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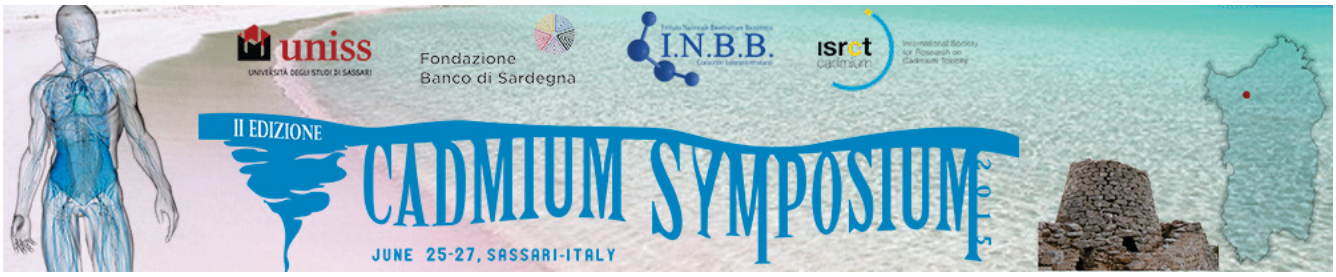

Cadmium Symposium 2015

ICS 2015

Congress Proceedings

Sassari | June 25-27, 2015

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Dear Friend and Colleague,

It's a great pleasure to welcome you to the "II Cadmium Symposium 2015".

Cadmium is a heavy metal with a high toxicity, even at very low dose, showing acute and chronic effects on human health and high impact on environment.
With a wide spectrum of presentations about the main aspects of Cadmium biology as well as its clinical implications, the meeting counts four key sessions:

Cadmium and Environment
Cadmium and Cell Biology
Cadmium and Diseases
Cadmium and Agronomics, Botany and Veterinary

Participants have the opportunity to exchange ideas with worldwide experts in the field and highly distinguished international speakers from different scientific areas related to biological and medical aspects.

The University of Sassari is a small but prestigious University which in 2012 celebrated 450 years since its foundation. The University was founded by Alessio Fontana in 1558, a distinguished gentleman of the town of Sassari and member of the Imperial Chancellery of Emperor Charles V. The official opening dates back to month of May 1562.
Sassari is located in the northwest of Sardinia, a region rich in natural and cultural attractions, with old traditions, beautiful sceneries and excellent cuisine. The area offers many itineraries to people interested in archeology, art, history, wine and food. The weather in late spring is usually very pleasant climate, an ideal time to visit one of the most beautiful location in the Mediterranean.

We hope that you will enjoy the Symposium and have a good time in Sardinia.

Yours sincerely,
Roberto Madeddu
Chairman

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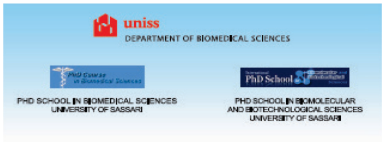

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ICS 2015

Program

Thursday, June 25

14.30-15.15 Registration
15.15-15.30 Welcome Greetings
15.30-15.40 **Michael P. Walkees** NIH, USA *Open greetings*
15.40-17.20 **CADMIUM AND ENVIRONMENT 1st part**
Co-Chairs
Paolo Castiglia, Vesna Matović

15.40-16.00 Associations between Cadmium exposure and risk of chronic diseases: Results from large population-based studies in Sweden
Agneta Åkesson *Karolinska Institutet, Sweden*
16.00-16.20 Combination of Cadmium and high cholesterol levels as a risk factor for hearth fibrosis
Barbara Messner *Medical University of Wien, Austria*
Discussion
16.30-16.50 Dietary Cadmium intake and breast cancer risk: a meta-analysis of longitudinal studies and results of the ORDET study
Marco Vinceti *University of Modena-Reggio, Italy*
16.50-17.10 Puberty onset, oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to Cadmium and living in the Milazzo, Valle del Mela area (Sicily, Italy)
Francesco Squadrito *University of Messina, Italy*
Discussion

17.20-17.40 ***Coffee break***

17.40-19.20 **CADMIUM AND ENVIRONMENT 2nd part**
Co-Chairs
Andrea Montella, Claudia Blindauer

17.40-18.00 Cadmium, Van Gogh and the European REACH regulation
Franz-Georg Simon *BAM Federal Institute for Materials Research, Berlin, Germany*
18.00-18.20 Geo-chemistry of Cadmium in Sardinia: regional scale studies
Alessandro Sanna *University of Cagliari, Italy*
Discussion
18.30-18.50 Metabolic adaptation to exercise and minerals loss in athletes
Michael Kalinski *Murray State University, KY, USA*
18.50-19.10 Dietary exposure to Cadmium and risk of breast cancer among postmenopausal women: a systematic review and meta-analysis
Geneviève Van Maele-Fabry *Université Catholique de Louvain, Brussels, Belgium*
Discussion

Friday, June 26

8.40-11.10 **CADMIUM AND CELL BIOLOGY 1st part**
Co-Chairs
Vincenza Bianchi, Jean Marc Moulis

8.40-9.00 Can metallothioneins display metal specificity?
Claudia Blindauer *University of Warwick, UK*

9.00-9.20 The environmental pollutant Cadmium alters human osteoblasts homeostasis in vitro by alteration of wnt/β-catenin pathway
Roberto Scandurra *University of Rome "La Sapienza", Italy*
Discussion

9.30-9.50 Spatio-temporal dynamics of acid sphingomyelinase in life-death decisions of renal proximal tubule cells exposed to Cadmium
Wing-Kee Lee *Memorial Sloan Kettering Cancer Center New York, USA and Witten/Herdecke University, Germany*

9.50-10.10 Interference by Cadmium with the cellular response to DNA damage: Impact on redox regulation and gene expression profiles
Andrea Hartwig *Karlsruher Institut für Technologie (KIT), Germany*
Discussion

10.20-10.40 Cadmium interference with ERK1/2 and AhR signaling cascades without evidence for cross-talk
Catherine Jumarie *University Québec à Montréal, Canada*

10.40-11.00 Roles of gluthatione and mitochondria in acute defense and adaptation to Cadmium-induced oxidative stress and toxicity of the kidney proximal tubule in vitro and in vivo
Frank Thévenod *Witten*/Herdecke *University, Germany*
Discussion

11.10-11.30 ***Coffee break***

11.30-12.00 **OPENING CEREMONY AND GREETINGS AUTHORITIES**

12.00-13.00 **PANEL DISCUSSION**
Moderator
Simona De Francisci (Journalist)
Speakers
Agneta Åkesson
Mario De Tullio
Andrea Hartwig
Roberto Madeddu
Marco Vinceti

13.00-14.00 ***Lunch***
14.00-15.00 **POSTER SESSION**

15.00-16.15 **CADMIUM AND CELL BIOLOGY 2nd part**
Co-Chairs
Roberto Scandurra, Catherine Jumarie

15.00-15.20 Prolonged Cd and PCBs co-exposure and thyroid function in rats: Is the liver relevant point of their interactions?
Vesna Matović *University of Belgrado, Serbia*

