triamino pentaacetic acid (DTPA) in the presence of calcium chloride and triethanol amine buffered at pH 7.3 [54]. This method is generally recommended for alkaline soils. The soil-extractant ratio is 1:2.

DTPA is widely used in USA and is rather applied to predict plant uptake. The procedure reported in the ISO 14870 [55] was followed. The results are reported in table 10. Although this procedure is suggested for alkaline soils, it has been applied on all soils and compost under study in the present work (except for Bovisa soil). The repeatability of the procedure is studied by replicating the extraction on three independent sub-sample of both Saliceto and Millesimo soils (table 11).

For acidic soils, the extraction with a solution of etilen diamino tetracetic acid (EDTA) at pH of 4.75 is widely used [7]. The soil-extractant ratio is in this case 1:5. The lower solid-liquid ratio renders this procedure easier to apply with respect to DTPA test for which the high mass/volume ratio limits the volume collected after the extraction step. The procedure reported in the Italian Ministerial decree (DM 13/9/99) was followed. The results are reported in table 12. Although this procedure is suggested for acidic soils, it has been applied on all soils under study in the present work and described in section 1 (except for Bovisa soil). The repeatability of the procedure was studied by replicating the extraction on three independent sub-sample of both Saliceto and Scarlino 1 soils (table 13).

It is assumed that EDTA extraction enables a complete extraction and mimics the mobility of trace metals from soils. The choice of extractant must be determined by the objectives of the study.

Determination of elements content

The EDTA and DTPA extracted solutions were analysed for metals content on an Agilent technologies 7500c ICP-MS equipped with a collision cell to minimize the polyatomic interferences and matrix effects. In particular, Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb were determined in both EDTA and DTPA extracted solutions. In addition, Fe was determined in the DTPA extracts, since this complexant is recommended for this metal. Rhodium at a concentration of 10 □g/L was added as internal standard to the extracted solutions to minimize the instrumental signal fluctuation and matrix effects.

Results and discussion

From Tables 11 and 13 appears that EDTA extraction procedure is more repeatable than DTPA. In fact, high relative standard deviations (RSD%) of three independent extraction replicates were found for this latter method. This is particularly marked in case of Ni in the Millesimo sample (22.6%) and for Zn in the Saliceto one (20.6%). This feature is probably due to the substantial instability of the DTPA-extracts, well documented especially in case of Zn [2]. The EDTA procedure achieved precise results for all elements under investigation, attaining RSD% below or equal to 9.4%. Table 10 shows that DTPA extraction procedure is not recommended for the determination of Cr. Actually, almost all the Cr concentrations are below the detection limit of ICP-MS.

It is easily recognizable from the results in Table 10 and 12 that DTPA procedure generally extract less than EDTA. This feature is well reported in literature [2].

However, it is interesting to note that for the soils characterized by low pH (Scarlino n°1-2) the DTPA and EDTA extraction procedure achieved very similar results, particularly in case of Mn, Co, Cd, Ni (Scarlino 2), and to a less extent for Zn. These results could be explained considering the typical increased solubility of metals at low pH, which renders the metals more available to be complexed also by relatively weak chelators such as DTPA.

By comparing the total metal concentrations as determined in section 3, up to 1-2 orders of magnitude differences are observed between the concentration in the extract and the total metal amount.

The results for EDTA and DTPA extraction procedure applied to Scarlino 1 and Millesimo samples are also reported in section 5 and “positioned” at the corresponding pH, on the curve of metal leached concentrations as a function of pH. For further details see section 5.

Sample code	Cr	RSD	Mn	RSD	Fe	RSD	Co	RSD	Ni	RSD	Cu	RSD	Zn	RSD	As	RSD	Cd	RSD	Pb	RSD
	mg/kg	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*
MILLESIMO	<LD		6.40	1.44	26.40	1.59	0.06	0.76	0.25	1.00	1.47	0.83	2.88	8.98	0.03	2.64	0.05	2.96	5.66	0.39
SALICETO	<LD		7.90	0.80	18.98	0.93	0.05	0.64	0.72	0.92	2.38	0.83	0.94	1.41	0.01	6.21	0.06	2.45	3.86	0.64
SCARLINO 1	<LD		254.04	1.20	662.52	0.74	6.13	0.64	0.45	0.32	9.18	0.70	229.65	0.58	0.01	13.36	1.20	0.73	6.07	0.52
SCARLINO 2	0.92	1.10	138.80	1.72	7679.51	1.04	10.54	0.50	1.04	0.71	41.25	0.13	208.93	0.55	3.33	1.12	0.84	0.96	1.09	1.56
ERSA	<LD		25.68	1.79	28.96	1.10	0.02	6.44	0.21	1.96	5.52	0.75	1.91	0.57	13.54	5.73	0.16	1.93	2.69	1.18
COMPOST	<LD		121.66	0.17	406.56	0.59	0.47	0.25	0.56	0.86	3.39	0.39	72.08	0.67	0.07	6.13	0.12	1.83	15.02	0.46

Table 10. DTPA extraction procedure applied to the soils and compost samples.

Sample code	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
	RSD%	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*
MILLESIMO	-	6.3	0.6	12.4	22.6	9.5	12.0	1.3	2.0	0.7
SALICETO	-	8.7	10.9	11.7	7.5	10.1	20.6	6.6	2.9	3.5

Table 11. Repeatability of DTPA extraction expressed as relative standard (as percentage) of three independent extraction procedures on Scarlino soil.

Sample code	Cr	RSD	Mn	RSD	Co	RSD	Ni	RSD	Cu	RSD	Zn	RSD	As	RSD	Cd	RSD	Pb	RSD
	mg/kg	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*	*mg/kg*	*%*
ERSA	0.10	5.46	503.34	0.50	2.44	0.22	2.42	2.06	13.51	1.13	5.29	1.20	0.14	3.79	0.32	3.05	12.15	1.14
MILLESIMO	0.14	2.96	91.56	0.63	1.32	0.37	1.00	2.02	3.35	1.43	8.28	0.22	0.77	1.16	0.07	4.65	10.67	0.20
SALICETO	0.17	5.79	152.77	0.38	1.85	1.55	3.48	2.11	6.20	0.70	2.98	0.55	0.49	0.08	0.09	6.42	12.88	1.51
SCARLINO 1	0.41	6.18	255.32	0.74	6.90	0.53	0.74	5.05	29.23	1.14	246.32	0.96	0.11	6.54	1.28	1.41	377.63	1.88
SCARLINO 2	0.98	1.13	139.53	1.36	10.74	1.21	0.95	3.84	33.66	1.35	159.62	1.14	0.16	9.13	0.81	1.96	16.68	2.12
COMPOST	0.19	1.48	164.98	0.74	0.61	1.71	1.05	0.47	10.05	0.38	84.40	1.38	0.42	4.74	0.27	1.82	36.74	0.24

Table 12. EDTA extraction procedure applied to the soils samples and compost.

Sample code	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb
	RSD%	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*	*RSD%*
SCARLINO 1	8.5	2.2	4.2	3.9	9.4	4.3	5.6	4.1	9.4
SALICETO	7.8	2.0	4.4	1.2	0.6	3.9	2.4	4.4	1.5

Table 13. Repeatability of EDTA extraction expressed as relative standard (as percentage) of three independent extraction procedures on Scarlino and Saliceto soils.

SECTION 5. Leaching tests

Introduction

In recent years, it is increasingly being accepted that risk-assessment of contaminated environments should be based on the *mobile fractions*, rather than total concentration levels, of contaminants in soils, sediments or solid waste materials. This realisation has led to the development and application of various leaching and extraction procedures, which aim at the measurement of environmentally-relevant subfractions of contaminants.

In particular, in matrices that contain organic matter, both particulate organic matter and dissolved organic matter play a key role in the potential for either mobilisation or sorption of constituents [56].

The main release controlling processes are common to all materials - soil, sludge, sediments, compost, industrial and municipal wastes and a wide range of construction materials, including wood and metal products [6].

Leaching can occur in the field by exposure of material to natural infiltration or precipitation (for example a natural soil exposed to rainwater infiltration) or in laboratory during leaching/extraction tests. Generally, leaching/extraction tests are designed to reflect a field exposure situation.

Worldwide a great number of leaching tests have been developed for both research and regulatory controls to evaluate the influence of environmental parameters on different types of material and the impact of the use of these materials in the environment. There are short and long term leaching tests using different leachants and low or high liquid/solid (L/S) ratios, tests performed with pH control or not, different ways of stirring and so on. Each of these tests provide different information and it is necessary to select the most appropriate leaching test on the basis of the specific environmental scenario. As reported in the figure 6, batch tests can quantify contaminant emissions (i.e. the leached amount) from contaminated soils and sediments as a function of the extent of contact with water that is the L/S ratio and/or time.

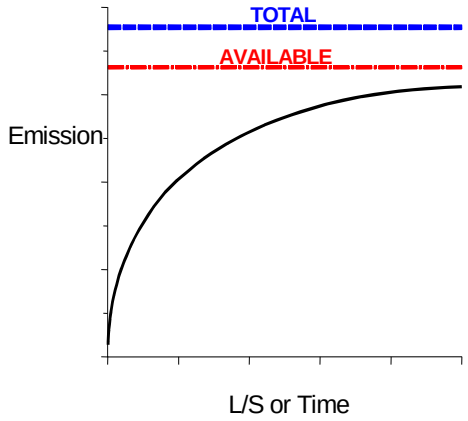


Figure 6. contaminant emission by leaching from contaminated sample as a function of L/S ratio and/or time and comparison with total contaminant amount

Considering that long term leaching behaviour of soils can not be obtained by experimenting in realistic time frames, the combination of modelling and accelerating certain aspects of leaching in batch or column tests can help to simulate such long term scenarios. This can be accomplished by increasing the volume of liquid used in batch tests or the throughput of liquid through columns.

In this context, the liquid to solid ratio is dramatically different at the laboratory scale (between 1 to 100 and more often 10) compared with the field situation (far below 1), simulating naturally occurring precipitation over a longer time frame.

Batch leaching methods are those in which a sample is placed in a given volume of leachant solution for a set period of time. Most of these methods require some type of agitation to insure constant contact between the sample and the leachant. At the end of the leaching period, the liquid is removed by filtration and analysed. In serial batch test, the sample is leached successively with fresh aliquots of the same leaching liquid. This method is intended to eliminate the effect of concentration on solubility and to simulate long term exposure to the leachant solution.

The driving force influencing the release of constituents from soils are the solubility of substances, the diffusion rate of the constituents inside the matrix and the wash off of the substances which lay on the surface of the matrix.

When risk-assessment is based on mobile, rather than total, metal concentrations, it is important to be able to assess the long-term leaching and

mobility of these contaminants in the environment. For that purpose, leaching and speciation measurements should be supported by geochemical models for the prediction of long-term contaminant mobility. Modelling will contribute to the interpretation and selection of the most appropriate methods for mobility measurements in different environments.

1. pH –dependence leaching tests

In the present study, a pH-dependence leaching test, called pH-stat test, has been selected for determining the influence of pH on the leachability of metals from the soils and compost samples described in section 1. As pH is the most important environmental variable controlling leaching, the test is considered to be a valuable tool to predict leaching in different environments. A pH-stat leaching test can also be used for the identification of the underlying (geochemical) processes that control contaminant leaching and provides valuable input for risk-assessment modelling.

In addition, a comparison of APAT – ECN (the Energy research Centre of the Netherlands) leaching data obtained on the same soil samples is reported.

ECN is strongly involved in leaching method development and standardisation at both national and international level and has been involved for many years in standardisation bodies such as CEN technical committees. An intercomparison exercise on leaching with ECN offers the possibility of identifying possible source of errors and method reproducibility among different laboratories.

The collaboration with ECN falls within the framework of the SOILEACH project, coordinated by APAT and focused on the definition of guidelines for the selection of leaching and extraction procedures of metals in soils and wastes.

Material and methods

pH-static leaching test is based on continuous pH-control and has been performed according to the procedure reported in the CEN TC292 WI 292032 standard [57]. The pH was monitored and adjusted to set point in 250-mL polyethylene reactors simultaneously at eight pH_S values in the range of 2-12. Among these eight pH_S, the natural pH of soils were monitored. Suspensions of the

granular samples were prepared in MilliQ water at a liquid/solid (L/S) ratio of 10 L/kg. The reactors were stirred continuously during a 48-hours equilibration period, using a teflon-coated magnetic stirring bar, at 20±1 ^{o}C. The reactors were closed with a special Teflon cap on which 3 openings were performed to introduce the electrode and tubes. In this way, it was possible to reduce the uptake of atmospheric CO_2 which causes pH changes.

The pH of the suspensions was continuously monitored by a computerized pH-stat system and was automatically adjusted to setpoint by addition of 1 M and 5 M HNO_3 or NaOH (analytical grade), when the measured pH deviated by more than a preset value from the setpoint. After a reaction time of 48-hours, the suspensions were pre-centrifuged at 3000 rpm for 0.5 h in 200 mL polycarbonate centrifuge tubes to facilitate the separation of leachate and particulate matter. Subsequently, the leachates were filtered through previously acid washed 0.45 μm membrane filters. The leachates were analysed for total trace metal content by ICP-MS (7500c Agilent Technologies). Rhodium at a concentration of 10 □g/L was used as internal standard to minimize the signal fluctuation and matrices effect.

The pH-stat test has proven to be particularly valuable [4] in providing geochemical "fingerprints" of the processes that control the solubility and speciation of contaminants in soils, particularly in combination with geochemical modelling programs, such as for example MINTEAQ2 or ORCHESTRA. These programs are based on thermodynamic data for soil components and phases. The distributions of metals between various solid phases can theoretically be determined by these calculations, although the models are dependent on the reliability of the data incorporated in the program. However, these programs are capable of providing some predictions of metal speciation in soil solutions and could support the interpretation of the results of metal speciation studies.

Results and discussion

The results of soil analysis for samples collected in Scarlino (Tuscany) and Millesimo (Piemonte) are shown in the figures 7-14 and 15-23, respectively. As

shown in these figures the leaching of trace elements is strongly dependent on pH (note the logarithmic concentration axes). Therefore, the pH-stat procedure provides geochemical "fingerprints" of the underlying processes that control the solubility and leaching of elements.

The plateaus in the leaching curves at low and/or high pH provide estimates of the chemical availability of elements in the soil.

The results for Scarlino are shown in Figures 7-14, Appendix 2.

Two features can be observed in the pH-stat leaching curves (Figure 7-14):

1. Increasing concentrations towards low pH, with maximum around pHs 2-4 (Ni, Zn, Co). This feature represents the typical increased solubility of metals at low pH. The maximum concentration at low pH can be taken as the maximum availability of the contaminants for release from the solid matrix.

2. V-shaped concentration curves, with maximum at pHs 2-4 and/or pH 10-12 (As, Cu, Cd, Pb). This feature is typical for metals which form soluble complexes with ligands such as OH^- and CO_3^{2-} at high pH. The plateaus in the leaching curves at low and/or high pH provide estimates of the availability of elements in the soil.

The figures 7-14 report also the EDTA and DTPA extractable metals and the total metal concentrations as determined by ICP-MS (see section 3 and 4). As it is easily recognizable from the figures, EDTA results do not fit the pH pattern as EDTA extraction is almost pH independent. Except in the case of As and Pb, the EDTA results correspond to the maximum concentration leached using pH-stat procedure. In particular, in case of Pb, EDTA results able to solubilize the largest fraction of metal.

Comparison of the plateaus in the leaching curves with the total concentrations shows the relevance of leaching tests. For most elements, up to 1-2 orders of magnitude differences are observed between the maximum concentration leached and the total amount. These results imply that only <1-10% of the total amount of these contaminants are available for leaching, confirming that the determination of total metals concentrations in soils could greatly overestimate the metal fractions which are effectively available for release from the solid matrix. The total

composition is mostly irrelevant for leaching. The chemical speciation of the metals and the chemical conditions in the leachate, dictated by the major elements in the sample, govern the release.

Comparison of APAT – ECN leaching data

The following figures (15-23) report leaching data for the pH-stat test performed on Millesimo soil sample by APAT and ECN laboratories. The ECN data for each metal are labelled as *pH stat ECN* in the figures.

As evident from the figures, APAT and ECN results show the same trend, although APAT results tend to be higher than that of ECN. This could be due to a better extraction, to a possible contamination or to analytical problems. Another possible cause of difference among the two set of results could depend on a dis-homogeneity of samples. The analytical uncertainty seems the most probable cause of difference between the two set of determination. This can be further investigated by performing pH-stat on the same soil reference material and by analysing the same aqueous reference material. In the first case it is possible to greatly reduce the dis-homogeneity of samples, being reference material highly homogenous and stable. In the second case, it is possible to investigate the capacity of the two labs of making accurate measurement by ICP-OES and ICP-MS.

However, it has to be noted that in the context of leaching, the trend of results vs pH give more information of geo-chemical speciation than the absolute values of metal concentrations.

Conclusive remarks

Further measurements will be focused on the determination of the dissolved organic carbon (DOC) in the eluates coming from the pH-stat test. Actually, the organic matter has an important influence on the concentration, availability and transport of metal ions in the environment. Organic matter lowers the metal concentration by its strong affinity, while it enhances the total concentration of soluble metals when a fraction of the organic matter is dissolved in water [4]. The increase in solubility of DOC with pH is generally observed and could be visually

observed by the colour of the leachate with changes from yellowish to light brown or dark brown.

All the experimental data determined in the eluates could be introduced as input parameters in the geochemical speciation program MINTEAQ2, freely available from web, in order to get helpful information on metals speciation.

2. Serial Batch leaching test

A batch leaching test which can quantify contaminant emission from contaminated soils as a function of L/S ratio was also applied on soils. The selected batch leaching test is a serial batch test, the 2-step compliance test EN 12457-3 standardized by the European Committee for Standardization (CEN) [58] which provides information on leaching under a liquid to solid ratio of 2 l/kg in a first step and subsequently of 8 l/kg in a second step.

Materials and methods

The test was applied on soils from Saliceto, Millesimo and Scarlino. This test is based on the assumption that equilibrium or near equilibrium is reached between the solid and liquid phases during the test duration. The first step is based on a contact time of 6 hours, the second of 18 hours, providing a total contact time of one day. The test is not under pH control.

Results and discussion

The two stage serial batch test allows the recognition of leaching behaviour of constituents as reported:

- the constituents are so readily soluble that they are almost completely leached out after the first leaching cycle. In this case the amount released is the total available for leaching;
- the leaching of the constituents is controlled by the solubility. In this case the concentration in the eluate is about equal in both extractions and the amount released increased proportionally to the liquid solid ratio (L/S) until the leachable fraction is depleted;

- the constituents show a sharp increase in concentration in the second cycle. This delayed release has to be understood since indicates the potential of uncontrolled conditions.

From the two step procedure the leaching behaviour can be identified by taking the ratio of the cumulative release at L/S = 2-10 to that at the release at L/S =2.

$Q = A_{10}/A_2$

where A_{10} = cumulative release at L/S = 2-10 and A_2 = release at L/S = 2.

A possible way of interpretation of the results is the following:

If Q is low (e.g. Q<2), the contaminants are washed out largely within L/S = 2 or the retention of the constituents in the matrix is extremely high. As reported in the following tables, this case is generally observed for almost all metals, except for Pb (Scarlino soil).

A distinction of the two situations is possible by the analysis of the potential leachability, which can be derived from the plateau value at low pH obtained by pH stat test. Actually, the maximum concentration at low pH can be taken as the maximum "availability" of the contaminants for release from the solid matrix.

In case of high solubility, the cumulative release at L/S = 10 equals the potential availability which represents the maximum quantity that can be released for the constituents.

Comparing the results with those obtained by pH stat test on Scarlino soil, it is possible to recognize that metals are not highly retained by soils matrix. Actually, the concentrations at pH 2 derived from pH-stat on Scarlino are very similar to those obtained for cumulative release at L/S = 10 in batch leaching test (Table 14-15). In particular, quite good agreements are obtained for Mn, Fe, Se, Cu, Zn, Co, Ni, Cr Cd. In addition, considering the low pH of Scarlino soil, an high degree of metal immobilisation is unexpected. In case of high retention of metals in the matrix, the concentrations are generally low and the difference between potential leachability and cumulative leachability may be several orders of magnitude.

If Q is intermediate (2<Q<6), as in the case of lead, leachability is largely controlled by the solubility. The concentration in the eluate is constant and the release as a function of L/S increases until the leachable fraction is depleted.

Comparison of APAT – ECN leaching data

The following figures (Figures 24-26) report leaching data for the 2-step CEN compliance test performed on Scarlino soil samples by APAT and ECN laboratories. The eluates were analysed by APAT using ICP-MS and by ECN using ICP-OES. The ECN data for each metal are labelled as Me ECN in the figures.

From the figures is evident the very good agreement achieved for Mn, Cu and Co. For As, Cr and Ni, our results are higher than that of ECN, even though the slopes of the curves that allow the recognition of the leaching behaviour of constituents are quite similar.

For Se, the opposite occurs, meaning that ECN results are higher than APAT results. This probably depends on the different instruments used by the two labs. For Se, APAT uses ICP-MS equipped with a collision cell pressurized with hydrogen to minimize the Ar dimer interference on Se, which generally cause an overestimation of Se.

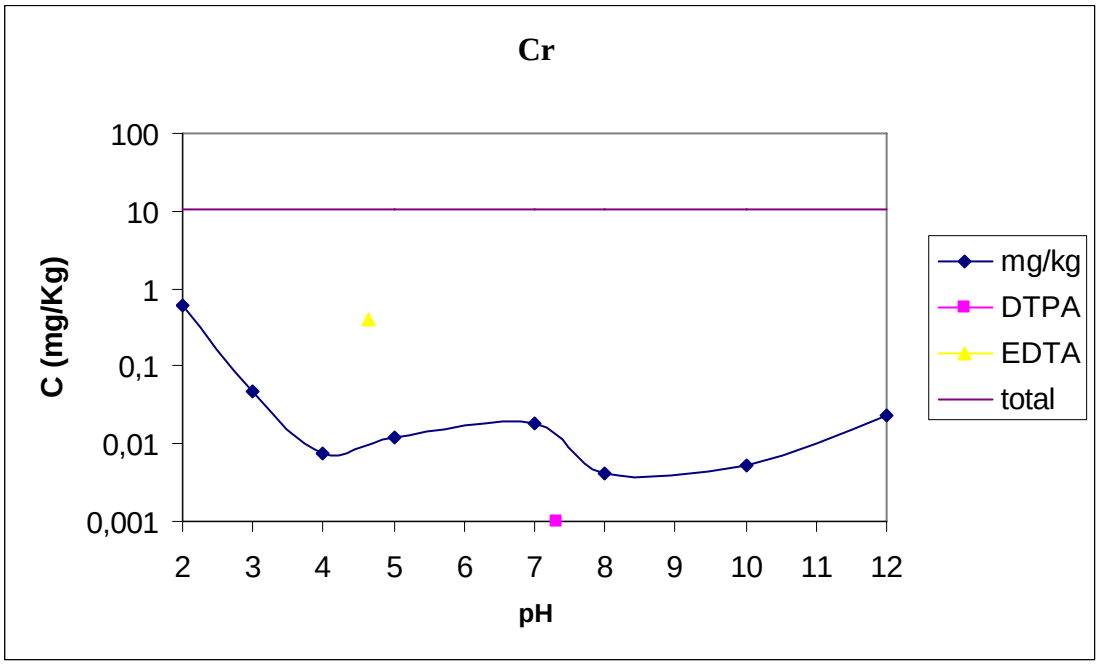
Finally, in case of lead the difference between APAT and ECN results mainly concerns the first measurement at L/S of 2, causing a big difference in the slope.

From our data results that leachability is largely controlled by the solubility. A possible explanation of the higher values of Pb measured by ECN could depend again on the different instruments used or on possible contamination which frequently occurs for lead.

Another possible explanation is a better contact between leachant and soil obtained by ECN. However it could be expected that a similar trend must be obtained for all the target elements.

Appendix 2

Leaching test results



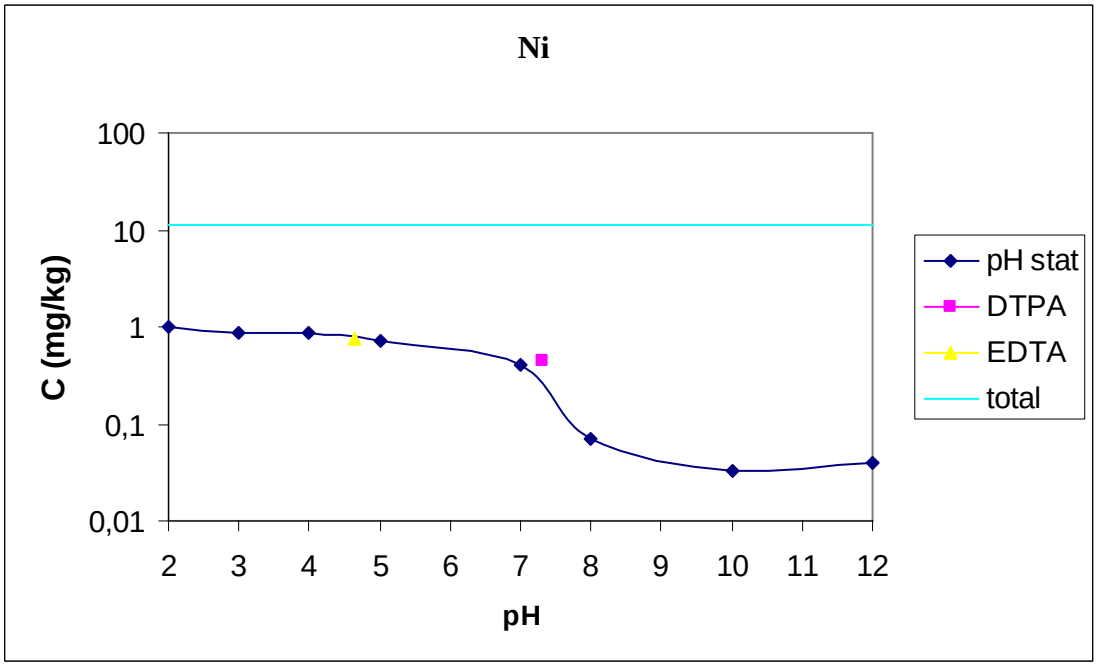
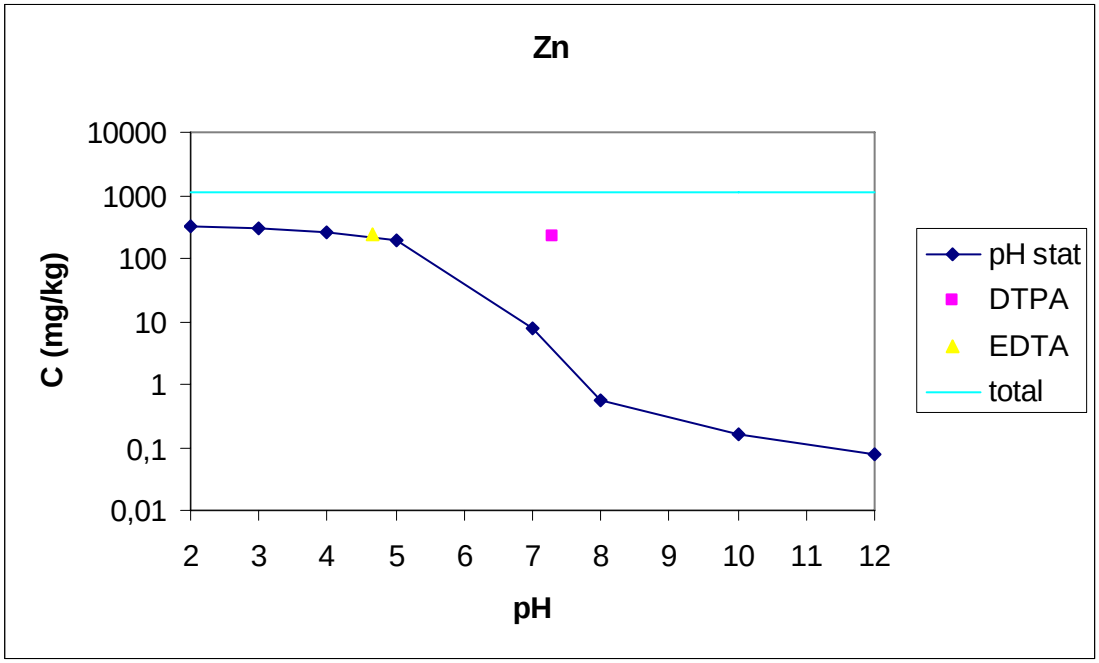


Figure 7-8. Leached concentrations (pH-stat) of Cr and Ni from Scarlino 1, as a function of pH. The figures report also the metal total concentrations and EDTA and DTPA extractable metals (see section 3 and 4).



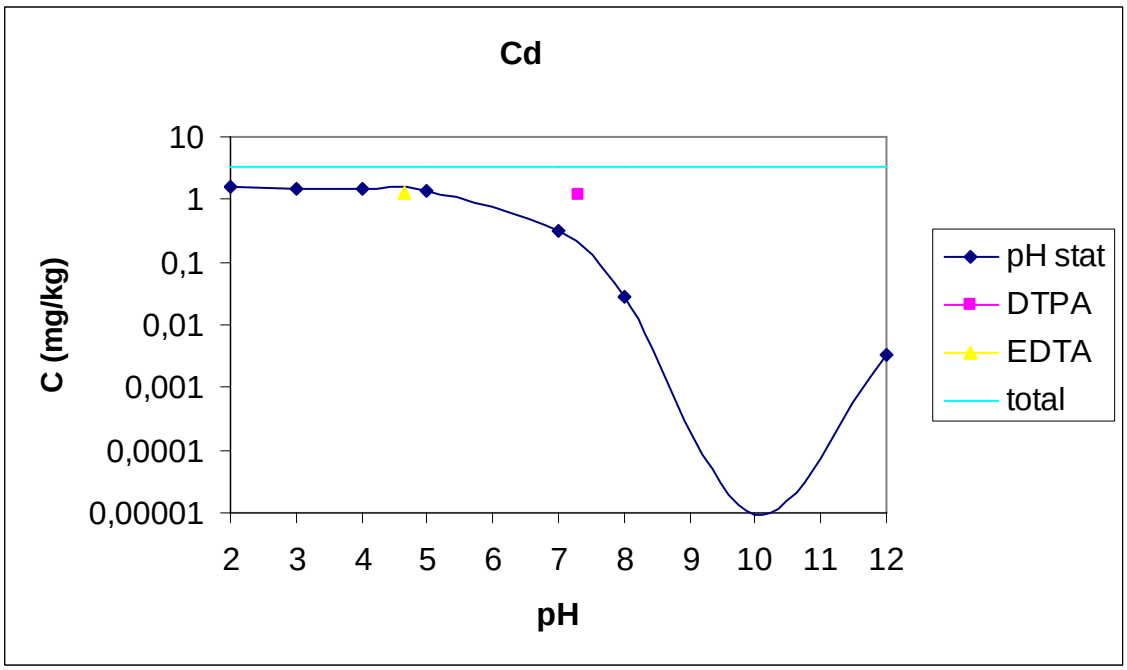
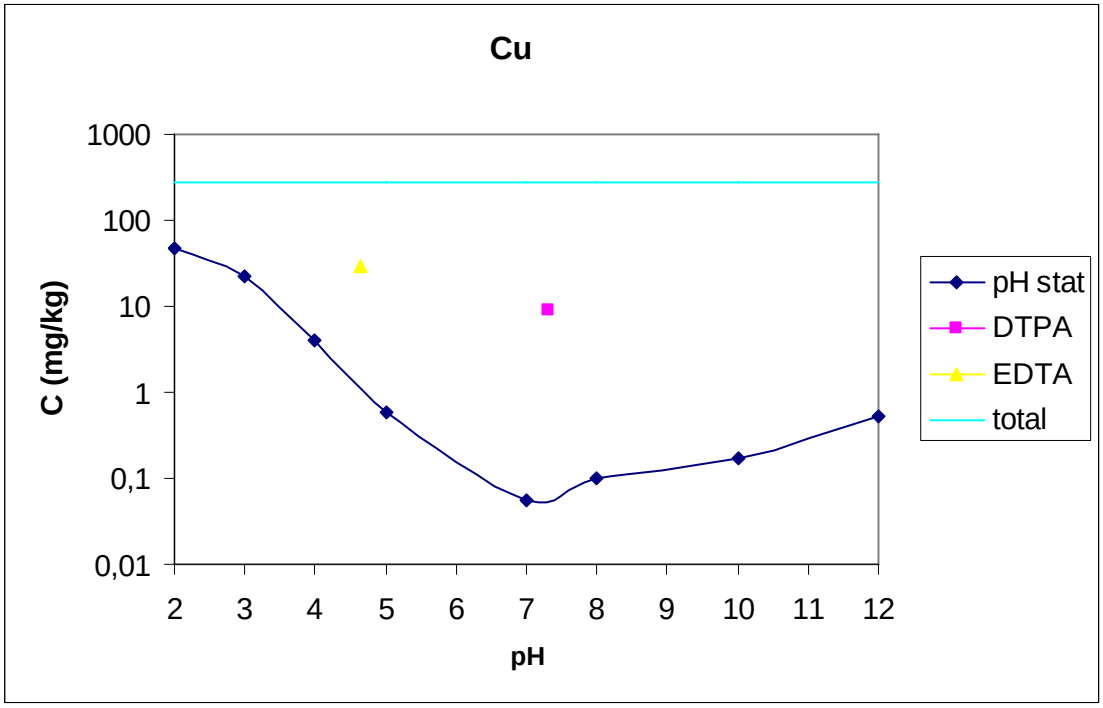


Figure 9-10. Leached concentrations (pH-stat) of Zn and Cd from Scarlino 1, as a function of pH. The figures report also the metal total concentrations and EDTA and DTPA extractable metals (see section 3 and 4).



