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3rd International IUPAC Symposium on Trace Elements in Food

Istituto Superiore di Sanità
Rome, Italy
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ABSTRACT BOOK

Edited by
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Silvia Ciardullo and Marina Patriarca

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Edited by Francesco Cubadda, Federica Aureli, Silvia Ciardullo and Marina Patriarca
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Aim of this interdisciplinary IUPAC (International Union of Pure and Applied Chemistry) symposium is to gather experts with different background to discuss all aspects of trace elements in relation to food and feed, with special emphasis on their biological effects. The topics covered include essentiality, toxicity, bioaccessibility, bioavailability, speciation, sources and transfer in the food chain, effects of processing, food and feed fortification, supplementation, international legislation and standards, analytical developments, analytical quality assurance and reference materials. Special emphasis is placed on research and development efforts which have taken place over the last few years as well as on emerging issues in the area.

Key words: Trace elements, Food, Analytical techniques, Toxicology, Risk assessment, Nutrition, Human health

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Lo scopo di questo simposio interdisciplinare IUPAC (International Union of Pure and Applied Chemistry) è quello di riunire esperti con diversa formazione ed esperienze per discutere tutti gli aspetti degli elementi in traccia in relazione alla loro presenza negli alimenti e nei mangimi, con particolare riferimento ai loro effetti biologici. I temi trattati includono l'essenzialità, la tossicità, la bioaccessibilità, la biodisponibilità, la speciazione, le fonti e il trasferimento lungo la catena alimentare, gli effetti dei processi di trasformazione, la fortificazione, l'integrazione della dieta, la legislazione e le norme internazionali, gli sviluppi in campo analitico, l'assicurazione della qualità analitica e i materiali di riferimento. Particolare enfasi viene data ai più recenti progressi della ricerca in questo ambito e agli aspetti emergenti.

Parole chiave: Elementi in traccia, Alimenti, Tecniche analitiche, Tossicologia, Valutazione del rischio, Nutrizione, Salute umana

Symposium Chair: Francesco Cubadda, Istituto Superiore di Sanità, Rome, Italy

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NOTE FOR THE READER

This volume gathers all the contributions presented at the symposium. Abstracts are divided into oral and poster presentations. For easy consultation, oral presentations are listed in the order of the programme. However, for each session, the invited lectures are presented first, followed by the contributed lectures.

Posters are listed after the oral presentations and divided by session. The poster abstracts are numbered with a code including the initial of the day of presentation (W for Wednesday, T for Thursday) and a progressive number.

At the end of the volume, the authors' index is provided for the reader's convenience.

PREFACE

The diet is the main source of trace elements and exposure to dietary trace elements has a direct impact on the health of hundreds of millions worldwide. Insufficient intake of essential trace elements is a global issue. The deficiencies of iron, zinc, iodine and selenium result in millions of people being affected by various diseases, with very serious consequences in those countries where malnutrition is widespread. On the other hand, the impact of toxic element species such as inorganic arsenic and methylmercury on whole populations has come to the fore again as a priority for both the scientific community and health authorities. The presence of trace elements in feeds, either as contaminants or as additives aimed to improve animal health or food properties, and their transfer across the food chain to humans also pose questions to the scientific community and regulators.

The different facets of trace elements, from essentiality to toxicity, are addressed throughout the symposium. Advancements in the different areas are discussed and special attention is paid to the means in order to prevent adverse health effects on those individuals and populations most vulnerable to trace-element inadequacies, excesses or imbalances.

This meeting follows two previous ones, organized in Warsaw, Poland (2000), and in Brussels, Belgium (2004). Alike the two previous meetings of this series, the symposium is held under the auspices of IUPAC and aims at being a suitable forum for new ideas and experiences to be exchanged among researchers in the trace element area with a view to providing an evidence base for policy, advice on the development of improved foods and feeds and the implementation of risk-management tools to protect public health.

At the time of writing, 220 participants, representing 40 countries, have joined the event. In addition to the 15 invited lectures, delegates from Europe, Asia, Africa, North and South America enriched this edition with 20 oral and 135 poster contributions, thus providing an excellent opportunity for participants from different cultures and backgrounds to convene and share their views. Insofar as this happens, the meeting is expected to be a fruitful and rewarding experience for everyone.

Francesco Cubadda
Symposium Chair

Oral Session 1

Advances in trace elements analysis in food matrices

Chairpersons

Ryszard Lobinski, Kevin Francesconi

STATE-OF-THE-ART ANALYTICAL TECHNIQUES FOR ELEMENT SPECIATION STUDIES IN FOOD AND FOOD SUPPLEMENTS

Joanna Szpunar
Laboratory of Analytical, Bio-Inorganic and Environmental Chemistry, CNRS UMR 5254, Pau, France

The safety and nutritional quality of food are determined by the total concentration levels and the speciation of trace elements in foodstuffs. Since the element bioavailability is highly variable between chemical forms, information on trace element speciation is essential to provide better understanding of the mechanisms of absorption of essential nutrients and potentially toxic species. Therefore, there is a need for the development of accurate, reproducible and precise methodologies suitable for speciation-related characterization of commercial food and food supplements. Analytical approaches proposed include: 1) fractionation (usually in simulated gastrointestinal conditions) in order to estimate the toxicity/bioavailability of elements of interest; 2) quantitative determination of toxic (*e.g.*, As(III), MeHg) or essential species (*e.g.*, selenomethionine to assess the quality of Se-supplements): 3) quantitative determination of all the species of the element of interest for comprehensive characterization of foodstuffs; 4) identification of unknown species of the element of interest; 5) speciation blueprinting or detection of specific markers of the origin of commercial products. The presentation will highlight the state-of-the-art of analytical methodology for speciation studies in foodstuffs addressing the above issues. The advantages and drawbacks of the currently used analytical approaches will be discussed with a focus on selenium, the element present in food in a large variety of forms with a complex chemistry and nutritional significance.

DETECTION AND CHARACTERISATION OF INORGANIC NANOPARTICLES IN LIFE SCIENCE STUDIES

Erik H. Larsen, Bjørn Schmidt, Katrin Löschner
National Food Institute, Technical University of Denmark, Søborg, Denmark

Nanoparticles are defined as particles having at least one dimension below 10^{-9} meter (nm). The chemical and physiological properties of nanosized particles may differ from their bulk counterparts because the smaller the particle, the larger is the number of uncoordinated atoms on its surface. The associated surface chemistry properties may be responsible for toxic effects observed for nanosized matter, and which are not seen for the corresponding bulk material. Formation of Reactive Oxygen Species (ROS) catalysed by TiO_2 nanoparticles present in biological systems is an example of such an adverse effect. The large ratio of surface area to mass of nanoparticles however, may also be beneficial because they constitute a source of soluble and bio-accessible essential elements in biology. Examples of nanoparticles causing formation of ROS, and examples of beneficial effects of nanoparticles of essential elements will be given. The experimental study of Engineered inorganic Nanoparticles (iENPs) in biological systems requires methods of analysis that provide information on metrics such as size distribution, agglomeration state, elemental composition and mass concentration. To acquire results on these variables, an analytical platform involving Field Flow Fractionation (FFF) was coupled with Multi Angle Light Scattering (MALS) detection for size determination and on-line with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for elemental detection and quantification. This platform was used for analysis of nano-clays, silver, gold and selenium iENPs. Results from stability studies of iENP stock suspensions demonstrated that agglomeration did occur. The platform was applied also for analysis of migrates from clay-reinforced polylactide, or a future "green" plastic as food contact material. The nano-sized clay was characterised in terms of size and the natural content of rare elements such as zirconium and cerium were used for their selective ICP-MS detection. Finally, the potential usefulness of additional imaging techniques in nanoscience studies will be outlined. Such techniques include electron microscopy or synchrotron radiation X-ray fluorescence.

SPECIATION AND ISOTOPE PATTERN DECONVOLUTION FOR ICP-MS QUANTITATIVE STUDIES OF MINERAL METABOLISM AND SUPPLEMENTS

Alfredo Sanz-Medel, Héctor González Iglesias, María Luisa Fernández-Sánchez
Department of Physical and Analytical Chemistry, University of Oviedo, Oviedo, Spain

Breast human milk could be considered the "ideal" food for the correct development of the newborn baby. When, due to whatever reason, infants are not breast-fed formula milks have to be used. Of course, formula milks' composition should be ideally as similar as possible to maternal milk. Apart from other micronutrients, an adequate supply of essential trace elements (*e.g.* Fe, Zn, Cu, Se, I) is required for infant appropriate development, particularly so in premature babies. Moreover, it is accepted today that food trace element absorption, retention and bioavailability depends strongly on their chemical speciation (*i.e.* chemical form in the food). In particular, trace element speciation information, rather than the common total determinations, is urgently needed these days for a better understanding of mineral metabolism and supplements optimization. We will document first, by using HPLC-ICP-MS, the alarming qualitative differences observed in the elemental speciation of human breast milk whey as compared to Spanish commercial formula milks speciation. On the other hand, quantitative analysis of such elements and their species (*e.g.* in milk) are mandatory. In this vein, the use of stable isotopes in connection with ICP-MS detection offers today a most convenient alternative to the radioisotope techniques widely used before in metabolism and nutritional studies. In particular, we will refer to the use of two different "isotope-enriched" spikes in combination with "Isotope Pattern Deconvolution" (IPD) to differentiate and quantitate endogenous (natural) and exogenous (supplemented) essential elements (*e.g.* Se or Fe). Results of successful application of such ICP-MS based techniques in Se-supplemented lactating rats, all along 14 days of supplementation, will be discussed. This IPD analytical tool has also been extended to quantify selenospecies, of endogenous and exogenous Se, in the experimental rats' urine. Selenium absorption and distribution in different element-species in those urine samples have been measured quantitatively both, in control and in Se-supplemented animals. Intriguing information about quantitative Se biotransformations, kinetics and final catabolism of the element, via quantitative data of endogenous and exogenous Se in the urine of rats during the two weeks of supplementation, will be discussed.

INTERNAL AND EXTERNAL QUALITY CONTROL FOR THE DETERMINATION OF TRACE ELEMENTS IN FOOD

Maria Beatriz de la Calle
Institute for Reference Materials and Measurements, Joint Research Centre, European Commission, Geel, Belgium

Generally speaking, Quality Control (QC) is a process used to ensure a certain level of quality in a product or service. When applied to analytical chemistry, QC is one of a number of concerted measures, internal or external, that can be taken to ensure that the data produced in the laboratory are fit for their intended purpose. Internally, the process of quality control starts with the development of a new analytical method in some cases, or with the implementation of an existing standardised method. In the first case, validation of the method must be performed to ensure the fit-for-purpose of the method in terms of specificity, selectivity, sensitivity and accuracy. In the second case a verification process is needed to warranty that the potential performance characteristics of the standard method, as described in the standard, are actually met in a particular laboratory. At international and national level several external quality control mechanisms have been put in place to help laboratories to evaluate the fit-for-purpose of their analytical processes. Several are the fundaments of the external quality control, among others accreditation, standardisation and metrology. Ideally these elements are brought together in the form of reference laboratories that through the execution of interlaboratory comparisons and providing laboratories with traceable reference materials establish the bridge between external and internal quality control. The system of Community Reference Laboratories (CRLs), recently established by the European Commission in the area of food and feed, is a perfect example of how analytical systems should work. In this lecture all the elements previously mentioned will be presented and discussed.

CHARACTERIZATION OF IODINE BIOMOLECULES IN MICROALGAE FUNCTIONAL FOODS

Elisa Gomez De Las Heras, Macarena Gonzalez-Fernandez, Fernando Moreno-Roldan, Tamara Garcia-Barrera, Jose Luis Gomez-Ariza
Department of Chemistry, Universidad de Huelva, Huelva, Spain

Functional food is becoming increasingly interesting in the last decade to provide additional physiological benefits beyond that of meeting basic nutritional needs. Some essential elements such as selenium and iodine are biotechnologically incorporated to foods using element-enriched microalgae biomass (mainly, *Chlamydomonas reinhardtii* and *Spirulina platensis*). An important question is the chemical forms in which the heteroelement (Se, I) is involved, because metabolites represent a set of active biomolecules with potential health effects (metabolomics). Therefore, a suitable analytical approach based on the use of these heteroelements as biomolecule tags, using ICP-MS coupled to multidimensional chromatographic techniques and assisted by 2D electrophoresis, constitutes a powerful tool for this purpose. In this work, *Chlamydomonas reinhardtii* and *Spirulina platensis* microalgae have been used for iodine-biomolecule enrichment with a biotechnologic production platform. Iodine-containing metabolites were extracted from the biomass produced and characterized with a metallomics approach which involves iodine SEC-ICP-MS profiling and latter purification of SEC-ICP-MS fractions by HPLC-ICP-MS chromatography. Purified I-biomolecules were characterized by tandem mass spectrometry. 2D electrophoresis was used in parallel to monitor the purification process. Fractions with low molecular mass (under 300 Da) were additional studied with chiral HPLC-ICP-MS, to check the presence of biologically active chiral iodine molecules. The latter were subsequently characterized by MS/MS spectrometry.

THE DEVELOPMENT OF A GUIDELINE TO IMPROVE RELIABILITY OF PUBLISHED ANALYTICAL DATA ON TRACE ELEMENTS IN FOODSTUFFS

Lars Jorhem
Research and Development Department, National Food Administration, Uppsala, Sweden

There are many papers published worldwide every year on the content of trace elements, such as lead and cadmium, in food. It is often indicated that the foods are not suspected of being contaminated by environmental or production activities. Quite often the results of such surveys cover a wide range, from the LoD (Limit of Detection) and up to considerable quantities, sometimes several mg/Kg. It is often difficult for the reader to elucidate if the reported results are reliable or not, and it has been shown that the reliability of the reported results in many publications is very low. CEN is the European standardisation organisation. Its Technical Committee (TC) 275 develops analytical standards for food control, and Working Group (WG) 10 works specifically on standards for trace elements and heavy metals. CEN works in close cooperation with ISO. CEN/TC 275/WG 10 has initiated the development of a guideline for authors, editors and reviewers/referees. It is based on common quality control criteria, such as certified reference materials, control of contamination and interferences *etc*. This guideline should, when ready, be easy to use, hopefully not only for trace elements, but also in other areas of food chemistry.

ISOTOPIC AND ELEMENTAL FINGERPRINTS TO PROVE AUTHENTICITY AND ORIGIN OF FOOD AND FEED BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

Thomas Prohaska (a), Marion Brunner (a), Siegfried Swoboda (a), Micha Horacek (b), Zsolt Stefanka (c), Robert Katona (c), Carla Rodrigues (d), Cristina Maguas (d)
(a) Department of Chemistry, University of Natural Resources and Applied Life Sciences, Vienna, Austria
(b) Austrian Research Center, Seibersdorf, Austria
(c) Institute of Isotopes, Hungarian Academy of Sciences, Budapest, Hungary
(d) Science Faculty Center for Environmental Biology, University of Lisbon, Lisbon, Portugal

Proof of provenance of agricultural products has become a major concern in anti fraud and consumer protection for the past few years. Elemental and isotopic fingerprinting by mass spectrometry are increasingly applied in order to map the geographical origin of food and proof authenticity of food and food products. The arise of MC-ICP-MS has augmented significantly the capability of analyzing isotopic fingerprints allowing both, the assessment of a variety of different isotopic pattern of the periodic table and the throughput of a statistically relevant number of samples. The Sr isotopic system has turned out to have a denotative potential for authentication and provenance studies since its concentration and variation is sufficient throughout most agricultural products. Moreover, no significant annual or seasonal variation could be observed. The major source of Sr originates from leachable Sr from the surrounding geology or ground water. The S isotopic system proofed to be another major system providing reliable results. We observed the availability and concentration of isotopic systems of other elements within the samples as major limitation even though they comprise potential viable indicators for provenance studies (*e.g.* Pb, Ca). Thus, additional systems are investigated carefully for each specific sample material for the potential as isotopic tracer. At least the combination of isotopic systems (including light elements) often allows a standardized and validated setup for the reliable proof of provenance. Additional effort concerns the variation of isotopic signatures of processed products by contamination or possible fractionation. Finally, direct analysis is increasingly requested in order to proof authenticity without sample preparation. These efforts include direct laser ablation analysis. First results showed clearly the challenges and limitations of this technique with respect to signal intensity, uncertainty and interference correction. We have applied trace element pattern as well as isotopic pattern for proof of authentication of agricultural products such as asparagus, paprika, coffee or wine in order to identify source and origin of products in both plain agricultural products as well as processed products.

CHEMICAL SPECIATION AND FRACTIONATION OF METALS IN WINE

Krystyna Pyrzynska
Department of Chemistry, Warsaw University, Warsaw, Poland

The growing concern about human exposure to different mineral components provided the impetus for studying their content in food and beverages. Wine is one of the most widely consumed beverages in the world and could be an important source of several metal ions. The presence of metals can influence the wine making process or change its taste and quality. Analysis of certain elements in wines is of special interest due to their toxicity in case of excessive intake. A typical example is copper, which is both an essential and a potentially toxic element for humans when in excess. Several elements, including Cu, Fe, Al and Zn, contribute to haze formation and sometimes taste effects. Determination of other elements, such as Pb, As or Cd is of considerable importance due to their potential toxic effects. Moreover, the content of some metals can be used to identify the geographic region in which the grapes were grown due to the direct relationship with soil composition. The research has been mostly focused on the determination of total content of metals regardless of what species were present. However, there is a considerable evidence that metal bioavailability and toxicity is mainly governed by their chemical forms. Metals may exist in wines as free ions, as complexes with organic acids as well as species with large molecules of pectic polysaccharides, peptides, proteins and polyphenols. This presentation will discuss the main developments in chemical speciation and fractionation analysis of metals in wine samples.

SIMULTANEOUS DETERMINATION OF TOTAL S, P, Se AND As AND ELEMENTAL SPECIES BY HPLC COUPLED TO ORS ICP-MS USING SPECIES-SPECIFIC ISOTOPE DILUTION MASS SPECTROMETRY

John Entwisle
Mass Spectrometry, LGC Ltd, Teddington, United Kingdom

The biochemical interactions and balance of elements such as sulfur, selenium, arsenic and phosphorus are currently of particular interest but are difficult elements to measure by ICP-MS. Their relative poor ionization in the ICP and much spectral interference affects their detection. A promising approach for simultaneous determination of these elements is the use of interference-reducing ICP-MS (*e.g.* collision/reaction cell). As a first step, we studied the performance of an octopole reaction cell (ORS) using a Xe/H_2 mixture for the simultaneous detection of S, P, As and Se in microwave acid digests of food CRM's (Certified Reference Material). Using optimal ICP-MS conditions and with the addition of methane to the plasma in order to enhance the As and Se signal by charged transfer effect, limits of detection of 22 mg/Kg (for ^{31}P), 47 mg/Kg (^{32}S), 0.003 mg/Kg (^{75}As) and 0.022 mg/Kg (^{78}Se) were achieved (0.5 g digested with 4 mL nitric/2 mL H_2O_2 and diluted to 25 g). The accuracy of the methodology for total elements was assessed by analysis of four unfortified CRM's including rice flour SRM 1568a, bovine liver SRM 1577b and whole egg SRM 8415 from NIST (National Institute of Standards and Technology,) and a white clover BCR402 from IRMM (Institute for Reference Materials and Measurements,). Quantification was performed using external calibration. Average recoveries were better than 100±10% (n=15) for S and P and 100±15% (n=15) for As and Se. As a second step, we evaluated the potential of the developed ORS ICP-MS in combination with liquid chromatography for the simultaneous accurate quantitation of methionine (Met) and selenomethionine (SeMet) in food supplements using Species-Specific Isotope Dilution Mass Spectrometry (SSIDMS). S and Se, often coexist in the same biomolecule (*e.g.* proteins) as the amino acids methionine (Met) and Selenomethionine (SeMet). To perform SSIDMS ^{34}S-enriched Met and ^{76}Se-enriched SeMet were "in house" synthesised and characterised. A rapid microwave-assisted hydrochloric acid hydrolysis procedure was developed and digests analysed by reversed-phase HPLC coupled with ORS ICP-MS (in Xe/H_2 mode). The procedure was validated by the analysis of the NRCC CRM SELM-1 with certified Met content of 5,758+/-277 mg/Kg and a SeMet 3431+/-157 mg/Kg an average recovery of 98±2.5% (n=3) was obtained for Met and a detection limits were 0.26 mg/Kg (for Met as ^{32}S). Similar recovery and detection limits were obtained for SeMet. The potential of the developed methodologies for the future characterisation of food reference materials will be highlighted.

AFFORDABLE SYNTHESIS OF $SeCys_2$-CONTAINING OLIGOPEPTIDES FOR QC PURPOSES IN SELENIUM SPECIATION

Mihaly Dernovics (a), Anna Magyar (b)
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Quantitative determination of selenocysteine (SeCys or SeC) content from biological samples (including tissues and different kinds of food) has become a target in the field of selenium speciation. Quantification of this analyte may suffer from several analytical pitfalls arising from sample preparation steps, low quality HPLC separations, *etc*. Especially, the stability of SeCys is a critical point during the whole analysis. Ideally, the analytical Quality Control (QC) of quantitative SeCys analysis should monitor all the sample preparations steps that directly act on SeCys, namely reduction, derivatisation, proteolytic or acidic protein digestion, clean-up methods and final analytical determination. Up to now, the only authentic way for this purpose has been the use of a commercially available selenoenzyme, bovine glutathione peroxidase. However, as SeCys is present in this enzyme in reduced form, the complete analytical procedure including the reduction of Se-S and Se-Se bridges cannot be monitored. Therefore, a $SeCys_2$-containing synthetic oligopeptide could theoretically serve as a matching QC material. While the synthesis of selenomethionine (SeMet)-containing peptides is considered straightforward, addressing $SeCys_2$-containing peptides is far more challenging. The presence of the dimer of SeCys in peptides can be the result of either the synthesis of SeCys-containing peptides and their subsequent oxidation into diselenide-bridged compounds or the direct incorporation of $SeCys_2$ into peptides. The previous technique requires either Solid Phase Peptide Synthesis (SPPS) protocols through SeCys derivatisation and sophisticated (site-directed) oxidization or the direct modification of bridged Cys residues into bridged SeCys through an admittedly difficult stepwise conversion technique. The second way, *i.e.*, the direct incorporation of $SeCys_2$ into peptides is also considered difficult due to the inadequate solubility of $SeCys_2$ in common organic solvents such as dimethyl-formamide and ethyl-acetate. In our study, we succeeded in applying the classical liquid phase peptide synthesis through an active ester formation (with pentachlorophenol) to couple a commercially available Boc-protected peptide (Boc-Gly-Gly-Phe-Gly, Boc-P) to $SeCys_2$. The synthesis resulted in a 5:1 mixture of $(Boc\text{-}P)_2\text{-}SeCys_2$: $Boc\text{-}P\text{-}SeCys_2$ with 68% yield. The purity and integrity of these compounds were verified with ICP-MS and ESI-MS/MS. The overall procedure including coupling, clean-up and individual purification of the two oligopeptides could be carried out with the means of a general analytical laboratory, thus offering the possibility to reproduce it in a laboratory usually dealing with elemental (selenium) speciation. The developed protocol can be easily modified for the synthesis of other oligopeptides as well.

Oral Session 2

Sources and transfer of trace elements in the food chain

Chairpersons

Alberto Mantovani, Barbara Szteke

FOOD CHAIN SELENIUM AND HUMAN HEALTH: THE COMPLEXITY OF OPTIMAL INTAKE

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Following the publication of the landmark trial of Clark and colleagues in 1996 that appeared to show that Se could reduce the risk of cancer, awareness of the importance of selenium (Se) to human health has markedly increased. As a result, there is now much more aggressive marketing of Se supplements and functional foods, even in situations where additional consumption of Se is inappropriate. The presentation addresses how Se gets into the food chain, the wide variability in Se content of foods and the very different levels of intake between countries and regions. Though it is clear that there are adverse consequences for health of both deficient and excessive intake, health effects at intermediate levels of intake are less certain. Thus it is difficult to define optimal intake which depends on a large number of factors, such as which functions of Se are most relevant to a particular disease state, which species of Se is most prominent in the Se source, which health condition is being considered, the adequacy or otherwise of intake of other nutrients, the presence of additional stressors, and lastly whether the ability to make selenoproteins may be compromised. These complexities need to be understood, particularly by policy makers, in order to make informed judgments. Potential solutions for increasing Se intake, where required, include agronomic biofortification, genetic biofortification or for individuals, increased intake of naturally Se-rich foods, functional foods or supplements. The difficulties of balancing the risks and benefits in relation to Se intake are highlighted.

INSIGHTS ONTO SELENIUM FERTILIZATION ON TEA PLANT AND RICE AND CHEMOPREVENTION OF Se-ENRICHED FOOD

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Selenium (Se) is a non-metallic essential trace element for animals and the human body and has received considerable attention for its possible role as an effective, naturally occurring, anti-carcinogenic agent. Epidemiological studies reveal that selenium intake was conversely related to incidences of cancer and diseases. However, Se concentration of a particular food may be variable and dependent on the geographic origin of the raw agricultural product with regard to the soil where the agricultural crops are grown. Around the world, there are the regions that are so Se poor that overt deficiency syndromes are endemic. Se deficiency is still a very serious nutritional and health problem in China. The total human Se intake was reported by China Nutrition Society (CNS) to only 26-32 μg/day, with some regions below 10 μg/day. Therefore, the supplementation of Se to livestock through forage and to human beings through food has been a safe and effective strategy to increase this mineral's intake. Rice is one of the leading food crops of the world and is the staple food of over half of the world's population; and tea is the second most common beverage next to water in terms of the worldwide popularity. Unfortunately, the Se content of rice and tea was lower, and the mainly rice-based diet contributed to an inadequate amount of Se for Chinese inhabitants. Therefore, our work is aimed at elevating Se status in rice and green tea by foliar application of selenium fertilizer and thus to enhance human Se nutritional level. Results showed that foliar enrichment of Se was able to significantly increase the selenium content both in rice and green tea. Meanwhile, the yield, the sensory quality, chemical qualities and preservation qualities of green tea were significantly enhanced by Se spraying. Speciation of selenium in the rice and green tea rich in Se by foliar spraying was performed using ion paring reversed chromatography with interfaced Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results indicate that the major selenospecies found in rice and green tea was SeMet, while inorganic selenium in regular ones, suggesting that Se as inorganic selenium taken up into plant roots from soil still existed as inorganic selenium predominately. However, with little increase of inorganic selenium, SeMet was enriched dramatically in the rice grain and new tea shoots after foliar treatment. According to the similarities of Se and S metabolism, it could be concluded that S can be easily replaced by Se via plant leaves pathway, because of higher S content of leaves than that of roots. We further evaluated the biological properties of Se-enriched food and our data showed that: 1) Antioxidant capacity of the aqueous and ethanolic extract from Se-enriched rice and tea was significantly higher compared with the regular ones; 2) Se-enriched green tea could inhibit the cyclophoshamide-induced mutagenicity and mitomycin C induced chromosomal aberrations in spermatocytes in mice; 3) Extracts from Se-enriched green tea presented significantly higher antiproliferative activities in human lung cancer

A549 and hepatoma HepG2 cells than regular green tea or selenium alone as well as regular green tea + selenium; 4) Polyphenols from Se-enriched green tea (SGTPP) could retard cell cycle progression in human HepG2 cells by induction of cell cycle arrest in S-phase and induce apoptosis, which might imply the possible synergistic effect of Selenium and bioactive components from Se-enriched green tea.

INORGANIC AND ORGANIC SELENIUM IN CHICKEN FEED; UPTAKE, DISTRIBUTION AND BIOACCESSIBILITY OF Se IN Se-ENRICHED CHICKEN MEAT

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Selenium (Se) is a crucial trace element for human health, being an important component of several selenoproteins and enzymes required for such functions, as antioxidant defence, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility and reproduction. Thus, increased concentration of bioavailable Se in enriched feed for animal products should represent Se enriched food for human consumption that would additionally increase the population Se intake. The distribution of Se in male chickens (*Gallus gallus*) fed with feeds fortified with selenite, Se enriched yeast or Se enriched wheat (organic Se) for 31 days were investigated. The feeds having Se concentrations of 0.83, 0.73 and 1.0 mg Se/Kg, respectively. Thereafter, the bioaccessibility of Se in the breast muscle was assessed by digesting freeze dried and grinded meat with (1) protease and lipase or (2) Human Ventricle Juice (HVJ) and Human Duodenal Juice (HDJ). The total breast and leg meat Se concentration increased from 0.44 to 1.7 mg Se/Kg Dry Matter (DM) by feeding organic Se compared to selenite. The liver Se-concentration increased from 3 mg/kg DM using selenite to 4 mg/Kg using organic Se. The blood Se-concentrations increased from 0.3 mg/L (WM) feeding selenite to 0.4 mg/L feeding organic Se. These results clearly show that organic Se is more efficient in raising the meat, liver and blood Se concentration than selenite. To obtain information on the bioaccessibility of Se, chicken meat was digested at 37ºC (1) protease and lipase or (2) HVJ and HDS. Preliminary results indicate a different chromatographic Se profile by SEC-HPLC-ICP-MS followed by RP-HPLC-ICP-MS by using human juices compared with protease XIV and lipase. After digestion with protease XIV and lipase one single peak of Se-methionine (Se-met) occurred, whereas HVJ and HDJ gave three distinct peaks, one peak was Se-met and the other peaks were not identified. These results indicate that larger molecules such as di- and three peptides might be present in the human digest. During the digestion samples were collected after 3, 9, 15, 60 minutes with HVJ, with HDJ an additional sample was collected after 2 hours. In this way transformation processes taking place in the stomach and intestine could be followed. In the present paper, focus will be put on the organic Se enriched meat, Se speciation and degradation in the GI tract.

SELENIUM SPECIATION OF MILK AND MILK PRODUCTS FROM DAIRY CATTLE FED DIETS CONTAINING GRADED ADDITIONS OF ORGANIC AND INORGANIC SELENIUM

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A meta-analysis was conducted on total selenium (Se) and Se speciation data (selenomethionine [SeMet], selenocysteine [SeCys] and selenium IV [SeIV]) of milk and milk products (curd and cheese) that were derived from 3 separate studies in which dairy cattle were offered diets containing graded additions of dietary Se derived either from sodium selenite (SS) or selenoyeast (SY [Sel-Plex produced by *Saccharomyces cerevisiae* CNCM I-3060) containing 63% SeMet (Alltech, Nicholasville, KY)]). Treatments in all studies included a negative control ([Con] background Se only), background plus additional Se as either SS or SY to achieve 0.3 mg/Kg DM total Se (SS 0.3 and SY 0.3) or 0.45 mg/Kg DM total Se (SS 0.45 and SY 0.45). Diets within each study were offered for a period of 140 days (T140) followed by a wash out period of 56 days (T196). Milk samples were taken at T0, T140 and T196 and milk products manufactured from milk taken at T140. Data were analysed by analysis of variance using a general linear model. There were 90 observations for each parameter measured in milk (total Se, SeMet, SeCys, and SeIV) and 12 observations for each milk product. The model for milk comprised study (2 d.f.), treatment (4 d.f.), time point (2 d.f.) and time by treatment interaction (8 d.f.). The model for milk products comprised study (1 d.f.) and treatment (2 d.f. [Con, SS 0.45 and SY 0.45 only]). There were significant effects of treatment ($P<0.001$), time ($P<0.001$) and treatment by time interaction ($P<0.001$) on total Se, SeMet, SeCys and SeIV content of milk; total Se, SeMet, SeCys and SeIV contents were similar between treatments at T0 but significantly greater in the milk of SY supplemented animals at T140, irrespective of dose, when compared to SS, and greater in SS supplemented animals when compared to Con. Similarly total Se, SeMet, SeCys and SeIV content of both cheese and curd from SY 0.45 supplemented animals were significantly greater ($P<0.001$) than those of both Con and SS 0.45 which had similar total Se, SeMet, SeCys and SeIV values.

BIOACCESSIBILITY OF SELENIUM IN Se-ENRICHED WHEAT

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Selenium (Se) is a crucial trace element for human health, being an important component of several selenoproteins and enzymes required for functions such as antioxidant defence, reduction of inflammation, thyroid hormone production, DNA synthesis, fertility and reproduction. Since Se enters the food chain through plants, it is essential to increase the agricultural Se-fertiliser efficiency to common food plants such as wheat and to assess the Se-bioaccessibility. Hydroponically grown spring wheat were exposed to 75selenate (gamma-emitter) for three days during vegetative growth (four leafs developed and stem elongation) to investigate the effect of the nutrient solution nitrogen/sulphate-ratio on the re-distribution of Se to the grains. Delaying the Se-fertilisation from the four leaf stage to stem elongation together with a high nutrient solution N/S-ratio increased the re-distribution of the total plant Se to grains from approximatively 15 to 50%. This knowledge was then used to enrich field grown spring wheat with Se. Selenate was fertilised together with 25% of the N at heading, grains were milled at a milling plant and separated into flour and bran. The total wheat grain Se concentration was 1.1 mg/Kg. The flour and bran Se-concentration was 0.98 and 1.38 mg Se/Kg DM, respectively. Thirty one and 26% of the total flour and bran Se concentration was extracted in 0.9% NaCl (pH 5.5) by a ball mill (3 min, 30 Hz) and analysed by SEC-HPLC-ICP/MS. The chromatograms of Se protein profile showed that the wheat flour contained larger Se-proteins than the bran. o assess the bioaccessibility of Se in wheat flour and bran, both were digested at 37ºC in (1) protease and lipase, (2) amylase, pepsin, cellulase-protease-lipase and (3) human ventricle and duodenal juice. The Se-concentration in the extracts did not differ much, showing that the bioaccessibility of Se in the flour is higher than in the wheat bran. These results show that it is possible to increase the Se-fertiliser efficiency and thus the bioaccessibility of Se in wheat by adjusting the agricultural practise to fertilise with selenate at heading and using a split N-fertiliser strategy. The study also shows that the bioaccessibility of Se in wheat is higher from the flour than the bran, but because of the higher Se concentration in the bran it seems like the nutritional Se-efficiency is the same from these two unprocessed Se-enriched wheat fractions.

IRON BIO-FORTIFICATION MORE THAN SIX-FOLD IN THE ENDOSPERM OF GENETICALLY ENGINEERED RICE BY SYNERGISTIC ACTION OF TWO TRANSGENES

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About a third of the world population, largely women and children, suffer from iron malnutrition and its consequences like, *e.g.*, anemia or distortions of the intellectual development. Iron fortification of food is difficult, since the soluble forms add an awful taste or are unstable and the non-soluble forms can hardly be taken up. Bio-fortification by genetic engineering is therefore a promising approach worth to be explored. We used rice as a model plant, because rice feeds about half of the world population, among them most of the iron undernourished people. Up to now, in genetic engineering approaches, single genes have been used to increase the iron content in plants including rice but the success was limited and reached in the best case a maximum of 3.7 fold in the polished endosperm of rice, the part of the grain which is eaten. We used in our approach two transgenes: (1) a constitutive promoter driven nicotianamine synthase which increases the amount of nicotinanamine, a chelator which should facilitate transport of iron in the plant and (2) the gene for the iron storage protein ferritin under the control of the glutamin promoter, which is particularly expressed in the endosperm of the seeds. Over-expressing simultaneously two proteins involved in iron homeostasis, we have been able to increase the iron content of rice endosperm up to 6.3-fold. We conclude that these two transgenes genes provide a synergistic effect for the iron biofortification of rice endosperm. As shown by laser ablation inductively coupled plasma mass spectrometry, the iron in the endosperm of the genetically engineered plants is not equally distributed, but accumulates in hot spots. We are confident that the iron content of rice endosperm can be further increased by accumulating more genes involved in iron homeostasis and by the use of streamlined promoters.

ZINC BIOFORTIFICATION OF CEREAL GRAINS THROUGH APPLYING ZINC FERTILIZERS

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Zinc (Zn) deficiency is a common micronutrient deficiency affecting both crop production and human health, globally. In recent years, Zn deficiency has received increasing attention in public health, and appears to be, together with vitamin A deficiency, the most serious micronutrient deficiency, globally. It is estimated that increases in food prices will result in a significant decline in daily intake of Zn and other micronutrients and consequently in a worse micronutrient malnutrition problem. Monotonous consumption of cereal-based foods with very low levels and bioavailability of Zn seems to be the major reason of Zn deficiency. Nearly 50% of the cereal-cultivated soils, globally, are low in plant available Zn. Cereals are inherently very low in Zn concentrations, and growing them on potentially Zn deficient soils would further lower the grain Zn concentration. It is, therefore, not surprising that in most cases, the regions with Zn-deficient soils are also the regions where Zn deficiency is increasingly observed in human-beings. Generally, the grain Zn concentration in wheat grown on Zn-adequate soils ranges between 20 and 30 mg/Kg, whereas it ranges between 8 and 15 mg/Kg for wheat grown on Zn-deficient soils. Use of Zn fertilizers or Zn-enriched NPK fertilizers (*e.g.*, agronomic biofortification) offers a rapid solution to Zn deficiency problem. Increasing evidence is available showing that applying Zn fertilizers to soil and/or foliage is highly effective in improving grain Zn concentration up to 3- to 4-fold. A combination of soil and foliar application is particularly effective to maximize root uptake and grain accumulation of Zn. Foliar application of Zn fertilizers increases also the endosperm Zn concentration, that is the most commonly-eaten part of grain. Application of Zn-containing fertilizers represents a strong potential to increase Zn concentrations of cereal grains and to alleviate Zn deficiency-related problems in human beings. Use of micronutrient-enriched fertilizers is now an increasing practice, already implemented in Turkey for Zn and in many Scandinavian countries for selenium. Application of Zn-enriched fertilizers can be easily implemented and practiced also in the other many target countries to contribute to public health.

PHYTOAVAILABILITY OF Cd FROM REPEATED ANNUAL APPLICATIONS OF PHOSPHORUS FERTILIZER

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Field studies have been conducted since 2002 at seven locations across the Canadian prairies to evaluate the cumulative impact of application of increasing rates of monoammonium phosphate fertilizer varying in cadmium (Cd) concentration on seed Cd concentration of durum wheat (*Triticum turgidum* L.) and flax (*Linum usitatissimum* L.). Cadmium concentration was higher in durum wheat than flax and varied with location and season, indicating a strong effect of soil type and environment on Cd phytoavailaiblity. Cadmium concentration in the seed of both crops increased with application of P fertilizer even when the fertilizer contained only trace concentrations of Cd, indicating that P fertilization directly influenced Cd concentration of crops apart from the effect of Cd addition. Seed Cd concentration was higher when the fertilizer contained greater Cd concentrations, particularly when rate of fertilizer application was also high. The impact of Cd concentration in the fertilizer became more apparent with repeated applications over time. Cadmium concentration in crops was directly proportional to the total amount of Cd applied over time, but the effect of fertilizer application varied with soil characteristics. After six years of application of 80 Kg P/ha as monoammonium phosphate containing 210 mg Cd/Kg, Cd concentration in flaxseed was from 40% to 230% higher than that of the unfertilized control, depending on the location. Highest availability of Cd added in P fertilizer was on a light-textured, slightly acid soil, while availability of applied Cd was lower on heavier-textured, higher pH soils. Therefore, soil characteristics that affect phytoavailability must be taken account when assessing the risk of transfer of Cd into the food chain from P fertilization. Risk of Cd accumulation in crops and soils can be reduced by reducing the Cd concentration of fertilizer and by improving P use efficiency to minimize fertilizer application.

Oral Session 3

Toxicology and risk assessment

Chairpersons

Philippe Grandjean, Erik H. Larsen

PITFALLS IN ASSESSING ADVERSE HEALTH EFFECTS OF METHYLMERCURY IN SEAFOOD

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Assessment of the imprecision of exposure biomarkers usually focuses on laboratory performance only. Unrecognized imprecision leads to underestimation of the true toxicity of the exposure. We have assessed the total imprecision of exposure biomarkers and the implications for calculation of exposure limits. Using data from a large birth cohort study, total mercury concentrations in cord blood, cord tissue, and maternal hair were used as biomarkers of prenatal methylmercury exposure. We determined their mutual correlations and their associations with the child's neurobehavioral outcome variables at age 7 years, an age where neurotoxic effects may be detectable by feasible clinical tests. We found that the exposure biomarkers correlated well with one another, but the cord blood mercury concentration showed the best associations with neurobehavioral deficits. Due to the availability of at least three exposure parameters available, factor analysis and structural equation modeling could be applied, and it then became possible to determine the total imprecision of each biomarker. The estimated imprecision could then be applied to adjust benchmark dose calculations and the derived exposure limits. We found that the total imprecision of the cord-blood parameter was about 25-30%, and almost twice as much for maternal hair. These biomarker imprecisions much exceed normal laboratory variability (which in this case had a coefficient of variation of about 5%). Such imprecision causes an underestimation of dose-related toxicity and therefore must be considered in the data analysis and when deriving exposure limits, which will otherwise be inflated. Adjusted calculations resulted in an exposure limit 50% below the level recommended by the U.S. National Research Council. Future studies should ideally include at least three exposure parameters to allow independent assessment of total imprecision. In the absence of such information, sensitivity analyses should incorporate a realistic imprecision to ascertain its likely impact on the dose-effect relationship.