15.20-15.40 The mammalian metabolism of Cadmium: dynamic and structural aspects
Juergen Gailer *University of Calgary, Canada*

15.40-16.00 Chemical imaging and overall assessment of Cadmium distribution in the human body
David Bernhard *Innsbruck University, Austria*
Discussion

16.15-19.20 **CADMIUM AND DISEASES**
Co-Chairs
Salvatore Dessole, Frank Thévenod

16.15-16.35 The protection of Selenium against Cadmium toxicity in chicken lymphocytes via selenoproteins and heat shock proteins
Wen-chao Zhao *Northeast Agricultural University of Harbin, China*

16.35-16.55 Immunotoxicology of Cadmium: insight from acute intraperitoneal and intermediate period of oral exposure of rats
Milena Kataranovski *University of Belgrade, Serbia*
Discussion

17.10-17.30 ***Coffee break***

17.30-17.50 Impact of persistent low-level Cadmium exposure on pancreatic beta-cells
Jean-Marc Moulis *University Grenoble Alpes, France*

17.50-18.10 Chronic Cadmium exposure in rats produces pancreatic impairment and insulin resistance in multiple peripheral tissues
Eduardo Brambila *University of Puebla, Mexico*
Discussion

18.20-18.40 Cadmium, atherosclerosis and cardiovascular diseases
Lars Barregard *University of Gothenburg and Sahlgrenska University Hospital, Sweden*

18.40-19.00 Age-related accumulation of cadmium in retinal tissues: implications for AMD, a major form of blindness
Alex Langford-Smith *University of Manchester, UK*

19.00-19.20 Cadmium and Fever
Michele Malaguarnera *University of Catania*, *Italy*
Discussion

21.00 ***Social dinner – RISTORANTE "L'ASSASSINO"*** Sassari

Saturday, June 27

9.00-10.40 **CADMIUM AND AGRONOMICS, BOTANY AND VETERINARY 1st part**
Co-Chairs
Maria Grazia Cappai, Juergen Gailer

9.00-9.20 Toxic effects of Cadmium on reproductive function in chicken
Zi-wei Zhang *Northeast Agricultural University of Harbin, China*

9.20-9.40 Cadmium minimization in rice grain – Phytomanagement
Narasimha Majeti Varaprasad *University of Hyderabad, India*
Discussion

9.50-10.10 Cadmium accumulation and effects in two important crop plants for humans consumption (tobacco and carrot)
Luigi Sanità di Toppi *University of Parma, Italy*

10.10-10.30 Molecular dissection of Cd tolerance and accumulation in "normal" plants and Cd hyperaccumulators
Stephan Clemens *Bayreuth University, Germany*
Discussion

10.40-11.00 ***Coffee break***

11.00-13.00 **CADMIUM AND AGRONOMICS, BOTANY AND VETERINARY 2nd part**
Co-Chairs
Luigi Sanità di Toppi, Stephan Clemens

11.00-11.20 Cadmium in honey production: an overwiew on a possible marker for sustainability
Maria Grazia Cappai *University of Sassari, Italy*

11.20-11.40 Effects of Cadmium in Apis Mellifera: possible role in hive collapse
Panagios Polykretis *University of Florence, Italy*
Discussion

11.50-12.10 The mechanism of chicken embryo neuron apoptosis induced by Cadmium chloride in vitro
Guangliang Shi *Northeast Agricultural University of Harbin, China*

12.10-12.30 Cadmium and horses: past studies and future perspectives
Joana Nery *University of Torino, Italy*
Discussion

12.45 **CLOSING REMARKS**

15.00-23.30 Excursion to Alghero

ICS 2015

Oral communications

ASSOCIATIONS BETWEEN CADMIUM EXPOSURE AND RISK OF CHRONIC DISEASES: RESULTS FROM LARGE POPULATION-BASED STUDIES IN SWEDEN

Agneta Åkesson

Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

The aim was to explore to what extent long-term low-level exposure to cadmium via food plays a role in development of diseases of major public health importance.

Data from two large prospective population-based cohorts consisting of 100 000 women and men were used. The dietary cadmium exposure was estimated and incident cases of kidney stones, chronic kidney disease, cardiovascular diseases, fractures and hormone-related cancers were ascertained via linkage to national disease registers considered almost complete. Cadmium in urine and blood, circulating levels of sex-hormones and bone mineral density (BMD) was assessed in a subgroup.

Both dietary and urinary cadmium was significantly associated with lower BMD and increased risk of osteoporosis and fractures. Dietary cadmium exposure was associated with a statistically significant increased relative risk of cancer of the endometrium, breast, and prostate (39%, 21% and 13% respectively) but not with ovarian cancer comparing the highest tertile of dietary cadmium exposure with the lowest. We observed no association between dietary cadmium exposure and kidney stones, chronic kidney disease or cardiovascular diseases in women or men.

Low-level cadmium exposure from food was associated with increased risk of osteoporosis and fractures and hormone-related cancers, diseases with importance for the public health.

COMBINATION OF CADMIUM AND HIGH CHOLESTEROL LEVELS AS A RISK FACTOR FOR HEART FIBROSIS

Adrian Türkcan, Bernhard Scharinger, Gerlinde Grabmann, Barbara Messner

Cardiac Surgery Research Laboratory, Department of Surgery, Medical University of Wien, Austria

The deleterious effects of increased cadmium (Cd) serum levels on the cardiovascular system are proven by epidemiological and basic science studies. Cd exposure of animals and humans is known to impair myocardial function, possibly leading to heart failure. This study aims at investigating the effect of Cd treatment on the cardiac system with emphasis on the combined effect of Cd and high serum cholesterol levels as an important cardiovascular risk factor. Detailed analyses of Cd-induced effects on the heart of ApoE−/− mice fed a high fat diet (HFD), ApoE−/− mice fed a normal diet (ND), and C57BL/6J mice fed a ND revealed proinflammatory and fibrotic changes in the presence of cellular hypertrophy but in the absence of organ hypertrophy. Hypercholesterolemia in ApoE−/− mice alone and in combination with Cd treatment resulted in significant cardiomyocyte cell death. Based on further analyses of heart sections, we conclude that severe hypercholesterolemia in combination with ApoE−/− genotype as well as Cd treatment results in necrotic cardiomyocyte death. These data were supported by in vitro experiments showing a Cd-induced depolarization of the mitochondrial membrane and the permeabilization of the plasma membrane arguing for the occurrence of Cd-induced necrotic cell death. In summary, we were able to show for the first time that the combination of high cholesterol and Cd levels increase the risk for heart failure through cardiac fibrosis. This observation could in part be explained by the dramatically increased deposition of Cd in the hearts of ApoE−/− mice fed a HFD.