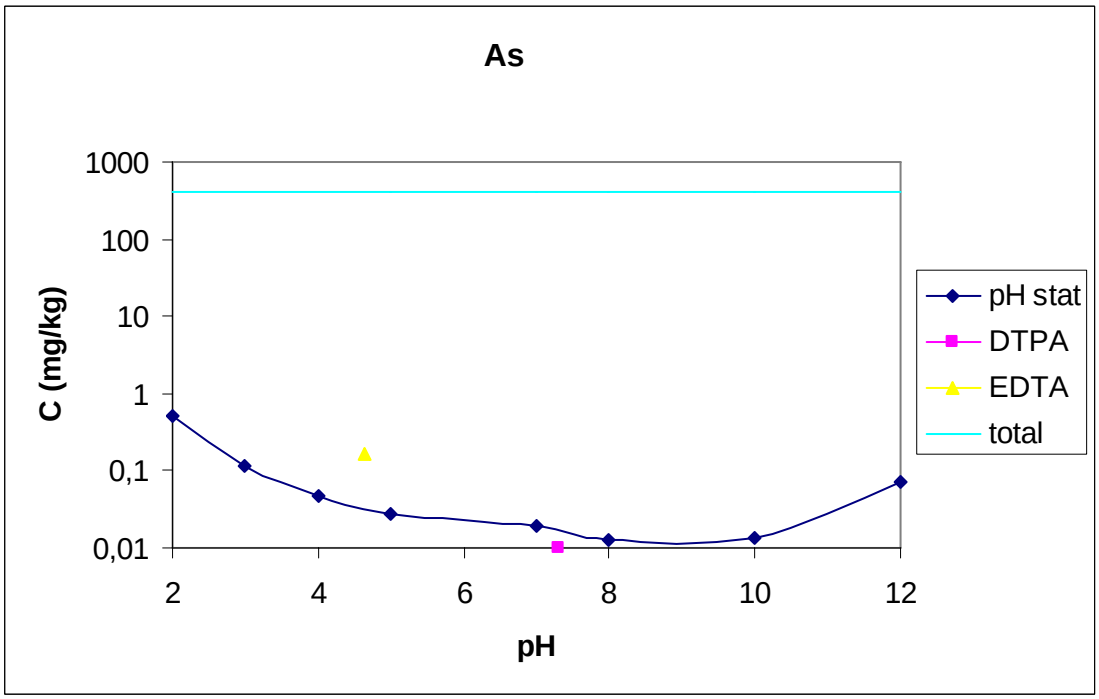
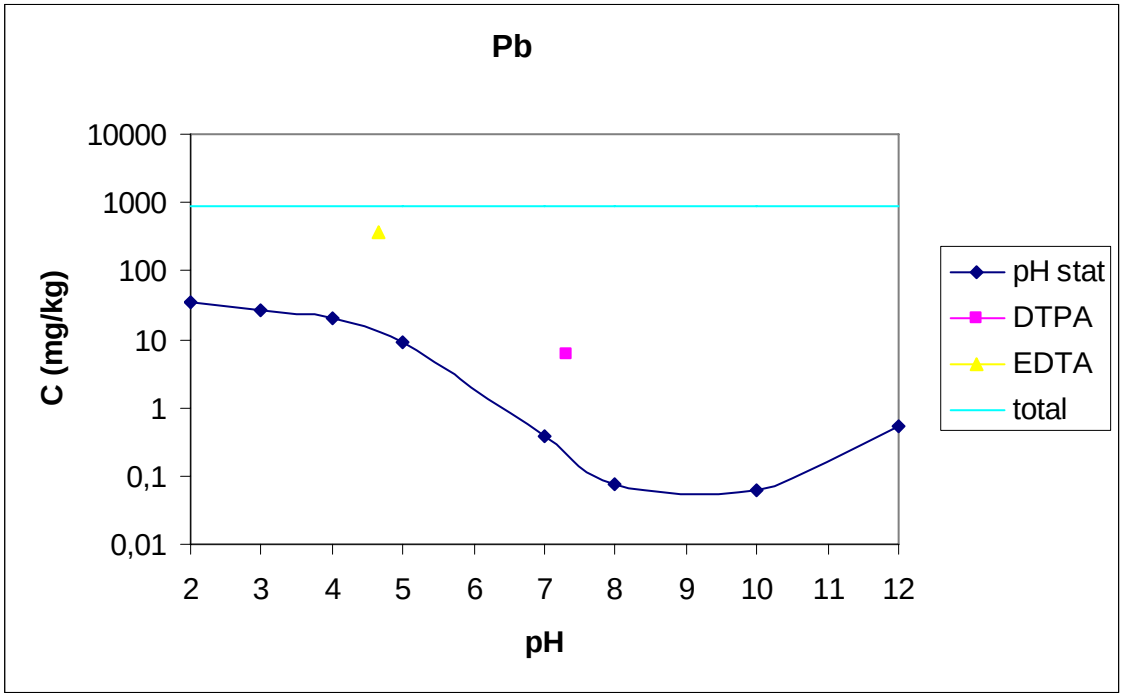


Figure 11-12. Leached concentrations (pH-stat) of Cu and As from Scarlino 1, as a function of pH. The figures report also the metal total concentrations and EDTA and DTPA extractable metals (see section 3 and 4).



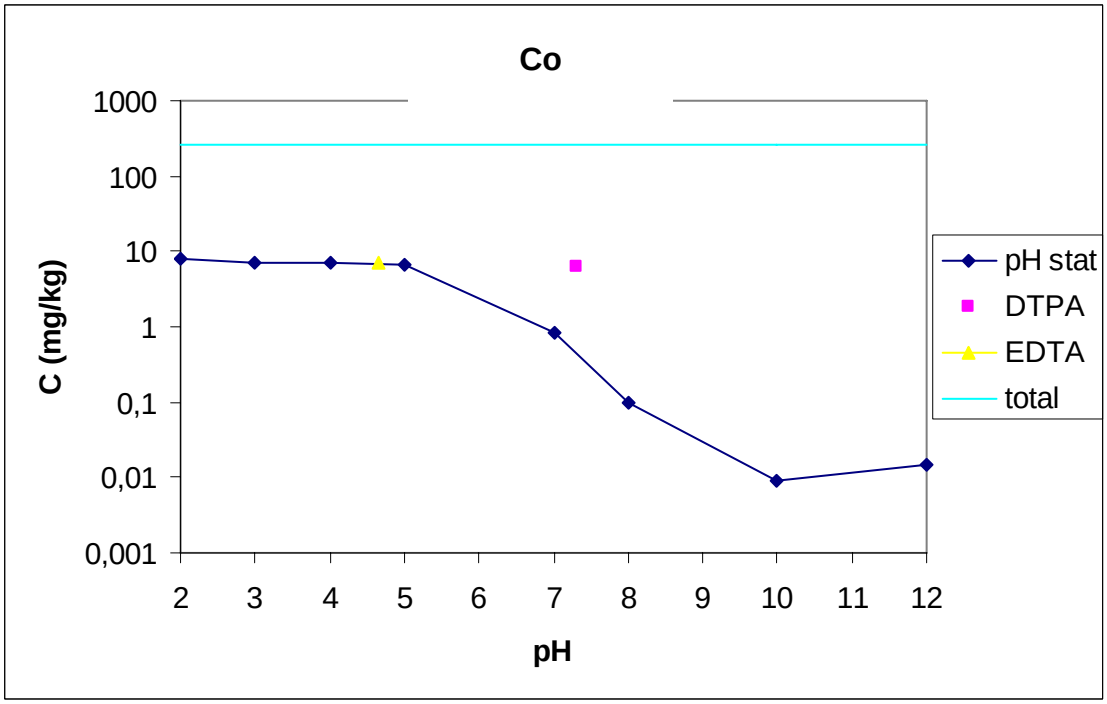
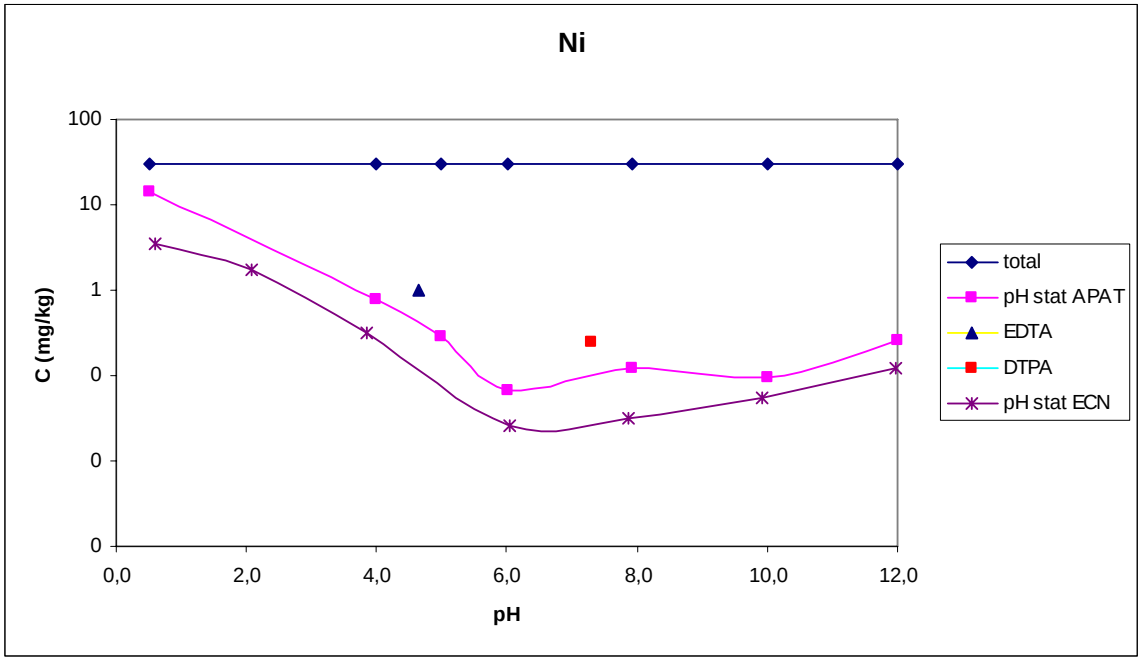


Figure 13-14. Leached concentrations (pH-stat) of Pb and Co from Scarlino 1, as a function of pH. The figures report also the metal total concentrations and EDTA and DTPA extractable metals (see section 3 and 4).



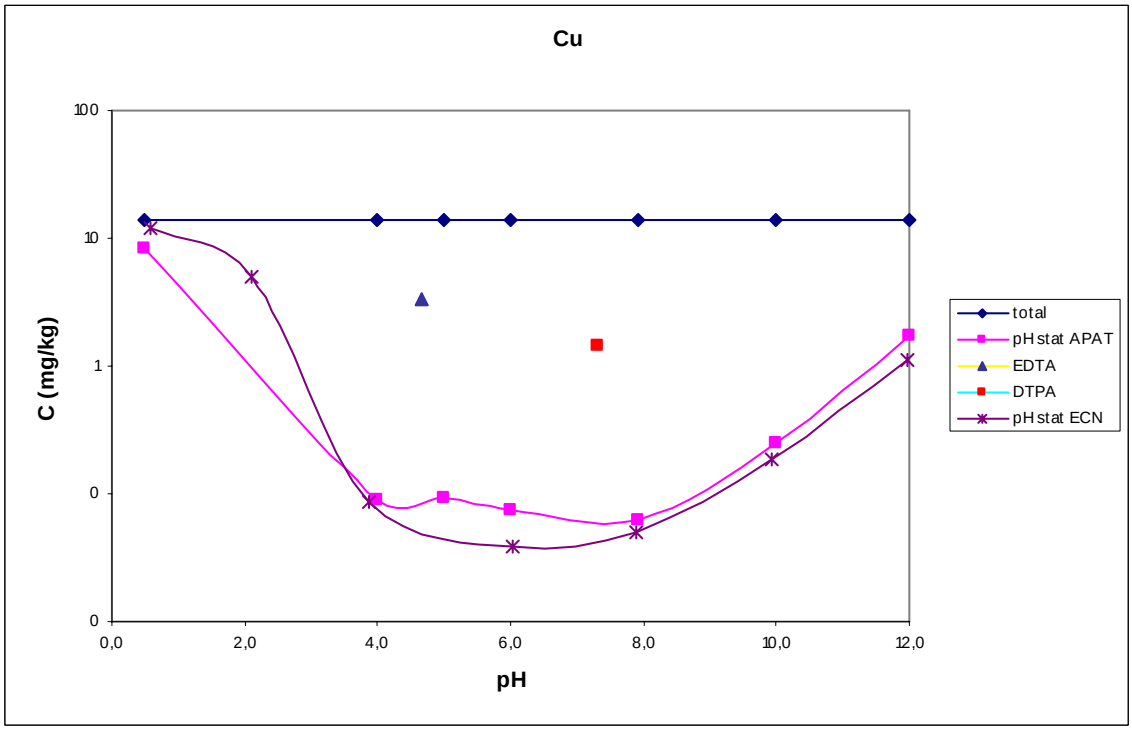
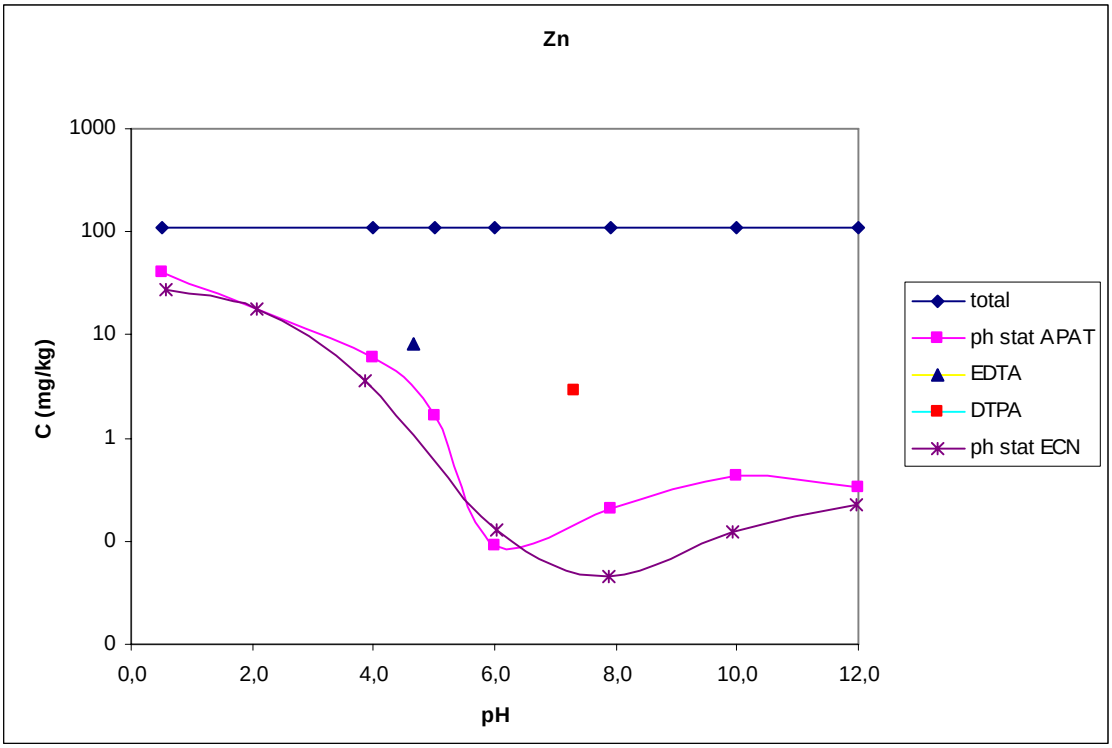


Figure 15-16 Leached concentrations (pH-stat) of Ni and Cu from Millesimo, as a function of pH measured by APAT and ECN. The figures report also the metal total concentrations and EDTA and DTPA extractable metals.



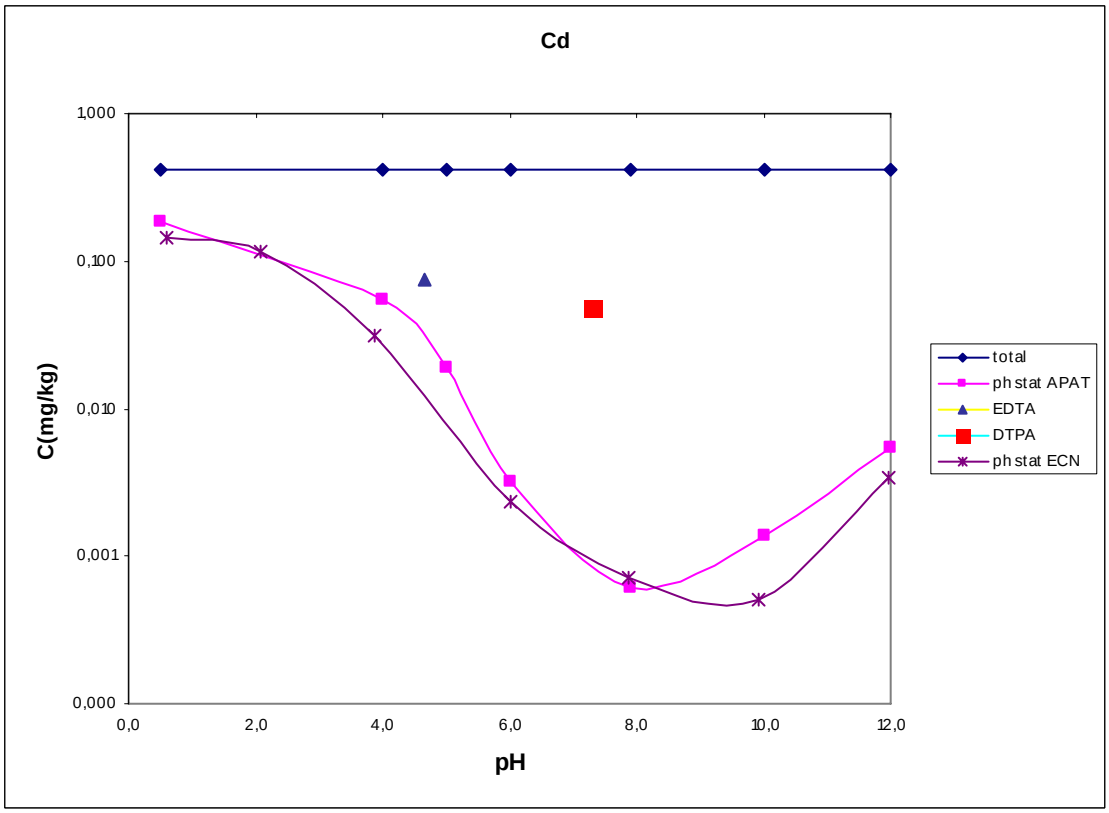
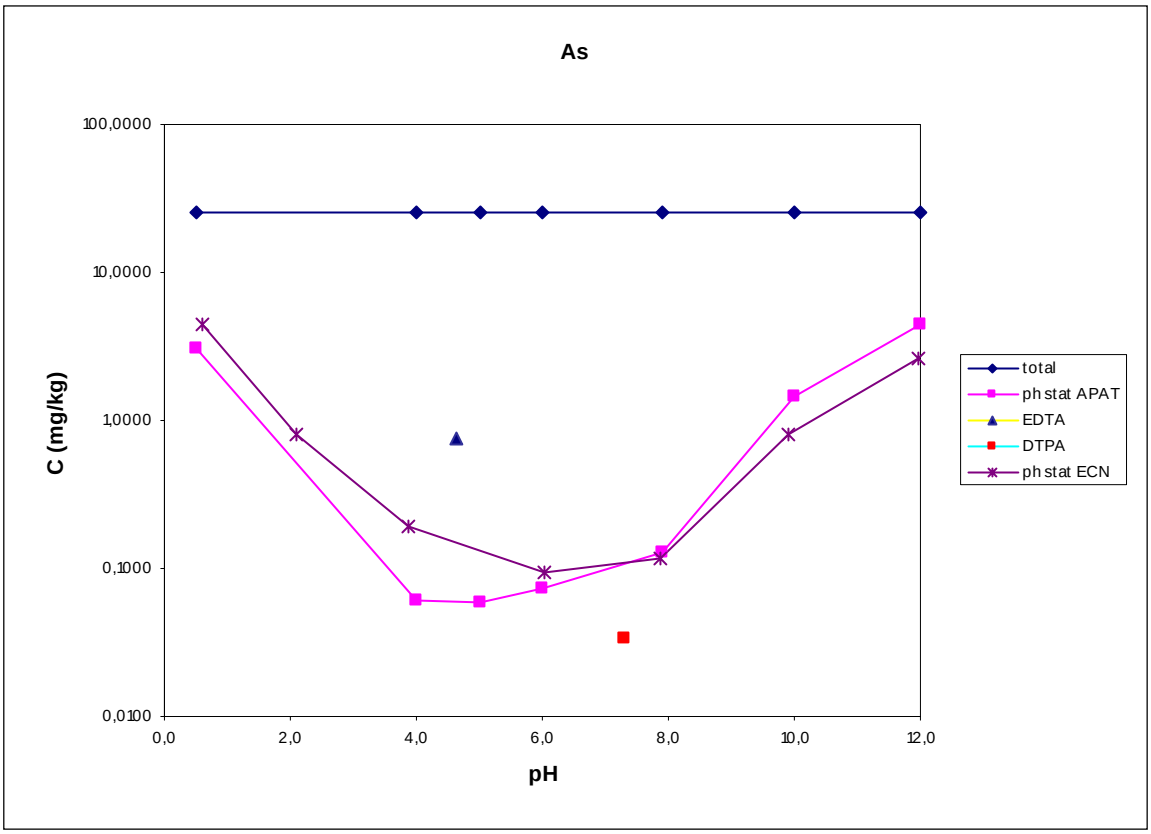


Figure 17-18. Leached concentrations (pH-stat) of Zn and Cd from Millesimo, as a function of pH measured by APAT and ECN. The figures report also the metal total concentrations and EDTA and DTPA extractable metals.



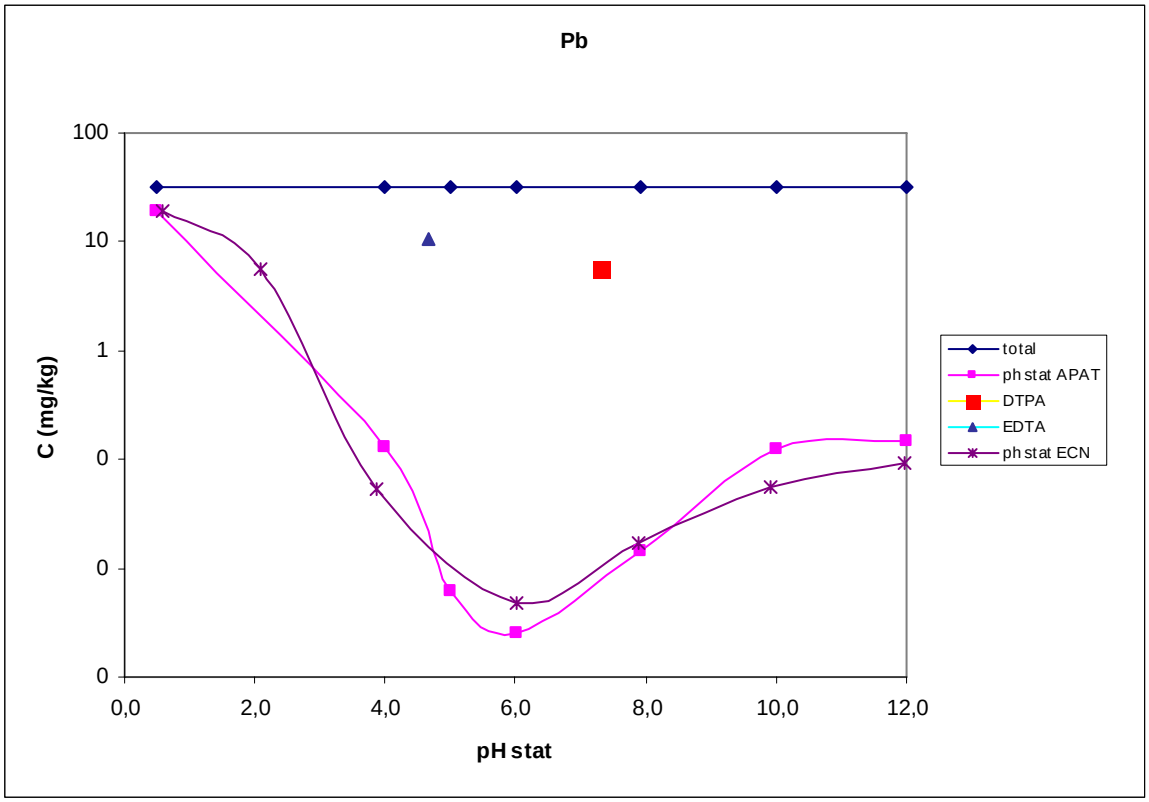
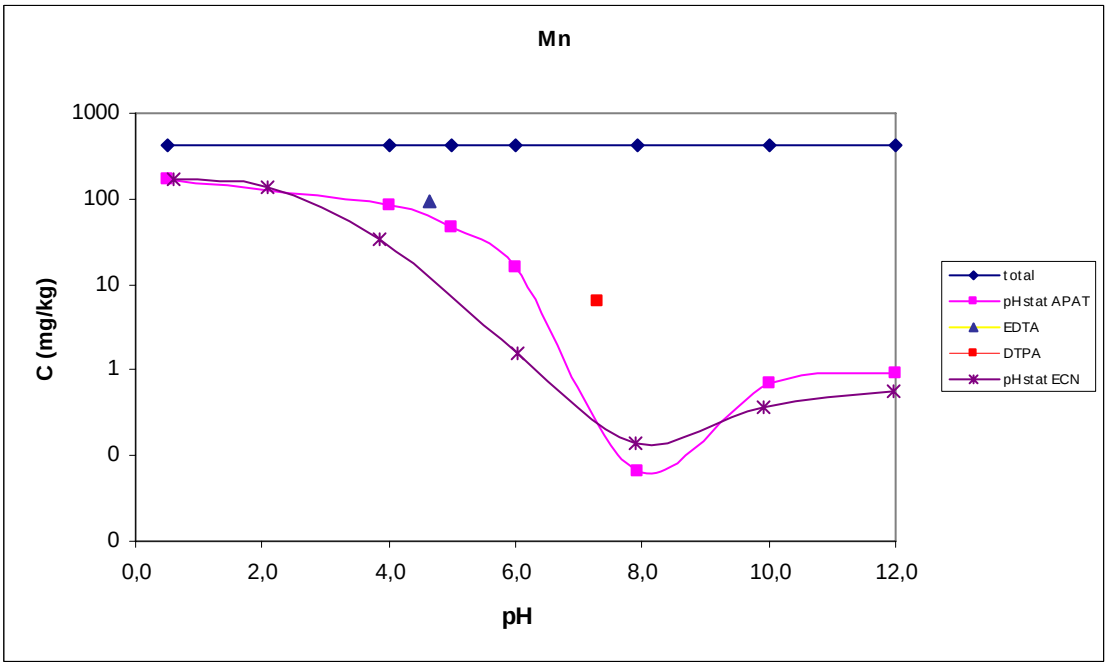


Figure 19-20. Leached concentrations (pH-stat) of As and Pb from Millesimo, as a function of pH measured by APAT and ECN. The figures report also the metal total concentrations and EDTA and DTPA extractable metals.



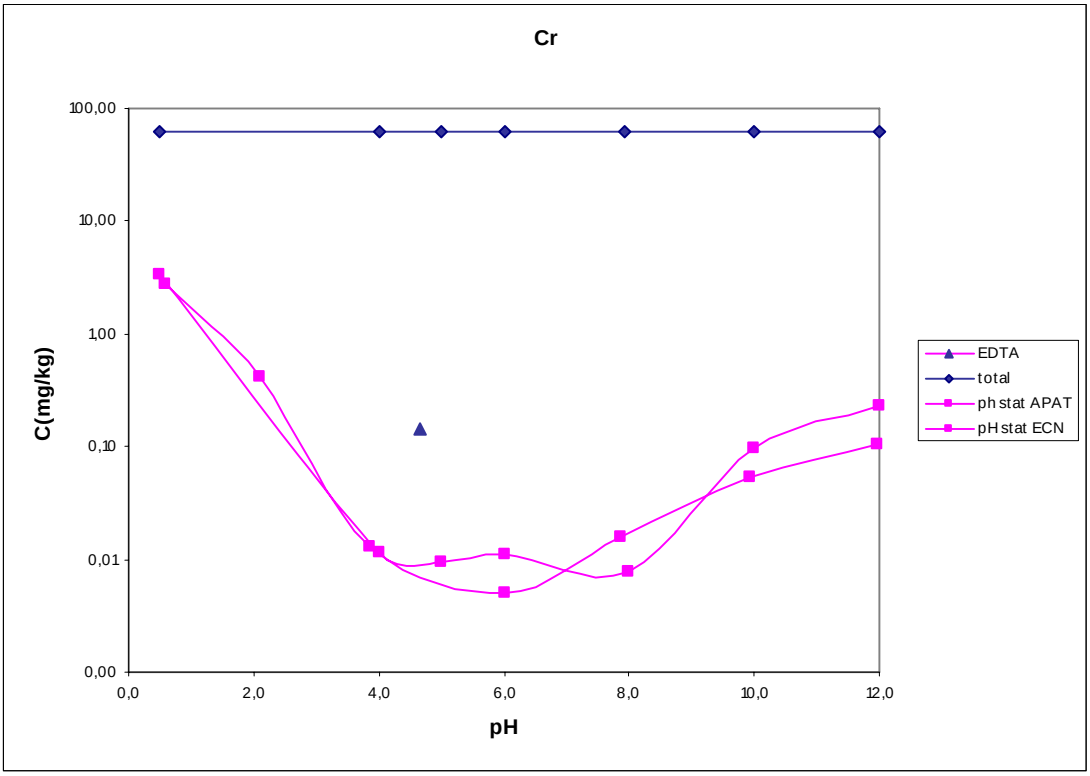


Figure 21-22. Leached concentrations (pH-stat) of Mn and Cr from Millesimo, as a function of pH measured by APAT and ECN. The figures report also the metal total concentrations and EDTA and DTPA extractable metals.

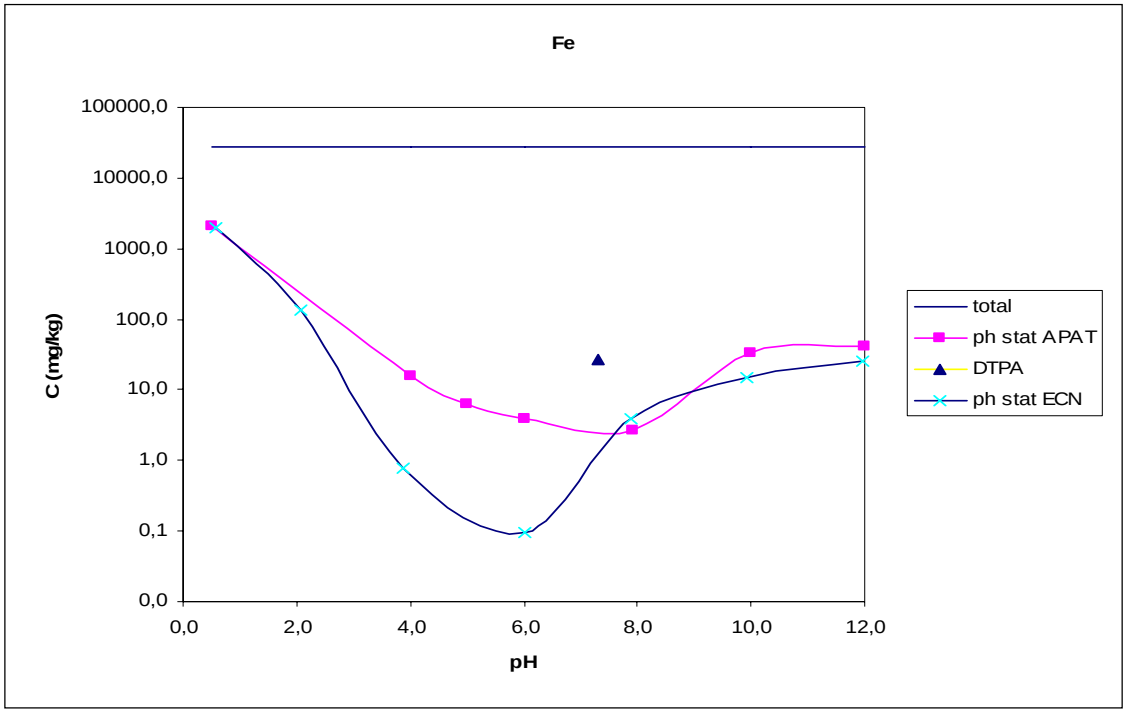


Figure 23. Leached concentrations (pH-stat) of Fe from Millesimo, as a function of pH measured by APAT and ECN. The figures report also the metal total concentrations and EDTA and DTPA extractable metals.