GENDER DIFFERENCES IN SUSCEPTIBILITY TO CADMIUM IN FOOD

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There is increasing evidence that cadmium-related health effects are more common among women than among men. Probably, this reflects both the observed higher body burden of cadmium in women compared to men, and differences in sensitivity to toxic effects. Besides smoking, the diet, especially fiber rich foods like cereals, vegetables and shellfish, is the main source of environmental cadmium exposure, which implies that people are exposed all the life. In fertile age, women tend to have increased intestinal absorption of cadmium, related to nutritional status of essential divalent metals. Since long, renal tubular toxicity has been considered the critical effect of cadmium. Previous health risk assessment was mainly based on data for occupationally exposed men. Obviously, risk assessment up-dates for cadmium exposure via food should include risks for all population groups. Recently, effects on both tubular function and glomerular filtration rate were observed in women at lower exposure levels than previously shown to affect men. In addition, several studies have indicated adverse effects on bone at low-level cadmium environmental exposure (mainly food), especially in women. This is in line with the classical cadmium effect, the Itai-itai disease with multiple fractures, which affected almost exclusively elderly, malnourished, multiparous women, exposed via contaminated rice in Japan. Support for bone effects of cadmium from experimental studies includes effects on calciotropic hormones and calcium metabolism, more so in female than male rats. The increasing experimental-based evidence of endocrine effects of cadmium, especially estrogen, implies additional important mechanisms of gender-specific cadmium toxicity. Probably, such mechanisms are involved in the recently observed associations between cadmium and hormone-related breast and endometrial cancer; the latter concerning exposure mainly from cereals and vegetables. Prenatal exposure main be of particular concern, as indicated in experimental studies. Cadmium was also shown to exert androgen-like activity, resulting in effects on the prostate gland, including cancer.

ARSENIC SPECIES IN SEAFOOD: ORIGIN AND HUMAN HEALTH IMPLICATIONS

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The presence of arsenic in marine samples was first reported over 100 years ago, and shortly thereafter it was shown that common seafood such as fish, crustaceans, and molluscs contained arsenic at exceedingly high concentrations. It was noted at the time that this seafood arsenic was probably present as an organically-bound species because the concentrations were so high that if the arsenic had been present as inorganic species it would certainly have been toxic to the humans consuming these seafood. Investigations in the late 1970s identified the major form of seafood arsenic as arsenobetaine [$(CH_3)_3As^+CH_2COO^-$], a harmless organoarsenic compound which, following ingestion by humans, is rapidly excreted in the urine. Since that work, however, over 50 additional organoarsenic species have been identified in marine organisms, including many important food products. For most of these arsenic compounds, the human toxicology remains unknown. The current status of arsenic in seafood will be reviewed, in particular, the possible origin of these compounds will be presented and the implications of their presence in our foods will be discussed.

APPLICATIONS FOR THE FOOD INDUSTRY AND TOXICOLOGICAL ISSUES SURROUNDING NANOTECHNOLOGY

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With the rapid development of nanotechnology, the presence of Nanoparticles (NPs) in commercially available products is becoming more and more common. The field of food nanotechnology has experienced significant growth over the last five years. Food security, packaging materials, disease treatment, delivery systems, bioavailability, new tools for molecular and cellular biology and new materials for pathogen detection are examples of the important items that are linked with nanotechnology within the food production chain. Among the manufactured nanomaterials already contained by products, nanosilver is the most materials used and occupies almost 45% followed by carbon nanomaterials, zinc oxide, titanium oxide and gold nanoparticles (http://www.nanotechproject.org). A number of products use nanosilver in medicine and water purification. Mostly nanosilver is used in processing/storage materials for its anti-bacterial properties. TiO_2 nanoparticles are widely used as food and cosmetics additives. There is increasing attention on have emphasized the potential impacts of nanomaterials on human and the environment of nanomaterials in association with the increasing possibility of exposure. Exposure is likely highest for workers, although product specific evaluations should consider consumer uses, disposal. All of these activities will bring on the environmental release and fate for products containing nanomaterials should be considered. The disposal of products may bring the release of nanomaterials to ecological, food and agricultural systems, in particular the hard degradable materials. In this review, we will focus on the following topics arising around related to nanotechnology in food industry:

- a review of both natural and anthropogenic element NPs;
- an overview of physicochemical characteristics of NPs highlighted as determinants of biological activity during *in vivo/in vitro* experiments;
- entry trough the gastrointestinal tract and target tissues and other different routes for uptake of engineered nanoparticles;
- concepts and progress of present nanotoxicology, including the need for targeted *in vitro* and *in vivo* studies.

ORGANIC FORMS OF TRACE ELEMENTS AS FEED ADDITIVES: ASSESSMENT OF RISKS AND BENEFITS FOR FARM ANIMALS AND CONSUMERS

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Adequate intakes of essential trace elements are required for health and welfare of farm animals, especially under intensive husbandry conditions. Accordingly, many trace elements (Cu, I, Mn, Se, Zn, etc.) are used as nutritional feed additives to counteract or prevent subclinical deficiencies. Some other non-essential trace elements (*e.g.*, As, Cr) have been proposed to support and improve farm animal production. Since 2003, in Europe the risk assessment of feed additives is carried out by the FEEDAP Panel within the European Food Safety Authority. In the last years the FEEDAP is confronted with an increasing number of organic forms of trace elements, including organic chelates, yeast-based additives, *etc*. Organic form of trace elements may present specific aspects deserving careful evaluation. Organic forms are expected to have higher bioavailability; if actually proven, this may lead to lower levels in feeds needed in order to meet farm animal requirements. Also, higher bioavailability may lead to a lower excretion into the environment, which is desirable particularly for some elements, *e.g.*, Cu. On the other hand, higher bioavailability might mean enhanced retention in edible tissues, hence, increased potential exposure of consumers. Also, more data are required to assess whether specific organic forms of trace elements might lead to a different distribution in edible tissues or products as compared to inorganic forms. Higher potential for consumer exposure might be especially relevant for essential trace elements with known potential for human toxicity (*e.g.*, Se). Therefore, risk assessment should be based on adequate retention studies in relevant farm animal species (including fish, dairy cows, laying hens). If possible, the resulting consumer exposure scenarios should: i) be based on conservative but realistic consumption figures; ii) consider age-related consumption patterns, and especially; iii) consider the intake levels of the trace element from background in foods or other sources (*e.g.*, supplements). Exposure scenarios should be compared with up-to-date tolerable upper intake levels (ULs) for both adults and children; when ULs are not available (*e.g.*, for Mn), a conservative estimate should be made whether the organic form in feeds might lead to a significant increase of background, unavoidable human exposure. Therefore, with the support of more robust database, maximum allowable contents of organic form of trace elements in feeds would be lower than those presently allowed for the inorganic forms; in its turn, this will require analytical methods able to distinguish organic forms from the trace element background in feeds.

RICE, THE MAJOR SOURCE OF INORGANIC ARSENIC: ASPECTS OF PLANT PHYSIOLOGY, ANALYTICAL CHEMISTRY AND TOXICOLOGY

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Rice is the major global staple food but may contain elevated level of arsenic, mainly in the form of inorganic arsenic and Dimethylarsinic Acid (DMA). The arsenic concentration can vary between 0.1 and 1 mg/Kg. Arsenic is taken up actively both as arsenate and arsenite. While arsenate uptake from soil into the rice plant is inhibited by the presence of sufficient amounts of phosphate in the soil, arsenite is not inhibited due to the uptake through aquaporin channels. Since rice grows in paddy soils, which is characterised by a reducing environment, and arsenite is the major arsenic species in the pore water, rice can take up large amounts of arsenic, while barley and wheat do not accumulate arsenic to this extent. The arsenic concentration and also speciation shows regional differences. Indian and Bangladesh rice growing in the wet season show low arsenic concentration, but that growing during the dry period contains enormous amounts of arsenic, when arsenic containing water is used for irrigation. On the other hand, US rice growing in the Central South areas contains large amounts of arsenic due to the traditional use of arsenic containing herbicides. Taking the rice consumption and the inorganic arsenic concentration of rice into account the excess cancer risk of eating rice will be discussed for different countries. Finally, it will be discussed why no Monomethylarsonic Acid (MMA) but only DMA and inorganic arsenic was found in the grain using hyphenated mass spectrometry and phytochelatin arsenic complexation and where arsenic is accumulating in grain on the basis of the results of investigations carried out by means of micro-XANES and laser ablation ICP-MS mapping.

METABOLISM OF METAL(LOID)S BY INTESTINAL MICROORGANISMS

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Methylation and hydrogenation of metal(loid)s by microorganisms are widespread and well-known processes in the environment, by which mobility and in most cases toxicity are significantly enhanced in comparison to inorganic species. Though the human gut contains a highly diverse and active microbiocenosis, little is known about the occurrence and importance of this process in the human intestine. Therefore, both *in vivo* and *in vitro* studies were conducted to elucidate the metabolism of metal(loid)s by intestinal microorganisms. First, *in vivo* studies with human probands were conducted. Following administration of bismuth subcitrate, trimethylbismuth was detected by GC-ICP-MS in blood, exhaled air as well as fecal matter. Both the relative distribution of trimethylbismuth as well as the kinetic of the methylation process indicated that the methylation predominantly occurs in the human intestine. In order to compare the capability of intestinal microorganisms towards volatilization of different metal(oid)s (Ge, As, Sn, Sb, Te, Hg, Pb and Bi) as well as the nonmetal selenium, further studies were conducted using an *in vitro* gastrointestinal model, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), due to both ethical and experimental considerations. Comparative experiments using fresh fecal matter were conducted. These experiments clearly showed that intestinal microorganisms are capable to volatilize As, Se, Te, Sb and Bi from inorganic species. In dependence on the element concentration and the part of the large intestine simulated, different species were detected. In addition to methylated species of Se, Te, Sb and Bi, surprisingly the formation of the highly toxic arsine (AsH_3) was found. In addition to these compounds, a range of high-boiling arsenic and selenium species was detected. By simultaneous elemental (ICP-MS) and molecular detection (EI-MS) hyphenated to gas chromatography, these compounds were identified as mixed Se/S, As/S as well as As/Se compounds. Five of these species have not been described in environmental or human matrices before. These results suggest that the intestinal microbiota can significantly increase the mobility and toxicity of orally ingested metal(loid)s. We therefore conclude that the role of the intestinal microbial community in metal(loid) biotransformation needs to be further addressed to assess to what extent this metabolic potency may pose health hazards to the human body. Further studies are necessary to investigate the extent of this process as well as the availability of metal(loid)s from different sources for microbial transformations.

CHARACTERIZATION OF INTESTINAL ABSORPTION OF As(III), As(V), MMA(V) AND DMA(V) USING Caco-2 CELL LINE

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Water and food are the main sources of exposure to arsenic for millions of people. Chronic arsenic exposure has been associated with a higher prevalence of some types of cancer and other diseases as type II diabetes, cardio and cerebrovascular pathologies and some skin lesions. Many toxicological studies have been conducted with arsenic species in target organ cell lines; however, although the epithelial cells of the gastrointestinal tract constitute the first barrier to the absorption of toxins, studies using intestinal cells are scarce. The aim of the present work was to study absorption through the intestinal epithelium, using Caco-2 as a model system. This cell line differentiates spontaneously into a monolayer that expresses most of the characteristics of mature small-intestinal enterocytes. The most toxic arsenic species present in foods [As(V), As(III), MMA(V), DMA(V)] were assayed. The results obtained for the pentavalent species (0.3-670 μM) showed changes in transport depending on arsenic species, concentration and culture conditions. In DMEMc medium, the gradation that the transport showed after 24 h was MMA(V) (3-11%)≈As(V) (3-10%)>DMA(V) (4-6%). The apparent permeabilities (measurement of absorption rate) indicate that the methylated species have low absorption, whereas As(V) could be considered as a compound with moderate absorption. The existence of paracellular transport (between cells) was shown for all the pentavalent species, with greater significance in the methylated species than in As(V), probably since transcellular transport exists in absorption of As(V). Indeed, absorption of As(V) is inhibited by 10 mM phosphate, and therefore a phosphate transporter may take part in intestinal absorption of this species. Acidification of the medium produces a notable increase in permeability of As(V) and DMA(V) (4-8 times). This makes it necessary to consider the possible existence of absorption in the proximal intestine and even in the stomach, where the environment is more acid, or else the participation of an H^+-dependent transporter. The trivalent species, As(III), has a very different behaviour. Its transport through the epithelium is greater (29%) at 4 hours. The apparent permeability indicates that it is a high-absorption compound and its transport may be mainly transcellular. The mechanisms involved in its absorption were studied by using inhibitors of the various transporters present in the small intestine (Glut, SLG1, OATPs, AQPs). The results obtained are the basis for future research on the mechanisms involved in the entry of As and its species to the organism, a decisive step for the exercise of this element's toxic action in the various target organs.

IGF-I AND PROGESTERONE RELEASE BY PORCINE OVARIAN GRANULOSA CELLS AFTER *IN VITRO* LEAD ADMINISTRATION

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The aim of the experiment was to examine possible effect of lead (Pb) administration on porcine ovarian granulosa cells in relation to insulin-like growth factor I (IGF-I) and progesterone release. Follicular fluid was aspirated from 3-5 mm follicles of gilts. Granulosa cells were isolated by centrifugation for 10 min at 200xg followed by washing in sterile DMEM/F12 1:1 medium and resuspension in the same medium supplemented with 10% fetal calf serum and 1% antibotic-antimycotic solution at a final concentration of 106 cells/mL of medium. Ovarian granulosa cells were incubated with same supplements and with/without acetate lead administration (18 hours) as follows: group Max (0.5 mg Pb/mL), group A (1:1 dilution), group B (1:5 dilution), group C (1:7 dilution), group D (1:10 dilution) and control group without Pb addition. Release of IGF-I and progesterone by cultured granulosa cells into the culture medium was assessed by RIA. Similar release of IGF-I was found in case of groups Max (32.42±7.00 ng/mL) and A (32.36±6.60 ng/mL) with the highest Pb administration in comparison with control (32.14±3.79 ng/mL). Granulosa cells in other experimental groups (B, C, D) released lower concentrations of IGF-I than in control, and groups C (23.92±3.13 ng/mL) and D (23.36±2.28 ng/mL) significant decreases in comparison with control were found. No significant differences between groups in release of steroid hormone progesterone by granulosa cells were found. The highest amount of the progesterone was released by ovarian cells of group Max (5.56±1.65 ng/mL) with the highest lead administration. Ovarian cells of groups A (3.88±0.81 ng/mL), B (3.82±1.12 ng/mL), C (3.58±0.29 ng/mL), D (3.68±0.58 ng/mL) released similar concentrations of progesterone in comparison with control (4.04±1.35 ng/mL). The present data obtained from *in vitro* studies indicate that different doses of lead differently affect the release of growth factor IGF-I and progesterone by porcine granulosa cells. The lowest lead concentration used in this study significantly decreased release of IGF-I by ovarian cells of gilts. On the contrary particularly higher concentrations of lead with reference to the present study may stimulate the release of progesterone by porcine ovarian granulosa cells. These observations suggest possible involvement of heavy metals on release of hormonal substances by porcine ovarian cells.

Oral Session 4

Trace elements in nutrition and human health

Chairpersons

Marina Patriarca, Munehiro Yoshida

ALTERNATIVE PATHWAYS FOR IRON ABSORPTION FROM FOODS

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Traditionally, iron has been believed to be absorbed from foods in two major forms, heme iron and non-heme iron. Iron status as well as dietary factors known to affect iron absorption has little effect on heme iron absorption, whereas inhibitors of iron absorption like phytate, tannins and calcium and enhancers such as ascorbic acid have pronounced effects on absorption of non-heme iron. The enterocyte transporter responsible for uptake of non-heme iron, divalent metal transporter 1 or DMT1, has been well characterized and its expression is strongly up-regulated during iron deficiency and down-regulated during iron overload. A transporter for heme iron, heme carrier protein 1 or HCP1, was recently characterized and it is, like DMT1, present on the apical membrane of enterocytes. Iron is subsequently released intracellularly from heme by the action of heme oxygenase. Two other pathways for iron absorption have also been discovered, and these may serve to facilitate the uptake of iron from two unique iron-binding proteins, lactoferrin and ferritin. Lactoferrin is the major iron-binding protein in human milk and has been shown to survive digestion in the gastrointestinal tract. It is likely to mediate uptake of iron in breast-fed infants through endocytosis via a specific lactoferrin receptor. Recently, lactoferrin has also become popular as a food additive and it may therefore enhance iron status in several age groups. Ferritin is present in meat, but also in low concentrations in a wide variety of plants, such as legumes, and beans. It is possible to enhance the ferritin content of plants by conventional breeding or genetic engineering, and thereby increase the iron intake of populations consuming predominantly plant-based diets. Ferritin may not be completely digested in the upper gastrointestinal tract and can serve as a bioavailable source of iron, as shown in recent human radioisotope studies. Dietary factors do not affect cell-mediated uptake of iron bound to ferritin, and recent studies show that ferritin can be taken up by intestinal cells via an endocytosis pathway, suggesting a receptor-mediated mechanism. In conclusion, iron may be taken up from foods by several different mechanisms, each being affected by various conditions.

SELENIUM BIOMARKERS AND IMMUNE FUNCTION: RESULTS OF A HUMAN INTERVENTION STUDY

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The results of a 12 week dietary intervention undertaken at the Institute of Food Research, Norwich, in 6 groups (n=20) of older adult men and women (50-64 y) who habitually consumed low intakes of Se, as indicated from their plasma Se concentration, will be presented. Using a double-blind design, volunteers in 4 of the groups took capsules containing supplements of 50, 100 or 200 µg Se (Se-yeast) or placebo (control group) every day, and the other two groups (again double-blind) consumed meals containing normal onions (<1 µg Se/onion) or Se-enriched onions (60 µg Se/onion) grown for the study by the University of Nottingham. Each volunteer in the two onion groups consumed 3 meals per week, which provided the equivalent of 50 µg Se/d (Se-enriched onions) or <1 µg Se/d (normal onions). Blood samples were taken periodically to measure the expression of Se-responsive genes (RT-PCR) and selenoproteins, enzyme activities, and plasma Se concentration. Changes in these biomarkers in the different groups were monitored and compared over the 12 week period. An influenza vaccine was given at week 10 and cellular and humoral immune responses were measured over the following 2 weeks. There were significant effects that related to both the dose and form of Se taken by the volunteers.

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USING STABLE ISOTOPES TO STUDY TRACE MINERAL METABOLISM IN CHILDREN

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Mineral requirements in young children are poorly understood. The current recommendations from the Institute of Medicine rely heavily on data collected in older children, and even in adults. Our objectives were to assess the relationship between mineral (Ca, Mg, Fe, Zn and Cu) intake in young US children, and mineral absorption, excretion and retention; and to use these data to re-examine the most recent EARs (Estimated Average Intake). Thirty children, 1-4 years old, were studied on their usual diet. After 7 days of home adaptation they were admitted for either a 2 days or 5 days metabolic study where mineral absorption (2-d study), or absorption and excretion (5-d study) were assessed using stable isotopes. Multiple Ca, Mg, Fe, Zn and Cu stable isotopes were given orally and intravenously, and mineral absorption, excretion and balance were measured from urine, blood and fecal collections. Fractional (%) of Ca and Mg were inversely related to dietary intake, while % absorption of Fe and Zn were unaffected by intake. Absolute (total) absorption and net retention of Ca, Mg, Fe, Zn and Cu increased as their intake increased. The main sites of regulation of mineral homeostasis in response to differences in mineral intake were changes in fractional absorption (Ca and Mg), fecal excretion (Zn), or whole body mineral status (Fe). Few mineral-mineral interactions were identified, although higher Zn intakes appeared to reduce Fe absorption. Our results support the current EARs for 1-4 y old children for Mg and Fe, but suggest that the EARs for Ca and Zn are too low. We conclude that direct measurements of mineral absorption and homeostasis in young children are possible using stable isotopes; fractional absorption is the main site of mineral homeostasis for some minerals (Ca, Mg) and fecal excretion is the main regulated site for others (Zn); and that the current EARs for Ca and Zn may be too low for 1-4 y old children.

ZINC DEFICIENCY IN INFANTS AND YOUNG CHILDREN: CURRENT PERSPECTIVES ON SUSTAINABLE SOLUTIONS

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On the basis of numerous large scale intervention studies conducted in developing countries, zinc deficiency is now recognized to be very common in older infants and young children. Deficiency of this micronutrient has been proposed to be one of the leading preventable causes of childhood morbidity and mortality. In developed countries, for those infants breastfed for the first year of life, as is recommended, complementary feeding practices are frequently not consistent with micronutrient needs. The result is a surprisingly high rate of zinc and iron deficiencies, even in resource-rich settings. Salient aspects of zinc supplementation RCT include general lack of response in infants less than 6 months of age; strong positive effects of supplements for low birth weight infants; variable effects on linear growth; and quite consistent positive effects on morbidity from diarrhea and pneumonia. Discussion will briefly address plausible explanations for these observations. Why is zinc deficiency so common in young children? In the simplest of terms, deficiency results when dietary intake is marginal relative to needs. A combination of relatively rapid growth, and therefore high physiologic requirements, and low intake from human milk and from traditional complementary foods provide a perfect context for development of zinc deficiency. Data from zinc homeostasis studies have now also convincingly demonstrated that there is limited ability of the host to adapt to low intake, with the major adaptation apparently a reduction in losses. Strategies to treat and prevent zinc deficiency in infants and young children range from large scale supplementation programs to dietary diversification, including cellular animal protein, through education initiatives. Centralized fortification of local food staples and home micronutrient fortification strategies are also advocated. Although the most feasible strategies may vary among settings, common principles can be applied, with holistic solutions having at least theoretical appeal over more reductionist approaches.

USE OF ENRICHED STABLE ISOTOPES FOR QUANTITATIVE SPECIATION OF ENDOGENOUS AND EXOGENOUS Se OR Fe IN RATS BY IPD-HPLC-ICP-MS

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Adequate mineral intake is crucial for human health and well being. The dietary mineral availability and absorption of the mineral elements has been determined using stable isotopes of the studied element. Although the most frequent application of stable isotopes was to perform absorption studies, the versatility of stable isotope tracer has increased to include measurement of endogenous excretion, kinetic measures of mineral turnover and body pools, resulting from compartmental modelling. Stable isotopes of mineral elements in connection with ICP-MS detection offer today increasingly employed approach in studies of nutrition and metabolism. On the other hand, measuring isotope ratios by ICP-MS and using appropriate mathematical calculations, based on the isotope dilution methodology, may also provide quantitative data about endogenous and exogenous essential or toxic elements and their metabolism. Isotope Pattern Deconvolution (IPD) has proved to be an important tool in such studies and has recently been applied in depth to total elemental analysis of endogenous and exogenous Se. In this work we explore the application of IPD to the quantitative speciation of endogenous and supplemented Fe and Se in rats, using a collision cell ICP-MS coupled to an HPLC separation. Such set-up in combination of two different enriched isotopes (Fe or Se)-a "metabolic" tracer and a post-column "quantisation" tracer-allows the accurate quantitation of natural and exogenous selenospecies and Fe species in the samples. The potential of these new analytical approaches for mineral metabolism studies will be discussed.

BIOACCESSIBILITY OF IRON, CALCIUM AND ZINC FROM BREAD AND BAKERY PRODUCTS DETERMINED BY TWO *IN VITRO* METHODS

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Epidemiological studies have identified correlations between habitual consumption of whole grain cereals and reductions in the prevalence of certain diseases. Fibre-rich bread and cereals are recommended as part of a healthy diet since they are valuable sources of dietary fibre, vitamins and trace elements. However, the presence of phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate), a common constituent of most cereals, could partly limit their benefits because it decreases the mineral bioavailability due to its chelating properties. The dephosphorylation of phytate to the lower phosphate esters can be achieved by fermentation during dough making. The aim of this study was to investigate the effect of breadmaking on iron, calcium and zinc bioavailability by two *in vitro* methods: measurement of the mineral fraction that dialyses across a semipermeable membrane and uptake and transport (intracellular retention + basolateral transport) by Caco-2 human intestinal cells. Breadmaking increased mineral dialysability of iron, calcium and zinc, whereas iron transport by Caco-2 cells did not show a significant increase from bread and bakery products as compared to the raw cereals. The fractional apical-to-basolateral calcium and zinc transport was significantly higher ($p<0.05$) for bread (51.6-71.9% for calcium and 53.1-94.7% for zinc) and other bakery products (88.3-158.6% for calcium and 88-132.8% for zinc) as compared to the corresponding raw flours (18.9% for calcium and 86.2% for zinc). Despite dialysability and total cell uptake and transport did not show a parallel trend, our results demonstrate that baking and fermentation during dough making is beneficial for mineral bioavailability.

ZINC, METALLOTHIONEINS AND LONGEVITY: INTERRELATIONSHIPS WITH NIACIN AND SELENIUM

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Ageing is an inevitable biological process with gradual and spontaneous biochemical and physiological changes and increased susceptibility to diseases. Some nutritional factors (zinc, niacin, selenium) may remodel these changes leading to a possible escaping from diseases with subsequent healthy ageing, because they are involved in improving immune functions, metabolic harmony and antioxidant defence. Experiments performed *in vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old mice or young mice fed with low zinc dietary intake) showed that zinc is important for immune efficiency (innate and adaptive), metabolic harmony (energy utilization and hormones turnover) and antioxidant activity (SOD enzyme). Of interest, zinc also lowers the chronic inflammatory status but at the same time increases the acute inflammatory immune response against external insults. Niacin confers NAD^+ substrate for the activity of DNA repair enzyme PARP-1 and, consequently, is important in maintaining genomic stability. Selenium provokes zinc release by Metallothioneins (MT), via reduction of glutathione peroxidase. This fact is crucial in ageing because high MT levels may implicate reduced capacity to release zinc ions with subsequent low intracellular free zinc ion availability for immune efficiency, metabolic harmony and antioxidant activity. Taking into account the existence of zinc transporters (ZnT and ZIP family) for cellular zinc efflux and influx, respectively, the association between zinc transporters and MT is crucial in maintaining satisfactory intracellular zinc homeostasis in ageing. Improved immune performance, metabolic harmony, antioxidant defence occur in elderly after physiological zinc supplementation, which also induces prolonged survival in old, nude and neonatal thymectomized mice. The association "zinc plus selenium" improves humoral immunity in old subjects after influenza vaccination. The study of "zinc plus niacin" in elderly is actually in progress.

CDC BIOMONITORING OF IODINE IN THE US POPULATION

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Biomonitoring the nutritional status of trace elements is an essential part of preventing disease. Iodine is one such trace element and is involved in the metabolism of thyroid hormones, important for development and normal physiological function. Iodized salt and seafood are the major dietary sources of iodine. The United States iodizes salt with potassium iodide, this iodized salt is used by about 50-60 percent of the US population. In the United States, iodine deficiency was eliminated in the twentieth century after the introduction of iodized salt and the inclusion of iodine in other foods. Urinary Iodine (UI) concentrations directly reflect dietary iodine intake because over 90 percent of dietary iodine appears in the urine, and consequently is recommended for assessing iodine status worldwide. Urinary iodine excretion is often expressed as a concentration (µg/L) or in relationship to creatinine excretion (µg iodine/g creatinine). In populations with adequate general nutrition, urinary iodine concentration correlates well with the urine iodine/creatinine ratio. The World Health Organization cut-off points for urinary iodine levels used to define nutritional iodine sufficiency/deficiency are: excessive iodine intake >300 µg/L; more than adequate intake 200-299 µg/L; optimal intake 100-199 µg/L; mild iodine deficiency 50-99 µg/L; moderate iodine deficiency 20-49 µg/L; and severe iodine deficiency <20 µg/L. Since 1971, National Health and Nutrition's Examination Survey (NHANES) has measured urinary iodine. The NHANES III survey (1988-1994) showed a sizable decrease in urinary iodine concentrations compared with concentrations measured during NHANES I (1971-1974). This decline may have been due to the dairy industry's effort in the mid-1980s to reduce the iodine residue in milk from feed supplements and iodophor sanitizing agents. Ever since 2000, CDC (Centers for Disease Control and Prevention) began using a new method (Inductively Coupled Plasma Mass Spectrometry) to determine urinary iodine levels. Using the new method, a 2000, 2001-2002, and 2004 NHANES surveys indicate that the US median urinary iodine concentrations have stabilized since the initial drop that had occurred from NHANES I to NHANES III. This funding confirms the stability of the US iodine intake and continued adequate iodine nutrition for the country generally. The contribution of dietary intake to the UI level, however, also supports the continued need to monitor the iodine status in the population because changes in patterns of dietary habit and iodine content in the food supply may negatively affect populations at risk for iodine deficiency disorders.

Poster Session 1

Advances in trace elements analysis in food matrices

W01 AFFF-MALS-ICP-MS AND ELECTRON MICROSCOPY FOR THE CHARACTERIZATION OF NANOPARTICLES IN BIOLOGICAL STUDIES

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A well defined characterization of Engineered Nanoparticles (ENPs) is essential for studying the influence of nanoparticles on biological systems *in vitro* and *in vivo*. For the determination of all relevant parameters, *e.g.* aggregation state, elemental composition, shape, size and size distribution, solubility, surface charge, a combination of different detection methods is required. One important precondition for long-term nanotoxicity experiments is the stability of the stock solutions of ENPs. Both, agglomeration and dissolution of the ENPs can produce misleading results. We studied the stability of silver (uncoated as well as dextran-coated) and selenium nanoparticles in stock solutions of different concentrations over a time period of several weeks. The size distribution, the state of dispersion, and the zeta potential, were determined by Asymmetric Field Flow Fractionation (AFFF) with Multi Angle Light Scattering (MALS) coupled to Inductively Coupled Plasma Mass Spectrometry (ICP-MS), or with Electron Microscopy (EM). Zeta potential measurements were carried out using Laser Doppler Electrophoresis (LDE). Because ENPs have a tendency to adsorb to surfaces the recovery of the ENPs following AFFF separation was evaluated. Furthermore, the soluble fraction of the ENPs could be obtained by collection of the filtrate from the AFFF membrane. The particle characterization by AFFF-MALS-ICP-MS and EM combined the advantages of both methods and their drawbacks canceled out each other. EM is a single particle imaging method that provides information on the particle's primary structure in high resolution, but the sample preparation (drying) might lead to artefacts concerning the agglomeration state. In contrast, AFFF is a particle population method where the disturbance of the sample is kept to a minimum by directly analyzing the particle suspension.

W02 THE EVALUATION OF CRUCIAL PARAMETERS IN Se SPECIATION ANALYSIS IN BIOLOGICAL SAMPLES

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Since food is a major source of Se for the general population, the content of Se in different food sources, both natural and Se-enriched, are of particular interest. The growing awareness of the strong dependence of the anti-oxidative function of Se upon its chemical forms has lead to an increasing interest in the determination of specific Se species. Se speciation has therefore become an important topic of present-day analytical research. Therefore, in this presentation several crucial steps, concerning extraction and stability of Se species, are discussed. Before chromatographic separation of Se species, the analytes must be extracted from the food sample. The most frequently used preparation method for samples of different origin is enzymatic hydrolysis. For these purpose several commercially available, non-specific and specific, enzymes can be used. On the other hand, no data on the Se content of such enzymes exist. Regarding this, the purity of commercially available enzymes is reported. Moreover, mass balance calculations are often eliminated from speciation analysis. Most authors consider only the Se species in the soluble part after enzymatic extraction and pay no attention to the residue. Here, mass balance was investigated on a real sample basis (plant samples). Further, to assure qualitative and quantitative results, Se standards (Se-methionine, Se-cystine, Se-cysteine, selenite, selenate) should not be prepared in water but in the extracts under analysis to assure comparable signal response and retention times. Therefore, the importance of the stability of Se species in the matrix under analysis was assessed. The critical parameters affecting Se speciation analysis of plant samples were identified and will be discussed.

W03 CHARACTERIZATION OF COMMERCIALLY AVAILABLE SELENIZED YEAST FOOD SUPPLEMENTS

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The increased interest in selenium (Se)-rich food products and the establishment of selenized-yeast as a valid and efficient low-cost source of selenium for supplementation raise concerns about the quality, safety and origin of preparations available on the market. The commercial products seem to vary considerably with respect not only to selenium concentration but also in terms of its speciation. Selenomethionine is the most frequently evoked species and its content is often considered as a parameter confirming the "organic character" of a yeast. However, it accounts rarely for more than 60% of total Se, other selenium chemical forms remaining usually unknown. Analytical methodology is required for the characterization of selenized yeast in terms of the total selenium, selenomethionine concentration, and the presence of unique selenium-containing metabolites which can serve as a fingerprint of the yeast origin. In this work, comparison of eight commercially available selenized yeast samples coming from the leading producers located in Europe, the US, Canada, Brazil, Mexico and China is presented. The samples were characterized in terms of total Se and selenomethionine concentration, as well as content of water soluble selenium fraction and its molecular weight distribution [assessed by size-exclusion LC-ICP-MS (Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry)]. Low molecular Se-rich fractions were characterized in order to designate Se-containing markers characteristic for given Se sources. Total Se content in the investigated samples varied from ca. 600 to ca. 2,500 μg/g, corresponding to 3 groups of yeasts with a nominal Se content of 500, 1,000 and 2,000 μg/g. The selenomethionine fraction in most preparations accounted for more than 60%, with two outliers with SeMet concentration of less than 50% of total Se. Water soluble selenospecies constituted ca. 10% of the total Se with the exception of one sample, where almost 30% of Se was water soluble; it should be noted that the same preparation showed extremely low selenomethionine content. The morphology of the molecular weight patterns of the water soluble Se-rich fractions was similar with, however, two exceptions lacking some peaks characteristic for the rest of the samples studied. The identification studies by HPLC-ES MS/MS (High Performance Liquid Chromatography-Electron Spray Mass Spectrometry) were focused on the low molecular selenium metabolites. A potential marker molecule, characteristic of the selenized yeast of one of the producers and absent in all the other samples, was detected.

W04 DETERMINATION OF SELENOMETHIONINE AND SELENOCYSTEINE IN EDIBLE TISSUES OF ANIMAL ORIGIN

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Selenium is an essential element playing a vital role in many metabolic functions and showing some cancer preventive properties. The main source of Se intake is food. However, the concentration of food production in regions with low soil selenium content has led to decline in the amount of this element in human food supply which poses potential health risk. Among selenium food sources, particular attention is paid to edible animal tissues of animal origin such as meat, milk and eggs due to their nutritional importance. Strategies of Se supplementation aimed at increasing its uptake by animals were proposed resulting in higher Se content in respective foodstuffs. The interest in species selective determination of selenocompounds results from their different bioavailability to humans and potential importance for the stability and quality of the marketed products. The study presents the development of analytical methodology for speciation analysis of selenoaminoacids in meat, cow milk and chicken eggs from animals fed with selenium enriched (both inorganic, sodium selenite, and organic, Sel-Plex®, that is a selenium-enriched yeast derived from a specific strain of *Saccharomyces cerevisiae* CNCM I-3060, from Alltech, Nicholasville, KY) and control diets. The techniques developed include size-exclusion HPLC-ICP MS of sequential foodstuffs extracts to monitor the molecular weight distribution of selenium and to clean up the selenoaminoacid fraction. Reversed-phase HPLC-ICP MS was developed to quantify selenomethionine and selenocysteine after proteolytic digestion and size-exclusion cleanup of the meat, milk and egg samples. The method was applied to the discrimination of the contribution of selenoproteins (containing SeCys) and other Se-containing proteins (containing SeMet) in edible tissues of animals during supplementation studies and to follow the evolution of selenium speciation in milk during cheese-making.

W05 LA-ICP MS AND NANO HPLC-COUPLED TO MASS SPECTROMETRY FOR THE DETECTION AND IDENTIFICATION OF SELENIUM-CONTAINING PROTEINS IN SELENIUM-RICH YEAST

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The essential trace mineral selenium is of fundamental importance to human health. Selenium (Se) occurs in staple foods, such as corn, wheat, and soybean, as selenomethionine (SeMet), the organic Se analogue of the amino acid methionine. Selenomethionine is generally considered to be the best absorbed and can be incorporated non-specifically into body proteins in place of methionine and serve as an endogenous Se source during times of sub-optimal Se intake. Selenium supplements come in two principle forms, inorganic salts which include sodium selenite and selenate, or organic forms such as Se enriched yeasts. These yeasts may contain as much as 1,000 to 2,000 µg of Se/g and much of the Se in these yeasts is in the form of SeMet. This form of Se was used in a large scale cancer prevention trial in 1983 which demonstrated that taking a daily supplement containing 200 µg Se/day could lower the risk of developing prostate, lung, and colorectal cancers. However, some yeasts may contain inorganic forms of Se which are not absorbed and incorporated as well as SeMet. Despite a wealth of analytical literature on the characterization of selenium-rich yeasts, the speciation of Se within yeast is poorly understood. Only recently have studies allowed an insight into the Se-containing protein fraction. A new analytical strategy has been developed combining classical proteomic approaches with LA-ICP MS and nanoHPLC coupled to mass spectrometry, allowing the analysis of Se-containing proteins in selenium-rich yeasts. Firstly, 2D gel electrophoresis was applied to the total protein extract (~45% of the total Se content) resulting in approximately 50 protein spots. Selenium-specific analysis of the gel by laser ablation -ICP MS revealed 14 selenium containing spots which were subsequently excised and enzymatically digested. NanoHPLC-ICP MS of the tryptic digest allowed for sensitive and specific selenopeptide mapping showing the existence of selenium-containing peptides within the digests before their identification by nanoHPLC-ESI MS/MS. This novel approach allowed for a deeper insight into the identity of 17 selenium-containing proteins and revealed, for example, the existence of selenium-containing glyceraldehyde-3-phosphate dehydrogenase 3 in yeast.

W06 FRACTIONATION OF Cr, Fe AND Zn IN METAL-ENRICHED YEAST FOR BIOAVAILABILITY STUDIES

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Chromium, iron and zinc are essential nutrients. Their deficiencies in the diet are known to perturb physiological functions and to contribute to a number of diseases including, among others, impaired glucose tolerance (Cr deficiency), anemia (Fe deficiency) and diarrhea (Zn deficiency). As a result, the use of Cr-, Fe- and Zn-enriched nutritional supplements is becoming increasingly widespread. Traditionally, trace mineral supplementation has been achieved by the addition of inorganic salts, such as sulfates and chlorides. However, recent research has clearly indicated an enhancement of the element bioavailability when it is added in the form of chelates with amino acids or peptides. Yeast grown in the presence of metal salts is increasingly used for supplementation. The growing commercial availability of such yeast preparations creates a need for the development of analytical methods suitable for their characterization. As the information about total elemental concentrations is insufficient to understand the bioavailability, activity and possible toxicity of Cr, Fe and Zn, the interest in fractionation and speciation analysis of the above metals in yeast has increased significantly. The purpose of this work was to investigate the fractionation and bioavailability of Cr, Fe and Zn in different samples of commercially available metal-enriched yeasts. The extraction procedures targeted water-soluble compounds, polypeptide and polysaccharide complexes and compounds soluble in simulated gastric and gastrointestinal conditions. The extracts and digests were analyzed by ICP-MS. The results showed that only a low percentage of the elements could be recovered by extraction with water and driselase. On the contrary, significant metal amounts were liberated by simulated gastrointestinal digestion (>40% of the total amount of metal present), indicating high bioavailability of metals from the yeast-based nutritional supplements. These results provided evidence of metal-enriched yeast being an effective source of minerals, however, the bioavailability of each element largely depended on the yeast source.

W07 As SPECIATION IN FRESHWATER FISH: FOCUS ON EXTRACTION AND MASS BALANCE

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Arsenic (As) speciation in marine fish has been widely investigated over the last two decades. Several studies contributed to current understanding about the presence of As compounds in these organisms, consisting in high concentrations of total As with a predominance of nontoxic arsenobetaine. On the other hand, the limited data on freshwater fish make it difficult to define a typical pattern of As species in this case, even though evidence exists of a more variable As speciation compared to marine fish. An obstacle to a better understanding of As speciation in freshwater fish is the low mass balances generally found, related to both low extraction efficiency and column recovery. In this study, As speciation in muscle tissues of freshwater fish was investigated with special emphasis on extraction yields and overall mass balance. The species studied were eel (*Anguilla anguilla* L., Anguillidae), common grey mullet (*Mugil cephalus* L., Mugilidae), chub (*Leuciscus cephalus* L., Cyprinidae), and carp (*Cyprinus carpio* L., Cyprinidae). For the quantification of water-soluble As compounds, samples were extracted using a 1:1 (v/v) methanol/water mixture under mechanical agitation. Various extraction parameters, such as extraction volume, number of additional extractions, intermediate and final volume in evaporation and freeze-drying procedures, were optimized so as to improve the extraction efficiency. Arsenic compounds were determined by HPLC with online Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS), using both cation and anion-exchange separation. Total As concentrations were measured by means of ICP-MS. Mean total As concentrations in fish species ranged from 354 to 1804 µg/Kg dry weight. Extracted As referring to the percentage of total As ranged from 82% for grey mullet, to 86% for eel, to 90% for chub. However, only 64% of total As in carp could be extracted. Extraction of lipid-rich eel with *n*-esane recovered some additional 3% of total As. Arsenic speciation analysis showed that arsenobetaine was the dominating As compound, but several other arsenicals, including arsenous acid (AsIII, traces), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), arsenocholine ion (AC), tetramethylarsonium ion (TETRA), oxo-arsenosugar-glycerol (AS1), oxo-arsenosugar-phosphate (AS2), thio-arsenosugar-phosphate (ThioAS2), and two unknown As compounds, were found. However, the pattern of As species in carp was different, and a lower proportion of arsenobetaine along with a high contribution of AS2 and ThioAS2 were found. In order to obtain sound mass balances, the recovery of As from chromatographic columns was studied as well. Column recoveries were 116%, 113%, 99% and 100% for eel, common grey mullet, chub and carp, respectively, indicating that no As was retained during chromatography.