DIETARY CADMIUM INTAKE AND BREAST CANCER RISK: A META-ANALYSIS OF LONGITUDINAL STUDIES AND RESULTS OF THE ORDET COHORT STUDY

Marco Vinceti[1], Sabina Sieri[2], Tommaso Filippini[1], Vittorio Krogh[2]

[1]Center for Environmental, Genetic, and Nutritional Epidemiology CREAGEN, Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Reggio Emilia, Italy
[2]Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Cadmium is a heavy metal with estrogenic activity and established human carcinogenicity, but uncertainties exist about the amounts of exposure and the cancer types involved. In particular, the possibility that dietary cadmium may increase breast cancer risk was suggested by one cohort study, but results of the other four longitudinal investigations were inconsistent. We meta-analyzed these studies using a random-effects model, and we computed the summary relative risk (RR) of breast cancer along with its 95% confidence interval (CI) in subjects with the highest versus the lowest cadmium intake category. Summary RR was 1.00 (95% CI 0.87-1.15), while limiting the analysis to estrogen-receptor positive breast cancer RR was 1.05 (0.94-1.16). We also investigated the relation between cadmium intake and breast cancer risk in the ORDET cohort, a prospective study of 9,343 healthy women of Varese province, Northern Italy, recruited between 1987 and 1992 and followed for cancer occurrence until December 2009. Dietary habits and the corresponding estimated cadmium intake were assessed at baseline via a food frequency questionnaire. During 158,190 person-years of follow-up, 419 breast cancer cases occurred. In a multivariate Cox regression model adjusting for several potential confounders, the hazard ratio (HR) of breast cancer increased with increasing quintiles of cadmium intake, with values of 1.19, 1.23, 1.36 and 1.66, respectively (P trend=0.019) compared with bottom category. HR associated with 1-unit increase of Cd intake was 1.12 (1.03-1.21). After stratifying the analysis according to estrogen-receptor, human epidermal growth factor-receptor 2 and progesterone-receptor status, the receptor-positive breast cancer subtypes showed the strongest association with cadmium intake. Overall, these results suggest that dietary cadmium increases breast cancer risk, particularly for some disease subtypes, though the possibility of unmeasured confounding must also be considered.

PUBERTY ONSET, OXIDATIVE STRESS AND DNA REPAIR AND DETOXIFICATION GENE EXPRESSION IN ADOLESCENTS EXPOSED TO CADMIUM AND LIVING IN THE MILAZZO-VALLE DEL MELA AREA (SICILY, ITALY)

Squadrito F, Bitto A, Pizzino G, Interdonato M, Altavilla D

Department of Clinical and Experimental Medicine, University of Messina, Italy

Pubertal onset, pituitary-gonadal axis hormones, oxidative-stress, and DNA repair and detoxification genes were studied in 111 males with increased urinary levels of Cd, aged 12-14 years, living in the Milazzo-Valle del Mela area. A control age-matched population (n = 60) living 28-45 km far from the industrial site was also enrolled. Cadmium (Cd) levels were significantly higher in adolescents living in the Milazzo-Valle del Mela area, compared to both age-matched subjects living far from the industrial plants and the reference values. Our population showed also a delayed onset of puberty, a smaller testicular volume and lower testosterone levels. Urinary 8-hydroxydeoxyguanosine (8OHdG) concentration was greater in exposed than in controls ($p = 0.01$), and it was correlated with cadmium levels. Moreover, cadmium levels showed a robust correlation with OGG1(DNA repair gene), and MT1A (detoxifying gene) gene expression levels. Finally, OGG1 and MT1A were over-expressed in adolescents from Milazzo-Valle del Mela area compared with controls ($p = 0.0005$; $p < 0.0002$, respectively). In male adolescents, Cd burden is associated with delayed onset of puberty, impaired testicular growth, increased oxidative DNA damage and impaired expression of DNA repair and detoxification genes.

CADMIUM, VAN GOGH AND THE EUROPEAN REACH REGULATION

Franz-Georg Simon and Nicole Bandow

BAM Federal Institute for Materials Research, Berlin, Germany

Cd sulfide and selenide pigments are used since the 19th century for artists' paints. It is known that Van Gogh, Gauguin, Degas and other old masters had Cadmium yellow, orange and red on their palettes. Still today professional and hobby artists appreciate paints with Cd pigments due to their lightfastness, opacity and tinting strength. However, the pigments are possibly introduced to sewage treatment plants by cleaning used brushes with water. After settling together with the sewage sludge in the sedimentation basin, the pigments may be transferred to soils as sewage sludge is often used as fertilizer in agriculture. It is well known that Cd sulfides are sparingly soluble. However, the cadmium can be mobilized by oxidizing or acidic conditions. Mobilized Cd can be transported to the groundwater or be introduced in the human food chain after uptake by plants. For this reason the Swedish Chemicals Agency KEMI submitted a restriction proposal according to annex XV of the REACH regulation to ban the use and placing on the market of artists' paints with Cd pigments.

Meanwhile, the two scientific committees of the European Chemicals Agency ECHA decided not to support the restriction proposal after intensive discussions. In an experimental investigation leaching tests were performed to measure the mobilization potential of Cd under realistic environmental conditions. The results are discussed in the presentation.

GEOCHEMISTRY OF CD IN SARDINIA: REGIONAL SCALE STUDIES

Alessandro Sanna, Salvatore Pretti, Alberto Marcello, Paolo Valera

DICAAR - Department of Civil-Environmental Engineering and Architecture, University of Cagliari, Italy

For more than thirty years, the DICAAR researchers have been leading studies in Sardinia, whose territory has a long history of mining exploitation, begun before the Bronze Age. Through the sampling of several environmental media supports and their analytical data, this research activity led to the creation of a large database, populated by thousands of data concerning soils, rocks and stream sediments. Sampling and analytic surveying are currently finished. A new phase is starting up to transform the stream sediment database, which was previously used for the aim of industrial mining, as a new source of environmental information. The deep experience in mining, with the help of modern technology, allowed to refine techniques for the detection of ore deposits up to make them extremely sensitive. By transferring these experiences in the environmental field, it is possible to identify and to delimit areas presenting pollution and/or high values of harmful elements; moreover, it also allows to detect the background in studied lithologies, even when significantly hidden by anthropic activity. Geochemical data were geo-referenced with a geographic information system (GIS) and now are available for further studies. In the present study, the geochemical data of Cd was analyzed in order to evaluate the Cd contents in Sardinian soils, in relation to the Italian law limits.

METABOLIC ADAPTATION TO EXERCISE AND MINERALS LOSS IN ATHLETES

Kalinski MI

Murray State University, KY, USA

This review concerns metabolic adaptation to exercise and the loss of various minerals and trace elements, including cadmium, in athletes. Minerals, major or trace, serve as constituents of enzymes, hormones and vitamins and are involved in many aspects of energy metabolism. Excessive sweating during strenuous exercise produces a considerable loss of minerals such as sodium, potassium, calcium and magnesium and triggers release of the hormones vasopressin and aldosterone. Exercise may also increase excretion of chromium, copper, manganese, zinc. Emerging data are pointing out to the advantageous effects of regular exercise to urban residents who are exposed to cadmium. Even so the sources of literature to this effect are scarce and incomplete, available data suggest that excretion of urine toxic metals from athletes are higher compared to non-training sedentary subjects. This implies that regular exercise may counteracts the cumulative effect of toxic environment by increasing the urine excretion of toxic metals in athletes.

