

**ISTITUTO SUPERIORE DI SANITÀ**

**XIII Italian-Hungarian Symposium  
on spectrochemistry:  
environmental contamination  
and food safety**

University *Alma Mater Studiorum*  
Bologna, Italy  
April 20-24, 2008

**ABSTRACT BOOK**

Edited by  
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**XIII Italian-Hungarian Symposium on spectrochemistry: environmental contamination and food safety. University Alma Mater Studiorum, Bologna, Italy, April 20-24, 2008. Abstract Book.**

Edited by Paola Bottoni and Sergio Caroli  
2008, xv, 157 p. ISTISAN Congressi 08/C3

This edition of the Symposium focuses primarily on the impact of environmental pollution on food safety. With about 90 contributions, equally distributed between oral and poster presentations, some of the major challenges posed by anthropic activities to food commodities are discussed. The role of modern analytical techniques in this context is highlighted particularly as regards the importance of reliable experimental information in the decision-making process. The Symposium features seven oral sessions, devoted, respectively, to basic research, manufacturers, water, soil and sediment, atmosphere, food and research in Antarctica. Presentations in the poster sessions are grouped likewise. The variety of issues dealt with in this conference give ample evidence of the progress made so far by analytical sciences in this field and set the stage for further innovative research.

*Key words:* Spectrochemistry, Analytical techniques, Food, Water, Soil, Sediment, Environmental contamination, Antarctica

Istituto Superiore di Sanità

**XIII Simposio italo-ungherese di spettrochimica: contaminazione ambientale e sicurezza alimentare. Università Alma Mater Studiorum, Bologna, 20-24 aprile 2008. Riassunti.**

A cura di Paola Bottoni e Sergio Caroli  
2008, xv, 157 p. ISTISAN Congressi 08/C3 (in inglese)

Questa edizione del Simposio è dedicata soprattutto alle conseguenze della contaminazione ambientale sulla sicurezza degli alimenti. Alcune delle sfide più rilevanti poste dalle attività umane alla integrità dei prodotti alimentari sono discusse in circa 90 contributi, equamente distribuiti tra relazioni orali e poster. Vengono particolarmente messe in luce l'importanza delle attuali tecniche analitiche per produrre informazioni sperimentali attendibili e la rilevanza di queste ultime nel processo decisionale. Le sette sessioni orali sono dedicate, rispettivamente, alla ricerca di base, ai costruttori di strumentazione, alle acque, ai suoli e sedimenti, all'atmosfera, agli alimenti ed alla ricerca in Antartide. Le presentazioni poster si articolano in modo analogo. L'ampiezza dei temi esposti dimostra concretamente l'innovazione ad oggi apportata in questo settore dalle scienze analitiche e pone le basi per ricerche ancora più avanzate.

*Parole chiave:* Spettrochimica, Tecniche analitiche, Alimenti, Acque, Suoli, Sedimenti, Contaminazione ambientale, Antartide

*Organized by:* Istituto Superiore di Sanità (ISS), Rome, Italy; University Alma Mater Studiorum (UAMSB), Bologna, Italy; La Sapienza University (ULSR), Rome, Italy; Società Chimica Italiana (SCI), Rome, Italy; National Council of Research (CNR), Bologna, Italy; Eötvös Loránd University (ELTE), Budapest, Hungary; Central European Initiative (CEI), Trieste, Italy

*Chairpersons of the Symposium:* Sergio Caroli (Istituto Superiore di Sanità, Rome, Italy) and Gyula Záray (Eötvös Loránd University, Budapest, Hungary)

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# TABLE OF CONTENTS

<b>Programme</b> .....	iii
<b>Preface</b> .....	xv
<b>Oral Session 1</b> Basic research <i>Sergio Caroli, Ibolya Molnár-Perl</i> .....	1
<b>Oral Session 2</b> The point of view of manufacturers <i>Gyula Záray, Roger Fuoco</i> .....	13
<b>Oral Session 3</b> Water <i>Paola Bottoni, Viktor Gábor Mihucz</i> .....	21
<b>Oral Session 4</b> Soil and sediment <i>Maurizio Forte, János Lakatos</i> .....	33
<b>Oral Session 5</b> Atmosphere <i>Sergio Caroli, József Posta</i> .....	39
<b>Oral Session 6</b> Food <i>György Heltai, Giampiero Pagliuca</i> .....	43
<b>Oral Session 7</b> Research in Antarctica <i>Erika Ribechini, Éva Bertalan</i> .....	57
<b>Poster Session 1</b> Basic research <i>Mihály Óvári</i> .....	65

<b>Poster Session 2</b>	
Water	
<i>Pietro Paris</i> .....	77
<b>Poster Session 3</b>	
Soil and sediment	
<i>Michaela Zeiner</i> .....	99
<b>Poster Session 4</b>	
Atmosphere	
<i>Enrico Veschetti</i> .....	105
<b>Poster Session 5</b>	
Food	
<i>Anikó Vasanits-Zsigrai</i> .....	113
<b>Poster Session 6</b>	
Research in Antarctica	
<i>Clara Turetta</i> .....	125
<b>Authors' addresses</b> .....	131
<b>Authors' index</b> .....	155

# PROGRAMME

## Sunday, April 20, 2008

19.00 Welcome reception

## Monday, April 21, 2008

8.00 Registration of participants

9.00 *Opening ceremony of the Symposium and welcome address*  
**Sergio Caroli, Gyula Záray**

### Oral session 1

#### BASIC RESEARCH

*Chairpersons: Sergio Caroli, Ibolya Molnár-Perl*

9.20 *Biochemical studies by X-ray techniques*  
**Gyula Záray**

9.45 *Development of an analytical procedure for the quantification of volatile compounds in human breath*  
**Fabio Di Francesco, Massimo Onor, Sara Tabucchi, Silvia Ghimenti, Alessio Ceccarini Roger Fuoco**

10.10 *Spectral and trace elemental characterization of selected textile dyes*  
**Michaela Zeiner, Iva Rezić, Ilse Steffan**

10.35 *Indirect determination of organic and inorganic trace compounds with Atomic Absorption Spectrometry*  
**József Posta, Fazwi Mohamed El Saih, Sádor Kapitány, Anita Preszner, Dávid Nagy**

11.00 Coffee break

11.20 *Formyl- and acetyldols: vibrational spectroscopy of an expectably pharmacologically active compound family*  
**Ferenc Billes, Monica Toşa, Ildikó Mohammed-Ziegler, Hans Mikosch, Cornelia Majdik**

11.45 *Elemental analysis of cancer cells by Total Reflection X-ray Fluorescence*  
**Norbert Szoboszlai, Zsófia Polgári, Zsuzsa Szabó, Andrea Réti, Barna Budai, Judit Kralovánszky, Gyula Záray**

- 12.10 *Investigation of Cr species transformation in low and high temperature processes*  
**János Lakatos**
- 12.35 *Speciation studies as a basis for the interpretation of in vivo Pt-induced cytotoxicity in Balb/3T3 cell line*  
**Massimo Farina, Salvador Fortaner, Riccardo Del Torchio, Enrico Sabbioni**
- 13.00 *Speciation studies in in vitro assays on Cr-induced embryoletality. Teratogenicity and carcinogenic potential by advanced analytical spectrochemical and radioanalytical techniques*  
**Salvador Fortaner, Stefano Bosisio, Massimo Farina, Riccardo Del Torchio, Enrico Sabbioni**
- 13.25 Lunch break

## **Oral session 2**

### **THE POINT OF VIEW OF MANUFACTURERS**

*Chairpersons:* **Gyula Zárny, Roger Fuoco**

- 14.25 *Novel sample handling techniques in Gas chromatography to enhance detection limits and improve performance and reliability*  
**Ariovaldo Bisi, Massimo Santoro**
- 14.50 *ICP-MS as a chromatographic detector in food safety and environmental applications*  
**Torsten Lindemann, Shona McSheehy, Meike Hamester**
- 15.15 *Polyatomic ions: no longer a problem for routine multi-element analysis of variable matrix samples, using ICP-MS*  
**Ed McCurdy, Glenn Woods, Gianfranco Fiabane**
- 15.40 *A comprehensive approach to microwave sample preparation*  
**Camillo Pirola, Angelo Agazzi**
- 16.05 *Dedicated assistance tools to favour the use of multiline analysis in CCD-based ICP-AES*  
**Jean-Michel Mermet, Cendrine Dubuisson, Emmanuel Fretel, Olivier Rogerieux, Sébastien Velasquez, Sophie Lebouil**
- 16.30 Closure of day 1

**Tuesday, April 22, 2008**

**Oral session 3**

**WATER**

*Chairpersons:* **Paola Bottoni, Viktor Gábor Mihucz**

- 9.00 *Advances in the sample preparation. Gas chromatographic identification and quantification of water soluble pollutants*  
**Ibolya Molnár-Perl**
- 9.25 *Italian monitoring plan of pesticides in water. Technical background and results for 2003-2005*  
**Pietro Paris, Dania Esposito**
- 9.50 *Occurrence and distribution of As in groundwater of a large volcanic area in central Italy*  
**Laura Achene, Emanuele Ferretti, Luca Lucentini, Paola Pettine, Enrico Veschetti, Massimo Ottaviani**
- 10.15 *The Water Framework Directive: a challenge for analytical chemistry*  
**Stefano Polesello, Mario Carere**
- 10.40 *Speciation analysis of As in shallow groundwaters from Hungary by plasma spectrochemical methods*  
**Éva Bertalan, András Bartha, Éva Horváth, Mária Ballók, Teodóra Szócs, István Horváth**
- 11.05 Coffee break
- 11.25 *Complexation of Cd and Cu by fluvial humic matter and effects on their toxicity*  
**Gabriele Capodaglio, Fabiana Corami, Clara Turetta, Marcantonio Bragadin, Nicoletta Calace, Bianca Maria Petronio**
- 11.50 *Environmental aspects of riverbank filtration on the Szentendre island (Danube river, Hungary)*  
**Ferenc Homonnai, Ferencz Kaszab, Csaba Szabó**
- 12.15 *Environmental occurrence and microbial degradation of selected acidic pharmaceuticals and their metabolites in waste water*  
**Anikó Vasánits-Zsigrai**
- 12.40 *A novel slurry sampling analysis of Pb in different water samples by electrothermal Atomic Absorption Spectrometry after coprecipitation with Co/pyrrolidine dithiocarbamate complex*  
**Asli Baysal, Suleyman Akman, Ferah Calisir**

13.05 *The use of fluorescence in situ hybridization method on soil and water ecosystems: a new approach to study the effect of xenobiotics on bacterial community*  
**Anna Barra Caracciolo, Paola Grenni**

13.30 Lunch break

#### **Oral session 4**

##### **SOIL AND SEDIMENT**

*Chairpersons: Maurizio Forte, János Lakatos*

14.30 *Development of new fractionation methodologies for characterization of environmental mobility of heavy metals in soils, sediments and gravitation dusts*  
**György Heltai, Karol Flórián**

14.55 *Characterization of metabolic activity of depth-related microbial community in lake sediment*  
**Jun Yao, Fei Wang, Huilun Chen, Rensheng Zhuang, Ke Chen, Yong Zhou, Haiyan Chen, Gyula Záray, Brunello Ceccanti, Thomas Maskow**

15.20 *Reactivity investigation of layer-structured minerals by spectroscopic methods*  
**János Kristóf, Ray L. Frost**

#### **Oral session 5**

##### **ATMOSPHERE**

*Chairpersons: Sergio Caroli, József Posta*

15.45 *Comparison of the trace element concentration in airborne dust between Budapest and the region of Buenos Aires*  
**Mihály Óvári, Gábor Muránszky, Patricia Smichowsky, Gyula Záray**

16.10 *Urban air pollution. particulate matter in Bologna*  
**Luciano Forlani, Alberto Legnani, Paolo Zani**

16.35 Closure of day 2

**Wednesday, April 23, 2008**

**Oral session 6**

**FOOD**

*Chairpersons:* **György Heltai, Giampiero Pagliuca**

- 9.00 *Radioactivity in food and environment: a historical overview*  
**Maurizio Forte**
- 9.25 *The role of sampling and sample preparation in food risk analysis strategies*  
**Marina Miraglia, Barbara De Santis, Francesca Debegnach, Elisabetta Prantera, Carlo Brera**
- 9.50 *Element removal from washed and cooked rice studied by SF-ICP-MS and synchrotron radiation-based confocal micro-XRF*  
**Victor Gábor Mihucz, Geert Silversmit, Imre Szalóki, Tom Schoonjans, Enikő Tatár, Björn De Samber, László Vincze, István Virág, Jun Yao, Gyula Záray**
- 10.15 *Metabolism, toxicity and carcinogenicity of dietary metals*  
**Enrico Sabbioni, Salvador Fortaner, Massimo Farina, Riccardo Del Torchio**
- 10.40 Coffee break
- 11.00 *Examination of factors determining quality of Egri Bikavér (Bull's Blood of Eger)*  
**László Rácz, Csaba Csutorás, Szabolcs B. Tóth, Zoltán Szilágyi**
- 11.25 *Determination of the antioxidant capacity of different types of bread and flour using a superoxide dismutase biosensor*  
**Riccardo Antiochia, Luigi Campanella, Paola Lipone**
- 11.50 *PDBE levels in food and dietary exposure in central Italy*  
**Cristianna Guerranti, Michela Mariottini, Silvano Focardi**
- 12.15 *Cyclodextrins as fluorescence enhancers for mycotoxin detection in food*  
**Gianni Galaverna, Chiara Dell'Asta, Arnaldo Dossena, Rosangela Marchelli**
- 12.40 *Past food: a multi-analytical approach for the characterization of commodities in an archaeological jar from Egypt*  
**Maria Perla Colombini, Erika Ribechini**
- 13.05 Lunch break
- 15.00 Guided tour of Bologna

19.00 Social dinner

22.00 Closure of day 3

## **Thursday, April 24, 2008**

### **Oral session 7**

#### **RESEARCH IN ANTARCTICA**

*Chairpersons:* **Erika Ribechini, Éva Bertalan**

- 9.20 *Spatial and temporal trends of organic pollutants in Antarctica*  
**Roger Fuoco, Stefania Giannarelli, Massimo Onor, Silvia Ghimenti,  
Carlo Abete, Sandro Francesconi, Marco Termine**
- 9.45 *Set-up of a Sector Field ICP-MS method for the determination of trace elements  
and REEs in soil and oceanic sediment samples*  
**Francesco Rugi, Silvia Becagli, Emiliano Castellano, Massimo Chiari,  
Franco Lucarelli, Federica Marino, Silvia Nava, Mirko Severi,  
Rita Traversi, Roberto Udisti**
- 10.10 *Studies on Cd bioaccumulation in the liver of the Antarctic teleost Trematomus bernacchii*  
**Silvia Illuminati, Cristina Truzzi, Anna Annibaldi,  
Elisa Bassotti, Giuseppe Scarponi**
- 10.35 *A twelve thousand years records in the occurrence of trace metal  
in the sediment of the Ross Sea (Antarctica)*  
**Gabriele Capodaglio, Clara Turetta, Elisa Morabito, Vania Gaspari,  
Carlo Barbante, Nicoletta Calace, Bianca Maria. Petronio, Federico Giglio**
- 11.00 Coffee break
- 11.20 *Volatile chlorinated hydrocarbons in Antarctica. Analytical aspects  
and environmental evaluation*  
**Susanna Insogna, Lelio Zoccolillo, Luca Amendola**
- 11.45 *The certification project for trace elements in Adamussium colbecki*  
**Sergio Caroli, Paola Bottoni**
- 12.30 Closure of the Symposium
- 13.00 Lunch break
- 14.00 Closure of day 4

## **Poster session 1**

### **BASIC RESEARCH**

*Coordinator:* **Mihály Óvári**

*The removal and preconcentration of Pb from water solution by using flow injection techniques*

**Dilek Bakircioglu, Muhammet Kuscu, Yasemin Bakircioglu**

*Comparative evaluation of the determination of some phyto-hormones in plants by GC-MS and HPLC-MS*

**Stefania Giannarelli, Beatrice Muscatello, Roger Fuoco, Patrizia Bogani, Marcello Buiatti, Antonio Rosati**

*Culture medium components as factor influencing the effective bioavailability of metal ions in vitro: the example of Co<sup>2+</sup> in cell transformation assay (Balb/3T3 cell line)*

**Riccardo Del Torchio, Salvador Fortaner, Massimo Farina, Enrico Sabbioni**

*Determination of polycyclic aromatic hydrocarbons (PAHs) in virgin and recycled elastomeric materials*

**Igor Fochi, Vittorio Abate, Silvia de Luca, Nicola Iacovella, Anna Laura Iamiceli, Alessandro di Domenico**

*Removal of metal pollutants from environmental cotton samples by ultrasound assisted extraction*

**Iva Rezić, Ilse Steffan**

*Automated indirect determination of fluoride ions by ICP-AES*

**Máté Kovács, Pál Halmos, János Borszéki, Riccardo Magarini**

*A new method for utilization of red mud*

**Attila Lengyel, Janos Lakatos, Gabriella Lengyel, Viktor Baranyai**

*Novel decomposition method for direct total X-ray Fluorescence analysis*

**Zsófia Polgári, Zsuzsa Szabó, Norbert Szoboszlai, Mihály Óvári, Gyula Záray**

*The OECD activities for the control of chemicals in the international trade*

**Sergio Caroli**

## **Poster session 2**

### **WATER**

*Coordinator:* **Pietro Paris**

*Adsorption of humic substances on melamine formaldehyde urea resin from different natural source waters*

**Ayca Ozdemir, Birsen Demirata Ozturk**

*Leaching of Sb from polyethylene terephthalate bottles into mineral waters and soft drinks*  
**Szilvia Keresztes, Victor Gábor Mihucz, Enikő Tatár, István Virág,  
Jun Yao, Gyula Záray**

*Advances in the Gas Chromatographic - Mass Spectrometric analysis  
of pharmaceuticals present in the aquatic environment*  
**Ágnes Sebők, András Helenkár, Anikó Vasanits-Zsigrai, Gyula Záray,  
Ibolya Molnár-Perl**

*Effect of distribution networks on metal occurrence in Italian drinking waters*  
**Enrico Veschetti, Laura Achene, Emanuele Ferretti, Luca Lucentini,  
Giovanni Citti, Massimo Ottaviani**

*Semi-quantitative determination of organoleptic properties of raw waters disinfected  
with peracetic acid or sodium hypochlorite*  
**Daniela Maresca, Enrico Veschetti, Emanuele Ferretti, Luca Lucentini,  
Massimo Ottaviani**

*Degradation of trichloroethylene in groundwater by aerobic cometabolism*  
**Beatrice Muscatello, Cristina Di Leo, Alberto Bittoni**

*Risks of water-borne disease outbreaks after extreme events*  
**Stefania Marcheggiani, Camilla Puccinelli, Simone Ciadamidaro,  
Valentina Della Bella, Mario Carere, Monica Francesca Blasi,  
Enzo Funari, Laura Mancini**

*A molecular approach for the impact assessment of faecal pollution in river ecosystems*  
**Laura Mancini, Marcello Iaconelli, Stefania Marcheggiani, Anna Maria D'Angelo,  
Elio Piedominici, Paolo Formichetti, Michele Equestre, Bruno Floris, Paola Rosselli,  
Patrizia Ammazalorso, Marco Le Foche, Ennio Zaottini, Claudio Fabiani**

*Implementation of WFD 2000/60/EC in Italy: diatoms as biological indicators of waters*  
**Camilla Puccinelli, Valentina Della Bella, Stefania Marcheggiani, Francesca Ciutti,  
Maria Elena Beltrami, Cristina Cappelletti, Laura Mancini**

*Antibiotic resistance in bacteria isolated from freshwater fish farms*  
**Laura Mancini, Claudia Marinilli, Simone Ciadamidaro, Valentina Della Bella,  
Stefania Marcheggiani, Camilla Puccinelli, Lorenzo Tancioni,  
Francesca Anna Aulicino**

*Determination of non-steroidal anti-inflammatory drugs as trimethylsilyl derivatives  
in Danube river by Gas-Chromatography-tandem Mass Spectrometry*  
**András Helenkár, Anikó Vasanits-Zsigrai, Ibolya Molnár-Perl, Gyula Záray**

*The environmental quality standard of the priority substances in the context of the European Water Framework Directive*

**Mario Carere, Stefano Polesello**

*MALDI-TOF and Q-TOF Mass Spectrometry characterisation of a new microcystin produced in a cyanobacteria bloom in the Averno lake*

**Pasquale Ferranti, Serena Fabbrocino, Milena Bruno, Luigi Serpe, Pasquale Gallo**

*Pharmaceuticals as priority water contaminants*

**Paola Bottoni, Sergio Caroli, Rosanna Fidente**

*Environmental fate of terbuthylazine and of its metabolite desethylterbuthylazine and their significance as surface water and groundwater pollutants*

**Paola Bottoni, Paola Grenni, Angiolo Martinelli, Anna Barra Caracciolo**

*Enhancing the quality of drinking water. The experience of an italian twinning project in poland*

**Massimo Ottaviani, Monica Paganelli, Elena Stefanoni, Luca Lucentini, Emilia Aimo, Flavio Trotti, Andrea Bertolo, Lucia Bonadonna, Emanuele Ferretti, Enrico Veschetti, Saveria Spezzano**

### **Poster session 3**

#### **SOIL AND SEDIMENT**

*Coordinator: Michaela Zeiner*

*Optimization of subcritical (H<sub>2</sub>O/CO<sub>2</sub>) extraction for soils, sediments and gravitation dusts*

**Gábor Halász, Radoslav Rusnák, Ilona Fekete, Márk Horváth, Karol Flórián, György Heltai**

*Triad-approach in the bioremediation of polluted environmental matrices*

**Veronica Bianchi, Brunello Ceccanti, Jun Yao, Thomas Maskow**

*Acceleration of BCR sequential extraction procedure by ultrasonic treatment for soils, sediments and gravitation dust samples*

**Radoslav Rusnák, Vladislava Boková, Ilona Fekete, Gábor Halász, Dagmar Remeteiová, György Heltai, Karol Flórián**

### **Poster session 4**

#### **ATMOSPHERE**

*Coordinator: Enrico Veschetti*

*Exhaust emission of PAHs from gasolines with different composition: relevant variables influencing results and the problem of blanks*

**Luigi Turrio Baldassarri, Maria Luisa Casella, Anna Laura Iamiceli, Walter Quattrocchi, Chiara Laura Battistelli**

*Optimization of a high-resolution ICP-MS internal standard method with a signal drift correction for determination of the soluble fraction of trace metals in aerosol samples*

**Francesco Rugi, Silvia Becagli, Emiliano Castellano, Massimo Chiari, Franco Lucarelli, Andrea Morganti, Silvia Nava, Desiré Manganelli, Mirko Severi, Omar Cerri, Roberto Udisti**

*Elemental analysis of PM<sub>10</sub> fractions of urban aerosol collected in Budapest between 2004 and 2007*

**Gábor Muránszky, Mihály Óvári, István Virág, Gyula Záray**

*Exposure to Pt for different groups of the Viennese population*

**Michaela Zeiner, Mihály Óvári, Gyula Záray, Ilse Steffan**

*Trace elemental characterization of fly ash*

**Michaela Kröppel, Irene Lahoz Muñoz**

## **Poster session 5**

### **FOOD**

*Coordinator: Anikó Vasánits-Zsigrai*

*Gas chromatographic mass spectrometric study on the simultaneous analysis of the constituents of black and green teas (flavonoids, catechins, sugars, carboxylic and amino acids)*

**Zsófia Füzfai, János Tóth, Ibolya Molnár-Perl**

*Total antioxidant capacity of pomegranate (*Punica granatum*) using spectrophotometric method (CERAC)*

**Dilek Ozyurt, Birsen Demirata Ozturk**

*Analysis of micotoxins FB1, FB2, HFB1 and HFB2 in pig liver by liquid chromatography coupled to tandem mass spectrometry*

**Teresa Gazzotti, Elisa Zironi, Barbara Lugoboni, Ester Grilli, Andrea Piva, Giampiero Pagliuca**

*Effect of microwave and oven heating on the antioxidant content of rosehip infusion (herbal tea)*

**Birsen Demirata Ozturk, Dilek Ozyurt**

*Analysis of micotoxin content of reform nutrition*

**Szabolcs B. Tóth, Csaba Csutorás, László Rácz, Attila Kiss**

*Analysis of ochratoxin-A content of wines originating from the Mediterranean and Hungary*

**Szabolcs B. Tóth, Csaba Csutorás, László Rácz**

*Infrared spectroscopic study of the fatty acid - bacterium interactions*

**Viktor Molnár, Ferenc Billes, Ernő Tyihák**

*Organohalogen compounds in food: does eating organic make a difference?*

**Cristiana Guerranti, Silvano Focardi**

*Perfluorinated compounds in Italy: results of a two-year study on levels in humans, the environment and food samples*

**Guido Perra, Cristiana Guerranti, Simonetta Corsolini, Silvano Focardi**

*Assessment of performance of instrumental techniques for trace elements in food: a new graphical approach to improve the exposure estimation*

**Chiara Frazzoli, Piotr Robouch, Sergio Caroli**

## **Poster session 6**

### **RESEARCH IN ANTARCTICA**

*Coordinator:* **Clara Turetta**

*SWASV determination of Cd, Cu and Pb in a hydrofluoric acid solution of siliceous spicules of marine sponges from the Ligurian Sea and the Ross Sea (Antarctica)*

**Cristina Truzzi, Anna Annibaldi, Silvia Illuminati, Elisa Bassotti, Giuseppe Scarponi**

*Levels of polybrominated diphenyl ethers (PBDEs) in eggs of Antarctic penguins*

**Nicoletta Borghesi, Simonetta Corsolini, Pim Leonards, Sicco Brandsma, Jacob de Boer, Silvano Focardi**

*Major, minor and trace element profiles in two Antarctic marine sediment cores from Ross Sea*

**Edoardo Mentasti, Mery Malandrino, Agnese Giacomino, Claudia Elena Casalino, Ornella Abollino, Enrico Dinelli, Laura Tositti**

*Biophysiological changes observed in members of the Italian expeditions to Antarctica*

**Rosalba Mattei, Antonio Peri, Gianna Taviani, Fabio Ferretti, Emanuele Paolini, Alessandra Grosso, Salvatore Petralia, Silvia Ciardullo, Sergio Caroli**



## PREFACE

The thirteenth edition of Italian-Hungarian Symposium on Spectrochemistry is held in Bologna (Italy), April 20-24, 2008 under the auspices of the University *Alma Mater Studiorum*, of the Italian National Council of Research and of the Italian Ministry of Foreign Affairs. As a part of the bilateral governmental programme for scientific and technical co-operation between Italy and Hungary, this series of biennial events are a well established tradition which provides scientists from both countries with a permanent forum to discuss the outcome of their studies, primarily in the field of human health and environmental protection. The XIII Italian-Hungarian Symposium on Spectrochemistry deals with one of the most serious threat to human health, *i.e.*, the consequences of environmental pollution on food safety. In this regard, the major challenges posed by anthropic activities to food integrity are taken into account, *e.g.*, the presence in the environment of potentially toxic elements released by motor vehicles, the emission to the atmosphere of persistent organic pollutants from industrial plants and the contamination of water bodies with metabolites of pesticides and pharmaceuticals. In this context, the availability of fit-for-purpose, innovative and sophisticated analytical (mostly spectrochemical) techniques is of paramount importance and will thus permeate every aspect of the conference. The backbone of the Symposium consists of approximately forty oral presentations. Poster presentations, in turn, will be displayed for the entire duration of the event. Last, but not least, a number of oral presentations are given by prominent scientists from countries other than Italy or Hungary so as to further stimulate the mutual exchange of views and gain information on other possible approaches adopted to assess and preserve the integrity of food.