Appendix 3

Sample	Leaching test	pH	As		Se		Cd		Pb		Zn		Cu		Ni		Co		Fe		Mn		Cr	
			A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10	A2	A2-10
Scarlino	2 step -Batch	-	7.2	6.2	0.24	0.23	0.99	0.98	0.21	0.57	216	209	48.8	47.5	1.09	1.10	12.4	12.2	10314	10151	153	151	1.24	1.22
Scarlino	pH stat	pH = 2.4		0.58		0.27		0.88		1.23		192		47.3		0.86		10.0		8363		157		0.95

Table 14. Comparison of concentrations at pH 2 derived from pH-stat on Scarlino to those obtained for cumulative release at L/S = 10 in 2-step CEN leaching test.

	As	Se	Cd	Pb	Zn	Cu	Ni	Co	Fe	Mn	Cr
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
Scarlino	0.9	1.0	1.0	2.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 15. Ratio of the cumulative release at L/S = 2-10 to that at the release at L/S =2. Q = A_{10}/A_2 where A_{10} = cumulative release at L/S = 2-10 and A_2 = release at L/S = 2.

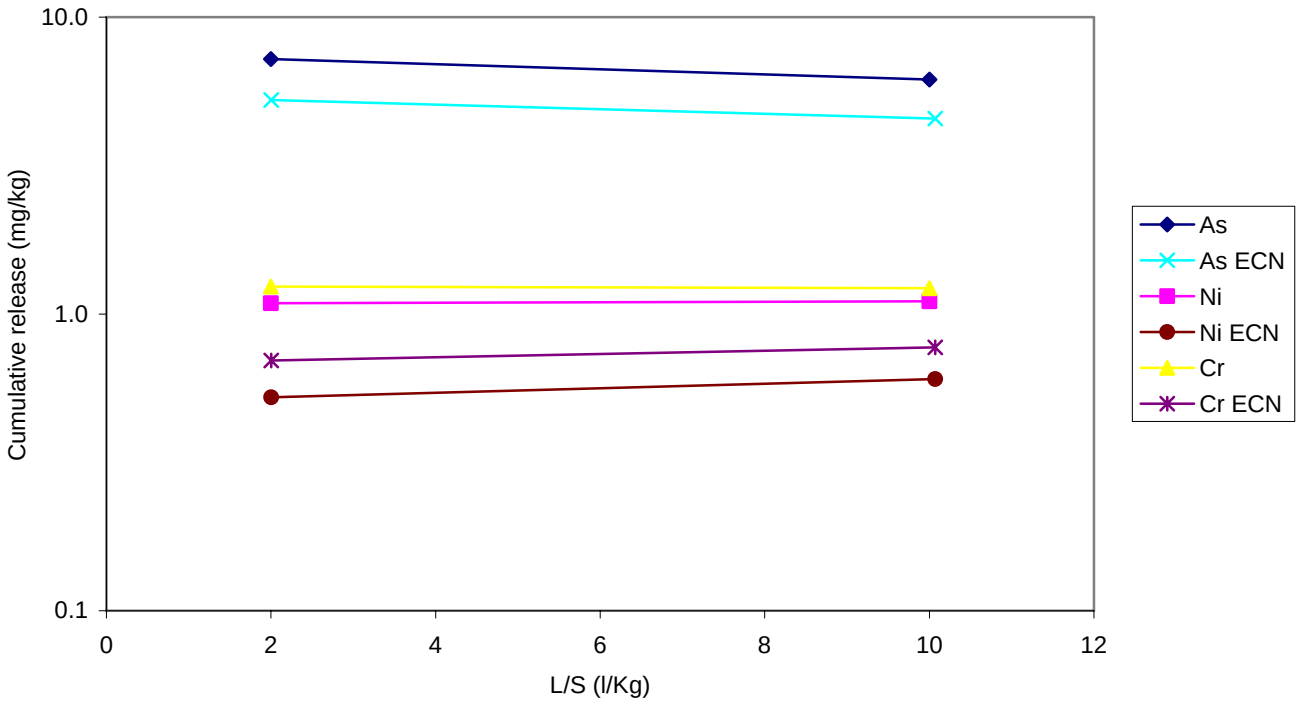


Figure 24. As, Ni and Cr concentrations as measured in the 2-step CEN compliance test performed on Scarlino soil sample by APAT and ECN laboratories.

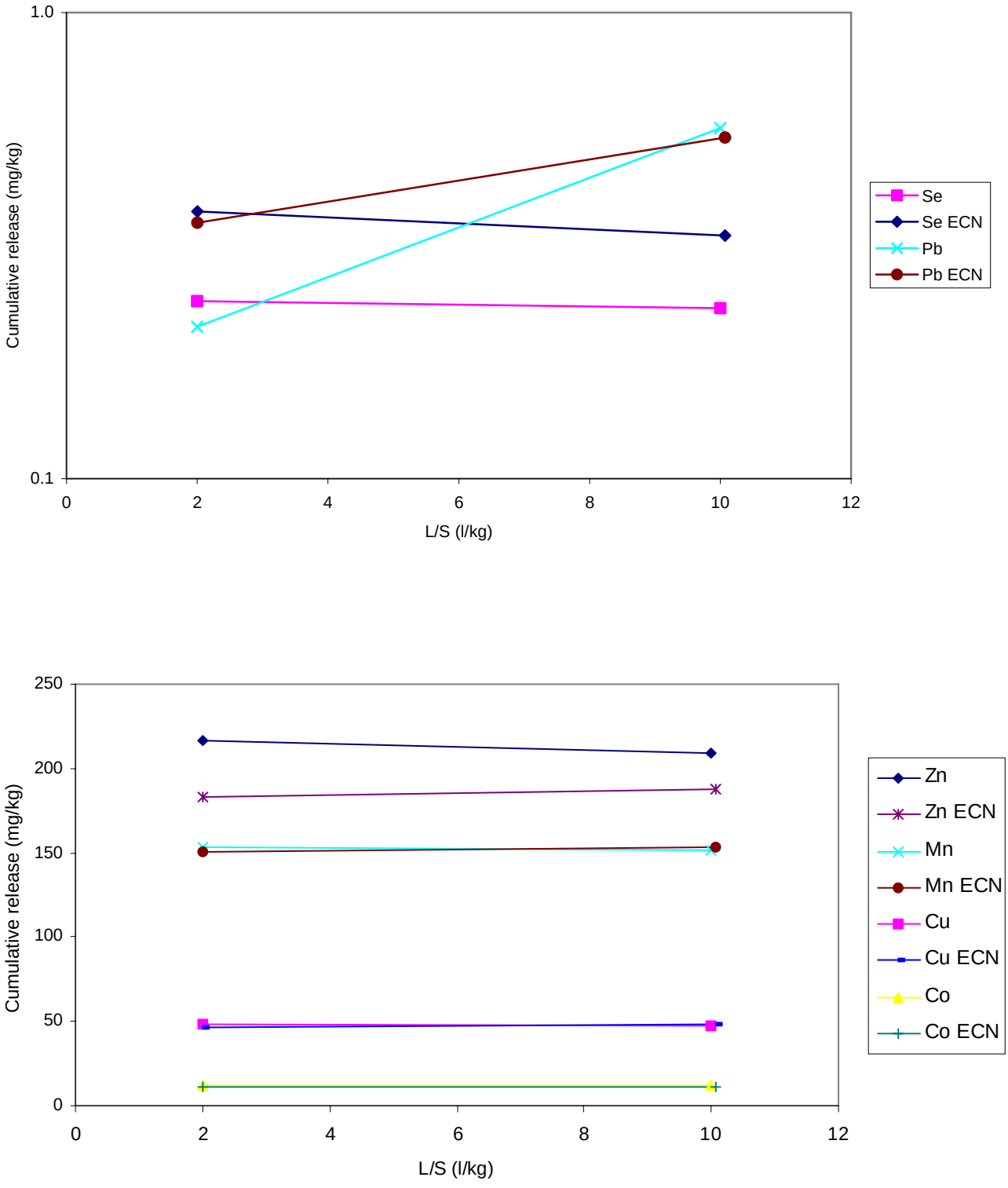


Figure 25-26. Se, Pb, Zn, Mn, Co and Cu concentrations as measured in the 2-step CEN compliance test performed on Scarlino soil sample by APAT and ECN laboratories.

SECTION 6. Diffusive Gradients in Thin Films Technique - DGT

The DGT technique is a recently developed technique capable of in situ measurement of labile species in water [5, 8, 9]. It was developed in Lancaster by Bill Davison and Hao Zhang in 1994. Up today, more than 70 publications and 10 PhD theses have been made on this technique.

DGT plastic devices accumulate dissolved substance in a controlled way, standing on diffusive gradients in thin films, and could be used for measuring trace metals, phosphate, sulphide and radionuclides.

In 1994, the DGT technique was firstly developed to be applied in natural waters and in 1995 its use was extended to soils and sediments [10, 11]. The interpretation of DGT measurements in soils and sediment is based on the theoretical background of DGT application in waters, whereas it is not as straightforward in soils as in solution. In fact, the well mixed conditions that exist in solution can not be achieved in soils and sediment pore waters. The next sections firstly illustrate the DGT theory in waters and then the additional implications necessary to understand the DGT behaviour on soils.

In the present work, the DGT technique was applied for measuring trace metals in four soil samples and for the first time on a compost sample.

DGT application in waters

Theory

The DGT technique is based on a simple device which accumulates solutes on a binding agent after passage through a well defined diffusion layer (Figure 27). A binding agent such as a resin, selective to the target ions in solution (e.g., Chelex 100 resin for trace metals, see Appendix 4), is immobilised in a thin layer of hydrogel (binding-gel). It is separated from the solution by an ion permeable hydrogel layer (diffusive gel) of thickness □g [1]. The outer surface of the diffusive gel is covered by a 0.45 □m membrane filter in order to protect from adhering particles. The gel

and resin are encased in a moulded plastic assembly which is robust and small (Figure 28-29). The solution must be well stirred.

Between the diffusive gel and the bulk solution there is a diffusive boundary layer (DBL), of thickness □. where transport of ions is solely by diffusion. Ions diffuse across the DBL and then through the diffusive gel and filter and are concentrated on the resin. Small ions can diffuse freely through the effectively 2-5 nm pores of gel with effective diffusion coefficients, D, indistinguishable from those in water. The gel matrix excludes very large molecules, such as colloidal species or complex bounded metals. Within a few minutes of deployment, a steady state linear concentration gradient is established between the solution and the binding-gel.

The flux, J, of an ion through the gel is given by Fick's first law of diffusion (Equation 1), where dC/dx is the concentration gradient.

$$J = -D \frac{dC}{dx} \ (\text{mol cm}^{-2}\,\text{s}^{-1}) \qquad (1)$$

If diffusion coefficients of ions in the diffusive gel are the same as in water, the flux is given by Equation 2, where C is the bulk concentration of an ion and C' is the concentration at the boundary between the binding-gel and the diffusive gel.

$$J = D\frac{(C - C')}{\Delta g + \delta} \qquad (2)$$

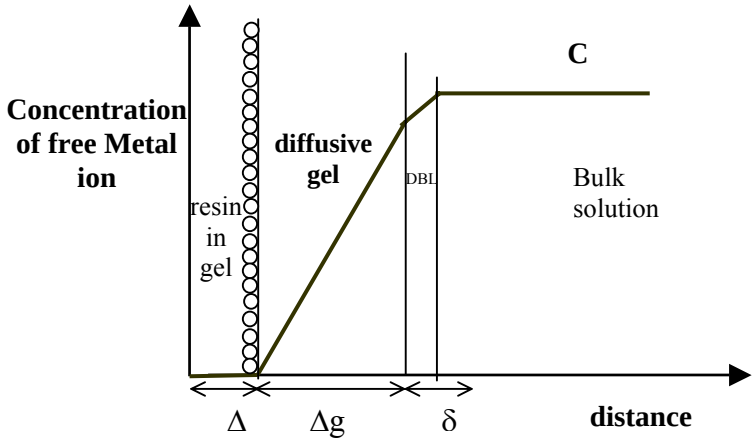


Figure 27. Representation of the steady state concentration gradient of a solute through a DGT assembly deployed in a well stirred solution with solute concentration C.

If the free metal ions are in rapid equilibrium with the resin, with a large stability constant, the concentration C' is effectively zero, providing the binding agent is not saturated. In well stirred solutions the boundary layer thickness, □, is negligible compared to the thickness of the diffusive layer, □$_g$ of ~1 mm. Equation (2) then simplifies to Equation (3).

$$J = D \frac{C}{\Delta g} \tag{3}$$

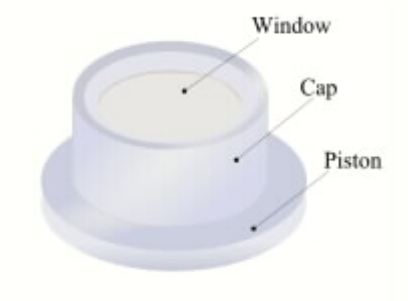
In practice, the DGT device is deployed for a fixed time, t. On retrieval, the binding-gel layer is peeled off and the mass M of the accumulated ions in this layer is measured. M can be used to calculate the flux through the known area of the exposed diffusive layer, A (Equation 4).

$$J = \frac{M}{A \cdot t} \tag{4}$$

Equating (3) and (4) and rearranging gives Equation (5), which demonstrates that the concentration in the bulk solution can be calculated from the known values of □g, D and A, the measured deployment time, t, and accumulated mass, M.

$$C = \frac{M \cdot \Delta g}{D \cdot A \cdot t} \qquad (\text{mol cm}^{-3}) \tag{5}$$

This feature of DGT whereby concentration is calculated from the measured mass and deployment time make it ideal for *in situ* use. The relationship of external concentration to measured mass is determined by the values of □g and A which are simple, fixed geometric quantities and the diffusion coefficient in the gel which can be measured for each temperature.



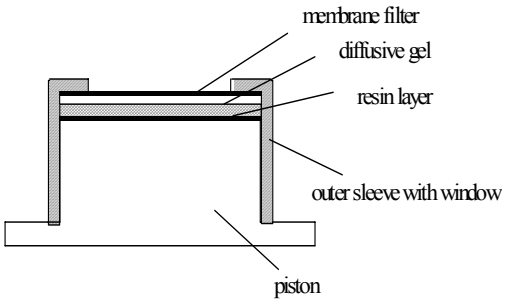


Figure 28. DGT unit

Figure 29. Cross section of DGT

Clearly, as values of diffusion coefficients change with temperature, the DGT response is temperature dependent. If the structure, chemical reactivity and dimensions of the gel do not vary with temperature, the DGT temperature response should solely depend on the diffusion coefficient. This has been demonstrated to be the case [15].

DGT assemblies equipped with Chelex resin as binding agent can be used to routinely measure Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn.

DGT units can be assembled in laboratory, following standard procedures. They can be also purchased from the University of Lancaster (DGT Research Ltd.) or, as in our case, from the ExposmeterAB, Sweeden. In the present work, the thickness of the diffusive layer, □g, is 0.094 cm and the area of the exposed diffusive layer, A, is 3.14 cm^2.

DGT validation

The relationship between metal trapped on DGT and the metal concentration in the surrounding medium have been fully verified in the laboratory as well documented in literature [8]. Zhang and co-workers found that when gel assemblies with a single diffusive gel layer thickness were deployed in stirred $CdCl_2$ solutions for various times, their measured mass increased linearly with time (Figure 30-a). Similarly when different diffusive layer thickness were used and deployment time was fixed, the measured mass was inversely proportional to gel layer thickness (Figure 30-b). In both cases the experimental data agreed with the theoretical response calculated from the known concentration in solution, C, using Equation (5).

In addition, the concentrations of metals measured by DGT in the field agreed with those obtained by other techniques, as the anodic stripping voltammetry [5].

Effect of solution composition and pH

DGT technique can be used in almost all aqueous environments including industrial discharges, lakes, estuaries and sea. Actually, DGT response has been shown to be independent of ionic strength from 1 mM to 1M, which embraces the salt content of all normal natural waters. There are some data that suggest that there are problems when making measurements on very dilute solutions with an ionic strenght less than 0.2 mmol l^{-1} [20, 59]. These are exceptionally diluted freshwaters and for most freshwaters there are no problems [21, 22].

The pH range that can be used depends on the measured metals. Figure 31 shows the pH operational range of Chelex resin for some trace metals (Cd, Cu, Zn,

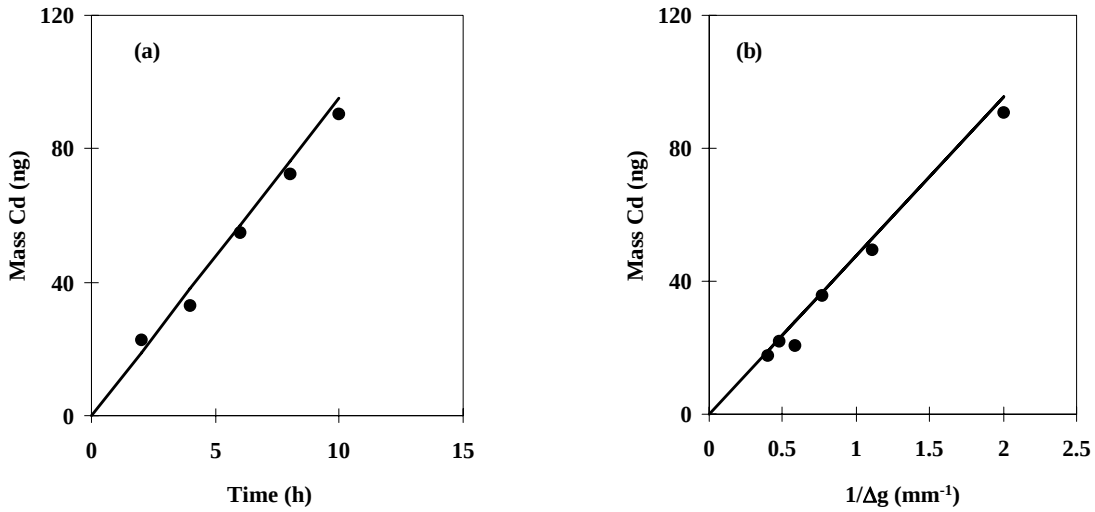


Figure 30. Measured mass of Cd in the resin layer for DGT assemblies as reported in literature [2]: a) immersed in a stirred solution (1 µg/L Cd) for different time periods; b) with different gel layer thickness exposed to a stirred solution (1 µg/L) for 10 hours. The solid lines are the theoretical responses predicted by equation 5.

Co, and Mn). The figure reports the ratio of metals concentration in solution determined by direct analysis of ICP-MS (C_{soln}) and the concentration measured by DGT (C_{DGT}) versus pH.

The lower pH limit is determined by the competition between metals and hydrogen ions for the binding agent [22]. The different behaviour of DGT with respect to the different trace metals depends on the resin-trace metals bond strength, which follows the order Cu > Pb >>> Ni > Zn > Cd. Cu binds very strongly to the resin and can be measured accurately down to pH 2, while Cd is bound relatively weakly and its determination is possible down to 5. For other metals (as Co, Mn, Zn), DGT measurements are usually accurate down to pH 3.5. For most metals, the upper limit of pH range is about 11, above which there are gel stability problems.

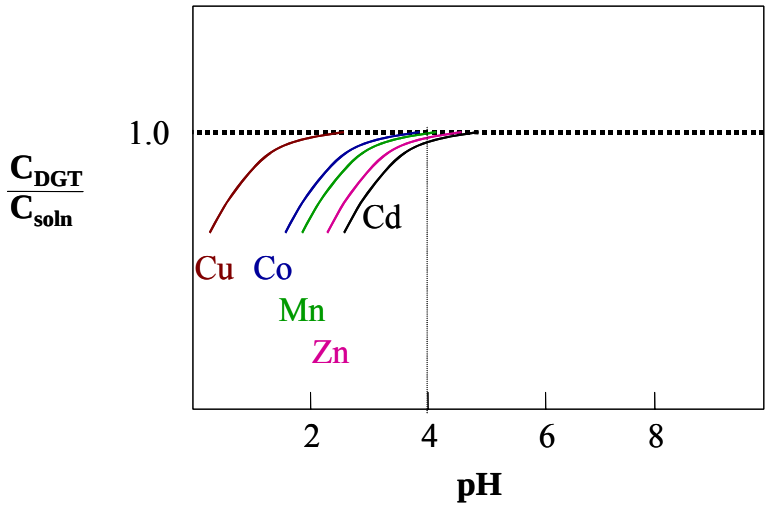


Figure 31. pH operational range of Chelex resin for Cd, Cu, Zn, Co, and Mn. Note: C_{soln} = Concentration of metals in solution determined by direct analysis by ICP-MS; C_{DGT} = Concentration measured by DGT.

However, it has to be noted that there is likely to be little metals in solution at pH values between 8 and 11 because of adsorption and solubility evidences.

Deployment time

Deployment times can vary from as little as 1h to several months. The capacity of DGT is limited by the capacity of the resin layer. It is controlled by the capacity of the Chelex-100 resin and the amount of Chelex-100 used in the resin layer. In practice, the quantity of Chelex-100 is limited to the quantity necessary to form a monolayer adjacent to the diffusive gel layer. The capacity of DGT device

can be measured by immersing gel assemblies for fixed time at various concentrations of metals. It can be demonstrated that the mass of metals in the resin layer increases linearly with increasing the metal concentration in the bulk solution reaching a *plateau value*, when saturation is reached.

The capacity of the resin should allow 3 months immersion in relatively contaminated coastal waters before saturation is reached so that the DGT technique has the potential to be used for long term (weeks or months) deployment to obtain an integrated record of trace metals concentrations [5].

Deployment of DGT in situ and in laboratory

It is possible to use DGT in different ways, according to the different needs. For example, a little hole placed on DGT plastic assembly enable to attach the unit to weighted fishing lines tied to concrete base or buoys, directly in situ.

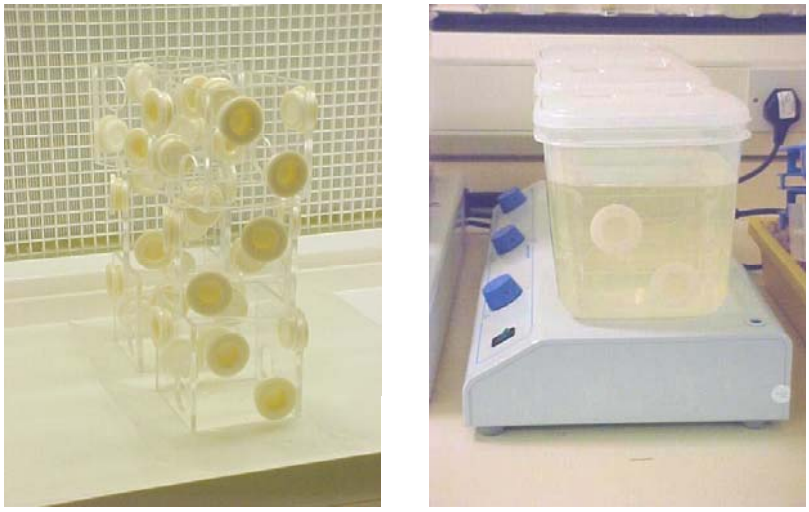


Figure 32. Device to fix the DGT unit in the container in laboratory.

For a laboratory use, the plane of the filter should be vertical, parallel to the container walls and facing towards the centre of the container. To this end, it is necessary to design a device to fix the unit in the container, as illustrated in Figure 32. On recovery, the DGT assemblies are rinsed well with MQ water and immediately placed in clean, plastic, zip-lock bags. As soon as possible, the caps of the DGT assemblies are carefully prised off and the resin layers are retrieved using

plastic tweezers and processed for analysis. The accumulated mass can be measured directly in the resin layer by drying it and using a beam technique like laser ablation ICP-MS. More commonly, ions in the binding-layer are eluted with a known volume V_e of 1 or 2 M HNO_3 (usually 1 ml) and the concentrations of ions in the eluent, C_e are measured by any suitable analytical technique, such as ICP-MS or AAS, after appropriate dilution. The elution in batch enable to retrieve only a fraction of the bound ions; the ratio of the eluted to bound metal is known as the elution factor, f_e. Values of f_e of 0.8 have been reported for most metals when using 1 or 2 M HNO_3 to elute them from Chelex resin [8].

Taking the elution factor *fe* into account, the accumulated mass (moles) can be calculated from equation (6) where V_g is the volume of the binding gel (typically 0.16 ml).

$$M = C_e (V_g + V_e) / f_e \qquad (6)$$

The accumulated mass M is used in equation (5) to give out the concentration C of metals in solution.

Metals speciation

The pore size of the diffusive gel of standard DGT assemblies (open pore size) permits free metal ions and inorganic and small organic metal complexes to diffuse through the resin. It excludes particles and large colloids which will not be measured. DGT assemblies using restricted gel (restricted pore size) for measuring labile inorganics only are also commercially available and enable further discrimination of species. DGT distinguishes between species not only by size (whether they can pass through the diffusive gel layer) but also kinetically (according to their lability). For metal complexes to be measured they must dissociate during their transport through the diffusive layer. Therefore, only labile complexes that can dissociate on time scale less than minutes are measured. This includes metal-fulvic complexes, but excludes complexes with very strongly binding ligands, such as EDTA.

Comparisons among measurements of metals by DGT and total dissolved metal concentrations have been made in rivers [21]. In all cases, DGT measured metal concentrations were reported to be smaller or equal to the total dissolved metal concentrations. Generally, ratio of DGT metal/dissolved metals lower than 1 is

consistent with strong complexation by humic substances or the presence of colloidal material. Actually, metal complexes by lower molecular weight humic substances can pass through the resin gel, but strong complexes and large colloidal species can not.

DGT application in soils

The principles of using DGT in soils are quite different from those in solution. Indeed the well-mixed conditions that exist in solution enable the interpretation of DGT measurements as concentrations. Pore waters of soils are not well mixed and as a result the concentrations of metal ions in the soil adjacent to the DGT device are lowered (Figure 33). This can induce supply of metal ions from the solid phase to solution in the layers of soils near to the device [3, 12, 13]. If there is no mechanism of supply of solutes other than diffusion, the zone of depletion adjacent to the device becomes progressively larger with time. The flux to the DGT device, which is driven by the concentration gradients through the gel layer, progressively decreases with time. It has been shown [60] that in absence of any resupply of solutes to the pore water, after 24 hours deployment, the interfacial pore water concentration between the soils and DGT device (C_a) is about 0.06 C, where C is the labile pore water concentration in the bulk solution ($C_a \approx 0.06C$). In practice C_a is usually much greater than this [12], implying that significant resupply of solutes is occurring. The local depletion in pore water concentrations induces remobilisation of solutes from the soil solid phase.

DGT measures directly the mean flux of labile species to the device during the deployment. This can be interpreted directly as the mean concentration of labile metal at the interface between the device surface and the soil. For the situation where supply from soil particles to solution is rapid, this interfacial concentration is the same as the concentration of metal in bulk pore water.

The concentration calculated from the measured DGT mass, C_{DGT}, will be less or equal to the concentration of labile species in the pore water C. Where independent measurements of C are available, DGT measurements can be interpreted in terms of ratio R.

$$R = \frac{C_{DGT}}{C} \qquad 0 < R < 1 \qquad (7)$$

R may be obtained experimentally and used to characterise the deployment as one of the three cases described below.

(a) Fully sustained case (R>0.95). Ions removed from the solution by the DGT device are rapidly resupplied from soil solid phase; the concentration in solution is buffered to a constant value. The DGT measured concentration can be interpreted as the concentration of labile metal species in the pore waters.

(b) Unsustained case ($R = R_{diff}$). There is no resupply of solutes to the pore water. The DGT device is supplied only by the diffusion of solutes through the pore waters, which becomes progressively depleted. The exact value of R_{diff} depends on the solute diffusion coefficient in the soil, the design of DGT device and the deployment time. Harper et al [60] estimated that for a typical sampler design in high porosity soil, $R_{diff} = 0.1$ (*i.e.* $C_{DGT} = 0.1$ C) for a 24 hours deployment.

(c) Partially sustained case ($R_{diff} < R < 1$). There is some resupply of ions from soil to solution but is insufficient to sustain the initial bulk concentration and to satisfy fully the DGT demands.

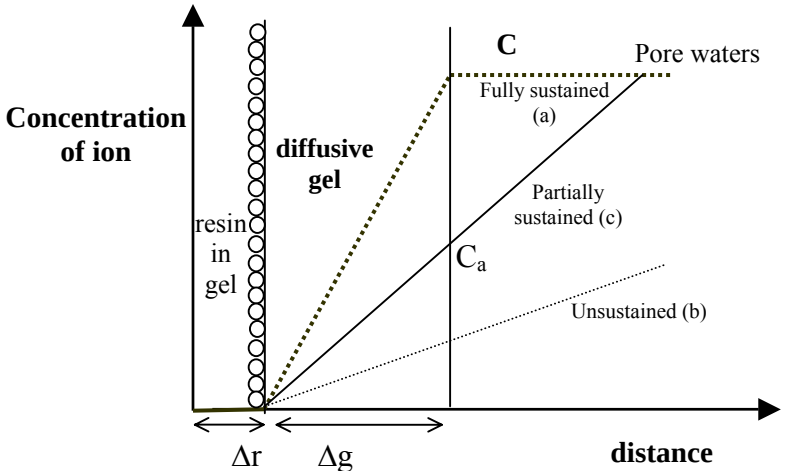


Figure 33. Schematic representation of the concentration gradient in a DGT assembly in contact with pore waters were the concentration is fully sustained (case a), partially sustained (case c) or unsustained (case b) by resupply from the solid phase in soil.

The precise value of R is a quantitative measure of the ability of the solid phase to resupply the pore water in response to the depletion induced by the DGT sink. To further interpret DGT measurements, the concentration directly measured by

DGT (C_{DGT}) can be converted to an effective concentration (C_E) introduced by Zhang [10] using equation 8:

$$C_E = C_{DGT} / R_{diff} \quad (8)$$

C_E represents the concentration of metal that is effectively available from both soil solution and solid phase labile pool. R_{diff} is the ratio of the theoretical metal concentration at the DGT surface for the diffusional-only case (i.e. no resupply from the solid phase) to the concentration in soil solution. In other words, it expresses the extent of concentration depletion at the interface of the device and the soil for the diffusion-only case. R_{diff} can be calculated using a dynamic, numerical model known as DIFS (DGT induced fluxes in soils) [60, 61]. DIFS is freely available for general use on the web (http://www.es.lancs.ac.uk/wdgroup/aquach.htm). DIFS is a software tool for investigating and interpreting trace metal measurements made by DGT. DIFS implements a numerical model of trace metal reaction and transport in soils and sediment which takes into account the factors affecting the response of DGT devices. It can be used either to simulate DGT deployments, or estimate trace metal resupply parameters. DIFS considers at any location and point in time, diffusion of metal ions in the soil solution in response to induced concentration gradients.

Simulations using DIFS for a typical soil have shown how concentration gradients of a metal through the DGT diffusive layer and the soil solution change with time [62]. If there is no resupply of metal from the solid phase the metal concentration at the DGT surface declines appreciably in 24 hours. When there is resupply from the solid phase the concentration does not decline so much. There is effective buffering by the solid phase.

The exchange of metal ions between solid phase and solution is assumed to be a 1st order reversible process between a dissolved phase (pore water concentration, C_d) and a solid phase (sorbed concentration, C_s), described by Equation 9 where k_1 and k_{-1} are the rate constants for sorption and desorption respectively.

$$DGT \leftarrow C_d \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} C_s \quad (9)$$

Higher values of C_s or k_{-1} provide a better buffer.

Simulations using DIFS for a typical soil show how concentration gradients of a metal through the DGT diffusive layer and the soil solution change with time.

R_{diff} can be calculated using DIFS dynamic numerical model of the DGT-soil system. Input parameters of the particle concentration (Pc), soil porosity, (ϕ) and the diffusion coefficient of the metal in the soil (Ds) were calculated using equations (Harper, 1998):

$$P_c = m/V \quad (10)$$

$$\phi = d_p/(P_c+d_p) \quad (11)$$

$$D_s = D_o/(1-2\ln\phi) \quad (12)$$

Where m is the total mass of all soil particles; V is the pore water volume in a given volume of total soil; D_o is the diffusion coefficient in water; and d_p is the density of the soil particles, which is commonly assumed to be 2.65 g cm^{-3} in soils [61]. Note that the effective diffusion coefficient in soil is lower due to tortuosity.

DGT mimics of plant uptake

Risks associated with heavy metal contamination in soils are difficult to assess. Researchers have well recognized that the total metal content of soil embraces large fractions which are unavailable to plants, microorganisms or soil fauna. Extraction of soil solution from soil isolates the aqueous phase to which plant roots and microorganisms are exposed, and there is some evidence that measurements of free ion activities in soil solution provide a better indication of metal availability [10]. However, measurements of metals in soil solution fail to account for the ability of the soil to sustain the solution concentration following depletion by uptake.

A proportion of metal will only be available from the solid phase if plant uptake is fairly rapid compared to diffusional supply [3]. Then depletion of its concentration in solution in the immediate vicinity of the roots allows transfer from solid phase to solution. For the component associated with the solid phase to contribute to plant uptake, it must be capable of rapid transfer to solution. This solid phase metal is then said to be kinetically labile. Some plants may actively mobilize nutrients, such as Fe, Cu and Zn, from the solid phase under deficiency condition. Under non-deficiency or excess conditions, however, it is soil rather than plant properties that determine what fraction of the metal responds to local depletion.