DIETARY EXPOSURE TO CADMIUM AND RISK OF BREAST CANCER AMONG POSTMENOPAUSAL WOMEN: A SYSTEMATIC REVIEW AND META-ANALYSIS

Geneviève Van Maele-Fabry[1], Noömi Lombaert[2], Dominique Lison[1]

[1]Louvain center for Toxicology and Applied Pharmacology, Université catholique de Louvain, Brussels, Belgium
[2]International Zinc/Cadmium Association, Brussels, Belgium

With tobacco smoking, diet is the main source of cadmium exposure in the general population. His carcinogenic and estrogenic activities make Cd a contaminant of particularly high concern for hormone-dependent cancers including breast cancer. Postmenopausal women are the most appropriate population to investigate the impact of exogenous factors with potential estrogenic activity on breast cancer.

We systematically reviewed available studies on the association between dietary exposure to Cd and breast cancer focusing on postmenopausal women. We performed a meta-analysis combining the risk estimators and tried to identify potential sources of the between studies heterogeneity. Relative risk estimates were extracted from 6 selected studies. Meta-rate ratio estimates were calculated according to different meta-analysis models. Meta-analyses were performed on the whole set of data and separate analyses were conducted after different stratifications.

No statistically significant increased risk of breast cancer was observed when all studies were combined but there was a high heterogeneity and inconsistency among studies. The results were not substantially modified after stratifications and several sources of heterogeneity and inconsistency were identified, including smoker status, HRT use, BMI, zinc and iron intake. Inconsistency was also strongly reduced for ER-, PGR-, studies from USA and from Japan.

This study does not provide support for the hypothesis that dietary exposure to Cd increases the risk of breast cancer among postmenopausal women. Misclassification in dietary Cd assessment is a major concern that could have biased the results towards a finding of no association. As the number of available studies estimating the dietary Cd intake in relation to breast cancer is limited, additional epidemiological studies among other populations and experimental studies are required to confirm the lack of association and to better understand the underlying mechanisms.

CAN METALLOTHIONEINS DISPLAY METAL SPECIFICITY?

Claudia A Blindauer, Hasan T Imam

University of Warwick, UK

After reviewing some fundamental coordination chemistry principles pertaining to metallothioneins, the talk introduce our recent work on type 4 plant metallothioneins (MTs).

Plant type 4 MTs are found in reproductive tissues such as embryos and pollen, but also aleurone layer of seeds, and in vegetative tissues of resurrection plants. It is likely that they play important roles in zinc supply to nascent proteins after re-hydration of dormant, desiccated tissues.

We have studied type 4 MTs from both a mono- and a dicotyledonous plant, namely wheat and two isoforms from Arabidopsis thaliana. They display very similar metal-binding properties and protein folds, and their Zn6 forms are very well folded. All six zinc ions can be easily displaced by cadmium; however, the second domain in the resulting Cd6 species is structurally disordered. Previous mutagenesis studies have shown the crucial importance of two conserved histidine residues, and their involvement in a mono-nuclear Cys2His2 zinc site, for governing structural order in domain II. Formation of this site is thermodynamically disfavoured in the presence of equimolar cadmium, and the resulting alternative metal-sulfur clusters formed do not lead to ordered protein structure.

We hypothesise that cadmium-induced disordered protein may also form in vivo, but that such disordered forms are prone to degradation, promoting remobilisation of cadmium, to prevent excessive storage of this toxic element in the plant embryo.

THE ENVIRONMENTAL POLLUTANT CADMIUM ALTERS HUMAN OSTEOBLASTS HOMEOSTASIS IN VITRO BY ALTERATION OF WNT/β CATENIN PATHWAY

Vincenza Papa[1], Anna Scotto d'Abusco[2], Laura Politi[2], Silvia Migliaccio[1], Roberto Scandurra[2]

1Department of Movement, Human and Health Sciences, Section of Health Sciences, "Foro Italico" University of Rome Italy
[2]Department of Biochemical Sciences, "Sapienza" University of Rome

Background: It is known that the toxic pollutant Cadmium (Cd) is widespread in the environment and can cause significant alterations in several tissues. Interestingly, Cd also causes bone tissue damages altering bone cell homeostasis. Thus, aim of the study was to investigate the potential molecular and intracellular alterations induced by Cd in human osteoblasts.

Results: Cd (10μM) induced osteoblast cells a nuclear traslocation of -catenin and an increased expression of Wnt/-catenin target genes uponr 6hrs of exposure. However, prolonged exposure to the same Cd concentration (10M) induced cell apoptosis. A following series of experiments was performed to evaluate whether Cd could induce a modulation of canonical Wnt/-catenin target genes by RT-PCR and Western-blotting analysis. A transient TCF-1 and LEF-1 protein increase was observed upon 6hrs of exposure, but after 15 and 24hrs TCF-1 and LEF-1 expression was significantly reduced as compared to untreated control. Moreover, c-Myc protein expression was increased after 6hrs of Cd exposure and started to decline after 15 and 24hrs of treatment. In addition, to further characterize the intracellular events of the effect of Cd exposure on actin filaments, and proteins associated to cytoskeletal actin, was evaluated. Interestingly, Cd exposure (15, 24 hrs) reduced LIM proteins expression and induced actin filaments destruction, indicating a disruption of cytoskeleton arrangement.

Conclusion: In conclusion, our study shows for the first time that short-term exposure of osteoblasts to Cd induces cell proliferation enhancement through a Wnt/β-catenin dependent mechanism, likely for a compensatory mechanism to toxic damage, whereas longer Cd exposure to the same concentration induces cells apoptosis by cytoskeletal disruption.

SPATIO-TEMPORAL DYNAMICS OF ACID SPHINGOMYELINASE IN LIFE-DEATH DECISIONS OF RENAL PROXIMAL TUBULE CELLS EXPOSED TO CADMIUM

W-K Lee[1], K Becker-Flegler[2], J-S Bellingrath[1], W Al-Hamdani[1],J-K von Sivers[1], I Diebels[1], E Gulbins[2], F Thévenod[1]

[1]Chair of Physiology, Pathophysiology & Toxicology, Centre for Biomedical Education and Research, Witten/Herdecke University, Germany
[2]Institute of Molecular Biology, University Duisburg-Essen, Germany

Cadmium (Cd2+) causes apoptosis, partly involving sphingolipids, in the kidney proximal tubule (PT). In autophagy, organelles and long-lived proteins are catabolized by lysosomal degradation and can contribute to cell survival or death depending on the cellular context. Recent studies indicate autophagy modulation by sphingomyelin (SM) and acid sphingomyelinase (ASM). The autophagy inducer rapamycin was also reported to prevent Cd2+-induced PT death, implying a pro-survival function. Here, in cultured rat kidney PT cells (PTCs) (WKPT-0293 Cl.2, NRK-52E), autophagy markers LC3 and p62 indicate rapid autophagy induction after 1h Cd2+ (5-10µM) that was accompanied by the formation of autophagic vacuoles labelled with monodansylcadaverine. Cd2+ exposure exceeding 3h resulted in p62 and LC3 accumulation and an increase of extra-lysosomal cathepsins, indicating decreased autophagy flux and lysosomal permeabilization. Cd2+ (6h) also increased cellular SM that was detected by lysenin staining that was associated with altered ASM activity and attenuated cell viability loss by 3-methyladenine (autophagy inhibitor). In contrast to previous reports, rapamycin (100nM) did not prevent Cd2+-induced cell viability loss after 24h. The data suggest a dual and temporal impact of Cd2+ on autophagy: A rapid activation (possibly via reactive oxygen species signaling) followed by a block of autophagy flux and increased SM, possibly resulting in lysosomal disruption and increased cell death.