*Sergio Caroli and Gyula Záray*



**Oral Session 1**

**Basic research**

*Chairpersons*

Sergio Caroli, Ibolya Molnár-Perl



# BIOCHEMICAL STUDIES BY X-RAY TECHNIQUES

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Due to its excellent micro and trace analytical capability Total-reflection X-ray Fluorescence (TXRF) spectrometry has become a widely used analytical method in biochemical research. In case of laboratory-scale spectrometers equipped with Mo or W X-ray tubes as excitation sources the absolute detection limits is in the range of ng to pg. By application of synchrotron radiation the absolute detection limits can considerably be improved achieving the fg range. Since the determination of low Z elements is hampered by absorption of low energy X-ray photons in the air, the development of the ATI WOBISTAX vacuum spectrometer equipped with Cr X-ray tube paved the way to analytical information from C to U.

For speciation of essential or toxic elements the X-ray techniques offer two different approaches, *i.e.*:

- *off-line hyphenated method.* The biological fluids (*e.g.*, xylem sap, urine) are introduced into a High Performance Liquid Chromatography (HPLC) or Overpressure Liquid Chromatography (OPLC) in order to separate the different species. Then the fractions of the separated molecules are transported by the eluent and collected in small PTEE tubes. For the TXRF determination solution volumes of about 100-500  $\mu\text{L}$  are requested. In this case both the organic and inorganic species of elements can be determined since the TXRF system is only used as an element specific detector;
- *K-edge TXRF-XANES.* This technique delivers information about the oxidation state of medium Z elements. It is a definitive advantage that biological fluids can be analyzed without need for prior separation; however, this method does not give information about the possible organic compounds of elements which are especially important for biological research.

In addition to these X-ray techniques the three-dimensional micro-X-ray Fluorescence tomography offers a new powerful way to study small biological objects (less than 1 mm). It is possible to determine the 2D/3D distribution of elements along an optional slice with trace level detection limits. The sample preparation methods and the analytical capabilities of X-ray techniques mentioned above are illustrated on the basis of the analysis of real samples.

# DEVELOPMENT OF AN ANALYTICAL PROCEDURE FOR THE QUANTIFICATION OF VOLATILE COMPOUNDS IN HUMAN BREATH

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Breath analysis has long been considered a promising technique for health monitoring as an alternative to traditional diagnostic techniques based on blood and urine samples. Due to the almost instantaneous equilibrium between alveolar air and capillary pulmonary blood, the concentrations of compounds in breath are proportional to concentrations in blood. In principle, any volatile substance absorbed by inhalation, *via* the skin or the gastrointestinal tract can be monitored in the exhaled air. For this reason, breath analysis can be used to assess the exposure to chemicals in indoor or outdoor environments and keep risks for health under control. The appeal of this approach to biological monitoring comes from non-invasiveness and the possibility of taking into account both multiple routes of exposure and individual variability, thus giving information on the real absorbed dose. Furthermore, the detection in breath of endogenous markers can provide useful insights into body metabolism and identify alterations of diagnostic interest.

An analytical procedure for the quantification of volatile organic compounds in breath is described involving collection in sampling bags, selective removal of water vapour, pre-concentration in absorption tubes, thermal desorption and GC/MS analysis. In the optimization of operating conditions, attention was focused on crucial steps such as the preparation of standard gas mixtures, the construction of calibration curves, the selection of a suitable desiccant for water removal and the determination of breakthrough volumes for the selected absorption tubes. The optimised procedure allows for the determination of many terpenes, linear and branched hydrocarbons, carbonyl compounds, alcohols, sulphur compounds, aromatic and other exogenous compounds at the ng/g concentration level with an average reproducibility of 5%. Results of analysis of representative breath samples are discussed.

# **SPECTRAL AND TRACE ELEMENTAL CHARACTERIZATION OF SELECTED TEXTILE DYES**

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In textile processing dyes play an important role. Many dyes applied nowadays are metal-complex or contain metal-complexes. During every day's use textile dyes may be extracted from the fabrics and then be absorbed by the skin. Nocuous skin reactions related to textiles coming into direct contact with the skin are raising more and more concern. Heavy metals can be very dangerous to the human population and their toxic effects are currently the subject of many scientific studies. Thus, it is an important issue to monitor heavy metals in substances used for textile production and processing, such as metal-complex dyes.

The main objective of this study was the trace elemental characterization of commonly applied dyes by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Additionally, UV-VIS analyses were performed by scanning over the UV/VIS region (200-900 nm). In this reasearch work 23 textile dye samples were analyzed.

# INDIRECT DETERMINATION OF ORGANIC AND INORGANIC TRACE COMPOUNDS WITH ATOMIC ABSORPTION SPECTROMETRY

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Promazine belongs to phenothiazines, an important family of compounds for use in medicine. Phenothiazine derivatives are widely used tricyclic antidepressants. Their frequent applications in medicine require analytical methods for their determination in body fluids and pharmaceuticals. Promazine dissociates in aqueous solution and gives place to a large, chemically active cation which reacts in acidic media with thiocyanate complexes of metals forming ion-association compounds which are insoluble in water, but can be quantitatively extracted into organic solvents, *e.g.*, chloroform, trichloroethylene or benzene. A 2 mL solution of promazine hydrochloride is added with 2 ml of aqueous ammonium reineckate and the mixture is diluted to a total volume of 5 mL with water and organic solvent. The mixture is stirred for 2-3 min. After separation of the two phases, the organic phase is analyzed by Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS) for Cr concentration. From the Cr concentration the concentration of promazine can be calculated.

A new method for the indirect determination of cocaine with a continuous liquid-liquid extraction is reported. The method is based on the formation of different ion-pairs between the drug and inorganic complex,  $\text{BiI}_4^-$  (Dragendorf reagent), followed by their extraction and the determination of Bi in the organic phase by ET-AAS. The optimal pH, complex concentration and continuous extraction parameters were determined. The method allowed cocaine at low and high concentration ranges (low ng/mL- $\mu\text{g}/\text{mL}$ ) to be determined. The formation and composition of dry aerosols in flames was systematically studied. Based on such data, some general patterns governing the composition of the dry aerosols during the drying process of droplets of solutions were identified. From a solution containing several mineral acids, the metal ion will form a salt in the dry aerosol with the acid having the lowest volatility. The following order of salt-formation holds:  $\text{CH}_3\text{COO}^- \rightarrow \text{Cl}^- \rightarrow \text{NO}_3^- \rightarrow \text{Br}^- \rightarrow \text{I}^- \rightarrow \text{ClO}_4^- \rightarrow \text{SO}_4^{2-} \rightarrow \text{PO}_4^{3-}$ . The initial composition of the dry aerosol can be changed only in the direction of acetate  $\rightarrow$  phosphate by adding another acid. Under extremely oxidative acetylene-air flame conditions ( $\text{C}/\text{O}=0.2$ ), narrow light beam ( $\text{O}=1$  mm), low observation height (1 mm of triple slot burner) of a Flame Atomic Absorption Spectrometry (FAAS) instrument, the absorbance of  $\text{Mg}(\text{ClO}_4)_2$  is 10 times higher than the signal of  $\text{MgSO}_4$ . If  $\text{H}_2\text{SO}_4$  is added at concentrations of  $0 - 5 \cdot 10^{-5}$  M to  $\text{Mg}(\text{ClO}_4)_2$  ( $\text{Mg}=1$   $\mu\text{g}/\text{mL}$ ), the absorbance of magnesium gradually fall to the signal of  $\text{MgSO}_4$  as a consequence of the conversion of dry aerosol along the  $\text{ClO}_4^- \rightarrow \text{SO}_4^{2-}$  direction. This technique is a very sensitive indirect method for the determination of  $\text{H}_2\text{SO}_4$  ( $\text{LoD}=0.15$   $\mu\text{g}/\text{mL}$ ), which was used successfully to determine  $\text{SO}_2$  and  $\text{H}_2\text{SO}_4$  in the indoor atmosphere of an accumulator recharging station.

# FORMYL- AND ACETYLINDOLS: VIBRATIONAL SPECTROSCOPY OF AN EXPECTABLY PHARMACOLOGICALLY ACTIVE COMPOUND FAMILY

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Indole derivatives are present in both animal and vegetable organisms. The most important compound in this group is triptophan a 3-substituted indole and an essential amino acid. Important alkaloids, such as serotonin and triptamin, are also indoles substituted in position 3. Indole-3-carbinol can be found in some vegetables (like cabbage) and its antitumoral and antioxidant effects are a current subject of research. It seems that the biological activity of the indole derivatives is in connection with the substituent in position 3 on the pyrrole ring. This makes of the 3-formyl and 3-acetyl derivatives of indole promising pharmaceuticals.

Seven different derivatives of indole were synthesised. Beside the effect of the aldehyde chain length in position 3, also the N-alkyl effect on the aldehyde derivatives is of interest. The optimised structures, atomic net charges and fundamental vibrational frequencies of these molecules have been calculated using the indole molecule as a reference. An advanced quantum chemical method was applied, namely, the DFT one, with the Becke3LYP functional and the 6-311G\*\* basis set. The substituent effect of the atomic net charges points to the priority of the atoms in intermolecular interactions. Based on the quantum chemical calculations both IR and Raman spectra of the individual molecules were simulated. By comparing the recorded IR and Raman spectra of these compounds to those calculated for the separated molecules it was possible to elucidate the intermolecular associations.

# ELEMENTAL ANALYSIS OF CANCER CELLS BY TOTAL REFLECTION X-RAY FLUORESCENCE

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Relationships among various chemical elements and different types of cancer have been studied for a long time. Especially numerous investigations have been devoted to monitoring of Cu, Fe and Zn levels in a variety of tumoral cells or tissues. One of the most powerful microanalytical techniques in this context is the Total Reflection X-ray Fluorescence (TXRF) spectrometry. This technique requires digested samples or highly diluted suspensions of cells resulting in about 5  $\mu\text{m}$  thick dry residue layer on the sample carrier plate.

This study aimed at developing a direct and rapid TXRF method capable of determining the possible largest number of elements in cancer cells from one single measurement. Therefore, a novel experimental set-up was developed for the determination of trace elements in human cancer cell lines. The cells were directly transferred to TXRF quartz plates by centrifugation in laboratory-modified tubes, followed by digestion on the plates. By using different primer X-ray sources, a significant number of elements could be excited with good efficiency. The measured concentrations are presented and discussed. Such data were also normalized to the total protein content or cell numbers, as customary in biological studies. The method thus developed was used for the determination of Ca, Cu, Fe, K, Mg, Na, P, S and Zn concentrations in different human colorectal adenocarcinoma cell lines (HT-29, HCA-7, SW-620, DLD-1, HCT-15).

# INVESTIGATION OF CR SPECIES TRANSFORMATION IN LOW AND HIGH TEMPERATURE PROCESSES

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Chemical speciation of elements is gaining importance in the life science. Direct speciation methods, however, are hampered by a low detection power as chemical species are quantified in their original form. Indirect speciation techniques, on the other hand, can provide adequate analytical performance, but require that the chemical species are transformed, separated and concentrated, thus increasing the risk of species modification. This investigation deals with Cr species transformation in solution as well as in the solid phase.

Different types of coals and biomass were selected as model compounds in aquatic media at different pH values. The goal of this study was to predict the behaviour of Cr in the presence of humic and fulvic acids in soils as well as in natural waters. Information on the behaviour of airborne C particles with various surface functionalities can be obtained using different types of coal. Negligible transformation of Cr(VI) was found in alkaline medium, whereas reduction was enhanced by decreasing pH. Opposite pH effects occur on the adsorption of Cr(III) thus formed. This leads to the conclusion that the conditions for Cr(VI) preservation in the presence of organics in aquatic media are quite critical.

Samples of biomass, coals, minerals, clays and soils were added with Cr(III) and Cr(VI) separately and heated up to 750 °C. The leachable fraction of Cr was quantified. It was found that Cr(VI) can be reduced to Cr(III) in the presence of organics even though the extent of this process depends on the type of the ignition residue formed. In the case of alkaline-type ashes Cr(III) can be reoxidized to Cr(VI) by the oxygen of the air. Similar conclusions for the Cr(III) transformation into Cr(VI) can be drawn in the case of interaction with inorganic substances at high temperature.

# SPECIATION STUDIES AS A BASIS FOR THE INTERPRETATION OF *IN VITRO* PT-INDUCED CYTOTOXICITY IN BALB/3T3 CELL LINE

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The increasing use of Pt and its potential contamination of the environment has raised the debate over the health risks posed by increased human exposure to this metal. This latter includes chronic industrial exposure (the anionic chlorocomplexes  $\text{PtCl}_4^{2-}$  and  $\text{PtCl}_6^{2-}$  and the cationic species  $\text{Pt}^{2+}$  and  $\text{Pt}^{4+}$ ), environmental exposure as a result of emission from car catalytic converters ( $\text{Pt}^0$  and soluble  $\text{Pt}^{2+}$  and  $\text{Pt}^{4+}$ ) and pharmacological exposure (use of coordination complexes, such as *cis*-diaminodichloro-Pt in chemotherapy). Diet makes a substantial contribution to the total Pt intake (about  $1\text{-}2\mu\text{g}\cdot\text{day}^{-1}$ ). The inorganic soluble Pt salts are responsible for respiratory and cutaneous hypersensitivity. However, there is increasing evidence that the biological effects of Pt depend on the chemical form of the Pt compound tested and on its biotransformation in mammalian cells.

The aim of this work is to show the role of chemical speciation in metabolic patterns of inorganic soluble Pt species in relation to their cytotoxicity induced *in vitro* in Balb/3T3 cell line. The study includes: (i) the assessment of the bioavailability of Pt to cells as investigated by High Performance Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Obvious changes of the original Pt species were observed in culture medium with the possibility of binding of the metal to medium components; (ii) the determination of the uptake of Pt and its intracellular distribution in relation to the cytotoxic effect measured by Colony Forming Efficiency (CFE) assay. The ranking of the amount of Pt incorporated into cells exposed to non-toxic doses of the Pt species was inversely proportional to the corresponding ranking of cytotoxicity. The intracellular distribution of Pt in cells exposed to non-toxic doses of  $\text{PtCl}_4^{2-}$  and  $\text{PtCl}_6^{2-}$  showed no significant differences at 4- and 72-hours exposure. However, at toxic doses of  $\text{PtCl}_6^{2-}$  the cytosolic Pt decreased with a corresponding increase of the element into the nuclear fraction. In addition, gel filtration of the Pt-containing cytosols from exposure to equimolar non-toxic concentration of both Pt chlorocomplexes shows similar chromatographic profiles of Pt. Results point to the critical role of the oxidation state of Pt in inducing the final biological effect.

# SPECIATION STUDIES IN *IN VITRO* ASSAYS ON CR-INDUCED EMBRYOLETALITY. TERATOGENICITY AND CARCINOGENIC POTENTIAL BY ADVANCED ANALYTICAL SPECTROCHEMICAL AND RADIOANALYTICAL TECHNIQUES

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Chromium exists mostly in two oxidation states in nature: hexavalent Cr(VI) and trivalent Cr(III). While Cr(VI) is a well proven toxin, mutagen and carcinogen, the trivalent species is acknowledged as a dietary supplement, being considered essential for proper insulin function and for normal protein, fat and carbohydrate metabolism. Since the two oxidation states have contrasting physiological effects in toxicological studies it is crucial to have available analytical methods to accurately define the individual quantity of both valence forms in biological systems. Several methods are reported in literature on Cr speciation in biological matrices. However, since Cr is present in biological samples at very low concentrations the methods are almost based on preconcentration procedures and separation of the two Cr species before the instrumental measurement. This may lead to the possibility that the original Cr species change during the analytical procedures.

The objectives of this work are connected with mechanistically-based *in vitro* studies on soluble Cr(VI) and Cr(III) species to assess their embryotoxicity and teratogenicity by Frog Embryo Teratogenic Assay on *Xenopus laevis* (FETAX), and to determine the carcinogenic potential by mouse fibroblasts Balb/3T3 cell line. Specific aims are to develop and apply:

- an on-line analytical method by HPLC-ICPMS, to determine the stability of the oxidation state of Cr in the FETAX medium used to expose *Xenopus laevis* embryos to soluble  $\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$  and  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ . The chromate showed a high stability in FETAX for all the duration of the assay (144h). Instead, changes between several forms of Cr(III) were detected but without any conversion from Cr(III) to Cr(VI);
- a radioanalytical method based on the use of  $^{51}\text{Cr}$  radiotracer in combination with solvent extraction and the use of liquid anion exchange resin:
  - (a) to analyze the stability of the carcinogenic  $\text{Na}_2[^{51}\text{Cr}] \text{CrO}_4 \cdot 4\text{H}_2\text{O}$  into culture medium (DMEM) used to growth Balb/3T3 cells. No change of the hexavalent state and no binding with DMEM components were observed during the time of the assay (72h)
  - (b) to determine the oxidation state in the highly purified Cr-DNA adduct isolated from Balb/3T3 cells by NucleoSpin procedure and gel filtration after exposure to the chromate salt for 72h. Chromium was recovered bound to DNA in the trivalent state indicating that a biotransformation of the original hexavalent state occurred into the cell.



**Oral Session 2**

**The point of view of manufacturers**

*Chairpersons*

Gyula Záray, Roger Fuoco



# NOVEL SAMPLE HANDLING TECHNIQUES IN GAS CHROMATOGRAPHY TO ENHANCE DETECTION LIMITS AND IMPROVE PERFORMANCE AND RELIABILITY

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The monitoring of many analytical samples requires sensitive and selective methods as many legislations are becoming more stringent every year. To meet these sensitivity demands the use of automated sampling techniques is of help to concentrate the samples and analyze them in unattended operations.

This talk presents several new applications developed with the use of an enhanced headspace autosampler that uses an on-board packed trap. This headspace trap is a new technological device that is capable of sampling up to 100% of the headspace by a pulsed-pressure headspace extraction process with analytes refocused on an adsorbent trap. The trap is then rapidly heated to desorb the trapped analytes and carrier gas sweeps them *via* an optional splitter as a narrow band onto the GC column. There are substantial gains in sensitivity as a result of the increased sample volume injected (up to 100x in some instances).

Other applications demonstrate the use of a new line of thermal desorption systems that have been significantly improved with over 30 new features including redesigned programmable pneumatics, sample recollection technology and system and sample integrity tests. These features allow the user to repeat the analysis from a single tube. A number of examples based on the use of a Mass Spectrometer detector are illustrated as regards the analysis of ambient air and some food samples.

# ICP-MS AS A CHROMATOGRAPHIC DETECTOR IN FOOD SAFETY AND ENVIRONMENTAL APPLICATIONS

Torsten Lindemann, Shona McSheehy, Meike Hamester  
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Over the last decade, the need to identify and quantify the chemical forms of trace elements has increased considerably. This is especially true for food and environmental samples where principally a difference in the toxicity of chemical species has generated interest in the potential risk or alternatively the bioavailability associated with the presence of certain elements. Development of speciation techniques has focused on the main analytical steps such as sample collection, sample preparation, the separation of species and their detection.

This talk describes the coupling of Gas Chromatography (GC) and Liquid Chromatography (LC) to Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Both analytical approaches have been effectively used for a number of years to answer questions about how trace elements are distributed in certain matrices. Some recent developments in chromatographic methodologies for the determination of trace elements in, *e.g.*, animal feeds, fish and blood are also described. The respective benefits of quadrupole ICP-MS with collision cell technology and Sector Field instruments are briefly discussed along with supporting figures of merit used to evaluate how these techniques address current demand and legislation.

# POLYATOMIC IONS: NO LONGER A PROBLEM FOR ROUTINE MULTI-ELEMENTAL ANALYSIS OF VARIABLE MATRIX SAMPLES, USING ICP-MS

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The rapid acceptance of collision and reaction cell technology as an enhancement to or replacement for conventional quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrumentation is an indication of the dramatic improvements that may be obtained in the analysis of certain interfered trace elements, which previously proved difficult to analyse at the required levels in certain sample matrices. For many routine laboratories, the main attraction of ICP-MS is its simple operation in a multi-elemental analysis role, largely combining the performance of, and therefore replacing, other atomic spectrometric techniques, such as Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS), Atomic Emission Spectrometry Inductively Coupled Plasma (AES-ICP) and specific techniques for the analysis of hydride elements and Hg. However, the potential for these multi-elemental capabilities of ICP-MS to be utilised in combination with the use of collision cell (CC) conditions has been a relatively under-studied area.

Rather than investigating further the range of specific interferences that can be addressed using specific reaction gases and specific cell conditions, where the relative reactivity and therefore the identity of the analyte and interference (*i.e.*, the matrix) must be known and consistent to allow an appropriate reaction gas to be selected, an approach has been developed which is based on the maintenance of the multi-element capability of the ICP-MS when variable and unknown matrix interferences are to be removed.

This is a more difficult analytical challenge than the selection of simple, specific conditions, appropriate only for single analyte-interference pairs in stable, consistent, known sample matrices. In the multi-element analysis of unknown samples, multiple analytes must be measured, without prior knowledge of the interferences that may occur on each analyte in a given sample matrix. This more accurately reflects the analytical needs of a routine contract analysis laboratory, where the composition of the samples is typically unknown, there is no time for specific method development for each matrix-analyte pair and a small set of consistent instrument conditions is used routinely for all sample types.

Using a comparison of the mass spectra obtained in standard mode (no cell gas) and a single set of He mode conditions (one gas type, one gas flow rate and one set of lens and cell voltages), the removal of multiple interferences derived from a complex, multi-component matrix (containing 1% each of HNO<sub>3</sub>, HCl, H<sub>2</sub>SO<sub>4</sub>, butanol, and 100 mg/L each of Ca and Na) was investigated for all isotopes of all elements in the mass range 50 to 84 amu. The effectiveness of the reduction of all interferences in this mass range was studied, with residual peaks in the blank matrix being found to be the result of trace level contamination in the matrix components. Good isotopic template fits were obtained for all elements in this mass range for spikes of 5ng/mL in the same matrix. Finally, the new High Matrix Interface for heavy matrix is described.