W08 DETERMINATION OF DEPLETED URANIUM IN FOOD SAMPLES FROM BOSNIA AND HERZEGOVINA

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Uranium is a naturally occurring, ubiquitous, weakly radioactive element. Natural uranium consists of three radioactive isotopes: the composition by mass is ^{238}U (99.27%), ^{235}U (0.72%) and ^{234}U (0.0054%). Uranium is used primarily in nuclear power plants; most reactors require uranium in which the ^{235}U content is enriched from 0.72% to about 3%. The uranium remaining after removal of the enriched fraction is referred to as depleted uranium (DU). DU typically contains about 99.8% ^{238}U, 0.2% ^{235}U and 0.0006% ^{234}U by mass. For the same mass, DU has about 60% of the radioactivity of uranium. DU may also result from the reprocessing of spent nuclear reactor fuel. Under these conditions another uranium isotope, ^{236}U, may be present in traces along with very small amounts of transuranium nuclides ($^{239+240}$Pu, ^{241}Am, and ^{237}Np) and the fission product ^{99}Tc. Due to its high density, which is about twice that of lead, and other physical properties, DU has been used in munitions designed to penetrate armour plate. Since its use in military conflicts in the Gulf and the Balkans, public concern has been raised about potential health consequences from exposure to DU. Concerns have been particularly for peacekeeping forces, humanitarian workers and local populations living and working in areas contaminated by DU following conflict. A potential exposure scenario to DU is ingestion of contaminated food. Within the context of a programme launched by the Italian Ministry of Health, aimed at determining whether the use of DU had any detrimental effect related to transfer of DU in the food chain in Bosnia and Kosovo, our laboratory developed analytical methods for the determination of DU in food. Quadrupole inductively coupled plasma mass spectrometry was used for the measurement of ^{235}U/^{238}U ratio. Signal intensity and isotope ratio precision were substantially increased by use of a high efficiency/micro-sample introduction system (APEX IR/ACM) and optimization of the acquisition parameters. Coefficients of variation $\leq 2\%$ were obtained for the majority of the 100 samples screened for possible presence of DU. In all measurements, isotopic reference materials with the composition of natural U (IRMM-184) and DU (IRMM-183) were used. Screening of ^{236}U, use of columns for preconcentration/matrix removal and of DRC reaction gases were also considered. Higher levels of total U and a lower ^{235}U/^{238}U ratio were found in analytical aliquots of a few plant samples from Bosnia. However, the other aliquots of the same samples had the isotopic composition of natural U, thus showing that when contamination occurred it was due to the deposition of DU particles and not to root uptake from soil.

W09 CDC ANALYTICAL METHODS FOR SERUM MULTIELEMENT-SELENIUM, ZINC AND COPPER-DETERMINATION

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Using advanced laboratory science and innovative techniques, the Division of Laboratory Science (DLS) at CDC has been in the forefront of efforts to assess the nutritional status of the U.S. population through monitoring nutritional indicators. CDC determines trace elements to include selenium, cooper and zinc in biological matrices, using Inductively-Coupled Plasma Dynamic Reaction Cell Mass Spectrometry (ICP-DRC-MS). The analysis of selenium is important because selenium deficiency is still a problem in many countries. Selenium deficiency in the United States, however, is rare. Deficiency of selenium is associated with severe gastrointestinal problems, such as Crohn's disease. In August 2008, DLS released the National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 1999-2002, the first CDC publication that offers reference information on a wide range of biochemical indicators in the U.S. population in a single document (www.cdc.gov/nutritionreport). These indicators include trace elements (selenium and iodine). CDC scientist measured these levels in the serum and urine of people who took part in CDC's National Health and Nutrition Examination Survey (NHANES) during all or part of the four-year period from 1999 through 2002. Other trace elements measured in our laboratory, zinc and copper, play crucial roles in maintaining regular homeostatic functions. In particular, zinc plays a central role in antioxidant mechanisms and zinc deficiency can cause detrimental oxidative stress. Our method at CDC is used to achieve rapid and accurate quantification of three elements of toxicological and nutritional interest and may be used to screen serum when people are suspected to be acutely exposed to these elements or to evaluate chronic environmental or other non-occupational exposure.

W10 CDC's EXTERNAL QUALITY ASSURANCE PROGRAM FOR TRACE ELEMENT DETERMINATIONS

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Iodine is a trace element and essential component of thyroid hormones, necessary for normal growth, development, and metabolism during gestation, infancy, and throughout life. Many people do not get all of the iodine that they need and Iodine Deficiency Disorders (IDD) are thought to affect more than a billion people worldwide. Today an estimated 70% of the world's edible salt is being iodized. Accurate laboratory tests can detect iodine deficiency. Urinary Iodine (UI) directly reflects the body's iodine status and UI analysis is the most common method used worldwide for biochemical assessment of the iodine status. In 2001, the Centers for Disease Control and Prevention (CDC), established a program, Ensuring the Quality of Urinary Iodine Procedures (EQUIP) to assist laboratories around the world assess the accuracy and precision of their urinary iodine procedures. The accuracy and precision of measurements of iodine is important not only to monitor the status of iodine nutrition of populations around the world, but it is also important for quality assurance to ensure accurate data is collected to document efforts to eliminate IDD. EQUIP urine iodine samples are prepared at CDC and assigned target values using CDC's ICP-MS methodology. 84 reference laboratories around the world use these materials to gauge the accuracy of their analysis. Most of the participating laboratories use spectrophotometric monitoring of the Sandell-Kolthoff reaction with sample digestion accomplished by using either ammonium persulfate or chloric acid. ICP-MS provides a measurement standard by which other laboratories can assess accuracy and precision when measuring UI. In the 8 years since EQUIP was established, our data shows the usefulness of the program, supporting the contention that inter-laboratory comparisons are an effective tool for laboratory performance improvement. There has been significant improvement in the quality of data from laboratories measuring iodine in different parts of the world. EQUIP will continue to be a tool available to laboratories by encouraging good laboratory practices, providing an assurance of quality data, and also assisting those who are establishing new laboratory or modernizing old ones.

W11 TOTAL Cd, Pb AND As AND EXTRACTABLE Cd AND Pb IN MINERAL FEED

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To overcome problems associated with a high metal content in feed, maximum levels for trace elements in several commodities have been laid down in Directive 2002/32/EC. The aim of the IMEP-27 Interlaboratory Comparison (ILC) is to test the competence of the routine laboratories to determine the total concentration of Cd, Pb and As and of extractable Cd and Pb according to the European legislation. The test material used in this exercise was a commercial mineral feed for pigs naturally contaminated. The reference values were provided by the Institute for Reference Materials and Measurements (IRMM) for total and extractable Cd and Pb and by the Studiecentrum voor Kernenergie (SCK-CEN) for total As. The uncertainties of the respective assigned values, u_{ref}, were calculated combining the analytical uncertainty, u_{char}, with a contribution for the between-bottle homogeneity, u_{bb}, and for the short term stability of the test material, u_{sts}. The laboratory performance was evaluated using z and zeta scores in accordance with ISO 13528. The standard deviations for proficiency assessment (also called target standard deviation) were calculated using the modified Horwitz equation and were between 15 and 16% for all the measurands. In this ILC most of the laboratories obtained satisfactory results for total and extractable Cd and for total As. For total and extractable Pb most results were negatively biased. From the information provided by the participants in the questionnaire, it was observed that most of the laboratories have optimised their method for the determination of total Cd, Pb and As using certified reference materials of organic composition. Organic matrices are easier to mineralise than matrices of mineral origin, which may easily result in an overestimation of the recovery and thus in an underestimation of the analyte concentration when the same method is applied to a mineral matrix. This would explain why results for Pb are negatively biased. The uncertainty was reported by 90% of the participants but the zeta scores obtained show that there are still some difficulties to provide a reliable estimate of the uncertainty of measurement.

W12 THE COMMUNITY REFERENCE LABORATORY FOR CHEMICAL ELEMENTS IN FOOD OF ANIMAL ORIGIN (CRL-ISS) AS PROVIDER OF PTs FOR THE NATIONAL REFERENCES LABORATORIES OF EU

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The Community Reference Laboratory for Chemical Elements in Food of Animal Origin at the Istituto Superiore di Sanità (CRL-ISS) is one of the Community Reference Laboratories for feed, food and the animal field. The function and duties of the CRL-ISS are described in Article 32 of Regulation (EC) no. 882/2004 of the European Parliament. One of the activities of the CRL-ISS is the organization of Proficiency Tests (PTs) for the National Reference Laboratories of the European Member States (NRLs), often called to solve analytical controversies regarding the determination of chemical elements at concentrations close to the "maximum permitted levels" set in the Commission Regulation (EC) 1881/2006. Thus, since 2006 the CRL-ISS has undertaken the preparation of some reference materials (RMs) for PTs, such as milk, fish and meat. The chemical elements considered in the preparation of RMs were arsenic, cadmium, mercury (fish) and lead. Milk RMs were produced both as freeze-dried and liquid materials; RMs from meat (bovine and porcine) and fish were prepared as freeze-dried powders. The preparation of the RMs from commercial foods required specific procedures according to the matrix considered. Meat, fish and milk were spiked with suitable amounts of arsenic, cadmium, and lead, and frozen-dried or sterilized in autoclave, respectively. As for mercury in fish, two different species (swordfish, hake) were mixed in order to obtain a suitable concentration of this element in the freeze-dried material from endogenous contents. Taking into account the small number of participants (n=21-27), all the factors, which can compromise robust estimates, were considered. The z-score approach was chosen for the scoring of results. The assigned value for each test material was derived from the robust mean of results (Huber H15, algorithm A). At first, a value derived either from the Horwitz equation (concentrations >120 µg/kg) or from the more stringent Thompson equation (<120 µg/kg) was adopted as standard deviation for PT assessment (σ_p); at present, in consideration of the high qualification requested to the participant laboratories, suitable lower and fit for purpose values are adopted, so as to match the requirements of the NRLs. After three years of activity (2006-2008), the general evaluation of the results is quite satisfactory. There is a high percentage of z-scores less than or equal to 2, specifically for Cd and Hg which show a percentage of values higher than 95% in the satisfactory class. As for arsenic, about 4.7% of z-score values resulted in the range $2<Z\leq3$, and only about 4% were >3; however, the frequency of these values was higher at the high concentrations usually found in fish. Thus, if we exclude the fish matrix, the percentage of satisfactory z-scores increases for arsenic up to

96%. With regard to lead, the results showed that the analysis of this element seems to be still the most difficult one. In the last year, however, the outcome of the PT improved, with a decrease from 9% to 2% in the unsatisfactory class.

W13 TRACE ELEMENT ANALYSIS OF DIETARY SUPPLEMENTS AND NUTRIENTS BY TXRF

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In the last decades, Total reflection X-Ray Fluorescence (TXRF) analysis was proven to be an easy and versatile method for the determination of trace elements. TXRF can be applied to different sample types, like solids in form of micro fragments, powders, suspensions, thin films or liquids. The required sample amount is in the low µg or µl range, respectively. In TXRF the samples are prepared as thin film or layer, thus matrix effects are negligible. Quantification is possible by means of the known concentration of an internal standard element. However, because of the large size and high operative costs, the application of this method was restricted to large research and development laboratories. But recently with the introduction of low-power benchtop instruments, this analytical method starts to get established even in small laboratories for research or routine applications. This has opened new doors in the rapidly growing field of Phytoforensic chemistry. This presentation will present a brief outline of the instrumental design and theory of TXRF followed by case studies demonstrating how the technique is utilized in every day laboratory analysis. In particular, heavy metal contamination, particle contamination and lot comparison profiling of dietary supplement products will be presented.

W14 SOLID PHASE EXTRACTION OF TRACE METALS IN FOOD SAMPLES USING RESIN FUNCTIONALIZED WITH AROMATIC DITHIOCARBAMATE AND DETERMINATION BY ICP-AES

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The direct determination of trace metals in food samples with conventional analytical techniques like AAS and ICP-AES may be difficult because of the very low concentrations of the metal ions and the complexity of the matrices of the samples to be analysed. The best way is to separate (and preconcentrate) the metal ions from the matrix constituent and then analyze the extracts using the most suitable analytical technique. In the present work the authors have developed a solid phase extraction method for the separation and preconcentration of selected trace metal ions from food samples, based on the functionalization of Amberlite XAD-2 resin with potassium benzoyl-hydrazine-N1-carbodithioate (a new aromatic dithiocarbamate), which was tested for the separation and preconcentration of Cr, Cu, Mn and Zn from leafy vegetable samples. The leafy vegetables were air dried followed by drying at 105ºC in hot air oven for 2 h and then powdered. Aliquots of each sample (1 g) were placed in a crucible and digested with 3 mL of concentrated nitric acid followed by dry ashing carried out in a muffle furnace at 600ºC for 4 h. The ashed samples were dissolved in 2 mL of HCl and diluted to 50 mL with doubly distilled water. A reagent blank was prepared following the same procedure without plant material. The samples were passed through the column loaded with the resin functionalized with potassium benzoyl-hydrazine-N1-carbodithioate. The trace metal ions that were retained by the resin by chelation were eluted with nitric acid and determined by ICP-AES. The optimum experimental conditions for the column solid-phase extraction of Cr, Cu, Mn and Zn ions have been investigated. All these metal ions were quantitatively retained by the functionalized resin at pH 5.0 and could be eluted with 2.0 M HNO_3. The detection limits, calculated on ash weight, were 6.48 µg/Kg for Cr, 8.62 µg/Kg for Cu, 7.56 µg/Kg for Mn and 2.28 µg/Kg for Zn, respectively. The developed method was applied for the determination of Pb and Zn metal ions in leafy vegetables namely Chukkaku (*Rumex vesicarius*), Palakura (*Ichnocarpus frutescens*), Thotakura (*Amaranthus tricolor*) and Cabbage (*Brassica oleracea var. capitata*) which are common in Indian diet.

W15 DETECTION OF MERCURY IN FEED AND FOOD: VALIDATION OF A TDA-AAS METHOD

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The aim of this study was to validate a method based on Thermal Decomposition Amalgamation-Atomic Absorption Spectrometry (TDA-AAS) to detect mercury in food and feed. It was applied to estimate mercury levels in complete feeding stuffs and fish. The TDA-AAS method directly expressed in µg/kg Hg concentrations in samples. No sample preparation is necessary with the direct mercury analyser; the samples are homogenized and then weighed directly in the cell instrument. Spectrophotometric measurements were performed at a wavelength of 253.7 nm. Calibration curves were obtained with Hg standard solutions at 25, 50, 100 and 150 µg/L or at 200, 300, 1,000, 2,000 and 5,000 µg/L. The validation process was performed according to the European Regulation 882/2004, considering the following parameters: linearity, specificity, Limit Of Quantitation (LOQ), repeatability and reproducibility, trueness and uncertainty of measurement. The uncertainty of measurement was estimated according to an internal Standard Operative Procedure that follows the "bottom up" approach described in ISO Guide to the Expression of uncertainty in measurement (1993), and applied to chemical analysis by EURACHEM/CITAC Guide "Quantifying uncertainty in analytical measurement" Second Edition (2000). This Guide assumes that the uncertainty evaluation requires the analyst to look closely at all relevant sources of uncertainty. The uncertainty components were: relative repeatability uncertainty-recovery, weighting relative uncertainty (standards and samples), relative uncertainty of the concentration of the standard solutions, relative volume uncertainty (pipette and flask). Every uncertainty contribution was indicated as standard uncertainty and stated as standard deviation. The relative compound uncertainty (u_c) was derived from the square root of the total variance (considering all contributions). The relative expanded uncertainty (U) was estimated by multiplying u_c by k (coverage factor), rounded up to 2 for 95% confidence level. The Limit Of Detection (LOD) was 0.030 mg/Kg; while the Limit Of Quantitation (LOQ) was 0.100 mg/Kg. The average method recovery was of 96.3%, with a relative standard deviation (RSD) of 5.1%.The relative expanded uncertainty of the method was 8.7%. This method, validated according to 882/2004 EU Regulation, is also consistent with the requirements of ISO/IEC 17025 and it is suitable for quantitative official analysis.

W16 COMPARISON OF THE PERFORMANCES OF ANALYTICAL TECHNIQUES BASED ON ATOMIC SPECTROMETRY FOR THE DETERMINATION OF TOTAL TIN FROM PPT TO PPM LEVEL IN CANNED FOODSTUFFS

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Different techniques for the determination of total tin in beverage and canned food by atomic spectrometry were compared. The performance characteristics of Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Hydride Generation Inductively Coupled Plasma Atomic Emission Spectrometry (HG-ICP-AES), Electrothermal Atomization Atomic Absorption Spectrometry (ETA-AAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) were determined in terms of linearity, precision, recovery, limit of detection, CCα and CCβ. Calibration ranges were covered from ppt to ppm level. Limits of detection ranged from 0.01; 0.05; 2.0 to 200 μg/L for ICP-MS; HG-ICP-AES; ETA-AAS and ICP-AES, respectively. Precision, calculated according to ISO 5725-2 for repeatability and expressed as Relative Standard Deviation (RSD), ranged from 1.6% to 4.9% and recovery, based on the decision 2002/657/EC, was found between 95 to 110%. Various procedures for the mineralization or extraction of total tin were evaluated. Wet digestion, sequentially, with nitric acid and hydrogen peroxide provided the best results. The influence of possible interferences present in canned food and in beverage was studied and no interference on the determination of tin was observed. Since maximum levels for tin established by the European legislation vary from 50 mg/Kg in infant food to 200 mg/Kg in canned food, ICP-AES was chosen as the preferred technique for routine analysis thanks to its good precision, reliability and easy to use. The accuracy of this routine method was confirmed by participating in 6 proficiency test schemes with z-scores ranging from -1.9 to 0.6. Several canned foodstuffs and beverage samples from local markets were analysed with this technique.

W17 MULTI-ELEMENT DETERMINATION OF Cu, Fe, Ni AND Zn CONTENT IN VEGETABLE OILS SAMPLES BY HIGH RESOLUTION CONTINUUM SOURCE ATOMIC ABSORPTION SPECTROMETRY AND MICROEMULSION SAMPLE PREPARATION

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The determination of trace metals content in vegetable oils is an important criterion for the assessment of oil quality with regard to freshness, keeping properties, storage and their influence on human nutrition and health. However, the accurate determination of metals in this kind of samples is still an analytical challenge, owing to their low concentration level and the difficulties that occur due to the characteristics of the matrix. In general, atomic spectrometric methods for metals determination in organic matrix present some disadvantages, such as the reduced stability of the analytes in the solution, the need of organometallic standards for calibration, the use of dangerous organic solvents or sample digestion with an acid or acid mixture. Alternatively, some methodologies use the modification of these organic liquid samples by formation of emulsions or microemulsions avoiding previous mineralization of the sample and making possible the use of aqueous standards for calibration. On the other hand, the applicability of Flame Atomic Absorption Spectrophotometry has been extended with the development of High-Resolution Continuum Source spectrometers. The main advantages of these equipments are the possibility of performing fast sequential multi-element determinations, measuring major and secondary atomic lines, adding absorbance of different lines for a given element, integrating the absorbance signal over the center pixel by including part of the line wings to extend the linear work range, and correcting background by means of mathematical algorithms. The aim of this work was to evaluate the microemulsification as sample preparation procedure for determination of Cu, Fe, Ni and Zn in vegetable oils samples by High-Resolution Continuum Source Flame Atomic Absorption Spectrometry (HR-CS FAAS). Microemulsions were prepared by mixing samples with n-propanol and aqueous acid solution, which allowed the use of inorganic aqueous standards for the calibration. To a sample mass of 0.5 g, 100 μL of hydrochloric acid and n-propanol were added and the resulting mixture diluted to a final volume of 10 mL. The sample was manually shaken resulting in a visually homogeneous system. The main lines were selected for all studied metals and the detection limits (n=10) were 6, 21, 39 and 6 μg/L for Cu, Fe, Ni and Zn, respectively. The Relative Standard Deviation (RSD) ranged from 5 to 11% in samples spiked with 0.25 μg/mL and 1.5 μg/mL of each metal, respectively. Recoveries varied from 85 to 110%. The proposed method was applied to the determination of Cu, Fe, Ni and Zn in soybean, olive and sunflower oils.

W18 DETERMINATION OF ARSENIC AND SILICON IN VEGETABLE OIL USING ATOMIC SPECTROMETRY METHODS

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In Brazil, a variety of vegetable oils are used in daily meals, thus being an important source of fatty acids in the Brazilian diet. More recently, vegetable oils such as soybean, sunflower, palm and almonds oils, are also being used as a primary product for biodiesel production. Used vegetal oils are also considered, due to their low cost and the advantage of waste recycling. Mineral composition influences the stability of the oil, as well as the further use of the technical products obtained. The determination of trace elements in vegetable oils is thus of importance, since their presence above certain levels can change their suitability for specific uses. The Brazilian current specification limits the maximum concentration for As and Si in foods to less than 1 mg/Kg, therefore, the development of analytical procedures for the determination of As and Si in vegetable oils is of extreme importance for the control of their quality. In the present work, procedures for the determination of As and Si in vegetable oils of different origins using atomic absorption spectrometry methods are proposed. For the determination of As, Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) was used. Samples were prepared by mixing appropriate volumes of the samples with propan-1-ol and nitric acid aqueous solution. An aliquot of 15 µL of this sample was injected directly in the graphite tube together with 5 µL of Pd (chemical modifier). Pyrolysis and atomization temperatures, as well as the sample dilution factor were investigated. The determinations were carried using external calibration with inorganic or organometallic As standards solutions for comparation. For Si, Flame Atomic Absorption Spectrometry was used: samples were diluted with xylene and the concentration of Si was determined directly using a High Resolution-Continuum Source (HR-CS FAAS) equipment. In this case, the calibration solutions were prepared in xylene with addition of base oil for viscosity matching. Since no adequate certified reference material was available, the trueness for both methods was assessed by recovery tests (90-117%). The Limits Of Detection (LOD) in the original sample under optimized conditions were about 0.3 for As and 0.7 mg/Kg for Si, which was adequate for the determination of these elements in vegetable oils according to the Brazilian legislation.

W19 MINERALS AND MACRONUTRIENTS IN FOOD PROCESS CHAIN: EXAMPLES OF METHODS USING ICP TECHNOLOGY FOR CONTROL AND LABELLING

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Minerals are essential nutrients that have various functions as supplements or additives in food chemistry. If they need to be supplied in sufficient quantities within the food during fortification, relative low controlled content is required when used as stabilisers, as preservatives or as processing agents. Macronutrients, such as calcium or magnesium, as well as trace elements, such as iron, copper, manganese, zinc, molybdenum, chromium and selenium, are important for nutritional-physiological aspects. ICP-AES and ICP-MS are the most accurate and sensitive techniques to ensure either compliance and confident nutrition labelling of fortified food products, or quality, safety and purity of additives like salts or processed foods like edible oils. A method using ICP-AES has been validated for the control of mineral impurities in raw materials like salts. Internal standardization and ion buffering are optimised to compensate for chemical and physical interferences and matrix effects. A method using ICP-MS after different acid digestions has been developed for the control of metal trace elements at the origin of oxidation during processing of edible oils. Dynamic Reaction Cell (DRC) technology is applied to avoid isobaric interferences for certain elements like chromium, iron and copper. The performance of the two methods, in terms of sensitivity, trueness, precision and specificity, will be presented and discussed.

W20 DETERMINATION OF COPPER AND NICKEL IN SEASONING SAMPLES BY ATOMIC ABSORPTION SPECTROMETRY AFTER CLOUD POINT EXTRACTION

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The determination of copper and nickel in selected foodstuffs by Flame Atomic Absorption Spectrometry (FAAS) after preconcentration using cloud point extraction is described. The method is based on the extraction of hydrophobic complexes formed by the metals and 4-(5-bromo-2-thiazolylazo) orcinol (Br-TAO). A sample mass of 0.1 g was digested, diluted to 50 mL, and mixed with 0.25 mL of Br-TAO. Dilution of the surfactant-rich phase with acidified methanol was performed after phase separation and the metal contents were measured by FAAS. Variables affecting the system, *i.e.*, concentrations of reagents, time and temperature of extraction and composition of acidified methanol for dissolution of the surfactant-rich phase, were optimized. Signals were measured as peak height by using instrument software. Under the experimental conditions defined in the optimization, the method allowed copper and nickel determination with detection limits of 0.8 and 0.6 μg/L, respectively. The enrichment factors calculated were 24 (Cu) and 23 (Ni). The effects of other potentially interfering ions were also studied. The accuracy of the procedure was checked by analysis of the certified reference material NIST 1570a, Spinach Leaves. Seasoning samples purchased from supermarkets were digested in an acid digestion bomb enclosing a Teflon sample cup. The digestion was carried out by conventional heating in an oven and the digests were submitted to the developed procedure. The method was successfully applied to copper and nickel determination in pepper, bay leaves and curry.

W21 APPLICATION OF A NEW SOLID SORBENT TO PRECONCENTRATION OF COBALT AND MANGANESE IN COMMERCIAL CONDIMENTS

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The use of chelating reagents loaded or incorporated onto solid sorbents has been largely employed for preconcentration of metallic species before their analytical determination. The determination of metal contents in food is very important because the diet is the main route of intake for these elements, which can have significant biological effects in humans. In this work, Polyurethane Foam (PUF) functionalized with 2-hydroxyacetophenone by covalent coupling through -N=C groups, was applied to an on-line preconcentration system for determination of cobalt and manganese in food samples. PUF was reacted with 2-hydroxyacetophenone in dioxane medium. The mixture was added to a reflux system and heated for 72 h at 90°C with continuous stirring. The solid sorbent was used in an on-line preconcentration system for the determination of Co and Mn. Analysis was performed by flame atomic absorption spectrometry. Chemical and flow variables affecting the preconcentration were studied. Under the optimized conditions, the enrichment factors obtained were 36 and 27, for cobalt and manganese, respectively. The detection limits of the proposed procedure were 0.8 (Co) and 1.0 (Mn) micrograms per liter. The accuracy of the proposed method was demonstrated by the analysis of the certified reference biological material NIST 1570a, Spinach Leaves. The method has been applied to the determination of cobalt and manganese in condiment samples, *i.e.*, black pepper, bay leaves and salt.

W22 MULTIVARIATE OPTIMIZATION OF AN ET AAS METHOD FOR THE DETERMINATION OF CADMIUM IN WINE AND ITS APPLICATION TO THE QUANTIFICATION OF CADMIUM IN BRAZILIAN WINES

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The optimization and validation of a direct method employing Electrothermal Atomic Absorption Spectrometry (ET AAS) for the determination of cadmium in wine is described. A sample volume of 20 microliter without dilution was used for analyses. The optimization step was carried out using a two-level full factorial design, involving the followings factors: pyrolysis time, pyrolysis temperature, atomization temperature and modifier mass. Palladium was used as matrix modifier. The optimized conditions were as follows: pyrolysis time=10 s, pyrolysis temperature=600°C, atomization temperature=1,300°C, and modifier mass=15 µg. Under the optimized conditions, a characteristic mass of 0.7 pg, and limits of detection and quantification of 0.030 and 0.100 µg /L, respectively, were obtained, using a sample volume of 20 µL. The precision (RSD) was 0.30, 0.37 and 0.49% for wines with cadmium concentrations of 1.286, 0.266 and 0.356 µg /L, respectively. The accuracy of the direct ET AAS method was assessed by comparison with a reference method based on sample acid digestion and ET AAS determination, using five wine samples. The proposed method was applied to the determination of cadmium in 30 Brazilian wine samples (dry red, dry white, sweet white and sweet red). The cadmium content ranged from 0.146 to 1.563 µg /L, values lower than the permissible maximum level stipulated by the International Organisation of Vine and Wine (OIV) as well as by the Brazilian Legislation, which is 10 µg /L.

W23 DIRECT DETERMINATION OF SELECTED TRACE ELEMENTS IN APPLE JUICE BY ICP-OES

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The knowledge of the mineral contents of fruit juices is important not only for nutritional aspects, but also for toxicological reasons. Due to the increased awareness of healthy lifestyle the consumption and relevance of apple juice in human nutrition is rising. Furthermore the change of the Austrian fruit juice guideline "Fruchtsaftverordnung" in 2004 permits supplementation of vitamins and trace elements to fruit juices. At the moment there is a lack of information about the mineral contents of fruit juices, especially concerning potentially toxic elements. A major problem of trace and especially ultratrace elemental analysis is the sample preparation procedure, since it may lead to contamination of the samples as well as to losses of analytes. Thus it is advisable to perform direct measurements of the metals instead of decomposing the sample prior to analysis. The trace elemental content of apple juices can be analysed directly by GF-AAS, but this method as single element method is time-consuming. ICP-OES however offers the possibility of simultaneous quantification of the elements of interest. In order to reduce interferences and problems caused by the high sugar content of the fruit juices, the samples were diluted 1:20 with diluted HNO3.The method was optimized and validated for the following elements: Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, and Zn.

W24 DETERMINATION OF COPPER IN MILK USING A PORPHYRIN BASED COPPER-SELECTIVE ELECTRODE

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Determination of trace metals, including copper, has a great importance and several analytical techniques are used for this purpose. Copper has a vital importance in many biological systems and it is used for many industrial, agricultural and domestic purposes. Copper is an essential nutrient for humans, but it is toxic at high concentrations; therefore its determination in medicinal, environmental and industrial samples is very important. In milk, a high copper content contributes to accelerated lipid oxidation. The potentiometric determination of ions offers advantages such as selectivity, sensitivity, good precision, simplicity and low cost. This is the reason why a copper-selective electrode based on 5, 10, 15, 20-tetrakis(3,4-dimethoxyphenyl) porphyrin as ionophore (I), made by us, was used for the selective determination of copper in milk. The sensor has a poly(vinyl chloride) polymeric membrane plasticized with bis(2-ethylhexyl)sebacate (DOS) and potassium tetrakis(4-chlorophenyl)borate (KTCIPB) as additive in the ratio: I:PVC:KTCIPB:Plasticizer=10:165:5:330. The working concentration range is 2.5×10^{-6} M-1×10^{-1} M with a slope of (27.8±1.5) mV/decade copper and a detection limit of 2×10^{-6} M. The results obtained by the potentiometric method were compared with those obtained by the sodium diethyldithiocarbamate spectrophotometric method used as the reference method, with relative errors within 2%.

W25 TRACE ELEMENT PROFILING IN INDIAN TEA SAMPLES USING TOTAL REFLECTION X-RAY FLUORESCENCE AND AND INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY

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Many of the metallic and non-metallic elements are essential for growth and good health of human body. However, beyond a certain limit, these elements can have toxicological effects on the body. There is also a growing awareness among consumers for quality of foodstuffs and herbal products with clear regional identity. Therefore, determination of elemental composition and geographical origin of food and related products are essential. Herbal tea products are widely consumed in India and worldwide for medicinal purposes. Several groups around the world have analyzed tea samples coming from different origins. In this connection, it was found that, besides multielement profiles, data on Sr and B isotopic composition can be useful for identifying the geographical origin of the sample. Known amount of tea samples were digested in Suprapure^{TM} (Merck) concentrated HNO_3. The elemental profiling of tea products produced/marketed in India was obtained using Total X-ray Florescence (TXRF) and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Elemental determinations were carried out by using Co and Sc as internal standards and an ITAL STRUCTURES Model TX-2000 instrument for TXRF measurements. Samples were loaded on to a quartz support and excited with radiation of W L_β and Mo K_α from a dual target X-Ray tube in two separate experiments. The data on the sensitivity factors for the different elements were obtained using multielemental standards after suitable dilutions. The concentration of the elements in the tea samples was obtained using the X-ray intensity data and the relative sensitivity factor using the EDXRF-32 software. The same samples were also analyzed by ICP-AES using a ULTIMA-II (Jobin Yvon) instrument. Calibration curves in the range of 0.1 μg/g to 10 μg/g were employed for the various elements using certified multielemental standards. Spectral lines for the different elements were selected in order to minimize spectral interferences from other elements present in the sample. The samples were analyzed after suitable dilution. The elemental composition of the different samples analysed is presented along with the statistical data analysis. Several elements such as Ca, K and Mn are present at the mg/g level, whereas elements such as Cr, Fe, Co, Ni, Cu, Sr and Zn are present at concentrations of few μg/g. Only one sample was found to give a high Pb concentration of 0.8 μg/g.

W26 TRACE CONTENT OF MACROELEMENTS IN CANE AND BEET SUGARS AS CRITERIA OF SUGAR AUTHENTIFICATION

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For over 200 years sugar beets have been used in Europe mainly as raw materials for white sugar production. Recently, European sugar industry underwent sugar market regime reform, which implied a considerable reduction of beet sugar production in Europe. This will result in Europe becoming an importer of cane sugar. Cane sugar will be present on the European market both as brown sugar and white sugar. Cane sugars appear in various assortments and may have different levels of quality. A specific group of cane sugars is constituted by plantation white sugars, which have similar organoleptic properties but a lower quality compared to refined white sugars. As a consequence of the reform, the significant increase of the presence of cane sugar on the European market makes quality assessment of cane sugar a particularly topical problem. From the point of view of consumer protection the evaluation of authenticity of sugars may be particularly important. This study is an attempt to use the trace contents of potassium, sodium, calcium and magnesium as criteria for the evaluation of the origin of sugar, differentiating between beet and cane sugars. Our analysis determined the trace content of the above elements in white and brown cane sugar samples in comparison with respective beet sugar samples. The analyzed cane sugars showed a statistically lower content of sodium in ash (about 1%) when compared to beet sugars (about 3%). The content of magnesium in ash in the analyzed cane sugars was about 3%, which means it was significantly higher with respect to beet sugars, in which the Mg content of ash was about 0.2%. Thus, our results show that the contents of Na and Mg in ash may be treated as auxiliary criteria in differentiating between cane and beet sugars.

W27 FAAS AND ICP-AES FOR THE ANALYSIS OF SELECTED BIOLOGICAL ACTIVE ELEMENTS IN COMPONENTS OF THE POLISH TYPICAL DIET

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All elements occurring in earth's crust can also occur in food. The contents of these elements in diet components always point out issues related to products quality. The monitoring of food quality is the basic way enabling both the evaluation of nutrition adequacy and the detection of xenobiotics appearance. Samples of twenty six different comestibles were collected: barley groats, white rice, grains, UHT milk, natural yoghurt, cheese, beef, rabbit meet, poultry liver, thin dry smoked pork sausage, poultry sausage, wall-eye pollock, pilchards in oil, chicken eggs, cherries, strawberries, mandarins, bananas, kiwi, parsley, carrot, red cabbage, broccolis, string bean, carrot juice, and bitter chocolate. Quantitative determination of biologically active elements (Ca, Co, Cu, Fe, Mg, Zn, Cd, Pb, V) was made by Flame Atomic Absorption Spectrometry (FAAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Samples were dry and wet mineralized. Both mineralization methods could be used for the determination of the above mentioned elements in the selected comestibles. Contents of Ca, Cu Fe, Mg, and Zn were analyzed from mineralized samples directly or after dilution, if it was necessary. Cd and Pb determination by ICP-AES was made directly on the sample after mineralization whereas for FAAS a previous enrichment of these metals by a suitable extraction method was required. For these two elements, the recovery values obtained after samples mineralization by the wet method were higher (about 95%) than for ashing (about 88%). Cu, Zn and Fe contents did not exceeded the maximum tolerated levels set by Polish legislation in the above mentioned food products. Cd and Pb contents in some products were near these limits. For quality control, certificated reference materials were used in parallel to check both mineralization and further analytical determinations. The analytical procedures worked out may be applied to the determination of biologically active elements, both essential and potentially toxic (*e.g.*, Cd, Pb), in comestibles of the Polish typical diet.

W28 DETERMINATION OF THE CONTENT OF SELECTED POTENTIALLY TOXIC ELEMENTS IN *CURCUMA LONGA* AND ITS PRODUCTS USING ICP-MS

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The concentrations of several inorganic toxic impurities such as Cr, Cd, As, Hg, Sb, Pb, Sn, *etc.* contained in the root of *Curcuma longa*, and its products, such as curcumine powder, have been quantitatively determined by ICP-MS. Fresh samples were washed carefully with distilled water and the thin outer layer of the roots was peeled away. The root was then dried at room temperature and finely ground. The powder was decomposed by wet digestion with a mixture of nitric acid and hydrogen peroxide in a microwave oven. The optimal conditions for sample digestion were identified and a suitable microwave operating program selected. A study of the efficiency of recovery during sample decomposition was carried out with a certified reference material (CRM). The curcumine powder (hydrophobic) produced from *Curcuma longa* was also dissolved under similar conditions. Sample solution was injected into the nebulizer of ICP-MS under the optimum working conditions. The content of each element was calculated on the basis of external calibration. The accuracy of trace concentration measurement of some elements by ICP-MS was improved by the use of suitable internal standards. The analytical results have been compared with the limits issued by the World Health Organization.

W29 EFFECT OF DIFFERENT WATER SOLUBLE CHEMICALS ON THE SIGNAL OF VARIOUS ELEMENTS USING ICP-MS

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In our laboratory, elemental analysis of different food-related samples (food raw material, food, feed, plant, soil, organic fertiliser, irrigation water, sewage, sewage sludge) is carried out by Flame Atomic Absorption Spectrometry (FAAS, Perkin-Elmer Aanalyst 300), Graphite Furnace Atomic Absorption Spectrometry (GF-AAS, Unicam 939 QZ), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Perkin-Elmer), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Elemental). Recently, many researchers seek to analyse lower and lower concentrations of elements. Of the above techniques, generally inductively coupled plasma mass spectrometry is capable to determine the lowest concentration of an element. The ICP-MS instrument features Collision Cell Technology (CCT), which has better detection limits (at least in the case of specific elements), up to 1-3 orders of magnitude lower compared to the "normal" ICP-MS. While analysing the above mentioned types of samples, several interference effects appeared, due to the effect of the various matrixes having different evaporation and nebulization properties as well as a different carbon content. In our research work, an X7 type (Thermo Elemental, Winsford, UK) ICP-MS instrument was used to study the effect of different water soluble chemicals containing various carbon sources. Both the effect of the different type of chemicals and that of the quantity of carbon contained in the solutions was investigated. Since ICP-MS is a multielemental technique, 16 different elements (Cr, Co, Ni, Cu, Zn, Ge, As, Se, Mo, Cd, Sn, Sb, Te, Hg, Pb and Bi) were tested and various isotopes where monitored when available. In our work, we determined the duration of matrix effects after the end of the analytical run and recorded the extent of the carbon effect on the signal of the different isotopes studied. Finally, we tried to develop a method which can eliminate (or minimise) the effect of the above interference, so that the extent of error in the analysis of the above elements (mainly selenium and arsenic) can be lowered.

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W30 DETERMINATION OF SELECTED HEAVY METALS IN MAIZE: COMPARISON BETWEEN WD-XRF WITH DETATA PRECONCENTRATION AND FLAME ATOMIC ABSORPTION SPECTROMETRY

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Atomic Absorption Spectrometry and Inductively Coupled Plasma Mass Spectrometry are established techniques for the analytical determination of heavy metals in food materials. This paper presents a comparison of two analytical methods - conventional FAAS and the rarely applied Wavelength Diffraction X-Ray Fluorescence Spectrometry with preconcentration of analyte. Preconcentration was achieved by use of cellulose filters containing diethylene-triaminetetraacetic groups (DETATA), which absorbed ions from solutions. The DETATA filters are produced by Moscow State University by the reaction between typical cellulose groups and the diethylenetriaminepentaacetic acid. This process is protected by 1702659 USSR patent. After mineralization of the maize samples with concentrated nitric acid and hydrogen peroxide by use a microwave apparatus, zinc, nickel, cobalt and lead were determined by use of an AAS (1N Carl Zeiss-Jena). FAAS analyses were carried out by certificate reference solutions containing the analyzed elements in 0.5M HNO_3. The multielement calibration curves had all determination factors higher than 0.99. Analysis with use of the DETATA filters depended on adsorption of the analyzed elements from buffer solutions, with a definite rate typical of these filters. The process occurred with use of polypropylene dishes produced by Byospektr. After the process of preconcentration, DETATA filters were dried at 40°C and placed in aluminium measurement cuvettes. WD-XRF analyses were carried out with use of several certified reference materials produced by China National Analysis Center for Iron and Steel, such as NCS ZC73010 Mealie, NCS ZC73011 Soy bean, NCS ZC73012 Cabbage, NCS ZC73014 Tea, DC73348 Bush Branches and Leaves. Recovery factors were determined for FAAS and were 99.8%, 101.3%, 100.7%, and 98.9% for Zn, Ni, Co and Pb, respectively. The limits of detection were as follows: 0.32; 0.43; 0.50 and 0.55 mg/dm^3, respectively. In the case WD-XRF with DETATA preconcentration, the determined recovery factors were 100.6%, 101.1%, 100.2% and 99.3%. The limits of detection were 0.13; 0.12; 0.11 and 0.09 mg/dm^3, respectively. A complete comparison of the two methods will be presented.