INTERFERENCE BY CADMIUM WITH THE CELLULAR RESPONSE TO DNA DAMAGE: IMPACT ON REDOX REGULATION AND GENE EXPRESSION PROFILES

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The carcinogenicity of cadmium has been recognized for some decades. While direct DNA damage appears to be of minor importance, the interference with antioxidative defense systems as well as interactions with DNA repair processes, tumor suppressor functions, and signal transduction pathways have been described in diverse biological systems. As one underlying mechanism, the interference with cellular redox regulation by reaction with redox-sensitive protein domains or amino acids may provide one explanation for cadmium-induced carcinogenicity. Particularly sensitive targets appear to be proteins with zinc binding structures, present in DNA repair proteins such as XPA, PARP-1 as well as in the tumor suppressor protein p53. Especially the combination of these multiple mechanisms may give rise to a high degree of genomic instability in cadmium-adapted cells, relevant not only for tumor initiation, but also for later steps in tumor development. These interactions are also evident in a newly established gene expression profiling approach, which enables the parallel and quantitative investigation of 96 samples on the expression of 95 genes, selected by their relevance for maintaining genomic stability. Here, among other interactions, pronounced upregulation of the oxidative stress response and downregulation of DNA repair factors was observed, revealing a toxicological “finger print”, which very well reflects the mode of action of cadmium compounds.

CADMIUM INTERFERENCE WITH ERK1/2 AND AHR SIGNALING CASCADE WITHOUT EVIDENCE FOR CROSS-TALK

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Cadmium (Cd) is a toxic metal that enters the food chain. Following oral ingestion, the intestinal epithelium may in part protect against Cd toxicity but is also a target tissue. Using human enterocytic-like Caco-2 cells, we have previously shown that Cd may induce a concentration and time-dependent increase in MTT (3-[4,5-dimethyl-2-thiazol-2-yl]-2,5-diphenyltetrazolium bromide assay) activity in differentiated cultures exclusively. This effect was not related to cell proliferation but to enhanced protein synthesis which involves ERK1/2 activation. Because some studies have suggested cross-talk between AhR and ERK signaling pathways we have hypothesized that Cd may indirectly lead to AhR activation. Western blot analysis and immunofluorescence data show that: i) contrary to Cd and insulin, dioxin (TCDD) or benzo[a]pyrene do not increase ERK phosphorylation; ii) TCDD and Cd both increase the nuclear translocation of AhR; iii) TCDD but not Cd increases CYP450 1A1 expression; iv) there is no correlation between phospho-ERK and AhR activation. In conclusion, the Cd-induced hormetic-like effect on MTT activity in the Caco-2 cells is a differentiation stage-specific phenomenon that is not observed with xenobiotics acting as AhR ligands. ERK phosphorylation is not prerequisite to AhR activation and it does not necessarily lead to AhR activation. Cd does not stimulate the transcriptional activity of AhR but Cd increases the nuclear translocation of AhR and may interfere with AhR signaling cascade.

ROLES OF GLUTATHIONE AND MITOCHONDRIA IN ACUTE DEFENSE AND ADAPTATION TO CADMIUM-INDUCED OXIDATIVE STRESS AND TOXICITY OF THE KIDNEY PROXIMAL TUBULE IN VITRO AND IN VIVO

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Cadmium (Cd2+) induced oxidative stress defines cell fate and pathology. Mitochondria are energy-producing organelles, but also contribute to reactive oxygen species formation, cell injury and death signaling. Oxidative signature and mitochondrial function and biogenesis were investigated in vitro in cultured rat kidney proximal tubule cells (KPTCs) (1-30μM CdCl2, 24h) and in vivo in Fischer 344 rats (1mg/kg CdCl2 s.c., 13d) representing acute and sub-chronic Cd2+ toxicity models, respectively. In KPTCs, 30μM Cd2+ caused ~50% cell viability loss whereas apoptosis peaked at 10μM Cd2+, along with lowered reduced glutathione (GSH) and an increased oxidized glutathione (GSSG)/GSH ratio. Quantitative PCR evidenced increased anti-oxidative enzymes (Sod1, Gclc, Gclm), pro-apoptotic Bax, and metallothioneins 1A/2A, but attenuated anti-apoptotic Bcl-xL and Bcl-w. The positive regulator of mitochondrial biogenesis Ppar □ and m (mtDNA) increased and cellular ATP remained unaffected with Cd2+ (1-10μM). In vivo, active caspase-3 and augmented Bax mRNA were detected in kidney cortex of Cd2+-treated rats. Bcl-2, Bcl-xL, Bcl-w were also upregulated. Cd2+ increased GSSG and GSH but not GSSG/GSH and induced anti-oxidative enzyme expression (Gpx4, Prdx2). Augmented mtDNA, mitofusin 2 and Ppar □ indicate mitochondrial biogenesis and fusion. These results demonstrate mitochondrial involvement in acute defense against Cd2+-induced oxidative stress in KPTCs as well as in adaptation to chronic renal Cd2+ toxicity.

PROLONGED CD AND PCBS CO-EXPOSURE AND THYROID FUNCTION IN RATS: IS THE LIVER RELEVANT POINT OF THEIR INTERACTIONS?

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Close to 800 chemicals are suspected to interfere with receptors, synthesis or hormone conversion, but only a small fraction has been thoroughly studied. Thus, data on cadmium (Cd) effects as thyroid disrupting chemical are limited, especially at low exposure levels. Our results showed that oral treatment of rats with different doses of Cd (ranging from 0.3-10 mg Cd/kg b.w.) during 28 days induced dose-dependent decrease of serum triiodothyroxine (T3) while statistically significant reduction of thyroxine (T4) was observed for doses ≥1.5 mg Cd/kg b.w. revealing that T3 hormone is more sensitive to Cd than T4. This finding suggests that Cd interferes with thyroid function predominantly at extrathyroidal level, liver being of special importance.

Since humans are exposed to complex mixtures of persistent pollutants, we investigated the effects of co-exposure to Cd and polychlorinated biphenyls (PCBs) on T3 and T4 and results indicated synergism. In order to elucidate the point responsible for this interaction, the following hepatic parameters were determined: albumin, aspartate aminotransferase (AST), alanine transaminase, alkaline phosphatase, as well as malondyaldehide (MDA).

Dose dependent raise caused by Cd or PCBs given alone was proved for albumin, AST and MDA. The analyses of differences in the slopes of dose-response curves for mixtures and Cd and PCB curves revealed no interactions within investigated parameters. Although literature data indicate that different processes in liver have important role in Cd and PCBs induced thyroid toxicity, these results give evidence that liver is not the relevant point of their interactions.