# **A COMPREHENSIVE APPROACH TO MICROWAVE SAMPLE PREPARATION**

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Since its development in the early 1980's, Microwave (MW) digestion has become the most popular sample preparation technique for elemental analysis. It is possible to estimate that nowadays over 25,000 instruments are installed worldwide. The technology has significantly evolved during all these years and today's state-of-the-art includes rugged MW hardware, sophisticated reaction sensors and safe and reliable pressure vessels.

The aim of this presentation is to offer an overview of what is currently available. The newest developments are shown and described, with particular emphasis on instrument operating software, temperature control and vessel technology. Several application examples and recovery studies on a wide range of samples are given to facilitate the understanding of these new features. Furthermore, it is time to forecast the future of MW sample preparation. Looking back to the past can be of great support in building the future.

# DEDICATED ASSISTANCE TOOLS TO FAVOUR THE USE OF MULTILINE ANALYSIS IN CCD-BASED ICP-AES

Jean-Michel Mermet (1), Cendrine Dubuisson (2), Emmanuel Fretel (2), Olivier Rogerieux (2), Sébastien Velasquez (2), Sophie Lebouil (2)

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In order to take full benefit of the available information obtained with multichannel detection and to improve the reliability of the results, several lines per element may be used so as to perform multiline analysis. In order to favour multiline analysis, HORIBA Jobin Yvon has developed a series of dedicated assistance software tools to be used with the Charge Coupled Device (CCD)-based ACTIVA Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) system. The aim is to facilitate method development. The first tool is dedicated to multiline selection and is called Multi-element Analysis Selection Tool for Enhanced Reliability (MASTER). MASTER makes use of an ICP-AES-based spectra database, the S<sup>3</sup>-base. This base has a double access, namely: (i) collection of single-element spectra; and (ii) information about wavelength, excitation energy, line width, sensitivity, Limit Of Detection (LOD), detector saturation, and background intensity.

Based on this information, it is possible to select lines that can be used over a given concentration range and to predict spectral interferences through a filtering procedure and an interactive display of the lines and their vicinity. There is obviously no need to prepare synthetic solutions and to perform tedious and time-consuming tests. Once the results are obtained, a detailed analysis of variance (ANOVA) is applied to the series of line concentrations so as to detect any possible outlier through the Statistics for Outlier Survey (SOS) software. A reliable, single concentration can be subsequently obtained for each analyte. Examples of the use of these tools are given.



**Oral Session 3**

**Water**

*Chairpersons*

Paola Bottoni, Viktor Gábor Mihucz



# ADVANCES IN THE SAMPLE PREPARATION. GAS CHROMATOGRAPHIC IDENTIFICATION AND QUANTIFICATION OF WATER SOLUBLE POLLUTANTS

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An overview is presented on recent proposals and experience gained so far on sample preparation and gas chromatographic identification/quantification of pollutants present in the aquatic environment, with special regard to the Non-Steroid Anti-Inflammatory Drugs (NSAIDs), including also other acidic drugs, estrogens, personal care products and pollutants of industrial origin, as well as their metabolites. Published material was classified and evaluated from an analytical viewpoint, with particular emphasis primarily on pollutants' enrichment procedures and secondly on Gas Chromatographic detection and quantification techniques. Recently, more and more attention has been devoted worldwide to the identification and quantification of pharmaceuticals, personal care products, compounds of industrial origin and their metabolites (in the following referred to as pollutants) present in the aquatic environment which can pose health risk to humans and animals as well.

Since pollutants' concentrations - in waste, riverine and drinking waters - can vary between  $10^{-6}$  and  $\ll 10^{-9}$  g/L, an enrichment step prior analysis is inescapable. This enrichment step plays a crucial role in the reliable and reproducible determination of pollutants. Among the major chromatographic methodologies, such as Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE), GC and HPLC are those primarily applied, GC being often the preferred option.

As HPLC techniques are complex and expensive, according to some research groups standardization of the pharmaceutical residue analysis for regulatory purposes can be more conveniently performed by coupling with Mass Spectrometry (MS) (GC-MS and/or GC-MS/MS). In fact, in order to simultaneously identify and quantify as many as possible basically different functional-groups-containing compounds (as required for the determination of water pollutants), GC separation combined with MS detection does provide several advantages, *i.e.*: (i) high selectivity of MS and/or MS/MS detection combined with chromatographic separation at the lowest cost; (ii) high detection power, often equivalent to and in some cases higher than that offered by HPLC; (iii) outstanding resolution of the GC capillary columns; (iv) availability and expediency of the silylation approach in terms of proper labeling of all active-proton-containing functional groups in a single step and of increase in the selectivity and sensitivity of the procedure. Last, but not least, the reasonable cost of the GC-MS/MS systems compared to the corresponding HPLC-MS/MS ones might be also attractive, thus paving the way to its acceptance in specialistic laboratories.

# ITALIAN MONITORING PLAN OF PESTICIDES IN WATER. TECHNICAL BACKGROUND AND RESULTS FOR 2003-2005

Pietro Paris, Dania Esposito

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About 150,000 tons of plant protection products (known also as pesticides) are used every year in Italy. The advantage for the agriculture deriving from these substances is doubtless, but their use also raises issues about possible concern for human health and the environment because most of them are potentially dangerous for living organisms.

Almost 400 active substances are present in products used every year in Italy. Many of these substances, moreover, are present in products used for non-agricultural purposes, for which enough information is not available and the relevant amounts used cannot be quantified. The Italian Environmental Protection Agency (APAT in the Italian Acronym) has been coordinating the national monitoring plan of pesticides in waters since 2003. The aims of the national monitoring plan are: (i) assessment of the occurrence of pesticides in surface and groundwater in order to detect effects not predicted during the authorization procedure; (ii) harmonization of the regional monitoring plan; and (iii) organization of a survey system and information management at the national level.

Before the development of the National plan, monitoring plans varied strongly at the regional level, in particular isofar as the selection of substances is concerned. As a matter of fact, only a few compounds, often not related to the agricultural uses (e.g., DDT), were considered. The plan provides the basis for harmonized regional monitoring programmes, focusing on the substances really used and ranked in relation to the environmental risk. APAT provides methodological documents and guidelines concerning priority substances, monitoring networks, sampling and analytical methods; moreover, APAT collects, processes and reports data.

During the monitoring period 2003-2005, over 29.000 samples were collected, for a total number of measurements exceeding 602.000. The monitoring data showed a widespread contamination mainly in the northern regions. On the other hand, it should be considered that monitoring did not cover completely the southern part of the country. Future developments will be the improvement of monitoring of pesticides in order to cover all the national areas of concern and updating of the plans to take into account new substances.

# **OCCURRENCE AND DISTRIBUTION OF As IN GROUNDWATER OF A LARGE VOLCANIC AREA IN CENTRAL ITALY**

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Arsenic is a naturally occurring element in the earth's crust and has been found throughout the environment. Water contamination by this element is mainly related to non-anthropogenic processes, such as weathering of As-rich geological forms, biological activities and volcanic emissions. Certain geological formations contain high levels of this element that can easily leach into groundwater and find its way into wells and other public water supplies. Ingestion of inorganic As from drinking water has raised public health concern since the International Agency for Research on Cancer set forth sufficient documented evidence of its carcinogenicity to humans. As a result, in 1998 the EU promulgated a new Drinking Water Directive (Council Directive 98/83/EC) containing stricter maximum acceptable concentrations for this element (10 µg/L) than ever before. Even though high concentrations of As have been reported in drinking waters for many areas all over the world, little information is still available on its occurrence and variability at levels below 50 µg/L due to the lack of adequate testing facilities. Therefore, monitoring activities are strongly required, particularly as regards small communities.

This study focused on As occurrence and distribution in groundwaters of a large volcanic area in the central part of Italy. Samples were collected from 100 springs and wells currently supplying drinking water to the province of Viterbo. A complete chemical and physical-chemical characterization of the samples was carried out. The concentration of the target element was greater than 10 µg/L in about 40% of the analyzed samples and greater than 3 times the legal limit in 20% of the sites (in the south-east area of the Vico lake). Moreover, 40% of the concentration values was in the range 2.0-5.0 µg/L. The presence of As is most likely due to the volcanic origin of the monitored area. Cluster analysis of the whole data set pointed to the existence of a significant correlation between As concentration and redox potential. The results obtained will be used to assess the weekly intake of the target element as well as the most suitable removal treatment where necessary.

# THE WATER FRAMEWORK DIRECTIVE: A CHALLENGE FOR ANALYTICAL CHEMISTRY

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The Water Framework Directive (2000/66/EC) (WFD) is the European instrument to achieve good chemical and ecological water status or good ecological potential in all EU Member States (MS). To assess the chemical status of water bodies a list of Priority Substances (PS) has been established. Environmental Quality Standards (EQS) that are derived from toxicological studies are envisaged for those substances. In the proposal for a Daughter Directive on PS they are set up as Annual Average concentrations (AA-EQS) and Maximum Allowable Concentrations (MAC) that have to be met in all water bodies. To determine whether water bodies comply with the EQS, analytical chemical methods must be available in the MS that allow priority substances to be determined at the required concentrations.

The two main performance requirements are tentatively put down in a legally binding Commission Decision under art. 21 of the WFD (QA/QC commission decision) which states that MS shall ensure that the minimum performance criteria for all methods of analysis applied are based:

- on a measurement uncertainty of 50% or below ( $k=2$ ) as estimated at the level of the relevant environmental quality standards; and
- limit of quantification equal or below a value of 30% of the relevant environmental quality standards.

Those two requirements can be met for the majority of the PS, whereas they are hard to meet for some substances as the draft EQS is low (this leading in turn to an extremely low LOQ) and/or because the substance is difficult to analyze due to its physical-chemical properties. The other challenging issue for analytical chemistry derives from the need to assess EQS and long-term trends in water quality by using sediment and biota as alternative or complementary matrices for lipophilic substances. As a consequence, monitoring plans will require the development and validation of robust sampling and analysis methods for the determination of PS in sediment and biota.

# SPECIATION ANALYSIS OF As IN SHALLOW GROUNDWATERS FROM HUNGARY BY PLASMA SPECTROCHEMICAL METHODS

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Arsenic is ubiquitous in the environment, *e.g.*, in the earth's crust, soil, sediments, water, air and living organisms. This element can be present in various forms of different toxicity. Arsenic can be easily released from rocks by weathering, meteorological phenomena, biological activity, anthropogenic influence and dissolution in rain, rivers or groundwater. Several regions and countries all over the world are endangered by the toxic effects of As, due to natural groundwater contamination as well as to anthropogenic sources related to industrial, mining or agricultural activity. In Hungary, in certain areas of the country, the As content of subsurface waters is considerably higher than the acceptable maximum concentration limit, *i.e.*, 10 µg/L. About 1,3 million people are affected by this problem. The present study forms a part of the project entitled *Genesis of the high arsenic content of shallow groundwaters in young basin* financially supported by the Hungarian National Research Fund (No. 67967).

Arsenic speciation analysis of samples was pivotal to the study. The As(III)/As(V) ratio, which is the key parameter of the accumulation and re-mobilization of As and a good geochemical tracer, can in fact be assessed by means of the analysis of As species. The efficiency of As removal processes can be optimized and the magnitude of the health risk can be better assessed if this ratio is known. During the As speciation study of groundwaters, four As species, namely, As(III), As(V), MA and DMA, could be detected. The presence of As(V) and As(III) in the groundwater is primarily natural, but the other two organic species (MA, DMA) are mainly due to anthropic activities. The toxicity of these four species is decreasing in the given order, *i.e.*, As(III), As(V), MA, DMA.

In order to minimize the possibility that the oxidation state changes prior to the analytical determination, a field separation method with easy-to-use ion-exchange columns was employed. Suitability, efficiency and reliability of the separation with different column types were checked. The role of sample pH was also clarified. By the field separation method, the As(III)/As(V) ratio can be recorded at the moment of the sampling and even the organic As species can be separated, if they are at all present. Thus, the speciation analysis does not require hyphenated techniques as these can be replaced with simple As determinations using any suitable approach, *e.g.*, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). This method was used throughout a study aimed at better understanding the origin of As-bearing waters in the outskirts of Kecskemét.

# COMPLEXATION OF Cd AND Cu BY FLUVIAL HUMIC MATTER AND EFFECTS ON THEIR TOXICITY

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The effects of river humic acids and fulvic acids on the bioavailability and toxicity of Cd and Cu were assessed in relation to changes in their speciation. Measurements of the complexing capacity of solutions containing these organic components were carried out by Differential Pulse Anodic Stripping Voltammetry (DPASV) measurements and toxicity tests were carried out by lysosomes isolated from rat liver. The complexing capacity of the solution containing 13 mg/L of humic acids, expressed as ligand concentrations, was 0.30 and 0.072  $\mu\text{mol/L}$  for Cd and Cu, respectively; the corresponding conditional stability constants were  $4.2 \times 10^{11}$  and  $1.3 \times 10^8$   $(\text{mol/L})^{-1}$ . The complexing capacities of the solution containing the same amount of fulvic acids were 0.33 and 0.164  $\mu\text{mol/L}$  for Cd and Cu, respectively and the conditional stability constants were  $3.2 \times 10^{11}$  and  $2.4 \times 10^7$   $(\text{mol/L})^{-1}$ .

The humic acids reduced the toxicity of Cd by five times: the  $\text{EC}_{50}$  changed from 4.4 to 20.4  $\mu\text{mol/L}$ . The dose-effect curve of Cu presented a bi-sigmoid trend and two  $\text{EC}_{50}$  values could be determined. The  $\text{EC}_{50(1)}$  in the presence of humic acids changed from 2.0 to 3.1  $\mu\text{mol/L}$ , while the  $\text{EC}_{50(2)}$  increased from 22.3 to 45.3  $\mu\text{mol/L}$ . The fulvic acids reduced the Cd toxicity by about the same amount as humic acids, from 4.4 to 18.6  $\mu\text{mol/L}$ , but they had no effect on Cu toxicity. Analysing the chemical speciation under toxicity test conditions of the two metals in the presence of humic components, we can hypothesize that more processes contribute to their toxicity reduction. The shape of the dose-effect curves obtained for the two metals suggest that the toxic mechanisms are different.

# **ENVIRONMENTAL ASPECTS OF RIVERBANK FILTRATION ON THE SZENTENDRE ISLAND (DANUBE RIVER, HUNGARY)**

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The objective of this study was to explore the benefits and the incidental disadvantages of riverbank-filtration. The relationship between the hydrological and water quality parameters of the Danube River as well as of four well-sections (Kisoroszi, Tahi I, Sziget-II, Balpart II) on the Szentendre Island (Hungary), was elucidated on the basis of data collected between 1990 and 2006. First, water quality variables of the river were compared to the hydrological and hydrometeorological parameters (*e.g.*, runoff, water and air temperature). A weak correlation was observed between runoff and other water quality components. The present results indicate that temperature has a strong effect on water quality.

In order to estimate the time it takes for water to get into the wells through the riverbank, water quality parameters taken from the Danube river were correlated to those measured in wells assuming a retention time of 0-3, 4-7, 8-11, 12-15 and 16-20 days. The Sziget II well-group is the nearest to the river (20-30 m). Hence, retention time of water is significantly shorter than in the case of the Kisoroszi wells, which have a distance from the river of about 100 m. It was also observed that the water parameters of Sziget II wells are probative of the shortest transport time (2-4 days), whereas the Kisoroszi reservoir is characterized by the longest flow and transport time (10-14 days).

# ENVIRONMENTAL OCCURRENCE AND MICROBIAL DEGRADATION OF SELECTED ACIDIC PHARMACEUTICALS AND THEIR METABOLITES IN WASTEWATER

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Pharmaceutical and Personal Care Products (PPCP), pesticides, herbicides, pharmaceutical drug residues can reach detectable concentrations in rivers and lakes, if their production and use are sufficiently large and the compounds show some mobility and persistence in the aquatic environment. These materials threaten the efficiency of the bank filtration process, which affects the drinking water quality. Acidic Non-Steroidal Anti-Inflammatory Drugs (NSAIDs, such as ibuprofen, naproxen, ketoprofen and diclofenac) are excreted unmetabolized, as parent drug (1-10% for ibuprofen, 1-15% for diclofenac, 30% for naproxen and ketoprofen), or as metabolites, *via* the urine and feces. The parent drugs and their metabolites are conjugated in the hepatic phase II. process, as either sulfates, or glucuronides and may make up to 90% of the excreted amount.

The removal of NSAIDs and their metabolites during the wastewater treatment process has been found to be incomplete and in some cases, the concentration of the parent drug has been increased in the sewage effluent. This may be because the glucuronide- and sulfate-conjugates of the drugs can be cleaved during the microbial degradation in the biological sewage treatment step. In order to study this phenomena NSAIDs in wastewater influents and effluents were measured by Gas Chromatography/Mass Spectrometry (GC/MS), with, or without hydrolysis. This indirect method utilizes the classical liquid-liquid extraction and a two step derivatization process (oximation and trimethyl-silylation). Comparative results of acidic- and alkaline hydrolysis and the calculated concentrations of conjugates will be given.

# **A NOVEL SLURRY SAMPLING ANALYSIS OF Pb IN DIFFERENT WATER SAMPLES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY AFTER COPRECIPITATION WITH Co/PYRROLIDINE DITHIOCARBAMATE COMPLEX**

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A preconcentration/separation technique based on the coprecipitation of lead with Cobalt/pyrrolidine dithiocarbamate complex and subsequently its direct slurry sampling determination by Electrothermal Atomic Absorption Spectrometry was described. For this purpose, at first, lead was coprecipitated with Cobalt/pyrrolidine dithiocarbamate complex formed using Ammonium Pyrrolidine Dithiocarbamate (APDC) as a chelating agent and cobalt as a carrier element. The supernatant was then separated and the slurry of the precipitate prepared in Triton X-100 was directly analyzed by Electrothermal Atomic Absorption Spectrometry with respect to lead concentration.

The effects of experimental conditions on coprecipitation of lead with gathering precipitate as well as homogeneity and stability of the slurry were investigated. After optimization of experimental parameters, a 100- fold enrichment of the analyte with quantitative recovery (>90%) and high precision (<10% RSD) were obtained. By using the proposed technique, the lead concentrations in heavy matrices of Certified Sea-Water and Waste Water samples could be practically and rapidly determined in the range of 95% confidence level. The detection limit of the described method for lead using sample-matching blanks was 1.5 ng/L ( $3\sigma$ , N=10).

# **THE USE OF FLUORESCENCE *IN SITU* HYBRIDIZATION METHOD ON SOIL AND WATER ECOSYSTEMS: A NEW APPROACH TO STUDY THE EFFECT OF XENOBIOTICS ON BACTERIAL COMMUNITY**

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Groundwater quality and pesticide contamination of aquifers have become of great concern in all countries in which populations rely on this resource for drinking water. The transport of pesticides from agricultural fields to groundwater bodies is mainly due to water infiltration through soil. The ability of soil and groundwater to recover from pesticide contamination depends primarily on the presence of a microbial community able to degrade them. Nevertheless, there has been little research on these microorganisms because it relies on methods able to identify and characterize their community structure and functioning. For example, most bacteria living in oligotrophic conditions are unable to grow on classic culture media.

The use of molecular methods makes it possible to overcome this kind of identification limitation. In the present work the Fluorescence *In Situ* Hybridization (FISH) method has been applied to both soil and groundwater samples treated with s-triazines (simazine and terbuthylazine). The bacterial community structure in the presence and in the absence of the herbicides have been compared. The use of 16S rRNA-targeted oligonucleotide probes, designed specifically for the main phylogenetic levels (*Archaea*, *Eubacteria*, *Alpha- Beta-Gamma*-subdivision of *Proteobacteria*, *Plantomycetes* and Gram-positive bacteria with a high DNA G+C content), and a DAPI stain made it possible to assess the structure of the bacterial community and its changing in the presence of the herbicides.

**Oral Session 4**

**Soil and sediment**

*Chairpersons*

Maurizio Forte, János Lakatos



# DEVELOPMENT OF NEW FRACTIONATION METHODOLOGIES FOR CHARACTERIZATION OF ENVIRONMENTAL MOBILITY OF HEAVY METALS IN SOILS, SEDIMENTS AND GRAVITATION DUSTS

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Recent approaches for risk assessment of heavy metal contamination in the soil-water-atmosphere system are based on chemical fractionation by sequential extraction/decomposition techniques. The mostly applied BCR (3+1)-step procedure was developed primarily for the characterization of mobility of heavy metals in aquatic sediments. This procedure follows a pure chemical philosophy and does not model the natural mobilization pathways. This procedure is very time-consuming and the results of fractionation may be influenced by interactions between extractants and solid phase, which can lead to changes in the pH and redistribution of dissolved metals in the liquid and solid phases during the long-term shaking. The BCR procedure can be applied to soils as well. On the other hand, its application to airborne dust samples is still problematic due because of the small amounts available.

Two approaches were developed to overcome the above mentioned problems, *i.e.*:

- improvement of the BCR methodology by accelerating the leaching steps through ultrasonic treatment. Optimization and validation of this procedure was performed using the sediment Certified Reference Materials (CRMs) 601 and 701 and soil and airborne dust working reference;
- application of a new dynamic sequential extraction scheme using supercritical (CO<sub>2</sub>), subcritical (H<sub>2</sub>O) and their mixture (H<sub>2</sub>O+CO<sub>2</sub>) in the given order. Using sediment, soil and gravitation dust reference materials the chemical interpretation and performance of this method were compared with those afforded by both the BCR and the accelerated BCR procedure.

The metal concentrations in the extracts were determined by Flame Atomic Absorption Spectrometry (FAAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Moreover, solid sampling Direct Current Plasma Atomic Emission Spectrometry (DCP-AES) and Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS) were applied to analyze the total element contents in the solid phase.

# CHARACTERIZATION OF METABOLIC ACTIVITY OF DEPTH-RELATED MICROBIAL COMMUNITY IN LAKE SEDIMENT

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Bacteria in the sediment constitute an important component of the benthic food web as well as of nutrient cycling and decomposition of organic matter. In this context, a series of experiments were carried out by TAM III multi-channel microcalorimetry, also in combination with routine microbiological methods. Samples were collected in the Honghu Lake (south part of the Hubei Province, 355 km<sup>2</sup>) by KC Kajak Sediment Core Sampler. The sediment columns were subdivided into short columns (5.0 cm each) which were in turn characterized by thermogenic curves (power-time curves). The total activity of each unit was determined by microcalorimetric technique at 25°C. Thermodynamics models were set up and parameters, such as growth rate constant ( $k$ ), total thermal effect ( $Q$ ) and maximum heat flow ( $P$ ), were obtained. In addition, the cultured microbes' number at different depths was estimated by the plate count method.

The number of bacteria was highest at the sediment surface and decreased with depth. Like the bacteria density, total bacterial metabolic activity decreased with depth. Bacterial activity declined with depth independent of the method used, although the slope was different. Direct microcalorimetry can provide non-specific estimates of the total metabolic activity of the sediment community. Thus, the microcalorimetric method is very useful in the case of sediments that contain mixed communities of anaerobes, fermenters and aerobes.

# REACTIVITY INVESTIGATION OF LAYER-STRUCTURED MINERALS BY SPECTROSCOPIC METHODS

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The reactivity study of layer-structured minerals (*e.g.*, kaolinites, hydrotalcites) is of considerable importance in the light of practical applications (as adsorbents, fillers, additives *etc.*). In addition, organo-clay complexes play a significant role in environmental protection as well. Kaolinite minerals can be fully expanded with low molecular weight organic compounds like formamide, dimethyl sulphoxide and hydrazine. The reagent molecules disrupt the H bonds holding together the kaolinite double layers, which consist of the two-dimensional arrangements of tetrahedral (siloxane) and octahedral (gibbsitic) sheets) and form new hydrogen bonds. This process is known as intercalation.

The intercalation compound is a nano-layered mineral since the mineral sheets are regularly separated with reagent monolayers. The rate of expansion can be studied by X-ray diffraction, while the elucidation of the structure (types of bonds, orientation effects) requires the use of high- and low temperature Raman and FTIR spectroscopic techniques.

The use of Controlled-Rate Thermal Analysis (CRTA) makes it possible to produce “pure” complexes, *i.e.*, containing only reagent molecules between the layers. Thermal deintercalation results in an increased surface reactivity of the mineral. Another methods of synthesizing surface active kaolinites is through mechanochemical activation by dry grinding. Mechanochemical treatment can cause deformation and breaking of O-H, Al-OH, Al-O-Si and Si-O bonds leading to partial dehydroxylation of the mineral. The combination of mechanochemical activation and intercalation/thermal deintercalation is a new method to produce super-active surface centers with tailored properties leading to the development of a potentially new adsorber. The structural variance of surface species can be studied by spectroscopic methods. The methodology developed for the study of kaolinite-type minerals can be used for the investigation of other layer-structured minerals like hydrotalcites or uranyl micas.