Characterization of these properties may provide an assessment of the potential hazard of a soil contaminated with heavy metals [3].

Like plants, DGT locally lowers metal concentrations in the soil solution and responds to metal re-supplied from labile species in solution and the labile metal pool in the solid phase.

Measurements of Cu as its DGT concentration, its soil solution concentration, by EDTA extraction and as free Cu^{2+} in soil solution were made on 29 different soils covering a large range of copper concentrations [3]. They were compared to Cu concentrations in the plant material of *Lepidium heterophyllum* grown on the same soils. Plant concentrations were linearly related and highly correlated with the concentration measured by DGT, but were more scattered and non-linear with respect to Cu^{2+}, EDTA extraction or soil solution concentrations. These results demonstrated that the dominant supply processes in these soils were diffusion and labile metal release, which the DGT-soil system mimics. DGT offers the possibility of a simple test procedure for soils. The kinetic perturbation of solute concentrations in the soil system is most likely similar to that occurring during plant uptake [3].

Experimental section

1.DGT measurement of metals resupply from the soils

DGT was applied on soils and compost to study the DGT applicability and capability of measuring flux of available metals from solid phase to solution.

Material and methods

Sample pre-treatment

DGT was applied to the following samples: Scarlino 1, ERSA, Saliceto and Millesimo and compost, briefly described in section 1. The soils were sieved at 2 mm prior to DGT deployment. The compost sample was sieved at 1 mm, trying to remove the large part of small wood residues. For each sample the Maximum Water

Holding Capacity (MWHC) [64] was determined prior DGT deployment (the results are reported in Appendix 6). Since DGT response depends on pH, the natural pH of the samples was determined in soil/water suspension, as reported in Appendix 6. The samples were well mixed to ensure homogeneity, placed in a plastic container and appropriate amounts of MilliQ (MQ) water were added to obtain 30% of the MWHC. The samples were then equilibrated for one week at room temperature. The water content of the samples was then raised to 80 % of the MWHC. The samples slurry were mixed using a glass rod and kept at room temperature for 24 h prior DGT deployment.

DGT deployment and retrieval

The shelf life of DGT units stated by the manufacturer is six months. After this period the DGT units have to be revived following a standard procedure reported in literature by Gimpel [22]. The DGT used in this work have been stored refrigerated for two years; before use, they were revived following the procedure reported in details in Appendix 5.

In addition, before using DGT units, it is necessary to test their performance in a solution of known concentration of metal (results in Appendix 5). The results in Appendix 5 show that even after such a long period of storage of 2 years, the DGT units work well, providing accurate results in test solutions of known concentration.

The DGT devices were pushed gently into the wetted samples, taking care that no air pockets remained between the device and the sample and that the sample did not dry out during the deployment (Figure 34). The containers were loosely covered with a lid. The plastic DGT devices were deployed for a known time at known temperature (Appendix 8) and then the mass of metal on the resin layer measured after elution with 1 ml of HNO_3 1M by ICP-MS (7500c, Agilent Technologies). The measured metals in DGT were Cd, Co, Cu, Cr, Ni, Zn. Lead was not measured due to its high concentration in the blanks, obtained by measuring the DGT resin gel after reviving and acid elution.

Time of deployment optimization

The deployment time for direct flux and concentration measurements is usually 1 day. If deployment is significantly extended beyond 1 day, there are risks associated with exhausting the available pool of solute (which reduces the flux to the DGT device), of saturating the resin if the concentration of metal is high, and of changing the redox conditions due to saturation. If less than a day, the DGT device may not attain a time invariant response, although if resupply from the solid phase is rapid a pseudo-steady state can be attained within 1 hour.

The sample Scarlino (n°1) is characterized by a high metal content as resulted from the Chelex resin saturation achieved after a 24 h deployment. In this case, a two hours deployment time was used to avoid saturation of the resin gel in the DGT devices [13]. Soils samples Saliceto and Millesimo have a relatively low content of metals and the deployment time was therefore raised from 24 to 27 hours. For the sample ERSA and for the compost one, the standard time of 1 day was used. The time of deployment for each sample are reported in Appendix 8.

Pore water

Soils and compost solutions were extracted from soil samples by centrifugation at 3000 rpm for 10 minutes. The solutions were filtered at 0.45 □m and acidified to about pH 2 using HNO_3 solution.

The solutions were analysed by ICP-MS (7500c, Agilent Technologies) to determine Cd, Co, Cu, Cr, Ni, Zn.

Figure 34. DGT deployment in soils.

Results and discussion

DGT measurements were done in duplicate for each sample. The mean values for trace metal results obtained by DGT and the soil solution concentrations are reported in Appendix 9. R values, calculated for each metal in each sample, are also reported in Appendix 9. In case of sample Scarlino (n°1), reliable DGT measurements could be attained just for Cu due to the low pH of the soil.

The concentration of particulate, labile, trace metal (C_s) available for release and the kinetics of the adsorption and desorption processes determine the efficiency with which trace metal concentrations are sustained in soil solutions relative to their initial levels.

Low R values were generally observed for all samples, meaning that small resupply from soil to solution occurred. A lower DGT response as compared to soil solutions can be explained by either a significant fraction of the metal species in solution being present as non labile complexes or as colloids or kinetic limitation providing only a partial resupply from solid phase to solution.

The compost sample is likely to be rich in organic fraction. This feature may affect as a limiting factor the metal availability from the solid phase. Consequently the proportion of labile metals in the solid phase (C_s) may be low. Therefore, the concentration of metals in the vicinity of the DGT device decreases and the flux to the DGT is less than the maximum possible. The mean concentration measured by DGT is inevitably lower than the initial concentration in the soil solution. However, it has to be noted that the very low R value found for Cu in compost is unrealistic (R = 0.003). Simulations with DIFS provide a R_{diff} for the diffusion-only case of 0.084. This high discrepancy derives from the relatively high uncertainty associated on the different kind of species measured by DGT and in the soil solution. Ideally the solution concentration used in R values should include only the species measured by DGT. Total dissolved concentration in soil solution may include colloidal forms and inert complexes. DGT does not measure trace metals bound on colloids or kinetically inert trace metal complexes in soil solution. This effect is particularly relevant for Cu, which it is well known to be strongly organically complexed. Where possible, the concentration of the solution should be measured by a technique which

discriminates species in similar way as to DGT, such as anodic stripping voltammetry. The effects would tend to result in R values smaller than the true value. In the case of sample collected from Scarlino, the low R value (R = 0.15) is probably more correlated to its low pH (2.9) rather than the organic content. Actually, the organic content is likely to be very low in such a soil deriving from a plant for the arsenopyrite minerals processing. In this case, the low pH could account for the small resupply from soil to solution. Actually, at low pH, a typical increased solubility of metals is generally observed for most metals. This is clearly shown by Figure 35, which represents the curve of leached Cu vs pH, (for further details see the section 5 on leaching). Figure 35 shows a maximum concentration of copper in solution at low pH, which can be taken as the maximum "availability" of the contaminants for release from the solid matrix. In other words, at such a low pH, Cu is almost totally dissolved and the limiting factor of resupply could be attributed to a limited reservoir of available metal (C_s) rather than slow desorption kinetics.

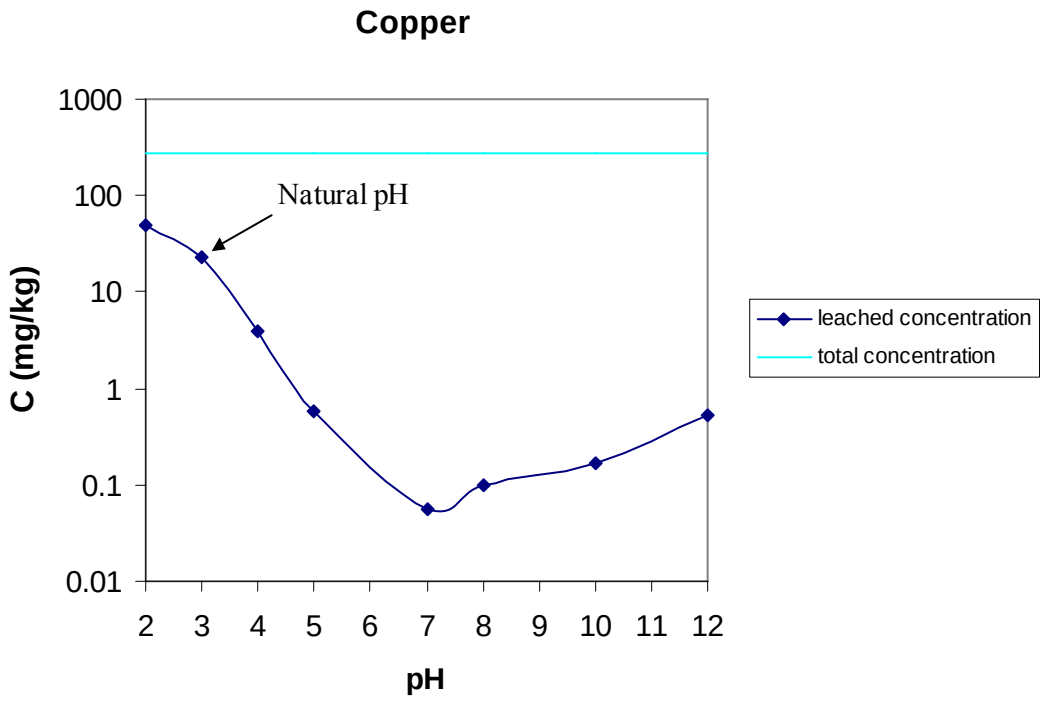


Figure 35. Leaching curve of Cu reporting the leachate concentration vs pH for Scarlino (n° 1). For more details see the section 5 relevant to leaching procedures.

The DIFS output illustrating the diffusive-only case for Cu in Scarlino soil are reported in the following Figures 36-37. Simulations with DIFS provide a R_{diff} for Cu of 0.082. The initial peak in R is due to the initial high flux to the diffusive layer (Fig. 36). The DGT device progressively depletes pore water concentrations, diminishing the flux to the DGT device. The DGT estimated concentration and R decrease, reaching zero at infinite time. The experimental R found for Cu is just a little higher than the theoretical R_{diff} confirming a small resupply and the diffusion of metal through the pore water is the dominating process.

If there is no resupply of metal from the solid phase, the metal concentration at the DGT surface declines appreciably in 24 h (Fig 37). Figure 37 reports the DIFS model distribution of Copper at three different times. Note the change in slope at the soil-DGT interface is consistent with different effective diffusion coefficients in the soil and gel.

Conclusive remarks

The interesting case of resupply from solid phase to solution induced by DGT was generally not observed for the target metals in the matrices under study. In this context, it will be significant to study the effect of variation on soils and compost particle size distribution, attainable by grinding, on the capacity of the solid phase to satisfy the DGT demands. So, further study will be focused on the assessment of the dependence of resupply from solid phase on the particle size distribution.

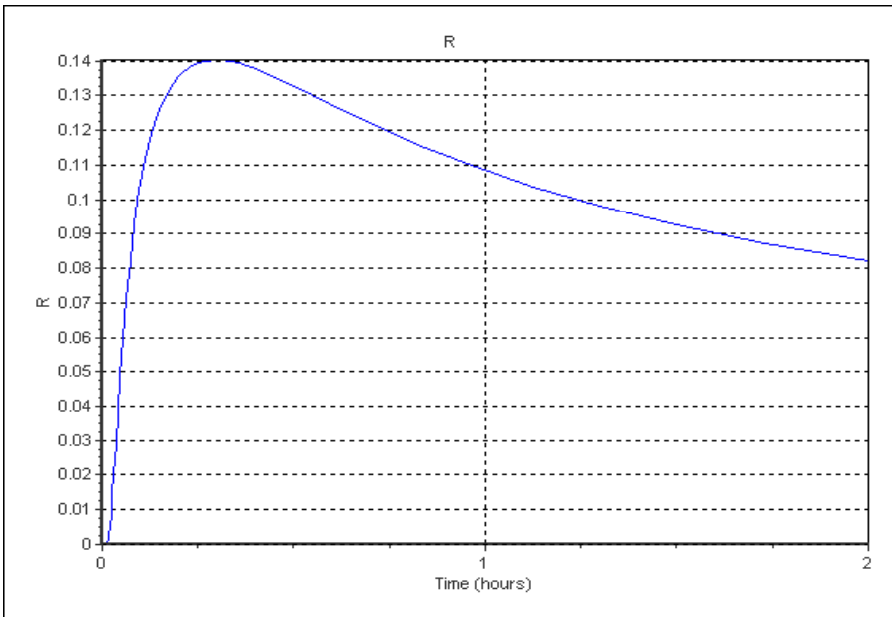


Figure 36. Simulations with DIFS for R in the diffusion-only case.

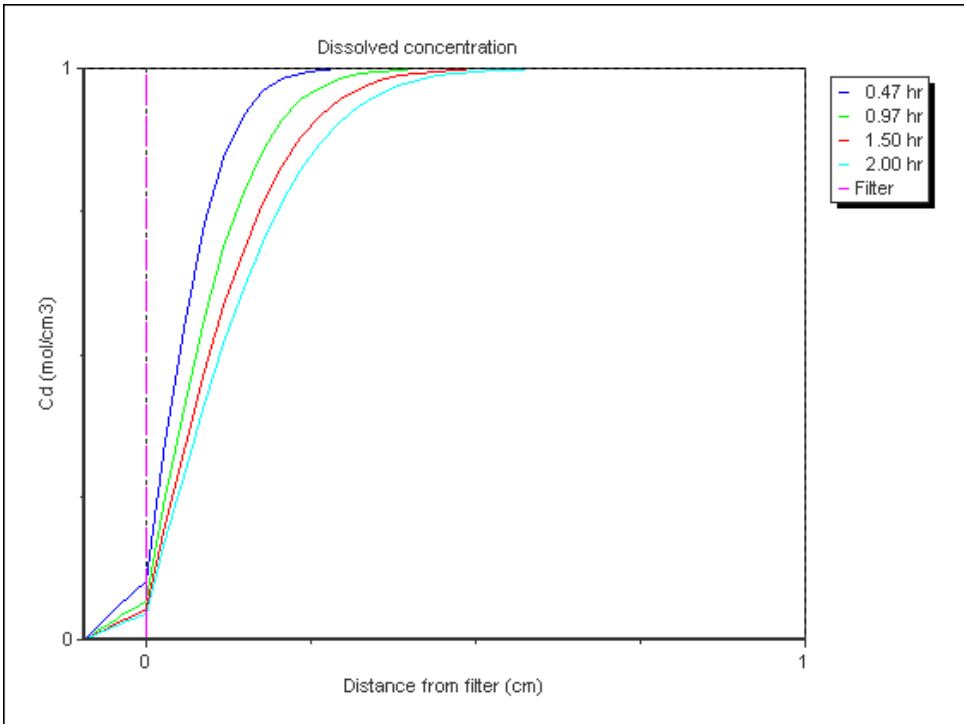


Figure 37. DIFS model distribution of Copper at three different times through the diffusive layer of the DGT device (negative direction) and the soil solution (positive direction).

2. DGT as a predictor of metal plants uptake

As already described in the previous section, to predict the availability of metals to plants, it is important to understand both solution and solid-phase processes in the soil, including the kinetics of metal release from its binding agent.

Like plants, DGT locally lowers metal concentrations in the soil solution and responds to metal re-supplied from labile species in solution and the labile metal pool in the solid phase. It is well recognized that depletion of metal concentration in solution in the immediate vicinity of the roots allows transfer from solid phase to solution.

DGT offers the possibility of a simple test procedure for soils. The kinetic perturbation of solute concentrations in the soil system is most likely similar to that occurring during plant uptake.

The aim of this study, conducted in collaboration with CNR (Pisa), was to compare techniques such as DGT and metal chemical extractions to assess their capabilities in predicting metal uptake by plants.

In this study, Zn and Pb concentrations measured in soils, soil solutions, through chemical extraction by $CaCl_2$ 1M and DGT were compared to the Zn and Pb taken up by the wheat and lupine plants shoots.

In particular, the present work examined the speciation and availability of Zn, Pb in three soils, two well-equilibrated soils and a Zn and Pb amended soil.

The first soil was the Saliceto soil (Piemonte), already under investigation. Due to its low contamination, it was stated to enrich it by Zn and Pb at concentrations similar to those found in a second soil sampled by CNR personnel in Bovisa (Lombardia). This gives the opportunity to study metal bioavailability in an amended soil compared to that found in natural well-equilibrated soils.

Material and Methods

Microcosms experiments

All soils in this study were collected from the A horizon (0-10 cm), dried at 40°C and sieved to <2 mm for chemical analyses and microcosm plant experiments.

Microcosms were prepared mixing 100 g of soil and 100 g of quartz <2 mm. The quartz improves plant shoot aeration, which could be prevented in strictly packed fine soils. The 1:1 ratio soil/quartz was optimised on the basis of preliminary DGT trials performed on soils mixed with various amount of quartz (5 replicates for each different S/Q ratio). Table 16 reports as example the relative standard deviations expressed as percentage for 1:1 and 1:2 S/Q ratio for some elements. Increasing the quartz amount, it was observed a general increase in the RSD% calculated on the 5 replicates for all elements. This probably derives from an increase in the variability of the system soil-quartz depending on the difficult to homogenise the two different phases.

It has to be noted that the preliminary experimental activity (before sowing) has been focused on the determination of various target metals (Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb), while the finally discussion of the results was focused just on Zn, Pb.

Microcosm	Mn	Co	Cu	Zn	Cd	Pb
	RSD%	RSD%	RSD%	RSD%	RSD%	RSD%
1:1 S/Q	13.62	1.96	3.99	11.44	4.11	10.00
1:2 S/Q	16.45	13.73	5.61	54.09	22.66	8.75

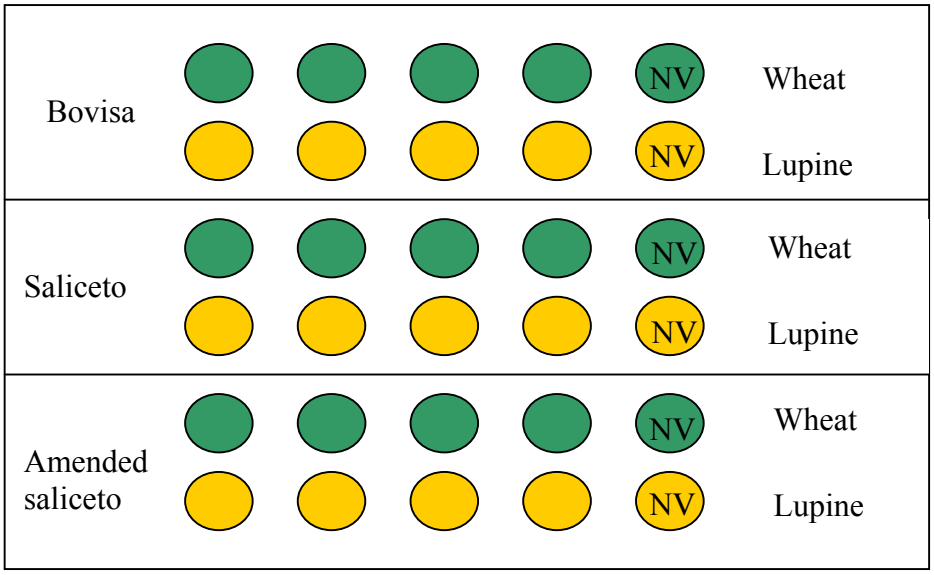
Table 16. Relative standard deviations expressed as percentage for 1:1 and 1:2 Soil/Quartz ratio for some elements.

The preliminary experiments focused on the optimisation of S/Q ratio and of time of DGT deployment were conducted in the APAT laboratory (Rome) as well as metal determinations using ICP-MS in all type of samples. The other experiments (DGT on microcosms, plant growth, chemical extractions) were conducted at CNR laboratory (Pisa) by CNR and APAT personnel.

Due to the intrinsic variability of plant growth, it was decided to perform 4 replicates plus a non vegetative control soil for each type of plant and soil. Considering the 3 soils and 2 type of plants, this results in growing 24 plant specimens and performing 6 blanks, for a total of 30 microcosms. As regards to plants, metals have been determined on both shoots and roots.

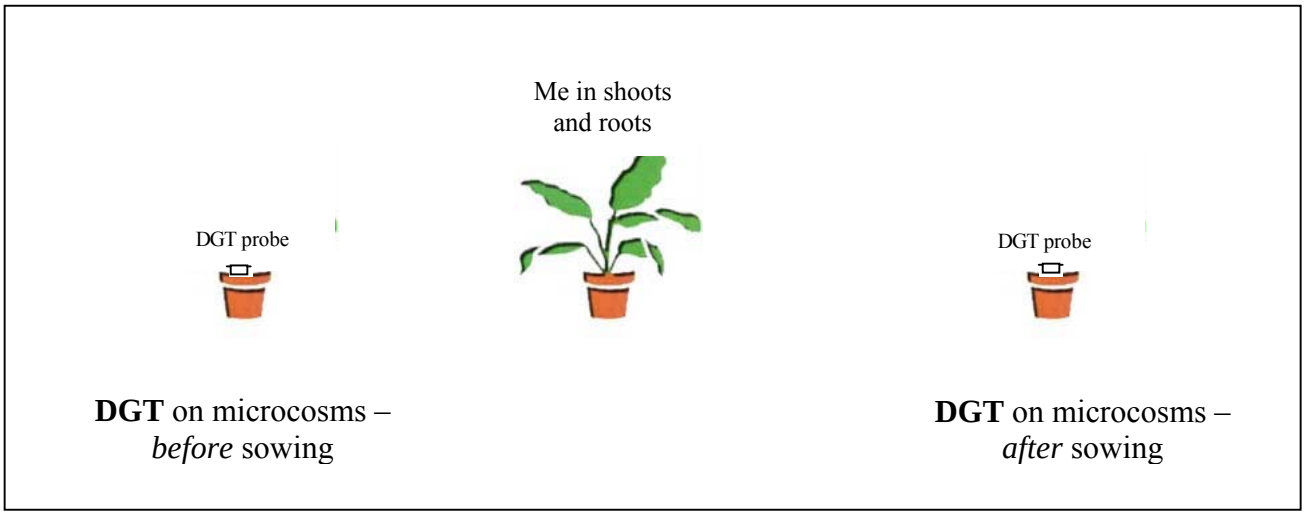
Soil moisture was maintained at 30% maximum water holding capacity (MWHC) with additions of deionised water for 6 days before planting for DGT deployment. Before sowing, soils were maintained at 70% MWHC for 24 h.

One standard DGT device with a sampling surface area of 2.54 cm^2 was deployed in each microcosm for 24 h, at an average temperature of 20°C. DGT was gently pressed onto the soil surface, ensuring that good contact between the device and the soil was attained. Metals in the binding-layer were eluted with a volume V_e of 1.1 ml of 1 M HNO_3 and the concentrations in the eluent C_e were measured by ICP-MS, after diluting to 3.5 ml with deionised water.



Scheme 1. Scheme of the number and type of soils and plants selected for the experimental activity.

Plants were grown in a growth cabinet with the following conditions: 14h day and 10 night, 20°C and 15°C day and night temperatures. Some pictures of plants growth in soils are then reported (figures 38-40). Twenty-two days after germination the aboveground plant material was harvested. The plant roots have been carefully removed and rinsed with water and ultrasounds. The plant material was dried at 40°C for 72h. Total metal concentrations in the plant samples using ICP-MS (7500c, Agilent Technologies) following microwave aqua regia digestion. DGT devices were also deployed after harvest as illustrated in the following scheme, and this time the soils were maintained at 100% MWHC for 24 h before DGT deployment. DGT deployment in soils with different moistures has been shown to comply with theory when used at or above 70% field capacity [64].



Scheme 2. Scheme of DGT experimental activity performed on soil before and after planting.

Total metals and total Carbon and Nitrogen

Total metal content in soils and quartz was determined by ICP-MS, after microwave assisted aqua regia digestion.

Microwave digestions were performed in CEM Mars 5 microwave oven (Matthews, NC).

For the three digestion procedures, test portions of about 0.1 g were weighed in 120 ml Teflon-PFA microwave digestion vessel after manually shaking the bottles for at least 1 minute.

After digestion, samples were quantitatively transferred into volumetric flasks (Brand) and diluted with MilliQ water to 100 ml. Rhodium at a concentration of 25 □g/L was added *on line* as internal standard to minimize ICP-MS instrumental signal fluctuations and matrix effects. The solutions were allowed to settle down and then analysed by ICP-MS. The digestions were performed in triplicates.

Mean results and percentage standard deviations are reported in table 17 for soils.

Sample		Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
		mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Bovisa	mean	401	25657	9.0	44.4	48.8	119	0.36	314
	RSD%	9.2	8.1	0.8	10.4	4.1	0.7	7.6	7.6
Saliceto	mean	565	23960	11.9	72.1	25.8	70.56	0.25	40.23
	RSD%	3	6	4.4	6.4	10.4	5.02	1.40	3.93
Amended	mean	626	30883	14.1	92.2	34.2	199	0.28	529
	rsd	4.1	4.3	3.2	5.5	8.6	7.9	2.9	10.0

Table 17. Mean results and percentage standard deviations for total metal concentration in soils.

Quartz was digested by using both aqua regia and HNO_3 as digestion solutions. As expected, HNO_3 provides a more bland metals extraction with respect to aqua regia. The results are reported in the following table 18. It is clear that metal release from quartz could be considered negligible with respect to metal release from soils, even using a strong acid mixture such as aqua regia.

Sample	Mineralization solution	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Quartz	Aqua regia	2.2	177.0	0.3	0.9	0.9	2.0	0.7	0.019	2.0
Quartz	HNO_3	0.6	32.0	0.1	2.4	<LD	0.7	0.2	0.02	<LD

Table 18. Concentration of metals in quartz determined by aqua regia and HNO_3 digestion.

Total carbon and nitrogen were determined by using CHN analyzer and the results are reported in table 19. The determinations were performed in triplicates. The table reports the mean and RSD% of the 3 replicates. The results are expressed as percentage and in mg/g.

Samples		% C	% N	C (mg/g)	N (mg/g)
Bovisa	mean	4.75	0.16	47.45	1.63
	RSD%	7.00	9.35	7.00	9.35
Saliceto	mean	1.39	0.08	13.93	0.83
	RSD%	4.08	6.93	4.08	6.93
Amended Saliceto	mean	1.41	0.14	14.07	1.37
	RSD%	7.05	15.23	13.97	15.23

Table 19. Total carbon and nitrogen determined in soils by using CHN analyzer.

Bovisa soil contains a slightly higher content of carbon with respect to Saliceto. This probably could enhance the growth of plants in microcosms based on soil from Bovisa. The same value of C found in Saliceto and Saliceto amended soil totally agrees with the expectation that amendment does not affect carbon content. On the contrary, the N difference found in the two soils could be ascribed to the minor precision and accuracy generally found for N determinations by HCN analyser in our routine activity.

Amendment of soil from Saliceto

As already mentioned, amendment was conducted on soil from Saliceto to increase Zn and Pb concentration at similar level to those in Bovisa. Soil was treated with standard metal solutions containing Zn and Pb and left to equilibrate for 1 month. The aim was to compare two soils with similar concentration but differing on metals way of binding to solid-phase. Actually, unless the amended soil was used after the equilibration time of 1 month, it was not affected by aging processes as natural soils. As regard to Pb, the amendment resulted in a greater Pb concentration (529 mg/kg) with respect to Bovisa aqua regia extractable content (314 mg/kg).

Soil solutions were extracted from soil samples by centrifugation at 3000 rpm for 10 minutes. The solutions were filtered at 0.45 □m and acidified to about pH 2 using HNO_3 solution. The solutions were analysed by ICP-MS (7500c, Agilent Technologies)

RESULTS AND DISCUSSION

DGT measured concentration before sowing

As already mentioned, the preliminary experimental activity (before sowing) has been focused on the determination of various target metals (Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb), while the finally discussion of the results was focused just on Zn, Pb.

DGT measured concentration results are reported in the following table 20. The table reports the mean values for the ten replicates performed for each soil. Before sowing the 5 replicates of lupine and of wheat can be pooled. The relative standard deviations expressed as percentage are also reported. In case of Bovisa, the results for Mn are considered unreliable due to the high rsd%. Amended saliceto results can be totally considered acceptable, being the rsd% under 14%.

For Saliceto amended soil, DGT metal concentrations are higher with respect to the other two and for this reason they are easier to measure. It is interesting to note the higher DGT measured concentrations found for all target elements, not just for Zn and Pb, in amended Saliceto with respect to Saliceto. This can be partly explained on the basis of a pH change caused by the amendment procedure. This was due to the acidity of the standard metal solutions added to the soil. The pH decreased from 8.47 to 7.16, causing a larger metals release from soil to the pore water. The pH decrease partly changes soil capabilities of metals release, but this aspect could represent an advantage considering the low metal concentrations present in the Saliceto soil, hardly measured by DGT. The pH of amended soil and soil from Bovisa (pH ≈ 8) can be considered quite similar for making considerations on changes in metal release relevant to different equilibration times and aging processes. Another possible explanation for the difference between Saliceto and amended Saliceto could be found in the possibility of a change in the grain size distribution of the two soils. The amended soil was kept under stirring in water solution to achieve metal enrichment. This could cause a reduction in the grain size. This hypothesis must be supported by grain size distribution analysis using laser grain size analyser.

Sample		Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
		□g/L	□g/L	□g/L	□g/L	□g/L	□g/L	□g/L	□g/L
bovisa	mean	8.5	177.6	0.1	1.6	8.8	5.3	0.1	0.7
	rsd%	18.4	9.4	7.8	5.8	6.7	13.2	7.2	21.1
saliceto	mean	2.3	152.9	0.05	2.0	3.8	5.4	0.03	0.1
	rsd%	26.7	13.5	22.8	8.1	14.5	18.7	20.0	11.8
Amended saliceto	mean	618.6	305.4	1.4	5.4	5.4	33.3	0.3	13.5
	rsd%	8.5	12.0	10.0	8.7	8.0	13.2	9.5	14.0

Table 20. DGT measured concentration and rsd% (mean values for the ten replicates) for each soil.

Figures 38-40. Plants growth in soils and growth cabinet

Metals accumulated by plants

The metals accumulated by plants taken into consideration were Zn and Pb. Figure 41 reports lead and zinc concentrations in wheat and lupine shoots for the three soils. The figure shows a minor lead uptake with respect to zinc in both lupine and wheat shoots. This outcome is unexpected if considering its higher concentration in soils (Table 17) and could be explained in view of Pb toxicity which could represent a limiting factor for its uptake.

The concentrations of lead in lupine and wheat plants grown on Bovisa soil are around a mean value of 3.9 mg/kg, for the amended soil being higher, 10.4 and 14.4 mg/kg respectively.

The mean quantity of Zn adsorbed by plants is 49 mg/kg, excepting for wheat grown on amended soil which is 73.1mg/kg.

For both metals, metals uptake is more effective in case of the amended soils, especially for wheat.

It is interesting to note that metals uptake of lupine on saliceto soil is often similar or slightly higher to that on bovisa, even though this soil contains a minor amount of metals in term of total concentration.

These results underline that the metal accumulation in plants depend on the combination of plant species, metals, their concentration ranges and metal bioavailability.

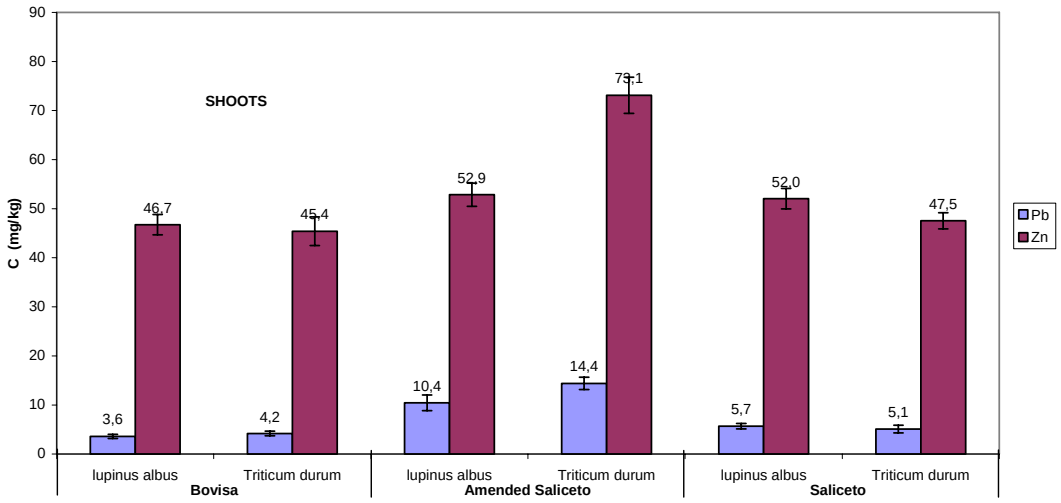


Figure 41. Lead and zinc concentrations in wheat and lupine shoots for the three soils.

Figure 42 reports lead and zinc concentrations in wheat and lupine roots relevant to the three soils.