THE MAMMALIAN METABOLISM OF CADMIUM: DYNAMIC AND STRUCTURAL ASPECTS

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The low level exposure of various human populations to the environmental pollutant cadmium is of increasing public health concern. While the exposure assessment of individuals can be based on the determination of the cadmium concentration in their bloodstream, the interpretation of the health relevance of the obtained results is often exceedingly difficult. In order to improve this undesirable situation, two principle problems need to be addressed: 1. A better understanding of the biomolecular mechanisms which link the exposure of mammals to cadmium with adverse health effects (e.g. cancer) and 2. The development of a cadmium-specific chelating agent for the treatment of afflicted populations. Progress to solve both of these toxicological problems fundamentally hinges on a better understanding of the mammalian metabolism of cadmium as well as its modulation. To this end, an overview will provided about results that we have recently obtained. Applying a metallomics-based LC-approach provided insight into a possible mechanism by which cadmium is translocated from the bloodstream to its target organs. Furthermore, we were able to structurally characterize the cadmium complexes that are formed with meso-2,3-dimercaptosuccinic acid and 2,3-dimercaptopropane-1-sulfonic acid.

CHEMICAL IMAGING AND OVERALL ASSESSMENT OF CADMIUM DISTRIBUTION IN THE HUMAN BODY

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The scientific interest in cadmium (Cd) as a human health damaging agent has significantly increased over the past decades. Despite enormous efforts in many areas of biomedical research particularly the histological distribution of Cd in human tissues is still scarcely defined. To our knowledge this study is the first human cadaveric study focusing on Cd distribution in the entire body and organs on the microscopic and tissue level. Using inductively coupled plasma-mass spectrometry (ICP-MS) and laser ablation (LA)-ICP-MS we determined the concentration of Cd in 40 different human tissues and provide spatial information (chemical imaging) on the microscopic distribution of Cd in 8 selected tissues. ICP-MS results suggest that the major Cd deposits of the human body are muscle (27%), kidney (27%), adipose tissue (12%), and liver (8%). LA-ICP-MS analyses show that defined histological structures are mirrored by sharp cut differences in Cd concentrations between neighboring tissues types, particularly in the rectum, testes and kidneys. The results of this study provide evidence that i) the relevance of some organs as Cd body pools may have been over- and others underestimated in the past, ii) the amount of blood perfusion of a tissue may grossly define its Cd concentration, iii) some tissues show dramatic Cd concentration gradients between neighboring cells and tissue layers, iv) and the routes of Cd uptake of some tissues may differ from current concepts.

THE PROTECTION OF SELENIUM AGAINST CADMIUM TOXICITY IN CHICKEN LYMPHOCYTES VIA SELENOPROTEINS AND HEAT SHOCK PROTEINS

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The aim of this study was to investigate the cadmium (Cd) toxicity and protection role of selenium (Se) via the four endoplasmic reticulum resident selenoproteins and the heat shock protein (HSP) pathway in chicken lymphocytes. Chicken splenic lymphocytes received Cd (10−6 mol/L), Se (10−7 mol/L), and the mixture of 10−7 mol/L Se and 10−6 mol/L Cd and were incubated for 12 h, 24 h, 36 h, 48 h,respectively. Next, we detected the mRNA expression of SelK, SelN, SelS, SelT, HSP27, HSP40, HSP60, HSP70 and HSP90 by using real-time PCR method. The present study indicated that Cd toxicity might relate to the reduced expression of selenoproteins and the increased expression of HSPs in chicken lymphocytes. And Se can serve as protection role by increasing the expression of selenoproteins and reducing the expression of HSPs. This indicated that the protective effect of Se against the toxicity of Cd might, be attributed to stimulation of the level of Seleproteins and HSPs.

IMMUNOTOXICOLOGY OF CADMIUM: INSIGHT FROM ACUTE INTRAPERITONEAL AND INTERMEDIATE PERIOD OF ORAL EXPOSURE OF RATS

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Conflicting data exist concerning cadmium effects on immune system depending on the experimental model, exposure or tissue/activity examined. In this study immunotoxicity of acute intraperitoneal and oral cadmium administration was investigated in rats. The use of the inflammation-prone inbred Dark Agouti (DA) and less reactive Albino Oxford (AO) rats showed differential (immune activity-related and/or strain-related) effects of cadmium (1 mg of Cd/kg, i.p.) on spleen immune responses. A decrease in ConA-induced proliferation (related to altered spleen cells responsiveness to IL-2) and of IFN-γ (independently of IL-4 and IL-10) was more pronounced in DA rats. Increased innate immunity splenocyte activity (granulocyte CD11b+ cells, iNOS mRNA and NO production, myeloperoxidase MPO activity, IL-1β mRNA and IL-1β protein product levels) were observed in both strains (some of them more pronounced in DA rats), while a decrease in respiratory burst (dihydrorhodamine/DHR oxidation) was similar. 30-day oral intake of 5 ppm and 50 ppm of cadmium by DA rats resulted in reduction of some probiotic bacteria, villous damage and intestinal inflammation [(increased levels of High Mobility Group Box1/HMGB1, antioxidant enzyme (superoxide dismutase/SOD and catalase/CAT) activity and proinflammatory cytokine (TNF, IL-1β, IFN-γ, IL-17) in gut homogenates]. Stimulation of both adaptive (increased cellularity, proliferation, IFN-γ and IL-17cytokine responses) as well as innate immune activity (increases in numbers of NK cells and M1-like macrophages, oxidative cell activities, IL-1β) of gut draining (mesenteric) lymph nodes was associated with decreased or unchanged antiinflammatory cytokine (IL-10) cell response. Differential (immunosuppressive and immunostimulatory) effects noted in the same tissue (spleen) should be taken into account when exploring immunotoxicity of this metal. Stimulation of gut immune responses imply dietary cadmium as health risk factor.

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IMPACT OF PERSISTENT LOW-LEVEL CADMIUM EXPOSURE ON PANCREATIC BETA-CELLS

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As it is also the case for other chronic diseases, epidemiological studies have raised the issue of association between exposure to cadmium and diabetes or pre-diabetic conditions in different human populations. But no definite consensus about the relevance of this association has yet been reached. The available epidemiological data are not balanced by enough suitable studies carried out with animal or cellular models. Important, but generally lacking, experiments should mimic environmental conditions with minute amounts of contaminants applied for long periods. In the latter conditions, a pending question to answer is whether cadmium-induced redox challenge readily impacts insulin-producing and insulin-sensitive cells or is a later effect occurring after accumulation of intra-cellular cadmium. A key point is not to address cell death, but rather impaired specialized function. Glucose homeostasis and insulin sensitivity under low-level long-term cadmium exposure are not thoroughly characterized. In this context, the most prominent cadmium sensitive canonical signaling pathways have been probed in order to sort out which of them contribute to adaptation or resistance to the cadmium insult. The available data will be presented in order to highlight future directions of work in the complex toxicology of cadmium.