W31 QUALITATIVE AND QUANTITATIVE METAL ANALYSIS IN SELECTED HERBS AND DIET SUPPLEMENTS BY EDX/EDS, WD-XRF WITH DETATA PRECONCENTRATION AND FAAS

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The human body requires metallic and non-metallic elements for healthy growth. Some elements are essential for plants, but some can be accumulated at very high levels and become harmful for man. Hence the determination of trace elements in all the compartments of the soil/food plant system (plant, soil, water) is very important. The main purpose of this study was the determination of Mg, Ca, Cr, Mn, Fe, Co, Cu, Zn, Pb in selected plants (*Inflorescentia tiliae, Rhizoma agropyri, Folium betulae, Fructus sorbi, Hippocastani cortex, Solidaginis herba*) and in commercially available diet supplements. EDX/EDS, WD-XRF and FAAS were used for analytical determinations. The studied samples were mineralized by microwave irradiation or simply homogenized depending on the analytical method applied. EDX/EDS analyses were performed on the powdered and homogenized samples. The analyses by WD-XRF were carried out on samples in the form of powders and solutions (*i.e.*, after mineralization), whereas FAAS analysis were made on solutions. The recoveries of the analytes in the diet supplements ranged from 95.2% to 100.1% (CV<5%) depending on the type of sample and the metal. The contents of trace elements in the herbs were as follows: 0.29-10.2 ppm for Pb, 7.33-15 ppm for Zn, 2.61-12.0 ppm for Cu, 0.01-0.02 ppm for Co, 1.27-40.2 ppm for Fe, 0.41-4.75 ppm for Mn, 0.05-0.07 ppm for Cr, and 4.35-25.4 ppm for Mg. The obtained results showed that the more precise and accurate method was FAAS. However, EDS/EDX and WD-XRF allowed for very rapid analysis without complicated samples preparation. Moreover, the latter methods provide more information about sample composition during one analysis.

W32 DETERMINATION OF TRACE ELEMENTS IN TOMATO SAUCES WITH SOLID SAMPLING

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In this work, lead, chromium, zinc, tin, manganese and copper were determined in tomato sauces using solid sampling atomic absorption spectrometry and the results were compared with those obtained by traditional GF-AAS after sample digestion. Concentrated HCl and HNO_3 were used in microwave wet digestion of the samples. Solid sampling has many drawbacks, such as microweighting, lack of solid standards for calibration, sampling inhomogeneity due to limited sample size, sample introduction difficulties, high background, limited precision. Besides these disadvantages, atomization of analytes directly from solid state offers some advantages, such as no need for sample digestion, no risk of analyte loss as well as contamination during sample preparation. Most importantly, since samples are directly introduced into the graphite furnace, much lower concentration can be detected. It has to be noted that the amount of sample introduced into the furnace is higher than that in solution technique. The method is fast, simple, cheap and several samples could be analyzed. The results obtained by two techniques were in the limit of 95% confidence level.

W33 DETERMINATION OF SELECTED METALS IN HEN EGGS BY SPECTRAL METHODS

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Nutritional compounds occurring in hen eggs play an important role in the human diet. They include proteins, lipids, vitamins and minerals. Among trace metals, some are needed for human health, but some can be harmful. For this reason, the knowledge of the concentration of potentially toxic metals in basic constituents of the human diet such as hen eggs is important. The main purpose of this work was the determination of selected metals in different parts of hen eggs, *e.g.*, shell, yolk and white. Chemical constituents of hen eggs depend primarily on aviculture practice. In Poland four breeding methods are commercially available and were considered in this study: ecological, hen-yard, bedding-bread and cage housing. For the analytical determination of Al, Se, Fe, Pb, Mn, Zn, Co, Cd, Ni, Mg and Ca, Wavelength Diffraction X-Ray Fluorescence Spectrometry (WD-XRF), Flame Atomic Absorption Spectrometry, and Spectrophotometric UV-Vis or Spectrofluorimetric methods were used. Samples were prepared in a different way depending on the analytical method used. WD-XRF method did not require previous sample preparation. In the case of spectroscopic and FAAS methods, sample mineralization was necessary. The mineralization was performed in a microwave apparatus. As the reference material, hen egg from ecological breeding was used. The concentration of the studied metals differed depending on the egg portion. The content of heavy metals (*e.g.*, Pb, Cd, Ni) in eggs from ecological farms was the lowest and amounted to <0.01 ppm (calculated on dry mass). For other egg types this content was higher, but did not exceed 1ppm. Moreover, the concentration of the other metals was significantly higher in yolk than in white (25 ppm and 1 ppm, respectively, for Zn). A statistical evaluation (t-Student test and F-test) or the obtained results was carried out. Results suggested that the best method for qualitative analysis was WD-XRF. The application of DETATA filters allowed determination of metal concentration at low levels. Moreover, considering both speed of analysis and cost of chemicals as well as consumables, the above method is very attractive. However, FAAS is more precise and accurate. As a general comment, the spectroscopic methods require time consuming sample preparation, which is a disadvantage in routine analysis or in mobile laboratories.

W34 OPTIMIZATION OF A MICROWAVE-ASSISTED EXTRACTION PROCEDURE FOR ZINC AND COPPER DETERMINATION IN FOOD SAMPLES BY BOX-BEHNKEN DESIGN

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Response Surface Methodology (RSM) is an empirical modeling technique devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results. A prior knowledge of the process is necessary to set up a statistical model. Basically this optimization process involves three major steps, *i.e.*, performing the experiments according to an appropriate statistical design, estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model. This work describes the application of RSM to the development of a procedure for zinc and copper determination in food samples by Flame Atomic Absorption Spectrometry (FAAS) after digestion in a microwave system. An aliquot (1 g) of sample was transferred to the extraction vessel with 10 mL HNO_3 (1 mol/L) as the digestion solvent. According to a preliminary experimental design, extractions were performed at various conditions of temperature ranging from 80 to 120°C, irradiation time from 5 to 15 min and irradiation power from 50 to 150 W. After digestion, the irradiated sample was transferred to a 10 mL volumetric flask and the volume was completed to the mark. The concentration of the analytes in this solution was determined by FAAS. A Box-Behnken matrix was used to find optimal conditions for the procedure through a response surface study. Three variables (irradiation power, time, and temperature) were regarded as factors in the optimization study. The working conditions were established as a compromise between optimum values found for each analyte taking into consideration the robustness of the procedure. These values were 97°C, 90 W and 9.0 min, for temperature, irradiation power, and time, respectively. The accuracy of the optimized procedure was evaluated by analysis of certified reference materials (SRM 1567a-Wheat Flour and SRM 8433-Corn Bran from NIST). The method was applied to the determination of copper and zinc in wheat flour and corn flour samples.

Poster Session 2

Sources and transfer of trace elements in the food chain

W35 EFFECTS OF SELENIUM SOURCE AND DOSE ON SELENOMETHIONINE AND SELENOCYSTEINE IN MILK OF DAIRY COWS

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The aim of this study (research funded by Alltech EU Regulatory Affairs Department, trial ref. SEL/DAI/EFF/04/0107/IT; the authors would also like to give special thanks to Ryszard Lobinski, UT2A laboratories, for performing the selenium analyses) was to determine the effect of dietary Se source and dose on selenomethionine (SeMet) and selenocysteine (SeCys) content of milk in dairy cows. The study involved 40 Italian Friesian lactating cows in mid lactation in a randomized complete block design (5 treatments with 8 animals per group): during the 140-day experiment all animals received the same basal diet, which differed only in Se source and dose: SY3 and SY5 (0.31 and 0.50 mg total Se/Kg DM respectively) containing organic Se (Se yeast) produced by *Saccharomyces cerevisiae* CNCM I-3060 (Sel Plex®); SS3 and SS5 (0.31 and 0.50 mg total Se/Kg DM respectively) containing inorganic Se obtained from sodium selenite; CTRL as negative control (0.09 mg Se/Kg DM). Prior to the start of the study (T0), at 112 (T112) and 140 (T140) d, samples of milk were analyzed (one pooled milk of 8 samples per treatment at T112; two pooled milk of 4 samples per treatment at T0 and at T140) to determine the proportion of total Se comprised as SeMet and SeCys. At T140 the SeMet content of milk was greater ($P<0.001$) in Se yeast supplemented animals (234 and 366 ng Se/g DM in SY3 and SY5 respectively) than CTRL (93 ng Se/g DM), and than those receiving comparable doses of selenite (96 and 112 ng Se/g DM in SS3 and SS5 respectively). A dose effect with Se yeast was also observed. Conversely, the SeMet content of milk from selenite treatments at T140 did not differ from CTRL. Consequently the proportion of total Se present as SeMet was greater ($P<0.05$) in Se yeast (56 and 60% of total Se in SY03 and SY05 respectively) than selenite (37 and 40% in SS03 and SS05 respectively) and CTRL (44%). At T140 the SeCys content of milk was greater in Se yeast supplemented animals (44 and 51 ng Se/g DM in SY3 and SY5 respectively) than selenite supplemented (32 and 36 ng Se/g DM in SS3 and SS5 respectively). At T140 the proportion of total Se present as SeCys was, on average, 13% of total Se, without significant differences between treatments. These results indicate that increases in the total Se content of milk obtained from Se yeast supplemented animals when compared to selenite supplemented ones was principally a consequence of the increase in the proportion of total Se present as SeMet, which accounted for 60% of total Se increase.

W36 PHYTOAVAILABILITY OF CADMIUM AS INFLUENCED BY NITROGEN RATE, SEEDING DATE AND SOIL TYPE

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Cadmium (Cd) is a trace element that has been associated with various human health problems. Cadmium may occur naturally in soil, while phosphate fertilizers and sewage sludges are anthropogenic sources of Cd. Cadmium enters plants, either by direct absorption through leaves or by uptake from soils, allowing Cd into the food chain. Cadmium accumulates in the human body over time and long-term consumption of foods high in Cd could lead to chronic toxicity. Therefore, it is vital to be able to predict potential Cd uptake by plants from soils. The availability of soil Cd for plant uptake is determined by various plant and soil factors, as well as by environmental conditions and agronomic management practices. Concentrations of Cd in field crops often vary significantly from year to year, due to variable environmental conditions. Changing seeding dates will change the environmental conditions that the crop experiences during growth. Therefore, field trials with high-Cd and low-Cd durum wheat cultivars were seeded at three different dates, with and without urea fertilizer. Data from six site-years were analysed to determine the interactive effects of N rate, seeding date and soil type on crop concentration of Cd. Effects on N, P and Zn were also determined. Cadmium and N concentration in straw and grain were strongly affected by N application, with only minor effects on N occurring on concentration and uptake of P and Zn. There were large differences in Cd concentration from year to year, and with seeding date indicating a strong environmental influence. Cadmium concentration in the grain was generally below proposed regulatory limits. Relationships between yield, protein content, and Cd accumulation were also identified.

W37 THE INFLUENCE OF ORGANIC AND MINERAL FERTILIZATION ON THE TRACE ELEMENTS CONTENTS IN THE FEED FROM THE HILL REGION OF BANAT (ROMANIA)

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This paper presents original studies about the influence of mineral and organic fertilization on trace metals (Fe, Mn, Zn, Cu, Co, Ni, Cr, Pb and Cd) in feed from a permanent meadow situated in a hill area of Banat County (west part of Romania). Permanent meadow was first fertilized in autumn of 2003. Mineral fertilizers used were NPK complex, ammonium nitrate, superphosphate and potassium salt. Fermented manure sheep was used as organic fertilizer. Permanent meadow was organized in ten experimental variants and five replicates for each variant, fertilized either with sheep manure only, or with sheep manure and NPK mineral fertilizers, or with NPK mineral fertilizers only. The mineral fertilization was made yearly, whereas the sheep manure was applied at each two years. The main plants in analyzed forages were *Festuca rupicola* and *Calamagrostis epigeios*. From the other botanical families there were: *Antohoxanthum odoratum*, *Briza media*, *Poa pratensis*, *Trifolium arvense*, *Trifolium medium*, *Genista tinctoria*, *Lotus corniculatus* and *Filipendula vulgaris*. Determination of trace metals in feed is very important both for the nutritional and potentially toxic effects of these elements and their compounds, in both plants and animals. Transfer through the food chain may ultimately affect the final consumer, *i.e.*, man. All metals have been analyzed using Flame Atomic Absorption Spectrometry (FAAS). The correlations between metal contents in feed and mineral or organic fertilizers are discussed. Both fertilizers influenced the metal content of feed, but in a different manner. Organic fertilizer had the smallest effect on the metal contents of feed.

W38 TRANSFER AND ACCUMULATION OF CADMIUM, LEAD AND ARSENIC IN THE FOOD CHAIN OF CATTLE FROM RURAL AND INDUSTRIAL AREAS IN BELGIUM

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Industrial and agricultural processes have resulted in the release of toxic trace elements into the environment. Cadmium, lead and arsenic (especially inorganic arsenic) are trace elements that give rise to most concern because of their transfer through the food chain, bearing a potential risk to animals and humans. Historical pollution of non-ferrous industries in northern Belgium have affected several areas, mostly with sandy soils that are, as such, subject to acidification. Although the natural content is low, the combination of enhanced trace element concentrations and low pH values can end up in accumulation of cadmium and other trace elements in fodder crops and hence their accumulation in cattle. The aim of our study was to evaluate the accumulation of the three toxic trace elements, *i.e.*, arsenic, cadmium, and lead, in 152 bovine animals raised in Belgium. The study was realized in collaboration with the Belgian Federal Agency for the Safety of the Food Chain, which carried out the samplings of meat, liver and kidney in the slaughterhouses. Altogether, 53 out of 152 animals originated from the polluted zones. The other 99 animals were raised in agricultural areas spread all around Belgium, which are believed to be non-polluted areas. They are considered as reference samples. For most of the animals originating from the polluted areas and for a selection of animals from the reference area, the farms were visited several times to sample farm grown fodder crops as well as the corresponding soils, groundwater and surface water if it was used as drinking water for the animals. Biomonitoring was also used to study the impact of atmospheric deposition that can be an additional source of pollution for fodder crops. Meadows were sampled in spring, summer and autumn and in addition also grass silage and hay were sampled. The main fodder crop was maize, which was sampled in the field and also as silage. The field samples were separated in cob's, stems and leaves in order to determine their relative contribution to the transfer of trace elements. If available, also fodder beet and cereals were sampled and analyzed. Commercial feed was not sampled as it can not be linked to the local environmental pollution. The soils were analyzed not only for their trace element content but also for their acidity and organic matter content. The mass flow of trace elements in the bovine food chain was studied in order to detect the proportion of the different components that contribute to the accumulation of trace elements primarily in kidney and liver.

W39 EFFECTS OF WITHIN NATURAL RANGE VARIATION IN CADMIUM AND LEAD LEVELS IN GENERAL AQUATIC ENVIRONMENT ON LOCAL FOODS AND BODY BURDEN OF LOCAL RESIDENTS: NATION-WIDE ANALYSES IN NON-POLLUTED AREAS IN JAPAN

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The present study was initiated to examine if variations of two ubiquitous hazardous elements, cadmium (Cd) and lead (Pb), within natural ranges in the general water environment may affect the corresponding metal levels in local foods and the body burden of local residents in non-polluted areas in Japan. Data were obtained through surveys in areas of no known man-made pollution. Recently in Japan, two nation-wide databases have been made available containing data on Cd concentration in locally harvested brown rice and on Cd and Pb concentrations in the sediments of local river beds. These data were combined with published ones reporting Cd and Pb concentrations in 24-hour food duplicates (including drinking water) and human biological samples (blood and urine) from the same individuals, and those in polished rice samples. Possible association among the levels was examined by correlation analyses. The results have shown that Cd concentrations in river sediments correlated significantly with Cd concentrations in food duplicates, blood and urine. Cd levels in food duplicates correlated with those in brown (unpolished and uncooked) and boiled (polished and cooked for consumption) rice. In contrast, the concentrations of Pb in the sediments either did not correlate or correlated only weakly with Pb levels in human biological materials or food duplicates. The possible implications of the difference between the behavior of Cd and that of Pb with regard to the intensity of correlation were discussed with reference to the different routes of exposure of the local residents to these hazardous elements. In conclusions, the Cd burden on residents in Japan was significantly influenced by Cd levels in water in the general environment, whereas water-borne Pb did not show a clear correlation with the Pb body burden.

W40 HEAVY METAL CONTENT OF CROPS IRRIGATED WITH WASTEWATER

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Heavy metals may enter the food chain as a result of their uptake by edible plants. Thus, determination of heavy metals in environmental samples is important. Some of these metals have significantly toxic and hazardous effects on human health. The present study was carried out to asses levels of different heavy metals such as lead, cadmium, chromium, copper and nickel, in crops irrigated with wastewater. Municipal or industrial wastewater in Tehran (capital city of Iran) is mostly used for irrigation of crops, mainly in periurban ecosystems, due to its easy availability, disposal problems and scarcity of fresh water. The wastewater irrigation area, more than one hundred thousands hectares of agricultural lands, is located south of Tehran, in Varamin plain. Samples of some commonly grown vegetables, *i.e.*, mint, cress, lettuce, watermelon and tomato were collected and digested with chloric and nitric acid. Heavy metals were determined by atomic absorption spectrophotometry. The result indicated a substantial build-up of heavy metals in crops irrigated with wastewater. The range of various metals in wastewater-irrigated plants was 0.5-3, 0.003-71, 0.01-1.7, 1-3.1, 0.001-0.09 mg/Kg for lead, chromium, cadmium, nickel, and copper, respectively. The highest levels of Pb, Cr, Cd, were detected, on average, in cress, whereas the levels of Ni and Cu were highest in lettuce. The lower levels of heavy metals were found, on average, in tomato and watermelon. Heavy metals concentration in some of the investigated crops was over the permissible levels. Therefore, this study showed the concern involved in the cultivation and consumption of vegetables irrigated with wastewater, a practice which may place at risk the health of urban population who consume these vegetables.

W41 MEASUREMENT OF HEAVY METALS BOUND BY POMEGRANATE (*PUNICA GRANATUM*) PEEL IN A MODEL SYSTEM

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Pomegranate fruits are an important sources of natural phenolic compounds and extracts from pomegranate peel have been investigated for use as antioxidants in food. In this study, the influence of pomegranate fruit peel on the binding of some heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) was established by means of Inductively Coupled Plasma Atomic Emission Spectrometry. The aim of this work was to ascertain the ability of pomegranate fruit peel to bind heavy metals in view of a possible use of this by-product of juice industrial production in other food industries. Pomegranate fruits were purchased from the local market in Konya (Turkey). All of the fruits were brought to the laboratory at the same time. Diseased, bruised and injured fruits were removed. Fruits of uniform size and appearance were randomly distributed into different lots. The pomegranate fruits were kept refrigerated (+4°C). Amounts of 1, 2, 3, 10, 20, 40 and 60 g of ground peel were used in this experiment. After drying in oven (70°C), the samples were weighed and placed into 500 cm^3 conical flasks, which were then treated with known amounts of the appropriate model solution, *i.e.* standard solutions containing known concentrations of heavy metals. The formulations for the three experiments (a, b and c) are given below:

a) 1 g ground material + 50 ml, 5 ppm standard solutions; 2 g ground material + 50 ml, 5 ppm standard solutions; 3 g ground material + 50 ml, 5 ppm standard solutions;
b) 10 g ground material + 200 ml, 5 ppm standard solutions; 20 g ground material + 200 ml, 5 ppm standard solutions;
c) 40 g ground material + 200 ml, 2 ppm standard solutions; 60 g ground material + 200 ml, 5 ppm standard solutions.

The concentrations of metal ions in the resulting solutions were measured by means of ICP-AES. The heavy metal content of solutions prepared with ground material decreased with increasing amounts of ground material. At the same time, the binding rate of heavy metals increased with increasing amounts of ground peel. The most bound was lead (Pb), with a 99.2% binding rate for the solution containing 20 g of ground sample. It was observed from the results, that the amount of metal ions bound by the ground material differed from one fraction to others.

W42 MINERAL CONTENTS OF KERNELS OF SOME IMPORTANT APRICOT (*PRUNUS ARMENIACA* L.) VARIETIES CULTIVATED IN TURKEY

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Oil from apricot kernels is used as a culinary flavouring and the kernels to make distilled liquors and for other food uses. The mineral contents of apricot (*Prunus armeniaca* L.) kernels were determined in this study. Kernels of five apricot varieties were obtained by hand processing from apricots growing in Malatya (Turkey) in August, 2007. Kernels were kept refrigerated in glass jars until analysis. In all stages of trials, dry and mature kernels were used. The content of minerals (Al, Ca, B, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P and Zn) in kernels was determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The kernels were dried at room temperature. The dried materials were then ground in a mortar and the ground samples sealed in bottles for storage until analysis. About 0.5 g of ground kernel samples was put into a burning cup with 15 ml of pure HNO_3. The sample was incinerated in a MARS 5 microwave oven at 200°C. Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all reagents, standards, and digested samples. After digestion, samples were filtrated through Whatman No 42. The filtrates were collected in 50 ml Erlenmeyer flasks and analysed by ICP-AES. All kernels contained high amounts of Ca, K, Na and P. Other elements were determined at minor levels. The P levels of kernels ranged from 4,968 (Kabaaşı) to 9,387 mg/Kg (Soğanoğlu). The highest minerals in all samples were K, P and Ca. While K content was highest (91,909 mg/Kg) in kernels from Soğanoğlu, P and Ca levels were 9,387 mg/Kg and 2,910 mg/Kg, respectively, in the same sample. These results are useful for the evaluation of the nutritional value of apricot kernels foods derived from them.

W43 THE INFLUENCE OF PLANTING TIME AND PLANTING CLOSENESS ON THE ELEMENTAL COMPOSITION OF GROUND ALMOND (*CYPERUS ESCULENTUS* L.)

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Ground almond (*Cyperus esculentus*) is one of the finest nuts from the tropics to the temperate regions. In this study the concentrations of some minerals and trace elements in ground almonds from three different populations, cultivated by The Institute of Agricultural Research in Çukurova (Turkey) and registered as Population 1, Population 2 and Population 3, were investigated. These populations were planted at different times (April 27th, May 17th and June 6th in 2006) and at different plant closeness (60x25cm, 60x40cm and 60x50cm). The element concentrations in ground almond were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry. Prior to analysis, samples were digested in a microwave digestion unit using a mixture of nitric acid and hydrogen peroxide as reagents. Measurements were accomplished by external calibration using aqueous multi-element standards. To check the accuracy of the analytical data, standard reference materials were analysed. The results (mean values±SD) for the following elements (on a mg per Kg dry weight basis) were: potassium (1,483.08±453.92-23,720.90±4,389.08), magnesium (78.13±22.20-2,774.11±452.13), manganese (0.00±0.00-21.07±5.11), zinc (1.33±0.70-28.62±7.11), copper (1.07±0.14-26.05±5.12), chromium (0.30±0.08-26.56±5.13), calcium (0.00±0.007-2,833.56±231.07), iron (4.96±1.85-866.92±75.11) and nickel (0.52±0.15-9.19±2.11). Concentrations of Ni, Mn and K were not affected by the different planting times and Na values did not seem to be affected by the planting closeness. A closeness of 60x40 cm lead to higher concentrations of all the elements investigated, except for Cu and K; the samples planted with 60x25cm closeness had higher values of these latter elements. Ground almond from specimens planted in June contained higher amounts of Al, B, Ca, Na and Zn than the specimens planted earlier. The differences between elemental compositions of the three populations of almonds were statistically significant ($P<0.01$). P1 had the highest values of macro elements such as Ca, K, Mg and Na. These findings may be useful for dietary information, which requires prior knowledge of the nutritional composition of edible cultivated nuts.

W44 MINERAL CONTENT OF THE SEED AND THE PERICARP OF SUMACH (*RHUS CORIARIA* L.)

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The content of major (Ca, K, Mg, Na and P) and minor (Al, As, B, Cd, Cr, Cu, Fe, Li, Ni, Sr, Ti, V and Zn) mineral constituents in dried pericarps and seeds of sumach (*Rhus coriaria* L.) was determined by means of Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The samples were collected from ten locations in Turkey, *i.e.*, Çanakkale, Hakkari, Hatay, İskenderun, Kastamonu, Manisa, Kahramanmaraş, Mersin (Mut), Mersin (Silifke) and Siirt in September and October 2002. About 0.5 g of ground sample were added with 15 mL of HNO_3 and incinerated in a MARS 5 microwave oven at 200°C. Distilled deionized water and high-purity commercial acids were used to prepare all reagents, standards, and samples. After digestion, samples were filtered through Whatman No. 42 filters. The filtrates were collected in 50 mL Erlenmeyer flasks and analysed by ICP-AES. The analytical results were checked by using standard reference materials. Ca, K, Mg, Na and P showed the highest concentration in the pericarp samples. The ranges of Ca, K, Mg, Na and P content in pericarps were 1,000-3,577, 8,307-17,361, 343-700, 731-1,249 and 781-1,635 mg/Kg, respectively. The highest values were observed in samples from Silifke (Ca), Hakkari (K), Hatay (Mg), Kahramanmaraş (Na) and Çanakkale (P). The content of the other elements was much lower in pericarps. Arsenic was not detected in the pericarp samples from Hakkari, Manisa, Kahramanmaraş, Mut and Silifke, neither cadmium in samples from İskenderun, Kastamonu and Manisa. The ranges of Ca, K, Mg, Na and P in seeds were 1,062-1,603, 2,803-7,242, 492-750, 510-742 and 1,530-3,172 mg/Kg, respectively. The K, Mg and P content of the seeds from Hakkari was the highest. The highest values of Ca and Na obtained in the seed samples were from Kahramanmaraş (1,603 mg/Kg) and İskenderun (742 mg/Kg), respectively. The arsenic content in seeds ranged from 2.13-4.28 mg/Kg. The results showed that the pericarp and seed of sumach are potential sources of Ca, K, Mg, Na and P.

W45 THE AFFINITY OF SOME MUSHROOMS SPECIES UBIQUITARIAN IN DAMBOVITA COUNTY ECOSYSTEMS FOR ABSORBTION AND STORAGE OF BIOMINERALS

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This study is about the capacity of mushrooms (macromycetes) to absorb biominerals from their substrate at its natural pH (6.04-6.76) and store them inside their tissues. Some ubiquitarian species were collected all over forestry ecosystems of Dambovita county (in mountains and in the valley along Dambovita river) and analyzed. It is a popular practice to harvest mushrooms from everywhere in nature, prepare and eat them. Our forestry ecosystems are very rich in edible mushrooms, which are commonly used as food. It is important to know the composition and nutritive value of each species, given the growing interest in the exploitation of different mushrooms as well as the lack of information about which could be especially valuable as food. The species investigated in this study belong to *Basidiomycetae* class, and included *Fistulina hepatica* and *Armillariella melea*, each of them harvested from two different sites. They were analyzed using elemental atomic techniques with a high detection power. In particular, the elemental profile of the samples was determined by Energy Dispersive X-ray spectrometry (EDXRF). The biomineral content of the studied mushrooms species was characterized and differences in element content among mushroom species were detected. A high concentration of potassium was detected in all samples along with a sizeable calcium concentration. Only some samples contained phosphorous, sulphur or silicon well above detectable levels. Some samples of *Armillariella melea* harvested from mountain locations contained traces of gold.

W46 CONTENT OF SELECTED ELEMENTS IN KING BOLETE (*BOLETUS EDULIS*) COLLECTED FROM THE SAME SITE OVER TWO YEARS

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Baseline data published by many authors on mineral composition of mushrooms did indicate that the concentrations of a given metal in a given species can vary considerably between specimens collected from spatially distant sites. Soil bedrock geochemical composition and soil pollution with metallic elements could impact mineral composition of mushrooms. However, site-to-site variations in metallic elements content of a given mushroom species could be related also to biological and environmental factors other than the parent soil geochemical composition, *i.e.*, year-to-year fluctuating weather condition etc. Aim of this study was to examine if there could be any variation in Al, Ba, Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Na, Sr and Zn content of the fruiting bodies of King Bolete mushroom collected from the same site over two years. Fifteen well-developed fruiting bodies of King Bolete, of approximately the same size, were collected in the northern part of Poland in 2000 and 2001. Mercury was determined by CV-AAS and other elements by ICP-AES with external standard calibration and yttrium (20 mg/L) as internal standard. Around 200-500 mg aliquots of the samples were pre-digested at room temperature and further digested under pressure in an automatic microwave digestion system type MARS 5 and using of analytical grade concentrated nitric acid. The analytical method was successfully checked through analysis of several certified reference materials and participation in inter-laboratory trials. The standard reference materials used included Dogfish muscle (DORM2; National Institute of Standards, Ottawa, Canada), Oriental Tobacco Leaves (CTA-OTL-1), Tea Leaves (INTC-TL-1) and Polish Herbal Blend (INCT-MPH-2). The caps of King Bolete collected from the same site over two years showed similar content of Cu, Fe, K, Mg, Mn, Na and Zn but different concentrations of Al, Ba, Ca, Cd, Hg and Sr ($p<0.05$; U Mann-Whitney test), while in the case of stipes statistically significant difference was noted only for Hg. These data imply that under real environmental conditions and stable geochemical composition of the soil substrate the biological factors, *e.g.*, age of mycelium or, more likely, year-to-year fluctuating weather conditions could cause variation in some metallic elements content of edible mushrooms.

W47 BIOACCUMULATION OF MERCURY IN SLIPPERY JACK (*SUILLUS LUTEUS*) MUSHROOM

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Total mercury content was determined in 120 samples of fruiting bodies of Slippery Jack and in samples of the soil substrate underneath the mushrooms, collected from eight spatially distant sites in Poland. Mercury was determined by CV-AAS after thermal decomposition of the sample matrix and further amalgamation and desorption of the analyte from gold wool. Analytical Control and Quality Assurance (AC/AQ) included routine analysis of the certified reference material CS-M-1 (dried fruiting bodies of mushroom Cow Bolete, *Suillus bovinus*) produced by the Institute of Nuclear Chemistry and Technology in Warsaw, Poland. The certified total mercury content of the reference material was 0.174±0.018 and our measurements in this trial found 0.167±0.044 µg/g dry weight (n=3). With every set of 10 mushrooms or soil samples one blank sample was included as well. The mean Hg content of the soil substrate ranged between 0.012±0.002 µg/g dw (Toruńska Dale) and 0.053±0.025 µg/g dw (Płocka Dale). Results of analysis of variance (Kruskall-Wallis test; $p<0.05$) indicated diversity of Hg content of soil substratum between certain sites. It was confirmed that the concentration of Hg in the Slippery Jack fruiting bodies was greater than that in the respective soil substrate samples. The caps of Slippery Jack from Giełczewska Upland had the highest Hg levels (0.28±0.07 µg Hg/g dw) when compared to other sites, whereas the lowest mean concentration (0.095±0.082 µg/g dw) was found in caps from Kurpiowska Plain. Total range of Hg concentration in all caps was 0.028-0.39 µg/g dw, whereas for stipes it was 0.025-0.18 µg/g dw. For stipes, mean Hg concentrations were between 0.045±0.018 (Kurpiowska Plain) and 0.13±0.02 µg/g dw (Giełczewska Upland). Both in case of caps and stipes, analysis of variance showed diversity in Hg content between the sites (Kruskall-Wallis test; $p<0.05$). The cap to stipe mercury concentration ratio was 2.5±1.0, and difference between caps and stipes mercury content was statistically significant (Wilcoxon Matched Pairs Test; $p<0.05$). Hg cap to soil concentration ratio (bioconcentration factor) ranged between 4.4±4.6 and 14±5, depending on the site. A meal consisting of 300 g of fresh caps of Slippery Jack picked-up at the Kurpiowska Plain could provide the consumer with 2.8 µg Hg, while the same figure is 8.3 µg for the Giełczewska Upland (14 and 40% of daily Hg reference dose, respectively). The amounts of fresh caps needed to exceed the Hg reference dose for a 70 Kg-body weight individual were 0.8 Kg at the Giełczewska Upland and 2.2 Kg at the Kurpiowska Plain sites.

W48 MERCURY AND ITS BIOCONCENTRATION POTENTIAL BY RED ASPEN BOLE (*LECCINUM AURANTIACUM*)

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This communication reports data on the baseline mercury contamination of Red Aspen Bolete mushroom and the soil substrate layer (0-10 cm) underneath the fruiting bodies collected from eight spatially distant sites across Poland. The carpophores, after removal of plant material and particles of soil with a plastic knife followed by an initial air-drying at room temperature in a well-ventilated place for 2-3 days, were further oven dried at 80°C to constant weight, and finally ground in an agate mortar to fine powder. Soil substrate samples, after removal of visible organisms, small stones and leaves, were air dried in room temperature for 10 weeks and then sieved through a pore size of 2 mm. Mercury content of caps, stipes and soil samples were determined by CV-AAS after thermal decomposition of the sample matrix and further amalgamation and desorption of Hg from gold wool (Mercury analyzer type MA-2000, Nippon Instruments Corporation, Takatsuki, Japan). Analytical Control And Quality Assurance (AC/AQ) of the method included routine analysis of the certified reference material CS-M-1 (dried fruiting bodies of mushroom Cow Bolete, *Suillus bovinus*) made by the Institute of Nuclear Chemistry and Technology in Warsaw, Poland. The certified mercury content of the reference material was 0.174±0.018 and our measurements in this trial found 0.171±0.008 µg/g dry weight (n=3). In addition, with every set of 10 mushrooms or soil samples one blank sample was included. At seven of eight sampling sites, total mercury concentration in soil substrate varied between 0.011±0.006 and 0.028±0.007 µg/g dw, which could be considered as a baseline level. The arithmetic means of mercury concentration in Red Aspen Bolete caps varied, depending on the site, between 0.27±0.07 and 1.3±0.6 µg/g dw. At the site nearby the town of Ożarów, where the highest mercury concentration in soil substrate was observed, the mean content of caps was 0.64±0.14 µg Hg/g dw, indicating a relatively low mercury bioconcentration factor (BCF) of 9±2. Much greater BCF values could be obtained for other sites, ranging from 15±4 to 110±13 and 130±66, values obtained at the two sites showing the lowest mercury content of soil- In general, BCF values in caps showed a downward trend with increasing mercury content of soil. A meal consisting of 300 g of fresh caps of Red Aspen Bolete could provide the consumer with 8.1 µg Hg in the Aleksandrów Kujawski region and 39 µg in the Lubelska Upland (39 and 186% of daily Hg reference dose, respectively). The amounts of fresh caps needed to exceed the Hg reference dose for a 70 kg-body weight individual were 0.78 Kg in the Aleksandrów Kujawski and 0.16 Kg in the Lubelska Upland regions.

W49 TRACE ELEMENTS IN WILD EDIBLE MUSHROOMS: *BOLETUS EDULIS*

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There are many factors that can influence body trace element concentrations, such as state of nutrition, gender, age, element retention percentage, chemical form and bond sites. Food is the main route involved in trace element transfer from environment to human body. In literature, it is described that wild edible mushrooms may accumulate some heavy metals. The usual contents in non-accumulating species from unpolluted (background) areas are <1 µg/g for As, 0.5-5 µg/g for Cd, 0.1-2 µg/g for Cr, 1-5 µg/g for Pb, 0.5-5 µg/g for Hg, 0.4-2 µg/g for Ni, 1-5 µg/g for Se (all data on a dry matter basis). However, the contents are higher by one and exceptionally two orders of magnitude in accumulating species growing in highly polluted sites, such as in recent or historical areas of mining and processing of heavy metals, inside cities, *etc*. The ability to accumulate some element(s) is species-dependent. Relatively high selenium content in a group of *Boletus* mushrooms seemed to be promising for a possible use of this species as source of this nutritional element. However, its availability was very low. The aim of our investigation was to characterize the concentration of trace elements in *Boletus edulis* collected in the east of Europe (90% in Romania, the others in Hungary, Bulgaria, Turkey, Serbia and Macedonia). The fresh samples were dried overnight at 100°C. About 1.0 g of dry mushroom was broke up and sonicated for 60 min, in an ammonium acetate buffer at pH 4.65 to destroy the cells. The solution diluted 10 times was analysed with an ICP-MS Perkin Elmer ELAN DRC II. The limits of detection were 0.2 µg/L (0.002 µg/g dry) for Hg and Pt and 0.5 µg/L (0.005 µg/g dry) for Pb, Co, Se, Cr, Ni, Sb, Rh, Cd and As. The uncertainties related to the calibration repeatability and recovery were 32% for Cd and As, 31% for Cr, 37% for Se, Hg and Sb, 28% for Pb and Ni, 26% for Co, 33% for Rh and 36% for Pt. Altogether, 97 Mushrooms (85 specimens of *Boletus edulis* and specimens of 12 *Amanita cesarea*) were collected from the middle of June to the middle of November. The concentration of the elements ranged from the limits of detection to 3.8 µg/g for Pb, to 8.2 µg/g for Cd, to 13.6 µg/g for Hg, to 2.3 µg/g for Co, to 1.4 µg/g for Cr, to 146.9 µg/g for Se, to 4.3 µg/g for As, to 2.4 µg/g for Ni (all data on a dry matter basis), and were below the limits of detection for Pt, Rh and Sb.

W50 DETERMINATION OF HEAVY METAL CONTENT OF MACROMYCETES (MUSHROOMS) AND THEIR SUBSTRATE BY EDXRF SPECTROMETRY AND AA SPECTROMETRY

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Metabolism of macromycetes, commonly called mushrooms, is substantially different from that of green vascular plants. The determination of heavy metal concentration in the fruiting bodies of mushrooms is essential in dietary intake studies, because mushrooms form a non-negligible part of the diet in many countries, especially for certain population groups. Different heavy metals are toxic, such as As, Cd, Hg; on the other hand many elements are essential for the human body, such as Fe, Zn, Se. Both biominerals and heavy metals can be accumulated in mushrooms, and this accumulation is generally species-dependent and also strongly affected by the chemical composition of the substrate from which mushrooms get their nutrients. The aim of this work was to determine the heavy metal content of the fruiting bodies of *Lycoperdon perlatum and Pleurotus ostreatus* and their respective substrates. The samples were collected from Dambovita County Romania, at various distances from of a metal smelter. The Cr, Mn, Fe, Ni, Cu, Zn, Se, Cd concentrations were determined using Energy Dispersive X-ray spectrometry (EDXRF) together with Atomic Absorption Spectrometry (AAS). The EDXRF measurements were made using the Elvax spectrometer having an X-ray tube with Rh anode and a solid state Si-pin-diode X-ray detector with a 140 μm Be window and 200eV at 5.9 KeV (^{55}Fe line) energy resolution. The AAS measurements were performed using an Atomic Absorption Spectrometer, equipped with an AVANTA GBC flame and Hollow Cathode Lamps (HCL). A highest accumulation of Fe, Cu and Zn from substrate was observed for all the analyzed mushrooms samples. Furthermore, a high accumulation of Pb was observed in mushrooms growing in the vicinity of the metal smelter.

W51 IODINE CONTENT IN KOMBU (EDIBLE KELP) PRODUCTS CONSUMED IN JAPAN

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Iodine is an essential trace element in human nutrition as a component of thyroid hormones. However, excess intake of iodine may induce hypothyroidism. In the Dietary Reference Intake established in USA and Canada, the upper limit of iodine intake has been set at 1.1 mg/d to prevent hypothyroidism. Kombu is an edible kelp from the genus *Laminaria* widely eaten in Japan and Korea. Since kombu contains iodine at a level of more than 1 mg/g dry weight, an excessive ingestion of iodine due to kombu consumption is feared in the Japanese and the Korean. However, since a sizeable amount of iodine is eliminated from kombu during cooking, it is believed that the iodine intake from kombu doesn't reach a level of any health concern. In the present study, we determined the iodine content in kombu products sold in the Japanese market and examined the iodine intake from kombu products in Japanese. Samples of kombu products and other edible seaweed products (nori, hijiki, mozuku, aosa and wakame) were purchased in local retail stores in Kyoto city. The seaweed product samples (0.5 g) were extracted by a mixture of 5 mL of pure water and 1 mL of 25% tetramethylammoniumhydroxide for 3 h at 90°C. Iodine in the extracts was determined by inductively coupled plasma-mass spectrometry. Among the seaweed samples, most of kombu products showed high iodine values (more than 3 mg/g dry weight), but the other seaweed products were lower in iodine (less than 0.5 mg/g dry weight). Among the kombu products, the ones that were boiled during the manufacturing process tended to show a low concentration of the iodine.

W52 MERCURY IN FISH PRODUCTS FROM THE FISH MARKET IN GENOVA

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Fish is one of the most common food in the Italian diet. It is a great source of proteins, polyunsatured fatty acids, and micronutrients. However, as a result of a contaminated environment, different pollutants can be found in fish and fish products. Heavy metals, *e.g.* mercury, can be a concern for the consumers, as this pollutant can be accumulated through the food chain, especially in predators, as tuna fish or swordfish. The aim of this study was to evaluate mercury contamination levels in fish, molluscs and shellfish caught in Mar Ligure, for human consumption and collected in the town fish market of Genova. From March to May 2008, 95 fish tissue samples were clustered together from every single species in 41 pools, and 36 portions of shellfish/molluscs were grouped in 12 pools according to their species. Mercury was detected by atomic spectrometry, using a DMA80 analyzer in all pools collected. The limit of quantification of the applied method was 0.1 mg/Kg. The mean mercury concentration in fish samples was 0.453 mg/Kg, whilst in shellfish and mollusc samples was 0.551 mg/Kg. Twelve samples out of 41 (29.3%) showed a mercury level above the legislation limit, stated in the EC Regulation 1881/2006. Of these positive samples, 27.8% were fish and 28.6% were shellfish/molluscs. Industrial activities are the main source of pollutants in the marine environment. These toxic substances can affect sea organisms and, indirectly, human health. The results of this monitoring study show that a significant amount of the catch in Mar Ligure (35.8%) had a mercury content above the legislation limit. Therefore, a certain percentage of the population could be exposed to mercury and thus exceed the provisional tolerable weekly intake (PTWI), which is 5 µg/Kg b.w., of which less than 1.6 µg/Kg b.w. as methyl mercury. People, in particular children, eating considerable amount of fish as a regular part of their diet, *i.e.* those ones who live near the coast, could be at higher risk. Further investigations are necessary to assess the actual level of contamination of individual species and the risk for the population based on the actual consumption of fish, molluscs and shellfish.