Lead accumulation is greater for plants growth on amended saliceto, mainly in case of wheat (140 mg/kg). In Bovisa soil, lead accumulation is lower even though a similar trend is observed (39.4 and 64.5 mg/kg for lupine and wheat respectively). In case of Saliceto, further decrease is detected getting to 11.5 and 14.6 mg/kg. For both

amended saliceto and saliceto, practically the same ratio (about 1.25) between the concentrations in wheat and lupine was found.

The greatest accumulation of zinc in roots is observed in case of lupine on amended saliceto attaining 466 mg/kg, while a 1.7 times less accumulation is observed for wheat (274 mg/kg). The same trend is observed in Bovisa soil, on which wheat uptake was 1.75 times less than that of lupine.

In case of Saliceto soil, a similar amount of zinc is absorbed by the two plants (249 mg/kg for lupine and 283 mg/kg for wheat). Afterwards, lupine zinc uptake from this soil was not so effective as that on the other soils.

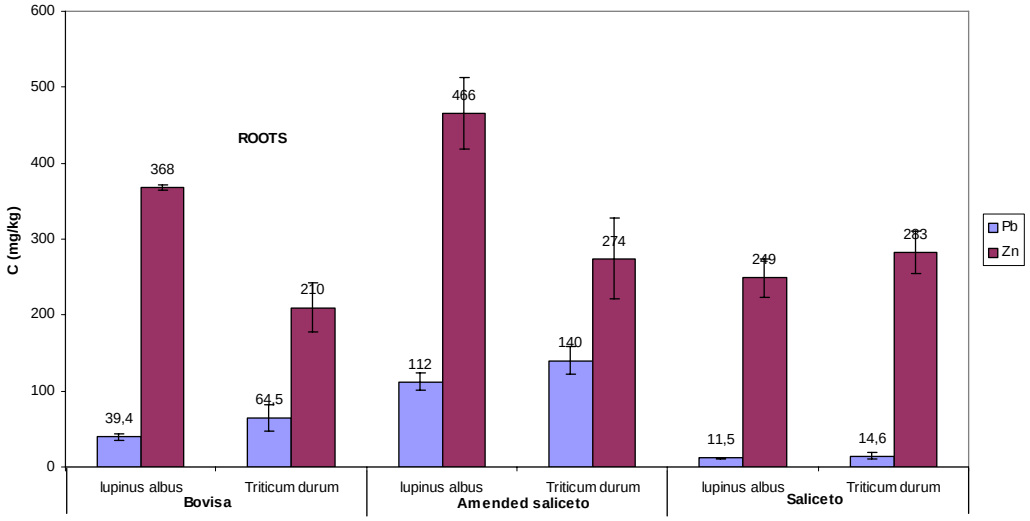


Figure 42. Lead and zinc concentrations in wheat and lupine roots relevant to the three soils.

Figure 43 reports the metal translocation factors expressed as the ratio of metal quantity accumulated by shoots and that found in roots. This parameter enable to examine the capacity of plant species to absorb and transport metals from roots to shoots. These factors are quite low, especially in case of lead in the amended and Bovisa soils. Concerning these soils, lead is transported to a minor extent with respect to zinc, getting a mean translocation factor of 0.09; zinc is transported more effectively by wheat, twice as high as lupine. On the contrary, in case of Saliceto soil, the situation is just the opposite being lead transported to a greater extent with respect to zinc. In Saliceto soil, lupine translocation factors are higher than that of wheat, in particular 1.4 and 1.2 times higher for lead and zinc respectively.

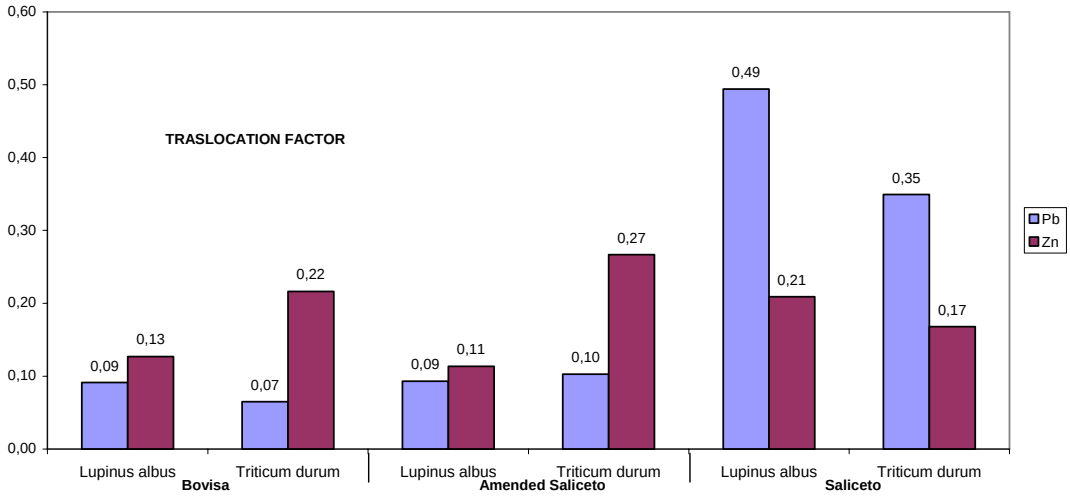


Figure 43. Metals translocation factors expressed as the ratio of metal quantity accumulated by shoots and that found in roots.

Figure 44 reports plant biomass yields for lupine and wheat. Concerning lupine, the biomass yields for Bovisa and Saliceto soils are almost equivalent and approximately twice as high as biomass yield on the amended Saliceto. Lupine has more difficulties on growing on the amended Saliceto than wheat. Biomass yield is variable owing to inherent differences in the type of plants and in the toxicity and fertility of the different soils. In case of triticum durum, plant biomasses developed in the more contaminated soils (Bovisa and amended Saliceto) are almost identical and about 30% less than that on Saliceto. Biomass yield on Saliceto soil is practically the same for the two plant species.

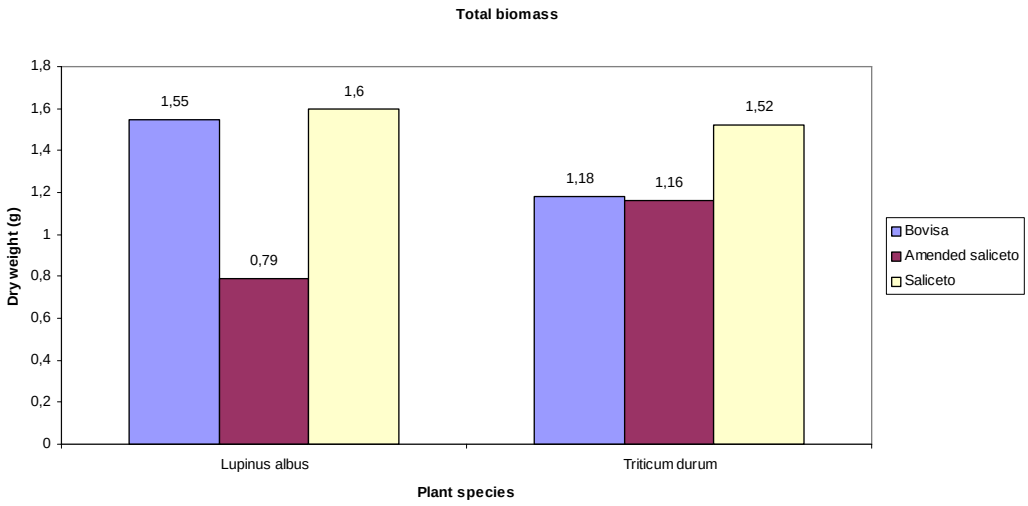


Figure 44. Plant biomass yields for lupine and wheat.

Chemical extraction

Figure 45 reports the $CaCl_2$-extractable zinc and lead concentration relevant to the three soils. Furthermore in the figure the limits of quantification (LQ) of the target metals are reported.

The extractable Zn in Bovisa and Saliceto soils is not detectable (< LQ) meaning that chloro-complexation and Ca competition are too weak for Zn in Bovisa and Saliceto soils, even though the high salt concentration of $CaCl_2$. Bovisa and Saliceto soils release the same amount of $CaCl_2$-extractable lead, though their different total metal content. The amended soil exhibits the higher contamination in term of total metal concentration. In addition, the way of binding to solid phase of Zn and Pb should be weaker than in the other two natural soils owing to the amendment. Consequently, the metals in the amended soil are probably more easily exchangeable.

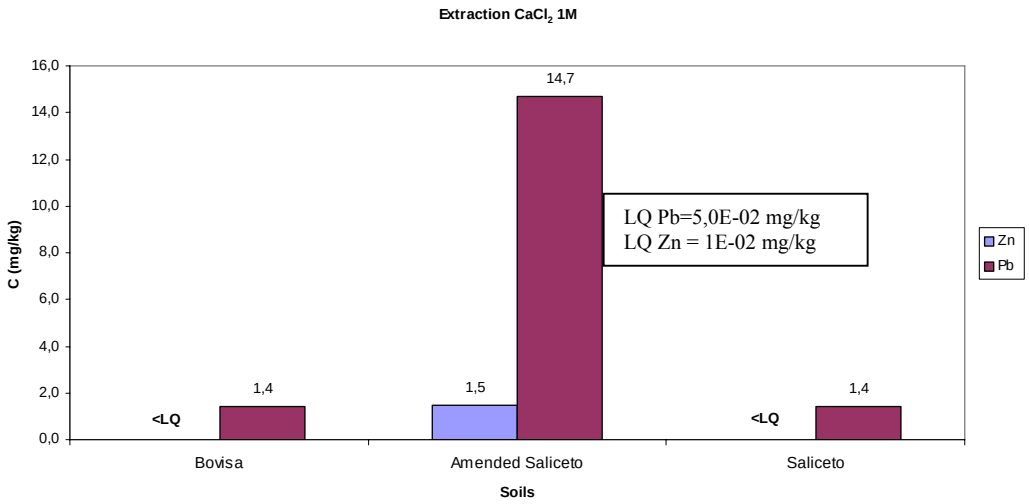
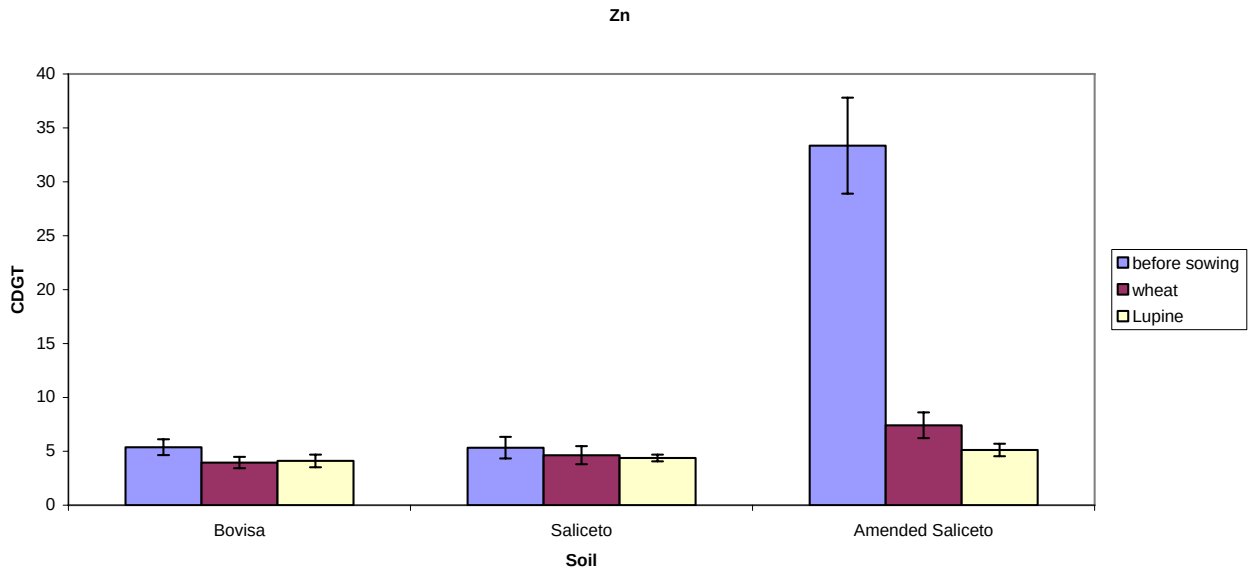


Figure 45. $CaCl_2$-extractable zinc and lead concentration relevant to the three soils. The limits of quantification (LQ) of the target metals are also reported.

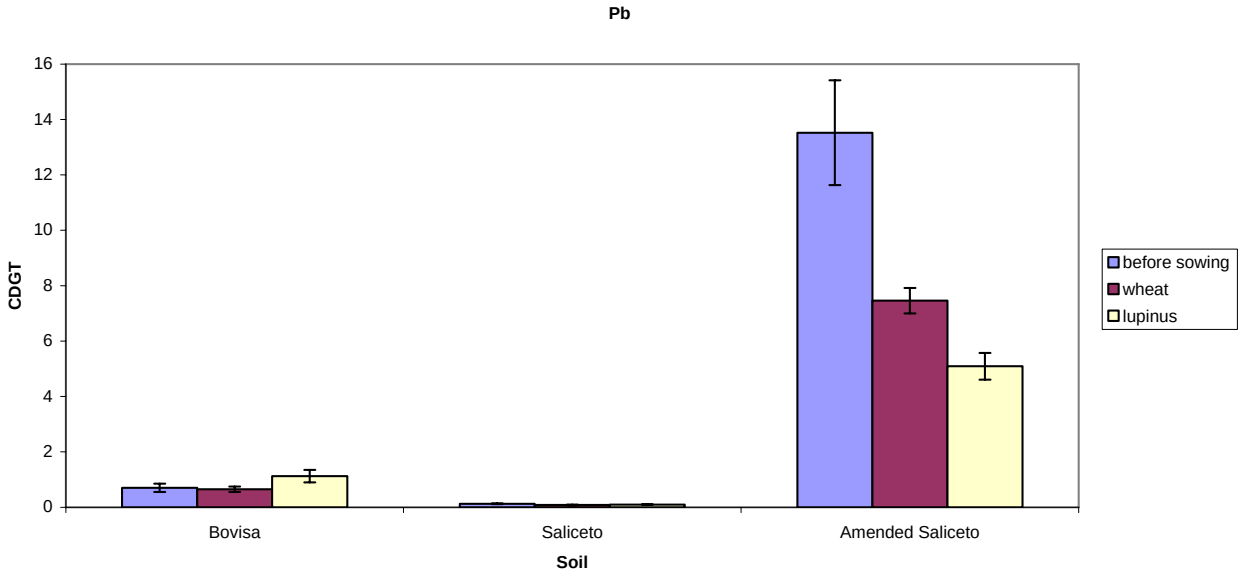
DGT measured concentration after sowing and a comparative analysis with metals in plant tissue

Figures 46-47 report the DGT measured concentration for Zn and Pb measured in soils before and after plant growth.

Notably, C_{DGT} concentrations for Zn measured after plant growth were lower than those before growth. This is confirmed by experiments conducted by Nolan et al. [14]. The most significant diminution was found for the amended soil. The same issue was observed for lead C_{DGT} concentrations measured after plant growth on the amended soil. In the natural soils, an evident trend was not observed. As reported by Nolan, it is therefore important to consider when these and other measurements are conducted.



Figures 46-47. DGT concentrations (□g/l) for Zn and Pb measured in soils before



and after plant growth.

Figure 48 reports plant Zn concentration vs the effective concentration C_e derived from DGT measurements (eq.8) after plants growth. From a predictive perspective, the Zn concentrations in the wheat and lupine across all soils correlate quite well with the effective concentration C_E applying a linear fit (R^2=0.86). As the figure reports single values, no error bar is shown.

Figure 49 reports the mean values of Zn concentrations for the 4 microcosms for lupine and wheat on the three soils (regression coefficient of 0.95). Error bars give the standard deviation of 4 replicate DGT devices. A linear relationship was found also by Nolan for Zn in a range of contaminated soils using wheat. Figure 50 reports plant Zn concentration vs the soil pore water measured after plants growth. Poor relationship (R^2 = 0.67) for Zn with respect to pore water solution was found indicating that the kinetically labile solid-phase pool of metal, which is included in the DGT measurement played an important role in Zn uptake by plants along with the labile metal in solution. This issue can be also outlined considering that DGT measurements can be interpreted in terms of ratio R ($\frac{C_{DGT}}{C}$ eq. 7). The concentration calculated from the measured DGT mass, C_{DGT}, is less or equal to the concentration of labile species in the pore water C. R values obtained experimentally for Zn relay in the partially sustained case (R_{diff} <R<1), meaning that there is some resupply of ions from soil to solution even though insufficient to fully satisfy the DGT demands.

Figure 51 and 52 report the dependence of Pb concentration in plant tissue on the C_E concentration after growth measured for each microcosms and on the C_E after growth as mean of 4 independent microcosms. The Pb concentrations in the wheat and lupine across all soils correlate very well with C_E using a curved/quadratic regression (R^2 = 0.93 - figure 51 and R^2 = 0.95 - figure 52). Error bars give the standard deviation of 4 replicate DGT devices. A number of soil or plant factors can influence the relationship between soil solution free ion activities and metal accumulation in plants so that a linear relationship is not necessarily expected [14]. The correlation coefficient is high although the results are not well distributed. The correlation proceeds across all soils appearing independent from the two plants species.

Figure 53 reports plant Pb concentration vs the soil pore water measured after plants growth.

Plant Pb concentrations well correlate to soil pore water concentrations using a curved/quadratic regression (R^2 = 0.96). Plant Pb is highly related to soil pore water concentrations measured by DGT indicating that supply from the solid phase may not be so important for Pb.

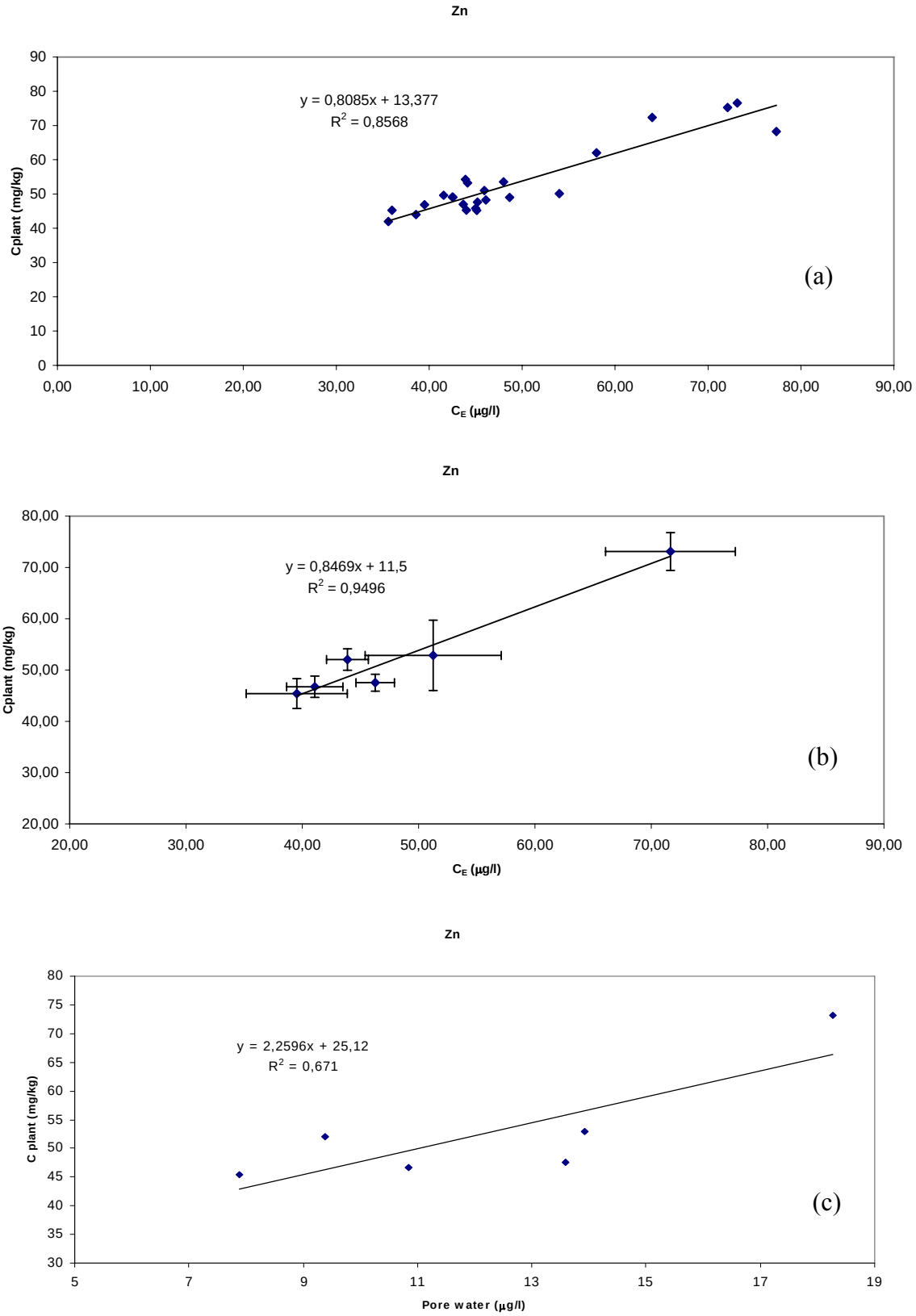


Figure 51-52-53. Dependence of Zn concentration in plant tissue on the a) C_E concentration after growth measured for each microcosms; b) C_E as mean of 4 independent microcosms and c) soil pore water measured after growth (mean values).

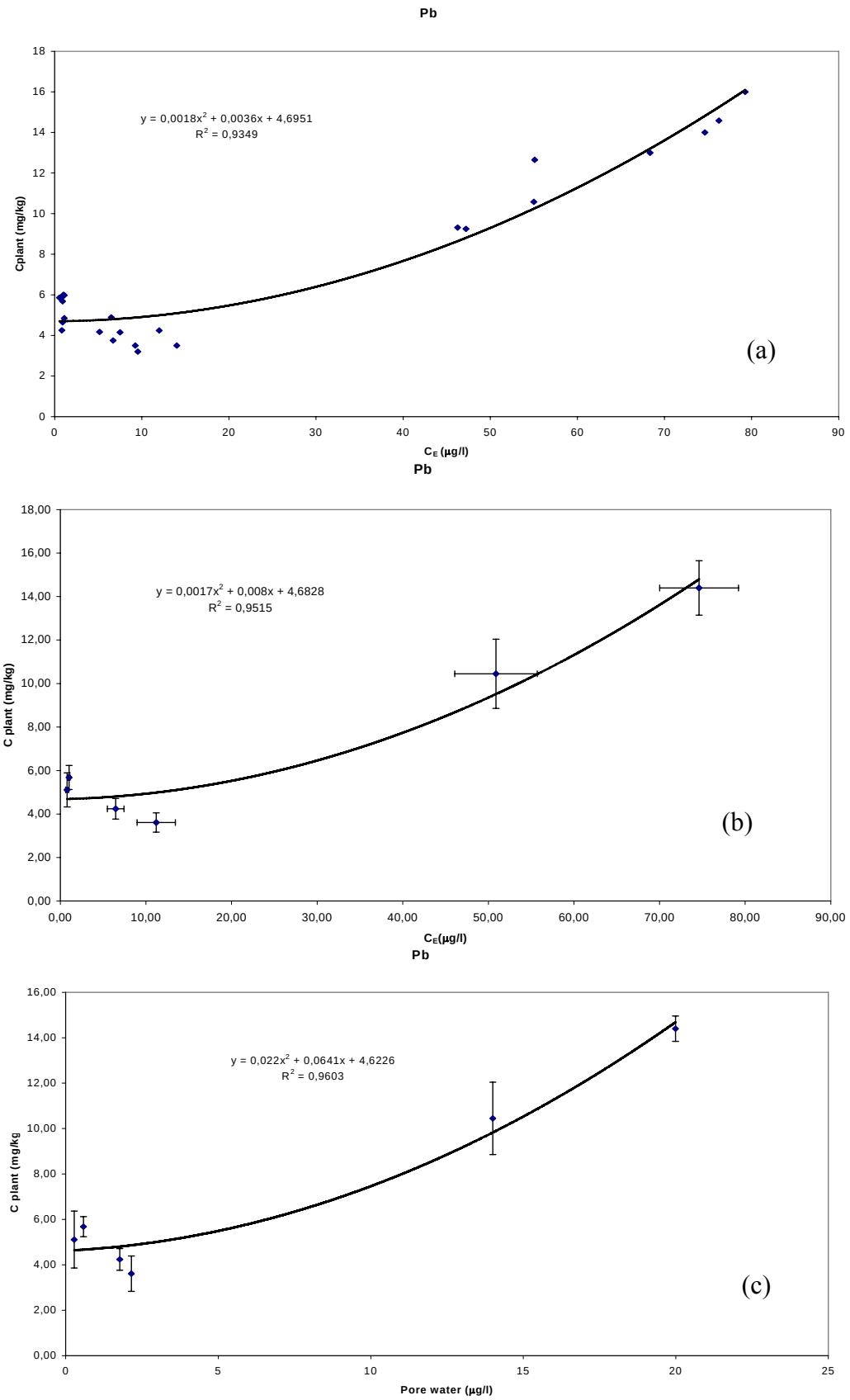


Figure 54-55-56. Dependence of Pb concentration in plant tissue on the a) C_E concentration after growth measured for each microcosms; b) C_E as mean of 4 independent microcosms and c) soil pore water measured after growth (mean values).

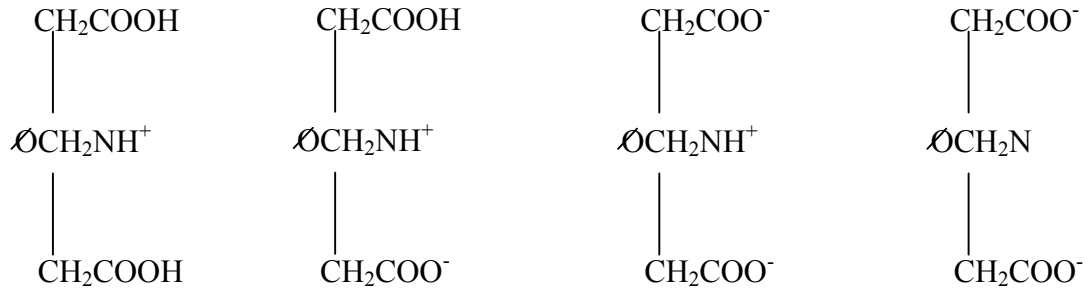
Conclusions

The distinguishing feature of the DGT measurement is that incorporates the kinetics of metal supply from solid phase to solution, and our results indicate that this supply may be important for Zn in these soils. Plant Pb concentrations were highly related to both soil pore water concentrations and to the effective concentration (C_E) measured by DGT indicating that supply from the solid phase may not be so important for Pb. To conclude the present study has reported the ability of DGT to potentially predict the Zn and Pb phytoavailability for lupine and wheat within metal contaminated soils.

Appendix 4

CHELEX 100 cationic exchange resin

Chelex is a styrene lattice with iminodiacetic acid exchange groups which act as chelating groups in binding polyvalent metal ions. It prefers divalent cations. Chelex is classed with weakly acidic cation exchange resins by virtue of its carboxylic acid groups, but differs from the ordinary exchangers because of its high selectivity for metal ions and its much higher bond strength. It operates in basic, neutral and weakly acidic solution of pH 4 or higher. At very low pH the resin acts as an anion exchanger.



pH 2.21 pH 3.99 pH 7.41 pH 12.30

The quantity of cations exchanged is a function of pH. Exchange is very low below pH 2, increases sharply from pH 2 to 4, and reaches a maximum above pH 5. Any metal removed from the solution is replaced by an equivalent amount of the ions originally on the resin. The resin is supplied in the sodium form. These weakly held ions allow other ions to be readily adsorbed. The most effective agents to elute the metals from the resin are acids.

Appendix 5

DGT performance evaluation in water

The DGT units used in the present work were stored in the fridge for 2 years before being used. For these reasons, they had to be revived before use, following standard procedure reported in Gimpel [22].

Revival procedure

DGTs were placed for 6 hours in a MilliQ bath, followed by soaking in 0.01M $NaNO_3$ (ultrapure grade) solution overnight.

The DGT units were deployed immediately after they have been revived, to test their performance in a solution of known concentration.

Performance Test

DGT replicates have been immersed for increasing times in 3 L of water spiked with 10 ppb of Cd (pH = 6, 0.01M $NaNO_3$). The temperature of the solution was monitored with a probe (T =25°C).

Blanks values

3 DGT units after reviving procedure have been analysed as blank.

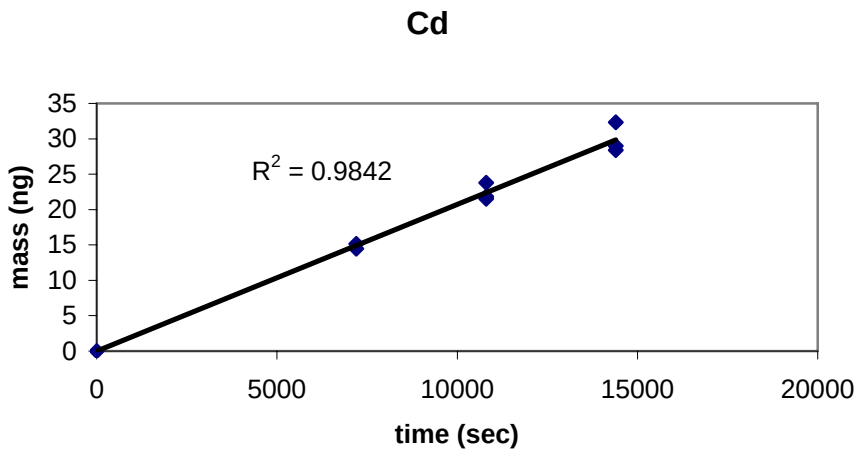
replicates	Cd (ng/ml)
DGT Blank 1	0.2132
DGT Blank 2	0.2298
DGT Blank 3	0.1228

Table 21. Determination of blanks values

Test solution

DGT units have been immersed in the test solution and collected at intervals of 2-3-4 hours. The DGT resins were eluted with 1 ml HNO3 1 M for 24 h. The concentration C_e of Cd in the eluted solution, determined by ICP-Ms, is used to determine the mass accumulated in the resin and the concentration of bulk solution.

The following plot represents the accumulated mass of Cd versus time of deployment.



For each time of deployment, the mean values of the DGT replicates were taken.

The calculated concentration for Cd, for each time of deployment, is reported in Table 21.

During DGT deployment, 3 aliquots of test solution were collected from the container and directly analysed for Cd determination. The results are reported in Table 22.

Time (sec)	DGT Concentration C (ppb)	Solution Concentration (ppb)
0	-	10.1
7200	9.9	10.1
10800	10	10.0
14400	10	9.9

Table 22. DGT measured concentration and solution concentration directly determined by ICP-MS.

The results provided by DGT are in good agreement with the results determined directly in the test solution, meaning that DGT performance is accurate.

Conclusion

The previous Tables show that, even after such a long period of storage of 2 years, the DGT units work well, giving accurate results in test solution of known concentration.

Appendix 6

Maximum Water Holding Capacity (MWHC)

The MWHC is determined by placing 30 g of dried soils in a funnel with a Whatman N°40 filter paper (110 mm diameter) [64]. The funnel is held in place in a container of MQ, further MQ is added to saturate the samples and the samples allowed to stand for 120 minutes. After this time, the funnel is removed from the container and allowed to drain for 120 minutes. The weight difference between the dry soil and the wet soil, minus the weight of the wet filter paper (≈ 3.5 g) is taken as the Maximum Water Holding Capacity (MWHC)

$$MWHC\ \% = (Soil_{\ wet\ weight} - 3.5\ g_{\ wet\ filter\ paper} - Soil_{\ dry\ weight}/Soil_{\ dry\ weight}) \times 100$$

Sample	Matrix	MWHC %
SALICETO	Agricultural soil	40
MILLESIMO	Agricultural soil	31
ERSA	Agricultural soil	36
COMPOST	Compost	198
SCARLINO	Contaminated soil	21

Table 23. Maximum water holding capacity (MHWC) of soils and compost.

Appendix 7

Natural pH of samples

The natural pH of the samples was measured in a suspension 1:10 of samples : water. The samples were allowed to equilibrate for 20 minutes under stirring and then the pH was measured.

Sample	Weight soil (g)	Volume water (ml)	pH	Equilibrating time
SALICETO	5.0046	50 ml	8.47	20 min
MILLESIMO	5.0046	50 ml	8.62	20 min
ERSA	5.0146	50 ml	8.16	20 min
COMPOST	4.8452	50 ml	7.80	20 min
SCARLINO	5.0068	50 ml	2.94	20 min

Table 24. Natural pH of soils and compost.

Appendix 8

DGT deployment

Sample	Mean Temperature (°C)	Time of deployment (hours)
SALICETO	24	27
MILLESIMO	24	27
COMPOST	24	24
ERSA	24	24
SCARLINO 2	25	2

Table 25. DGT deployment parameters (mean temperature and time of deployment).