**Oral Session 5**

**Atmosphere**

*Chairpersons*

Sergio Caroli, József Posta



# COMPARISON OF THE TRACE ELEMENT CONCENTRATION IN AIRBORNE DUST BETWEEN BUDAPEST AND THE REGION OF BUENOS AIRES

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A study was undertaken to assess the content of major, minor and trace elements in airborne particulate matter collected in Budapest, the capital of Hungary, and San Nicolás, an industrial city, in Argentina. The particulate matter fraction PM<sub>10</sub> was collected in both cities using high volume samplers. The samples in Budapest originate from a very busy traffic junction. The sampling was carried out for three years, with one 4-day sample in each month. The samples in San Nicolás originate from an industrial region, covering 4 sampling sites. Sampling was carried out during one week in winter and one week in summer. Twenty-three trace elements namely, Ag, B, Be, Cd, Co, Cr, Cu, Fe, Ga, Li, Mn, Mo, Ni, Pb, Pt, Rb, Sb, Sn, Te, Tl, U, V and Zn were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Moreover, the concentration of both organic and inorganic carbon was determined. In order to estimate whether the presence of a certain element in the PM<sub>10</sub> fraction was mainly from natural or anthropogenic origin, enrichment factors (EFs) were calculated.

It has been found that traffic related elements (*e.g.*, Sb, Pt) are present at elevated concentrations at both sampling sites. The concentration of industry-related elements (*e.g.*, Fe, Mn, Zn, Ni) was found to be higher in San Nicolás. A detailed comparison of the experimental results is presented.

# URBAN AIR POLLUTION. PARTICULATE MATTER IN BOLOGNA

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Among the air pollutants in urban areas, the Particulate Matter (PM) deserves intensive attention, as indicated by frequent warning from local and international health agencies. For many years, PM<sub>10</sub> were the most extensively monitored PM. Now, these pollutants are considered less dangerous of the smaller sized PM, although their threshold level, up to now, was not prescribed by the UE. In more recent years, PM<sub>2.5</sub> began to be investigated, whereas little information about PM<sub>1</sub> is available.

Recently, the amounts of PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub> are being tested in the urban area of Bologna. The measurements are carried out with a portable aerosol spectrometer (Grimm, mod. 1.101, based on the laser light scattering principle) to check, for short times, the amount of PM. In the same place, during the same day, the mean of 24 hours of PM<sub>10</sub>, was found to be 84  $\mu\text{g m}^{-3}$  as measured by ARPA (Bologna). PM<sub>1</sub> and PM<sub>2.5</sub> turned out to be at very similar concentrations when these pollutants arise from urban traffic. It should be emphasized that this method is a very simple tool for data collection and can be used to convey information to the layman, in particular to undergraduate students, about the actual PM amount inhaled during daily activities.

**Oral Session 6**

**Food**

*Chairpersons*

György Heltai, Giampiero Pagliuca



# RADIOACTIVITY IN FOOD AND ENVIRONMENT: A HISTORICAL OVERVIEW

Maurizio Forte

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Nuclear energy burst onto human history in the middle of the XX century. Fermi switched on his “Chicago Pile 1” in 1942. In August 1945 two A-bombs laid Hiroshima and Nagasaki flat. In the following decades both civil and military applications of nuclear energy had an exponential growth. The first nuclear power plant was built in Sellafield (UK) in 1956 and since then nearly 500 more plants have been installed. On the other hand, nuclear bomb tests continued until a few years ago. Over 2000 nuclear tests have been performed overall, 543 of which in the atmosphere. The latter were partially banned in 1963 and stopped once and for all in 1980. Nevertheless they caused a worldwide contamination by artificial radionuclides which can still be detected ( $^{90}\text{Sr}$ ,  $^{137}\text{Cs}$ , Pu isotopes *etc.*) The maximum intensity of exposure due to nuclear tests took place in 1963 and reached 0.15 mSv (7% of the average natural exposition).

The need for a strong international cooperation both to develop the peaceful applications of nuclear energy and to control its military use became immediately apparent. The International Atomic Energy Agency (IAEA) was created for such a purpose in 1957. In the same year six European countries signed the Treaties of Rome giving birth to Euratom which was one of the very first founding acts of the European Union. While the 1960's and the 1970's were marked by the widespread fear of a nuclear worldwide war, in the 1980's the attitude of public opinion towards nuclear energy was affected by two major accidents in power plants: the first one took place in United States at Three Mile Island and the second, much worse, at Chernobyl in Soviet Union (1986). The Chernobyl accident had a serious health impact only in the surrounding area, but it caused an extensive contamination all over Europe. Chernobyl raised the attention of national and international institutions towards the risks of the “peaceful nuclear energy”. Several initiatives were undertaken both from the regulatory and environmental monitoring viewpoint, in particular by the European Community. The Radioactivity Environmental Monitoring (REM) network was created to provide data for radiological contamination of food and environment throughout the Europe. In addition, Member States set up monitoring programmes which include all most important foodstuffs and environmental matrices.

The IAEA set up the ALMERA network in 1999 with the participation of selected laboratories, worldwide distributed, capable of giving quick analytical support if an emergency occurs. In recent years much attention has been devoted to the study, the monitoring and the regulations concerning the natural radioactivity, in particular as regards:

- indoor Rn. Maximum acceptable levels for  $^{222}\text{Rn}$ , which emanates from soil into houses or working places, have been established. Much work is currently being done to identify the most affected sites (Rn-prone areas) and to set up proper remediation or mitigation programmes;
- radioactivity in drinking water. Recent European directives and recommendations take into consideration the radioactivity in drinking water. Though actual limits are

not given for radionuclides, reference values are reported. Some countries adopt more stringent national regulations, especially when baby food is concerned;

- Naturally Occurring Radioactive Materials (NORM). Many manufacturing activities, not related to nuclear industry, produce wastes which are strongly enriched by natural radionuclides. This raises concerns as regards both workers and the environment;
- artificial radioactive materials for non-nuclear purposes. Radioactive materials (mainly artificial radionuclides) have a widespread use in many fields. They are used as thickness gauges and electrostatic dischargers, for industrial radiography etc. Moreover, in recent years, applications in medicine hugely increased. This is especially relevant since, except in radiotherapy, non-sealed sources are usually employed, with the ensuing much higher risk of environmental contamination.

Analytical techniques for radioactivity detection had probably a slower evolution than those applied in other fields. This seems to be especially true for radiochemical procedures, when direct quantification of radionuclides by gamma spectrometry cannot be achieved. Only in very recent years some efforts to apply a more modern chemical approach have been accomplished.

# THE ROLE OF SAMPLING AND SAMPLE PREPARATION IN FOOD RISK ANALYSIS STRATEGIES

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According to the risk analysis principles, food safety is implemented by the combined action of all the stakeholders involved in food science, control, production, distribution and food ruling with all the ensuing methodological/technological approaches and legal/economic implications. Therefore, food safety should be conceived and implemented with a holistic, cross-thematic and integrated approach, clearly identifying and harmonizing activities, roles and responsibilities of scientists, control authorities, producers, operators, traders and layers. An innovative concept to be associated to any risk analysis process is to provide new scientific tools for the implementation of systematic, reliable, statistically-based, pragmatic, cost-effective, user friendly and harmonized sampling programmes and plans for each step of risk analysis. The implementation of the risk analysis process requires reliable experimental data for the health, legal and socio-economic assessment by a multifactorially-driven process. Two critical factors which so far have not been focused on to obtain reliable concentration data are as follows:

- data should be generated taking into consideration the “fit-for-purpose” principle;
- appropriate and consistent metadata (where “metadata” means “data about data” or the structured information that describes, explains, locates or otherwise makes it easier to use or manage the experimental data to be generated) should be used.

In this context, one of the most relevant factors for obtaining as less as possible biased data is to guarantee the reliability of sampling procedures, especially if addressed to heterogeneously distributed contaminants and/or agents like mycotoxins, salmonella, radionuclides and GMOs. The full process of generating data can be described by four rather different steps, as follows:

- primary sampling programmes. At this stage the appropriate choice of samples (sites, types and times) is performed according to the statistically-based primary sampling programmes set up consistently with and according to: (i) the fit-for-purpose principle for any risk analysis target; (ii) a number of parameters that should be preselected and ranked for the collection of individual samples;
- secondary sampling plans. This step consists of the actual drawing of samples for analysis which are actually representative of the bulk. These have to be performed in agreement with the inference principle according to which the sample for analysis should be representative of the whole consignment or lot;
- sample preparation. The usually rather large sample taken according to the secondary sampling plans must be duly treated in order to obtain a reasonably small sample to be analyzed maintaining the representativeness of the large sample in the final analyzed sample;
- analysis through appropriate analytical methodologies.

# ELEMENT REMOVAL FROM WASHED AND COOKED RICE STUDIED BY SF-ICP-MS AND SYNCHROTRON RADIATION-BASED CONFOCAL MICRO-XRF

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Much attention has to be paid to the element concentrations in rice, a major staple food, the biggest producer of which is the People's Republic of China. Rice preparation procedures vary with cultural traditions and the type of dish to be prepared. Washing and boiling of rice prior to consumption results in the removal of several elements, among which also the toxic ones. In order to determine the extent of this process, rice grains from two Hungarian (*Oryza sativa* L. ssp. *Indica* Risabell and ssp. *Japonica*, Kőröstáj 333) and one Chinese rice (*Oryza sativa* L. ssp. *Indica* Zhenshan 97) species were washed and cooked in deionized water in a ratio of 6 : 1 water : rice (cm<sup>3</sup> : g). The raw, washed and cooked rice samples were subjected to Microwave-assisted digestion. The digested samples were analyzed for their As, Cd, Cu, Fe, Mn, Ni, Pb, Ti, V and Zn concentrations by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS). These results were compared with those obtained on the relevant rice grains by synchrotron radiation-based confocal X-ray Fluorescence analysis, a non destructive technique very well suited for analyzing small-sized biological samples. Moreover, the confocal X-ray Fluorescence analysis (with microscopic volume sensitivity) was performed on the surface as well as in the bulk of the washed and cooked rice grains in order to semi-quantitatively determine the element concentration distribution as weight percentages. For bulk measurements, absorption corrections had to be taken into account. A good correlation was found between the SF-ICP-MS and the X-ray Fluorescence results as regards the removal of the investigated elements from the surface of washed and cooked rice grains.

# METABOLISM, TOXICITY AND CARCINOGENICITY OF DIETARY METALS

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Metals are ingested daily by humans as part of the diet. Some of them are essential for human nutrition, while others are present in foodstuffs without a known biological role. Epidemiological evidence indicates that chronic environmental and occupational exposure to certain well defined physical-chemical species of metal compounds such as As, Cd, Cr and Ni, can cause systemic toxicity and increase the incidence of cancer in humans. In order to improve the scientific basis of health risk assessment associated with dietary metal exposure, the use of integrated strategies along with both *in vivo* data derived from human and animal studies and data from experimental models obtained by *in vitro* systems is required.

The aim of this work is to stimulate researchers in adopting a multidisciplinary approach based on the collaborations among chemists, biochemists and toxicologists in order to investigate *in vitro* the biological effects of trace- and ultratrace metals in food, which also lead to a reduction in the use of laboratory animals, for a better analysis of the associated health risk. In particular, the objectives are: (i) to review the existing experimental evidence on the toxicity and carcinogenic potential in humans and animals associated with dietary metals; (ii) to compare the data with results derived from a systematic study on metal compounds carried out by *in vitro* Cell Transformation Assay (CTA, mouse fibroblasts Balb/3T3 cell line) in conjunction with selected genotoxicity tests (comet and micronucleus assays). This aims at detecting metal carcinogens that act *via* genotoxic or non-genotoxic mechanisms; (iii) to show the importance of establishing *in vitro* an integrated toxicological profile of individual dietary metal species using different types of cells; (iv) to highlight the great potential that nuclear/radioanalytical techniques (neutron activation analysis and radiotracers with high specific radioactivity) in combination with advanced spectrochemical techniques, such as Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS) and High Performance Liquid Chromatography (HPLC) combined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS), have in speciation and metabolic studies as determinants of the interpretation of metal toxicity/carcinogenicity induced by dietary metal compounds in cell cultures; (v) to identify research priorities in the field of potential toxicity and carcinogenicity of dietary metal compounds.

# EXAMINATION OF FACTORS DETERMINING QUALITY OF EGRI BIKAVÉR (BULL'S BLOOD OF EGER)

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Egri Bikavér (Bull's Blood of Eger) is the "flagship" of the Eger wine region in Hungary. Overall quality assessment was done from the viewpoint of wine making and chemical analysis. Wine is not just only an alcoholic beverage, but a much more complex drink. What actually gives to wine its value? Alcohol, acid, sugar or fruity properties? Grapes is closely connected to its surroundings, *i.e.*, the wine region with all its inorganic and biological characteristics. The inherent value of wine is determined by the *terroir*, (a fashionable word today), which includes in practice geographic location, position, basic rock and the soil formed on it and climate even at the microscale.

The Eger wine region is made up of partly brown forest soil formed on rhyolite tuff of volcanic origin and pebbly rocky brown forest soil formed on sedimental sea limestone. Egri Bikavér superior is produced so as to comply with the regulated yield of maximum 0,8 kg/m<sup>2</sup>. The traditional Hungarian varieties of grapes are mostly used, namely, Kékfrankos, Kadarka, Blaugurger, Nagyburgundi and Zweigelt along with wine varieties from other countries, such as Caberne sauvignon, Cabernet franc, Pinot Noir and, Merlot. In this study several Kékfrankos-based (but similarly blended) Egri Bikavér and Egri Bikavér superior wine coming from the same territory were investigated by determining alcohol, sugar, sugarfree extract, titrable acid, volatile acid, colour intensity, polyphenol contents, jelly index, hydrochlorid acid and various other acid components of these wines.

# DETERMINATION OF THE ANTIOXIDANT CAPACITY OF DIFFERENT TYPES OF BREAD AND FLOUR USING A SUPEROXIDE DISMUTASE BIOSENSOR

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The analysis of antioxidant compounds and radical species is currently an issue of great interest. Recently many studies have allowed the role of Reactive Oxygen Species (ROS) in the aging and in degenerative chronic pathologies such as cardiovascular diseases, coronary artery diseases, diabetes, cancer and Alzheimer's disease, to be clarified suggesting that the amount of antioxidant compounds in the diet can be inversely correlated to the onset of the illness mediated by ROS. For this reason many scientific studies are being conducted to measure the antioxidant capacity of several kinds of food. In this work some varieties of common bread as well as particular bread types and the relevant flours have been analyzed using a Superoxide Dismutase (SOD) biosensor recently developed by the present authors and successfully used to determine the scavenging properties of different kinds of fruit, vegetable and other food matrixes.

Measurements were carried out by comparing the biosensor response to the concentration of superoxide radical produced in solution using a xanthine/xanthine oxidase system in the presence and in the absence of the antioxidant sample considered. Precision of the antioxidant capacity measurements for crust and crumb of the different breads was found to be adequate ( $RSD\% \leq 8\%$ ) and acceptable for the aqueous suspensions and filtrates of the different flours studied ( $RSD\% \leq 12\%$ ).

The results obtained showed that in general flours possess antioxidant capacity values higher than the corresponding breads and that crust has always an antioxidant capacity definitely larger than the crumb. Lastly, the antioxidant capacity values were compared with those of almond, red pepper and strawberry, three foods containing powerful natural antioxidants.

# PBDE LEVELS IN FOOD AND DIETARY EXPOSURE IN CENTRAL ITALY

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Organohalogen compounds are a class of organic contaminants that pose potential risks to human health. Due to their persistence, PBDEs are considered as Persistent Organic Pollutants (POPs). Consumption of food is considered as the major source of non-occupational human exposure to most organohalogen compounds, with food of animal origin accounting for more than 90% of the human body burden, and meat, dairy and fish products being the main contributors. In spite of the great interest in evaluating the presence of these chemicals in food, only limited sets of comparable data on the concentration of PBDEs in various foodstuffs are available. In Italy most of the existing information concerns fish and fish products, which are easy to sample and have often been used to evaluate environmental contamination. No specific data are available for Tuscany or central Italy.

A large number of food samples, which most contribute to the daily intake, were analyzed in this study. Results on the levels and compound patterns of PBDEs in a broad variety of food products are presented. An estimation of dietary intake is carried out in order to evaluate the level of exposure of the general population in central Italy. To this end, the results of this investigation have been compared with literature data in other areas. Determination of 19 PBDEs was performed on 487 pools of foodstuff samples from supermarkets in Siena. The average daily consumption for food items was derived from a survey performed by the Italian National Institute for Research on Food and Nutrition (INRAN). Fish and fish products were found to be the items with the highest contents of contaminants. The estimated intake of PBDEs through food items was calculated, meat, fish and dairy products being those of major importance.

# CYCLODEXTRINS AS FLUORESCENCE ENHANCERS FOR MYCOTOXIN DETECTION IN FOOD

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Mycotoxins are toxic secondary metabolites produced by several moulds belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* in a wide range of food commodities supporting fungal growth, such as cereals, dried fruits, oil seeds, spices and pulses.

Over the last years, an increase in mycotoxin contamination of foods caused concern among producers and consumers. Therefore, the availability of reproducible and sensitive methods for screening foodstuffs is essential. Complexation by cyclodextrins *via* formation of inclusion (host-guest) complexes induces significant changes in the physical and chemical properties of mycotoxins as guest molecules, effects that can be used in a variety of analytical techniques. Changes in chromatographic and electrophoretic properties were applied to set up new separation methods. The other particularly significant effect is the enhancement of the mycotoxin fluorescence upon inclusion, phenomenon which provides a simple and convenient method to significantly increase the sensitivity of fluorescence-based trace analysis.

The study of the inclusion mechanism is not only interesting to clarify the observed effects, but it is also useful for the design of new modified selectors with a higher recognition and complexation ability. The design and synthesis of tailored selectors with increased selectivity may be used in the near future to set up very efficient detection methods, also suitable for rapid screening of food and feed products. The fluorescence enhancement results obtained for aflatoxins, ochratoxin A and zearalenone using native and modified cyclodextrins are discussed. Moreover, the mechanism of complexation is described by means of spectroscopic measurements, such as fluorescence, circular dichroism and Nuclear Magnetic Resonance (NMR). Finally, these results can be used for the development of an analytical method for detecting mycotoxins in food.

# PAST FOOD: A MULTI-ANALYTICAL APPROACH FOR THE CHARACTERIZATION OF COMMODITIES IN AN ARCHAEOLOGICAL JAR FROM EGYPT

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Ceramic collections and pieces of pottery are often investigated by archaeologists by means of functional studies to understand how they were used and to obtain information about ancient civilizations in terms of their daily activities, trade and technological level and food consumption. Functional studies usually consist of information coming directly from the archaeological context as well as information from the shape of the item of pottery and the technology used. Nevertheless, this kind of approach can be problematical due to the complex relationship between the information obtained, on one hand, from the shape and technology and, on the other hand, from the archaeological contexts. For this reason, important information regarding the use and function of the vessels can be obtained by independent analytical-chemical, morphological and histological tests. This information allows for a thorough evaluation of the remains, such as amorphous residues, organic material absorbed in the ceramic matrix or as structured remains (seeds, fruits and roots) found in archaeological ceramic vessels.

In particular, the chemical characterization of archaeological materials, the identification of their constituents and the residues contained in ancient vessels is one of the recent methods used in studying how ceramics were used and is very valuable in terms of what it reveals about ancient diet. For instance, it is well known from historical records that grapevine was grown and wine was produced in the Mediterranean basin already for some 5000 years. Yet, little more is known. Only recently the application of Liquid Chromatography (LC) coupled to Mass Spectrometry (MS) in the tandem mode (LC-MS-MS) for the identification of traces of wine markers in ancient Egyptian vessels revealed that they had been used for storage of red wine. A common wine marker is tartaric acid, while syringic acid is the product derived from malvidin, a red wine pigment. Studying residues from archaeological findings, especially those of an organic nature, is a dynamic field, which is increasing in terms of its popularity and objectives and poses a challenge to analytical chemistry. This is due not only to the complexity of the chemical composition of the organic and inorganic substances that may be present, but also to changes in their chemical composition due to natural ageing and/or human activities. For all these reasons, the use of multi-analytical approaches providing complementary information and enabling the inorganic and organic materials in the object to be reliably recognized are thus particularly suitable for the chemical study of complex and degraded materials such as those from archaeological findings.

This paper describes a multi-analytical chemical study performed on the original, almost totally conserved, content of a small ceramic jar from the Antinoe archaeological site (V-VII centuries AD, Roman Egypt) and now belonging to the archaeological collection of the Istituto Papirologico “Girolamo Vitelli” (Florence, Italy). Scanning

Electron Microscopy (SEM) with an Energy Dispersive X-ray (EDX) spectrometer, Fourier Transformed Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) spectrometry were all used to characterize the inorganic components of the archaeological material. The organic substances were analyzed using FTIR, Direct Exposure Mass Spectrometry (DE-MS) and Gas Chromatography-Mass Spectrometry (GC-MS).

The inorganic component essentially consisted of halite (NaCl). Among the NaCl crystals, lamellar elements were identified. They were chemically characterized by carbonate-apatite and showed a concentric morphology typical of the scales of small fish. The most prevalent organic constituents of the sample were monocarboxylic acids,  $\alpha,\omega$ -dicarboxylic acids and cholesterol and its oxidation products. The organic material composition was consistent with the occurrence of lipids of animal origin. In addition, diterpenes related to pine pitch were also identified. The overall results suggest that the material recovered in the small ceramic jar found in Antinoe is a residue of fish-based pickles such as *garum*, *muria*, *allex* and *liquamen*, which were commonly used in Roman times.



**Oral Session 7**

**Research in Antarctica**

*Chairpersons*

Erika Ribechini, Éva Bertalan



# SPATIAL AND TEMPORAL TRENDS OF ORGANIC POLLUTANTS IN ANTARCTICA

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The basic objective of environmental monitoring is to detect and measure changes in the environment by collecting time series of data for defined purposes and by observing spatial and temporal trends in the selected variables. The Antarctic environment is still almost uncontaminated by human activities and is thus as an unparalleled natural laboratory for research into the problems of global change and global pollution. The Polar Chemistry Project of the Italian Research Programme in Antarctica (PNRA in the Italian acronym) studies these processes by monitoring organic and inorganic pollutants in different environmental components, elucidating the relevant diffusion and distribution processes, and evaluating their change in the short- and long-term by means of sample of those matrixes that retain this information.

In particular, spatial and temporal trends of a few classes of Persistent Organic Pollutants (POPs) and their diffusion on a large area of Antarctica were investigated during several Italian Expeditions from 1996 to 2005. Samples of snow and firn were collected at various distances from the sea, at different altitudes and at different depths in several coastal and plateau sites. Organic compounds linked to sea aerosol show a fractionation that depends on the altitude and the distance from the sea according to their volatility and affinity to aerosol particles. Finally, temporal trends which were evaluated in the short- (10 years) and medium-term (100 years) are discussed.

# SET-UP OF A HIGH RESOLUTION ICP-MS METHOD FOR THE DETERMINATION OF TRACE ELEMENTS AND REES IN SOIL AND OCEANIC SEDIMENT SAMPLES

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In the framework of the international projects European Project for Ice Coring in Antarctica (EPICA) and Antarctic Drilling Project (ANDRILL), a Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS) method for the determination of trace elements and Rare Earths Elements (REEs) was set up. The analytical procedure, after complete acid digestion of the solid matrix, was optimized for the geochemical characterization of soil and oceanic sediment samples. The choice of the isotopes used for the quantitative analysis and the selection of the suitable mass resolution have been evaluated as a function of the relative isotopic abundance and the possible interferences present in real samples for each element of interest. Particular care was devoted to the selection of the best method for the analytical quantification among the methods of external calibration, internal addition and internal standard with drift correction.

The performance and reliability of the different methods were evaluated by analysis of Certified Reference Materials (CRMs). In particular, three different CRMs were used, namely, the Basalt, Hawaiian Volcanic Observatory (BHVO-1), the Japanese Andesite (JA-2) and the Antarctic Marine Sediment (MURST-ISS-A1). Because of the low concentrations of some elements in the solutions obtained after acid digestion, particular care was paid to the study of the blanks and to the optimization of the operative conditions. Finally, in order to provide a complete geochemical characterization of soils and sediments, an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) method for the determination of the composition in major elements after acid digestion was also set up.