W53 MERCURY AND METHYLMERCURY CONTENT, FATTY ACIDS PROFILE, AND PROXIMATE COMPOSITION OF CONSUMED FISH IN CANANÉIA, SÃO PAULO, BRAZIL

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Given the fact that chemical constituents vary greatly from species to species, as well as, from individual to individual of the same species due to differences in seasons, locations, habitat, gender, age and feeding habits, it is important to know the composition and nutritional value of some Brazilian fish species consumed by coastal populations. Cananéia city is surrounded by an important estuarine system that supports a diversity of aquatic life and represents an important food source to the local community. In this study, 53 individuals of the four most commonly consumed fish species by the Cananéia city population, State São Paulo, Brazil, were analyzed to determine contaminants, *i.e.*, total mercury (Total Hg) and methylmercury (MeHg), as well as proximate composition and fatty acid profile. The samples were acquired from local fisheries. The muscle of three carnivorous species, namely, *Centropomus parallelus* (Robalo Peba), *Macrodon ancylodon* (Pescada) and *Micropogonias furnieri* (Corvina), and one planktivorous species, *i.e.*, *Mugil platanus* (Tainha), were analyzed. The determination of MeHg and Total Hg was performed by using Cold Vapour Atomic Absorption Spectrometry (CV-AAS). The analytical methodology was validated by means of certified reference materials, and precision and accuracy were determined accordingly. The fatty acid profile was determined by Gas Chromatography (GC), whilst AOAC methods were used for proximate analysis. The Total Hg averages and ranges for the species analyzed were: 193 µg/Kg (61-712), 191 µg/Kg (56-456); 1,071 µg/Kg (516-2,008) and 33 µg/Kg (<10-98) for *Centropomus parallelus*, *Macrodon ancylodon*, *Micropogonias furnieri* and *Mugil platanus*, respectively. MeHg averages and ranges for these species were: 88 µg/Kg (22-399); 171 µg/Kg (37-452); 652 µg/Kg (227-1,487) and <10 µg/Kg, respectively. Total polyunsaturated fatty acid contents varied from 21.9% in Corvina to 26.4% in Tainha; and total saturated fatty acids from 28% in Pescada to 41.8% in Tainha. These species presented similar proportions of polyunsaturated fatty acids of the ω-3 family (average of 17.8%) and of the ω-6 family (average of 7.0%), while the Corvina species presented the lowest contents of these fatty acids (15.8 and 6.1%, respectively). The results for proximate composition and MeHg and Total Hg for the four fish species analyzed were treated: 1) considering the individuals of the same specie; 2) considering the four species groups. Since Hg in fish muscle is mainly present in its organic lipid soluble form (MeHg), the correlation between MeHg muscle levels and fatty acid profile for each fish species is discussed.

W54 MERCURY, LEAD, CADMIUM AND ARSENIC CONCENTRATIONS IN SEAFOOD FROM THE NORTH ADRIATIC SEA

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Seafood is the main source of exposure to heavy metals and arsenic, though fish consumption is encouraged for its undoubtedly beneficial effects. During 2006 and 2007, 130 seafood samples were taken from a seafood market placed in the North Adriatic Sea. Except for mussels, all the animals were of wild origin. Ten samples were taken for each of the following 13 different species: 4 mollusc species (*Chamelea gallina, Tapes decussatus, Mytilus galloprovincialis*, and *Sepia officinalis*), 2 crustacean species (*Penaeus kerathurus, Squilla mantis*) and 7 fish species (*Merlangius merlangus, Merluccius merluccius, Sardina pilchardus, Solea vulgaris, Mullus barbatus, Engraulis encrasicholus* and *Mugil cephalus*). The species were chosen for their representativeness, being the most consumed and caught in the considered area. After microwave assisted acid digestion the samples were analysed to determine cadmium and lead concentrations by means of graphite furnace atomic absorption spectrometry and by hydride generation inductively coupled plasma atomic emission spectrometry to determine arsenic and mercury concentrations. Cadmium contamination was low in almost all considered species and the mean concentration was only 0.017±0.018 mg/Kg. Only clam, cuttlefish and mullet had higher concentrations of Cd, *i.e.* 0.147±0.058 mg/Kg, 0.134±0.032 mg/Kg and 0.106±0.023 mg/Kg, respectively, within their groups. Lead concentrations were low in each sample of each examined species showing that pollution from this metal is no longer of concern in the Adriatic Sea (overall mean concentration 0.022±0.015 mg/Kg). Even the crustacean and the bivalve molluscs included in this study showed low levels of lead contamination (mean 0.098±0.023 mg/Kg and 0.121±0.034 mg/Kg respectively). Even if the mean concentrations of mercury were quite low (0.257±0.248 mg/Kg), high levels were found only in mullet (*Mullus barbatus*) samples (mean concentration 0.722±0.315 mg/Kg within this species), and in two cases the concentrations exceeded the legal limit set by EU Regulation no. 1881/2006 at 1 mg/Kg for this species. Total arsenic concentrations were in many cases high and the mean concentration was therefore high (11.4±8.46 mg/Kg). In particular, crustacean species had very high levels of this element as shown by squill and prawn (mean 23.3±3.28 mg/Kg and 18.6±4.73 respectively), but it has to be stressed that this element is present mostly in organic, non toxic species in seafood. The results show that seafood is still a relevant source of exposure to some heavy metals, but a good choice of species, made possible by scientific knowledge and risk communication, can significantly reduce this exposure.

W55 MERCURY CONTAMINATION IN MARINE ORGANISMS AT DIFFERENT TROPHIC LEVELS IN THE "MAR PICCOLO" OF TARANTO (IONIAN SEA, SOUTHERN ITALY)

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Starting from the 60's, Taranto has gone through an intense industrialization process and today it represents an important commercial and industrial harbour. The Italian Navy Yard, the Naval Station, mussel farms and industrial settlements (iron and steel factories, petroleum refineries, shipyards) are located there. For these reasons, the city has been identified as a "high risk of environmental crisis" area (M.D. no. 349/86). Later on, law #426/98 classified it as "Site of National Interest" included in the "National Project of Environmental Restoration". In this work we directed our attention to mercury pollution, a serious environmental problem that raised much international concern. The research focused on a particular coastal marine ecosystem, the "Mar Piccolo" of Taranto, an inner, semi-enclosed basin with lagoon features, divided into two inlets (first and second Inlet).

The aim of this study was to investigate the bioaccumulation and biomagnification via combined sediment analysis and stable isotope determinations ($\delta^{13}C$ and $\delta^{15}N$) on different marine organisms, including edible and non-edible species. The starting point of this work was the study of mercury contamination in sediments and the application of speciation techniques in order to identify the different chemical forms and obtain useful information about bioaccessibility. The obtained results showed a serious contamination problem. In fact, comparing the data with the "chemical action levels" (ISPRA Institute), which are specific for Taranto area, mercury raised serious concerns especially in the first Inlet, near the Italian Navy. Speciation analysis indicated that mercury was mainly in elementary form "Hg (0)" (50-70%) and bound to sulphides. Only a little amount was in the form of methyl-mercury (0.1-3%). These species, in the "Mar Piccolo" environmental condition (undisturbed environment, low redox potential, high content of organic matter) are less bioaccessible, but changes of these conditions might determine serious metal release. The bioaccumulation study showed that the gastropod mollusc *Hexaplex trunculus* and the bivalve mollusc *Chlamys varia* had higher mercury concentrations than the bivalve mollusc *Mytilus galloprovincialis.* High percentages of methyl-mercury (50-80%) were found in gastropod molluscs and fishes. The results of the stable isotope analysis suggested that the main contamination route might be represented by sediment ingestions and direct uptake from polluted compartments (*i.e.*, interstitial water) because a higher bioaccumulation was found in benthic species which live in close association with polluted sediments.

W56 MERCURY AND METHYLMERCURY IN SEDIMENTS AND SOFT TISSUE OF *MYTILUS GALLOPROVINCIALIS* FROM TARANTO GULF (IONIAN SEA, SOUTHERN ITALY)

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Mercury is the most toxic heavy metal and its determination in environmental and biological matrices is of utmost importance in order to assess possible risks for environment and man. It is well known that Hg exists in the environment in a large number of physical and chemical species, characterized by different properties which influence and determine both the distribution in an ecosystem and the toxicity towards biota. Although all forms of Hg are toxic, the ecological and human health effects are generally related to the environmental transformations in sediments of inorganic Hg to methyl mercury (MeHg). MeHg is the most toxic form, with bioaccumulation and biomagnification features. Several organisms, including mussels, have been used as indicator of environmental exposure, based on the assumption that mercury contents in the soft tissue of mussels are related to the mercury concentration in the corresponding environment. "Mar Piccolo" of Taranto (Ionian Sea, Southern Italy) represents an example of a marine ecosystem whose biological balances have been progressively modified as a result of anthropic pressure. "Mar Piccolo" is a semi-enclosed basin subject to pollution phenomena, due to both a large industry settlement and to its peculiar morphology, which causes a scarce water circulation. "Mar Piccolo" is a noteworthy economic resource, being the site of intensive mussel farming. Therefore, Hg and MeHg monitoring in sediments and mussels is very important for studying the transfer of the metal and its organometallic form from abiotic to biotic. The aim of this work was to study both the total mercury and methylmercury distribution in sediments and accumulation in mussels (*Mytilus galloprovincialis*) collected at the ten sites of the "Mar Piccolo" with different degrees of contamination, in order to predict the fate of mercury accumulated in the bottom sediment. In sediments, the analytical results showed a high contamination of the basin but a low methylation capacity when compared with the published results concerning other coastal areas of the Mediterranean Sea. Moreover, sediment quality guidelines, such as TEL (Threshold Effect Level) and PEL (Probable Effect Level), were used to evaluate possible toxic effects in the benthic species and will be discussed along with the likelihood of health risks for humans due to mussel consumption. Although an high mercury contamination was determined in sediments, mussels did not show serious levels of the metal, and thus risks for human health due to mussels consumption are very low. The reason appears to be mercury speciation, because the element occurs in sediment principally as a not bioavailable forms.

W57 RISKS AND BENEFITS OF FRESH AND CANNED FISH ON THE SLOVENIAN MARKET

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Fish are an important source of proteins, certain vitamins, minerals and especially n-3 polyunsaturated fatty acids. However, they also contain pollutants such as mercury and polychlorinated biphenyls (PCBs). In order to provide information on the risks and benefits of consumption of fresh and canned fish from the Slovenian market, their levels of total mercury (THg), methyl mercury (MeHg), PCBs, selenium (Se) and n-3 fatty acids were determined. The analyses of THg, MeHg and Se content were performed on all 52 fresh fish and 76 canned fish samples bought on the Slovenian market, while the analyses of fatty acid composition were performed on 20 fish samples and analyses of PCBs were performed on 51 fish samples. THg was determined by chemical digestion and atomic absorption spectrometry using Cold-Vapour Atomic Absorption Spectrometer (CVAAS). The method for MeHg determination was based on isolation of MeHg by evaporation onto cysteine-impregnated paper in micro diffusion cells, followed by extraction into toluene and quantification by Gas Chromatography-Electron Capture Detection (GC-ECD). Chemical digestion and Hydride Generation Atomic Fluorescence Spectrometry (HG-AFS) was used for TSe determination. For identification and quantification of n-3 acids the in situ transesterification method and Gas Chromatography-Flame Ionization Detection (GC-FID) were used. Seven "indicator polychlorinated bipenyls" (congeners 28, 52, 101, 118, 138, 153 and 180) were extracted by the Soxhlet method, lipids were removed by concentrated sulfuric acid and the florisil column was used for cleaning-up. The PCBs were identified and quantified by GC-ECD. The median (Me) levels in fresh and canned fish were 259 mg/100g for n-3 fatty acids, 17 ng/g for PCBs and 82 ng/g for THg. Methyl mercury comprised over 40% of mercury in fish. The percentages of MeHg were highest in organism at the top of the food chain. Selenium levels in fish were reported (Me=421 ng/g), not only because selenium is an essential trace element in human nutrition, but also because it is thought to have a protective role regarding mercury exposure. Although no significant correlation between selenium and mercury levels was found, an excess of selenium in relation to mercury was observed. These results represent an important contribution to the more extensive study on mercury exposure within the framework of the EU-founded project PHIME.

W58 ESSENTIAL AND TOXIC ELEMENTS DETERMINATION IN BRAZILIAN CULTIVATED MUSSEL PERNA PERNA

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Mariculture is an economic activity that is experiencing rapid growth worldwide due to the depletion of natural stocks. In the case of mussel culture, in several countries, like Brazil, Chile, Korea, Spain and Australia, the amount of cultivated organisms is becoming greater than that of those captured in nature. The *Perna perna* is the biggest Brazilian Mytilidae mussel, usually found in the Atlantic coast of South America from Venezuela to Uruguay and one of the most consumed by the Brazilian population. In the present paper, cultivated *Perna perna* mussels from a mussel farm at Cocanha Beach, in the city of Caraguatatuba, state of São Paulo were acquired and analyzed in the four seasons of the year, from 2005 to 2006, in order to assess the levels of some essential and toxic elements and to compare them with the limits prescribed by the Brazilian legislation. To determine the concentration of the studied elements, the following techniques were applied: instrumental neutron activation analysis (arsenic, bromine, chromium, selenium and zinc), epithermal neutron activation analysis (uranium), electrothermal atomic absorption spectrometry (cadmium and lead), and cold vapour atomic absorption spectrometry (mercury). For quality control, the NIST Standard Reference Material 1566b, Oyster Tissue, was analyzed. The results obtained have shown that the concentrations of arsenic, chromium and selenium were above the limits established by the legislation, whereas those of cadmium, lead, mercury and zinc were compliant. There is no specified legal limit for the uranium content in foodstuffs in Brazil. As for the seasonal variation, it was verified that the concentrations of cadmium, selenium and zinc were higher in winter, and those of mercury, in autumn and winter.. Arsenic and chromium were mostly accumulated in springtime and summer. For uranium the content was very similar in most seasons, except for springtime, when the accumulation was lower.

W59 TRACE OF HEAVY METALS IN SMOKED "PROVOLA" CHEESE FROM CALABRIA (ITALY)

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In our previous investigation residual levels of heavy metals in milk and in "Provola" cheese samples from different farm of Calabria were evaluated, to monitor whether the consumption of dairy products can contribute to heavy metal intake in human diet. The aim of this study was to evaluate the presence of heavy metals (Cd, Pb and As) in "Provola" cheese from Calabria, produced using different smoking technologies, with natural methods and with commercial buffered smoke, to evaluate the effect of smoking on heavy metal concentration in cheese. In particular, a comparative study of the residual levels of heavy metals in different portions (rind, exterior zone, core and slice) of non-smoked, naturally and buffered smoked "Provola" cheese samples was carried out. Quantitative determination of heavy metal concentration was carried out by graphite furnace atomic absorption spectrometry, for Cd and Pb, and hydride generation atomic absorption spectrometry for As. The results showed that the highest concentrations of Cd and As was found in naturally smoked "Provola" cheese while for Pb the highest levels were found in buffered smoked "Provola" cheese samples. In all different types of "Provola" cheese samples analyzed, the exterior part was the most contaminated, followed by core and rind; whereas intermediate values were found in the slice (constituted by all portions considered). In addition, a comparative analysis was conducted on the heavy metals concentrations in naturally smoked "Provola" cheese samples, obtained using different wood materials for combustion (oat straw, beech shavings, shrubs, heath, laurel and myrtle, poplar shaving, olive and orange wood). The comparative analysis of the different smoking processes for "Provola" cheese showed that the use of controlled commercial buffered smoke is preferable to traditional smoking procedures for greater food safety; however a standardization of the traditional smoking procedures with natural wood combustion would be advisable.

W60 MONITORING OF POCs, PCBs AND MINERAL COMPONENTS IN DONKEY'S MILK FROM SICILIAN FARMS

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Milk is a basic food in the human diet, both in its original form and as various dairy products. It can carry numerous xenobiotic substances (pesticides, drugs, metals and various environmental contaminants), which constitute a technological risk factor for dairy products, for the related commercial image and, above all, for the health of the consumer. Donkey milk can be used for infants suffering from hypersensitivity to milk protein of some milk animal species (cow, sheep and goat's) including some formulas containing soy or hydrolyzed protein; donkey' milk could be used in case of multiple hypersensitivity. In Sicily the production of donkey's milk for this use is growing. In this preliminary study, the contamination by POCs, PCBs and potentially toxic metals was evaluated in donkey milk collected in autumn 2008 from various farms of Sicily (Italy). The quantitative determination of POCs and PCBs was optimised and performed by HRGC-ECD, whereas HRGC-MS was used for confirmation: most analyzed donkey milk samples showed levels of these contaminants lower than the limits of quantification. Cd (II), Cu (II), Pb (II), Mn (II), Zn (II) and Se (IV) levels were determined using derivative Potentiometric Stripping Analysis (dPSA). In the optimised electro-chemical conditions the accuracy, expressed as obtained recoveries from cow milk, was in the range 89-93%. Metal levels were comparable between cow and donkey milks. Moreover, lead content was lower than the legal limit established for cow milk (EC Regulation no. 1881/2006). EC Regulation doesn't set any limit for residual of toxic substances for donkey' milk. In view of its particular destination, an integration with some limits should be advisable, after sound scientific researches on the topic. Anyway, according to the results of this research, donkey milk could be considered safe from the toxicological point of view.

W61 TRACE ELEMENTS IN SAMPLES OF DIFFERENT TYPES OF STOCK CUBES AND BROTH GRANULATES

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The study was carried out to investigate on the presence of "toxic" and "essential" metals in 28 different samples of cubes and granulates for broth from retail sale. In particular, bovine, poultry and fish meat cubes and granulates samples were analyzed. Quantitative analysis of Cd, Pb, Sn, Cu, and Se, was performed using graphite furnace atomic absorption spectrophotometry; Zn was determined by flame atomic absorption spectrophotometry. As regards "toxic" heavy metals, the highest mean values (MVs) were those of Sn (MV 1.50 µg/g and 1.10 µg/g for granulates and stock cubes samples, respectively); intermediate values were found for Pb (MV 485 ng/g and 141 for granulates and stock cubes samples, respectively), while lower concentrations of Cd were detected (MV 7.11 ng/g and 7.70 ng/g for granulates and stock cubes samples, respectively). With regard to "essential" metals, Zn (MV 1.69 µg/g and 7.19 µg/g for granulates and stock cubes samples) showed the highest concentrations. Lower concentrations were detected for Cu (MV 452 ng/g and 162 ng/g for granulates and stock cubes samples, respectively) and Se (MV 69.9 ng/g and 46.4 ng/g for granulates and stock cubes samples, respectively). At present there are no specific MLs for heavy metals in stock cubes and broths granulates; the EC Regulation no. 1881/2006 only establishes a limit for Pb (ML=0.10 mg/Kg for meat and 0.30 mg/Kg for fish muscle) and Cd (ML=0.050 mg/Kg for meat and fish muscle). For food safety, it would be advisable to establish MLs for the various metals also in derived products such as stock cubes and broths granulates, after some fit-for-purpose scientific researches.

W62 TRACE ELEMENT CONTENT OF NATURAL FOOD RED COLORANT FROM POKEBERRY AND GREEN TEA

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Recently, the number of synthetic food colorants approved for use in food industry has been decreased as a consequence of government safety regulations and toxicity testing advancements. Allergic reactions or intolerant responses have been reported for most azo colorants and in some countries their use in food industry has been restricted and natural colorants are used more often. Therefore, intensive research has been carried out all over the world to identify plant raw materials and to develop technology of production for natural food colorants from them. In addition to pigments, natural colorants may contain valuable biologically active compounds, such as amino acids, aromatic substances, carbohydrates, as well as beneficial trace elements, improving the quality of the final product. The purpose of this work was to determine the chemical constituents, including trace elements, in the red colorant/composite from pokeberry and green tea as well as to investigate its antioxidant activity in order to use it as a biologically active food red colorant. The colorant/composite was prepared by the following procedures: green tea leaves were extracted with water at 70°C; juice of berries of pokeberry (*Phytolacca americana*) was boiled twice to inactivate toxic substances and mixed with the green tea extract. The obtained mixture was filtered, concentrated up to 25% dry matter and spray-dried. The trace element content was determined by flame atomic absorption spectrometry. Antioxidant activity of the colorant was estimated according to the method of the ferric reducing ability of plasma (FRAP). The natural colorant of plant origin is red dry powder with content of dry matter 95%. It was found to consist of phenolic compounds-15.8%, pectin substances-18.0%, free aminoacids-16.3%, reducing sugars-27.0%, organic acids-7.8% and minerals-8%. This plant composite was shown to contain small amounts of toxic elements and radionuclides, in particular 0.02 mg/Kg mercury, 0.04 mg/Kg arsenic, 0.18 mg/Kg cadmium, 32 Bq/Kg cesium-137 and 24 Bq/Kg strontium-90. It also contained 9.5 mg/Kg zinc, 27.3 mg/Kg copper, 400 mg/Kg iron, and 500 mg/Kg manganese. The colorant revealed high antioxidant activity-only about 6 times less antioxidant potential than ascorbic acid. Thus, the natural red colorant from pokeberry and green tea extract is rich in biologically active compounds and beneficial trace elements such as iron, zinc, manganese. It may be used in food industry as a biologically active food red colorant both to colour food products and to increase their nutritional value.

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W63 LEAD EXTRACTED FROM CERAMICS UNDER HOUSEHOLD CONDITIONS

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Lead exposure from ceramics is an old and well-known health hazard. In order to investigate the lead leakage from ceramics at more realistic test conditions and with more realistic test simulants like beverages, the following tests were carried out: A number of white mugs with a volume of 230 ml were prepared with an enamel colour containing lead, mimicing a lead glaze. Red wine, orange juice, coffee, tea with lemon and water were used as test simulants and compared with the standard test solution of 4% acetic acid. Extraction times were chosen to mimic those of normal contact time. Unexpected results were found for *e.g.* tea with lemon, which extracted approximately the same amount of lead as 4% acetic acid in a short time at high temperature. The implications of these tests are that 4% acetic acid in most cases is a realistic worst case extractant, although attention should be paid to other, more realistic, test simulants.

W64 THE EFFECT OF CADMIUM, LEAD AND ZINC AQUATIC POLLUTION ON TOTAL ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN FRESHWATER FISH

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Due to the widespread occurrence of environmental pollution, aquatic biotas are often exposed to anthropogenic factors such as metals, Reactive Oxygen Species (ROS) and pesticides. As fish absorb oxygen from water, they simultaneously take up ROS and toxic compounds polluting this water. This study aimed at finding relationships between total antioxidant status or lipid peroxidation and levels of certain metals (Cd, Pb, Zn) in fish organs and tissues. Additionally, relationships between metal levels in water and in fish tissues were determined. The study encompassed three species of freshwater fish: rainbow trout (*Oncorhynchus mykiss* Walbaum), common carp (*Cyprinus carpio* L.), and Siberian sturgeon (*Acipenser baerii* Bandt). A total of 120 fish comprised 40 individuals of each species, aged from 18 to 24 months, weighing from 776.7 to 1,554.1 g and measuring from 44.1 to 75.6 cm. The fish were cultured in north-western Poland (Western Pomerania region) and were fed an Aller Aqua pelleted feed. The samples submitted for analysis included blood, liver, kidney and dorsal muscles. After blood and tissue samples were wet mineralised in concentrated HNO_3 in a microwave oven, cadmium and lead concentrations were determined by means of flameless graphite furnace atomic absorption spectrometry, whereas concentrations of zinc were measured by means of inductively coupled plasma atomic emission spectrometry. Prior to biochemical analyses, organ and blood samples were homogenized. Total antioxidant status was determined by the $ABTS^{\bullet+}$ reduction assay. Lipid peroxidation was estimated on the basis of malondialdehyde (MDA) concentrations measured by the thiobarbituric acid reactive substances assay. Total protein content in tissue homogenates and blood plasma was determined with the Bradford reagent containing Brilliant Blue G in phosphoric acid and methanol. The accuracy of the methods applied, tested on reference materials and standards, ranged from 90 to 94%. In the examined fish samples, metal levels ranged from 0.002 to 0.022 mg/Kg wet weights (ww) for cadmium, from 0.002 to 0.019 mg/Kg ww for lead and from 11.6 to 98.5 mg/Kg ww for zinc. Total antioxidant capacity ranged from 1.72 to 8.73 nmol Trolox eq.$\bullet$mg^{-1} protein. Concentrations of MDA were within 0.68-6.91 nmol/mg protein. This study revealed that muscle and blood metal levels were positively correlated with ABTS activity and MDA levels (r from 0.989 to 0.998, $P<0.05$, one-way ANOVA). On the contrary, in the liver and kidney, metal and MDA levels were negatively correlated with ABTS activity (r from -0.952 to -0.997, $P<0.05$, one-way ANOVA). The results indicated that aquatic pollution with cadmium, lead and zinc reduces fish antioxidant defence measured by ABTS activity and simultaneously increases lipid peroxidation, especially in the liver and kidney.

W65 EFFECT ON Cu, Mn AND Zn ABSORPTION OF SUPPLEMENTARY *ROSA CANINA* AND PHYTASE TO DIETS FED TO WEANED PIGLETS (10-30 KG)

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A 35 day study on 8 piglets evaluated the effects on Cu, Zn and Mn absorption of using supplementary *Rosa canina* in piglet diets containing phytase. Eight piglets, of average initial weight of 12 kg, kept in individual metabolic cages, were assigned to two groups (C and E) and received the same basal diet (corn and soybean meal) with 18.27% crude protein (CP) and 3300 Kcal metabolizable energy (ME). The diet for group E contained 3% supplemental *Rosa canina* and 0.01% phytase. Both the ingesta and excreta were recorded throughout the experimental period. For each piglet, average weekly samples of the ingesta and faeces were collected and assayed for Cu, Zn and Mn. For Cu, the coefficients of apparent absorption were significantly different, with higher values in group E (C: 53.17%±4.63 and E: 59.58%±2.09, p=0.0453). No significant differences were noticed for Mn (C: 30.84%±4.74 and E: 33.13%±4.32) or for Zn (C: 65.14%±5.25 and E: 64.59%±2.41). Observing the evolution of the experimental group, one can notice the stabilization of the amounts of zinc excreted through faeces after 10 days of treatment, which implicitly stabilized the amounts of Zn absorbed by the animal organism. For the control group, the sinusoidal shape of the chart is characterized by a polynomial equation of the third degree. The conclusion of the study was that the supplemental *Rosa canina* and the phytase improved copper absorption in the organism and stabilized the amount of eliminated zinc leading to a constant absorption of zinc after 10 days of treatment.

W66 QUALITY OF Se-ENRICHED EGGS UNDER THE CONDITIONS OF USING DIFFERENT LEVELS OF DIETARY E VITAMIN

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In order to produce selenium (Se)-enriched eggs, a diet with 2,770 kcal/Kg metabolisable energy and 19.0% crude protein, composed of corn and soybean meal, was supplemented with Se-rich yeast associated to two different levels of dietary vitamin E. The experiment used 80 Lohmann Brown layers (30-36 weeks) assigned to 4 groups (G1-G4, 20 layers per group) housed in batteries. The layers had free access to feed and water. The diets differed by Se and vitamin E levels, as confirmed by chemical analysis of the feeds (for Se and vitamin E, respectively: 0.27 ppm and 26 ppm [G1]; 0.48 ppm and 27 ppm [G2]; 0.28 ppm and 81 ppm [G3]; 0.47 ppm and 82 ppm [G4]). In order to evaluate the physical and chemical characteristics of the eggs, every two weeks, 18 eggs per group were collected randomly and evaluated for the average egg weight; Hugh unit; freshness; yolk and egg white pH; whole egg (egg white+yolk) Se; yolk vitamin E. The experimental data were processed with StatView software. Layer performance (laying percentage, egg weight, feed intake) didn't differ significantly between groups irrespective of the dietary Se or vitamin E level. Egg Se was significantly ($p \leq 0.05$) higher in groups 2 and 4 (0.505±0.08 and 0.511±0.06 ppm Se), which had the highest dietary Se levels, compared to groups 1 and 3 (0.351±0.03 and 0.381±0.04 ppm egg Se). There was a positive correlation between egg Se and dietary Se ($R^2=0.99$). The same strong correlation was also noticed between egg vitamin E and dietary vitamin E ($R^2=0.943$). Only for the level of 81 ppm dietary vitamin E we may say that the dietary Se influenced egg yolk vitamin E level. When using the higher level of vitamin E in the diet, the ratio of the ingested selenium and the egg selenium was 1/39 for G3 and 1/30 for G4. Our results did not show significant differences between groups in terms of Hugh unit or egg freshness.

W67 COMPARISON OF SELENIUM CONCENTRATIONS IN EGGS AND MILK FROM ANIMALS FED SELENITE OR SELENIUM-ENRICHED PROBIOTICS

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The present studies were conducted to compare the effects of an inorganic Se source (sodium selenite) with an organic Se source (selenium-enriched probiotics) on the Se concentrations in whole-eggs from laying hens and milk from dairy cows. Both Se sources were added to the basal diet of laying hens at concentrations of 0, 0.2, 0.5 and 1.0 mg/Kg of Se in experiment 1. Seven hundred, 68-week old, Rohman laying hens were allocated randomly into seven groups and fed with the 7 different diets mentioned above for 30 days. During the experiment, eggs from each group were collected every 7 days. In experiment 2, both Se sources were added to the basal diet of cows at concentrations of 0 and 0.3 mg/Kg of Se. Twenty seven multiparous Holstein cows were divided randomly into 3 groups and fed with the 3 different diets mentioned above. The experiment period was from 1 month prepartum to 3 months postpartum. Milk samples were collected every month. Se assay of the egg and milk samples was performed by Hydride Generation Atomic Fluorescence Spectrometry (HG-AFS). The results showed that the addition of Se from either source caused a significant increase in whole-egg and milk Se concentrations ($p<0.01$). Selenium-enriched probiotics was more effective than sodium selenite in increasing the Se concentrations in whole-eggs and cow's milk. It was concluded that eggs from hens and milk from cows fed with selenium-enriched probiotics are a potential source of Se for humans.

W68 THE EFFECT OF NICKEL PERORAL ADMINISTRATION ON GROWTH, EGG QUALITY AND BIOCHEMICAL BLOOD PARAMETERS IN LAYING HENS

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In this study the effects of nickel administered in drinking water on laying hen body weight, egg production, egg quality and the concentrations of selected blood biochemical parameters (calcium, phosphorus, magnesium, sodium, potassium, glucose, total cholesterol, total proteins, triglycerides, alanine aminotransferase ALT, aspartate aminotransferase AST, gamma glutamyl transferase GGT and glutamatdehydrogenase GLDH) and their correlations in blood serum of Isa brown breed after nickel administration were analyzed. Animals were divided into four groups (K, P1, P2, P3). Experimental hens (5 in each group) received nickel ($NiCl_2$) per os in drinking water (P1-20 mg $NiCl_2$/L; P2-200 mg $NiCl_2$/L; P3-2,000 mg $NiCl_2$/L of drinking water) for 28 days; the control group K (n=5) did not receive nickel. Blood collection was realized at day 0, day 7, day 14, day 21 and day 28. Growth parameters and total egg production during the experiment were significantly decreased mainly in the group with the highest nickel concentration. Egg weight was mainly affected in P3 group. Albumen weight and albumen content were significantly decreased in groups with the highest nickel concentration versus control group. Egg yolk analysis detected significantly decreased yolk weight in P3 group. As for yolk colour, a significant difference was detected between the group receiving 20 and 200 mg $NiCl_2$/mL. Eggshell compactness was increased in all experimental groups, which could be induced by altered mineralization of eggshell. Significant decreases ($P<0.05$) of magnesium and triglyceride between control (1.74±0.28 mmol/L and 20.92±8.13 mmol/L) and P3 group (0.91±0.37 mmol/L and 8.04±8.49 mmol/L) were found. Nickel had only slight effects on other parameters such as energy, enzymatic and mineral profile (statistical results were not significant). A positive high correlation between Ca-ALT (r=0.71), P-ALT (r=0.74) and total proteins (TP)-ALT (r=0.77) in the control group was detected. A negative high correlation between P-K (r=-0.75) and Mg-K (r=-0.73) in group P1 and a positive high correlation between P-cholesterol (r=0.74), Na-ALT (r=0.69) and cholesterol-triglycerides (TG) (r=0.87) in group P1 was found. A positive high correlation between Ca-ALT (r=0.71) and cholesterol-TG (r=0.91) in the group P2 was found along with a positive high correlation in P3 group concerning Ca-TG (r=0.69), Mg-TG (r=0.67), Na-AST (r=0.69) and glucose-AST (r=0.71). Our results may contribute to an evaluation of the reference levels of the analyzed parameters as well as to monitor the health and nutritional status of nickel-administered hens.

T01 RESEARCH ON THE HYPOCHOLESTEMIC EFFECT OF HIGH LEVELS OF Cu GIVEN TO LAYERS

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Even if egg is regarded as the "perfect natural food", particularly due to its high level of quality protein (adequate essential amino acids profile) and to the high digestibility of its nutrients, the fear of cholesterol still affects the average individual consumption. As a consequence, scientists continue their efforts to obtain low cholesterol eggs (below the usual 230 mg cholesterol/egg). Nutrition is one of the approaches. In this direction, we conducted a trial on two groups (C and E) of layers (20 layers per group). The Experimental Diet (ED) was supplemented with copper (Cu) and it had a lower dietary energy level than the Control Diet (CD). Pearce in 1983 demonstrated that copper supplements decreased plasma lipids, 17β-estradiol, liver lipid concentrations and hepatic lipogenic enzyme activities. Mean liver glycolytic and amino acid metabolizing enzyme activities were affected by dietary Cu additions. The experiment had a duration of 4 weeks and it used Lohman Brown layers with the initial age 43 weeks, housed in cages (4 layers per cage) with a 16 hours/day regimen of lighting with light bulbs. The layers had free access to the feed and water. The formulation of both diets was based on corn and soybean meal and had similar protein levels (18, 12 g% and 18, 20 g%, respectively). ED was supplemented with 200 ppm Cu (as CuSO4×7 H2O) and its energy level (metabolisable energy) was 2,453 Kcal compared to 2,700 Kcal for the CD. Throughout the experiment, eggs were collected weekly, in a random manner, and used to form 6 samples (with 3 eggs per sample), assayed for cholesterol and copper level. During the experiment we also monitored feed intake, the laying percentage, eggs weight, layer health state. According to the experimental data, the concentration of yolk cholesterol didn't differ between groups and it also was not significantly lower than the level of 230 mg cholesterol/egg. The egg Cu level was significantly ($p \leq 0.05$) higher in the experimental group (7.354 ppm) compared to the control group (5.069 ppm). To produce eggs with lower cholesterol levels, it might be necessary to add higher levels of Cu supplements and to use a diet with energy level of about 2,100-2,200 Kcal/Kg feed.

T02 RESPONSE IN ACTIVITY OF SELENOENZYME GLUTATHIONE PEROXIDASE TO FEED SUPPLEMENTATION WITH *CINNAMOMUM ZEYLANICUM* OIL IN POULTRY

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The prevention effects of essential oils against oxidative stress have been known for a long time. The aim of this study was to examine the effects of feed supplementation with *Cinnamomum zeylanicum* oil on selenoenzyme glutathione peroxidase (GPx) activity and parameters of oxidative stress in chickens. Thirty two Ross 308 hybrid broilers were divided into four groups with 8 birds in each and fed diets supplemented with 0%, 0.1%, 0.05% or 0.025% cinnamon oil from hatching up to day 38. At the end of the experiment, the activity of GPx was found to be significantly higher in chickens fed the diet supplemented with 0.05% cinnamon in both liver (control *vs* 0.05%: 1.88±0.18 *vs* 2.24±0.27 U/mg protein, $P<0.05$) and kidney tissue (control *vs* 0.05%; 0.76±0.09 *vs* 0.97±0.08 U/mg protein, $P<0.05$). The activity of GPx in whole blood of chickens treated with 0.1% cinnamon was significantly elevated as well (control *vs* 0.1%: 131.9±18.67 *vs* 218.4±23.93 U/g Hb, $P<0.05$). Simultaneously, the intake of diet with 0.1% cinnamon resulted in reduced malondialdehyde level in blood plasma (control *vs* 0.1%: 0.32±0.04 *vs* 0.23±0.01 µmol/L, $P<0.05$) and in duodenal epithelium (control *vs* 0.1%: 0.54±0.03 *vs* 0.37±0.03 µg/mg protein, $P<0.05$). The present results show that feed supplementation with cinnamon oil is able to increase the GPx activity in broiler tissues and blood and by this way to possibly prevent oxidative stress.

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T03 SELENIUM STATUS OF RUMEN MICROBIOTA IN SHEEP FED DIET SUPPLEMENTED WITH SODIUM SELENITE

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The objective of this experiment was to investigate the effect of feed supplementation with sodium selenite on the concentration of selenium, activity of glutathione peroxidase (GPx) and concentration of malondialdehyde (MDA) in total, bacterial and protozoal fractions of rumen fluid in sheep. Ten sheep of Merino breed were randomly divided into two groups with 5 animals in each and fed experimental diets for 5 months. The analysed background content of Se in the Basal Diet (BD) for the control group of sheep was 0.07 mg/Kg Dry Matter (DM). The diet of the experimental group consisted of BD enriched with a Se dose of 0.3 $mg.kg^{-1}$ DM in the form of Na_2SeO_3, resulting in a total analysed Se content of 0.33 mg/Kg DM. The rumen fluid was collected in early morning from fasting animals after 5 months of feeding experimental diets. Long-term intake of the diet supplemented with selenite resulted in significantly increased selenium concentration in both bacterial (3.08±0.28 *vs* 9.72±1.73 µmol/Kg DM, $P<0.01$) and protozoal (5.06±0.54 *vs* 12.21±1.0 µmol/Kg DM, $P<0.001$) fractions of rumen fluid. No significant difference in Se levels was observed in total rumen fraction. The accumulation of Se in rumen microbiota was associated with significantly increased GPx activities in both bacterial (0.06±0.01 *vs* 0.13±0.01 U/mL, $P<0.01$) and protozoal fractions (0.06±0.01 *vs* 0.12±0.02 U/mL, $P<0.05$) of rumen fluid. Simultaneously, the decrease in MDA formation in both bacterial and protozoal fractions (from 2.23±0.03 to 1.62±0.06 nmol/mL, $P<0.001$ and from 2.51±0.14 to 1.1±0.11 nmol/mL, $P<0.001$, respectively) was observed. The results of this study demonstrate that long-term selenium supplementation of feed of ruminants increased the GPx activity and reduced the lipid peroxidation in rumen microbiota.

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T04 BENEFICIAL EFFECT OF DIETARY *CHLORELLA* AND ESSENTIAL OIL PLANT EXTRACTS ON ESSENTIAL TRACE ELEMENT STATUS IN WEANED PIGLETS

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In the last decade Natural Plants Extracts (NPE) have been proposed as possible alternatives to antibiotics as growth promoters for weaned piglets. The possible interference of natural substances with the absorption and metabolism of essential micronutrients represents a subject to be investigated. Within this context, our study evaluated the effect of several dietary Natural Plant Extract (NPE) supplements used as replacement antibiotics on the concentrations of essential trace elements (copper, iron, manganese and zinc) and immunoglobulin subsets (M, A, G) in plasma and organs (liver and spleen) of piglets after weaning. *Chlorella vulgaris* and a mixture of essential oils containing garlic, cinnamon, onion and caraway encapsulated (mixture 1) or not (mixture 2) were added, in proportion of 1% and 0.04%, respectively, to the starter diets providing minimal vitamins and minerals supplements (NRC 1998) (National Research Council). Mineral analysis was done by flame atomic absorption spectrophotometry and immunoglobulin subsets by ELISA. All data are expressed as mean±Standard Error of the Mean (SEM). Statistical differences between groups for mineral concentrations were determined using an ANOVA test. The effect of this addition was tested in an *in vivo* experiment carried out in piglets after weaning for 11 consecutive days. At the end of this period, the results of measurements of the trace element content indicated a positive effect of the investigated plant extracts especially on Cu and Fe. In comparison with controls, a higher accumulation was obtained in spleen>liver>plasma after 11 days of feeding with dietary NPE. Thus, the *ratio* between mineral content in spleen from piglets fed NPE or control diets was 1.33 for Cu and 1.19 for Fe (*Chlorella*), 1.48 for Cu and 1.37 for Fe (essential oil mixture 1) and 1.46 for Cu and 1.52 for Fe (essential oil mixture 2). Positive correlation with the total concentration of plasma IgG were also observed. The present study thus suggests a beneficial effect of dietary NPE supplementation in piglets after weaning, but the optimal rate of NPE inclusion in the diet and the duration of feeding with such supplements need to be further investigated.