CHRONIC CADMIUM EXPOSURE IN RATS PRODUCES PANCREATIC IMPAIRMENT AND INSULIN RESISTANCE IN MULTIPLE PERIPHERAL TISSUES

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Cadmium exposure has been linked with disturbances in carbohydrate and lipid metabolism. In our laboratory we investigate the level of Cd-exposure that produces changes on carbohydrates, lipids and insulin release. Also, with mathematical models, is studied the effect of cadmium on the insulin resistance and sensitivity of tissues such as liver, muscle, adipose tissue and cardiovascular tissue. Cadmium exposure (35.5ppm) in rats induces hyperglycemia, increased insulin release after a glucose load, and causes increases triglycerides, cholesterol, LDL-C and VLDL-C in serum, and a time-dependent decrease of HDL-C circulating. After cadmium exposure HOMA-IR increases, while the HOMA-S%, QUICKI and Matsuda-DeFronzo indexes of peripheral sensitivity shows significant decreases. At organ-specific level, a decrease of insulin sensitivity is shown in muscle (ISMI) and liver (HIS). Additionally, cadmium increases insulin resistance in liver (HIRI and LIRI), adipose tissue (IDA-IR) and cardiovascular system (IRIEV). The β-cell functioning, which was evaluated by HOMA-B% index and insulin disposition index, show decreases, while insulin generation index increases. In conclusion, cadmium exposure increases insulin release, induces hyperglycemia and alters lipid metabolism. These changes likely occur as a consequence of reduced sensitivity and increased insulin resistance in multiple insulin-dependent and non-dependent tissues, producing a biochemical phenotype similar to metabolic syndrome.

CADMIUM, ATHEROSCLEROSIS, AND CARDIOVASCULAFR DISEASE

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Background

Cadmium from diet and tobacco smoke may increase the risk of atherosclerosis and cardiovascular disease.

Methods

We examined atherosclerosis in the carotid arteries in a population-based cohort of about 5000 Swedish middle-aged women and men examined in 1991–1994. We also examined cardiovascular mortality, and incident myocardial infarction and stroke until 2010. Cadmium in blood was analyzed by ICP-MS. We studied cadmium in plaques (endarterectomy) and blood in clinical cases (N=37), and some plaques were subdivided according to vulnerability.

Results

The prevalence odds ratio for plaque was significantly increased (1.3 - 1.9 depending on model) in the 4th blood cadmium quartile after adjustment for other risk factors. No association was found in never-smokers. Hazard ratios for cardiovascular endpoints were consistently increased (about two-fold) in the 4th quartile (median 0.99 µg/L). Ratios were slightly higher in never-smokers. Cadmium in symptomatic plaques was 50-fold higher than in blood. Levels were highest in the upstream part where rupture usually occurs.

Conclusions

The results indicated that cadmium is a risk factor for atherosclerosis and cardiovascular disease even at moderate exposure levels. Smoking is a strong confounder, but for cardiovascular events the association was found also in never smokers. Preventive measures are warranted.

AGE-RELATED ACCUMULATION OF CADMIUM IN RETINAL TISSUES: IMPLICATIONS FOR AMD, A MAJOR FORM OF BLINDNESS

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Age-related macular degeneration (AMD) is the leading cause of blindness in developed nations, where a disease hallmark is the presence of particulate material (drusen) in the central retina, i.e. between the Bruch's membrane (BM) and retinal pigment epithelium (RPE). Multiple genetic and environmental factors have been identified (including ageing) and we hypothesised that an age-related accumulation of toxic metal ions may contribute to AMD pathology through increased oxidative stress. We analysed eyes from 135 human donors (11-88 years of age) and quantified the level of metal ions in the BM by inductively coupled plasma mass spectrometry (ICPMS). We identified a population of older donors with high levels of aluminium and a linear increase in cadmium and cobalt with age. Smoking, another AMD risk factor, also increased the amount of cadmium in the BM. Analysis of the RPE (from 83 donors) indicates that the oxidative stress and lipid peroxidation response gene, GPX4, and the complement gene, CFH, are expressed at higher levels in samples with high cadmium compared to those with no cadmium. This suggests that cadmium may contribute to pathogenic oxidative stress and complement dysregulation in AMD. Work is currently underway to investigate this further.

CADMIUM AND FEVER

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Cadmium is a heavy metal, known for its toxicity known for its toxicity in animals and man as it is not used in these species. This metal is often found in industrial discharge and electronic waste, for this reason Cadmium-related diseases are an increasing problem in developed countries as well as several other countries with economies focused almost exclusively on e-waste recycling.

Cadmium fever is characterized by flu-like illness, that develops after inhalation of metal fumes with symptoms beginning 3-10 hours after exposure. The initial symptoms include thirst and metallic taste in the mouth, chills, fever, myalgia and fatigue. These symptoms are self limited and disappear within 36 hours if treated. Bronchial and pulmonary irritation do not disappear and must be treated for a longer period.

Acute cadmium poisoning can occur in two ways: by inhalation of freshly generated cadmium fumes and by ingestion of cadmium compounds. The most common scenario that causes this metal fume fever involve workers exposed to metal oxide fumes, trough operation such as brazing, welding, galvanizing or smelting. Since acute cadmium oxide fume intoxication is often due to an occupational exposure, it is necessary to ask and obtain the patient's work history as precise as possible.

The treatment of acute poisoning by inhalation consists of oxygen therapy, ethylendiamine tetraacetic acid, broad spectrum antibiotcs, nebulized bronchial dilators and cortisone.

The diagnosis of this condition may be easily mistaken if the clinician does not know that the worker has been unawares overly exposed to cadmium fumes. His condition of metal fume fever may be misunderstood and underestimated.

TOXIC EFFECTS OF CADMIUM ON REPRODUCTIVE FUNCTION IN CHICKEN

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Cadmium (Cd) has toxic effects on reproductive function in animals. The aim of this study was to investigate the toxic effect of Cd on reproductive function in chicken. Fifty day-old male Hyline chickens were fed either a commercial diet or a Cd-supplemented diet containing 140 and 210 mg/kg. The ovary tissues were collected at 20, 40, and 60 days, respectively, and pathological observation, ultrastructure, oxidative stress, apoptosis and the level of FSHR and LHR mRNA expression in the ovarian were measured. The present study indicated that Cd caused reproduction organ pathological damage, suppressed the reproductive function, induced oxidative stress, destroyed enzyme activity, interfered FSHR, LHR mRNA expression, and promoted ovarian follicle granular cell apoptosis in chckens.

CADMIUM MINIMIZATION IN RICE GRAIN – PHYTOMANAGEMENT

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Rice grain accumulates cadmium. Cadmium toxicity is prevalent in populations of South East Asia where rice is the main staple food. The source of cadmium to rice paddies being application of phosphate fertilizers. A variety of agrochemicals, use of waste water released from mines for irrigation also enrich Cd. The fate of Cd in paddy soils depends on a wide array of factors such as availability of water, soil physiochemical properties, field management etc. Rice paddy is a wetland agroecosystem where intermittent wet and dry periods occur. This result in temporal change in reduction potential of paddy soil. Characteristic features of paddy soils offers specific field and crop management practices that allow us for Cd minimization. Rice root being unique in its structural and metabolic features, exert influence on the mobility of Cd and other TE in paddy soils. Organic farming decreases Cd uptake and remediates crop fields. Application of Azolla has dual benefit of phytoremediation and nitrogen fixation. Cd uptake in rice can possibly be controlled by gene families that code for putative metal transporters or metal chaperones and quantitative trait loci (QTL). Generation of Cd excluder rice is, perhaps the best possible solution. Field cultivation practices are also highlighted for better clarity.