# **STUDIES ON Cd BIOACCUMULATION IN THE LIVER OF THE ANTARCTIC TELEOST *TREMATOMUS BERNACCHII***

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Cadmium is regarded as a priority pollutant in many environmental norms. It tends to accumulate in tissues during organism growth (bioaccumulation) and often biomagnifies up the aquatic food chain. Organisms have developed different mechanisms to nullify or minimize the toxic effects of heavy metals. A basic role in these processes is played by Metallothioneins (MTT), *i.e.*, intracellular proteins that bind metals.

In this work, the variation of Cd concentration in the liver of the Antarctic teleost *Trematomus bernacchii* was studied over a 7-day period of exposure to Cd-contaminated seawater. During the XXI Italian Expedition to Antarctica (austral summer 2005-2006) twenty specimens of *Trematomus bernacchii* were caught close to the Mario Zucchelli Italian Station at Terra Nova Bay (Ross Sea). After an acclimatization period of 2-3 days at a temperature of  $-1\pm 0.5^{\circ}\text{C}$ , 10 specimens were exposed to a Cd concentration of 2.0 mg/L in seawater and 10 specimens (controls) were not exposed to Cd (aquaria with running seawater).

Organisms were taken for analysis after 0, 1, 3, 5 and 7 days from the beginning of the experiment (each time, two Cd-treated specimens and two control specimens, respectively). Samples of various organs and tissues (liver, gills, gut, muscle and gonads) were dissected and three sub-samples were stored at  $-20^{\circ}\text{C}$  for metal determination, while three other sub-samples were frozen at  $-80^{\circ}\text{C}$  for MTT determination. Once back to Italy, the liver samples were thawed, dried until constant weight and then digested under pressure with high purity  $\text{HNO}_3$  (~68%) in a Microwave (MW) system (CEM, Mars 5). The digested liver solution was then properly diluted for metal determination using the Differential Pulse Anodic Stripping Voltammetry (DPASV) technique.

Results show that while the liver Cd concentration remains practically constant in the control specimens during the experiment ( $8.7\pm 0.9 \mu\text{g/g}$ ), it increases almost linearly in the exposed organisms and over a week reaches values which are 3-times higher than those of the controls ( $31\pm 3 \mu\text{g/g}$ ). It is possible that a higher rate of increase may occur for much longer exposure periods (weeks or months), as observed in experiments carried out by other authors with different organisms and in different ecosystems.

# **A TWELVE THOUSAND YEARS RECORD IN THE OCCURRENCE OF TRACE METALS IN THE SEDIMENT OF THE ROSS SEA (ANTARCTICA)**

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Fifteenth major and trace elements were determined in one sediment core collected in one accumulation site of the Ross Sea (Antarctica) and covering the entire Holocene. Measurements were carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results show enhanced variability of concentration for most metals. The relative standard deviation ranged between 33% for Zn and 153% for Cd.

Multivariate statistical analysis pointed to three groups of elements: crustal elements the presence of which is associated to the atmospheric transport; elements for which the sedimentation process is controlled by the biological activity in the region; elements for which the distribution process is affected by both processes. The metal distributions along the sediment core are compared to that of tracer elements in one dated ice core covering the same time period and collected at one site about 2000 km from the coastal area (Dome Concorde), where the atmospheric transport is the prevalent process. Comparison of records and climatic proxies are used to hypothesize the effects of climate change on the fate of trace elements in the marine environment.

# VOLATILE CHLORINATED HYDROCARBONS IN ANTARCTICA. ANALYTICAL ASPECTS AND ENVIRONMENTAL EVALUATION

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Volatile Chlorinated Hydrocarbons (VCHCs) are considered to be markers of global contamination processes and cause of possible accumulation phenomena in Earth's cold areas characterized by reduced photochemical activity, where they can produce harmful ecotoxic effects. The first goal of this research project was the evaluation of environmental fate of VCHCs both in a global context, based on natural distillation cycles and in the specific context of Antarctica. Another target was the determination of VCHCs at air/water and air/snow interfaces, both with a temperate zones' climate and the Antarctic climatic conditions, so as to evaluate differences in distributions among environmental compartments. Fit-for-purpose analytical methodologies have been developed for years and VCHCs have been monitored in Antarctic aqueous matrices. VCHCs were found in the entire Antarctic aquatic environment with concentrations ranging from a few ng/L to hundreds of ng/L.

In this work sampling and analytical methods for VCHCs' determination in air were further developed. Air sampling was planned taking into account the operative difficulties posed by remote zones. The analytical system for VCHCs' analysis in air at ppt (v/v) level included a Cryofocusing Trap Injector (CTI) device coupled to a Gas Chromatography (GC) apparatus with Mass Spectrometry (MS) detection. During some Italian expeditions in Antarctica, air and aqueous matrices samples were gathered and analyzed. At the same time, samples of air, water and snow samples from temperate zones, such as Italy, were gathered and analysed. The results obtained confirmed VCHCs' environmental ubiquity. The atmosphere/aquatic environment concentration ratios were in favour of aquatic environment (*i.e.*, lower than 1). VCHCs tends to reside in aqueous matrices. In fact they contaminate all water body, both superficial and groundwater. Besides, once in atmosphere through distillation cycles and air masses circulation, VCHCs reach remote and cold zones of the Earth, where they tend to accumulate on snow *via* snowfalls' scavengers. Accumulation phenomenon of VCHCs in Antarctica is driven by an air/snow concentration ratio clearly in favour of snow.

Experimental data gained in this work can be used to model the environmental fate of VCHCs in polar climates as such studies have not yet been undertaken.

## THE CERTIFICATION PROJECT FOR TRACE ELEMENTS IN *ADAMUSSIUM COLBECKI*

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A certification project for a new candidate Certified Reference Material (CRM) for trace elements based on the Antarctic bivalve *Adamussium colbecki* (IRMM 813) was undertaken by the Istituto Superiore di Sanità (ISS, Rome, Italy) in cooperation with the Institute for Reference Materials and Measurements, Joint Research Centre of the European Commission (EC-JRC-IRMM), in the frame of the Italian National Programme of Research in Antarctica (Programma Nazionale di Ricerca in Antartide, PNRA). Samples were collected at Terra Nova Bay (Ross Sea) during the 2000-2001 expedition in Antarctica. The pre-treatment of the material was performed at the EC-JRC-IRMM, whereas homogeneity and short- and long-term stability tests were carried out at the ISS. The elements selected for the certification project were As, Cd, Cu, Cr, Fe, Mn, Ni and Zn.

Fifteen internationally renowned expert laboratories were selected for the accomplishment of the certification campaign. A variety of analytical techniques were used, such as Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Quadrupole Inductively Coupled Plasma Mass Spectrometry (Q-ICP-MS) with and without Dynamic Reaction Cell (DRC) and anodic stripping voltammetry. Experimental data submitted by each laboratory are being processed and it is expected that the project can be successfully closed within 2008.

**Poster Session 1**  
**Basic research**

*Coordinator*  
Mihály Óvári



# **THE REMOVAL AND PRECONCENTRATION OF Pb FROM WATER SOLUTION BY USING FLOW INJECTION TECHNIQUES**

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A flow-injection, solid-phase extraction, preconcentration method was developed for the determination of Pb in water samples by Flame Atomic Absorption Spectrometry (FAAS). Lead was adsorbed on the EGDMA-MA polymer. Several parameters were optimized such as sample acidity and volume, loading and elution flow rates and eluent type and volume. Lead adsorbed at pH 2-8 on the EGDMA-MA column was eluted with 500  $\mu\text{L}$  of 4.0 M  $\text{HNO}_3$  at 4.9 mL/min. Finally, the method was evaluated through spike recovery from aqueous samples.

# COMPARATIVE EVALUATION OF THE DETERMINATION OF SOME PHYTO-HORMONES IN PLANTS BY GC-MS AND HPLC-MS

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Two analytical procedures based on Gas Chromatography (GC) coupled with Mass Spectrometry (MS), namely, GC-MS and High Performance Liquid Chromatography (HPLC) coupled with MS in the tandem mode, namely HPLC-MS/MS, have been optimized, particularly for the determination of a few classes of phyto-hormones in plant samples. In this context, 3-Indoleacetic Acid (IAA) (an auxin which is mainly responsible for stem extension in plants) isopentenyladenine (iP), isopentenyladenosine (iPR), zeatin (Z) and Zeatin Riboside (ZR) (cytokinins that are mainly responsible for cell division and proliferation) have been taken into consideration. In particular, an extraction procedure was developed based on Bielecki modified solvent, which allows the enzymatic degradation of phytohormones to be blocked (in particular cytokinins) without extracting large quantities of lipids. GC-MS analysis entailed the derivatization of phytohormones in volatile and less polar compounds and subsequent two-step sample purification. On the basis of an experimental evaluation, N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) turned out to be the most versatile reagent, as it gave good calibration curves for all the compounds under test. On the other hand, HPLC-MS analyses were performed directly on the extract, as this technique does not need the preliminary stages of purification and derivatization.

The optimized analytical procedures were applied to the evaluation of the above phyto-hormones in both *Nicotiana glauca* and *Nicotiana langsdorffii* species. A comparative evaluation of the results obtained by GC-MS and HPLC-MS was done in terms of limits of detection and applicability to real samples.

# **CULTURE MEDIUM COMPONENTS AS FACTOR INFLUENCING THE EFFECTIVE BIOAVAILABILITY OF METAL IONS *IN VITRO*: THE EXAMPLE OF $\text{Co}^{2+}$ IN CELL TRANSFORMATION ASSAY (BALB/3T3CELL LINE)**

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Humans can be exposed to Co from occupational and consumer sources as well as by exposure from the environment, food being the most significant source of Co. Cobalt has both beneficial and harmful effects on human health. Dietary sources provide Co in the form of vitamin B<sub>12</sub>, a cofactor in catalyzing key biochemical reactions. Excessive exposure to Co has been associated with contact allergic dermatitis, cardiomyopathy and occupational asthma and lung fibrosis. One unsolved question is the carcinogenicity and genotoxicity of the inorganic soluble Co salts ( $\text{Co}^{2+}$ ). Although Co and its compounds are classified as possibly carcinogenic to humans (Class 2B) by the International Agency for Research on Cancer (IARC), the existing epidemiological studies are of uncertain relevance, if not equivocal, for evaluating whether exposure to soluble Co causes cancer.

The objective of this work is linked to a study on the *in vitro* determination of the carcinogenic potential of  $\text{Co}^{2+}$  by mouse fibroblast Balb/3T3 cell line, a well recognized two-stage model to assess the concurrent cytotoxicity and morphological transformation of chemicals. In particular, it is meant to show how the interaction of  $\text{Co}^{2+}$  with cell culture medium (DMEM) components can affect the bioavailability of Co to the cell. The study has been carried out by using radioanalytical ( $^{57}\text{Co}^{2+}$  radiotracer) techniques in combination with ion exchange and gel filtration chromatography. Aspects investigated cover the kinetic of complexation of  $\text{Co}^{2+}$  with culture medium components, the characterization of the electronic charge of the complexes formed (presence of negative, positive, neutral complexes and free ions), the identification of the sequestering agents able to complex  $\text{Co}^{2+}$  (albumin and histidine) and the measurement of the saturation point of such complexes by  $\text{Co}^{2+}$ .

Results show how the components of the culture medium reduce the effective bioavailability of  $\text{Co}^{2+}$  to cells. Thus, the value of the IC<sub>50</sub> (concentration of Co causing 50% of cell survival, index of basal cytotoxicity) derived from the dose-effect relationship is underestimated. This is of a crucial importance considering that the IC<sub>50</sub> is the fundamental parameter to select the  $\text{Co}^{2+}$  concentrations on which the subsequent morphological transformation assay is based.

# DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN VIRGIN AND RECYCLED ELASTOMERIC MATERIALS

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In the winter of 2006 the Italian National Institute of Health (Istituto Superiore di Sanità) was involved in a study on rubber granulate used for playground surfaces of football fields made of synthetic turf. Synthetic turf is generally constituted of plastic fibres of polyethene, polypropylene or nylon attached to a plastic web of polypropylene or polyester. Sand and rubber granulate is filled between the fibres in order to hold the plastic web in place and provide elastic properties. Although virgin elastomeric material is also used, the principal source of rubber granulate is recycled waste tyres. Recent studies have demonstrated the presence in this material of chemical substances of toxicological concern, such as Polycyclic Aromatic Hydrocarbons (PAHs), phthalates, and certain metals. The possible release of these toxic substances from recycled tyres has caused a discussion in several European countries concerning the properties of synthetic turf and the potential health and environmental effects linked to its use.

The study carried out by the Italian National Institute of Health covered different types of rubber granulate in use in some Italian football fields, including virgin elastomeric material, waste tyres, “nobilitated” waste tyres, vulcanized rubber waste and material from shredding of seals. Each type of granulate was analyzed with regard to the content of PAHs, chlorinated aromatic and aliphatic compounds, nitrated and chlorinated benzenes and metals.

This paper describes the results obtained from the analysis of carcinogenic PAHs with 4-6 rings, including benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, chrisene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pirene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[c,d]pyrene and pyrene. As expected, PAH levels in samples of rubber granulate originating from virgin thermoplastic material were in general found to be lower than those measured in samples from recycled wastes. In addition, in some of these samples PAH concentrations were found to exceed the Italian regulatory values established by the Legislative Decree 152/2006.

# REMOVAL OF METAL POLLUTANTS FROM ENVIRONMENTAL COTTON SAMPLES BY ULTRASOUND ASSISTED EXTRACTION

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Ultrasonic Extraction (UE) is today recognized as a useful method for the extraction of elements from different biological and environmental samples. One of the first applications of UE of metals prior to multielement determinations was previously performed. For this purpose, a NIST SRM standard material was subjected to ultrasonic irradiation. In a more recent study, the use of UE for the determination of metals in several European reference materials was investigated. Numerous other studies have demonstrated the usefulness of ultrasound for an effective extraction and single or multielement determination of heavy metals. UE is a fast, inexpensive and efficient alternative to conventional leaching processes. The industrial usage is spread from the food industry to textile manufacturing with different applications: biopreparation, dyeing, laundry and textile finishing. It is expected that the use of UE for sample preparation purposes in environmental analytical chemistry will become widespread due to its simplicity, easy applicability, speed and enhanced safety compared to other, more traditional sample preparation methods.

Textile raw materials like cotton, flax or hemp contain different metals. They are able to adsorb large amounts of metals from the environment depending on the conditions of growth (soil, water and air). Exposure of plants to high metal concentrations can be toxic for the plant. On the other hand such plants can be used as natural bio-absorbers. Metals present may also pose problems in the textile industry, *e.g.*, in yarn manufacturing, bleaching and dyeing.

This work presents the UE efficiency of 23 elements (Al, As, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Si, Sn, Tl, and Zn) extracted from standard cotton adsorptive material after previous adsorption of metals. The elements were extracted from the samples for 5-45 min in an ultrasonic bath, afterwards they were dried, weighed and digested in a Microwave oven in 10 mL of 7 M HNO<sub>3</sub> at a temperature not exceeding 200°C. The total concentration of metals in the resulting solutions after digestion was determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

An application of this approach to cotton material was illustrated. The results obtained point to better efficiency, less solvent consumption, shorter extraction time as well as ecological and economical advantages of the UE method.

# AUTOMATED INDIRECT DETERMINATION OF FLUORIDE IONS BY ICP-AES

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The direct atomic spectroscopic determination of F encounters several difficulties. Excitation of the F atom requires high energy, which is difficult to provide by an Ar plasma. An additional obstacle is that the wavelengths of the resonant lines are below 100 nm, thus requiring a sophisticated optical system. Only a few papers deal with F determination in gaseous samples at the non-resonant line (F I 685.602 nm) by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using direct injection *via* a gas sampling loop or a Gas Chromatographic apparatus. A direct F determination method in aqueous samples by ICP-AES was reported using the electrothermal vaporisation sample introduction system.

The indirect method developed in this study is based on the fact that F ions form a stable precipitate with Ce(III) ions according to the reaction  $\text{Ce}^{3+} + 3 \text{F}^- = \text{CeF}_3$  ( $\lg K_s = 16.1$ ). After the separation of the Ce(III) fluoride precipitate, the Ce concentration of the clear solution can be measured by ICP-AES. The determination of the excess Ce concentration by ICP-AES provides a high precision method for F determination in various samples. This method can be easily automated using a simultaneous ICP-AES spectrometer and a Flow Injection Analysis System (FIAS) unit. The FIAS and the ICP-AES spectrometer can be connected quickly and readily. The combination of ICP-AES and FIAS unit has been used for determination of Hg and hydride-forming elements and limits of detection achieved for these elements are better than without FIAS.

The precipitate-forming reagent concentration and the peristaltic pump speeds were optimized. In this work the analytical performance of the automated and the batch method was compared as well. The chemical background of the proposed determination, *i.e.*, a complex analytical study of the precipitation and complex formation reactions, was presented. The applicability of the technique for the study of groundwater monitoring of a fluoride contaminated area was demonstrated.

# A NEW METHOD FOR THE UTILIZATION OF RED MUD

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Red mud is a dangerous waste which forms in the Bayer process during production of alumina. This sludge has composition characteristics which hinder its recycling. Consequently, huge dump sites are needed all over the world to store it. In the Bayer process the bauxite is treated with NaOH. During the treatment several Al compounds can be transformed into water soluble forms except the ferrous minerals which gave place to an Fe rich residue with still considerable Al content. Typical composition of the red mud is (in %): Al<sub>2</sub>O<sub>3</sub>, 16-18; Fe<sub>2</sub>O<sub>3</sub>, 33-48; SiO<sub>2</sub>, 9-15; TiO<sub>2</sub>, 4-6; Na<sub>2</sub>O, 8-12; MgO, 0.3-1; CaO, 0.5-3.5; V<sub>2</sub>O<sub>5</sub>, 0.2-0.3; P<sub>2</sub>O<sub>5</sub>, 0.5-1.0. Removal of the rest of the Al content in the red mud can open new ways for its exploitation.

In this investigation two types of experiments were conducted:

- extraction by different aqueous salt and acidic solutions;
- Microwave (MW)- and ultrasound-assisted acidic and alkaline dissolution.

For the selective extraction a range of solution with increasing acidity were used: distilled water, MgCl<sub>2</sub> 1 M, acetic acid 1 M, 0 hydroxylamine chloride 1 M+acetic acid 1 M, nitric acid 1 M+20% (w/w) H<sub>2</sub>O<sub>2</sub> etc. These extraction experiments proved that the mineral transformations in the Bayer process improve the Al solubility and decrease the solubility of Fe-containing minerals in the weak acidic media. Using weak acids most of the Al can be selectively extracted from the mud.

The extraction process was facilitated by ultrasound and MW irradiation. In this experiment the reagents were aqueous solutions of HCl, acetic acid and NaOH at concentrations of 0.5, 1, 2, 3 and 4 M. Both approaches can increase the dissolved amount of Al and Fe by mineral and acetic acid. However, the dissolution remains unselective. In case of NaOH both the efficiency and selectivity can be improved as Fe is insoluble. By the ultrasound-assisted extraction in an open air vessel the efficiency improvement was only 20% for Al and practically zero for Fe. By the MW-assisted extraction most of the Al can be dissolved with the 2-3 M NaOH liquors during the one-hour treatment if the temperature is higher than 100°C and the liquor/solid phase ratio is larger than 20. The MW-assisted extraction can be considered an efficient method for the selective removal of Al from the deposited red mud providing new perspectives for its utilisation.

# NOVEL DECOMPOSITION METHOD FOR DIRECT TOTAL X-RAY FLUORESCENCE ANALYSIS

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Total Reflection X-ray Fluorescence (TXRF) spectrometry represents a powerful tool for the analysis of mass-limited biological samples. In the case of direct methods, the biological material is placed on the quartz plate and measured with or without preliminary digestion. The clear advantages of direct methods are minimization of contamination, speed of treatment and no dilution of the samples. The aim of this work was to develop a digestion method for direct TXRF analysis using biological materials. The thickness of the dry residue on the carrier surface is critical especially for the measurement of the low Z elements. Using an internal standardization method the thickness of the dry residue cannot exceed 5  $\mu\text{m}$ .

For developing the method, four different Certified Reference Materials (CRMs) were chosen, two plants, *i.e.*, IAEA-331 (spinach) and IAEA-359 (cabbage), and two of animal origin, *i.e.*, NIST 1577a (bovine liver) and MURST ISS-A2 (antarctic krill) with different elemental composition. About 1 mg ( $\pm 20\%$ ) of each CRM was weighed on quartz plates, 100-200 ng of the Ti internal standard were added and the plates were then put on quartz stands, covered by quartz caps and digested with vapour phase  $\text{HNO}_3$  using a Microwave (MW) digestion system. The quartz tools were prepared in the laboratory. The MW programme was optimized by changing the pressure and the digestion time parameters of the MW-assisted vapour phase acidic digestion procedure. The selected internal standard was appropriate for quantitative analysis of biological matrices applying Cr or Mo X-ray tubes for the determination of low and high Z elements, respectively.

# THE OECD ACTIVITIES FOR THE CONTROL OF CHEMICALS IN THE INTERNATIONAL TRADE

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An overview is given of the degree of implementation of the OECD Principles of GLP in Member and Non-member Countries. Such activities allow the risk-to-benefit ratios of new chemicals (primarily industrial chemicals, pesticides, cosmetics, food and feed additives and pharmaceuticals) to be assessed, and promote better co-operation among Monitoring Authorities (MAs), Regulatory Authorities (RAs) and Test Facilities (TFs) in respect to Part II of the 1989 Council Decision and the Mutual Acceptance of Data (MAD). This results in better understanding and interaction among all partners and, in the ultimate, in a more effective protection of human health and the environment.



**Poster Session 2**

**Water**

*Coordinator*

Pietro Paris



# **ADSORPTION OF HUMIC SUBSTANCES ON MELAMINE FORMALDEHYDE UREA RESIN FROM DIFFERENT NATURAL SOURCE WATERS**

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Humic substances are the most common natural polymers on the Earth. These biopolymers can be found in animals, plants, soils and especially in natural waters. Humic substances originating from plants and soil, mixed with water from natural sources may change the color of water, complex with metal ions and produce trihalomethanes reacting with  $\text{Cl}_2$ . For this reason, humic substances should be removed from waters in order to better protect human health.

In this study, the adsorption of humic substances from natural waters onto melamine formaldehyde resin was studied. The IR spectrum of the newly synthesized material was evaluated. The adsorption of humic substances onto melamine formaldehyde urea resin was carried out by both batch and column method under acidic conditions at room temperature. The optimum pH for adsorption process was found to be 3.5. Adsorption isotherm analysis data under various initial concentrations of humic substances led to a model fitting well into the Langmuir equation. The optimum flow rate for the column method was found to be 1 mL/min. The regeneration of the resin can be performed with sodium acetate.

# LEACHING OF Sb FROM POLYETHYLENE TEREPHTHALATE BOTTLES INTO MINERAL WATERS AND SOFT DRINKS

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Antimony trioxide is the most important catalyst used in the manufacture of Polyethylene Terephthalate (PET). In a recent study, it was shown that water can be contaminated during storage because of Sb leaching from PET. The PET bottles contain hundreds of mg/kg of Sb, whereas the abundance of Sb in crustal rocks is in the order of 0.5 mg/kg. The Sb concentration of commercially available Hungarian and Chinese mineral waters as well as their respective PET bottles were determined by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS) in order to evaluate the extent of Sb leaching from PET and dissolution into water. About 200 mg of samples taken from PET bottles with a ceramic scalpel were placed into quartz closed vessels and subsequently subjected to Microwave (MW)-assisted digestion at a maximum power of 800 W (nominal value). In each of the 6 digestion vessels containing the samples 5 mL of high purity HNO<sub>3</sub>, further purified by subboiling distillation, were added. Soft drink samples underwent MW-assisted digestion before the SF-ICP-MS measurements.

Mineral water samples were investigated after a 10-fold dilution and filtration through 0.22 µm pore-sized Millex Millipore filters. For the SF-ICP-MS measurements, In was used as the internal standard. Under these conditions, the limit of detection for Sb was 3 pg/mL. The average concentration of Sb in the PET bottles was about 300 mg/kg, while the Sb concentration of waters reached 0.3 ng/mL. Further investigations will include the study of Sb leaching as a function of pH (still vs. sparkling mineral water), storage (before and after storage at various times) and temperature (room temperature vs. 40-60°C).

# ADVANCES IN THE GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF PHARMACEUTICALS PRESENT IN THE AQUATIC ENVIRONMENT

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This paper reports a sample preparation optimizations study of the most commonly found waste water's constituents: *i.e.*, the four non-steroidal, anti-inflammatory drugs, such as ibuprofen, naproxen, ketoprofen and diclofenac. Derivatizations have been compared with the most popular four silylation agents in order to select the most proper one, taking into account analytical and financial points of view, equally.