Sample	Cr	R	Co	R	Ni	R	Cu	R	Zn	R	Cd	R
	microg/L	*%*	*microg/L*		*microg/L*		*microg/L*		*microg/L*		*microg/L*	
MILLESIMO	<LD		0.058	0.045	0.724	0.128	2.048	0.086	2.726	0.215	0.098	0.110
SALICETO	<LD		0.048	0.057	1.681	0.209	3.125	0.125	4.145	0.657	0.069	0.288
ERSA	0.040	0.060	0.043	0.070	0.380	0.135	3.230	0.117	Not reliable results		0.090	0.225
COMPOST	0.616	0.007	2.220	0.077	5.287	0.045	3.426	0.003	55.714	0.101	0.086	0.047
SCARLINO 1							9621	0.150				

Appendix 9

Table 26. DGT metals concentration determined for soils and compost

Note: 1. In the ERSA sample, the two DGT replicates provided different results for Zn and therefore the results are considered unreliable.
2. LD = Limit of detection of ICP-MS.

Sample	Cr	RSD	Co	RSD	Ni	RSD	Cu	RSD	Zn	RSD	Cd	RSD
	microg/L	*%*	*microg/L*	*%*	*microg/L*	*%*	*microg/L*	*%*	*microg/L*	*%*	*microg/L*	*%*
MILLESIMO	1.683	4.6	1.27	2.4	5.647	0.57	23.9	0.7	12.69	1.52	0.8944	4.3
SALICETO	1.669	4.8	0.8364	3.5	8.045	1.24	24.92	0.24	6.309	1.51	0.2411	4.8
ERSA	0.7199	8.6	0.6453	1.8	2.788	2.61	27.5	0.65	3.988	1.44	0.3992	4.8
COMPOST	82.83	1.41	28.91	0.67	117.4	0.9	1062	0.11	551.1	1.17	1.821	1.81
SCARLINO 1	152.2	8.56	40070	0.45	4564	0.73	62890	0.42	1767000	0.64	8087	1.58

Table 27. Pore water metals concentration determined for soils and compost.

PART 2. METAL BIOAVAILABILITY IN WATERS

SECTION 7. Metals Bioavalibility in river waters and metals bioaccumulation in fish

1.DGT labile metals and 0.45 - 0.2 μm filterable fraction of metals: a comparison in a river compartment

The problems of determining concentrations of metals that are truly in solution from analysis of water samples are well documented in literature. Metals speciation may change during sampling and storage. Removal of a volume of water from an aquatic system can alter the finely balanced physical and chemical processes responsible for the distribution of metal species. For example, Laxen and Chandler [65, 66] found concentrations of filtered metals changed within a 2h delay between sampling and filtration.

Techniques that perform a separation in situ offer a good means of accurately determining the components present in solution, as it circumvents any problems associated with post-sampling changes, such as aggregation or oxidation.

Few in situ techniques exist that are easy to use. The simplest, most common technique is the separation into “dissolved” and “particulate” metal fractions by in situ filtration. Apart from its simplicity, the main advantage of this approach is that most water quality guidelines provide guideline values in 0.45 μm filterable or total metal concentration.

Typically, the fraction that passes through a 0.45 μm filter is defined as “dissolved”, while the fraction collected by the filter is termed “particulate”. "Dissolved" is operationally defined, and in a strict sense is incorrect, as small particulates (i.e., <0.45 μm) will pass through the filter membrane. Rather, the term "filterable" is a more correct term. In practice, the “dissolved” component includes metal species that are truly dissolved, including inorganic species (free metal ions and inorganic metal complexes) and organically complexed metals, but also includes “colloidal” metal species. By definition, colloids are particles which range in size from <0.01 to 10 μm, and are typically represented by clays, Fe-oxyhydroxides, amorphous silica and calcium carbonate [67]. The free metal ion concentration often

represents only a small portion of the total dissolved metal concentration. A significant fraction exists as complexes with assorted inorganic ligands such as CO_3^-, OH^-, Cl^- and SO_4^{2-} ions. For example, cadmium is known to form chloro complexes, whereas Co, Cu, Ni, Pb and Zn tend to form complexes with carbonate ion [66]. These species are often readily available to biota, because the complexes are typically weak and dissociate rapidly to the free metal ion. Until fairly recently, the speciation of dissolved metals was widely believed to be dominated by inorganic metal species. In recent years, however, there has been an increasing awareness that for many metals in natural waters, organic metal species predominate with a wide variety of ligands involved [69-73]. In most cases, complexation with organic ligands reduces metal bioavailability.

However, artifacts associated with filtration can highly affect speciation as well documented in literature. Horowitz [74] investigated many factors that led to over- or underestimation of the dissolved metals concentration in water samples. Even for a constant pore size, it is sufficient to vary the brand or diameter of the filter or the volume of water filtered to alter significantly the filtrate concentration. Processes thought to be responsible are listed adsorption/desorption of trace metals from the filter or from solids retained by it, inclusion/exclusion of colloidally associated trace metals or filter clogging. Moreover, the filter pore size could affect the results. In this work it is firstly investigated the influence of different pore size filters on the filtrate concentration of metals by using filter of porosity 0.45 μm and 0.2 μm.

The primary aim of the present work is to compare the results obtained from on site filtration using 0.45 and 0.2 μm filters to those obtained by DGT. As already mentioned in the previous section, DGT represents a relatively recent approach to water sampling which could be deployed directly in situ for determining the concentration of labile (potentially bioavailable) metal species in aquatic systems.

Increasing evidence suggests that the biological response of aquatic organisms in the face of dissolved metal concentrations is proportional to the activity of the free ions. Strongly complexed (thus, non-labile) and particulate metal species are less available [75]. DGT makes possible to investigate the metal complexation and speciation, focusing on the fraction of metal really available to biota. As reported in literature by

Denney [21] and Gimpel [22], a low ratio of DGT-metal/dissolved metal shows a strong complexation by humic substances or a presence of colloidal material. If good agreement exists between DGT measured metal and the dissolved metal, strong complexation by organic matter is not occurring and colloidal metal is negligible. These considerations are useful to quantify the metals fraction available for biota uptake. It is important to note that implicit within DGT approach is the notion of time integration. DGT integrates the concentration of labile metals in the test solution throughout the deployment period and provide a continual time-integrated response to changes in trace metal concentration. Under quiescent conditions, when variability in water is low, the data afforded by DGT are comparable to a more conventional sampling approach like on site filtration which is instantaneous in nature. If the variability is high as in this case, direct comparisons of DGT and filtration can be accomplished only through high frequency sampling during DGT deployment.

Material and Methods

The selected sampling site is the Sitzerri River (Sardinia, Italy), which is a river located next to the closed mine of Montevecchio. The extractive activity of the mine concerned mainly Pb and Zn minerals. In the past, contaminated material was discharged directly in the river and at present metal contamination derives from leaching of metals from discharged material and depends on meteorological conditions.

Figure 57. Sitzerri River (Sardinia).

The work was conducted in collaboration with the Regional Environmental Protection Agency of Sardinia (ARPA).

Sampling

The sampling starting time was scheduled for 24 September 2005. However, an unexpected dryness of the little river caused a delay in the sampling campaign which has been procrastinated on January. The first DGT sampling campaign was conducted on 10 January 2006.

The following matrices were sampled:

- metals bioavailable fraction dissolved in water by using DGT
- filtered waters (at 0.45 μm and 0.2 μm)

The second DGT sampling campaign was conducted on 22 May 2006.

The following matrices were sampled:

- metals bioavailable fraction dissolved in water by using DGT
- filtered water (at 0.45 μm)

Some river water parameters can be considered adequate for DGT sampling:

- pH : 7.3.
- Flow: 0.5 m/s

Sampling strategy

Five DGT units for each sampling time (5 replicates) were used to keep under control the environmental variability and that associated with DGT. The dependence of DGT on deployment time represents an advantage as a range of deployment time can be used. Sampling period of about 1.5h, 3.5h, 4h, 20h and 24h have been selected on the basis of metal concentration data provided by ARPA (not shown). Totally, 25 DGT units were used. A Plastic sampling apparatus to locate 5 DGTs was constructed by ARPA (fig.58). During the working day, water samples of 100 ml were collected in clean acid washed LDPE bottles. The samples were immediately filtered trough 0.45 μm and 0.2 μm pore size membrane filter (nitrocellulose, Millipore) using a field filtration apparatus and acidified with concentrated suprapur

HNO_3 to 2 ml per liter of sample. Upon returning to the laboratory each sample was refrigerated (<4°C) and analysed as soon as possible.
During sampling, pH, dissolved oxygen, and temperature were recorded in situ with a multiprobe analyzer.

Figure 58. Plastic Apparatus to locate 5 DGT units (ARPA).

Metals determination
Metals have been determined by APAT using ICP-MS equipped with a quadrupole detector and an octopole reaction cell. Recoveries found for multielement certified reference materials were comprised between 95 and 105%.

RESULTS AND DISCUSSION

First sampling campaign (January 2006)

The following plots (Figure 59-65) represent the accumulated mass of the target metals (Mn, Ni, Co, Cu, Zn, Cd, Pb) versus deployment time. According to the theory, the mass of metals in the resin layer increases linearly with time providing good correlation factors (R^2). No metals reached a plateau value, demonstrating that saturation did not occur.
Figures 66-69 report the DGT metals concentration integrating on different periods. Quite good agreement is found among the results demonstrating that during sampling (24 h) the river is characterized by low variability.

Figure 70-76 show the concentrations of the target metals measured by DGT and by on site filtration using filters of different pore sizes (0.45 - 0.2 μm). DGT results are shown by a line that reflects the whole deployment time (20h). Error bars give the standard deviation of 5 replicate DGT devices. The filtration results are shown as a single bar at the sampling time. As they represent single samples, no error bar is shown. In particular, the figures report the direct comparisons of DGT and filtration occurred at t = 0h, t = 2h and t = 20h during the 20 h DGT deployment.

Good agreement between Mn, Ni, Co, Zn, Cd, concentrations measured by DGT and by 0.45 - 0.2 μm filtration suggest that most of the metals is present as simple inorganic species readily available to biota.

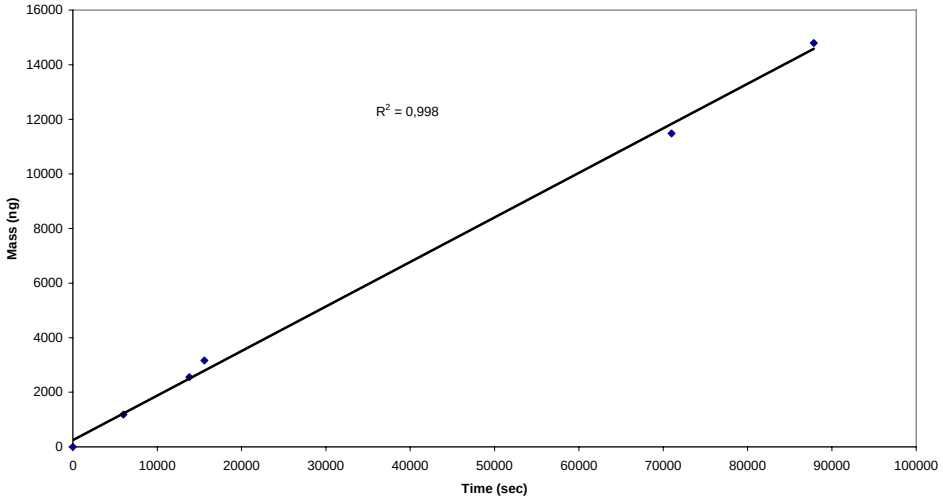
However, DGT labile measurements as a fraction of 0.45 - 0.2 μm filterable concentrations were found in case of Cu. DGT measured only a third of the Cu present indicating that a substantial proportion might be present as strong complex. Cu-complexation is often reported in literature for freshwater compartment since Cu tends to form organic complexes with various ligands. Cunis also present in waters as hydroxide and as inorganic complexes which are generally weak and tend to dissociate fast.

DGT measured only a third of the Pb measured after 0.45 μm filtration and about 3/5 of Pb after 0.2 μm filtration. Firstly, it has to be noted that only in case of Pb there is a significant difference between the concentration measured after 0.2 μm and 0.45 μm filtration. This discrepancy must be attributed to an exclusion of some proportion of metal between 0.2 and 0.45 μm by the restricted pore size filter, to a 0.2 μm filter clogging or to a lead contamination of samples using 0.45 μm filtration. Lead contamination of sample frequently occurs in laboratory and it is often not easy to keep under control.

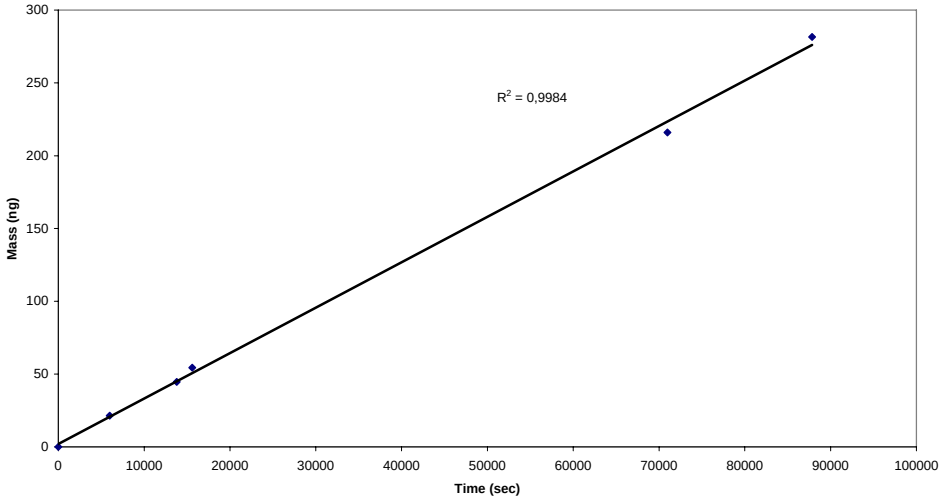
	Mn		Co		Ni		Cu		Zn		Cd		Pb	
time	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C D
sec	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg
6000	1187	1636	6.6	9.0	21.5	29.7	2.6	3.7	7723	10560	41.1	56.1	2.1	2.
13800	2553	1484	14.0	8.1	44.5	25.9	4.2	2.4	16967	9783	88.6	50.9	3.1	1.
15600	3163	1720	17.3	9.4	54.3	29.6	4.8	2.6	20484	11039	108.2	58.3	3.7	1.
70980	11480	1338	66.2	7.7	215.9	25.2	10.3	1.1	80766	9336	417.0	48.1	22.2	2.
87840	14799	1393	87.9	8.3	281.6	26.6	14.9	1.4	104441	9751	536.5	50.0	27.8	2.

Table 28. January sampling campaign. DGT deployment time, accumulated mass of the target metals (Mn, Co, Ni, Cu, Zn, Cd, Pb) and calculated DGT concentrations.

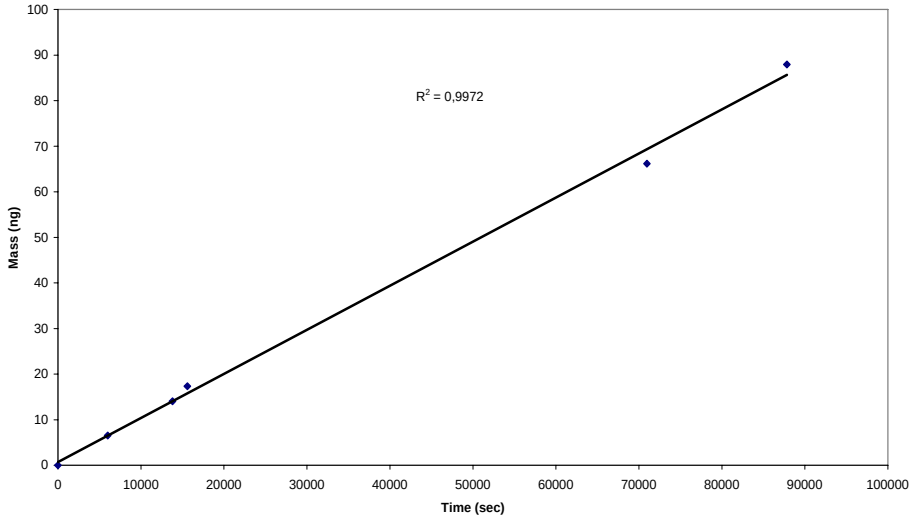
Mn
January 2006



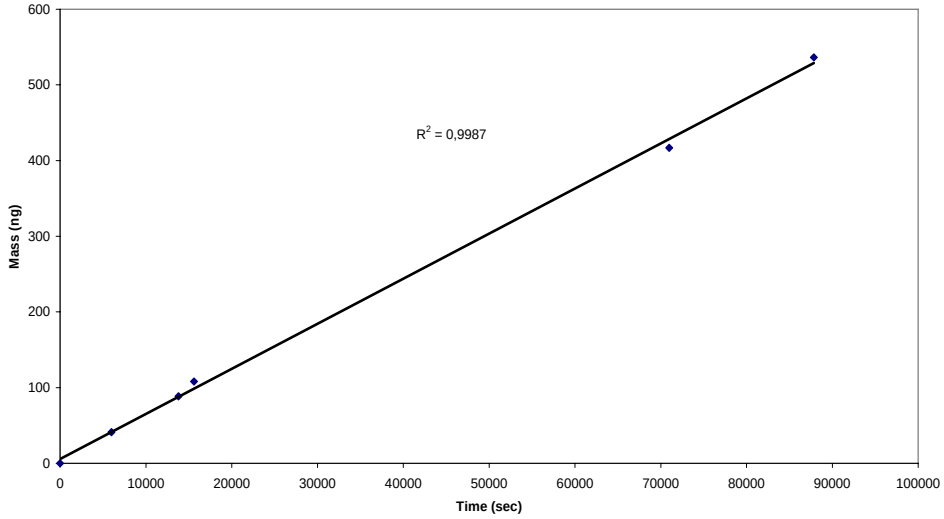
Ni
January 2006

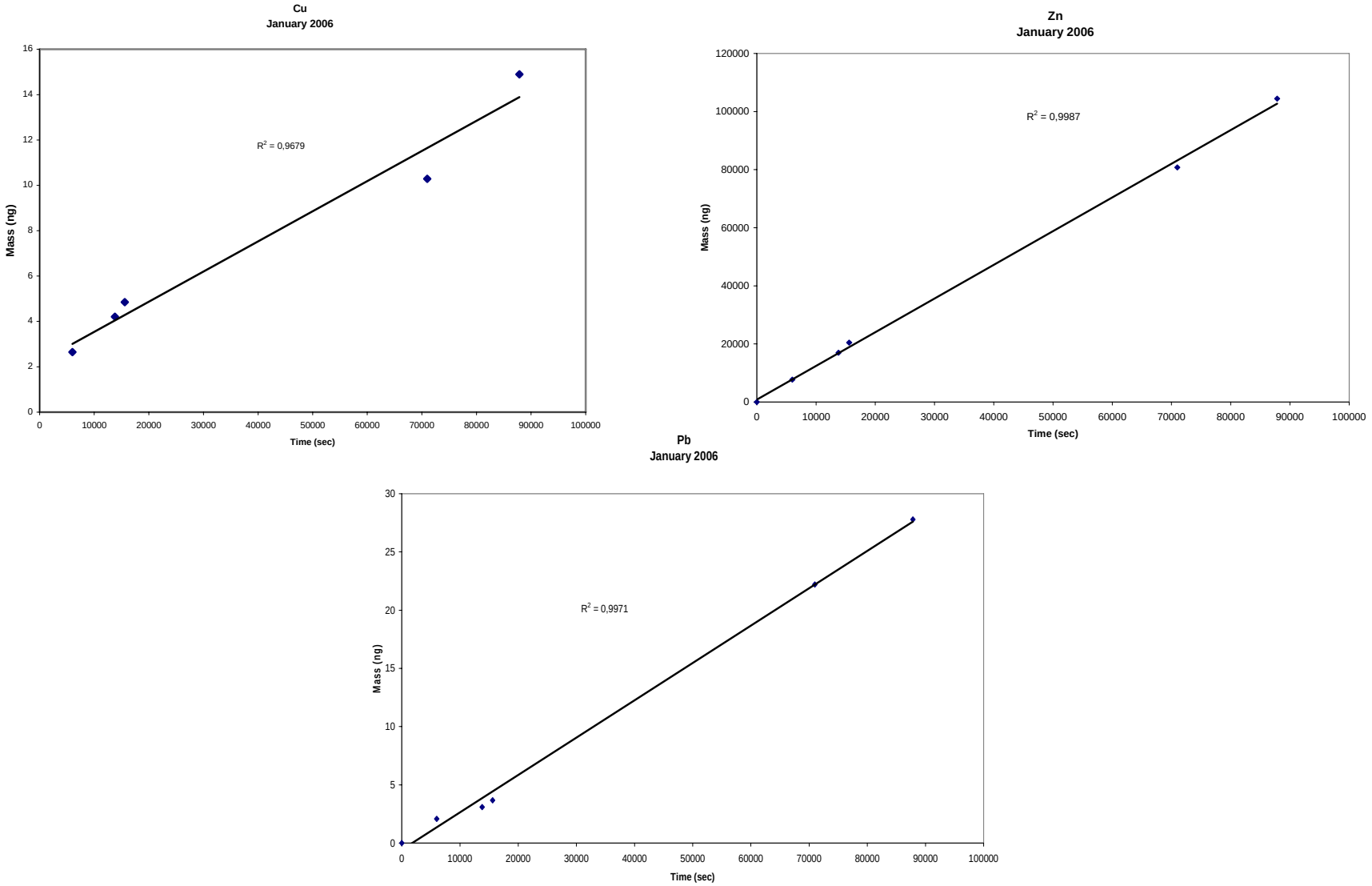


Co
January 2006

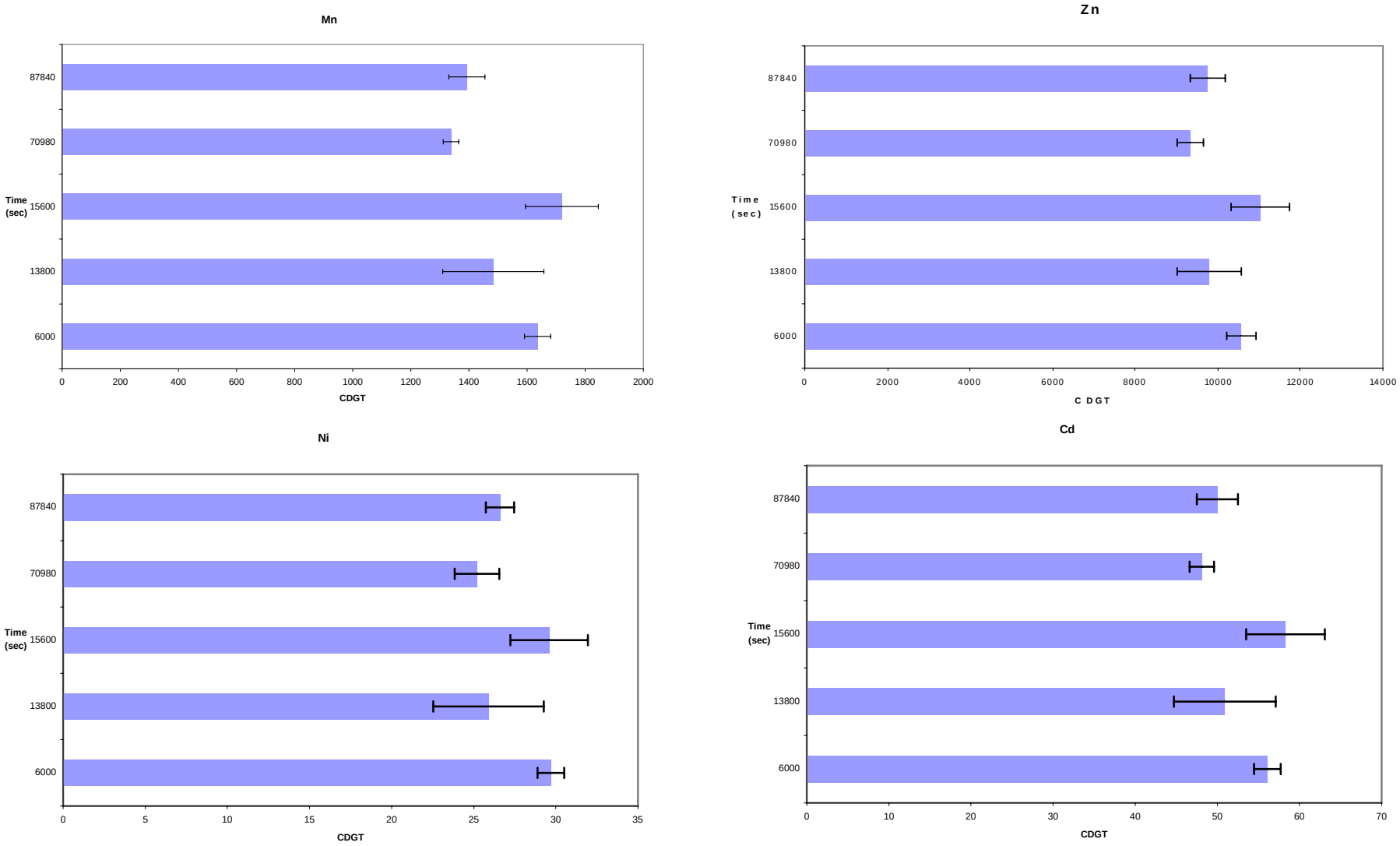


Cd
January 2006

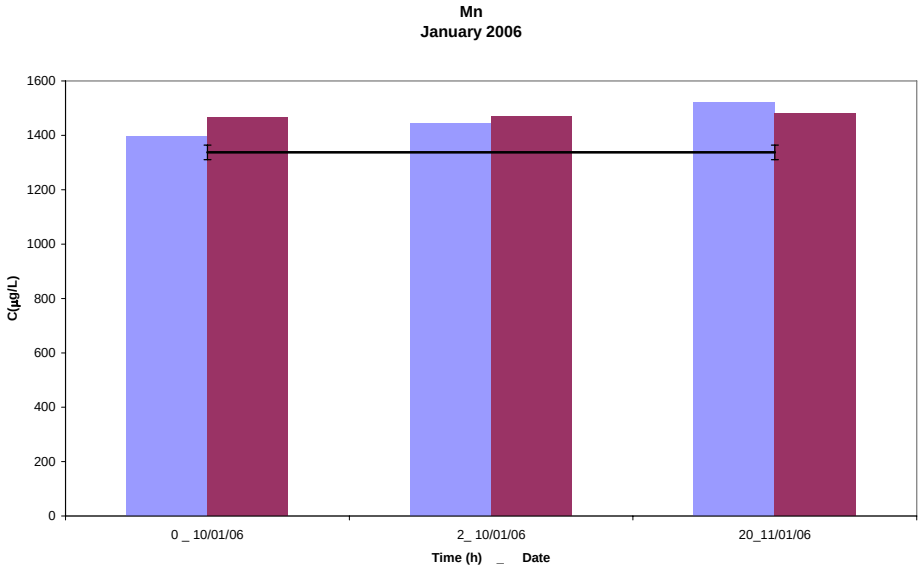


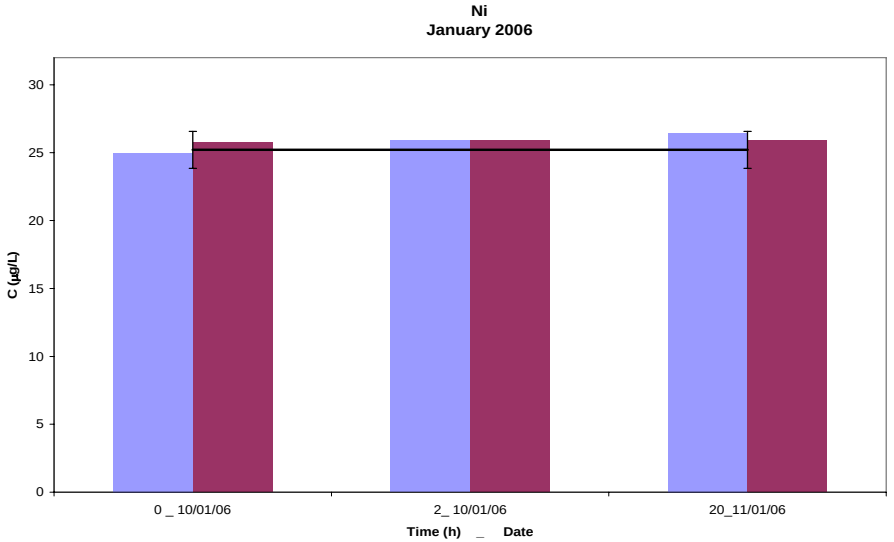


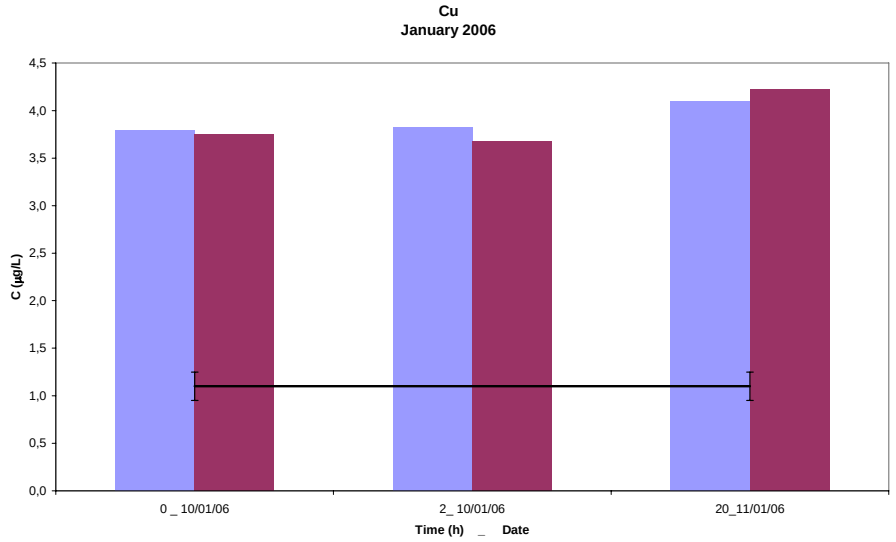
Figures 59-65. Accumulated mass of the target metals (Mn, Ni, Co, Cu, Zn, Cd, Pb) versus deployment time. The correlation factors are reported.

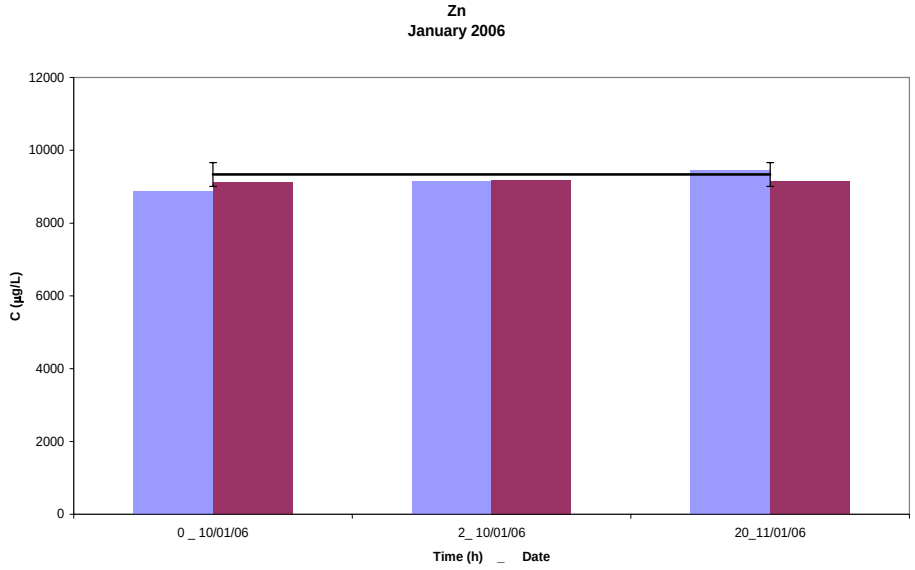


Figures 66-69. Calculated DGT metals concentration integrating on different period.