T05 SCREENING OF *RHODOTURA RUBRA* STRAINS RESISTANT TO MANGANESE, ZINC AND COPPER FOR THE PRODUCTION OF PROBIOTICS FOR ANIMAL NUTRITION

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The aim of this study was to investigate the effect of salts of various divalent metals, *i.e.*, copper, manganese and zinc, on strains of the yeast *Rhodotorula rubra*. Different concentrations of these trace elements were tested and the mode they were added was optimized. In this way, after the manufacturing process, yields of more than 20 g/L DCW (Dry Cell Weight) of dry biomass, and of 524.17 μg/g dry cells of total carotenoids pigments, expressed as β carotene, were obtained. After isolation of the strains, it was studied which yeasts specialized for producing probiotics were capable to grow in presence of various concentrations of copper, zinc and manganese. A previous study showed that *R. rubra* ICCF 384 tolerated high concentrations of copper, zinc and manganese and formed a static culture on solid medium (preinoculum), which was used to obtain a liquid inoculum, developed at 28°C, for 44 hours, and had an optical density of 22.54 at λ=590 nm. Starting from this inoculum, the probiotic, a yeast enriched with optimal concentration of copper, manganese and zinc as sulfate salts, was produced in 500 mL-flasks with 100 ml medium, glucose as carbon source, an optimal pH (5.0-6.5), incubation at 28-32°C, for 44-48 h. Copper and zinc inhibited the production of the carotenoids pigments when used alone, but they had a positive influence when added along with manganese and iron into the fermentation media. From the experimental data it was observed that the utilization of copper sulfate, zinc sulfate and manganese sulfate did not lower the growth speed, which was approximately the same than without adding the three trace elements. The minerals were non-toxic towards the yeast cells when added in optimal concentrations into the cultivation media. Overall, it was observed that the biomass increase was influenced by the mineral concentration, the mode of addition and by experimental parameters.

T06 OBTAINING CHROMIUM AND SELENIUM ENRICHED YEAST UNDER BIOCONVERSION OF INORGANIC FORMS

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The effect of chromium chloride and sodium selenite on yeast cells during cultivation was studied, in order to obtain a chromium/selenium enriched yeast biomass with a high rate of bioconversion, using *Saccharomyces cerevisiae* strains. The screening model for improving chromium assimilation by *S. cerevisiae* strain comprised cultivating live yeast cells in malt extract suspension containing chromium or selenium at high concentrations, and selecting the best formed yeast colonies by means of the determination of the viability (CFU/mL) of cultures with higher optical density. At the same time the toxic levels of concentration were identified. For the determination of the total selenium and chromium concentrations in Cr- and Se-enriched yeast biomass a Perkin Elmer Elan DRC-e Inductively Coupled Plasma Mass Spectrometer was used. Sample preparation consisted in microwave digestion of 0.5 grams yeast powder with nitric acid. By determining and monitoring the growth cell rate and the carbon source uptake, we succeeded in establishing optimal parameters for obtaining chromium and selenium enriched yeast, *i.e.*, 28°C, agitation at 240 rpm, rate of inoculation 15% and pH 5.0. The best manner for addition of chromium and selenium was at 0 h and 15-16 h, respectively. An automatic Linux based backup system was established in order to prevent data loss for critical analyses. The total chromium concentration in yeast ranged from 200 to 700 mg/L and, for selenium, from 500 to 2,500 mg/L, both accumulated in the same biomass. The addition of selenium during the fermentation had a positive influence on the accumulation of chromium in the yeast. The test substance, orally administered to rats in a single 5,000 mg/Kg body weight dose, didn't cause lethality during a 14 days observational period, so the LD50 value could not be obtained at the end of the experiment.

T07 STUDY OF THE INFLUENCE OF POTASSIUM PHOSPHATE ON *LACTOBACILLUS* STRAINS USED FOR PROBIOTIC PRODUCTION AND CHARACTERIZATION OF THEIR Cu, Zn, Co, Cd AND Fe CONTENT

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This paper presents the biotechnological studies concerning the influence of potassium phosphate added to the cultivation media in order to obtain a high rate of cellular multiplication of selected *Lactobacillus* strains. A set of chemical and microbiological analyses was established, in order to substantiate scientifically also their lactic-acid biosynthetical potential. The previous studies concerning the multiplication of a *Lactobacillus* probiotic strain on media containing honey and milled /non-milled pollen were carried out on a laboratory scale and indicated that milled pollen provided the best results. In order to obtain high yields of bacterial biomass with some selected *Lactobacillus* strains, various cultivating conditions, primarily the composition of culture media, were studied. The purpose of this paper is to present the findings concerning the influence of potassium on the bioprocess results. For the preparation of the starter culture, a single colony from *Lactobacillus* slant agar was transferred into a 200 mL flask containing a 50 mL inoculum medium and left at temperatures of 37-40°C. After 48 h of static cultivation, 10 mL of *Lactobacillus* liquid culture were pipetted aseptically into various culture media (750 mL flask containing 200 mL of liquid medium). All the media were sterilized for 20 min at 120°C. The preinoculum medium contained malt extract, the inoculum and production media contained glucose, peptone and yeast extract. This study focused on the influence of various concentrations of potassium phosphate on *Lactobacillus* biomass production, demonstrating also lactic-acid production on a cheaper media adequate for industrial scale. Moreover, the copper, zinc, cobalt, cadmium and iron total concentrations were determined in the biomass by inductively coupled plasma spectrometry using a Perkin Elmer Elan DRC-e instrument. The obtained results were compared to the international recommended data, in order to establish the possibility of using these strains for a human treatment. Potassium phosphate was found to be an important parameter for the growth performance of cells and its concentration clearly affected this performance.

T08 DISCRIMINATION OF BRAZILIAN *COFFEA CANEPHORA* BY REGION USING TRACE ELEMENT COMPOSITION

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The most common coffee species in the world are *Coffea arabica* and *Coffea canephora*. The economic importance of *C. canephora* has recently increased due to its broad adaptation to tropical climate conditions, higher tolerance to diseases, productivity and amount of soluble solids than *C. arabica*. For these reasons, *C. canephora* is currently more profitable than *C. arabica,* representing about 36% of total global consumption, with an uprising trend (40%) in the forthcoming years. The mineral composition of coffee may vary considerably according to genetics, altitude/climate, soil, agricultural practices, and harvesting season. Moreover, mineral content along with other chemical components, characterize the beverage's flavor. Therefore, the trace element composition has been considered as a promising tool (*fingerprint*) for origin discrimination of *C. arabica*. The aim of this study was to investigate the possibility of using trace element composition to discriminate Brazilian samples of *Coffea canephora* produced in different regions. Thirty coffee samples from three regions (ten from each-North, Northeast and South) were collected. 40% of the samples from each region were harvested at the early season and 60% at the mid-season. The analyses of biologically essential and toxic elements (17 in total) were carried out by ICP OES. PCA and discriminant analyses were carried out to compare the variables. Statistical analysis revealed two distinct groups. Group 1 contained samples from the North (harvested at early-season) and Northeast regions, and group 2, contained samples from North (harvested at mid-season) and South regions. Higher concentrations of Sr, Zn, Ba, B, Bi and total trace elements were observed in group 1, compared to group 2. The Northeast and South regions have very different altitude, climate (temperature, humidity, *etc.*) and soil composition. Samples from group 1 grew under warm temperatures, while samples from group 2 grew at different temperatures, but under high humidity conditions. In conclusion, the trace element composition of Brazilian *Coffea canephora* differed by region and appeared to be associated with the climate and period of harvesting, and other environmental factors to be studied further. The implications of variations in trace element composition on the sensorial aspects of the coffee beverage should also be investigated.

T09 WHAT DO METALS TELL US ABOUT WINE?

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Wine is a product of yeast fermentation of natural sugars present in grapes. In fact, it is a complex matrix, which, besides water, sugar and alcohol, contains a great variety of components, inorganic as well as organic. Its composition depends on many diverse factors such as grape variety, soil and climate, culture, yeast type, winemaking practices, transport and storage. Since wine is one of the most widely consumed alcoholic beverages in the world, it is crucial to estimate its quality. Determination of food authenticity is one of the most important issues in food quality control and safety. It is possible to find parameters that could differentiate wines and one of such criteria is their metal content. Metals of primary natural origin come from soil on which vines are grown and reach wine through grapes. Therefore, it appears that minerals could be employed for the identification of geographical origin of wine. The purpose of our study was to verify the quality of the chosen red wines based on their mineral composition. Moreover, the human exposure to metals due to wine consumption was assessed. The total elements (Mg, Ca, Na, K, Zn, Cu, Fe, Mn, Co, Cr, Ni, Cd, Pb) contents in different types of red wines obtained at retail were analysed by flame atomic absorption spectrometry (F-AAS) using deuterium-background correction. Phosphorus was determined in the form of phosphomolybdate by a spectrophotometric method. Reliability of the procedures was checked by the analysis of the certified reference materials Tea (NCS DC 73351) and Cabbage (IAEA-359). Recoveries of the elements analysed varied between 74% and 105% and precisions for the reference materials were in the range 0.00-21.8%. The concentrations of the analysed macro- and microelements in red wines varied significantly among samples. Based on RDA estimated for essential elements, it was concluded that semi-sweet wines supply the body with higher amounts of bioelements than the other analysed wines. In the case of toxic elements, no health hazard associated with exposure to Cd and Pb via red wine consumption exists based on comparison with the Provisional Tolerable Weekly Intakes (PTWI) for the two elements. Multivariate analyses (Kruskal-Wallis ANOVA, factor analysis) of the multielemental data of wine indicated differences between particular samples and categorised them according to their type (dry, semi-dry, sweet and semi-sweet wine). It was also possible to notice the influence of the geographical origin of wine on Zn and alcohol content.

T10 THE APPLICABILITY OF CHEMOMETRIC TECHNIQUES IN ASSESSING THE DIFFERENCES IN QUALITY OF VEGETABLE PRODUCTS

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The term vegetable generally means the edible parts of plants. Vegetables are eaten in a variety of ways as a part of main meals and as snacks. Frozen and canned vegetables can be just as healthy as fresh ones because they are packaged at the peak of freshness and retain most of nutrients. However, canned vegetables may be loaded with sodium. Compositions of vegetables vary according to botanical variety, cultivation practices, and weather, but also change with the degree of maturity prior to harvest, and the condition of ripeness, which is progressive after harvest and is further influenced by storage conditions. Generally, vegetables are rich in water soluble vitamins like vitamins B and C, fat-soluble vitamins including vitamins A and D, and also carbohydrates and minerals. They are high in water content, low in protein and fat. The purpose of our study was to verify the quality of different vegetable products based on their mineral composition using chemometric techniques. The examined mineral components (Mg, Ca, Na, K, Zn, Cu, Fe, Mn, Co, Cr, Ni, Cd, Pb) were analysed by flame atomic absorption spectrometry, except phosphorus which was determined by a spectrophotometric method. Reliability of the procedures was checked by the analysis of the certified reference materials Tea (NCS DC 73351), Cabbage (IAEA-359) and Spinach leaves (NIST-1570). The recovery obtained varied between 85 and 103% and precisions ranged from 0.13 to 13%. Based on the analysed elements concentrations, the intakes of elements via vegetable products were calculated and compared to the RDAs and PTWIs. Differences between each group of products (fresh, processed) were estimated. Moreover, application of techniques such as Kruskal-Wallis ANOVA and factor analysis allowed us to categorise vegetables according to their type, botanical classification and level of technological processing.

T11 APPLICATION OF MULTIVARIATE METHODS TO QUALITY EVALUATION OF RICE

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Most of the rice that is consumed today is polished, which makes it to appear white in colour. There is however a growing trend for the consumption of the rice type commonly called brown rice. Every rice seed has a structure that is formed of a stiff outer layer called husk, an inner brown layer that is called bran and a white coloured seed under the bran. The husk is always removed from both white and brown rice and it is the inner bran layer, which is either retained fully or partially removed, that gives rice the brownish colour. Whole grains (such as brown rice) contain high amounts of fibre, antioxidants, phytonutrients, vitamins and minerals. There is evidence to suggest that regular consumption of cereals, specifically wholegrain, may have a role in the prevention of chronic diseases. The strength of evidence varies and although cause and effect have not currently been established, people who consume diets rich in wholegrain cereals seem to have a lower incidence of many chronic diseases, *e.g.* coronary heart disease and type 2 diabetes. The aim of this work was the application of multivariate statistical methods in order to evaluate the rice products quality based on their mineral composition. The examined mineral components (Mg, Ca, Na, K, Zn, Cu, Fe, Mn, Co, Cr, Ni, Cd, Pb) were analysed by flame atomic absorption spectrometry. Phosphorus was determined in the form of phosphomolybdate by a spectrophotometric method. Reliability of the procedure was checked by the analysis of the certified reference materials Tea (NCS DC 73,351) and Cabbage (IAEA-359). The recoveries obtained varied between 85 and 103% and precisions were in the range 0.41-19.4%. Application of techniques such as Kruskal-Wallis ANOVA and factor analysis was helpful for a deeper understanding of the distribution of selected elements in the analysed rice products. Based on factor analysis data, it was possible to record the influence of the analysed elements on the factorial distribution of particular object samples and classify rice samples according to their country of origin (China, Guyana, India, Japan, Pakistan, Thailand, USA, Vietnam, Italy) and type (basmati, long grain, short grain, red, wild, natural and parboiled rice).

T12 CHOCOLATE-IS IT ONLY A SWEET THING?

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The word "chocolate" comes from the Aztecs of Mexico, and originated from the Nahuatl word xocolatl, which is a combination of the words, xocolli, meaning "bitter", and atl, which is "water". The Aztecs associated chocolate with Xochiquetzal, the goddess of fertility. Chocolate is the product generally made from cocoa butter or its mixture with another fat (*e.g.* from milk), cocoa, sweeteners and in some cases from milk (*e.g.* powdered milk). The most important stages of the chocolate production technology are roasting of cocoa beans, conching, and tempering. The final product is characterized by a unique taste, flavour, and texture. Its attributes and physicochemical properties, including rheological ones, are considerably affected by specific features of the continuous phase of chocolate, *i.e.* cocoa butter, and the quality of cocoa beans. While chocolate is regularly eaten for pleasure, there are potential beneficial health effects of eating chocolate. It is a source of magnesium and many other biologically active substances such as polyphenols and tocopherols. On the other hand, eating large quantities of any energy-rich food such as chocolate increases risk of obesity. The aim of our study was to assess the quality of cocoa and different chocolates based on their mineral composition. The total elements (Mg, Ca, Na, K, Zn, Cu, Fe, Mn, Co, Cr, Ni, Cd, Pb) contents in different types of chocolates obtained at retail were analysed by flame atomic absorption spectrometry (F-AAS) using deuterium-background correction. Phosphorus was determined in the form of phosphomolybdate by a spectrophotometric method. Reliability of the procedure was checked by the analysis of the certified reference materials Tea (NCS DC 73351) and Cabbage (IAEA-359). The recoveries obtained varied between 85 and 103% and precisions were in the range 0.13-13%. Techniques such as Kruskal-Wallis ANOVA and factor analysis were used to classify products according to their type (cocoa, instant cocoa, bitter and milk chocolate) and origin (Poland, USA) based on the concentrations of the analysed elements. Due to factor analysis data, it was possible to record the influence of the analysed bioelements on the factorial distribution of particular object samples.

T13 TRACEABILITY OF ELEMENT CONTENT DURING PROCESSING OF COW MILK

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Milk and milk products are very important for humans mainly due to their mineral content. Since these minerals, especially calcium, are essential for our constitution and in case of deficiency different diseases can appear, regular consumption of milk products is recommended. In Hungary, milk products are produced mostly from cow milk. This is the reason why we decided to study how the element content changes during processing of cow milk. To make samples suitable for analytical determination, we applied an atmospheric wet digestion sample preparation method using concentrated HNO_3 and H_2O_2 solutions. The concentrations of elements were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). We have found that, after skimming, most of the micro and trace elements (*e.g.*, Al, Cd, Cr, Cu, Fe, Li and Mn) remained in the cream and the rest was found in the skimmed milk (*e.g.*, B, Ba, Sr, Ti and Zn). Examining the distribution of macro elements (Na, K, Ca, Mg, P and S), a significant difference appeared to the advantage of skimmed milk: most of each macro element remained in the skimmed milk. We also studied the patterns of element content during cheese production. In our experiment the two most suitable Hungarian types of cheese (Hajdú and Trappista) and the whey from their production were examined. We found that micro and trace elements, except B, accumulated in the cheese, while macro elements, except K, did so in the whey.

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T14 EFFECT OF MOLYBDENUM AND SELENIUM CONTENT IN MINERAL SOLUTION AND SOIL ON ELEMENT UPTAKE BY VARIOUS PLANTS

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Molybdenum is not a frequent element. This microelement ranks 38th in terms of abundance on earth. Its essentiality is known since 1938. Molybdenum is generally present in food or feed at low concentrations, however under certain circumstances molybdenum concentration can be increased and reaches potentially toxic levels. Selenium is one of the most investigated microelements, as it has an important role in many physiological processes, not least its antioxidant effect. Deficiency of selenium can result in serious diseases (including cancer). A large part of soils in Europe are low in selenium. To investigate the effects of molybdenum and selenium as anthropogenic pollution agents, we have carried out molybdenum/selenium-load experiments. These experiments were performed either using nutrient solutions (hydroponic), or in rhyzoboxes or in an element-load long-term field experiment. The aims of the research programme were: 1) to study the uptake of various elements by various plants applying increasing concentrations of molybdenum and selenium in soil or in nutrient solution; 2) to examine the transformation of selenite into selenate; 3) to study the leaching of molybdenum and selenium into the soil deeper layers. The plots of the field experiment were treated with molybdenum or selenium in different ways (4 levels, 2 replicates) using ammonium-molibdate and sodium selenite, respectively. Various edible and non-edible plants were grown in the field experiment: maize (1991), carrot (1992), potato (1993), peas (1994), redbeet (1995), spinach (1996), winter wheat (1997), sunflower (1998), garden sorrel (1999), winter barley (2000), rape (2001), poppy (2002), mustard (2003), alfalfa (2004). Moreover, a monocotyledonous (maize, *Zea mays* L.) and a dicotyledonous (sunflower, *Helianthus annus* L.) were grown in rhyzoboxes and hydroponic experiments and exposed to molybdenum. Altogether, nitrogen and selenium species, molibdenum, and other 45 elements were analysed in plant tissues by FIAS, HPLC-ICP-MS, ICP-OES and ICP-MS. On the basis of our results, different relationships among the examined elements were identified. Other effects, including transformation of selenite to selenate and leaching of selenium and molybdenum into deeper soil layers were evaluated and will be discussed.

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T15 INVESTIGATION OF MOLYBDENUM IN SOIL-PLANT SYSTEM

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Molybdenum is not a well-known microelement, but being a constituent of several important cellular enzymes it is an essential microelement. Molybdenum occurs in all foods, but at very low levels. There does not appear to be any particular foods or types of foods, which in the absence of extrinsic factors, naturally have high levels of molybdenum. However, environmental pollution, from natural or anthropogenic sources, can lead to high level of the metal in plants. Our study is based on the long-term field experiments of Nagyhörcsök (located between Lake Balaton and Danube River), where 'everyday' soil contamination conditions are simulated. Soil and plant samples were collected from the experiment station to study the behaviour of molybdenum: total concentration, available concentration, leaching, transformation, uptake by and transport within the plants, accumulation in different organs, phytotoxicity and effects on the quantity and quality of the crop. In this work we would like to focus on the experiments made with maize (grain and straw), and peas, as well as with the related soil samples. According to our data, molybdenum leaches from the topsoil at a medium rate and it appears in the deeper layers. In the case of plant samples, we found that the molybdenum concentration in the straw is several times higher than in the grain. The concentration of molybdenum in maize grain from the untreated calcareous chernozem was 0.16 mg/Kg, whereas maize grain in the plot with the highest level (810 Kg/ha molybdenum) contained 15.7 mg/Kg molybdenum. The respective molybdenum concentrations in the case of peas were 2.6 mg/Kg (untreated) and 181 mg/Kg (the highest level). Therefore, molybdenum is mobile both in soil and in plant tissues. Furthermore, molybdenum accumulated both in the vegetative and in the reproductive organs of plants.

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T16 TRACE ELEMENTS IN HUNGARIAN HONEYS

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Seven trace elements were determined in 75 honey samples by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). 85 percent of these honey samples come from different regions of Hungary, whereas the remainders come from different countries, including Greece, France, Croatia, Finland, and Turkey. The examined samples had different botanical origin (acacia, linden, rape, forest, coriander, chestnut, fruit, multifloral, *etc.*) and were collected from different beekeepers and hypermarkets. The aim of our study was to determine the levels of aluminum (Al), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn). Samples were digested with HNO_3-H_2O_2 and analysed by ICP-OES, using a Optima 3300 DV instrument. Iron, manganese and zinc showed the highest levels, with average concentrations ranging between 0.27-15.24 mg/Kg for iron, 0.02-14.15 mg/Kg for manganese and 0.45-12.20 mg/Kg for zinc. In case of aluminum, chromium, copper and nickel the average concentration was always lower than 1 mg/Kg. The contents of these elements were in the range of 0.57-4.42 mg/Kg, 0.02-0.47 mg/Kg, 0.19-1.47 mg/Kg, 0.02-0.84 mg/Kg for aluminum, chromium, copper, and nickel, respectively. Overall, chromium had the lowest concentration and iron was the most abundant element in Hungarian honeys as far as the examined elements are concerned.

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T17 HEAVY METAL CONTENTS AND PHYSICOCHEMICAL CHARACTERISTICS OF SAUDI ARABIAN FLORAL HONEYS

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In Saudi Arabia, honey is intrinsic to culture. Whether used for its medicinal value, as a restorative or simply for sweetening, a Saudi family consumes 1 Kg of honey per month, on average. While honey is used daily, consumption is greater during traditional, religious and festive occasions. Saudi statistics show that Saudi Arabia produces almost 90 tons of honey a year, but only 2-4% is consumed there. In the present work, the quality of floral honey produced in Saudi Arabia was evaluated. All samples were analyzed for selected heavy metals and physicochemical parameters like moisture content, acidity, ash, pH and conductivity. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was used to determine the content of heavy metals and analytical methods recommended by International Honey Commission (IHC) were used to measure physicochemical parameters. Among heavy metals, Fe and Zn were detected in all samples, with mean levels of 2.65 μg/g and 0.77 μg/g, respectively. Nickel was detected only in 20% of the analyzed samples. The heavy metal contents of Saudi Arabian floral honey were either comparable or lesser than internationally reported values. Therefore, it can be regarded to be safe for human consumption with regard to its heavy metal content. All samples showed proper maturity, considering the low moisture percentage. The total acidity, below 50 meq/Kg, in all samples indicated the absence of undesirable fermentation. Also, the mean pH around 4.0 was usual for floral honeys. The mean values for ash and conductivity (0.33% and 402 μS/cm) were within the European Community standards.

T18 THE INFLUENCE OF SUNFLOWER SEED ON THE ELEMENT CONTENTS OF SUPPLEMENTED WHEAT-BASED PRODUCTS

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The role of minerals in human health cannot be emphasized enough since they are necessary for major biological processes and they are essential for the maintenance of health. Unfortunately, it has been estimated that over 60% of world's population is iron deficient, over 30% is Zn deficient and about 15% is Se deficient. Deficiencies of Ca, Mg and Cu are also common. The problems of dietary malnutrition are usually addressed through supplementation, food fortification or dietary diversification. Wheat-based breads have been a staple food in the diet of over half the world's population for centuries and this nearly ubiquitous consumption of bread places it in a position of global importance in international nutrition. It could be said that bread represents an irreplaceable food for humans without risk of overconsumption regardless of the sex and the social status of each individual. Therefore, it could be an ideal supplier of nutrients either in the case of nutrients fortification or supplements of plant origin. However, it is questionable that the fortified nutrients are as natural or health promoting as the nutrients that are taken out of the wheat during the refinement process. To improve the nutritional composition of bread, particularly of the one made from white flour, various supplements of plant origin (oil seeds, flaxseed, bran, germ) are being included in the basic formulation; such diversity in bread types makes them an ideal supplier for various nutrients. Although sunflower seeds have been evaluated as food ingredient in numerous studies, detailed information on the improvement of the nutritional profile of the supplemented products are rather scarce. The objective of this study was to assess the effect of high-oleic sunflower seeds supplementation of bread and cookies made of white and wholegrain wheat flour on the element content in the final products. The obtained results were compared with literature data and the relevant maximum allowable contents set in Serbia and EU. The adequacy of these products in meeting the Recommended Dietary Intakes for minerals (K, Mg, Ca, Fe, Cu, Zn, Se) was also discussed. Apart from being a superior nutrient source, sunflower seed has been reported as one of the crops that tend to naturally accumulate cadmium. To assess the potential risk consumers might face when consuming sunflower seed supplemented products, content of heavy elements (Cd, Pb, As, Hg) in the supplemented cookies was also studied.

T19 ESSENTIAL AND NON-ESSENTIAL TRACE ELEMENTS IN SAUSAGES AND EGGS

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Foodstuffs of animal origin are frequently controlled for their content of potentially toxic trace elements, such as As and the metals for which maximum permissible levels have been set within the EU (Cd-Pb-Hg). The digestates from previous analyses carried out for the official control of foodstuffs have been used for the determination of several other elements as well. Samples from former studies, *i.e.*, uncooked meat, liver and kidney, were investigated. Furthermore, the investigation was extended to sausages and hens' eggs. Eggs were separated into egg white and egg yolk, and both matrixes were freeze-dried. Since these samples were homogenous, subsamples could be easily obtained and digested with aqueous $KClO_3$-HNO_3 in pressure bombs by microwave heating. In addition, the feedstuffs of the laying hens were analyzed as well in order to detect whether any clear transfer of trace elements occurred. The sausage samples were homogenized by an Ultra-Turrax mixer and digested with HNO_3-H_2O_2-$HClO_4$ in open vessels. The sausages were grouped into 4 categories according to the Codex Alimentarius Austriacus. Referring to the daily allowances issued by the German Society for Nutrition, 1 Kg of meat of any kind fulfils the requirements for zinc and cobalt. The same for molybdenum from liver, On the other hand, the input of Ca-Cu-Fe-Mg-Mn is low. Sausages contained more Na, Li and Sr than meat, liver and kidneys (Na was 0.95% in dry mass on average). Egg white and egg yolk yielded significantly different element patterns. Eggs contained significantly more Ba, Sr, Cu, and Mn than meat. Iodine in egg white constantly was at a low level, whereas iodine increased in egg yolk with increasing iodine content of the feedstuff.

T20 TOXIC ELEMENTS AND THEIR SPECIES ASSOCIATED TO FOOD CONSUMPTION

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Chronic exposure to high concentrations of heavy metals may affect human health, thus causing several diseases. One of the major tasks of our laboratory is to evaluate the heavy elements contamination of foodstuffs. Examples of the most commonly analyzed among foodstuffs are herbal remedies, such as slimming tea, bread, sweets, food supplements, and drinking waters. Analyses are performed by using ICP-MS and ICP-OES for the determination of total element content, and HPLC-ICP-MS for the determination of element species. Samples preparation, when required, is carried out by using pressurized microwave digestion, and for each digestion batch Certified Reference Materials (CRMs) are included in order to keep control of the full analytical procedure. The laboratory works under QC protocols and routinely participates to FEPAS tests. Several of the herbal samples tested were found to contain high concentrations of arsenic, lead, cadmium, and mercury. Some items contained high levels of only one toxic element, but some were found to contain all the toxic elements at substantial concentrations. Some of the bottled drinking waters and well waters were found positive to heavy metals and also to bromates. Bromates are disinfection by-products that are formed when ozone reacts with naturally occurring bromide in drinking water. Bromate is a known animal carcinogen, also listed as a group 2B toxin, *i.e.*, probable human carcinogen. Bromates in bottled drinking waters and well waters were determined by HPLC-ICP-MS. Samples were injected directly without any further pretreatment or dilution. Bromates were found to be present in about 60% of the tested samples, and some of them at concentrations in the range 2 to 20 times the US maximum allowed concentration (10 µg/L).

T21 DYNAMICS OF NUTRIENT ELEMENTS UPTAKE BY GARDEN STRAWBERRY (*FRAGARIA X ANANASSA* DUCH.) DURING THE VEGETATION PERIOD

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Fruits have their irreplaceable position in a balanced human diet. Vitamins and antioxidants are most frequently mentioned when the health contribution of fruits is discussed. Garden strawberry belongs to those species with a high content of vitamin C in their fruits (300-1,000 mg per Kg of the fresh fruits). Moreover, low contents of all types of sugars (especially sucrose) make them attractive for diabetic people. However, strawberry plants are characterized by high demands on water and nutrients during vegetation. A suitable amount of available nutrients is necessary especially in the period from the spring burst till the fruit harvest. This period represents from 2 to 3 months in Central European conditions. In our experiment the dynamics of macro- and micro nutrient uptake (B, Ca, Cu, K, Mg, Mn, N, P, Zn) was investigated between the beginning of spring burst till the end of the harvest. Three varieties of strawberry (Korona, Elsant, and Darselekt) were studied in field conditions in two following vegetation periods. The aboveground biomass was sampled in different vegetation stages and ripe fruits were collected, as well. The dynamics of dry matter development are related to the dynamics of nutrient uptake by strawberry plants. The uptake of all the nutrients increased significantly during the vegetation period. The strawberry fruits were the main sink of nitrogen, phosphorus, and potassium. Whereas the predominant portion of calcium at the end of harvest was contained in leaves, magnesium was incorporated equally in the leaves and the fruits. The highest uptake was reported for potassium (1,200 mg per plant), followed by nitrogen (1,100 mg per plant), and calcium (560 mg per plant). Significant differences in nutrient uptake were observed among the individual varieties, as well. The effect of soil conditions, especially pH, was studied in relation to element uptake by strawberry plants.

T22 SELECTED ELEMENTS IN INFUSIONS OF TEA, COFFEE AND OTHER PLANT SPECIES

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The most widely consumed drink after water, which plays a major role in the intake of a number of nutritional and potentially toxic trace elements in humans, is tea. The only element presented in significant dietary amount in tea is manganese. Coffee and milk are considered as the 2 top contributors of boron in human nutrition, representing up 12% of its total intake. Therefore, we decided to evaluate the amounts of B, Cu, Fe, Mn, P, Zn, and Ca, K, Mg in beverages by inductively coupled plasma optical emission spectrometry and atomic absorption spectrometry. Hot water infusions prepared from 35 samples of common (tea and coffee) and less common (mate, rooibos, honeybush, and chamomile) plant preparations as well as other plant sources (hibiscus, rose hip, ginkgo and lemon grass) were studied. Maté and hibiscus infusions showed the highest amounts of elements (B, Mg, Mn, Ni, Zn, B, Ca, Cu, Fe, K, Mg, Mn), followed by chamomile and ginkgo (B, Ca, Cu, Fe, P and B, Ca, Cu, Mg respectively). Compared with tea, the contents of all elements analysed were quite low in rose hip, lemongrass rooibos and honeybush samples. Maté was found to be a rich source of elements in the human diet and its content of Mn (over 15 mg/L) can cover the whole recommended daily allowance (RDA; 2-5 mg/day). High concentrations of B were also found, which exceeded the levels found in coffee and could provide the body with amounts of this element equal to several times its RDA. The detected amounts confirm that the daily consumption of about 1 litre of certain infusions (*e.g.* maté green or yerba) could provide higher amounts of elements than tea or coffee and help to meet the human RDAs of certain elements.

T23 ALUMINIUM IN TEA AND OTHER PLANT STIMULANTS AND THEIR INFUSIONS

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The tea plant (*Camellia sinensis*) is a natural aluminium accumulator. Consumption of infusion prepared from its leaves is one of the major sources of Al in natural human diet and can more than double an individual's intake. During the last decades, tea is often replaced by other plant stimulants available from the world market. Therefore we decided to evaluate the amounts of Al in dry materials and infusions of 35 samples of traditional plant stimulants (tea and coffee), non-traditional plant stimulants (maté-*Ilex paraguariensis*, rooibos-*Aspalathus linearis*, honeybush-*Cyclopia intermedia* and chamomile-*Matricaria chamomilla*) and certain tea supplements (hibiscus-*Hibiscus sabdariffa*, rose hip-*Rosa canina*, ginkgo-*Ginkgo biloba*, lemon grass-*Cymbopogon citratus*, *Aloe vera* and ginseng-*Panax ginseng*). The aluminium content was determined using inductively coupled plasma optical emission spectrometry with axial configuration. The results show that the contents of all species were significantly different from that of tea. The highest amounts in the raw materials (1,860 and 1,000 mg/Kg) were determined in coffee and chamomile, respectively. The conversion seems not to be as dependent on the raw material content as on the plant species, because tea (exceeding 2 mg/L) followed by *A. vera* and hibiscus (both around 1 mg/L) were the plants found at highest concentrations in infusions. A high daily consumption of certain infusions prepared from plant material researched during this study could reach the maximal safe upper intake Al limit, set by WHO at 5 mg/L per day.

T24 ELEMENTAL COMPOSITION OF DIFFERENT PAKISTANI WHEAT VARIETIES

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In Pakistan wheat varieties are grown over a wide agro-climatic range and are expected to exhibit yield and quality differences in term of elemental composition. This study reveals important differences in elemental composition of wheat varieties available in Pakistan. Thirteen wheat varieties were collected from different ecological regions and were analyzed for minerals such as Ca, Mg, Na, K, and P, and trace elements such as Zn, Fe, Ag. The main analytical techniques were flame photometry, UV-Visible spectrophotometry and atomic absorption spectrometry. Results showed that wheat grains of different varieties contained 77.65~84.25 ppm of K, 7.70~35.90 ppm of Na, 29.75~42.95 ppm of Zn, 65.45~214.95 ppm of Fe, 1.65~2.5 ppm of Ag, 383~1488.5 ppm of Ca, 72~1324.5 ppm of Mg, and 0.24~0.84 ppm of P. Results suggest that different wheat varieties vary significantly in regards to their Na, K, P, Zn, Fe, Ca and Mg content, while little variation is observed among the varieties regarding Ag. The relation between grain hardness and elemental composition is also investigated.

T25 EVALUATION OF THE EFFECT OF INDUSTRIAL POLLUTION ON THE MICROELEMENT PROFILE OF WALNUT AND HAZELNUT CULTIVARS AND IMPLICATIONS FOR HUMAN HEALTH

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The effect of industrial pollution on the microelement profile of walnut and hazelnut cultivars grown near the "Lead and Zinc Complex Plc" plant (Kardzhali, Bulgaria) was studied. The investigated varieties of walnuts were Pedro (American cultivar), Shejnovo (Bulgarian cultivar), and Oreshinovo (Bulgarian cultivar, local form). The analysed hazelnuts were of the following types: *Corylus avellana* L. (Common hazel, Tonda Gentile and Uebov), *Corylus maxima* Mill. (Filbert, Badem), *Corylus pontica* C. Koch (European Hazel, Furfulak and Anna), and *Corylus colurna* L. (Turkish Hazel). The samples were lyophilized and digested with nitric acid (65%, m/v) and hydrogen peroxide (30%, m/v). The analytical techniques used were flame Atomic Absorption Spectrometry (AAS), electrothermal AAS, hydride generation AAS, and atomic emission spectrometry with inductively coupled plasma. The applied procedures were interlaboratory validated. The influence of environmental pollution on the different nut varieties was assessed. The mean contents of lead, zinc, cadmium, arsenic, antimony, iron, copper, manganese, nickel, chromium, arsenic and selenium were compared with the respective concentrations in walnuts and hazelnuts harvested in uncontaminated regions. The implications of the consumption of the studied nuts for human health are discussed as concerns both the importance for human nutrition and the potential toxicity.

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T26 DETERMINATION OF TRACE AND MAJOR ELEMENTS IN SOME MEDICINAL HERBS

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The use of medicinal plants as healing agents is as old as the presence of human being on earth. Nowadays, medicinal plants are still consumed all over the world, although it is possible for us to prepare almost every kind of drugs industrially. According to the World Health Organization, medicinal herbs are been widely used even in the industrial countries as a complementary way to cure and prevent diseases. It is well known that trace elements play a very important role in the formation of the active chemical constituents present in some medicinal plants. Therefore, they may be responsible for their medicinal properties, but depending on the element, its concentration and its bioaccessibility, they can exert toxic properties as well. In any case, these elements show high biological interest, because some of them are essential for normal growth and development of humans and the others can be toxic. This work focuses on the content of trace elements in some widely consumed herbs in Greece, such as *Tilia cordata Mill.*, *Matricaria chamomilla L.*, and *Salvia officinalis L.*, in an effort to evaluate their nutritive value. These are most popular herbs and were analyzed by Instrumental Neutron Activation Analysis (INAA), which is a very convenient method for analyzing trace elements in all types of samples. Therefore, samples from two different brands of every medicinal herb, bought from the local market, were irradiated for 5 minutes in the research reactor of the Nuclear Research Center "Dimokritos" in Athens, using a thermal neutron flux of $1.2x10^{12}$ $n.cm^{-2}.s^{-1}$. The precision and the accuracy of the results were evaluated by analyzing the reference materials Peach Leaves SRM 1,547 and Orchard Leaves SRM 1,571, both provided by NIST. Two hours after the irradiation, the corresponding gamma-spectrum for each of the activated samples and standards was recorded for 3 s, using an HPGe detector, (CANBERRA, GC2818-7500SL), coupled to a multichannel analyser and a PC. The concentration of the trace element manganese (Mn), and the major elements sodium (Na), potassium (K) and chlorine (Cl), was determined in the herbs. The use of the two certified standards allowed to check the accuracy and precision of the method, which was estimated better than 80.3% for each trace element in every sample. It will be discussed as the element concentrations in herbs and their infusions at their prescribed dosage cover an inadequate percentage (0.16-0.30%) of the daily needs of an adult, proposed by WHO, FDA and the Institute of Medicine of American National Academy of Sciences.

Poster Session 3

Toxicology and risk assessment

T27 IMPACT OF THE MILITARY CONFLICTS AND USE OF DEPLETED URANIUM ON THE FOOD CHAIN IN BOSNIA AND HERZEGOVINA AND KOSOVO: RISK ASSESSMENT OF URANIUM AND OTHER POTENTIALLY TOXIC TRACE ELEMENTS

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At least 3 and 10 tons of Depleted Uranium (DU) munitions were fired by NATO during the air operations in Bosnia and Herzegovina (1994-1995) and Kosovo (1999), respectively. The United National Environment Programme in their post-conflict environmental assessment of DU concluded that detectable ground surface contamination was limited to areas within a few metres of DU penetrators or penetrator impact and there was no significant risk related to these contamination points in terms of possible contamination of air, water or plants. Although studies at such sites have been useful for establishing the distribution of uranium immediately following dispersal, they provided little, if any, information about the longer term mobilisation and distribution of uranium. In 2004, the Italian Ministry of Health launched a programme aimed at determining whether military operations and use of DU had any detrimental effect related to transfer of DU and other potentially toxic trace elements (As, Cd, Hg, Pb) in the food chain in Bosnia and Kosovo. Even though DU has both toxicological and radiological health risks, the focus was on potential chemical toxicity following dietary exposure. A network of three laboratories, coordinated by the Istituto Superiore di Sanità (ISS), was given the task of determining contamination levels and assessing potential health risk for consumers. Some 70 samples of imported food products were sampled at their entry into Italy. Moreover, about 350 food samples were collected in Bosnia in two successive campaigns (2005-2006). The analytical strategy established by ISS involved validation of the analytical methods to be used by the participating laboratories, training of all the personnel involved in analytical determinations, implementation of Analytical Quality (AQ) assurance procedures including internal (certified reference materials) and external (proficiency testing) AQ control. Concentration thresholds separating background and anomalous uranium concentrations were identified for the various food groups on the basis of an extensive literature search. Samples with total uranium levels above these thresholds were sent to ISS for detection of possible DU contamination by measurement of the $^{235}U/^{238}U$ ratio. About 100 samples were found to be above the concentration thresholds and screened for possible presence of DU. Overall, no evidence of transfer of DU from dust and soil to plant and animal food was found. However, low-level contamination of some samples with DU particles was identified, which showed that the environmental impact of military operations is clearly detectable in Bosnia after ten years from the end of the conflict. It has to be emphasized that very few samples from Kosovo, the most heavily impacted area, were collected and analysed. Therefore, further studies are ongoing to find out whether the same general conclusion does apply to Kosovo, too. The significance of these findings in terms of public health will be discussed.

T28 TOTAL SELENIUM AND SELENIUM SPECIATION IN FOOD CROPS FROM A SELENIFEROUS AREA OF PUNJAB

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Pockets of seleniferous soils have been identified in India especially in Northeastern parts of Punjab and Haryana states (Northern India). In specific areas, adverse effects on livestock feeding on fodder from high-Se soils have been detected. Limited observations on human population have also shown signs of Se toxicity mainly due to consumption of grains and vegetables harvested from Se-rich agricultural soils. Se accumulation in crops has severe repercussions on the farming community since farmers are unable to consume or sell their produce. This study had two main aims. The first one was to determine total selenium concentrations in locally grown crops for risk characterization of dietary selenium exposure. The second one was to carry out selenium speciation in order to explore the opportunities for using locally grown grains for fortification of low-Se grain batches or producing naturally enriched products as Se supplements for human and animal nutrition. Samples of wheat (*Triticum aestivum*), corn (*Zea mays*), mustard (*Brassica spp.*) were collected in two villages in the Nawanshahr-Hoshiarpur Region (Punjab). Two sampling campaigns were carried out in September and February, respectively. Both grain and seeds, on the one hand, and vegetative plant tissues, on the other hand, were sampled and analysed. Total selenium concentrations in grains, determined by ICP-MS after microwave digestion, were 4.65 µg/g for corn and 29-185 µg/g for wheat (dry weight). These appear to be the highest Se concentrations ever recorded in grains for human consumption. Mustard, a known Se-accumulator, showed Se concentrations in the range 295-640 µg/g (dry weight). Intake estimates were carried out assuming a 20% reduction in Se concentration as a result of wheat milling. Consumption of 100 g of flour gave a selenium intake 7- to 43-fold the EC tolerable upper intake level (UL) for adults (300 µg Se/day). Selenium speciation was carried out after enzymatic extraction using an ultrasonic probe. Extraction efficiency was as high as 80% for wheat but averaged 54% only in corn. Speciation studies of corn and wheat grain extracts were carried out by HPLC-ICP-MS with different separation mechanisms. The accuracy of the determination of total Se and Se-methionine was controlled by analyzing suitable reference materials. The preliminary results of this study indicate that actions to limit the exposure of the population to excessive Se are urgently needed along with human biomonitoring in the severely impacted areas. On the other hand, it is clear that a proactive approach to tackle the problems of the local agricultural system is needed and it will continue to be the subject of research.