Out of Hexamethyldisilazan+Trifluoroacetic Acid (HMDS+TFAA), Bis-Trimethylsilyltrifluoroacetamide (BSTFA), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA), have been tested. Varying reaction time and temperature, taking also into consideration of molar responses of compounds in question, HMDS+TFAA proved to be the method of choice. Responses have been followed both on Total Ion Current (TIC) and Selective Fragment Ion (SFI) values. SFI responses have been evaluated on two basis: extracting the corresponding *m/z* masses from TIC elutions and from SIM elutions, in parallel.

Silylation optimization study related to reproducibilities in wide concentration range of derivatives: including the high ng and low pg level of injected species. As a main requirement, remaining on the safe and reliable side, it is to be underlined that blank tests are to be performed in parallel, in all cases, without exception: applying all reagents and absorption accessories used under the entire sample preparation procedure.

Drug enrichment was optimized with Solid Phase Extraction (SPE). Recovery studies, in the first step were performed with the primarily suggested Oasis HLB (Waters) cartridges. The utility of the optimized derivatization and drug enrichment method will be presented on monthly basis, at the first time in Hungary, by the identification and quantitation of the non-steroidal anti-inflammatory drug content of a Hungarian Waste Water Treatment Plant concerning its corresponding influent and effluent samples. The basic study has been extended to numerous pollutants belonging to different type of compounds present in the aquatic environment, such as other acidic drugs, estrogens, cholic acids, personal care products, pollutants of industrial origin and their metabolites, in total 40 compounds.

# EFFECT OF DISTRIBUTION NETWORKS ON METAL OCCURRENCE IN ITALIAN DRINKING WATERS

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Drinking water contamination by metals occurs throughout the source-to-tap delivery network. The EU Drinking Water Directive (98/83/EC) sets mandatory health-related standards for six metals (Cd, Cr, Cu, Hg, Ni and Pb) and three metalloids (As, Sb, Se) and more discretionary limits for further four elements (Al, Fe, Mn, Na). The Directive's point of compliance is at consumers' taps. This implies a need for control of all drinking water distribution system components, including pipes and fittings within consumers' premises. The stricter requirements that have been imposed by the 98/83/EC Directive indicate a need for a severe scrutiny of the leaching characteristics of currently available metallic products. A number of Member States (MSs) operate National Acceptance Schemes (NASs) for products and/or materials in contact with drinking water. These schemes involve testing of materials and products and/or assessment of evidence for product acceptability, which may include recognition of test results from other countries. The objective of all NASs is to ensure that products in contact with drinking water do not pose significant risks to the consumers' health, or cause unacceptable effects on drinking water quality, thus affecting taste and odour.

In the light of the circumstances stated above, an investigation aiming at the determination of variations in drinking water quality as an effect of the contact with metallic materials in the distribution systems was carried out. In Italy 15 main water suppliers and 13 state laboratories distributed homogeneously over the country (with the exception of Sardinia) were involved in the research project. 6000 samples were taken from 3800 sampling sites, selected according to specific criteria and specific sampling procedures. The samples were analyzed for Cr, Cu, Fe, Ni, Pb and Zn and these experimental data were completed with information on buildings, pipes *etc.* The major problems with respect to the metals originated from Fe, Ni and Pb. High Fe concentrations could be related to corrosion processes, Ni to tap materials and Pb to lead pipes in old buildings. With respect to the sampling procedures (random without flushing, stagnation for 30 min or 240 min after 5-min flushing), the statistical analysis showed that the first approach gave the same results as sampling after a stagnation time of 4 hours, this holding true for all types of metals.

# SEMI-QUANTITATIVE DETERMINATION OF ORGANOLEPTIC PROPERTIES OF RAW WATERS DISINFECTED WITH PERACETIC ACID OR SODIUM HYPOCHLORITE

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Drinking water disinfection is a common practice applied to reduce microbial load from raw water and to control algal re-growth in distribution systems. This practice also guarantees consumers' health in the case of water leaks in pipes and fittings. For a long time NaClO has been preferred to other agents due to its good disinfectant properties and low cost. Recently, improvements in analytical techniques have highlighted the risk of by-product formation, in particular of toxic and mutagenic compounds such as trihalomethanes. Moreover, NaClO imparts nasty odour and taste to drinking water when its concentration is in the range prescribed by the European Directive 98/83. This is why other chemicals, such as ClO<sub>2</sub>, O<sub>3</sub>, peracetic acid and KMnO<sub>4</sub>, have recently been tested as alternative water disinfectants. In particular, peracetic acid has proved to be one of the most suitable agents from a toxicological point of view as no dangerous by-products have so far been identified.

In this study the organoleptic properties of aliquots of surface and ground waters collected in Latium (Italy) from Lake Bracciano and waterworks Peschiera, respectively, were compared before and after disinfection with NaClO or peracetic acid. Every test was carried out according to the European Standard EN 1622 on the determination of threshold odour and flavour numbers. Assessors were selected by means of preliminary screening tests based on the evaluation of few reference compounds (citric acid, NaCl and sucrose for flavour; *n*-butanol and NaClO for odour) added to a mineral water with a low content of salts. A series of successive dilutions were prepared and assessed by every candidate applying paired test and forced choice procedures. Afterwards, selected assessors were asked to perceive odour and flavour of the investigated surface and ground waters added with increasing concentrations of NaClO, peracetic acid, acetic acid or H<sub>2</sub>O<sub>2</sub>. In this case paired tests were evaluated by both forced and unforced choice.

No significant differences were detected between the threshold numbers of the two aqueous matrices per each examined organic compound. NaClO was perceived much more strongly than peracetic acid with a threshold odour number (TON) of 0.04 and 6 mg/L, respectively and a threshold flavour number (TFN) of 0.23 and 11 mg/L, respectively. The two chemicals which are present in the peracetic acid solution (*i.e.*, acetic acid and H<sub>2</sub>O<sub>2</sub>) did not contribute considerably to the organoleptic properties of the two waters disinfected with peracetic acid. The use of the latter could eliminate nasty odours and tastes imparted to drinking water by residual free Cl<sub>2</sub> at the 0.2-mg/L level.

# DEGRADATION OF TRICHLOROETHYENE IN GROUNDWATER BY AEROBIC COMETABOLISM

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The effectiveness of aerobic cometabolism for the bioremediation of groundwater contaminated with chloroorganics was tested. Groundwater was treated in laboratory scale reactors with methane, oxygen and nutrient solutions to stimulate methane-oxidizing bacteria growth. Methanotroph cells reached a satisfactory concentration in 20 days and were stored at 4°C. These cells were used in degradation trials of Trichloroethylene (TCE), one of the most frequently chlorinated solvents detected in groundwater. The effects on TCE degradation of the presence or absence of the primary substrate and of the initial cell concentration were evaluated. Simulations were also made on saturated soil to confirm any biological activity and to prove biomass adhesion to soil. Cometabolic degradation kinetic coefficients were calculated for the in situ application.

# RISKS OF WATER-BORNE DISEASE OUTBREAKS AFTER EXTREME EVENTS

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An increase in water-borne disease outbreaks after extreme events are observed worldwide. Infection and transmission of epidemic illnesses can occur in different ways: they may be caused by technological disasters (*e.g.*, accidental transport) or natural catastrophes. High precipitation rates can increase the density of chemical and microbiological agents in surface water. The consumption of contaminated water represents a risk for the human health both in developed and developing countries. Several studies, carried out in the UK, USA and Asia, demonstrated a significant association between the enhancement of gastrointestinal symptom incidence and the occurrence of heavy rainfalls. Outbreaks of Cryptosporidiosis, Giardiasis, Campylobacteriosis and other infections were triggered by extreme events. However, the relationships between human health and problems regarding water quality are complex and predicting the potential impacts of climate change on water-related diseases is still rather difficult.

This study aims at providing a preliminary description of the Italian planning about outbreaks of water-borne diseases after flooding. In Italy, the Ministerial Decree of December 15<sup>th</sup>, 1990, prescribes the mandatory registration of illnesses that can constitute an epidemiological risk. The Ministry of Health publishes collected data concerning these illnesses in the Italian Epidemiological Bulletin on the website [www.ministerosalute.it](http://www.ministerosalute.it). It provides data on case number per year of infectious diseases, both at the regional and national level, from 1993 to date. In this study, available data on water-related diseases with high frequency and/or connectible to interventions of control (class II) were selected, namely, Legionellosis, Salmonellosis, Hepatitis, Skin and Visceral Leishmaniasis, Leptospirosis and Infectious Diarrhoea.

These data were analyzed at the national level over several years and correlated with national flood data. The relevant plots showed that the disease trends are not monotonous. In fact, there is documented evidence of spikes as regards illnesses such as Hepatitis A, Leishmaniasis and Infectious Diarrhoea in correspondence or immediately after significant floods both in the same years and region. These diseases, occurring worldwide, but more commonly in temperate or tropical climates, were related to poor sanitation and hygienic conditions and to extreme climatic events in developing countries. In developed countries, like Italy, despite several improvements in public health and economic welfare, the incidence of intestinal infections remains high and continues to be an important clinical problem, although mortality has been falling sharply during recent decades. In Italy a relationship seems to exist between these infectious diseases and floods, although further investigations should be carried out to support this assumption.

# A MOLECULAR APPROACH FOR THE IMPACT ASSESSMENT OF FAECAL POLLUTION IN RIVER ECOSYSTEMS

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Faecal bacterial indicators such as *Escherichia coli* and *Enterococci* are the most common organisms to detect faecal pollution and the presence of other pathogens in the context of monitoring programmes of sanitary surveillance of freshwater ecosystems. However, traditionally faecal indicators provide information on the recent state of water contamination, while complete information on water bodies can be gained by means of other faecal pollution indicators. *Clostridium perfringens* (spores) is suggested as an alternative faecal indicator as its natural habitat (sediments) works as a trap for pollutants. Thus, *Clostridium perfringens* provides evidence of historical faecal contamination

This work aims at assessing the genetic variability of the anaerobic microbial community in river sediments as a preliminary step for future possible assessment of its ability to relate to different faecal pollution sources. This can be obtained through a combined approach involving microbiological (UNI-ISO 7667, 1997) and molecular (16S rRNA) analysis of isolated bacteria. A comparison of concentrations of *Escherichia coli* with those of *Clostridium perfringens* was performed. The study was conducted on riverine systems (Foglia, Tevere, Astura and Sitzerri) located in three Italian regions (Marche, Latium and Sardinia).

The comparison between the concentrations of clostridia and *Escherichia coli* strains did not provide meaningful correlations, even if the small number of samples obviously affects the statistical reliability of the experimental data. The frequency of observed haplotypes results to be remarkably different in the four sampling areas. *Clostridium perfringens* was found to be the only ubiquitous species while *Clostridium bifermentans* was observed only in samples collected in Sardinia and Marche; *Clostridium thiosulforeducens* was identified only in some Latium (Astura river) and Sardinian

collecting sites; *Clostridium sporogenes* was isolated exclusively in the Sitzerri basin, where streams running in a disused mining area for the extraction of minerals like blende (containing Zn), galena (containing Pb), copper and other ones are present. The *Providencia stuartii* and *Proteus mirabilis* strains, optional anaerobic microorganisms, phylogenetically distant from the genus *Clostridium*, were exclusively observed in the Pontini samples. The phylogenetic relationships among the species obtained from the comparison of the different genetic sequences made it possible to identify six homogeneous clusters, among which five are attributed to the genus *Clostridium* and one to the genus *Bacillus*.

The results of this study suggest a possible correlation between different typologies of impacts and the presence of particular species of anaerobes. The research for sulphite-reducing clostridia in river sediments could therefore have an essential role in the query for complete information on the quality state of river ecosystems.

# IMPLEMENTATION OF WFD 2000/60/EC IN ITALY: DIATOMS AS BIOLOGICAL INDICATORS OF WATERS

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The Water Framework Directive (WFD) establishes a framework for the protection of all water bodies and one of its objectives is to reach a good ecological status by 2015. Ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters. Biological elements of aquatic ecosystems required by the WFD are phytoplankton (diatoms), macrophytes, macroinvertebrates and fish. Diatoms are unicellular eukaryotic algae. Their biological and ecological characteristics make them good indicators of water quality. Their use is widespread and well developed for evaluating the quality of flowing and standing waters. Analysis of diatom communities constitutes a valuable tool for general water quality monitoring and for the evaluation of more specific phenomena such as eutrophication and acidification.

In Italy the Implementation of the WFD is still going on: groups of experts produced guidelines for monitoring biological elements and they are working to the intercalibration process of biological elements. The aim of the intercalibration process is the comparability of the experimental data of the monitoring systems for biological quality. In order to achieve this, each Member State of the EU is required to establish Ecological Quality Ratios (EQRs) for the boundaries between high (H) and good (G) and between good (G) and moderate (M) status, which are consistent with the WFD legal definitions of those class boundaries given in Annex V of the WFD.

The Italian Mediterranean Rivers Diatoms Intercalibration Process started in March 2007. Mediterranean rivers are classified in five types, *i.e.*, (RM-1 to RM-5) depending on the river's characterization, catchment areas, geology, altitude and geomorphology. Moreover different hydroecoregions have been characterized depending on orology, climatology, geology. This study is an overview of the work carried out to satisfy intercalibration requirements. At present, reference sites have been selected and diatom samples were collected from reference and non-reference sites so as to analyze boundary derivation and harmonization. Different Diatom Indices were calculated, mainly derived from other European countries; next step will be to identify an official WFD compliant method for diatoms.

# ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM FRESHWATER FISH FARMS

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The use of veterinary medicines in aquaculture is not centrally ruled in the European Union, so that each Member State (MS) differently responds to the needs of the sector. In Italy, possession, use, prohibition and definition of veterinary medicinal products are defined by the Legislative Decree 193 (April 6<sup>th</sup>, 2006), based on the previous EEC Regulation 2377/90. Before this regulation, antimicrobial drugs were massively used against numerous pathogens of fish, leading to the appearance of antibiotic resistant strains. This work aims at detecting the presence of antibiotic resistances in *Escherichia coli* strains, that were collected in sites located upstream and downstream several fish farms in central Italy. These farms were selected in the central Apennine Mountains. Antibiotics, supplied directly with the feed, were chosen according to their use in aquaculture. In fact, isolated colonies of *Escherichia coli* were analyzed to detect the development of resistance to four antibiotics, namely, Amoxicillin, Flumequine, Tetracycline and Chloramphenicol. Antibiotic resistance analyses highlighted the illegal possible use of Chloramphenicol (4% of the total isolated colonies), even if a generally environmental diffused presence of drug resistance was detected (28% of Tetracycline resultant colonies, 18% of Amoxicillin resistant colonies and 3% of Flumequine resistant colonies). However, further analyses are necessary.

The adoption of a “Code of Conduct for Responsible Fisheries” for best management practices (API, Associazione Piscicoltori Italiani, *i.e.*, Italian Association of Fish Farmers) may strongly mitigate all adverse impacts of fish-breeding, *e.g.*, through the use of highly digestible foods and more environmentally friendly medical therapies. This study aimed at giving a contribute to the improvement of the existing knowledge on environmental impacts of river aquaculture, focusing on the region of central Apennines, where it is still in a developing state. Furthermore, it provides the so-called environmental value of some antibiotic resistances in natural Apennine rivers.

# **DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AS TRIMETHYLSILYL DERIVATIVES IN DANUBE RIVER BY GAS-CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

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Pharmaceuticals have become one of the major targets in environmental chemistry due to their presence in waste, surface, ground and even drinking water. Concentrations of acidic Non-Steroid Anti-Inflammatory Drugs (NSAIDs), such as ibuprofen, naproxen, ketoprofen and diclofenac have been measured at the low ng/L level in different European rivers. This study was undertaken to further develop an original Gas Chromatography (GC) Mass Spectrometry (MS) Single Ion Monitoring (SIM) method in order to improve the Limits Of Quantification (LOQ). The applied analytical method is based on Solid Phase Extraction (SPE) with Oasis HLB cartridges, followed by GC tandem MS (GC-MS/MS) of the NSAIDs as their trimethylsilyl (oxime)-ether/ester derivatives.

This hyphenated analytical technique allows only one ion to be isolated from the matrix. Secondary ions are formed from the parent ion by Collision-Induced Dissociation (CID) using He in the ion trap. Conditions for the dissociation of the selected parent ions were optimized by means of the Automated Method Development (AMD) in the resonant mode (Varian Saturn GC-MS/MS software). The signal-to-noise ratio is 2-12 times better than that afforded by the GC-MS SIM method. As a result of this ion generation technique, not only the detection power increased, but also the reliability of the spectrum identification was improved. A detailed spectrum study was performed and fragmentation pathways were given. The LOQ depended on the parent ion chemical structure and were in the range 0.20-1 ng/L. The method was successfully applied to the analysis of Danube River, well-waters and drinking waters.

# THE ENVIRONMENTAL QUALITY STANDARD OF THE PRIORITY SUBSTANCES IN THE CONTEXT OF THE EUROPEAN WATER FRAMEWORK DIRECTIVE

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The publication of the proposal for a Directive of the European Parliament and of the Council on Environmental Quality Standards (EQS) in the field of water policy is an important step for the achievement of the good chemical status set by the Water Framework Directive 2000/60/EC for all European surface water bodies. The EQS are derived for all the priority substances in the water column and for hexachlorobenzene, hexachlorobutadiene and Hg also in biota. There are two different kind of EQS: the annual average EQS based mainly on chronic effects on the aquatic organisms and the maximum acceptable concentration EQS based on acute effects. The EQS have to protect aquatic organisms of different trophic levels and also human health in relation to the consumption of drinking water and fish products; the procedure for arriving at the final EQS was rather complex as various objectives of protection had to be taken into account.

The final version of the proposal after the first reading gives to Member States (MSs) the possibility to adopt environmental quality standard also for sediment in certain categories of surface water. The possibility of obtaining EQS for the sediment is particularly important for certain priority substances that tend to accumulate in this matrix and are not detectable in the water column. According to the Directive the sediment EQS have to guarantee the same level of protection as for the water column.

# MALDI-TOF AND Q-TOF MASS SPECTROMETRY CHARACTERIZATION OF A NEW MICROCYSTIN PRODUCED IN A CYANOBACTERIA BLOOM IN THE AVERNO LAKE

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Cyanobacteria (blue-green algae), occurring worldwide within water blooms in eutrophic lakes and drinking water reservoirs, produce several different biotoxins. Among these, Microcystins (MCs) are a group of cyclic heptapeptides showing potent hepatotoxicity and activity as tumour promoters. The general structure of MCs is cyclo (D-Ala-X-DMeAsp-Y-Adda-D-Glu-Mdha). X and Y are variable L-aminoacids that identify the biotoxin as MC-XY. At least 75 MCs from different cyanobacteria genera have been isolated and characterized. The occurrence of MC-containing blooms in freshwaters has been involved in several animal and human poisoning outbreaks worldwide. The analysis of MCs is of growing interest for water surveillance authorities because they can contaminate drinking water reservoirs or lakes intended to be used as recreational areas. Therefore, reliable analytical procedures should be available to public authorities to monitor and characterize cyanobacteria blooms on the basis of the biotoxins produced.

The characterization of the biotoxins produced by a bloom of *Planktothrix rubescens* in the Lake Averno, near Naples, using both Matrix-Assisted Laser Desorption/Ionization (MALDI) - Time-Of-Flight (TOF) and Q-TOF Mass Spectrometry (MS) is described. By MALDI-TOF-MS analysis it was possible to detect several compounds and three major substances. The analysis by Q-TOF/MS allowed Anabaenopeptin B and Anabaenopeptin F to be identified, while the third biotoxin could not. On the other hand, the study of the fragmentation pattern of the unknown compound proved that it was a Microcystin never describe before, thus allowing its complete molecular structure to be elucidated and recognized as a variant of MC-YR. The results of this work give new information about the algae biotoxins detectable in Italian lakes and improve existing knowledge on the contamination by cyanobacteria.

# PHARMACEUTICALS AS PRIORITY WATER CONTAMINANTS

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Human and veterinary pharmaceuticals represent a wide category of water contaminants which are poorly detected by routinely monitoring of surface and ground water. Evidence of the presence of some pharmaceuticals in water bodies, due to point contamination, has been frequently obtained over these last twenty years. Generally, antibiotics, analgesics, antidepressants, hormones, estrogens, haematic lipid regulators, disinfectants, anti-inflammatory and anti-epileptic drugs among others, have been found at the level of ng- $\mu$ g/L, both in surface water and ground water and up to hundreds of mg/kg in sediments. Yet, the significance of these findings has not been adequately highlighted. The side effects observed in aquatic biota, such as the development of antibiotic resistance in pathogenic microorganisms, the appearance of morphological alterations and sex impairment on aquatic organisms and fish due to antibiotics, hormones and contraceptives, respectively, are supportive of the relevance of pharmaceuticals as priority water contaminants. At least 157 substances have been recognized to be of importance for the aquatic environment in Europe and some would be probably candidate for future environmental legislation. Pharmaceuticals include hundreds of heterogeneous molecules with very different chemical-physical properties and chemodynamic properties, such as soil and water half-lives and soil partition constants ( $K_{oc}$  and  $K_d$ ), still largely unknown. Hence, the possibility to define their environmental behaviour is mostly unfeasible.

Some pharmaceuticals are known for their persistence in soil and water or for their resistance to biodegradation in sewage treatment plants (X-ray contrast compounds, cyclophosphamide, clofibrac acid, ivermectin, oxolinic acid, flumequine, oxytetracycline). Moreover, pharmaceuticals have a wide spectrum of important biological properties which, as a rule, are considered to be a benefit in terms of protection of human and animal organisms. So far, they have not been considered for possible side effects on environmental species, but only recently it has been assumed that some compounds, at the concentrations commonly detected in waters, may pose a risk to aquatic fauna (ibuprofen, acetylsalicylic acid, mefenamic acid, aminotryptiline, amoxicillin, dextropropoxyphen, fluoxetine, oxytetracyclin, paracetamol, propranolol and tiordazine). As regards indirect exposure of man through drinking water, a comparison of levels of pharmaceuticals in drinking water and therapeutic doses does not point to an actual risk for human health.

# ENVIRONMENTAL FATE OF TERBUTHYLAZINE AND DESETHYLTERBUTHYLAZINE IN SOIL, SURFACE WATER AND GROUNDWATER

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The herbicide terbuthylazine is widely used in Italy and it is the first herbicide within the s-triazines category. The use of this substance noticeably increased since the early 1990's due to the ban of atrazine and the subsequent withdrawal of this compound from the Italian market. In 2001 terbuthylazine was sold at amounts higher than 320,000 kg (figure relevant to the active ingredient) primarily in the maize producing regions. Monitoring data from Italy and from other countries, *e.g.*, France, indicate that terbuthylazine and its main metabolite Desethyl-Terbuthylazine (DET) are found in surface water and in ground water at noticeable concentrations and frequencies of detection and, in several cases, above the Maximum Allowable Concentrations established for pesticides in the EC and national drinking water regulations (0.1-0.5 µg/L for single and total pesticides, respectively). Thus, due to their presence in water bodies and to their biological properties, terbuthylazine and DET may pose environmental and health problems for man and aquatic organisms. For these reasons, both terbuthylazine and DET are placed among the emerging substances by the EC. The widespread presence of terbuthylazine and DET in water bodies may be traced back not only to the high amounts of the parent compound used in agriculture, but also to agricultural practices, their intrinsic and chemiodynamic properties (mainly water solubility, soil and water half-lives, soil adsorption and  $K_{oc}$ ) and the hydrogeological characteristics of the receiving environment.

In this context, some results on soil degradation and mobility of these compounds under different conditions (influence of natural microorganisms, light and dark conditions, soil depths, nitrogen containing amendments, incubation temperatures, organic carbon content *etc.*) are discussed. Overall data indicate that terbuthylazine and DET possess a relatively high mobility in soil and a moderate to high persistence in soil and in water. In any case, the presence of active degrading bacterial populations is an essential prerequisite for s-triazines degradation. In this context, molecular probes were recently applied to the detection of bacteria populations involved in s-triazines degradation, by Fluorescent *In Situ* Hybridization (FISH).

# ENHANCING THE QUALITY OF DRINKING WATER. THE EXPERIENCE OF AN ITALIAN TWINNING PROJECT IN POLAND

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The European project PHARE - Twinning Poland *Extension of the Sanitary Supervision in the area of Water Quality* has recently reached completion. The project involved the Polish and Italian Ministries of Health and was aimed at improving the Polish sanitary supervision system in the frame of: (i) health threats which may result from the methods of water treatment, water storage and water distribution; (ii) health threats within the scope of the water used for recreation and sport purposes; (iii) water radioactivity monitoring. The structure of the project consisted of four interrelated components, arranged as task activities. The following main results were achieved and shared with the beneficiary country.

*Part C1. Treatment chemicals used in the production of drinking water and materials in contact with water during treatment, storage and distribution.*

Activities: (i) Construction Products Drinking Water (CPDW); (ii) Disinfectant and Disinfection By-Products (DBPs); (iii) coagulants, flocculants and other chemicals; (iv) distribution systems; (v) drinking water risks data model.