Mn
January 2006
C(µg/L)
1600
1400
1200
1000
800
600
400
200
0
0 _ 10/01/06
2_ 10/01/06
20_11/01/06
Time (h) _ Date


Ni
January 2006
C (µg/L)
30
25
20
15
10
5
0
0 _ 10/01/06
2_ 10/01/06
20_11/01/06
Time (h) _ Date


Cu
January 2006
C (µg/L)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0 _ 10/01/06
2_ 10/01/06
20_11/01/06
Time (h) _ Date


Zn
January 2006
C (µg/L)
12000
10000
8000
6000
4000
2000
0
0 _ 10/01/06
2_ 10/01/06
20_11/01/06
Time (h) _ Date

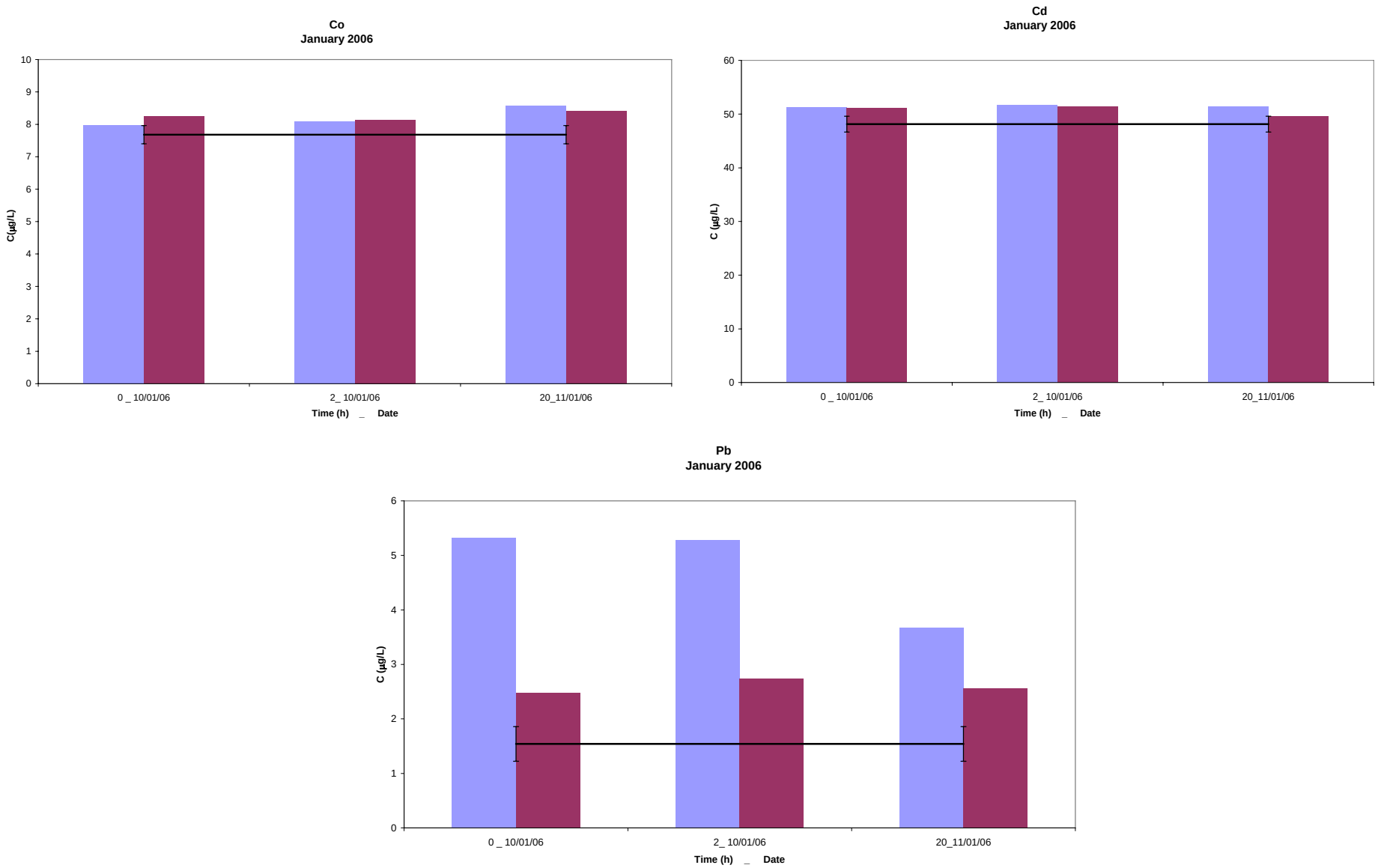


Figure 70-76. Metal concentrations measured by DGT (t=20 hours-represented by a line) and by on site filtration using filters of different pore sizes [0.45 µm (blue)- 0.2 µm (violet)]. Error bars give the standard deviation of 5 replicate DGT devices. The filtration results are shown as a single bar at the sampling time.

Second sampling campaign (May 2006)

Table 29 reports the accumulated mass of the target metals (Mn, Ni, Co, Cu, Zn, Cd, Pb) versus deployment time. According to the theory, the mass of metals in the resin layer increases linearly with time providing good correlation factors (R^2) except for lead (R^2 =0.88). Also in these cases, although the longer deployment time up to 2 days, saturation was not reached. Figures 77-82 show the concentrations of the target metals measured by DGT and by on site filtration using filters at 0.45 μm. DGT results are shown by a line that reflects the whole deployment time of 52 h. Error bars give the standard deviation of 5 replicate DGT devices (time≅52h). In particular, the figures report the direct comparisons of DGT and filtration occurred at t=0h, t=24h and t=52h during the 52 h DGT deployment.

Disagreement between Mn and Zn concentrations measured by DGT and by 0.45 μm filtration suggests that some problems have occurred since DGT metal concentrations should not be higher than filtrate concentrations, according to the theory. A possible explanation could be metal contamination of DGT samples or a sorption of the metals to the filter or to solids retained by it.

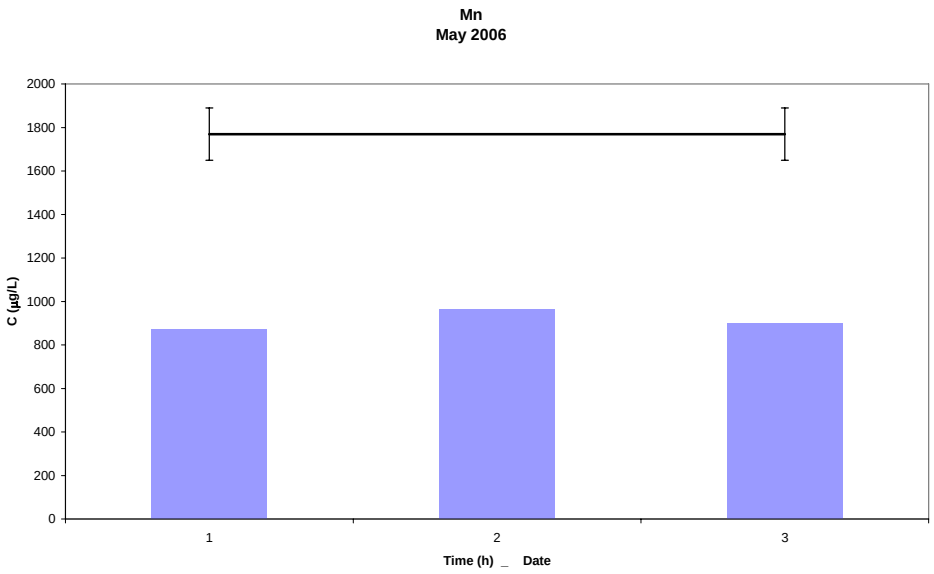
In general, a more significant complexation of metals is observed with respect to January sampling campaign.

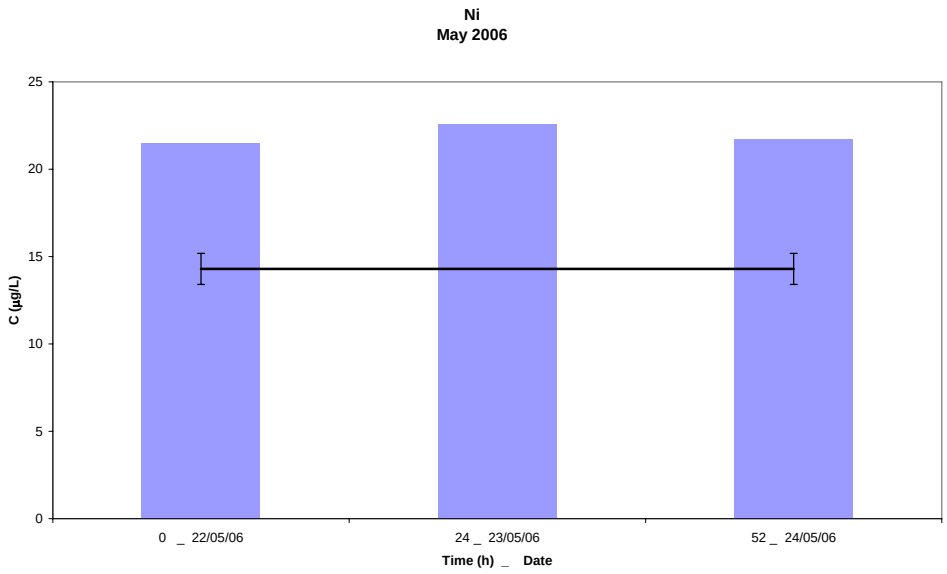
DGT measured only ¼ of the Cu present indicating also in this case that a considerable proportion might be present as complex with natural organic ligands. The filtrate concentrations reveal a change with time during the 52 hours, rising over 24 h and then falling again contributing to the disagreement with DGT results which integrates the metal concentration throughout the deployment period. A change with time is revealed also using DGT for various times of deployment indicating a great variability during time shown by Cu concentration in the river.

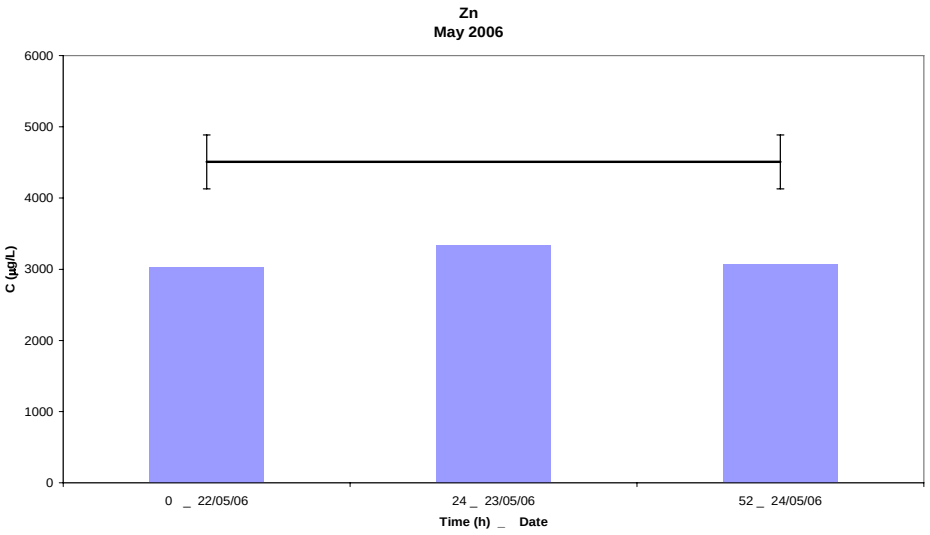
During the January sampling campaign a good agreement was found between Cd measured using DGT and filtration systems. On the contrary, in May, DGT devices measured about one order of magnitude less than filtration indicating in this case a considerable complexation.

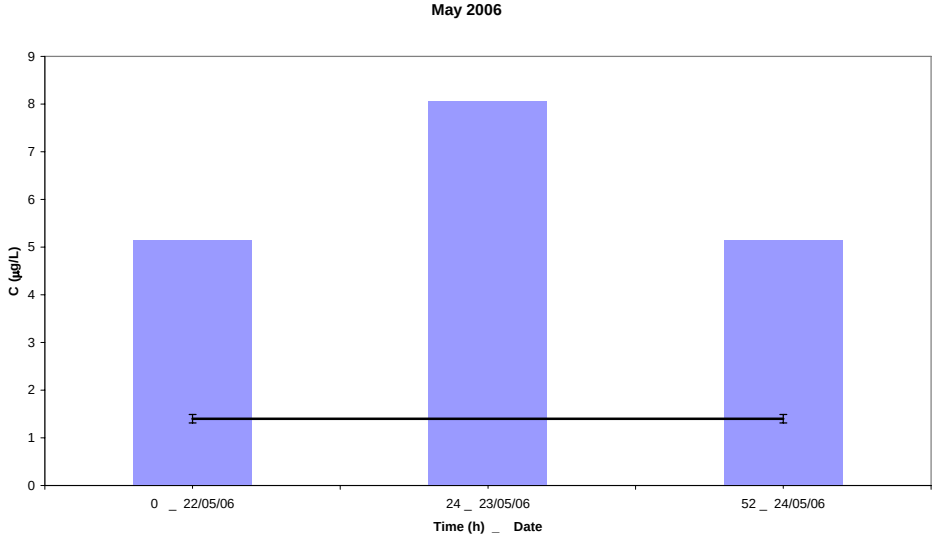
	Mn		Co		Ni		Cu		Zn		Cd		Pb	
Time	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C DGT	M	C D
sec	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg/L	ng	µg
20100	4688	1929	13.9	5.7	35.0	14.4	16.8	4.1	10584	4355	6.1	2.5	1.7	0.
84300	18369	1802	56.0	5.5	146.5	14.4	25.0	2.5	42916	4210	26.4	2.6	3.6	0.
105420	21762	1707	68.7	5.4	169.4	13.3	25.0	2.0	51953	4075	26.5	2.1	12.3	1.
168960	35410	1733	115.6	5.7	287.5	14.1	30.6	1.5	87709	4293	51.5	2.5	23.5	1.
188460	40331	1770	133.5	5.9	325.5	14.3	33.0	1.4	102734	4508	57.8	2.5	9.0	0.

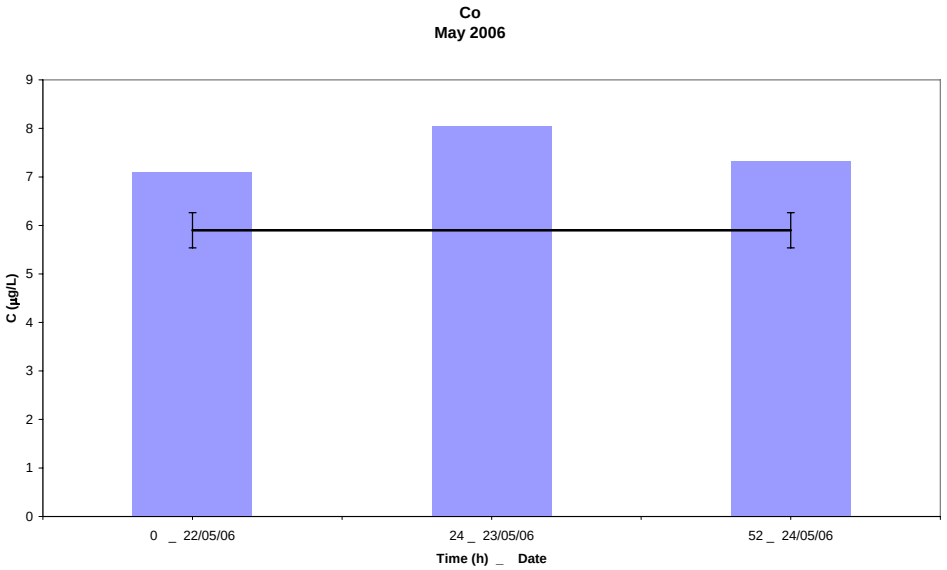
Table 29. May sampling campaign. DGT deployment time, accumulated mass of the target metals (Mn, Co, Ni, Cu, Zn, Cd, Pb) and calculated DGT concentrations.

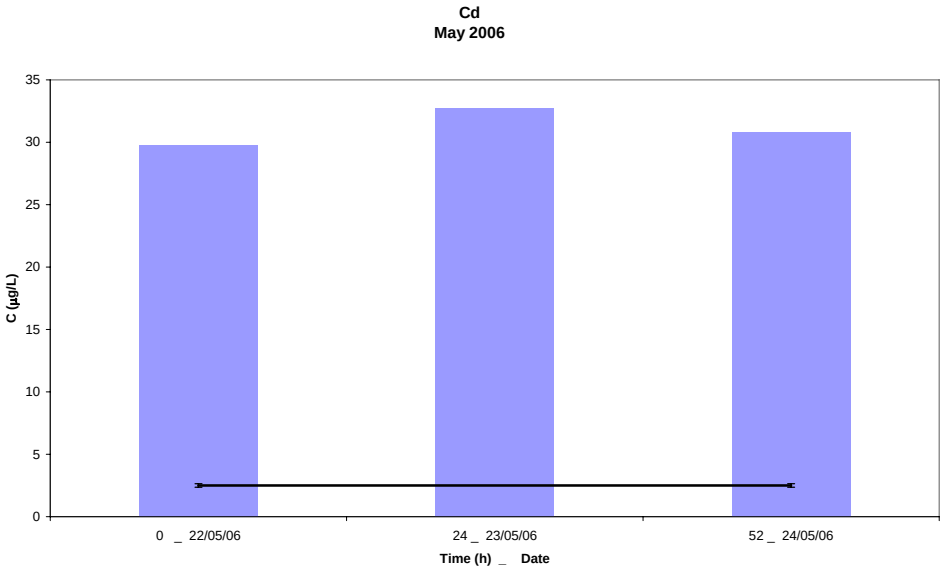

Mn
May 2006
C (µg/L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
Time (h) _ Date


Ni
May 2006
C (µg/L)
25
20
15
10
5
0
0 _ 22/05/06
24 _ 23/05/06
52 _ 24/05/06
Time (h) _ Date


Zn
May 2006
C (µg/L)
6000
5000
4000
3000
2000
1000
0
0 _ 22/05/06
24 _ 23/05/06
52 _ 24/05/06
Time (h) _ Date


Cu
May 2006
C (µg/L)
9
8
7
6
5
4
3
2
1
0
0 _ 22/05/06
24 _ 23/05/06
52 _ 24/05/06
Time (h) _ Date





Figures 77-82. Concentrations of the target metals measured by DGT (t=52 h represented by a line) and by on site filtration using filters at 0.45 µm. Error bars give the standard deviation of 5 replicate DGT devices. The filtration results are shown as a single bar at the sampling time.

Monitoring of metals concentration in the Sitzerri River

The river was monitored throughout an intensive sampling for about 4 months (from February to May). Water samples were filtered at 0.45 μm on site and analysed by ICP-MS. Table 30 reports the metal concentrations determined in the filtered river waters during the experiment:

Date	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
03/02/2006	2.3	738	197.3	9.9	32.1	20.2	2107	1.8	47.3	13.7
07/02/2006	1.1	1520	157.3	18.8	59.4	19.6	6557	2.7	105.7	15.6
14/02/2006	1.3	1628	304.4	20.5	66.3	10.8	9866	4.3	129.7	39.6
17/02/2006	0.9	1628	174.6	20.2	66.0	13.4	7657	2.9	115.9	17.5
23/02/2006	1.5	980	330.4	12.3	44.0	20.3	6974	6.0	78.0	36.9
27/02/2006	1.2	950	228.3	12.4	44.5	14.2	7414	6.3	75.5	16.5
06/03/2006	1.8	1344	282.3	19.6	57.3	10.7	5506	1.7	94.5	19.6
09/03/2006	2.9	549	760.9	8.8	29.3	18.5	4153	2.0	63.4	87.4
13/03/2006	2.5	509	586.9	8.1	27.0	16.6	3916	1.8	57.2	75.1
16/03/2006	1.6	676	343.6	11.0	34.7	11.9	5027	2.4	81.6	47.2
20/03/2006	0.8	684	187.2	11.0	34.8	9.7	3951	1.9	72.6	23.0
24/03/2006	1.2	1240	647.8	19.0	60.1	13.9	17060	7.4	193.2	98.6
22/03/2006	0.1	1172	151.5	17.0	51.6	11.0	5022	2.0	92.7	14.9
30/03/2006	0.1	1453	254.6	20.7	64.7	8.0	7978	3.4	130.8	24.1
02/04/2006	0.1	1428	217.9	20.0	62.7	7.6	7547	3.0	120.5	20.8
04/04/2006	0.1	1544	256.3	20.3	67.2	6.0	8519	3.7	119.9	18.9
06/04/2006	0.1	1376	161.4	20.0	59.8	11.2	6537	3.2	81.2	12.0
07/04/2006	0.3	1535	236.1	20.4	67.1	6.3	8368	3.5	113.4	16.5
10/04/2006	0.1	1524	198.1	20.2	66.0	8.7	7176	3.7	84.4	12.9
13/04/2006	0.1	1506	270.1	18.1	63.7	5.8	8285	5.7	100.7	15.5
18/04/2006	< DL	1435	66.6	16.3	60.8	11.5	6830	5.8	19.0	14.0
19/04/2006	0.8	1244	305.9	15.5	55.1	14.7	8936	6.8	83.0	26.8
21/04/2006	0.4	1422	202.5	17.7	60.4	9.7	7856	6.7	104.7	15.1
26/04/2006	0.3	1263	183.3	15.2	53.9	11.8	7578	5.7	66.7	14.0
02/05/2006	0.1	1199	74.6	14.0	48.9	10.3	3926	3.0	35.9	3.9
04/05/2006	0.1	1394	194.0	13.5	57.7	8.9	5868	3.9	50.0	10.7
05/05/2006	0.3	1368	214.3	13.6	57.5	14.0	7093	5.1	56.0	12.8
06/05/2006	0.2	1232	85.6	14.4	51.8	15.5	6560	5.9	41.5	4.7
15/05/2006	0.3	899	311.7	7.9	35.1	9.0	5275	9.1	44.6	25.8
19/05/2006	0.3	893	303.6	7.4	22.0	4.8	3188	12.7	31.8	29.0
22/05/2006	0.3	870	298.0	7.1	21.5	5.1	3034	12.4	29.8	26.4
23/05/2006	0.3	965	333.3	8.0	22.6	8.1	3333	12.2	32.8	30.9
24/05/2006	0.2	897	308.3	7.3	21.7	5.1	3072	11.7	30.8	28.6

Table 30. Metal concentrations determined in the river waters (from February to May 2006) after on site filtration.

Conclusions

In situ measurements of Mn, Ni, Co, Cu, Zn, Cd, Pb by diffusive gradients in thin films (DGT) in a river near a mine were compared to results from on site filtration. Two sampling campaigns (in winter and spring) were carried out in cooperation with ARPA Sardinia. During winter, the two techniques agreed for most metals, indicating an absence of colloids and negligible complexation by organic matter. Substantial differences between DGT and on site filtration were found for Cu and were consistent with complexation by organic matter.

In general, a more significant complexation of metals is observed during spring with respect to winter sampling campaign. During this period, Co and Ni measured by on site filtration were slightly higher than DGT measurements, appropriate to only partial complexation.

Some technical hitches occurred for Mn and Zn providing that DGTs were greater than the filtrate concentrations in contrast to the DGT theory. Significant differences between DGT and on site filtration were found for Cu and Cd meaning that a considerable proportion of metals might be present as complex. Variable extent of metal complexation and availability was found during the two sampling campaigns.

The river was monitored throughout an intensive on site filtrations during 4 months demonstrating substantial variability in metal concentration as well as in metal complexation as revealed trough DGT measurements. This aspect could be predictable if considering the variability intrinsic to a river compartment near a mine as a potential source of heavy metal inputs.

This work demonstrates the potential of parallel in situ measurements though in term of cost, simplicity and risk of contamination the on site filtration is slightly superior to DGT.

While the use of two in situ techniques provide useful information, further refinements are required to complete characterize the natural water. The use of DGT with a more restricted gel that excludes complexes with humic substantives should provide additional complementary information to in situ filtration. Speciation models like WHAM could be useful to predict the extent of metal complexation and to provide information to which compare the in situ determinations.

2. Metals bioaccumulation in fish

The bioavailability and toxicity of metals to aquatic biota have been examined extensively using a variety of test organisms including phytoplankton, bivalves, crustaceans and fish. In metal contaminated sites, metals can accumulate within cells and tissues of organisms, which could result in effects deleterious to cellular function. Metal coordination sites in cells are rarely entirely specific for a single metal, and therefore surface sites designed to bind nutrient metals will also bind no-essential and potentially toxic metals with similar ionic radii and coordination geometry.

The deleterious effects are variable, but are generally expressed as mortality, decreased growth rate, decreased fecundity and decreased metabolic activity.

The importance of understanding the mechanisms behind the bioaccumulation of toxic chemicals in biota is generally recognized among scientists. However, the mechanistic explanations for these processes are highly debated, and currently unresolved.

The potential of a chemical to bioaccumulate in organisms is dependent upon the properties of the chemical (e.g. hydrophobicity, lipophilicity, and resistance to degradation), biotic factors (growth, way of feeding, reproductive condition, metabolism, excretion), metals bioavailability, environmental factors (e.g. salinity, temperature, concentration of other organic chemicals and redox potential) [76-77].

For istance, concerning salinity, generally metals become more toxic as salt content of the water decreases. Cd (bound to chloride ion) becomes more toxic at low salinity because in this situation it is transformed into free ion. Temperature also affects the toxicity of some metals, and for instance lethality of Cd is higher at high temperatures. Bioaccumulation is also affected, high Cd uptake rates at 20ºC exceed those found at 15ºC in tissues of molluscs.

Material and methods

In this work, metals bioaccumulation in juvenile **Cyprinus Carpio** fish exposed to Sitzerri river waters was investigated.

Firstly, ARPA and APAT skilled personnel tried to locate Plexiglas cages containing fish directly in the Sitzerri river. The aim was to find correlation between metals bioavailability as measured by DGT and metals bioaccumulation in fish. Unfortunately, various technical hitches got out of working "in situ". Afterwards, tanks filled with Sitzerri river waters were located in the ARPA laboratory under controlled conditions. Fish were placed in the tanks and fed with the same commercial feed. Twice or once a week water collected from Sitzerri river was renewed in all tanks containing fish. In particular, twelve containers of 10 litres of capacity containing 7 fish each have been installed: 2 tanks containing 0.45 μm-filtered waters from Sitzerri river; 2 tanks containing not filtered waters from Sitzerri river; 2 tanks containing filtered waters from Sitzerri river diluted with not contaminated water (75%:25%); 2 tanks at 50%:50%; 2 tanks at 25%:50% and 2 tanks containing not contaminated waters (control blanks). Water properties were daily recorded in all tanks. The experiments last from February to the end of May 2006.

At the end of the experimentation, fish were caught, killed with a blow to the head. Metal content in fish tissue was determined by ICP-MS subsequent to freeze drying and acid mineralization. Three independent replicates were performed for each tank (6 replicates for each dilution level). The determination of metals content was performed according to a method validated in the APAT laboratory. Method validation was focused in particular on As, Se and Pb and is reported in the following section together with the uncertainty budget.

Results and discussion

The mean results together with the standard deviations of metal content found in fish tissues (dry weights) are reported in the following table 31. The results for each

dilution level were pooled (6 replicates). In case of Co and As the amount of accumulated metals does not increase with water contamination level. Co content show just a slight increase at 50% level and then it remains constant up to 100%. These outcomes could be attributed to a number of factors that can influence the accumulation of metals by organisms including excretion processes. Actually, the body content of a trace metal in a given organism results from the net balance between the processes of metal uptake and metal loss.

Cu exhibited an anomalous trend: at 75%-100% (not filtered), the accumulated metal is lower than the blank value. A possible explanation is difficult to assess on the basis of the available information. Further trials should be performed. Notably, DGT determinations on the river water revealed that a considerable proportion of copper might be complexed reducing Cu bioavailability.

Concerning Cd and Pb, fish show an increasing amount of the accumulated metals at increasing of the metal concentration in solution. In other words, fish bioaccumulate these metals in their tissues under the test conditions. Metal bioaccumulation could be attributed to the metals concentration in water, considering that the fish were fed with the same commercial feed, the fish age and exposure time being the same as well as the environmental conditions.

Metal concentrations in case of not filtered water are very scattered and exhibit high standard deviations. This could be attributed to the presence of particulate metal fractions in the not filtered water. In fact, it is well recognized that the suspended particulate matter represents an important vehicle of metal contamination. The presence of particulate matter in the water of tanks could be responsible for a great variability of the results for example due to metal adsorption/desorption processes or due to particulate fish ingestion. Due to the great standard deviations the results relevant to the not filtered waters for Cd and Pb must be managed carefully.

Sample	**Co**	**sd**	**Cu**	**sd**	**As**	**sd**	**Cd**	**sd**	**Pb**	**sd**
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Blanks	0.09	0.01	5.60	0.36	0.43	0.01	0.13	0.02	0.57	0.03
25%	0.11	0.02	6.39	0.37	0.58	0.04	1.18	0.01	0.65	0.14
50%	0.18	0.01	5.57	0.72	0.58	0.09	1.91	0.24	0.80	0.06
75%	0.17	0.01	4.14	0.37	0.48	0.03	2.39	0.27	0.97	0.03
100%	0.15	0.01	RSD>30 %		0.64	0.05	3.12	0.59	1.12	0.13
100% not filtered	0.19	0.06	3.03	0.14	0.68	0.11	4.35	1.86	1.94	0.82

Table 31. Mean results and standard deviations of metal content in fish tissues.

Particulate matter represents an important sink and vehicle of metal contamination even though its sampling in aquatic environment could represent a great source of uncertainty to the final analytical results.

Conclusions

Fish living in polluted waters tend to accumulate heavy metals in their tissues. Environmental metal level is not the only factor affecting the metal content of aquatic organisms. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions and intrinsic factors (fish age, feeding habits). In this work, juvenile fish of Cyprinus Carpio were maintained in tanks filled with the contaminated water at increasing concentration level. The time of exposure, environmental conditions, intrinsic factor and feeding were kept as much as possible homogeneous among the different tanks. Fish show bioaccumulation of Cd and Pb in their tissues under the test conditions that could be attributed to the metals concentration in water. For Co, As and Cu other processes like excretion seem to significantly influence the net metal accumulation in fish.

To correct evaluate and interpret environmental contamination and metal uptake in living organisms, data reliability is of crucial importance. In this context, a method to accurately determine metals in fish was validated (section 8).

SECTION 8. Method validation for metal determination in fish and uncertainty budget

Summary

As reported in section 7 (2), the importance of a validation of a method to accurately determine metals in fish tissue has been recognized.

Content of As, Se and Pb in fish has been determined by microwave oven digestion and analysis with ICP-MS equipped with a quadrupole detector and an octopole reaction cell. The method has been validated using 2 matrix certified reference materials ("BCR422-Trace elements in cod muscle" and "NRCC - Dorm-2 trace metals in dogfish muscle") and a Tuna fish reference material supplied by ENEA. Digestion conditions, repeatability, reproducibility, recovery, limit of detection, limit of quantification and robustness have been studied.

The validated method has been applied to a reference material based on salmon produced by Institute for Reference Materials and Measurements (IRMM - Belgium) and distributed to European Metrological Institutes during an intercomparison exercise (named CCQM P39-1 intercomparison).

Material and Methods

As, Se & Pb determination (microwave digestion and ICP-MS)

Content of As, Se and Pb in fish has been determined by microwave oven digestion and analysis with ICP-MS equipped with a quadrupole detector and an octopole reaction cell.

Reagents and Gases

All reagents deionized water (18 MOhm·cm), 70% nitric acid and 30% hydrogen peroxide were of ultrapure grade. Argon, hydrogen and helium were 99.999% in purity.

Instrumentation

Determination: Agilent 7500c ICP-MS equipped with octopole collision cell, Babington nebulizer, standard spray chamber, Cetac ASX 500 autosampler. Digestion: CEM MARS5 microwave oven.

Water Content

Sample portion of about 0.6 ± 0.1 g is weighed and dried in a ventilated oven at 102 ± 2°C for 24 hours. Cycles of drying and weighing have been repeated until a constant mass with difference less then 0.001 g is attained. The following equation has been applied:

$$water\ content\ (\%) = \frac{W_{fresh} - W_{dry}}{W_{fresh}} \times 100$$

Digestion

A sample of 0.500 ± 0.01 g has been weighed in a pre-cleaned Teflon vessel; then 6 mL of HNO_3 and 1 mL of H_2O_2 were added. Digestion blanks: 6 mL of HNO_3 and 1 mL of H_2O_2 in a vessel. The closed vessels has been placed in a CEM MARS5 microwave oven using the mineralization programme reported in Table 32.

Table 32. *Mineralization programme*

	Power	**Control pressure**	**Duration time (min)**
Step 1	250 W	200 psi	1:00
Step 2	0 W	200 psi	1:00
Step 3	250 W	300 psi	5:00
Step 4	450 W	400 psi	5:00
Step 5	650 W	530 psi	5:00

At the end, after cooling, sample has been recovered in a 50 mL class B, PMP volumetric flask by washing the vessel with ultrapure water. 0.5 mL of a rhodium solution (1 μg mL^{-1}) is added as internal standard (rhodium concentration in the sample =10 ng mL^{-1}). After this addition, ultrapure water is added until 50mL.

The rhodium solution of 1 μg mL^{-1} has been prepared from 1000 μg mL^{-1} stock solution of rhodium by dilution of 0.1 mL in a 100 mL volumetric flask.

ICP-MS Determinations

Calibration standard solutions are daily prepared from a working standard solution containing 5 μg mL^{-1} of As, 1 μg mL^{-1} of Se and 0.1 μg mL^{-1} of Pb. The working standard solution has been prepared from 1000 μg mL^{-1} stock solutions of arsenic, selenium and lead by dilution with ultrapure water of respectively 0.5 mL of As, 0.1 mL of Se and 0.01 mL of Pb in a 100 mL volumetric flask. Calibration curve has been determined on 6 points for each element, in a range from 0 to 200 ng mL^{-1} for As, 0 to 40 ng mL^{-1} for Se and 0 to 8 ng mL^{-1} for Pb.

The ICP-MS is optimized daily with a tuning solution at 10 μg L^{-1} of Li, Ce, Y, Tl. Optimization is performed using normal mode and collision cell mode.

A typical analytical run after optimization of the ICP-MS consists of calibration standards, blanks, samples, including CRM, and calibration standard again for checking any drift of the instrument.

The ^{75}As and ^{78}Se and ^{208}Pb isotopes were chosen for the analysis; further As and Se were analysed with octopole reaction cell mode in order to avoid isobaric interferences.