T29 DIETARY EXPOSURE TO POTENTIALLY TOXIC TRACE ELEMENTS IN NIGERIA: AN OVERVIEW

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Environmental contamination by Heavy Metals (HMs) seems to be a common place experience in Nigeria especially in the semi-urban and urban centers. Blood lead levels higher than 10 µg/dL in adult subjects not obviously exposed and in children aged between 2-6 years have been documented. Unfortunately heavy metals are the least considered culprits in poisoning by physicians in Nigeria. Metal analyses of foods are deemed necessary to be able to identify the source of these trace elements, though water and air pollution are often implicated. HMs survey in commonly ingested beverages revealed that Cd levels ranged from 0.003-0.081 mg/L for the canned and 0.006-0.071 mg/L for non-canned beverages. Lead in the canned beverages was 0.002-0.073 and 0.001-0.092 mg/L for the non-canned beverages. Investigation of HMs in paediatric syrups showed the following alarming findings: Ni in Magcid suspension (4.13 mg/L) and Gaviron (0.79 mg/L), Cr in Emvite (0.58 mg/L), while Ferobin and Jawaron Syrup plus had 28.23 and 4.37 mg/L of Mn. 2.45 mg/L Cd was seen in Magcid suspension whereas Maxiquine contained 0.09 mg/L Pb. Elevated levels of Cd, Cr, Fe, Mn, Pb, V and Zn in muscle of some fish species have been reported. The muscle of *Tilapia zilli* had the highest levels of Ni (6.82 mg/Kg) and Pb (0.60 mg/Kg). *Ethmaliosa timbriata* had highest Pb level, *i.e.*, 2.40 mg/Kg. *Clarias gariepinus* had the highest accumulation of the tested HMs in this order of ranking: Fe>Zn>Ni>V>Mn>Cr>Pb>Cd. Spinach (*Amaranthus Hybridus*), fluted pumpkin (*Telfairia occidentals*) and root crop, cocoa yam (*Xanthosoma sagittifolium*) have been found to contain high levels of HMs which correlated well with amount of these HMs found in the farm land. Other studies also showed high levels of HMs in indigenous and staple foods like cassava, local spices and sea foods. The various sources of contamination may range from soil where the farm produce is cultivated, handling, post harvest technology to water used in the processing of the foods, especially the beverages. It is feared that poverty coupled with environmental pollution could pose serious public health threat. The public health hazards from ingestion of these foods should be identified and disclosed by in-depth risk assessment studies.

T30 MERCURY, METHYLMERCURY AND FOOD: FOCUS ON RICE

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Mercury is a global pollutant and its environmental cycling, biotransformation and bioaccumulation has been an all-time favourite research topic, usually focussed on the aquatic environment. Mercury occurs in different species, and organic mercury compounds, especially methylmercury content in any type of marine food has been a major health concern due to its enhanced neurotoxicity. With regards to this, recommendations have since long been released by government authorities, for example the US EPA, to restrict fish consumption in young children or pregnant women. More recently, similar concerns have been raised regarding the terrestrial food chain, threatening wildlife and biodiversity. However, far less attention has been paid until now with regards to mercury and methylmercury contamination in plants or crops, *i.e.* food from terrestrial origin. Only recently, elevated methylmercury levels were found in rice grains from mercury contaminated Chinese paddy fields, contributing to a great extent to the methylmercury exposure of parts of the Chinese population. In plants, phytochelatins (PCs), small oligomers of aminoacids with the general formula $(\gamma\text{-GluCys})_n\text{Gly}$, are supposedly playing a major role for the resistance to heavy metal contamination. The main role of PCs is its ability to form metal complexes via Sulfur bonds to the cysteine function. Our research focuses on the direct determination of mercury phytochelatin complexes in rice exposed to inorganic mercury (Hg^{2+}) and methylmercury. We could show that inorganic mercury is greatly bound to phytochelatins, while there was no evidence for methylmercury complexes with phytochelatins. Moreover, the highest concentrations of mercury in plants exposed to inorganic mercury can be found in the roots, with low translocation into shoots and grains, translocation factors being <<1. In contrast, methylmercury uptake into the roots is much lower in rice plants, while translocation of MeHg into the grains is greatly enhanced with translocation factors >>1. Thus our results regarding the translocation behaviour of mercury and methylmercury in rice, give rise to the assumption that metal-phytochelatin complexes not only serve as a detoxifying agent, but they also greatly govern underlying transport processes in plants.

T31 DOES THE EDIBLE SEAWEED HIJIKI DEAL WITH ITS ARSENIC BURDEN VIA COMPLEXATION WITH PHYTOCHELATINS?

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Edible species of seaweed, such as Arame, Kombu and Hijiki, are considered delicacies in Japan and Asia, and are gaining in popularity within the western world. Seaweeds have long been established as aquatic hyperaccumulators of the highly toxic metalloid arsenic. To deal with this burden, biotransformation of arsenic to organic species such as arsenosugars occurs, resulting in a drastically lower toxicity. However, one major exception to this is Hijiki, which stores arsenic mainly as the highly toxic inorganic species arsenate (As(V), $[AsO_4]^{3-}$) and arsenite (As(III), $[As(OH)_3]$). Consequently, Food Standards Agencies in countries including Canada and the United Kingdom have advised against the consumption of Hijiki. Phytochelatins (PCs), peptides of generic structure $[\gamma\text{-Glu-Cys}]_{2\text{-}4}$-Gly, are synthesised by higher plants to bind toxic metal(loid)s such as cadmium, mercury and arsenic during cellular influx. This results in the formation of complexes such as Cd-PC, Hg-PC and As-PC, which reduces the toxicity relative to the free metal(loid). Recently, we have observed that species of brown and red algae are able to synthesise PCs. Interestingly, only one particular peptide, $[\gamma\text{-Glu-Cys}]_2$-Gly, ($PC_2$) has so far been found during our investigations. Here, for the first time, we perform 1% formic acid extraction on fresh and As-exposed samples of *Fucus spiralis* and Hijiki, a technique commonly used for the extraction of As-PC complexes from plants. Speciation analysis, via HPLC-ICP-MS-ESI-MS, shows that formation of As-PC complexes does not occur *in vivo* within seaweeds. We combine this data with the more traditional arsenosugar analysis, looking at the fate of arsenic in seaweed during short-term high-concentration As-exposure.

T32 Zn^{2+} AND PROSTATE-SPECIFIC ANTIGEN (PSA) AS PROSTATE FUNCTIONAL BIOMARKERS: PHENOTYPIC ANCHORING WITHIN THE REPROTECT PROJECT

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Within the EU project ReProTect (www.reprotect.eu), it has been set up a toxicogenomic approach aimed to identify specific gene expression profiles of environmental and food contaminants interfering with the Androgen Receptor (AR). The adopted strategy is based on two human prostate cell lines, PNT2 and LNCaP, both originating from prostate epithelium, responsive to androgens and secreting PSA but having different AR expression and different tumorigenic capabilities. Toxicogenomics has been performed using a few model compounds: the endogenous AR-agonist dihydrotestosterone/DHT, the synthetic AR-agonist 17α-methyltestosterone/MT, the pharmacological AR-antagonist 2-hydroxy-flutamide/2OH-FTA, the AR-antagonist and environmental contaminant linuron/LIN (a herbicide binding AR) and the antiandrogenic environmental contaminant di-*n*-butyl-phthalate/DBP (a plasticizer having the same antiandrogenic effects of LIN without AR binding). In order to link gene expression profiling to cell functionality ("phenotypic anchoring"), measurements of two biomarkers of effect have been performed on both human prostate cell lines: i) PSA secretion (by time-resolved fluorescence/DELFIA), a well-known clinical biomarker altered in different human prostate diseases (prostate cancer/PCa, benign prostate hyperplasia/BPH, prostatitis); ii) extracellular Zn^{2+} release (by inductively coupled plasma-mass spectrometry/ICP-MS), a prostate metabolic marker recently shown to be associated to PSA secretion-positively in BPH and prostatitis but inversely PCa-thus allowing a better screening of prostate cancer patients *versus* other prostate disease. In PNT2 cell line, no differences resulted in extracellular Zn^{2+} release upon the different treatments, whereas PSA secretion evidenced that the anti-androgenic compounds (2OH-FTA, LIN and DBP) had an effect on both free and total PSA (about 50% of reduction). On the contrary, in LNCaP cell line both extracellular Zn^{2+} release and PSA secretion were affected by the different (anti)androgenic treatments: as expected, the AR-agonists DHT and MT increased PSA secretion and reduced extracellular Zn^{2+} release, whereas the antiandrogenic compounds LIN and DBP decreased PSA secretion and reversed extracellular Zn^{2+} release (to control levels). Importantly, in LNCaP cell line, the AR-antagonist 2OH-FTA behaved as an AR-agonist at the tested concentration in both extracellular Zn^{2+} release and PSA secretion. Overall, the experiments of phenotypic anchoring confirmed that: a) (anti)androgenic compounds have specific effects depending on the AR status of the human prostate cell line used; b) both PSA secretion and extracellular Zn^{2+} release might be useful biomarker of effect in *in vitro* studies.

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T33 PRE- AND POST-NATAL EXPOSURE OF SPRAGUE-DAWLEY RATS TO THE ENVIRONMENTAL CONTAMINANT ETHYLENETHIOUREA (ETU) AFFECTS THE MALE REPRODUCTIVE SYSTEM ALTERING BOTH SEX STEROID HORMONE LEVELS AND PROSTATE Zn^{2+} SECRETION

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ETU (metabolite of ethylenebisdithiocarbamate fungicides) is a thyroid toxicant in adults. Thyroid influences growth, development, and metabolism of the reproductive system: hypothyroidism causes gonad dysfunction both in male and female rats. Since a few data are available on direct ETU effects on sex reproductive systems during critical phases of development, this study investigated the role of ETU administration on the development and function of male reproductive system mainly focussing on prostate. As a prostate functional biomarker we used extracellular Zn^{2+} release. Zn^{2+} is normally accumulated in prostate epithelium. Recently, a strong correlation between plasma Zn^{2+} levels and human prostatic diseases (*i.e.* cancer) have been shown, suggesting Zn^{2+} levels as a screening tool for prostatic pathologies. Pregnant Sprague-Dawley rats were treated with 0÷0.1÷0.3÷1.0 mg/Kg/bw/*pro die* ETU on gestational day 7-20, Post-Natal Day (PND) 1-22 until sexual maturity (PND62), in order to fully cover the programming window for development and morphogenesis of the male reproductive system. From PND40, F1 males were examined for preputial separation (biomarker of sexual maturation). Animals were sacrificed at PND62: blood samples were collected to evaluate sex steroid (testosterone/T, dihydrotestosterone/DHT and 17β-estradiol/E2) and Zn^{2+} serum levels; prostate, testis and adrenal glands were excised and weighted. Exposure at 0.1÷0.3 mg/Kg ETU increased prostate relative weight (+12,5% and +6,25%, respectively) and this is positively associated to increased Zn^{2+} levels (+5% and +15%, respectively). At 1,0 mg/Kg ETU, Zn^{2+} levels are comparable to controls, whereas the prostate relative weight decreased (-25%, p value$\leq$0,001). DHT levels dose-dependently decreased (38%, 51% and 52% at 0.1÷0.3÷1.0 mg/Kg ETU, respectively) as well increased the ratio T/DHT, suggesting the 5α-reductase (SRD5A) enzyme (T→DHT transformation) inhibition. At the same time, E2 levels increased while the ratio T/E2 decreased. Rodents exposed to SRD5A inhibitors (*i.e.* finasteride, dutasteride) showed the same effects of ETU on prostate relative weight. Thus, ETU administration might directly affect androgen metabolism since SRD5A is the main regulator of prostate DHT availability. Furthermore, ETU effects on the T/E2 ratio (decreasing at 0.1÷0.3 mg/Kg and increasing at 1.0 mg/Kg ETU) suggests aromatase (T→E2 transformation) inhibition at high concentration. Overall, Zn^{2+} serum levels seems to correlate with altered prostate relative weight and, together with measurements of sex steroids, represents a proper biomarker to detect altered male reproductive and prostate function(s) also in rodents.

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T34 BIOSENSOR-BASED APPROACH FOR THE ASSESSMENT OF THE RELATIVE ANTIOXIDANT CAPACITY OF BREAD

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The human body is provided with an efficient and complex endogenous system for defence against ROSs (Reactive Oxygen Species) and dietary exogenous molecules may contribute to the shielding from oxidative damage. Food items as fruits, vegetables and bread provide the human body with antioxidant substances which may interact with potential environmental food contaminants. It is remarkable indeed that trace elements (As, Cd) and other xenobiotics interfering with nuclear receptors (dioxin-like PCBs, bisphenol A) have been shown to increase ROS-producing pathways; in their turn, some bioactive natural compounds, either vitamins or phytoestrogens, may mitigate the ROS-increase elicited by xenobiotics. This study starts from literature data indicating flaxseed bread as a significant dietary source of Cadmium. The relative antioxidant property of aqueous and organic extracts of white and flaxseed bread samples was measured by an amperometric sensor coupled with bovine superoxide dismutase (SOD) enzyme. SOD-biosensors working in aqueous and organic solutions were used to measure the relative antioxidant capacity of the aqueous and organic extracts of the two types of bread. The test's precision was found to be acceptable (RSD≤5%). The relative antioxidant capacity of aqueous extracts was low and similar for both the bread types, whereas the values of organic extracts were markedly different. To compare with reference values of antioxidant capacity almond, strawberry and chilli were tested as reference antioxidant matrices.

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T35 HEALTH RISK ASSESSMENT OF SELECTED TRACE ELEMENTS IN INORGANIC FERTILIZERS POST APPLICATION: A BRAZILIAN CASE STUDY

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Trace Elements (TEs) are generally present in inorganic fertilizers as by-products or contaminants. There are however some TEs (*e.g.*, Zn, Cu, Fe, and Mn) that are plant nutrients and are intentionally included in fertilizer formulations. It is acknowledged *a priori* that exposure to high levels of TEs (nutrient or not) could pose a health risk to humans. Therefore, this evaluation tries to establish safe limits of TEs, referred to as Risk Based Concentrations (RBCs), in inorganic fertilizers that are applicable to Brazilian conditions. The methodology used to develop the RBCs is a back-calculation of health risks and is standard for a screening level risk evaluation. This work follows the same approach of the document prepared by The Fertilizer Institute (TFI, United States) for the US scenario, entitled "Health Risk Evaluation of Selected Metals in Inorganic Fertilizers Post Application" and aims to demonstrate how the development of a flexible reference tool based on risk assessment can be used to evaluate data on the presence of TEs in fertilizers, in order to determine whether or not the use of these products in the Brazilian agriculture poses a risk to human health. The major purpose of this first attempt of establishing guideline values (RBCs) for concentration of TEs in inorganic fertilizers in Brazil is to suggest limits (based on health risk assessment) that could be used as initial guidance for regulators seeking the protection of human health in the current scenario of fertilizers use in Brazil. The results obtained indicate that TEs do not cause harm to human health when considering post application of inorganic fertilizers in Brazil (based on information for phosphate as well as Zn-carrying fertilizers). This study also suggests that the limits currently established by the Brazilian legislation are safe in terms of health risk assessment. It should be highlighted the need to increase the database for the construction of the Brazilian scenario, especially with respect to information concerning the concentration of TEs in fertilizers marketed in Brazil. However, as more data are being collected, a new comparison with the RBCs suggested in this study could be done to make this scenario closer to the Brazilian reality. Similarly, as more data are generated with respect to the parameters that make up the equation used to calculate the RBCs for the Brazilian scenario, then, a review of currently estimated RBCs could be made.

T36 CADMIUM-INDUCED CHANGES IN TRACE ELEMENTS STATUS IN THE LIVER OF RATS IN RELATION TO DIETARY SELENIUM DEFICIENCY

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Selenium (Se) represents an essential element of fundamental importance for human health. Besides being a constituent of the key aminoacid selenocystein in many enzymes, it has been reported as an important detoxicant for several metals and metalloid elements, *e.g.* arsenic and mercury. Cadmium (Cd) is a serious industrial and environmental pollutant. It was classified as a group I human carcinogen. Several experimental studies have shown an interaction between cadmium and selenium, however, most of them examined short-term interactions. The present study (supported by the research grant MSM 0021620819) was designed to investigate the combined effects of dietary selenium deficiency, cadmium exposure and Cd-chelator treatment on tissue level of cadmium and key trace elements - copper, iron and zinc, in rats. Forty-one male and forty-two female Wistar weanling rats (60-70 g) were fed either Se-adequate (0.14 mg Se/Kg) or Se-deficient (<0.06 mg Se/Kg) Torula yeast-based diet (AIN 93G, ssniff® Spezialdiäten GmbH, Germany) for 12 weeks. Starting from week 9, each dietary group was further divided into two groups, who drank distilled water containing either 0 or 50 mg Cd/L as cadmium chloride till the end of experiment. On week 12, five males and 5 females from the Cd-treated groups received an additional treatment with the Cd-antidote Sodium N-(4-methylbenzyl)-4-O-(ß-D-galactopyranosyl)-D-glucamine-N-carbodithioate (MeBLDTC), (318 mg/Kg, sc), administered three times every 48 h. At the end of the 12-week experimental period, the concentrations of Cd, Cu, Fe and Zn in the liver were determined by Atomic Absorption Spectrometry (AAS). Samples of liver tissue (0.5 g) were placed in platinum crucibles and dry-ashed in a muffle furnace under temperature-controlled program overnight. The ash was solubilized with 3 M HCl and appropriately diluted for analysis. Analytical accuracy was monitored by assaying reference samples of NBS reference material 1577 Bovine Liver. The Se-deficient rats, both male and female, showed significantly lower accumulation of cadmium in the liver (by 35%) compared to the Se-adequate rats. Zinc concentration increased, and iron and copper levels decreased in the liver of Cd-treated rats. Selenium deficiency did not affect the alterations of zinc and iron hepatic levels. The hepatic concentration of copper decreased with Cd supplementation only in Se-adequate groups. The effects of Se-deficiency and Cd supplementation were equal in male and female rats. Under Se-adequate status, the mobilization of cadmium by MeBLDTC was more effective in male than in female rats (78% and 62%, respectively). In rats fed Se-deficient diet, no difference in the effectiveness of Cd mobilization was found between male and female rats (62%, both). The results demonstrate that the dietary intake of selenium can substantially influence the distribution of trace elements in the body. Direct Cd - Se and Cd - Cu chemical interactions are proposed as the main mechanisms of these interactions.

T37 A LONG-TERM ASSESSMENT OF THE MERCURY CONTAMINATION OF FOODS IN THE IRKUTSK REGION, RUSSIA

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The toxicology literature has clearly established the dangers of excessive exposure to mercury. An assessment of the exposure of the population in the Irkutsk Oblast, carried out through field surveys, provided relevant background information (*i.e.* characteristics of Hg sources, regional environment, Hg concentration in different foods). The determination of total Hg in samples was performed via cold vapour atomic absorption spectrometry. The primary anthropogenic sources of Hg are concentrated in the areas surrounding chemical factories that utilize Hg and in the Bratsk water reservoir. The assessment of mercury content (in food-more than 350 samples; in biosubstrates-more than 700) was carried out during two periods: the period from 1992 to 1998 (before the closing of the chemical plant that used Hg) and since 1999. The Bratsk water reservoir is strongly polluted by technogenic Hg. The Hg concentration in the muscles of fish (1,000-2,700 ppb) is up to a hundred-fold higher in comparison with the concentration in plankton. In the first survey, mercury concentration in fish muscles was found to exceed the legal limit by 2 to 4 times. Mercury content in cow milk was also found to be 20-30% higher than the relevant legal limit. Acceptable levels were determined in potatoes, cabbage, carrots and meat products (pork, beef and chicken). The average content of mercury in hair of the villagers was 2.11±0.3 mg/Kg as compared to the background level of 1.0 mg/Kg. The concentration of mercury in urine was found to be above background level in 77.9% and acceptable in 10.5% of the surveyed population. Among the population living near the sources of mercury, rural inhabitants, fishermen (HQ=7.2) and children are known to be at risk of unfavourable mercury effects, including mercury microcirculation in the organism and disturbances of the nervous-psychical and hormonal states. The concentration of mercury in fish has decreased by 1.5 to 2.5 fold since the closing of the chemical plant that used mercury for chemical processes. The average levels of mercury in the muscles of fish were shown to be 280-680 ppb in 2004-2008. Mercury content in meats and vegetables was found to be less than reference levels. Mercury concentration in urine was above the permissible level in 25% of the fishermen. Health risk based on the danger coefficient (HQ=4.1) has been assessed as medium. In conclusion, the closing of the chemical plant that used Hg did not solve the problem of mercury exposure in the Irkutsk Region. The main source of mercury exposure for the Irkutsk Region's rural residents is attributed to the consumption of fish caught in the Bratsk water reservoir. This finding dictates the necessity of organizing relevant continuous monitoring.

T38 METALLOIDS AND METALS IN FOOD SUPPLEMENTS

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The aim of the study was to determine the arsenic, cadmium, chromium, lead, mercury, nickel and zinc content in 14 vitamin or herbal preparations in order to estimate potential toxicity based on maximum possible bioaccumulation. Following microwave digestion (*ca* 0.25 g sample +5 mL 65% HNO_3 and 1 mL H_2O_2; 20 minutes; power output: 1400 W), the concentration of metals and metalloids was determined by various Atomic Absorption Spectrometry (AAS) techniques: graphite furnace AAS (Cd, Cr, Pb, and Ni), flame AAS (Zn), hydride generation AAS (As), and cold vapour AAS (Hg). Results showed that the preparations contained arsenic (0.10-0.19 mg/Kg, 3 samples), cadmium (0.12 mg/Kg, 1 sample), chromium (0.42-64.38 mg/Kg, 10 samples), lead (0.50-3.86 mg/Kg, 5 samples), mercury (0.04-0.12 mg/Kg, 14 samples), nickel (0.67-338.90 mg/Kg, 12 samples), and zinc (2.44-95.30 mg/Kg, 7 samples), expressed as mean value of three determinations in milligrams of analyte per kilogram of tested product. The observed results were compared to values of Maximum Allowed Amount (MAA) for food supplements (Cd, Hg, Pb,), and, in absence of specific regulative for food supplements, to stated MAA for grains (As), honey (Zn), oil (Ni) and hydrogenated oil (Cr). The observed values were above the specified limit for chromium and nickel (all samples), for lead (1 sample), for mercury (2 samples), and zinc (2 samples), while for cadmium and arsenic were lower. Calculated cumulative daily intakes compared to specific values of tolerable daily intake (TDI) ranged for lead 0.05-1.94%, for cadmium 0.17%, for arsenic 0.01-0.12%, for mercury 0.02-0.94%, for nickel 0.10-30.10% and for zinc 0.003-0.16%. Maximum possible bioaccumulation was calculated as the mass of analyte, present in the amount of vitamin/herbal preparation (as total number of dosage form units) ingested in a day, multiplied by the recommended administration period (from 30 to 365 days, depending on the preparation). The calculated ranges for maximum possible bioaccumulation were found to be relatively low: 7.30-18.25 μg for arsenic, 7.20 μg for cadmium, 19.80 μg-5.82 mg for chromium, 17.40-437.00 μg, for lead, 1.20-24.00 μg for mercury 27.00 μg-30.68 mg for nickel, and 0.17-4.72 mg for zinc. The results of this research showed low possible bioaccumulation, and pointed out the importance of defining MAA values for all toxic metals and metalloids in food supplements because of inadequacy of alternative product group values for proper risk estimation of the analysed preparations. It should be taken into account that intake of metals and metalloids by food supplement consumption is just one of the possible contributions to total daily intake, besides food, water, and air inhaling.

T39 DIETARY EXPOSURE ASSESSMENTS OF HEAVY METALS AND TRACE ELEMENTS IN CHINA

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In order to assess the safety and nutritional status of dietary trace elements in different areas in China, the dietary intakes of sixteen trace elements in Chinese adult males have been obtained by using the 2000 Chinese Total Diet Study approach. The Chinese Total Diet Study included twelve provinces, divided into four regions, namely, North One (Heilongjiang, Liaoning and Hebei), North Two (Henan, Shanxi and Ningxia), South One (Shanghai, Jiangxi and Fujian), and South Two (Hubei, Sichuan and Guangxi). The research steps of the Chinese Total Diet Study included food consumption survey, food aggregation, food sampling, cooking and preparation, sample analysis and dietary exposure assessments. The elements surveyed in this study were Fe, Zn, Se, Cu, Mn, Cr, Ni, B, V, Li, Mo, Pb, Cd, Hg, Al, total and inorganic arsenic. The dietary safety of these trace elements was assessed by using their respective Provisional Tolerable Weekly Intake (PTWI) recommended by WHO. The nutritional status of essential elements was evaluated by the RNI (Recommended Nutrient Intake) and AI (Adequate Intake) as established by the Chinese Nutrition Society. Chinese dietary Pb, Cd, Hg, Al, total arsenic and inorganic arsenic average intakes (% of PTWI) in adult males were 0.081 mg/d (36.1%), 0.022 mg/d (35.3%), 0.007 mg/d (15.2%), 22.9 mg/d (36.3%), 0.276 mg/d and 0.079 mg/d (58.6%), respectively. The main dietary sources of these harmful elements were cereals and vegetables. Chinese average dietary intakes of Fe, Zn, Cu, Se, Mn, Cr, Mo, Ni, B, V and Li (% of AI or RNI) in adult male were 13.0 mg/d (87.0%), 10.4 mg/d (69.3%), 1.7 mg/d (84.5%), 0.064 mg/d (128.6%), 3.838 mg/d (127.9%), 0.116 mg/d (233.8%), 0.257 mg/d (428.9%), 0.235 mg/d, 1.42mg/d, 0.034 mg/d, and 0.054 mg/d, respectively. The dietary intake of selenium was higher than the RNI in most provinces, but the intakes in Ningxia (0.037 mg/d) and Jiangxi (0.040 mg/d) were lower than the average level. Harmful elements in most food groups of the surveyed provinces were well below the National Standard Limit (NSL) of China except a few samples in some areas, such as lead in eggs in South 1 region (NSL exceeded by 8.1%) as well as cadmium in aquatic foods in regions of North 1 and South 1 (NSL exceeded by 49.0% and 27.6%, respectively). The results indicate that dietary lead, cadmium, total mercury, total arsenic and inorganic arsenic intakes were safe in the different regions. The dietary intakes of iron and zinc were insufficient in Chinese people.

T40 ESTIMATION OF THE DIETARY INTAKE OF CADMIUM BY A BRAZILIAN TOTAL DIET STUDY

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Estimates of toxic element dietary intakes from Total Diet Studies (TDS) have already been carried out in many countries. However, this kind of study is still rare in Brazil. In the present study, the cadmium concentration was determined in 30 food groups of a Market Basket in São Paulo State. The food consumption data source used was a Household Food Budget Survey (IBGE-POF 2002-2003), which contained 5,441 food items. Seventy-one foods were grouped in 30 composites, which resulted in a Market Basket. The criterion of selection was food consumption above 2g/day/person. The food items were collected, prepared table-ready at restaurants of the University of São Paulo (raw, washed, peeled and cooked). The inedible parts were discarded. After preparation, the food items were weighed and grouped in accordance to the percentage that they represent in their food composites. Each food composite was then homogenized in a domestic blender, frozen and freeze-dried for about 48 hours. Thereafter, the composite was again homogenized in a blender. Cadmium was determined by Graphite Furnace Atomic Absorption Spectrometry. Certified reference materials (NIST SRM Total diet, SRM Oyster Tissue, SRM Bovine Liver, SRM Apple Leaves) were analyzed to evaluate accuracy. The sea fish group presented the highest Cd concentration (50.6 ng/g wet weight), followed by tuberous vegetables (30 ng/g ww) and biscuits groups (13.3 ng/g ww). The daily intake of Cd was estimated at 1.4 µg, which does not represent any risk to the population studied.

T41 MERCURY CONTENT IN TWO FISH SPECIES, HAKE (*MERLUCCIUS MERLUCCIUS*) AND CARP (*CYPRINUS CARPIO*): ESTIMATED WEEKLY INTAKE IN SERBIAN POPULATION

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The nutritional benefits of fish are mainly due to the content of high-quality proteins, vitamins, elements and omega-3 polyunsaturated fatty acids. However, increased fish consumption may simultaneously increase the contaminant intake to levels of toxicological concern. Total mercury concentrations were measured in the muscle tissue of two fish species of great importance in the diet of the Serbian population: hake (*Merluccius merluccius*), imported to Serbia from Argentina, and carp (*Cyprinus carpio*), farmed in Serbia's fish ponds. After microwave digestion with nitric acid and hydrogen peroxide, the mercury content was measured by atomic absorption spectrophotometry using the cold vapour technique with reduction by $SnCl_2$. Analytical quality control was achieved by using certified reference material BCR 186. Replicate analyses were in the range of the certified values. Mercury concentrations in hake (75 samples) were in the range of 0.005-0.111 μg/g, with a mean value of 0.030 $\mu g/g^{-1}$. Mercury concentrations in carp (25 samples) were in the range of 0.001-0.023 μg/g, with a mean value of 0.015 μg/g. All samples contained mercury below the maximum level fixed by the European Commission regulation (0.5 μg/g wet wt). The estimated weekly intake of total mercury for both species was below the established provisional tolerable weekly intake (5 μg/g body weight), recommended by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives in 2003.

T42 INORGANIC ARSENIC IN SEAFOOD: AN UNDERESTIMATED ISSUE?

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Fishing activities in the Santa Gilla lagoon, in southern Sardinia (Italy), represent an important source of income and food for the population living in the metropolitan area of Cagliari (about 500,000 inhabitants). In the 1970's the lagoon was heavily polluted by industrial wastes, raising concerns for the high levels of mercury and lead in the seafood. Fishing was prohibited up to the 1990's, when relevant restoration works were carried out. This work presents the outcomes of the risk assessment of arsenic in seafood carried out according to the process recommended by EPA (2000) for cancer and non-cancer toxicity (hazard identification; dose-response assessment; exposure assessment; risk characterization). Hazard identification was mainly based on publicly available data resulting from an extensive field survey carried out by the regional environmental protection agency between 2006 and 2007 (ARPAS, 2007). The results pointed out concentrations of total arsenic up to 3 mg/kg (fresh weight), both in shellfish (*Mytilus galloprovincialis, Ruditapes decussatus*) and in muscle and liver of *Mullus barbatus* and *Mugil sp*. Inorganic arsenic was not determined. It is well known that arsenic in seafood is mostly organic arsenic, which has been considered to be metabolically inert and nontoxic. Maybe for this reason no concentrations limits have been set in EU and in the majority of the countries for arsenic in seafood. Moreover inorganic arsenic, which has been determined to be a human carcinogen based on epidemiological evidence, is generally found in seafood at concentrations ranging from 1 to 4% of the total arsenic, although concentrations up to 20% have been reported. EPA guidance for assessing chemical contamination data in fish advisories (EPA, 2000) recommend that inorganic arsenic rather than total arsenic should be determined, otherwise in risk assessment procedures 10% of total arsenic in seafood should be considered cautiously as inorganic arsenic. Based on this assumption the dietary exposure to inorganic arsenic of both general population and fishermen of the lagoon was evaluated. Risk characterization indicated that both non-carcinogenic and carcinogenic adverse effects due to the exposure to the element were not negligible. The results suggest that the potential human health risks for the exposure to inorganic arsenic through the consumption of seafood could be underestimated and support the importance of speciation analysis for risk assessments for arsenic exposure from marine biota.

T43 SIMPLE BUT SOUND RECOMMENDATIONS TO REDUCE ARSENIC INTAKE IN INDIA

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The chronic poisoning by arsenic of more than one hundred million people in Bangladesh and India is one of the world's worst health problems. Arsenic reaches the population not only through drinking water but also through foodstuff, such as rice irrigated with water from the same wells. In addition, the worst toxicological scenario is found because arsenic in groundwater and vegetables is mainly present under "inorganic" species, which are classified within Group I of human carcinogens by the International Agency for Research on Cancer. There are presently several studies on the arsenic crisis in the region, but the chronic poisoning in all areas affected has not yet been solved. Simple but sound recommendations to reduce arsenic intake in villagers are presented here. These include choosing the most convenient method of dehusking and cooking rice, as both of them are related with the arsenic concentration in cooked ready-to-eat rice, and by peeling roots and tuber, as several authors demonstrated that arsenic accumulates mainly (over 50%) in the skins of these sorts of foodstuff. Mechanically dehusked rice, "atab rice", presents significantly lower arsenic concentrations than "boiled rice", which is first soaked in water, boiled lightly, and finally mechanically dehusked. Arsenic concentrations in paddy, boiled and atab rices were 373±2, 332±1 (11.0% reduction) and 290±16 µg/Kg (22.3% reduction), respectively; water containing 40 µg As/L was used. There are three common methods of cooking rice. In the "traditional method" rice is washed until washings become clear, the washings are discarded and rice is boiled in excess water until cooked, finally the remaining water is discarded. Rice cooked following the "intermediate method" is washed as above but is boiled with less water until no water is left to discard. Finally, in the "contemporary method" unwashed rice is boiled with low water volume until no water is left to discard. The cooking method significantly affected total arsenic (t-As) in cooked rice, with concentrations being 258±14, 365±14 and 387±15 µg kg^{-1} for the traditional, intermediate and contemporary methods, respectively; water containing 40 µg As/L was used. A food questionnaire administrated in Kasimpur (West Bengal) showed that the mean daily intake of water, rice and vegetables were 1,760 mL, 385 g and 205 g, respectively. With these consumption values and choosing the most convenient method of dehusking and cooking rice among described above, simple but sound recommendations can be provided to produce at least a 21.3% reduction in the t-As daily intake.

T44 DETERMINATION OF TOTAL AND INORGANIC ARSENIC IN CANNED AND FROZEN SEA PRODUCTS AND FISH COLLECTED AT A SPANISH MARKET

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Several dietary studies carried out in various countries show that sea products are the most significant source of arsenic in the diet, although a great variability exists, ranging from 10 µg/day, for inhabitants of the city of Mumbai, to 345 µg/day for the population of Japan. The vast majority of arsenic in seafood is in organic forms and is mainly present as arsenobetaine, a non-toxic compound, but it also contain small amounts of inorganic arsenic forms (i-As), known human carcinogens, as well as DMA and arsenosugars which are mainly present in algae (seaweed) and might pose, at least, a theoretical risk. The number of studies on arsenic speciation in sea products and food in general are increasing. Even though they require expensive analytical systems (HPLC-ICP-MS, HPLC-HG-AFS, *etc.*), the data they provide are necessary to estimate the i-As daily intake and to evaluate whether a toxicological stage exists. Reported data of i-As in sea products from previous studies are not necessarily representative of the level of i-As existing in all the various types of fish or sea products; therefore, further studies on arsenic speciation on seafood are required. This work provides information on total arsenic (t-As) and i-As in a range of samples of canned and frozen sea products, bought from an important chain of supermarkets in Spain, to evaluate whether they pose a toxicological risk for Spanish consumers. Canned products analysed showed lower t-As concentration than frozen products, which could be associated with processing. Canned and frozen sea products showed ranges of 0.49-1.94 µg/g and 0.03-0.07 µg/g for t-As and i-As, respectively. The percentage of i-As was up to 11.3% in canned cockles. In conclusion, the i-As concentration and the average fish consumption are low enough not to pose a toxicological concern for Spanish seafood eaters. Since t-As does not guarantee the harmlessness of the products, it would be necessary to establish a new legislation for arsenic in sea products, taking into consideration the amount of i-As.

Poster Session 4

Trace elements in nutrition and human health

T45 EFFECTS OF INCREASED FISH INTAKE ON SERUM SELENIUM: RESULTS FROM THE FISHGASTRO HUMAN INTERVENTION STUDY

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The trace element selenium (Se) has received increasing attention as a possible cancer preventing substance. Epidemiological evidence suggests that Se status is inversely correlated with colorectal cancer. Fish is an excellent, highly bioavailable source of Se. The FISHGASTRO intervention study was conducted to assess the impact of two types of fish, salmon and cod, on gastrointestinal health. Participants (n=242), either apparently healthy or at increased risk of cancer were recruited for the study through gastroenterology clinics across The Netherlands and the UK. They were randomised onto one of three dietary interventions: salmon (300 g/week), cod (300 g/week) or no additional fish in the diet for six months. Dietary advice was given to all three groups and dietary intake was assessed throughout the study using Food Frequency Questionnaires (FFQs). Blood samples were collected at baseline and after 6 months dietary intervention. Serum Se concentration, for a sub-set of 175 paired samples, was measured using an Agilent 7,500ce ICP-MS following UV assisted wet digestion in a Metrohm 705 UV digester. The Se content of fish (cod and salmon) supplied to volunteers was also measured. At baseline the mean serum Se concentration was 91.7 ng/mL for all volunteers, which is considered low and so may potentially increase the risk of developing colorectal cancer. The 300 g of fish per week provided by the intervention accounted for 11-12 µg of additional Se per day. Despite the quite modest dietary contribution of Se from fish, serum Se concentrations were significantly increased following the intervention in the cod group (5.1±1.9% increase) (p=0.02), but not in either the salmon or dietary advice groups. No statistically significant differences were observed between interventions. To some extent, results from the FFQs support the evidence that adding fish to the diet increases Se intake, however these data did not correlate with serum Se concentrations. This study confirms that fish, particularly cod provides a good source of bioavailable selenium. However, insufficient additional Se was consumed to ameliorate the effects of a diet generally low in selenium. Higher levels of supplementation, probably only achievable with the addition of organic Se compounds or Se-enriched fish would potentially be required to assess the impact of Se on gastrointestinal health.

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T46 EFFECT OF RIBOFLAVIN STATUS ON THE BIOAVAILABILITY OF NON-HAEM IRON

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There is reasonably good evidence from a variety of sources to suggest that riboflavin deficiency interferes with the normal metabolism of iron. This study was designed to investigate whether riboflavin status has an effect on dietary Fe bioavailability and iron status utilising state of the art methods for the measurement of iron absorption. Thirty-six female volunteers aged 19-26 were randomly allocated to two groups. One group was given a placebo for eight weeks; the other group was given 4 mg/d of riboflavin as a supplement for eight weeks. Blood samples were taken pre- and post-intervention and analysed for a range of dietary and iron status (including Hb and ferritin) variables. In addition, a small dose (4 mg) of ^{58}Fe was taken orally by the volunteers pre-intervention and, again, post-intervention to measure their iron absorption by erythrocyte incorporation. The percentage absorption of iron from the test dose, post intervention, in the placebo group was 6.8±3.3% and that in the riboflavin group was 5.4±4.8% (P=NS). There was a significant association between (post-pre) change in riboflavin status (as measured by EGRAC) and (post-pre) change in Hb concentration (P=0.014; slope=-1.12 g/dL per unit change in EGRAC; adjusted R^2=0.14). There was a significant increase (P=0.023) in red blood cell count (post-pre) due to the intervention (placebo group: -0.08±0.24 x10^{12}/L; riboflavin group: 0.05±0.16 x10^{12}/L). There was a borderline association of (post-pre) change in riboflavin status on change (post-pre) in serum ferritin concentration (P=0.078; slope=17.2 ng/mL per unit change in EGRAC; adjusted R^2=0.07). There was no effect of the intervention on iron absorption as measured by Fe erythrocyte incorporation indicating that it is unlikely that riboflavin plays a direct role in influencing intestinal Fe absorption. Improvements in riboflavin status lead to a greater Hb concentration by increasing the number of red blood cells. The increase of circulating red blood cell iron appears to come from a reduction of iron in the liver stores as evidenced by a decrease in serum ferritin concentration.

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T47 MILK OR CASEINOPHOSPHOPEPTIDES ADDED TO FRUIT BEVERAGE TO IMPROVE FERRITIN SYNTHESIS?

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Iron deficiency is one of the most important nutrient deficiencies, and its more severe stages are associated with anemia. An effective strategy for preventing nutritional iron deficiency could be iron fortification, fruit beverages being a good vehicle in this context, since they lack certain iron absorption inhibitors such as phytates or oxalates. Caseinophosphopeptides (CPPs) are bioactive peptides derived from milk casein that can be released by enzymatic hydrolysis, gastrointestinal digestion, or during food processing. They present a structural cluster containing a sequence of three phosphoseryls residues followed by two glutamic acid residues that constitute the binding sites for iron, and thus play an important role in iron bioavailability. This property could justify the use of CPPs as functional ingredients for improving iron bioavailability. Fruit beverages (Fb) (all of them containing grape, orange and apricot) with iron (Fe as sulphate 3 mg/100 mL of fruit beverage), with/without zinc (Zn as sulphate 1.6 mg/100 mL of fruit beverage) and with/without skimmed milk (M) (11% v/v) were subjected to *in vitro* gastrointestinal digestion. CPPs obtained from whole casein subjected to simulated gastrointestinal digestion and isolated by selective precipitation were added to the soluble fraction of fruit beverages (bioaccessible fractions) obtained after digestion: FbFe+CPPs, FbFeZn+CPPs. The bioavailability of iron in these samples was estimated by ferritin synthesis in Caco-2 cells, determined via an enzyme-immunoassay (ELISA). Ferritin synthesis for the FbFe+CPPs sample was significantly ($p<0.05$) and two-fold higher versus FbFeZn+CPPs, and of the same order as in the case of the FbFeM and FbFeZnM samples. In beverages fortified only with Fe, the addition of milk or CPPs improves ferritin synthesis to an equal extent. The presence of milk counters the possible negative Fe-Zn interactions to a greater extent than CPPs.