Results:

- guidelines and proposals for regulatory purposes on CPDW;
- procedures concerning management of disinfection practices and microbial risk, risk and monitoring of DBPs;
- procedures on management of treatment practices, monitoring of coagulants and flocculants;
- report and proposal for research and control approach to the risk assessment related to migrations from DW distribution systems;
- report describing conceptual analysis and including the DWR related to CPDW and treatment data-model.

*Part C2. Water used for recreation and sport purposes.*

Activities: (i) knowledge; (ii) elaboration of standards for supervision of recreational water quality (monitoring); (iii) elaboration of a management system; (iv) proposal of a new act.

Results:

- reports on the asset of recreational waters management system;
- identification of chemical and microbiological parameters;

- report on the administrative competences and the responsibilities;
- report including database assessment, a data-model proposal and National Water Data Base framework interface;
- guidelines reporting standards of microclimatic parameters for supervision of the facilities and describing the competences and the responsibilities involved;
- draft regulation concerning the quality of recreational waters environment according to the Polish institutional asset.

*Part C3. Water radioactivity monitoring.*

Activities: (i) knowledge; (ii) settlement of analytical procedures; (iii) support in setting up foreseen methodologies; (iv) definition of the monitoring system and execution of a pilot project.

Results:

- report describing the Polish radioactivity monitoring system;
- report describing criteria for analytical techniques selection;
- testing radioactivity in selected water samples;
- report describing the Italian analytical procedure for the detection of radioactivity in water (gross  $\alpha$  and  $\beta$  activity, U isotopes concentration,  $^{226}\text{Ra}$  concentration,  $^3\text{H}$  concentration);
- report describing criteria for analytical techniques selection;
- report describing guidelines and procedures to manage an effective sanitary system for monitoring drinking water radioactivity;
- report describing the planning of a monitoring campaign in a selected waterworks;
- guidelines elaborated for logical and conceptual data model.

*Part C4. Risk factors affecting water safety in public buildings.*

Activities: (i) knowledge; (ii) training; (iii) *Legionella*; (iv) risk analysis integrated system; (v) study visit in Italy.

Results:

- reports describing decision-making system on water quality monitoring activities for microbial risks;
- reports describing procedures for sampling and analysis of pathogens with particular concern on Protozoa in water;
- guidelines on the information system able to control the microbial risk related to water;
- guidelines on evaluation and management of risk assessment, sampling and monitoring *Legionella* in water;
- guidelines on setting a system for prevention and control of legionellosis taking into account the Italian surveillance system;
- report describing data processing approach to microbial and chemical hazards monitoring, including *Giardia*, *Cryptosporidium*, *Legionella*;
- representatives of involved institutions trained in the ISS laboratories.

Some figures of the Project are as follows:

*Duration:* 14 months (Nov 2006-Dec 2007) Total working days: 458.

*Polish beneficiary:* Ministry of Health, Chief Sanitary Inspectorate, National Institute of Hygiene, Sanitary Epidemiological centers.

*Italian Partners:* Ministry of Health, National Institute of Health (ISS), Regional Agency for Environmental Protection of Veneto (ARPAV) and Lombardia (ARPAL), Italian training and study center (Formez).

*Experts involved:*

Project Leader, Resident Twinning Advisor, 4 Component Leader, 40 Short term experts, supporting personnel.

*Training:*

10 workshops in Poland, 1 International workshop, study visit in Italy, more than 2.000 trained representatives. The Project gave an essential contribution to the full implementation of Directive 98/83/EC on quality of water for human consumption in Poland.



**Poster Session 3**  
**Soil and sediment**

*Coordinator*  
Michaela Zeiner



# OPTIMIZATION OF SUBCRITICAL (H<sub>2</sub>O/CO<sub>2</sub>) EXTRACTION FOR SOILS, SEDIMENTS AND GRAVITATION DUSTS

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A three-step sequential extraction technique modelling the chemistry of the most frequent mobilization pathway of heavy metals in soils was proposed in former studies. The procedure was based on sequential application of supercritical (CO<sub>2</sub>) extraction, subcritical (H<sub>2</sub>O) extraction and a mixture of (H<sub>2</sub>O+CO<sub>2</sub>) in the same supercritical extractor. In all steps a 60 min extraction time was adopted. It was thus possible to separate an organic-bound, a water-soluble and a carbonate-bound heavy metal fraction in soils and sediments of moderate carbonate content

Extension of the application of this procedure to higher carbonate containing samples (soils, sediments and airborne dusts), however, required an optimization of the procedure. This was achieved by a kinetic study in which Hungarian soil reference samples with different lime content (4, 5 and 20%) were investigated. The samples were weighed directly in the supercritical fluid extraction (SFE) column after mixing with quartz at a 1:1, 1:20 and 1:100 ratio and then they were extracted with subcritical mixture of (H<sub>2</sub>O+CO<sub>2</sub>) for 4 hours. Extract samples were collected every 10 min. The dissolution of carbonates was detected by Flame Atomic Absorption Spectrometry (FAAS) measurements of the Ca concentration. In the case of the 1:20 soil/quartz mass ratio the total dissolution of carbonates was achieved in 90 min.

# TRIAD-APPROACH IN THE BIOREMEDIATION OF POLLUTED ENVIRONMENTAL MATRICES

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Soils and sediments can act as a repository for a whole array of organic and inorganic contaminants either directly or accumulated by airborne particulate depositions, rainfall and water courses. The exposure risks for humans and ecosystems depends on many factors, e.g., solubility in water, bioavailability, persistence, time and way of contact, among others. The so-called Triad approach is based on the concomitant actions of plants-microorganisms-earthworms in a complex matrix. There are no simple solutions to achieve and maintain sustainable and efficient remediation techniques. Environmental matrixes, such as soils and sediments, are made up of a myriad of complex ecosystems that are affected by natural and anthropic variables causing organic and inorganic contamination. Among the conventional remediation techniques (soil washing, solvent extraction, heating *etc.*), the natural ones are preferred as they permit the material to be possibly reused after remediation.

The Triad System works in both a direct and an indirect way. In the phyto-bioremediation technique, plants produce root exudates that stimulate the microbial community to degrade contaminants through their enzymes. In addition, plants through phytoexudates can chelate and extract metals from the solid matrix. Microorganisms in the rhizosphere are directly responsible for the degradation of organic compounds, such as hydrocarbons, while indirectly, they improve the release of heavy metals from organically-bound matrices. The earthworms stimulate the microbial activities as they mix and homogenize soil, increase permeability and aeration, improve water retention capacity and root elongation by means of the channels they dig and enhance nutrients releasing from soil.

# ACCELERATION OF BCR SEQUENTIAL EXTRACTION PROCEDURE BY ULTRASONIC TREATMENT FOR SOILS, SEDIMENTS AND GRAVITATION DUST SAMPLES

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The BCR sequential extraction scheme was originally developed for fractionation of heavy metal content in aquatic sediments. During validation and production of the Certified Reference Materials (CRMs) sediment 601 and 701, some critical aspects of the BCR methodology like the pH-instability at the second step and the danger of the redistribution of dissolved metals into the solid phase during the long-term shaking were observed. Extension of the BCR fractionation approach to airborne dust samples is particularly hindered by the above problems.

In the present study an attempt was made to improve the BCR methodology by speeding up the time-consuming (16 h) leaching steps by means of ultrasonic treatment. A kinetic study was performed using in-house sediment, soil and dust reference materials. The effectiveness of dissolution of carbonates by acetic acid was followed during the first step of the BCR procedure by measuring the amount of dissolved Ca. As a result of this study the 3x16 h leaching time was reduced to approximately 3x10 min depending on the nature of the samples. Validation of the accelerated procedure was performed by CRM 601. Concentrations of Ca and other metals in the extracts were determined by Flame Atomic Absorption Spectrometry (FAAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).



**Poster Session 4**

**Atmosphere**

*Coordinator*

Enrico Veschetti



# **EXHAUST EMISSION OF PAHS FROM GASOLINES WITH DIFFERENT COMPOSITION: RELEVANT VARIABLES INFLUENCING RESULTS AND THE PROBLEM OF BLANKS**

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In a study on the influence of gasoline composition on toxic compound emission from passenger cars, one of the goals was to evaluate the catalyst efficiency towards the various toxic species. Different biological and chemical assays were used. For Polyaromatic Hydrocarbons (PAHs), classical chemical analyses were performed. PAHs were collected on polyurethane foam, one the most used adsorbent to collect vapour-phase semi-volatile compounds, and analyzed by Gas Chromatography (GC) - Mass Spectrometry (MS) with isotope dilution. Cars without catalytic converters displayed well defined and measurable PAH levels, well above blank levels, whilst cars equipped with catalytic converters displayed PAH levels very close to the blanks. While this provided obviously sound evidence of the efficiency of the catalyst, the problem arose when trying to define exactly the level of emission of catalytic converters equipped cars because, in a set of 15 blanks associated to 15 analytical batches of samples the levels of the blanks for 8 PAHs varied over one order of magnitude, notwithstanding the most scrupulous effort to normalize the procedures and the materials used.

A measurable blank level is not an unusual problem in trace analysis. Most of the laboratories performing trace analyses cope with the problem by comparing the level of their analytes in a batch of samples to the level of a single blank associated with that batch. This is a very reasonable practice, as the blank reproduces accurately the random, slight different conditions each batch of samples is subject to. However, it may also be misleading when differences as high as one order of magnitude are dealt with. Several cases taken from the study on the emission from car catalytic converters are discussed. Evidence is also provided that PUF contributes significantly to excessive blank levels.

# OPTIMIZATION OF A SECTOR FIELD ICP-MS INTERNAL STANDARD METHOD WITH A SIGNAL DRIFT CORRECTION FOR DETERMINATION OF THE SOLUBLE FRACTION OF TRACE METALS IN AEROSOL SAMPLES

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Accurate and reproducible determinations of metals in environmental samples and discrimination between natural and anthropic contributions are pivotal to understand the impact of metal-cycle components on human health and ecosystems. In this regard, the development and optimization of analytical methods capable of reliably and simultaneously quantifying the concentration of several metals at the sub ng/g level play a key role in environmental studies. Hence, a study was set up to optimize methods for the simultaneous determination of 14 trace elements (Al, As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, V and Zn) in aerosol samples at concentrations ranging from a few pg/g to some ng/g by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS). The methods were applied to the analysis of aerosols collected on one quarter of 47 mm Teflon filters by impactors working for 24 h at a flow of 2.3 m<sup>3</sup>/h (EN 12341 Directive) with cut-off heads PM<sub>10</sub>, PM<sub>2.5</sub> and PM<sub>1</sub>.

The metal fraction soluble at pH=1.5, as an indicator of the easily mobile fraction in the environment, was determined by SF-ICP-MS. These results were compared with those obtained by the analysis of the total content of each metal as determined in the same filter by Particle Induced X-ray Emission (PIXE). It was thus possible to evaluate the percentage contribution of the soluble fraction of metals emitted in the atmosphere by natural and anthropic sources in different ecosystems. Aerosol measurements were carried out on several hundreds of filters collected in 10 sites with different anthropic impact located in Tuscany (Central Italy) and in the Lampedusa Island (Sicily Channel, Mediterranean Sea, Italy). The possibility of selecting three different mass resolution (300, 4000 and 10,000) allowed the most suitable isotopic species to be chosen for each metal on the basis of isotopic abundance, isobaric interferences and expected concentrations.

Method accuracy for Al, As, Cd, Cu, Ni and Pb was evaluated by analysis of the Certified Reference Materials (CRMs) 609 and 610 for low and high concentrations, respectively. Accuracy for As, Cd, Cr, Cu, Hg, Mn, Pb and Zn was also checked by using the ERM-CE278 CRM. For Ba, Fe, Mo and V, accuracy was evaluated by analyzing certified standard solutions and by comparison with Electrothermal Atomization Atomic Absorption Spectrometry (ET-AAS) measurements. Errors were always found to be lower

than 10%. Measurements on aerosol samples were carried out by an internal standard method with four internal standards ( $^{72}\text{Ge}$ ,  $^{103}\text{Rh}$ ,  $^{115}\text{In}$  and  $^{185}\text{Re}$ ) and signal drift correction. Limits Of Detections (LODs) ranged from 1.6 pg/g (Cd) to 71.4 pg/g (Zn). Due consideration being given to the procedures adopted for air sampling as well as to sample pretreatment, LODs were at least two orders of magnitude lower than concentration values measured in real samples. Filter blanks, in turn, showed that atmospheric concentrations were at least 10 times higher than blanks.

# ELEMENTAL ANALYSIS OF PM<sub>10</sub> FRACTIONS OF URBAN AEROSOL COLLECTED IN BUDAPEST BETWEEN 2004 AND 2007

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Airborne particulate matter has important health implications. The goal of this investigation was to offer an overview about the metal and Total Organic Carbon (TOC)/Total Inorganic Carbon (TIC) concentrations in the PM<sub>10</sub> aerosol fraction in Budapest, to monitor monthly changes during the sampling period, and to check whether there is a seasonal tendency in the pattern of concentrations. The sampling took place downtown in Budapest, Hungary, between September 2004 and August 2007, where heavy traffic conditions occur. For the sampling, a high volume aerosol sampler equipped with a PM<sub>10</sub> head was used. The samples were collected onto quartz fiber filters, which were previously pre-treated at 550°C in order to eliminate organic contamination. Each month two samples were collected, one at 24 h and the other at 96 h. The air intake was 500 L/min. To quantify the total and water soluble element concentrations, Microwave (MW) - assisted *aqua regia* digestion and water extraction by sonication were employed, respectively. Solutions were analyzed using a Thermo Fisher Element 2 Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS). The elements under test were Ag, B, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Pt, Rb, Sb, Sr, Te, Tl, U, V and Zn.

According to the data obtained from the *aqua regia*-based digestion procedure, the measured trace elements could be divided into four groups as a function of their concentrations: (i) Fe concentration less than 2500 ng/m<sup>3</sup>; (ii) Cu, Mn, Pb, Sn, Sb and Zn (10-100 ng/m<sup>3</sup>); (iii) Cd, Cr, Li, Mo, Ni, Rb, Sr and V (1-10 ng/m<sup>3</sup>); (iv) Ag, Bi, Co, Ga, Pt, Te, Tl and U (not detectable - 1 ng/m<sup>3</sup>).

The biological availability of the trace elements based on the water extraction followed the order: (i) Tl and Zn (70-100%); (ii) Cd, Co, Cu, Li, Mn, Ni, Rb, Sb, Sr, Te and V (26-50%); (iii) Cr, Mo and U (15-25%); (iv) Ag, Bi, Fe, Ga, Pb and Sn (1.5-10%); (v) Pt (below the limit of detection).

During this time period, no evidence of any seasonal change of trace element concentrations was found. In terms of trace element concentration, the health criteria guideline values were never exceeded during the sampling period, although the PM<sub>10</sub> mass concentration exceeded quite often the guideline values. The analytical data were subjected to chemometric analysis. It was found that Cu and Sb had very similar concentration profiles and this points to the fact that their emission source might be the same, namely the wear of the brake pads. For the measurement of the TOC/TIC, a LECO Co. (USA) RC-412 type C analyzer was used. The TOC ratio (30-50%) in the PM<sub>10</sub> fraction showed a seasonal profile with an increase in the winter period due to the heating.

## EXPOSURE TO Pt FOR DIFFERENT GROUPS OF THE VIENNESE POPULATION

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Platinum is emitted into the environment by Automobile Catalytic Converters (ACCs), hospitals, noble metal refineries and other industries. Due to abrasion of the ACCs, Pt is released with the exhaust fumes and can thus be found in the urban and rural atmosphere. To assess human exposure to this element its concentration in urine was determined. Urine is a biological fluid often used for monitoring studies, as it plays an important role in the elimination of various substances from the body and in addition can be easily collected. It is supposed that the Pt intake depends on the mobility of a person, *i.e.*, the amount of air inhaled and of food consumed. Subjects with and without one or two hip-endoprostheses were selected for this monitoring study. The median values obtained for the Pt concentration in urine of these three groups were 3.3 µg/g, 3.3 µg/g and 2.1 µg/g, respectively. By applying a Wilcoxon-test to compare the sets of experimental data, a *p*-value of 0.051 for the groups with n=0 and n=2 and a *p*-value of 0.052 for the groups with n=1 and n=2 were obtained, where n is the number of prostheses.

# TRACE ELEMENTAL CHARACTERIZATION OF FLY ASH

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The purpose of this study was the determination of heavy metal concentrations in ash samples of biomass incinerators. Ash samples of different biomass incinerators in Upper Austria were compared. The ash samples were taken from the filters of the gas cleaning system. As the biofuel used for the incinerators contains heavy metals they can be found also in the ashes. Heavy metals are released along with the effluents. The amount of metals strongly depends on the composition of the input material (*e.g.*, wood chips, bark and used wood) as measurements clearly show. Knowledge of the concentrations of heavy metals is therefore important to make decision on the further treatment or utilization of the ashes.

In an incineration process it is important to ascertain the distribution of the emitted metals from the input material. Some of the metals can be found in the gas phase (*e.g.*, Hg), while others can be found on the fly ash particles or on the bottom ash of biomass incinerators. In this investigation fly ashes were analyzed primarily for heavy metals. In the case of one incinerator the heavy metal contents were also measured in the bottom ash fraction. Prior to analysis the fly ash samples were at first dissolved in a Microwave (MW) digestion unit using HNO<sub>3</sub> and HCl. Afterwards the elements Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sb, Sr, Ti, Tl, V and Zn were quantified by Inductively Coupled Atomic Emission Spectrometry (ICP-AES). The highest concentrations in the fly ash samples were those of Al, Fe, Mg, Mn and Zn.

**Poster Session 5**

**Food**

*Coordinator*

Anikó Vasánits-Zsigrai





# **TOTAL ANTIOXIDANT CAPACITY OF POMEGRANATE (*PUNICA GRANATUM*) USING SPECTROPHOTOMETRIC METHOD (CERAC)**

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The human diet contains a great variety of natural antioxidants, such as fibres, polyphenolic compounds, flavonoids, isoflavones, tocoferols, ascorbic acid *etc.* Antioxidants may act through the generation of Reactive Oxygen Species (ROS). The generation of ROS is associated with environmental pollution, UV radiation and several normal metabolic processes. The role of ROS in various human diseases is becoming increasingly recognised, such as DNA damage and mutation (and promotion), that may be related to cancer, heart disease and aging. In addition to the endogenous defence, the consumption of dietary antioxidants, such as tocoferol, ascorbic acid, carotenoids and phenolic compounds, play a vital role in protecting against ROS.

In this study, a simple, flexible, reproducible and low-cost spectrophotometric method was developed which allowed for total antioxidant capacity assay of dietary polyphenols, flavonoids and ascorbic acid in plant extracts. This method, called the Ceric Ion Reducing Antioxidant Capacity (CERAC) assay, is based on the room temperature - oxidation of antioxidant compounds with Ce (IV) sulfate in diluted H<sub>2</sub>SO<sub>4</sub> solution and measurement of the absorbance of unreacted Ce(IV) at 320 nm. Pomegranate (*Punica granatum*) contains substantial amounts of polyphenols, flavonoids and ascorbic acid. The antioxidant capacity of pomegranate peel, seed and a byproduct of the juice were assayed. Antioxidant-rich fractions were extracted from pomegranate peels and seeds using water, 1:1 (v/v) ethanol-water mixture and pure ethanol.

The extracts were screened for their potential as antioxidants using various methods, such as CERAC and CUPRAC. All the pomegranate extracts exhibited marked antioxidant capacity, but the pure ethanol extract had the lowest value. Among these solvents, 1:1 (v/v) ethanol-water extract gave the highest CERAC yield for the dried peels, fresh peels, fresh seeds and juice. Pure ethanol gave the lowest antioxidant yield for all samples. The data also indicated that both the varieties of pomegranate and the various parts of the fruit could significantly influence the antioxidant activity.

# **ANALYSIS OF MICOTOXINS FB1, FB2, HFB1 AND HFB2 IN PIG LIVER BY LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY**

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Mycotoxins are heterogeneous chemical compounds characterized by a low molecular weight and synthesized by the secondary metabolism of different molds. Fumonisin is a water-soluble mycotoxin produced by *Fusarium* species spoiling corn and derived products. These mycotoxins can enter the food chain also through the consumption of meat of exposed animals. Fumonisin and their metabolites have been associated with several animal and human diseases. They are suspected risk factors for esophageal and liver cancers, neural tube defects and cardiovascular problems. Furthermore, fumonisins inhibit ceramide synthase disrupting sphingolipid metabolism because of their structural similarity to the sphingoid bases. Improved methods are needed to accurately assess fumonisin concentrations in food from vegetable and animal origin to prevent acute and chronic human exposure.

The aim of the present work was to develop a sensitive and selective method for quantification and unambiguous identification of fumonisin B1 (FB1), fumonisin B2 (FB2) and their completely hydrolyzed products (HFB1 and HFB2) in order to determine their accumulation in liver of weaned piglets fed a FB1 and FB2 contaminated diet. HFB1 and HFB2 could originate by endogenous hydrolysis of the Tricarballic Acid (TCA) side chains at carbon 14 and 15, which are replaced by hydroxyl groups. All the analytes were isolated simultaneously from the animal matrix, by a single Solid Phase Extraction (SPE) step and they were detected in spiked samples of pig liver using Liquid Chromatography (LC) coupled with tandem Mass Spectrometry (MS/MS) in positive Electrospray Ionization (ESI+). Under ESI+ conditions, the precursor ions are easily formed by protonation on the amine N. The analysis was carried out in the Multiple Reaction Monitoring (MRM) mode using the two main product ions. Losses of TCA groups and water dominate the spectra of the product ions of FB1 and FB2. For HFB1 and HFB2 the product ions originate from water losses. The analytical performance of the method was evaluated on standard samples. All the analytes were detected at amounts of 5 pg injected. The signal-to-noise ratios were quite adequate. Linearity was assessed on six calibration levels for each analyte over the range 0-500 pg of injected amount, with correlation coefficients ( $R^2$ ) better than 0.99. The ion suppression of the matrix was also evaluated. The good performances of the proposed method can assure a correct detection of fumonisins in liver even at relatively low concentrations.

# EFFECT OF MICROWAVE AND OVEN HEATING ON THE ANTIOXIDANT CONTENT OF ROSEHIP INFUSION (HERBAL TEA)

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Oxidative processes in organisms can produce free radicals which can easily react with different molecules and damage cells. An antioxidant is a molecule capable of slowing or preventing the oxidation of lipids, proteins and similar molecules. Antioxidants can terminate chain reactions initiated by free radicals *via* removal of radical intermediates. The protective effect of fruits and vegetables against chronic diseases is ascribed to their phytochemical content and the relevant antioxidant activity.

The objective of this study was to evaluate the effect of thermal processing on the antioxidant activity of herbal (such as rosehip) tea by total antioxidant capacity measurement of the raw and thermally processed rosehip. The Ceric ion Reducing Antioxidant Capacity (CERAC) [Ce(IV)-reducing] and the CUPRAC [Cu(II)-reducing] antioxidant capacity methods were used to measure the antioxidant properties of thermally processed products. The results showed that unheated and Microwave (MW) - heated rosehip fruit had similar antioxidant capacity. On the other hand, oven heating was characterized by a decrease in this property. The samples heat-treated for a longer time of 24 h at 85 °C showed the greatest decrease in ascorbic acid content. Oven heating had much higher adverse effect on the antioxidant capacity of rosehip than MW treatment.

# ANALYSIS OF MICOTOXIN CONTENT OF REFORM NUTRITION

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Nutritional habits have gone through revolutionary changes over the last 15 years. The bio and reform food and meals are gaining more and more territory within the culinary culture. Processing of basic materials has changed mainly in the field of the products made of cereals; furthermore, as an effect of the process the circle of consumer goods has increased. The earlier used plant protective materials were almost entirely disregarded in respect of treatment of the cultivated cereal seeds, primarily in the case of bio provisions. As a result the bio seeds are exposed to infections, which, due to pesticide treatment, remain cannot be detected. This is contradictory, because the non-resolvable and resistant fungus toxins are hundred, sometimes thousand times more toxic than fungicides and often elude the attention of producers and consumers. People who trust in bio products can be thus seriously affected. One dangerous form of cereal infection is that caused by fungus, and through fungus infection the external part of seeds can host the secondary metabolic products of fungus, *i.e.*, the toxins.