The equation used for analytes determination is:

$$C = C' \times D \times \frac{1}{R} \times F$$

where C' is the concentration of the sample solution as read from the calibration curve (in terms of weight-by-volume), D is the dilution factor applied to the sample (volume by weight), F is the dry mass correction as defined in chapter "ICP-MS Uncertainty budget", and R is the recovery factor (measured concentration in CRM divided by certified concentration in CRM). The recovery factor is a correction assumed to be 1 in the calculation of the analyte concentrations. The uncertainties in the correction factor will be included in the estimation of the overall uncertainty. The full results are reported in the next paragraphs.

ICP-MS Validation studies

As, Se and Pb determination in fish tissue has been validated using 2 matrix certified reference materials ("BCR422-Trace elements in cod muscle" and "NRCC - Dorm-2 trace metals in dogfish muscle") and a Tuna fish reference material supplied by ENEA. Digestion conditions, repeatability, reproducibility, recovery, limit of detection, limit of quantification and robustness have been studied.

Digestion

Different acid mixtures (HNO_3, HNO_3+H_2O_2, HNO_3+HCl) and different microwave digestion programmes have been tested on samples of 0.5 g of dogfish CRM. The best recovery has been obtained with the acid mixture 6 mL of HNO_3 and 1 mL of H_2O_2 and the digestion programme reported in Table 32.

Repeatability and Recovery

Repeatability and recovery have been evaluated by repeated independent analyses (including digestion and instrumental measurements) for each element by only one operator in the same day. Table 33 reports data from CRM measurements. The observed mean recovery (ratio of observed concentration by certified concentration) ranged from 101 to 103% for As and Se in both CRM, while for Pb in DORM-2 was 110%. However, the observed concentration value did not exceed the certified range of this CRM. Arsenic and Se repeatability ranges from 1.4 to 3.7%, while for Pb is about 6.0%. The lower repeatability observed for Pb could be due to the lower concentrations in both CRMs.

Table 33. *Repeatability and recovery data*

	DORM-2			BCR 422		
Element	**As**	**Se**	**Pb**	**As**	**Se**	**Pb**
Mean value (mg kg^{-1})	18.35	1.41	0.072	21.49	1.68	0.086
Standard deviation (mg kg^{-1})	0.495	0.053	0.005	0.298	0.03	0.005

Mean recovery (%)	102	101	110	102	103	101
CV(%)	2.7	3.7	6.5	1.4	1.7	6.0
N of measurements	13	13	11	9	9	8

Further the repeatability limit "r", reported in the following table, was calculated from the equation:

$r = 2.8 \times S_r$

where S_r is the standard deviation of the BCR422.

element	r (mg/kg)
As	0.834
Se	0.084
Pb	0.014

Reproducibility

Reproducibility has been evaluated by a sequence of repeated independent analyses for each element on BCR422 cod muscle by three different operators. Each operator performed the analytical measurements in a different day with respect to the others. CCQM salmon sample showed concentration level of Pb higher than BCR422. In order to have data for the same Pb concentration range of CCQM sample, a further step for reproducibility was done. So the same procedure applied for BCR422 has been repeated with a tuna fish RM (not certified). Table 34. reports coefficient of variations (CV%) for each operator.

Table 34. Coefficient of variations (CV%) for each operator.

	BCR422				**Tuna RM**	
operator	**As**		**Se**		**Pb**	
	CV (%)	**n**	**CV (%)**	**n**	**CV (%)**	**n**
A	1.4	9	1.7	9	3.5	10

B	1.4	7	3.1	7	1.8	7
C	1.6	11	2.0	11	3.0	7

Further the reproducibility limit "R" was calculated by the equation:

$R = 2.8 \times S_R$

where S_R is the standard deviation of reproducibility assessed in chapter "ICP-MS Uncertainty budget".

element	R (mg/kg)
As	3.421
Se	0.411
Pb	0.104

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) of the analytical method have been evaluated analysing ten independent digestion blanks and a 10 μg L^{-1} solution of every element; this type of blanks have been prepared by addition of 6 mL of HNO_3 and 1 mL of H_2O_2 in the vessels and after digestion they have been diluted to 50 mL in volumetric flasks with ultrapure water.

The LOD has been calculated as three times the standard deviation (σ) of signal counts of ten mineralization blanks divided by counts per unit concentration, based on analysis of a standard solution at concentration (C). The integration time was 10 seconds. This procedure has been done for As, Se and Pb.

The equation for calculating the LOD is reported below:

$$LOD = \frac{3\sigma * [C]}{S - B}$$

where :

(σ)= standard deviation in counts of ten readings of 10 digestion blank solutions

S= counts of ten readings of 10 μg L^{-1} solution

B= mean counts of ten readings of 10 digestion blank solutions

C= concentration of a standard solution of the element expressed in ppb (10 μg L^{-1})

The same equation with 10 σ instead of 3σ has been used for the evaluation of LOQ. The LODs and LOQ of the method are:

element	LOD (μg L^{-1})	LOQ (μg L^{-1})
As	0.0136	0.0450
Se	0.0146	0.0487
Pb	0.0170	0.0560

Robustness

Method robustness has been evaluated taking into account one of the main factors affecting the measurements: the dilution factor. Normally sample analysed with this method are diluted by a factor of 100 (volume/weight) after digestion. Different dilution factors have been applied on a BCR422 sample solution coming from digestion: after the normal dilution of the sample an aliquot has been diluted 1:3 for the analysis of As, Se and Pb. In this way it was possible to investigate the effect of different dilutions on measurements.

The results reported in Table 35 show that the dilution factors do not affect measurement results.

Table 35. *robustness data*

	As		Se		Pb	
DILUTION	1:100	1:300	1:100	1:300	1:100	1:300
Concentration CRM (mg kg^{-1})	21.1	21.1	1.63	1.63	0.085	0.085
Mean (mg kg^{-1})	19.83	21.35	1.526	1.673	0.069	0.072
Standard deviation (mg kg^{-1})	0.326	0.384	0.030	0.036	0.006	0.008
Relative standard deviation	1.64	1.80	1.98	2.17	8.99	10.57
N of measurements	11	11	11	11	8	7

ICP-MS Uncertainty budget

Identification of sources uncertainty

Concentration of each element is given by:

$$C = C' \times D \times \frac{1}{R} \times F \qquad (1)$$

where C' is the concentration of the sample solution as read from the calibration curve (in terms of weight-by-volume), D is the dilution factor applied to the sample (volume by weight), F is the dry mass correction and R is the recovery factor. This term is a correction factor assumed to be a unity in the calculation. Uncertainties in the correction factor must be included in the estimation of the overall uncertainty.

The sources of uncertainty for the method were identified by constructing a cause and effect diagram [78]. The 'effect', represented by the main horizontal branch in the diagram (Fig.83), is the result of the analysis, i.e. the concentration of arsenic (in terms of weight-by-weight). The main 'cause' branches represent the main parameters controlling the result.

Concentration – C'

The concentration branch C' shows two major contributions:

1. $f(I_{ref},C_{ref},I_{sample})$: represents the application of the calibration function obtained from a series of reference concentrations C_{ref} and observed intensities I_{ref}, to the observed sample intensity, I_{sample}, in order to obtain the interpolated concentration curve for the solution. The uncertainties associated with the calibration function are about the ratio counts for the standards and sample (I_{ref}, and I_{sample}, respectively) which are affected by the instrument performance. The instrument is calibrated for each batch of samples with a fresh set of calibration standards. Therefore, systematic effects relating to the instrument performances should cut out as they will be the same for both, the calibration standards and the sample solutions [79]. The calibration standards are prepared by diluting a working standard C_{dil} on a volume-by-volume basis.

   If a drift of >10% is observed, then the instrument is recalibrated and the samples reanalysed.

   The uncertainty associated with the maximum permitted drift needs to be included in the budget.

   The contribution to the overall uncertainty from the run-to-run variability of the instrument performance should be included in an estimate of the overall precision of the method.

2. C_{dil}: represents the concentration of the dilute working standard from which the calibration solutions are prepared. This solution is prepared by diluting the stock solution on a volume-by-volume basis. The only uncertainties associated with the concentration of the dilute working standard (apart from run-to-run variations in preparing the standard) which need to be considered are the uncertainties about the stock solution (C_{stock}) (w/vol) and the uncertainties about micropipette and the volumetric flask.

Precision – P

The precision branch collects terms which contribute to the random variability of the entire method. Estimates of precision are available from replicate analysis of samples. Three different operators performed replicate analysis of samples and it was evaluated the repeatability of each operator and the reproducibility within the laboratory.

Dilution factor – D

All the samples are diluted on a volume-by-weight basis. The uncertainties associated with the dilution factor are the uncertainty about the weight of the sample taken and the uncertainty about the final volume of the solution after dilution.

Recovery – R

The recovery comprises two components, R_m and R_s ($R = R_m \times R_s$).

R_m is the method recovery that is the recovery for the entire procedure, including sample treatment, preparation of calibration standards and any dilution of the sample. It is measured on a suitable reference sample or as a mean recovery over many materials and it represents a test of bias against a particular reference value.

R_s is the recovery for real samples, it represents the difference between the reference sample and a particular real sample. The uncertainty associated with R_s describes the variation in recovery between the different sample matrices and different analyte levels.

Dry mass correction - F

For correction of the measured values to dry-mass, water content measurements have been made on a portion of samples. Cycles of drying and weighing have been repeated until a constant mass. The uncertainty associated with the mass depends on the linearity of the balance (accounted for twice, once for the tare and once for the gross mass). The balance zero bias can be neglected because the mass by difference is done on the same balance over a very narrow range.

Calibration function

The only uncertainty that has been evaluated is the uncertainty associated with the drift. The drift is monitored by periodically analysing one of the calibration standards during a batch of analyses. If the standard reading differs by more than ±10% from the reading at calibration, the instrument is recalibrated and the samples reanalysed. For each sample, there is therefore a permitted variation of up to ±10% due to instrument drift. The distribution is assumed to be rectangular. The uncertainty associated with the instrument drift is obtained dividing by $\sqrt{3}$ the variation of ±10%. *u(drift)* is therefore estimated as 0.0577 (as a relative standard deviation).

Concentration of dilute working standard

The dilute working standard (nominal concentration: As 5µg/mL, Se 1µg/mL, Pb 0.1µg/mL) is prepared diluting 0.5mL of an arsenic stock solution (nominal concentration 1000.6µg/mL), plus 0.1mL of a selenium stock solution (nominal concentration 1000µg/mL), plus 0.01mL of a lead stock solution (nominal concentration 1000.2µg/mL) to 100mL.

The uncertainty in the concentration of the stock solutions is specified by the supplier and is assumed to be a rectangular distribution. The standard uncertainty $u(C_{stock(w/v)})$ is therefore obtained by dividing the stated uncertainty by $\sqrt{3}$ which gives:

$$\text{As} : u_{As}(C_{stock(w/v)}) = \frac{2\mu g/mL}{\sqrt{3}} = 1.155\mu g/mL$$

$$\text{Se} : u_{Se}(C_{stock(w/v)}) = \frac{3\mu g/mL}{\sqrt{3}} = 1.732\mu g/mL$$

$$\text{Pb} : u_{Pb}(C_{stock(w/v)}) = \frac{4.47\mu g/mL}{\sqrt{3}} = 2.581\mu g/mL$$

The concentration of the dilute working standard, C_{dil}, is given by:

$$C_{dil} = \frac{C_{stock(w/v)} \times V_{stock}}{V_{final}}$$

where V_{stock} is the volume of the stock solutions taken and V_{final} is the final volume of the dilute working standard.

$$C_{dil-As} = \frac{C_{stock-As(w/v)} \times V_{stock-As}}{V_{final-As}} = \frac{1000.6\mu g/mL \times 0.5mL}{100mL} = 5.003\mu g/mL$$

$$C_{dil-Se} = \frac{C_{stock-Se(w/v)} \times V_{stock-Se}}{V_{final-Se}} = \frac{1000\mu g/mL \times 0.1mL}{100mL} = 1\mu g/mL$$

$$C_{dil-Pb} = \frac{C_{stock-Pb(w/v)} \times V_{stock-Pb}}{V_{finaL-Pb}} = \frac{1000.2\mu g/mL \times 0.01mL}{100mL} = 0.1\mu g/mL$$

The uncertainty associated with the V_{stock} is evaluated on the basis of the micropipette certificate by the supplier. The distribution is assumed to be triangular.

$$u_{As}(V_{stock}) = \frac{0.2\mu L}{\sqrt{6}} = 0.082\mu L$$

$$u_{Se}(V_{stock}) = \frac{0.2\mu L}{\sqrt{6}} = 0.082\mu L$$

$$u_{Pb}(V_{stock}) = \frac{0.2\mu L}{\sqrt{6}} = 0.082\mu L$$

The uncertainty associated with the V_{final} is evaluated on the basis of the volumetric flask certificate by the supplier. The distribution is assumed to be triangular. According to the manufacturer, the volumetric flask has been calibrated at a temperature of 20°C, whereas the laboratory temperature varies between the limits of ±4°C. The standard uncertainty from this effect is calculated using the assumption of a rectangular distribution.

V_{final}=100 mL

$u(V_{final})$=0.1 mL

Combining these values with the uncertainty calculated for the concentration of the stock solution gives an uncertainty in the concentration of the dilute working standard $u(C_{dil})$ of:

$$u(C_{dil\text{-}As}) = C_{dil-As} \times \sqrt{\left(\frac{u_{As}(C_{stock})}{C_{As-stock}}\right)^2 + \left(\frac{u_{As}(V_{stock})}{V_{As-stock}}\right)^2 + \left(\frac{u(V_{final})}{V_{final}}\right)^2} = 0.007684 \mu g/mL$$

$$u(C_{dil}) = C_{dil-Se} \times \sqrt{\left(\frac{u_{Se}(C_{stock})}{C_{Se-stock}}\right)^2 + \left(\frac{u_{Se}(V_{stock})}{V_{Se-stock}}\right)^2 + \left(\frac{u(V_{final})}{V_{final}}\right)^2} = 0.002162 \mu g/mL$$

$$u(C_{dil}) = C_{dil-Pb} \times \sqrt{\left(\frac{u_{Pb}(C_{stock})}{C_{Pb-stock}}\right)^2 + \left(\frac{u_{Pb}(V_{stock})}{V_{Pb-stock}}\right)^2 + \left(\frac{u(V_{final})}{V_{final}}\right)^2} = 0.000865 \mu g/mL$$

Dilution factor

The dilution factor D is given by:

$$D = \frac{V_{final}}{W_{sample}} = 100 mL/g$$

where W_{sample} is the weight of the samples taken (0.5g) and V_{final} is the final volume of the sample after dilution (50mL). The uncertainty associated with the mass mainly depends on the linearity of the balance (accounted for twice, once for the tare and once for the gross mass). The balance manufacturer quotes ±0.3 mg for the linearity contribution. The linearity contribution is assumed to show a rectangular distribution.

$$u(W_{sample}) = \sqrt{\left(\frac{0.3mg}{\sqrt{3}}\right)^2 \times 2} = 0.245mg$$

The uncertainty associated with the V_{final} is evaluated on the basis of the volumetric flask certificate by the supplier. According to the manufacturer, the volumetric flask has been calibrated at a temperature of 20°C, whereas the laboratory temperature varies between the limits of ±4°C. The standard uncertainty from this effect is calculated using the assumption of a rectangular distribution.

$$u(V_{final})= \sqrt{\left(\frac{0.12mL}{\sqrt{6}}\right)^2 + \left(\frac{0.042mL}{\sqrt{3}}\right)^2} = 0.0547mL$$

Combining these values gives an uncertainty in the concentration of the dilute working standard u(D) of:

$$u(D)=D \times \sqrt{\left(\frac{u(V_{final})}{V_{final}}\right)^2 + \left(\frac{u(W_{sample})}{W_{samplel}}\right)^2} = 0.12mL/g$$

Precision

Three different operators performed replicate analyses of the same samples by using the same equipment and it was evaluated the repeatability of each operator and the reproducibility within the laboratory.

The repeatability and the reproducibility standard deviations have been evaluated as following:

Pb by using Tuna fish RM, not certified.

As by using Cod muscle BCR-422.

Se by using Cod muscle BCR-422.

It has to be noted that, in case of lead, the repeatability and reproducibility contribution are evaluated on a tuna reference material because its concentration is of the same order of magnitude of that found in salmon CCQM sample.

The reproducibility standard deviation is defined as:

$$S_R = \sqrt{S_L^2 + S_r^2}$$

where:

S_r^2 = arithmetic mean of the within-operator variances (estimate of the repeatability variance). This arithmetic mean is taken over the three operator taking part in the study.

S_L^2 = estimate of the between-operator variance

$u_{As}(P)$= 0.06 (as relative standard uncertainty)

$u_{Se}(P)$= 0.095(as relative standard uncertainty)

$u_{Pb}(P)$= 0.04 (as relative standard uncertainty)

Recovery

The estimate of R_m and $u(R_m)$ was obtained from digestion solutions prepared by the certified reference material BCR-422 "Cod Muscle".

The uncertainty associated with the reference values are quoted by the suppliers at the 95% confidence level and divided by the coverage factor k=2.

$u_{As}(C_{BCR}) = 0.25 \mu g / g$

$u_{Se}(C_{BCR}) = 0.035 \mu g / g$

Lead concentration in the BCR-422 is lower of more than one order of magnitude with respect to that found in the salmon CCQM sample.

For this reason, the uncertainty associated with lead recovery in the BCR-422 is not included in the estimation of the combined uncertainty.

However, as shown in the validation study, the mean recovery obtained for lead in BCR-422 is found to be satisfactory.

After dilution, the certified reference material was analysed in replicate in a single analysis run.

For arsenic the mean is 20.36 μg/g with a standard deviation of 0.37μg/g (n=7).

For selenium the mean is 1.494 μg/g with a standard deviation of 0.054μg/g (n=7).

The method recovery is:

$$R_m = \frac{C_{obs}}{C_{CRM}}$$

where C_{obs} is the mean of the result obtained from the replicate analyses of the CRM sample and C_{CRM} is the concentration of the reference material as quoted by the supplier. The uncertainty associated with R_m, $u(R_m)$, is obtained by combining the uncertainty in the reference value $u(C_{CRM})$ with the uncertainty in the mean of the observations:

$$u_{As}(R_m) = R_{m-As} \times \sqrt{\left(\frac{u_{As}(C_{CRM})}{C_{As-CRM}}\right)^2 + \frac{S_{As-obs}{}^2}{n \times C_{As-obs}{}^2}} = 0.0132$$

$$u_{Se}(R_m) = R_{m-Se} \times \sqrt{\left(\frac{u_{Se}(C_{CRM})}{C_{Se-CRM}}\right)^2 + \frac{S_{Se-obs}{}^2}{n \times C_{Se-obs}{}^2}} = 0.0233$$

The bias is calculated as follows:

$$\frac{|1 - R_m|}{u(R_m)}$$

Arsenic bias: 2.64

Selenium bias: 3.58

This ratio is compared with the coverage factor k (k=2, representing a confidence level of 95%), which will be used to calculate the expanded uncertainty of the overall method. A value >2 indicates that the recovery is significantly different from 1. However, in the routine application of the method, the difference is not considered to be of practical significance and no correction to the final result is applied. In such

cases, the uncertainty associated with the method recovery must be increased to take account of this uncorrected bias:

This value is calculated for each element:

$$u(R_m)' = \sqrt{\left(\frac{1-R_m}{k}\right)^2 + u(R_m)^2}$$

$u_{As}(R_m)' = 0.0219$

$u_{Se}(R_m)' = 0.0479$

The sample recovery R_s was investigated for each elements by diluting the reference material BCR-422 "Cod Muscle" to two different levels of concentration. Data show that the difference in analyte level does not contribute significantly to the variability in the recovery (for further details see the validation of method).

Dry mass correction

Cycles of drying and weighing have been repeated until a constant mass. The uncertainty associated with the mass mainly depends on the linearity of the balance (accounted for twice, once for the tare and once for the gross mass).

The balance manufacturer quotes ±0.3 mg for the linearity contribution. The linearity contribution is assumed to show a rectangular distribution.

$$F = \frac{W_{fresh}}{W_{dry}}$$

W_{fresh} is the initial mass (g), W_{dry} is the final mass (g) (constant mass).

Dry mass correction was made on all analysed samples. Following the uncertainty budget relevant to dry mass correction on reference material based on salmon distributed by IRMM is reported.

Sample n. CDAS1529714

W_{fresh}=0.6023g

W_{dry}=0.5886g

$$u(W_{fresh})=\sqrt{\left(\frac{0.3mg}{\sqrt{3}}\right)^2\times 2}=0.245mg$$

$$u(W_{dry})=\sqrt{\left(\frac{0.3mg}{\sqrt{3}}\right)^2\times 2}=0.245mg$$

$$u(F)=F\times\sqrt{\left(\frac{u(W_{fresh})}{W_{fresh}}\right)^2\times\left(\frac{u(W_{dry})}{W_{dry}}\right)^2}=0.00059$$

Sample n. CDAS1558612

W_{fresh}=0.5106g

W_{dry}=0.5003g

$$u(W_{fresh})=\sqrt{\left(\frac{0.3mg}{\sqrt{3}}\right)^2\times 2}=0.245mg$$

$$u(W_{dry})=\sqrt{\left(\frac{0.3mg}{\sqrt{3}}\right)^2\times 2}=0.245mg$$

$$u(F)=F\times\sqrt{\left(\frac{u(W_{fresh})}{W_{fresh}}\right)^2\times\left(\frac{u(W_{dry})}{W_{dry}}\right)^2}=0.00069$$

The following tables report as example the uncertainty budget relevant to As, Se and Pb determined in the reference material based on salmon distributed by IRMM (sample code: CDAS1529714).

UNCERTAINTY IN SALMON SAMPLE

ARSENIC

Parameter		Uncertainty as RSD
Method recovery	$u(R_m)$	0.0227
Sample recovery	$u(R_s)$	-
Precision	u(P)	0.06
Dilution factor	u(D)	0.0012
Concentration of dilute working standard	$u(C_{dil})$	0.001536
Instrument drift	u(drift)	0.0577
Dry mass correction	u(F)	0.00058

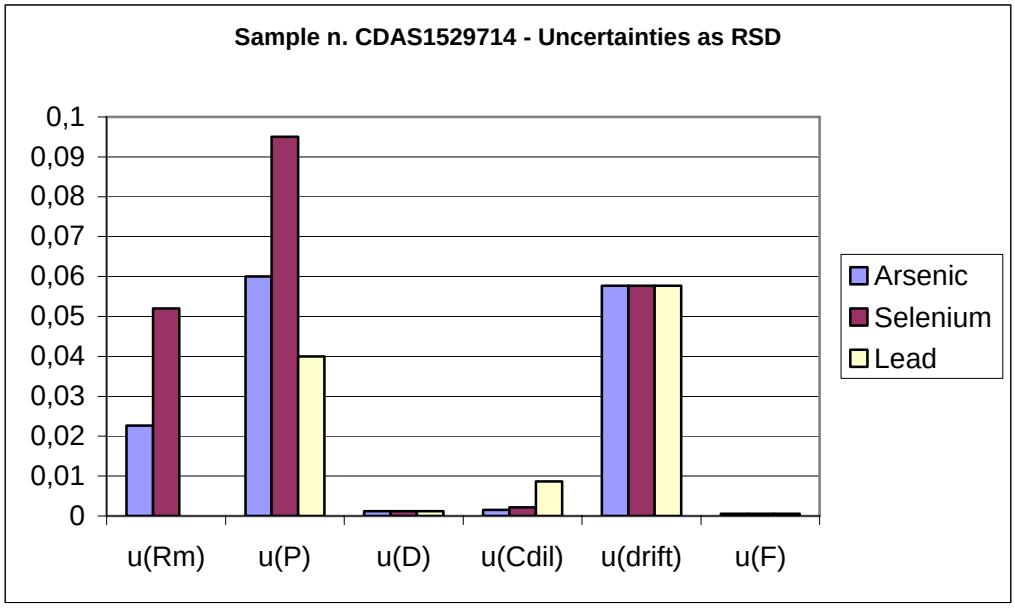
SELENIUM

Parameter		Uncertainty as RSD
Method recovery	$u(R_m)$	0.0523
Sample recovery	$u(R_s)$	-
Precision	u(P)	0.095
Dilution factor	u(D)	0.0012
Concentration of dilute working standard	$u(C_{dil})$	0.002162
Instrument drift	u(drift)	0.0577
Dry mass correction	u(F)	0.00058

LEAD

Parameter		Uncertainty as RSD
Method recovery	$u(R_m)$	-
Sample recovery	$u(R_s)$	-
Precision	u(P)	0.04
Dilution factor	u(D)	0.0012
Concentration of dilute working standard	$u(C_{dil})$	0.008654
Instrument drift	u(drift)	0.0577
Dry mass correction	u(F)	0.00058

Fig. 84. Illustration of contributions to the uncertainty budget


Sample n. CDAS1529714 - Uncertainties as RSD
0,1
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
u(Rm)
u(P)
u(D)
u(Cdil)
u(drift)
u(F)
Arsenic
Selenium
Lead

Calculation of standard and expanded uncertainty

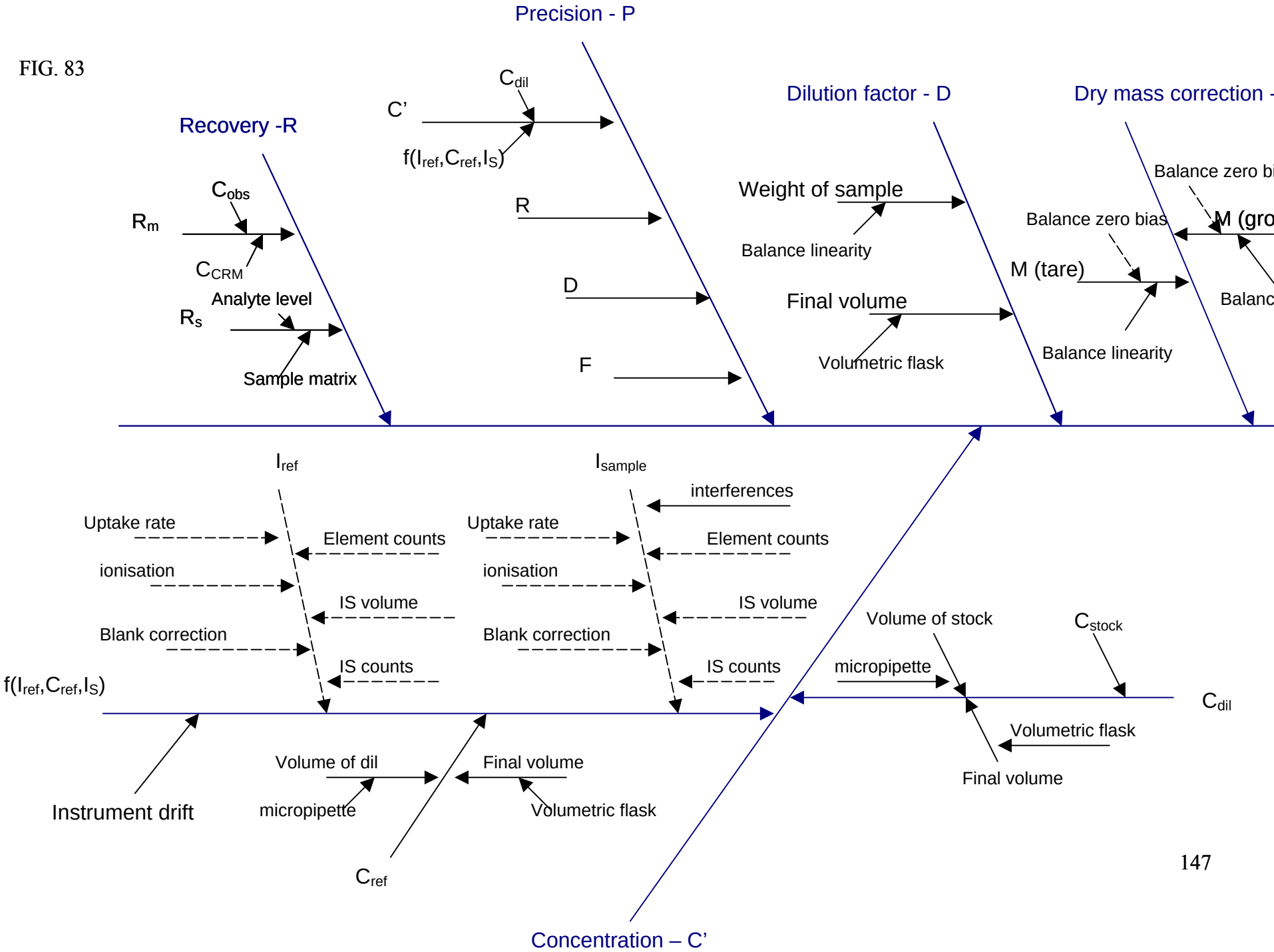
The combined standard uncertainty is calculated from the root sum of square of the individual components, according to the rules set out in the Eurachem guide [78]. The expanded uncertainty is calculated using the coverage factor of two which gives a level of confidence of 95%.

ICP-MS RESULTS

U= expanded uncertainty (k=2)

sample n CDAS1529714	As (mg/kg)	U_{As} (mg/kg)	Se (mg/kg)	U_{Se} (mg/kg)	Pb (mg/kg)	U_{Pb} (mg/kg)
misure 1	4.17	0.72	0.52	0.13	0.78	0.11
misure 2	4.24	0.73	0.51	0.12	0.89	0.13

FIG. 83


Precision - P
Recovery -R
Dilution factor - D
Dry mass correction -
C'
C_{dil}
$f(I_{ref},C_{ref},I_S)$
R
D
F
R_m
C_{obs}
C_{CRM}
Analyte level
R_s
Sample matrix
Weight of sample
Balance linearity
Final volume
Volumetric flask
Balance zero bias
M (tare)
Balance linearity
I_{ref}
I_{sample}
Uptake rate
Element counts
ionisation
IS volume
Blank correction
IS counts
interferences
$f(I_{ref},C_{ref},I_S)$
Instrument drift
Volume of dil
micropipette
Final volume
Volumetric flask
C_{ref}
Volume of stock
C_{stock}
micropipette
Volumetric flask
Final volume
C_{dil}
Concentration – C'

CONCLUSIONS

The present study examined trace metals bioavailability in a range of well-equilibrated contaminated and amended soils and in a river using several analytical techniques as a basis for inferring metal mobility in the environment and its uptake by biota.

Concerning soils, traditional techniques as leaching and extraction tests have been used in parallel to a relatively recently developed technique known as Diffusive gradients in thin films technique (DGT). These techniques have been developed for both research and regulatory controls. DGT technique is generally employed for research purposes, while the extraction and leaching tests are widely used also for environmental controls. For example, Regulators in some European countries have adopted operationally defined extractions using weak salt solutions instead of the total metals content in attempt to improve the assessment of metal bioavailability in soils.

Each of these techniques provided different information and the most appropriate techniques must be selected in relation to the particular environmental scenario or natural phenomenon under investigation. Leaching tests are particularly useful to provide information relevant to the geochemical metal speciation and to predict long term contaminant mobility. Considering that long term leaching behaviour of soils can not be obtained by experimenting in realistic time frames, certain aspects of leaching are accelerated in batch or column test to simulate such long term scenario. On the contrary, DGT could be deployed directly *in situ* reflecting field situations. In this work, leaching tests provided an estimation of the maximum availability of metals for release from the soil matrix offering a base of reference for the chemical extraction results. pH dependence leaching curves provided also a useful information to interpret DGT results, underlying the potential of parallel measurements to assess metal bioavailability.

The distinguishing feature of the DGT measurement is that incorporates the kinetics of metal supply from solid phase to solution and for this reason it could simulate metal uptake by plants. Our results indicated that this supply may be important for Zn uptake by wheat and lupine in well-equilibrated and amended soils.

Plant Pb concentrations were highly related to both soil pore water concentrations and to the effective concentrations (C_E) measured by DGT indicating that supply from the solid phase may not be so important for Pb. An advantage of DGT with respect to metal determinations in soil pore water is that, analytically, the concentrations of metals measured in DGT eluents were many times higher than those measured in soil pore water.

The present study has reported the ability of DGT to potentially predict metal phytoavailability for lupine and wheat within metal contaminated soils. Because DGT measurements are reported to be quite simple and inexpensive (about 8-10 € per unit), DGT could be used in risk assessment mapping of Zn contaminated sites after validation on a set of soils from the site.

In situ measurements of Mn, Ni, Co, Cu, Zn, Cd, Pb by DGT in the Sitzerri river (Sardinia) were compared to results from on site filtration. Two sampling campaigns (in winter and spring) were carried out in cooperation with ARPA Sardinia. The river represents a site of environmental concern due to the metal pollution from the closed mine of Montevecchio. In general, the two techniques agreed quite well for most metals, indicating an absence of colloids and negligible complexation by organic matter. Substantial differences between DGT and on site filtration were found for Cu (I and II campaigns) and Cd (II campaign) and were consistent with strong complexation.

This work demonstrates the potential of parallel in situ measurements even though in term of cost, simplicity and risk of contamination the on site filtration is slightly superior to DGT.

Metal bioaccumulation in fish was recognized to be an interesting topic of investigation in correlation with metal levels and bioavailability in waters. A specific experiment was designed to study metals bioaccumulation in Cyprinus Carpio fish and a method to accurately determine metals accumulated in fish tissues was standardized. In this work, fish show bioaccumulation of Cd and Pb in their tissues under the test conditions while for Co, As and Cu other processes like excretion seem to significantly influence the net metal accumulation in fish.

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