T48 IRON AVAILABILITY OF DIFFERENT TYPES OF INULIN ENRICHED INTEGRAL BISCUITS: STUDIES USING AN *IN VITRO* DIGESTION/Caco-2 CELL CULTURE MODEL

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Iron deficiency is the most prevalent nutrient deficiency among humans, mostly due to the low bioavailability of dietary iron, especially in widely consumed plant-based foods such as wheat, rice, maize *etc.* Therefore ways need to be found to increase the density and bioaccessibility of iron in these types of foodstuff. In the frameworks of our research wheat flour based biscuit composition was modified by addition of mixture of inulin with one of the following raw materials: soy flour, amaranth, carob, oat fiber and apple fiber. Modifications were conducted with the aim of generally improving its nutritive value and functionality while special emphasis has been laid on increasing the total content, solubility and absorption of iron from the final product. Iron content was assessed by means of ICP-AES, after microwave-aided wet digestion of the samples. Bioaccessible iron was determined in the soluble sample fractions obtained after an in vitro simulation of gastrointestinal digestion. Iron uptake was determined using a Caco-2 cell model, as the sum of iron absorbed and transported across the monolayer. Caco-2 cells have been proven suitable for assessing mineral utilization from different foodstuff since results obtained on Caco-2 monolayer correlate very well with human studies data. The results showed that total iron content increased from 2.2 (in the unmodified biscuits) up to 3.7 mg/100 g dry matter (biscuits with oat fiber). Significant increases of the iron content were also achieved using soy flour and amaranth for enrichment. The content of bioaccessible iron was significantly lower, ranging from 0.65 (samples with addition of apple fiber and carob), up to 1.75 mg/100g dry matter (sample with soy flour). The total iron uptake on Caco-2 cell monolayer was low, ranging from 7 to14% of the bioaccessible fraction. The highest uptake was from biscuits enriched with amaranth and soy flour (12.20 and 7.13%, respectively). The amounts of bioaccesible iron and iron absorbed on cell monolayer did not show a significant correlation, indicating the existence of other factors also involved in iron absorption (linkage and/or valence of iron; presence of other divalent metals competing for metal transporter *etc.*). Although all modifications of the biscuit composition resulted in significant increases of the iron content, improved bioaccessibility and uptake of iron was only achieved by using soy flour and amaranth. These results therefore emphasize that, in addition to its total content, iron bioavailability and uptake should also be determined when evaluating certain foodstuff as the nutritive source of this essential trace element.

T49 *IN VITRO* BIOACCESSIBILITY OF IRON AND ZINC IN FORTIFIED FRUIT BEVERAGES

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One of the main nutritional problems in the world is mineral deficiency. Although single mineral deficiency is possible, in most cases several minerals are lacking. An effective strategy for preventing nutritional mineral deficiency could be iron and zinc food fortification. In this sense, a frequent practice in the food industry is to enrich products with several biologically active compounds to improve their nutritional value, such as fruit beverages supplemented with milk, vitamins and/or minerals (*i.e.*, Fe and Zn). However, iron and zinc interactions when co-supplemented in any fortification strategy cannot be ruled out, since mineral elements with similar electron configurations can share absorptive pathways and interact and this situation could result in further lowering of the bioavailability of both minerals. The determination of mineral solubility is a prior and necessary requirement for further studies of trace element absorption. Since solubility in the intestinal tract is the first step towards bioavailability, solubility studies allow a first estimate of the bioavailability of Fe and Zn from different foods. The objective of this work was to assess the bioaccessible amounts of iron and zinc in 6 different fortified Fruit beverages (Fb) with/without iron (Fe as sulphate 3 mg/100 mL of fruit beverage) and/or zinc (Zn as sulphate 1.6 mg/100 mL of fruit beverage) and/or skimmed milk (11% v/v) (FbFe, FbZn, FbFeZn, FbFeM, FbZnM, FbFeZnM) using an *in vitro* solubility method consisting in two sequential steps (gastric at pH 2 with pepsin solution and intestinal at pH 6.5 with pancreatin-bile salt solution), and centrifugation to obtain the soluble (bioaccessible) fraction. Iron and zinc levels in the bioaccessible fractions were quantified by flame atomic absorption spectrometry. In the fruit beverages FbFe, FbFeM and FbFeZnM, iron bioaccessibility percentages were high (88-97%), whereas a lower value (53%) was observed for FbFeZn. In general, the bioaccessible zinc values were lower than those observed for iron. Zinc bioaccessibility from FbFeZn (79%) was the highest, with significantly higher values ($p<0.05$) than in the case of FbZn, FbZnM or FbFeZnM (68%, 69% and 48%, respectively). In contrast, FbFeZnM showed the lowest zinc bioaccessibility (48%). In view of these results, the effects of peptides derived from milk protein hydrolysis during digestion seem to benefit iron solubility rather than zinc solubility when these minerals are jointly present in the same sample, reducing the negative interaction between both minerals as seen in the FbFeZn sample.

T50 IODINE PROPHYLAXIS IN ITALY

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Elimination of Iodine Deficiency Disorders (IDD) is a most important health and social goal. This because iodine deficiency at critical stages during pregnancy and early childhood results in impaired development of the brain and consequently in impaired mental function. Therefore, iodine deficiency is the world's greatest single cause of preventable brain damage. In fact, when iodine intake is below the recommended levels, the thyroid-which plays a pivotal role in brain development-may no longer be able to synthesize sufficient amounts of thyroid hormones, resulting in different pathologies known collectively as IDD. The WHO recommended strategy for IDD control is based on increasing iodine intake at the population level and salt iodization has been recommended as the preferred strategy. Salt has been chosen as a vehicle because of its widespread consumption and the extremely low cost of iodization. Italy is still characterized by mild to moderate iodine deficiency. In our country the nation-wide strategy for IDD control has been established in force of a recent law (active in November 2005) promoting the utilization of iodized salt at the population level. This law requires retailers to make iodized salt always available and non-iodized salt has to be specifically requested by the customer. The law also foresees the possibility of using iodized salt in the food industry and in communal eating areas. Following application of the law to activate a monitoring program, a National Observatory for Monitoring Iodine Prophylaxis (OSNAMI) has been established at the Istituto Superiore di Sanità with the aim of monitoring and evaluating efficiency and effectiveness of the nation-wide iodine prophylaxis program. On the basis of the OSNAMI data, sales of iodized salt showed an increasing trend in the last years (27% in 2003; 31% in 2006; 35% in 2007). However consumption of iodized salt is still greatly lower than >90% of total salt consumption for food use recommended by WHO. Moreover, a preliminary analysis on neonatal TSH levels, as sensitive tool in the evaluation of iodine status of a population, has been performed in 3 Italian Regions (Lombardia, Marche, Calabria). In fact, according to WHO epidemiological criteria, if TSH values >5 microU/mL whole blood are present in 3-19.9% of the target population, this is considered exposed to mild iodine deficiency. The preliminary analysis showed the presence of a mild iodine deficiency in these Regions (Marche: 7.9%; Lombardia: 6.3%; Calabria: 3.6%). Our data confirm that in our country, although progress has been achieved by increasing the number of people with access to iodized salt, further efforts should done to make effective the Italian program of iodine prophylaxis.

T51 POSSIBLE EFFECTS OF PROFESSIONAL EXPOSURE TO THYROID DISRUPTORS ON URINARY IODINE CONCENTRATION IN AGRICULTURAL WORKERS: PRELIMINARY RESULTS OF AN EPIDEMIOLOGICAL STUDY

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Most iodine absorbed in the body eventually appears in the urine. Therefore, urinary iodine excretion is a good marker of very recent dietary iodine intake. In fact, when median urinary iodine levels in the target population are at least 100 µg/L and no more than 20% of values are below 50 µg/L that population is considered not exposed to iodine deficiency. Although urinary iodine concentration is commonly used to monitor nation-wide iodine prophylaxis programs, no studies have been performed to establish a possible effect of exposure to thyroid disruptors, especially those affecting Na^+/I^- symporter function, on urinary iodine excretion. The aim of this study was to verify a possible effect of professional exposure on urinary iodine excretion levels in a group of agricultural workers and non-exposed subjects. To this end, urinary iodine concentrations were measured in a group of agricultural workers (n=47; 21 men, 26 women) and in a representative sample of the general population (n=80; 37 men, 43 women) living in the Italian district of Maddaloni (Caserta province). Analytical determinations were carried out by inductively coupled plasma mass spectrometry. Analytical quality control included use of a matrix certified reference material and participation in an external quality assurance programme for urinary iodine determination. The comparison of the median urinary values observed in the agricultural workers and in the subjects of general population did not show any significant difference (127 *vs* 107 µg/L, respectively), indicating a sufficient iodine nutritional status for both groups of subjects. However, when the groups were stratified by sex, distribution of urinary iodine concentrations in women showed more frequent high values (>250 µg/L) among workers than among non-exposed subjects (30% *vs* 7%, P=0.05), while no significant differences were observed between the groups of men. It is important to note that both workers and non-exposed subjects lived in the same geographical area and no significant difference was observed in the frequency of using iodized salt among groups. In conclusion, these preliminary results seem to suggest that, especially in women, activities involving professional exposure to chemical compounds, such as pesticides and fertilizers, may affect the biological availability of iodine for thyroid hormone biosynthesis and that the observed increased urinary iodine concentrations in women may reflect this possible effect. The ongoing analysis of exposure biomarkers and the evaluation of thyroid function in the above mentioned subjects will contribute to verify this hypothesis.

T52 SELENIUM-ENRICHED MYCELIUM OF *LENTINULA EDODES* (BERK.) PEGLER-A SOURCE OF ORGANIC FORMS OF SELENIUM WITH A POTENTIAL CANCER PREVENTIVE ACTIVITY

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Preparations derived from *Lentinula edodes* (Berk.) Pegl. mycelium are worldwide used as dietary supplements containing compounds active as immune system enhancers and demonstrating chemopreventive and anticancer activity. Our goal was to obtain a new preparation with even higher anticancer activity, derived from selenium (Se)-enriched *L. edodes* mycelium. The *L. edodes* mycelial cultures were cultivated in Se-enriched media. Culture media were enriched by addition of sodium selenite, to selenium concentrations ranging from 0 to 100 μg/mL. Se concentrations in the harvested mycelium, expressed in mg/g of mycelial dry mass, rose from 0.001 mg/g to) to 50 mg/g in mycelia cultivated in medium containing 0.6 μg/mL and 100 μg/ml of selenium, respectively. Se bioavailability from Se-enriched mycelium was investigated *in vitro* and *in vivo*, using three different preparations of selenated mycelium cultivated in medium enriched with 10 μg/ml Se: dried mycelium, freeze-dried mycelium, and autolyzed freeze-dried mycelium. As reference in *in vivo* experiments, sodium selenite and Se yeast formulations were used at a Se-equivalent dose. *In vitro*, estimated Se bioavailabilities were 60%, 82%, and 98% for dried mycelium, freeze-dried mycelium, and autolyzed freeze-dried mycelium, respectively. Relative Se bioavailability from *L. edodes* autolyzed freeze-dried mycelium reached 0.55 compared to sodium selenite and 0.87 compared to Se yeast, suggesting a lower extent of Se absorption. Nevertheless, the rate of absorption for *L. edodes* mycelial formulations, reflected by time of maximum concentration in blood (t_{max}), maximum concentration in blood (Cmax), and absorption rate constant (K_a) was much higher than either reference compound. For freeze-dried mycelium and autolyzed freeze-dried mycelium the rates of elimination reflected by the elimination rate constant (K_{el}) was higher than that of sodium selenite and comparable to that of Se yeast. Preliminary speciation of selenium in Se-enriched mycelium of *L. edodes* was carried out by specific oxido-reduction reactions. Significant and dependent on the total Se content was the amount of elemental red selenium in tested mycelia. In mycelium cultivated in medium enriched with 10 μg/ml Se, most Se was present as Se^0 and Se^{IV}, either in an inorganic form or combined to lipid or carbohydrate structure. About 40% was present as Se^{II} in Se-amino acids or in other undefined water or alcohol soluble organic compounds. The differences in the pharmacokinetic data of *L. edodes* mycelial formulations and Se yeast-particularly the differences in t_{max}-are probably due to the presence of different Se species. In Se-yeast, almost 85% of Se is in the form of seleno-amino acids, but in the tested mycelial formulations, this species were less than 8%. The obtained pharmacokinetic data suggest that formulations of selenized mycelium of *L. edodes* could be used as Se dietary supplements.

T53 THE ROLE OF SELENIUM AND ZINC IN FORMATION OF ANTIOXIDANT QUALITIES OF PLANT FOOD

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As one of the reasons, human aging is attributed to the increasing concentration of high-reactive free radicals (superoxide anion radical, hydroxyl radical and others) in the organism. These active species of oxygen are formed in the process of normal metabolism, but their amount considerably increases under stress conditions (air pollution, low-grade food, irradiation, exposure to heavy metals, treatment with some forms of medicines and others). For compensating the negative effect of free radicals it is recommended to consume more natural antioxidants. However, the accumulation of antioxidants by plants strongly depends on the conditions of growth as plants sensitively react to changes in conditions such as light intensity, mineral availability, temperature and water availability. The purpose of this work was to study the influence of selenium and zinc on the accumulation of some low-molecular antioxidants by barley plants. 7-day sprouts of barley (*Hordeum vulgare* L.) were used as the object of study. A group of plants was cultivated on Hogland's nutrient solution containing all essential macro- and micronutrients excluding selenium and zinc. Three other groups were cultivated on the same nutrient solution with addition of selenium (sodium selenate, 1 µmol/L), zinc (zinc chloride, 2 µmol/L) or both. The total antioxidant activity and content of some low-molecular antioxidants (rutin, carotenoids, ascorbic acid, riboflavin, glutathione) were determined. Analytical determinations were carried out by amperometry (total antioxidant activity), potentiometric titration (rutin), spectrophotometry after extraction with acetone (carotenoids), photometry after reduction with 2, 6-dichlorphenolindophenol (ascorbic acid), photometry after extraction with 0.1 M HCl (riboflavin), and fluorimetry after reaction with o-phthalaldehyde (glutathione). It was established that addition of selenium (without Zn) to the nutrient solution led to a significant increase of the content of carotenoids (+43.4%), glutathione (+30.4%) and ascorbic acid (+26.5%) in the studied plants. The addition of zinc (without Se) led to a significant increase of the content of carotenoids (+57.6%), ascorbic acid (+39.14%), glutathione (+23.9%) and of total antioxidant activity (+23.2%). The influence of either selenium or zinc on the accumulation of rutin and riboflavin in barley plants was not significant. The maximum level of antioxidants was determined in the plants grown on the nutrient solution containing both selenium and zinc. In these plants, the levels of total antioxidant activity (+53.6%), ascorbic acid (+55.5%), carotenoids (+60.2%), glutathione (+42.5%) were higher than those observed in plants grown on a nutrient solution containing either selenium or zinc. Thus, the results prove the positive action of selenium and zinc on the content of antioxidant species in plant food.

T54 DOES A ZINC DEFICIENT DIET INDUCE IRON UPTAKE?

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Zinc stress has been reported as an inducer of iron uptake in plants. This would suggest that zinc deficiency may lead to increased iron uptake in animal tissues. In this study, 20 pregnant rats (hence in a stressful condition) were fed on a low-zinc diet whilst 20 pregnant rats were fed a control diet. The distribution and levels of iron and other trace elements in maternal and pup tissues were determined. Results were analysed to investigate micronutrient metal interactions. Feeding pregnant rats with a low zinc diet resulted in: decrease in maternal body weight, fetal resorption, suppression of fetal growth and low placental weight. In the low-zinc diet group a significant decrease of serum zinc and increase of serum iron were observed at late stage of gestation compared with the controls. Analysis of tissues disclosed significantly lower levels of zinc in maternal spleen and fetal liver, significantly higher concentration of iron in maternal liver, spleen and kidney and in fetal liver in the low zinc diet group, compared with the controls. The above findings suggest the presence of some interactions of micronutrient metals, especially those between zinc and iron in tissues.

T55 FRACTIONATION ANALYSES OF COPPER, IRON, MANGANESE AND ZINC IN HERBAL INFUSIONS

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Fractionation analyses are important to investigate the effects of dietary components on bioavailability of essential microelements. The chemical forms of Cu, Fe, Mn and Zn in chamomile (*Flores Chamomillae*), peppermint (*Follium Menthae*), hibiscus (*Hibiscus sabdariffa*), oregano (*Origanum vulgare*), and thyme (*Thymus serpyllum*) infusions were studied using inductively coupled plasma mass spectrometry and electrothermal atomic absorption spectrometry after using off-line fractionation. The fractionation scheme included ion-exchange, selective precipitation and liquid-liquid extraction procedures to separate the chemical forms of the micronutrients. It was applied to herbal infusions after simulating gastric and intestinal conditions. The extraction efficiency of the infusion process was estimated and the proportions of the elements content in the herbal tea as compared to the respective total contents in leaves were in the range 37-43% for Cu, 1.7-9.5% for Fe, 27-86% for Mn and 13-67% for Zn. The results indicate that at the acidity of gastric juices (pH 1.3), Cu, Fe, Mn and Zn exist in cationic form. At pH 7.6 (intestine conditions) copper, iron and zinc form neutral or negatively charged complexes with phytoligands in the infusions. Only manganese remained mainly in cationic form (80±6%) at pH 7.6. Hibiscus tea was found to be a rich dietary source of manganese (2.8-6.4 mg/L Mn). The weekly intake of bioaccessible essential elements when consuming herbal teas was estimated.

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T56 MINERAL CONTENT AND IRON SPECIATION OF TRADITIONAL TURKISH FRUITS' JUICE CONCENTRATE (PEKMEZ)

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Pekmez is a traditional Turkish food. Generally, fresh or dried grapes are used in the production of pekmez; but apple, watermelon, mulberry, apricot and locust bean can also be used. Pekmez is a rich source of major and minor minerals, especially iron. The nutritional value of a food for a given mineral depends not only on the amount, but also on its bioavailability for humans. It is generally accepted that only soluble non heme iron can be absorbed; thus, only a fraction of the soluble iron is available. So, it is well known that Fe^{2+} is more available than Fe^{3+}, because the latter has a low solubility in the gut. Generally, spectrophotometric methods are used for the determination of iron species but many chemical compounds in foods may interfere in the determination. Derivative spectrophotometry is an analytical technique of great utility for resolving mixture and overlapping spectra and has been applied to several analytical problems. In this study, four different traditional Turkish fruits' juice concentrate (Pekmez) were analyzed for elements (Ca, Cu, Fe, Mg, Mn and Zn) by Atomic Absorbtion Spectrophotometry (AAS). Moreover, a first derivative spectrophotometric method was developed for iron speciation of ferrous (Fe^{2+}), ferric (Fe^{3+}) and organic-bound iron in these samples. This method improves accuracy in determination and it may also be employed to estimate the availability for absorption of iron. The results of this study showed the following ranges for the Ca, Mg, Cu, Mn, and Zn concentration in grape, mulberry, locust bean and apricot based pekmez: 31.57-80.36, 10.11-59.17, 0.24-2.34, 0.21-2.18 and 1.24-219.00 mg/100 g, respectively. The total iron levels in pekmez samples were ranged from 7.24 to 18.99 mg/100 g. The percentage of Fe^{2+} and Fe^{3+} in total Fe of samples were 43.15 to 49.10% and 3.80 to 31.47%, respectively. The major findings of this study were the relatively high concentration of total and ferrous iron in the fruits' juice concentrate, indicating potentially high iron bioavailability. We suggest that the traditional Turkish fruits' juice concentrate (Pekmez) may be used instead of iron fortified food to increase iron intake for vegetarians.

T57 IRON AND ZINC IN FORTIFIED BRAZILIAN SOYBEAN JUICE

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Iron deficiency is the single most common nutritional disorder worldwide. Because of their high iron requirements, the most commonly affected groups are infants, children, adolescents and women of child-bearing age. The adverse effects of iron deficiency result from both impaired oxygen transport due to anaemia and the consequences of tissue iron deficiency that influences aerobic metabolism, protein synthesis, receptor function and many other metabolic processes. Zinc has catalytic, structural and regulatory functions and is a component of many enzymes. Skin lesions, anorexia, growth retardation, hypogonadism and suppression of immune function are caused by zinc deficiency. Food fortification is one of the components of a cost-effective strategy for addressing micronutrients deficiencies. The aim of this work was to evaluate iron and zinc content in fortified soybean juice commercialized in Brazil. The samples collected for this study had the following characteristics: 48 samples of soybean juice were chosen in an arbitrary way and divided in 16 different flavours. The analytical determinations were made by Atomic Absorption Spectrometer (Perkin-Elmer Aanalyst 300, equipped with a deuterium lamp background corrector). Samples of 0.60 g were taken and mineralized in a digester block with a mixture of concentrated HNO_3 and H_2O_2. Iron and zinc content in soybean juice ranged from 0.08 up to 1.75 mg/100 mL (in average, 1.00 mg/100 mL) and from 0.04 up to 0.69 mg/100 mL (in average, 0.40 mg/100 mL), respectively. The juices labels indicated 1.05 mg/100 mL for iron and 0.53 mg/100 mL for zinc. Iron concentration in fortified soybean juice met the label claim in 51% of the products tested, exceeded in 21% whereas in 28% was below the label. Zinc content was in compliance with the label claim in 51% of the samples, but exceeded it in 47%. The molar ratio iron:zinc for enriched juice was 3:1. The RDI (Recommended Dietary Intake) for iron and zinc for the Brazilian population is 14 and 7 mg/day, respectively When 200 mL of fortified juice are consumed, on average, 15% and 12% of iron and zinc RDI, respectively, are ingested. However, as showed, the products were heterogeneous as far as zinc and iron content is concerned.

T58 IRON IN COOKIES PREPARED WITH FORTIFIED FLOUR

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In 2002, Brazil issued a regulation, effective in 2004, requiring that corn and wheat flours be fortified by the addition of folic acid (150 µg/100g±20%) and iron (4.2 mg/100g±20%). However, the concentration of these nutrients in food containing enriched flours as ingredient is unknown. According to IBGE (Brazilian Institute of Geography and Statistics) the consumption of cookies for the Brazilian population is 2.5 Kg/people/year. Iron deficiency is the most common nutritional disorder in the word, and it is estimated that 3.5 billion people could be affected. Therefore, it is an important public health problem in both developed and developing countries. On the other hand, iron is implicated in many others reactions of potential biological significance, *e.g.*, cellular DNA damage, under pro-oxidant conditions, has been show to be mediated by iron. The aim of this work was to evaluate the iron content in cookies commercialized in Brazil. Samples had the following characteristics: 30 samples of cookies were chosen in an arbitrary way and divided in 6 different brands. The analytical determinations were made by Atomic Absorption Spectrometer (Perkin-Elmer Aanalyst 300, equipped with a deuterium lamp background corrector). Samples of 0.60 g were taken and mineralized in a digestor block with a mixture composed by concentrated HNO_3 and H_2O_2. Iron concentration ranged from 2.4 up to 7.4 mg/100 g (in average, 5.0 mg/100 g). The cookies labels indicated that the products were prepared with fortified flour. Approximately 3% of samples contained less iron than the amount added to flours, 47% presented adequate mineral levels and 43% of samples exceeded the Legislation for iron in enriched flours. The RDI (Recommended Dietary Intake) for iron for the Brazilian population is 14 mg/day. When 100 g of fortified cookies are consumed, in average, 36% of iron RDI is ingested. On the other hand, the results indicated that the iron concentration in the tested samples was heterogeneous and it was inadequate for several of them. Therefore, the aims of the campaign of flours fortification in Brazil could at least in part compromised.

T59 IRON AND ZINC IN SNACKS

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Iron deficiency has a significant impact on the well-being of individuals as well as the productivity of societies. The adverse effects of iron deficiency resulting from both impaired oxygen transport and many others metabolic process in which iron takes part, influence aerobic metabolism, protein synthesis and receptor functions. Zinc has catalytic, structural and regulatory functions and is a component of many enzymes. Some countries, including Brazil, issued a regulation requiring that corn and wheat flours be fortified by the addition of folic acid (150 μg/100g) and iron (4.2 mg/100g) and these flours are used for prepare read-to-eat-foods, including snacks. The aim of this work was to evaluate iron and zinc content in snacks prepared with flour fortified with iron, commercialized in Brazil. Samples were evaluated had the following characteristics: 30 samples of snacks were chosen in an arbitrary way, and divided in 6 different tastes. The analytical determinations were made by Atomic Absorption Spectrometer (Perkin-Elmer Aanalyst 300, equipped with a deuterium lamp background corrector). Samples of 0.60 g were taken and mineralized in a digestor block with a mixture composed by concentrated HNO_3 and H_2O_2. Iron and zinc content in samples ranged from 2.9 up to 6.9 mg/100g (in average, 4.4 mg/100g) and from 0.2 up to 0.8 mg/100 g (in average, 0.4 mg/100g), respectively. The snacks labels indicated 0.54 mg/100g for iron. Iron concentration in fortified snacks exceeded the label claim in 100% of the products tested. The RDI (Recommended Dietary Intake) for iron and zinc for the Brazilian population are 14 and 7 mg/day. When 100 g of snacks are consumed, in average, 31% and 6% of iron and zinc RDI, respectively, are ingested. However, the snacks samples evaluated were heterogeneous in the content of these minerals. The molar ratio iron:zinc for snacks was 13:1 and such a ratio could have a negative effect on zinc absorption.

T60 DETERMINATION OF MINERAL NUTRIENTS IN CEREAL FLAKES

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Nutrients are chemical substances that contribute to the normal functioning of the organism. Minerals are a group of nutrients that human organism needs but can not synthesize, therefore, they should be supplied by the diet. Nutritionists have recommended breakfast as an important meal, which is associated with an increase of energy and resistance, better attitude in school or work, maintenance of a constant glucose concentration in blood and prevention of hunger. People that consume cereals in the breakfast have a reasonably smaller ingestion of fats and cholesterol than those that consume other types of food. The present work aimed to determine the levels of Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Zn and Se in several types of cereals flakes, generally used at breakfast. Eighteen types and/or trademarks of cereal flakes were analysed-15 contained sugar, 21 were cereals flakes with chocolate, 18 were natural cereal flakes. Samples were treated by microwave irradiation: 0.6 g of sample was weighed, 2 mL of concentrated HNO_3, 0.5 mL of H_2O_2 30% v/v and 0.5 mL of water were added and the following microwave treatment applied: 15 minutes at 160 W, 5 minutes at 500 W and 10 minutes at 0 W. After cooling, the solution was diluted to 10 mL with water. The analytical determinations of the elements were made by ICP OES. The technique of multivariate analysis, using the method of pattern recognition, was adopted to study the relations between the samples analyzed and the results obtained. This allowed concluding that in general there were not significant differences concerning the mineral nutrients among the samples of cereals flakes studied. Furthermore, the data obtained allowed us to conclude that the samples of cereal flakes are good sources of Fe, Zn and Cu, important minerals for child and teenager development and the maintenance of a good health condition. They were also a good source of Na, K and P, but a poor source of Ca. Some care is needed considering the relatively high content of Na, since this mineral is present in a great variety of foods possibly leading to an excessive ingestion of this element.

T61 PECULIARITIES OF MINERALS IN IRANIAN DAILY DIETS

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The main results of a comprehensive study on trace elemental content of some typical daily diets of Iranians are presented. Some aspects of a study performed by the International Atomic Energy Agency (IAEA) in a few more countries are discussed as well. The actual purpose was to work out the intakes of nutritionally important trace elements via daily diets by certain population groups and/or by a reference man. This work was completed internationally in 1999, but it could be only accomplished in Iran very recently. The objective was not only a reassessment of the intake of 24 minerals by actual analytical measurements, but also to look at the peculiarities such as interrelations, bioavailability, interactions between and among trace elements (essential, toxic, radioactive ones). Some sort of crosschecking was also carried out. Moreover, energy intakes in mg(s) of trace elements per MJ per head are presented. In this recent work, a few population groups were added to the ones previously studied with the same general objectives and also to increase/verify the reliability of the applied methodologies. The diets were prepared by recording Dietary Regimes (RD), Duplicate Diets (DD) and Market Basketing (MB). The techniques for elemental measurement were mostly NNA, ICP-AES, AAS, whereas for the measurement of other parameters such as phytate, fiber and energy, the appropriate chemical methods were applied. Since there is plenty of results, apart from summarizing them in a few tables, the attempt is to present them individually and in aggregate form (where appropriate). The results were compared with those of other countries as well as with the Recommended Daily Allowances (RDAs). The outcome of these comparisons were generally fair, but some deficiencies of I, Se, Fe, Zn in a few Iranian population groups were detected. High or borderline exposure of a few toxic trace elements in other study groups were identified as well. The bioavailability and interrelations among trace elements such as Zn and Fe in presence of excess of phytate and Ca need to be thoroughly discussed. On the other hand, some interactions and imbalances among essential, toxic and counterpart radio elements were observed and should be taken into consideration as well.

T62 ESSENTIAL ELEMENTS IN PRE-TERM AND FULL-TERM HUMAN COLOSTRUM

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Deficiency of minor and trace elements can lead to various disorders in the early stages of child development. During early childhood trace element requirements, are more critical due to faster growth rates. Human milk is recommended as the only source of nutrients for infants up to 6 months. In this study, human colostrum samples from mothers of pre-term and term newborns were studied. Samples were collected by manual expression from the first to the fifth day after birth. After sampling, human colostrum samples were frozen and freeze-dried until the analyses. In this study, Ca, Cl, Fe, K, Mg, Mn, Na, Se and Zn were determined in 15 colostrum samples from each group by means of Instrumental Neutron Activation Analysis. About 100-200 mg of these powdered samples and the element standards were irradiated in the IEA-R1 nuclear research reactor at IPEN/CNEN-SP. For quality control of the results, NIST RM 8,435 Whole Milk Powder and NIST SRM 1549 Non Fat Milk Powder reference materials were also analyzed. The following results were obtained for the term and pre-term groups, respectively: Ca 224±81 µg/mL and 220±73 µg/mL; Cl 746±266 µg/mL and 951±294 µg/mL; Fe 0.52±0.36 µg/mL and 0.73±0.38 µg/mL; K 651±161 µg/mL and 786±244 µg/mL; Mg 16.6±8.6 µg/mL and 25.0±11.2 µg/mL; Na 632±424 µg/mL and 834±388 µg/mL; Mn 30±14 ng/mL and 48±23 ng/mL; Se 18.9±8.4 ng/mL and 37.9±19.2 ng/mL; Zn 8.0±2.7 µg/mL and 12.1±6.3 µg/mL. For each element large concentration differences were observed in both term and pre-term groups. Multivariate analyses were applied and the results were separated in two clusters. However the separation was not related to the corresponding gestational age.

T63 TRACE ELEMENTS IN HUMAN MILK

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The trace elements zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) were measured in human milk samples from ninety Sudanese mothers. Samples were collected from three cities, Khartoum, Khartoum north and Omdurman, from the second day of delivery up to the third month, when the milk reaches a relative stable level. Therefore, the samples represented different stages of lactation, *i.e.,* colostrum (1-3 days after delivery), transitional (up to 14 days after delivery) and mature milk. The main aim of this study was to measure the selected trace elements in relation to the stage of lactation and to compare the concentrations found with the literature. Atomic absorption spectroscopy and X-ray fluorescence were used to measure the analytes. These methods were found to be quite reliable as proved by the analysis of a standard reference material (Human Milk HM-1) set by WHO/FAO. Colostrum was found to have the highest amounts of trace elements. Fe mean concentration was 273 $\mu g/dm^3$ at the colostrum stage and it decreased to 146 $\mu g/dm^3$ in mature milk. Zinc decreased from 6,000 $\mu g/dm^3$ in colostrum to 1,300 $\mu g/dm^3$ in mature milk. Cu decreased from 370 $\mu g/dm^3$ to 117 $\mu g/dm^3$ and Mn decreased from 12 $\mu g/dm^3$ in colostrum to 2.9 $\mu g/dm^3$ in mature milk. These results show that Sudanese human milk samples were found to be at normal levels when compared with standard human milk, although some samples contained low levels and others contained high levels of trace elements, possibly due to individual trace-element inadequacies, excesses or imbalances.

T64 MINERAL AND TRACE ELEMENTS DISTRIBUTION PATTERN IN INFANT FORMULA AND RAW COW MILK

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The need of minerals and trace elements by growing infants has been relatively well studied. Mineral profile of infant formula is based on human milk. However, the mineral and trace element bioavailability from human milk in comparison with infant formula is not exclusively explained by total element composition since the ligand binding, therefore the distribution between casein and whey proteins, plays a role of special relevance. Casein may act as a limiting factor for the absorption of minerals and trace elements in the newborn with reduced digestive function. Both the total concentration and the distribution in fat, whey and centrifugable pellet (casein) fractions of minerals (Ca, P, Mg, Na and K) and trace elements (Zn, Fe, Cu, Mn, Se, Cr and Al) were investigated in raw cow milk (n=26) and different types of infant formula (n=50). Cow milk and reconstituted infant formula were fractionated by ultracentrifugation. Total and lipid fraction element content were determined by flame or graphite furnace atomic absorption spectroscopy in digested samples. Digestion was carried out with sub-boiling nitric acid in a closed, pressurized and high-throughput microwave digestion unit. Elements in the whey fraction were quantified by means of a direct slurry method. As expected, global mineral content was lower in infant formula than in raw cow milk (Na: 251.7 *vs* 413.9 mg/L; K: 753.4 *vs* 1,360.8 mg/L; Ca: 733.5 *vs* 1108.8 mg/L; P: 456.5 *vs* 913.8 mg/L; Mg: 74.5 *vs* 103.9 mg/L). On the other hand, infant formula supplied higher contents of trace elements, except for selenium, due to the supplementation in manufacture (Zn: 5.6 *vs* 4.2 mg/L; Fe: 8.33 *vs* 0.27 mg/L; Cu: 617.4 *vs* 62.7 µg/L; Mn: 250.9 *vs* 26.9 µg/L; Se: 9.5 *vs* 15.5 µg/L and Cr: 11.98 *vs* 3.88 µg/L). The electrolytes, sodium and potassium, as well as magnesium to a smaller extent, were mainly found in ionized form in the whey fraction (Na: 84.2 and 87.4%; K: 87.2 and 92.8%; and Mg: 55.8 and 65.9%, in infant formula and cow milk, respectively). Calcium and phosphorous exhibited a similar distribution pattern between casein (Ca: 54.4 and 47.8%; P: 62.7 and 66.3%) and whey (Ca: 45.3 and 49.7%; P: 29.4 and 28.9%) fractions. The larger proportion of copper (30.97 *vs* 17.6%), manganese (33.0 *vs* 12.9%) and selenium (54.4 *vs* 41.0%) found in the whey fraction for infant formula with respect to cow milk is also worth mentioning. Moreover, iron, zinc and chromium showed a relatively high association with the lipid fraction in both milk studied (Fe: 13.2 and 16.6%; Zn: 17.0 and 3.4% and Cr: 12.6% and 44.4%). High levels of aluminium found in whey fraction for infant formula (51.2% *vs* non detected in raw cow milk) gives an idea of its possible high bioaccessibility and potential risk for infants fed on. Overall, the findings of this study of mineral distribution might be useful to throw light on the bioavailability of the elements analyzed from different types of infant formulae.

T65 FRUITS AS AN ESSENTIAL PART OF A HEALTHY DIET

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Fruits are valued as nutritive foods that are pleasing to eat. They are eaten fresh, processed into canned, frozen and baked products or converted into juice or jam. Fruits are classified according to their botanical or geographical relationships, similarities in fruit type or manner of cropping and even culinary use. Fruits are irreplaceable in balancing the human diet due to the fact that they contain a number of essential vitamins and minerals that cannot be found in other types of foods or they contain higher levels of these nutrients than other foods. They play a significant role in human nutrition, especially as sources of vitamins C, A, E, thiamine, niacin, pyridoxine, folacin, some minerals, and dietary fiber. Fruits and vegetables in the daily diet have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke, and other chronic diseases. However, mean consumption rates vary widely depending on age, socio-economic class, and climate. The purpose of our study was to assess quality of different fruit products based on their mineral composition. The total elements (Mg, Ca, Na, K, P) contents in different kinds of fruit products obtained at retail were analysed by Flame Atomic Absorption Spectrometry (FAAS) using deuterium-background correction. Phosphorus was determined in the form of phosphomolybdate by a spectrophotometric method. Reliability of the procedure was checked by the analysis of the certified reference materials Tea (NCS DC 73351) and Cabbage (IAEA-359). The recoveries obtained varied between 85 and 103% and precisions for the reference materials were 0.41-7.26%. Based on the results obtained in the study, it was concluded that fruits differ significantly in their mineral composition according to type, botanical family as well as geographical provenance. The intake of the studied essential elements was estimated and it was found that 100 g of fresh fruits supplies to human body, on average, with 3.6% of the RDA of P, 2.82-3.53% of the RDA of Mg, 0.11% of the RDA of Na, 2.59% of the RDA of K, and 1.59-1.79% of the RDA of Ca.

T66 HEALTH BENEFITS RELATED TO CONSUMPTION OF WILD AND FARMED ATLANTIC SALMON

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With the worldwide expansion in aquaculture production, more and more farmed seafood is now marketed. It would appear that the concentration of most nutrients is relatively uniform as compared with wild fish; however the limited information available on farmed fishery products does not allow any general statements to be made. Vitamins and minerals are nutrients that are essential to life. However, providing the body with the correct quantity of vitamins and minerals is only possible when the diet is well-balanced and varied. Nutritional anaemia is the subject of special interest of the World Health Organization (WHO). International data suggest that about 1.5% of deaths worldwide is caused by IDA (Iron Deficiency Anaemia). The lack or excess of a single nutritional component, *i.e.* Fe, folic acid, protein, vitamins B12, B6, B2, as well as Cu, Se, Co, Zn, Mn or Mo cause disorders in erythrocyte and haemoglobin synthesis. According to literature data, mutual quantitative proportion of particular vitamins and mineral components plays an important role in anaemia prophylaxis. The aim of this research was to determine the concentrations of bioelements (iron, zinc and copper) and B vitamins (B12 and B6) in the muscle tissue of wild and farmed salmons. The vitamins were determined in the free form; an extraction step was performed prior to chromatographic separations by an isocratic HPLC method with coulometric electrochemical detection. The agreement between the analytical results obtained for the reference material (pig's liver - CRM 487) and the certified values was satisfactory, *i.e.*, the recoveries for vitamins B6 and B12 were 100.5% and 96.5%, respectively. Fe, Zn and Cu concentrations in salmons samples were determined in an air-acetylene flame by AAS using deuterium-background correction (Philips PU 9100X spectrometer). The quality of the method was checked and confirmed by the analysis of certified reference materials, *i.e.* Cabbage (IAEA-359) and Spinach leaves (NIST-1570). Recoveries of the elements analysed ranged between 88.1% and 96.5%. In conclusion, the study results show that the salmons analyzed exhibited high levels of vitamins B6 (0.45-0.762 mg /100g) and B12 (1.99-2.49 µg/100 g) and relatively low levels of Fe (0.16-0.31 mg/100 g), Zn (0.25-0.58 mg/100 g) and Cu (0.03-0.13 mg/100 g). Generally, consumption of salmons may significantly contribute to satisfying the human daily needs for vitamins B, especially B12. In spite of the relatively low content of iron, it has to be emphasized that its bioavailability from fish muscles, contrary to plant foods, is very high due to its presence mainly in the form of heme (Fe-protoporphyrin).

T67 IRON AND ZINC AVAILABILITY IN OPAQUE SORGHUM BEER

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Opaque sorghum beer is a significant component of the diet of millions of poor people in rural Africa. This study reports the effect of traditional brewing operations on its level of micronutrients, especially iron and zinc. The example of a West African sorghum beer, tchoukoutou, in Northern Benin was studied. The beer was characterized and the impact of process unit operations on phytate, phenolic compounds, and Zn and Fe *in vitro* solubility was evaluated. The major microorganisms involved in the beer fermentation were *Saccharomyces cerevisiae* and *Heterofermentative lactobacilli*. The manufacturing process reduces the phytate content by nearly 95%, particularly during germination, mashing-boiling and fermentation. The level of reactive phenolic groups increased as a result of germination and fermentation as well as a result of a shift in dry matter compensation. Simultaneously with these modifications, an increase of Fe solubility was observed, and an inverse correlation between phytate and Fe solubility ($R^2=0.85$) was established. No clear correlation could be established between the Zn solubility and the phytate content of the products. During beer manufacturing, significant losses of minerals occur particularly during soaking and mashing/filtration; thus the quantity of minerals available to consumers is restricted. Improvements aiming to minimize such losses are highly desirable.

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