The most important toxin-producers are *Fusarium*, *Aspergillus*, *Penicilium* and their various derivatives. Cereals can be infected by all of them, but toxicological problems are caused primarily by the first three. Recent investigations were centred on cereals like wheat, barley, maize, rye, corn flakes, oat flakes and fully extracted flour as found in the Hungarian bio- and reform food. Deoxyvalenol, Nivalnol, HT-2 toxin, Aflatoxin B1, B2, G1, G6 and Zearlenon toxins were assayed by High Performance Liquid Chromatography (HPLC) coupled to Mass Spectrometry (MS). The experimental data provided some evidence of the fact that food deemed to be safe and eaten with satisfaction is not always what it is thought to be. The amounts of toxins measured in some cereals can be, in fact, surprisingly high.

# ANALYSIS OF OCHRATOXIN-A CONTENT OF WINES ORIGINATING FROM THE MEDITERRANEAN AND HUNGARY

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Nowadays the healthy habit of wine consumption is regarded as an important activity of social life. During the past years when Hungary became a Member State of the EU the wine market has changed significantly, as foreign wines of different quality have appeared on the internal market. The largest part of the wines is exported from the Mediterranean regions to Hungary. Under the Mediterranean climatic conditions some of the parameters required for the growing of grapes (*e.g.*, number of sunny hours) are more favourable than those encountered in Hungary. As a result of the combined effect of some adverse parameters the plant becomes liable during the cultivation to some fungi that are able to multiply on the grapevine.

Earlier studies had shown that grapes and wine are the second most important sources of Ochratoxin-A (OTA). *Aspergillus carbonarius* and *Aspergillus nigris* fungi are responsible for the production of this toxin. It was concluded that the toxin content of the wine could easily reach highly dangerous levels. Red wines contain the toxin in larger quantity than the white wines. Furthermore, the content of the toxin increases moving southward through the wine districts. OTA causes tumors in animal kidney and in other organs and consumption in larger amounts can lead to human kidney diseases and to cancerous illness of the ureter. The Mediterranean climatic conditions favour the development of the fungi. Samples of wine to be analyzed were taken from retailers easily accessible to everybody, such as supermarkets. The determinations were carried out by High Performance Liquid Chromatography (HPLC) coupled to Mass Spectrometry (MS).

# INFRARED SPECTROSCOPIC STUDY OF THE FATTY ACID - BACTERIUM INTERACTIONS

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In the present work the interactions between fatty acids and bacteria were studied. The antibacterial properties of fatty acids are well known and have been thoroughly investigated. Nevertheless, there is no reassuring explanation about the mechanism of the antibacterial activity of these compounds. The results of the mentioned interactions can be different in the case of fatty acids having various structures. Earlier studies provided some evidence of the fact that even small structural changes could change their behaviour towards bacteria. Previous investigations with stearic acid (C18) pointed out that this molecule had no effect on the bacterial growth during the first few hours of the experiment. However, after 24 h a strong antibacterial effect was detected. On the other hand, if linolic acid (C18:2) was used, a strong antibacterial effect was observed in the first hours, although this effect totally vanished after 18 h. The direct bioautography was resorted to for clarifying the antibacterial effect of lipid acids as this method allows lipophilic compounds to be investigated.

Vibrational spectroscopy is a good approach for the examination of the structural changes in fatty acids. Changes in the spectra of the fatty acids reflect the interaction of fatty acids with the bacteria. In this way one can gain some information on the process. Great attention was devoted to the variations in double bonds as well as to the methylation - demethylation processes. These spectral changes may be of assistance in the elucidation of the probable mechanisms of the said interactions. The present study is part of a greater project which focuses on the study of antimicrobial properties of fatty acids and understanding of the mechanisms of the fatty acid - bacterium interactions.

# ORGANOHALOGEN COMPOUNDS IN FOOD: DOES EATING ORGANIC MAKE A DIFFERENCE?

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The consumption of organic food vs. conventional ones is presented by producers, and considered by most consumers, as the forerunner of many healthy effects. In fact, in organic breeding, the use of synthetic growth promoters, such as antibiotics, is not allowed, feed and fodder administered to animals should be organic and pasture should be the basis of diet. However, often the health benefits of organic products consumption are supported more by ideological beliefs and emotional factors than by results of scientific studies, thus creating the conditions for incorrect information. In 1991 the European authorities, through the Regulation 2092/91 on organic products marketing, had established that neither advertising nor label should suggest to the buyer that organic production methods were a guarantee of superior organoleptic, nutrient or healthy properties. As regards contaminants primarily related to the environment, such as organohalogen compounds, organic and conventional foods are equally at risk. From this viewpoint, food from organic production are not better and healthier than conventional food.

In this study, milk, cheese, eggs and meat samples from both conventional and organic production, were analyzed for their contents in PCBs, chlorinated pesticides, PCDDs, PCDFs and PBDEs. Results showed that also organic products are contaminated by the said compounds. In some cases the levels of contamination are higher for organic products compared with the conventional ones, even though there was no statistically significant differences between the two kinds of products. The results obtained support the hypothesis that organic food may be potentially contaminated in the same way as conventional food and that, despite the many benefits associated with organic food consumption, the presence of contaminants still represents a real danger.

# PERFLUORINATED COMPOUNDS IN ITALY: RESULTS OF A TWO-YEAR STUDY ON LEVELS IN HUMANS, THE ENVIRONMENT AND FOOD SAMPLES

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Perfluorinated Compounds (PFCs) are synthetic substances widely used in several fields for coating plastic tissues, plastic materials and electrical products, and as antispot compounds, non-stick coatings and photographic films. PFCs include compounds based on Perfluorooctanesulfonyl Fluoride (POSF) that, *e.g.*, when degraded by environmental factors or metabolized by organisms, leads to the formation of compounds of toxicological importance such as Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA), among others. The high stability of the molecules makes them able to accumulate in organisms and thus exert their adverse effects. PFOA and PFOS are on the list of Endocrine Disrupting Chemicals (EDCs) and some researchers are currently trying to highlight possible correlations between PFOS and PFOA exposure and the onset of some diseases. Despite the environmental importance of PFCs and the detection of these compounds in many countries around the world, little is known of their occurrence and distribution in Italy.

In this work the results of the first five studies on the distribution and levels of PFOS and PFOA in humans, the environment and food samples are reported. In all the samples analyzed only the presence of PFOS was found, as PFOA concentrations were below the limits of detection. Among the environmental samples, the highest concentrations of PFOS were detected in fish tissues from heavily polluted areas (petrochemical plants, Sicily). Fish and fish products were also the most contaminated samples among foodstuffs, although with low levels. PFOS were also found in a few samples of human blood out of all those analyzed, with concentrations between 12.93 and 127.61 ng/g.

# **ASSESSMENT OF PERFORMANCE OF INSTRUMENTAL TECHNIQUES FOR TRACE ELEMENTS IN FOOD: A NEW GRAPHICAL APPROACH TO IMPROVE THE EXPOSURE ESTIMATION**

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Food safety and the free movement of food commodities of animal origin in Europe is more and more based on the exchange and sharing of information and experience among the Member States. This results in an increase of mutual confidence as well as in large amount of experimental data generated from coordinated activities. In the frame of the Proficiency Tests Schemes conducted by the Community Reference Laboratory for residues and contaminants in food of animal origin, the network of National Reference Laboratories generated abundant experimental information on the quantification of toxic and potentially toxic trace elements in food. The measurement uncertainties of results obtained by specific analytical methods have different sources, remedies and consequences on the interpretation of data.

As recommended by the Commission's Environment and Health Strategy, the above mentioned dataset is being presently further evaluated using the *Naji Plot* graphical approach. The user-friendly graphical approach allows for the assessment and comparison of the two constituents of accuracy (bias and precision) of different instrumental techniques. The present work describes the application of this approach to assess the performance of major analytical instrumental techniques along with the identification of the main measurement uncertainty factors that can affect exposure estimates necessary for toxicological risk assessment.

**Poster Session 6**  
**Research in Antarctica**

*Coordinator*  
Clara Turetta



# SWASV DETERMINATION OF Cd, Cu AND Pb IN A HYDROFLUORIC ACID SOLUTION OF SILICEOUS SPICULES OF MARINE SPONGES FROM THE LIGURIAN SEA AND THE ROSS SEA (ANTARCTICA)

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Within the framework of the Italian National Programme for Research in Antarctica (PNRA in the Italian acronym), a recent project has been developed to explore approaches and means for biomonitoring chemical pollution. Among other organisms, sponges have been considered as bioindicators for the evaluation of the state of the marine ecosystem. As filter-feeders, they accumulate in their tissues a number of chemicals (hydrocarbons, organochlorinated compounds, metals) present in the water. In general, contaminants in the sponge body are determined, without considering the siliceous skeleton (spicules) that is present in some sponge groups and represents a considerable fraction of the total sponge weight (over 70% dry weight). No data have been reported so far on heavy metal traces in this matrix.

A new voltammetric method has been set up and optimized for the simultaneous determination of Cd, Cu and Pb in an HF solution of siliceous spicules of marine sponges directly in the HF solution. Square Wave Anodic Stripping Voltammetry (SWASV) was applied to the analysis of spicule solutions in  $\sim 0.55$  M HF (pH  $\sim 1.9$ ) using a Thin Hg Film Electrode (TMFE) plated onto an HF-resistant epoxy-impregnated graphite rotating disc support. The optimal experimental parameters, evaluated in terms of the signal-to-noise ratio, were as follows: deposition potential,  $-1100$  mV vs. Ag/AgCl, 3 M KCl, SW scan from  $-1100$  mV to  $+100$  mV, SW pulse amplitude 25 mV, frequency 100 Hz,  $\Delta E_{\text{tep}}$  8 mV,  $t_{\text{step}}$  100 ms,  $t_{\text{wait}}$  60 ms,  $t_{\text{meas}}$  3 ms. Under such conditions the metal peak potentials were Cd  $-654 \pm 1$  mV, Pb  $-458 \pm 1$  mV, Cu  $-198 \pm 1$  mV. The electrochemical behaviour was reversible for Pb, quasi-reversible for Cd and kinetically controlled (possibly following chemical reaction) for Cu. The linearity of the response was verified up to  $\sim 4$   $\mu\text{g/L}$  concentration for Cd and Pb and  $\sim 20$   $\mu\text{g/L}$  for Cu. The limits of detection were 5.8 ng/L, 3.6 ng/L and 4.3 ng/L for Cd, Pb and Cu, respectively, with  $t_d=5$  min.

This optimized voltammetric method was applied to the study of Cd, Cu and Pb concentration in spicules of two specimens of marine sponges (Demosponges) from the natural reserve of Portofino (Ligurian Sea, Italy, *Petrosia ficiformis*) and Terra Nova Bay (Ross Sea, Antarctica, *Sphaerotylus antarcticus*).

# LEVELS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN EGGS OF ANTARCTIC PENGUINS

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Contamination by Persistent Organic Pollutants (POPs) was first documented in Antarctica in the 1960's; among them, Polybrominated Diphenyl Ethers (PBDEs) are a class of POPs extensively used worldwide as flame retardants. Their global production has increased since the 1970's and nowadays they are found in the environment worldwide, including the Antarctic. PBDEs can cause toxic effects in organisms and previous studies in the Arctic reported that their rising levels have already threatened polar bears health. Gas Chromatography (GC)/Mass Spectrometry (MS) analysis revealed the presence of PBDEs in eggs of three species of Antarctic penguins, namely, Adélie penguin (*Pygoscelis adeliae*), Chinstrap penguin (*Pygoscelis antarctica*) and Gentoo penguin (*Pygoscelis papua*).

PBDE concentrations were 777 pg/g (wet weight), 1431 pg/g (wet weight) and 1,308 pg/g (wet weight) in Adélie, Chinstrap and Gentoo penguin, respectively. 2,2',4,4',5-pentaBDE (IUPAC no. 99) was the most abundant congener in all species of penguins. The congener concentration pattern was BDE99>BDE 47>BDE 85 in Adélie penguins; BDE 99>BDE 15>BDE 3+7 in Chinstrap penguins; and BDE99>BDE 85>BDE 47 in Gentoo penguins. Although these concentrations are lower than those reported for birds and marine organisms from temperate regions, the presence of PBDEs in Antarctic organisms confirms their global distribution and their ability to bioaccumulate in the tissues of organisms.

# MAJOR, MINOR AND TRACE ELEMENT PROFILES IN TWO ANTARCTIC MARINE SEDIMENT CORES FROM ROSS SEA

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Sediments are receptors of detrital matter deposited in the course of time as a result of the multiple processes occurring in the water column. As such, they behave as efficient environmental archives. The detailed characterization of sediment composition and the possibility of dating sediments can point out possible changes through time and is therefore very useful in the study of environmental variations both in a natural cycling perspective and in the light of the possible recent changes resulting from the profound influence of anthropic activities. In this study, the chemical composition of two sediment cores collected in Ross Sea was compared. One core (coded D) was collected in the polynya of Terra Nova Bay, at 75° 06' S and 164° 13' E, whereas the other one (coded H) was sampled on the outer continental Ross Ice Shelf, at 75° 56' S and 177° 36' W.

Aluminum, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Na, Ni, P, Pb, Sr, Ti, V and Zn were determined in the two cores both by Atomic Emission Spectrometry (AES) or Atomic Absorption Spectrometry (AAS) and by X-ray Fluorescence (XRF). Moreover, natural  $\gamma$ -emitters, namely  $^{210}\text{Pb}$ ,  $^{214}\text{Bi}$  and  $^{214}\text{Pb}$  ( $^{238}\text{U}$  series),  $^{212}\text{Pb}$  ( $^{232}\text{Th}$  series) and primordial  $^{40}\text{K}$ , were determined in core D by non destructive high resolution  $\gamma$ -ray spectrometry. The depth profiles of the above-mentioned elements were obtained and the results were treated with multivariate statistical techniques in order to detect similarities and differences among the layers of each core and between the two cores.

In general the average concentration levels for trace metals, but above all their vertical profiles, do not reveal variations ascribable to anthropogenic sources. In addition, highest concentration levels were found in deepest layers which are much older than the surface sections, suggesting that biogeochemical factors are far more important than anthropogenic ones. The trends of the depth profiles of the investigated elements (including the natural radionuclides for core D) show remarkable discontinuities and point out to likely differences in the properties of deposited materials and therefore of their mineral or biologic sources. The comparison of the data for cores D and H showed that they had several similar characteristics and allowed more general hypotheses on the formation and behaviour of Antarctic marine sediments to be made.

# BIOPHYSIOLOGICAL CHANGES OBSERVED IN MEMBERS OF THE ITALIAN EXPEDITIONS TO ANTARCTICA

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The objective of this study was to investigate whether food consumed by individuals working in Antarctica is adequate and sufficient, from the nutritional point of view, to sustain life in extreme environments. Malnutrition can be the cause of both health impairment and reduction in psychological and physiological efficiency. The project involved individuals from two subsequent expeditions in Antarctica, namely, 34 subjects (group A) from the Italian Mario Zucchelli Base (MZB) in October-November 2002 and 30 subjects (group B) from the Italian-French Dome Concorde Base (DCB) between November 2003 and January 2004. Each group of volunteers consisted of subjects with a controlled diet as well as of subjects with a free diet.

Just before the expeditions (with the purpose of setting the baseline) and during the last week spent in Antarctica blood and hair samples were taken and shipped to Italy to be analyzed, while the body composition and the hydration status were measured on the spot by Bio-Impedentiometric Assay (BIA). Anthropometric measurements were carried out weekly. The data obtained were statistically assessed by using the *t*-Test for correlated samples and showed, in all of the subjects, alterations of the hypothalamic-pituitary-thyroid axis, of blood parameters and of some components of the hair along with some variations of the psychological behavior. The variations observed can probably be ascribed to environmental conditions and a combination of factors such as isolation, atmospheric conditions and prolonged presence of daylight.

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## AUTHORS' INDEX

- Abate V.; 70  
Abete C.; 59  
Abollino O.; 129  
Achene L.; 25; 82  
Agazzi A.; 18  
Aimo E.; 95  
Akman S.; 31  
Amendola L.; 63  
Ammazzalorso P.; 86  
Annibaldi A.; 61; 127  
Antiochia R.; 51  
Aulicino F.A.; 89  
Bakircioglu D.; 67  
Bakircioglu Y.; 67  
Ballók M.; 27  
Baranyai V.; 73  
Barbante C.; 62  
Barra Caracciolo A.; 32; 94  
Bartha A.; 27  
Bassotti E.; 61; 127  
Battistelli C.L.; 107  
Baysal A.; 31  
Becagli S.; 60; 108  
Beltrami M.E.; 88  
Bertalan E.; 27  
Bertolo A.; 95  
Bianchi V.; 102  
Billes F.; 7; 121  
Bisi A.; 15  
Bittoni A.; 84  
Blasi M.F.; 85  
Bogani P.; 68  
Boková V.; 103  
Bonadonna L.; 95  
Borghesi N.; 128  
Borszéki J.; 72  
Bosisio S.; 11  
Bottoni P.; 64; 93; 94  
Bragadin M.; 28  
Brandsma S.; 128  
Brera C.; 47  
Bruno M.; 92  
Budai B.; 8  
Buiatti M.; 68  
Calace N.; 28; 62  
Calisir F.; 31  
Campanella L.; 51  
Capodaglio G.; 28; 62  
Cappelletti C.; 88  
Carere M.; 26; 85; 91  
Caroli S.; 64; 75; 93; 124; 130  
Casalino C.E.; 129  
Casella M.L.; 107  
Castellano E.; 60; 108  
Ceccanti B.; 36; 102  
Ceccarini A.; 4  
Cerri O.; 108  
Chen Ha.; 36  
Chen Hu.; 36  
Chen K.; 36  
Chiari M.; 60; 108  
Ciadamidaro S.; 85; 89  
Ciardullo S.; 130  
Citti G.; 82  
Ciutti F.; 88  
Colombini M.P.; 54  
Corami F.; 28  
Corsolini S.; 123; 128  
Csutorás Cs.; 50; 119; 120  
D'Angelo A.M.; 86  
Dall'Asta C.; 53  
de Boer J.; 128  
de Luca S.; 70  
De Samber B.; 48  
De Santis B.; 47  
Debegnach F.; 47  
Del Torchio R.; 10; 11; 49; 69  
Della Bella V.; 85; 88; 89  
di Domenico A.; 70  
Di Francesco F.; 4  
Di Leo C.; 84  
Dinelli E.; 129  
Dossena A.; 53  
Dubuisson C.; 19  
El Saih F.M.; 6  
Equestre M.; 86  
Esposito D.; 24  
Fabbrocino S.; 92  
Fabiani C.; 86  
Farina M.; 10; 11; 49; 69

Fekete I.; 101; 103  
 Ferranti P.; 92  
 Ferretti E.; 25; 82; 83; 95  
 Ferretti F.; 130  
 Fiabane G.; 17  
 Fidente R.; 93  
 Flórián K.; 35; 101; 103  
 Floris B.; 86  
 Focardi S.; 52; 122; 123; 128  
 Fochi I.; 70  
 Forlani L.; 42  
 Formichetti P.; 86  
 Fortaner S.; 10; 11; 49; 69  
 Forte M.; 45  
 Francesconi S.; 59  
 Frazzoli C.; 124  
 Fretel E.; 19  
 Frost R.L.; 37  
 Funari E.; 85  
 Fuoco R.; 4; 59; 68  
 Füzfai Zs.; 115  
 Galaverna G.; 53  
 Gallo P.; 92  
 Gaspari V.; 62  
 Gazzotti T.; 117  
 Ghimenti S.; 4; 59  
 Giacomino A.; 129  
 Giannarelli S.; 59; 68  
 Giglio F.; 62  
 Grenni P.; 32; 94  
 Grilli E.; 117  
 Grosso A.; 130  
 Guerranti C.; 52; 122; 123  
 Halász G.; 101; 103  
 Halmos P.; 72  
 Hamester M.; 16  
 Helenkár A.; 81; 90  
 Heltai Gy.; 35; 101; 103  
 Homonnai F.; 29  
 Horváth E.; 27  
 Horváth I.; 27  
 Horváth M.; 101  
 Iaconelli M.; 86  
 Iacovella N.; 70  
 Iamiceli A.L.; 70; 107  
 Illuminati S.; 61; 127  
 Insogna S.; 63  
 Kapitány S.; 6  
 Kaszab F.; 29  
 Keresztes Sz.; 80  
 Kiss A.; 119  
 Kovács M.; 72  
 Kralovánszky J.; 8  
 Kristóf J.; 37  
 Kröppel M.; 112  
 Kuscu M.; 67  
 Lahoz Muñoz I.; 112  
 Lakatos J.; 9; 73  
 Le Foche M.; 86  
 Lebouil S.; 19  
 Legnani A.; 42  
 Lengyel A.; 73  
 Lengyel G.; 73  
 Leonards P.; 128  
 Lindemann T.; 16  
 Lipone P.; 51  
 Lucarelli F.; 60; 108  
 Lucentini L.; 25; 82; 83; 95  
 Lugoboni B.; 117  
 Magarini R.; 72  
 Majdik C.; 7  
 Malandrino M.; 129  
 Mancini L.; 85; 86; 88; 89  
 Manganelli D.; 108  
 Marcheggiani S.; 85; 86; 88; 89  
 Marchelli R.; 53  
 Maresca D.; 83  
 Marinilli C.; 89  
 Marino F.; 60  
 Mariottini M.; 52  
 Martinelli A.; 94  
 Maskow Th.; 36; 102  
 Mattei R.; 130  
 McCurdy E.; 17  
 McSheehy S.; 16  
 Mentasti E.; 129  
 Mermet J.-M.; 19  
 Mihucz V.G.; 48; 80  
 Mikosch H.; 7  
 Miraglia M.; 47  
 Mohammed-Ziegler I.; 7  
 Molnár V.; 121  
 Molnár-Perl I.; 23; 81; 90; 115  
 Morabito E.; 62  
 Moranti A.; 108  
 Muránszky G.; 41; 110

Muscatello B.; 84  
 Nagy D.; 6  
 Nava S.; 60; 108  
 Onor M.; 4; 59  
 Ottaviani M.; 25; 82; 83; 95  
 Óvári M.; 41; 74; 110; 111  
 Ozdemir A.; 79  
 Ozturk B.D.; 79; 116; 118  
 Ozyurt D.; 116; 118  
 Paganelli M.; 95  
 Pagliuca G.; 117  
 Paolini E.; 130  
 Paris P.; 24  
 Peri A.; 130  
 Perra G.; 123  
 Petralia S.; 130  
 Petronio B.M.; 28; 62  
 Pettine P.; 25  
 Piedominici E.; 86  
 Pirola C.; 18  
 Piva A.; 117  
 Polesello S.; 26; 91  
 Polgári Zs.; 8; 74  
 Posta J.; 6  
 Prantero E.; 47  
 Preszner A.; 6  
 Puccinelli C.; 85; 88; 89  
 Quattrocchi W.; 107  
 Rácz L.; 50; 119; 120  
 Remeteiová D.; 103  
 Réti A.; 8  
 Rezić I.; 5; 71  
 Ribechini E.; 54  
 Robouch P.; 124  
 Rogerieux O.; 19  
 Rosati A.; 68  
 Rosselli P.; 86  
 Rugi F.; 60; 108  
 Rusnák R.; 101; 103  
 Sabbioni E.; 10; 11; 49; 69  
 Santoro M.; 15  
 Scarponi G.; 61; 127  
 Schoonjans T.; 48  
 Sebők A.; 81  
 Serpe L.; 92  
 Severi M.; 60; 108  
 Silversmit G.; 48  
 Smichowski P.; 41  
 Spezzano S.; 95  
 Stefanoni E.; 95  
 Steffan I.; 5; 71; 111  
 Szabó Cs.; 29  
 Szabó Zs.; 8; 74  
 Szalóki I.; 48  
 Szilágyi Z.; 50  
 Szoboszlai N.; 8; 74  
 Szócs T.; 27  
 Tabucchi S.; 4  
 Tancioni L.; 89  
 Tatár E.; 48; 80  
 Taviani G.; 130  
 Termine M.; 59  
 Toşa M.; 7  
 Tositti L.; 129  
 Tóth J.; 115  
 Tóth Sz. B.; 50; 119; 120  
 Traversi R.; 60  
 Trotti F.; 95  
 Truzzi C.; 61; 127  
 Turetta C.; 28; 62  
 Turrio Baldassarri L.; 107  
 Tyihák E.; 121  
 Udisti R.; 60; 108  
 Vasanits-Zsigrai A.; 30; 81; 90  
 Velasquez S.; 19  
 Veschetti E.; 25; 82; 83; 95  
 Vincze L.; 48  
 Virág I.; 48; 80; 110  
 Wang F.; 36  
 Woods G.; 17  
 Yao J.; 36; 48; 80; 102  
 Zani P.; 42  
 Zaottini E.; 86  
 Záray Gy.; 3; 8; 36; 41; 48; 74; 80; 81; 90;  
 110; 111  
 Zeiner M.; 5; 111  
 Zhou Y.; 36  
 Zhuang R.; 36  
 Zironi E.; 117  
 Zoccolillo L.; 63

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