CADMIUM ACCUMULATION AND EFFECTS IN TWO IMPORTANT CROP PLANTS FOR HUMAN CONSUMPTION (TOBACCO AND CARROT)

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Human exploitation of plant material derived from Cd-contaminated environments may lead to a noxious intake of this toxic element.

First, a possible source of Cd for humans is given by cigarette and cigar smoke. It is here postulated that an important role in reducing excessive Cd accumulation in leaves of Nicotiana tabacum L. (tobacco) plants might be played by arbuscular mycorrhizal (AM) fungi. Accordingly, the role of the AM fungus Funneliformis mosseae in protecting tobacco from Cd stress was investigated. Our findings proved that the AM symbiosis is effective in reducing Cd content in the leaves of adult tobacco plants, as well as in increasing the content of the antioxidant glutathione (GSH). Thus, the mycorrhization might be useful in diminishing the risks to human health due to the Cd present in smoking products.

Second, studies on potential localization of Cd in a crop largely employed for human and animal food, i.e. Daucus carota L. (carrot), were also performed. In general, carrot root could accumulate high concentrations of Cd, up to about 1000 ppm. A biphasic root response to Cd stress actually occurred: in the first phase (1 to 4 days of Cd exposure), the cytological and functional events observed can be interpreted as a plant acclimatory response aimed at diminishing the movement of Cd across the root. But, the prolongation of Cd exposure from 4 to 14 days led to a progressive root collapse and tissue disruption, given by the high Cd accumulation in this organ and the consequent oxidative and degenerative damage.

MOLECULAR DISSECTION OF CD TOLERANCE AND ACCUMULATION IN "NORMAL" PLANTS AND CD HYPERACCUMULATORS

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Arabidopsis halleri is an extreme organism with respect to Cd. We assessed A. halleri Cd hyperaccumulation in a comprehensive ionomic survey of over 160 European populations. The analysis of > 2000 leaf and soil samples identified tremendous natural variation with some plants showing leaf Cd levels of greater than 3000 ppm. Strong regional patterns exist, suggesting recent evolution of the Cd hyperaccumulation trait. Phenotyping under controlled conditions confirmed the variation which offers huge potential for the molecular elucidation of Cd accumulation in plants and the generation of low Cd crops through breeding and biotechnological approaches.

The isolation and analysis of metal hypersensitive *Arabidopsis thaliana* mutants represents a second approach we are following to molecularly understand Cd responses of plants. Such mutants enable the identification of genes affecting, for instance, uptake or storage of Cd.

One well-known Cd factor is the enzyme phytochelatin synthase. It synthesizes phytochelatins (Cd-binding peptides) upon exposure of cells to Cd ions. Phytochelatin-mediated sequestration of Cd constitutes a major Cd detoxification pathway in plants and also many animals and fungi. I will address the question as to why phytochelatin synthases are constitutively expressed in all plants and all organs even though their activity would only sporadically be needed in a small subgroup of plants potentially at risk of Cd exposure.

CADMIUM AND HONEY PRODUCTION: AN OVERVIEW ON A POSSIBLE MARKER FOR SUSTAINABILITY

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The bees, honey bees (*Apis mellifera*) primarily, can come into contact with contaminated air, soil, vegetation and water. In such cases, the heavy metal levels in honey can be used to express the level of contamination of colony's foraging areas. However, honey bee body from one same colony can be itself a reliable sample to reflect the level of exposure to Cd following environmental contamination. Different exposure routes can lead to heavy metal contamination, namely soil particles, or after contaminants uptake by plants which finally get pollen and nectar. Honey bees commonly forage within 1.5 Km of their hive and exceptionally as far as 10 to 12 Km, depending on their need for food and its availability. The foraging area is normally of 7 km^2. To such an extent honey bees have been used to assess the level of Cd load in the environment. It is worldwide accepted that honey can easily be contaminated with heavy metals, especially during processing and due to the location of hives. Honey bees showed to be effective biological monitors of environmental contaminants over large geographic areas. Experimental evidence was given when hives placed along a gradient of industrial As and Cd pollution showed the fewer bees and less honey in areas where the higher metal contamination of pollen and bees (2–20 ppm body burden) were. The reason behind the low yields and reduced colony size can reside in the fact that Cd can block the Ca^{2+} channel and impair muscular function in the contaminated bees. Methods have been developed to determine the level of several heavy metals. All of these findings indicate that actually bees can be considered as sentinels of Cd load and honey quality as a marker for sustainable production.

EFFECTS OF CADMIUM IN *Apis mellifera*: POSSIBLE ROLE IN HIVE COLLAPSE

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In previous researches we studied the role of cadmium-induced damage in several biological systems.

In Mammalians (and in particular in Humans) we demonstrated that Cadmium toxicity affects liver, kidney, central nervous system and endocrine system.

To better evaluate the possible role in immunosuppression, this in vivo study is focused in *Apis Mellifera* immunologic system: indeed, the immune system fall is considered the main responsible of the worldwide collapse of this species, and probably it is the result of a number of contributing factors. A possible stressor is represented by environmental pollution, and in particular heavy metals.

Our preliminary results show a substantial immunosuppression in Cadmium exposed bees, suggesting that in polluted areas the hives may have difficulties in dealing with pets and pathogens that threaten them.

Severe morphological disarrangements were also detectable in fat bodies, the organs involved in immunologic response of *Apis Mellifera* and producing the antimicrobial peptides.

THE MECHANISM OF CHICKEN EMBRYO NEURON APOPTOSIS INDUCED BY CADMIUM CHLORIDE IN VITRO

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The aim of this study was to provide a theoretical basis for mechanisms in cadmium-induced apoptosis of chicken embryo neurons. The embryo neurons model of chicken was established by added different concentration of into the culture medium. The mechanism of chicken embryo neuron apoptosis induced by $CdCl_2$ in vitro were revealed through detecting the activation of the neurons, the capability of adherence, apoptosis, anti-oxidative function changes. The results showed that neurons proliferation were inhibited by $CdCl_2$ and inhibition ratio in dose dependent manner. Through detection on anti-oxidative function dedicated that $CdCl_2$ can destroy the body's antioxidant defense system induced its apoptosis.

CADMIUM AND HORSES: PAST STUDIES AND FUTURE PERSPECTIVES

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Over the last few decades several authors assessed cadmium concentration in meat and offal of food-producing species. For both nutritional and physiologic factors, as well as age at slaughter, higher concentrations of cadmium were found in horses compared with other meat-producing species (Baldini et al. 2000). However few studies were done on the effect of cadmium accumulation in sport and leisure horses' (Gunson et al. 1992, Kowalczyk et al. 1986). The effect of cadmium toxicosis could be of particular importance during growth, as cadmium exposure was suggested to cause swollen joints, retarded growth and osteochondrosis (Gunson et al. 1992, Kowalczyk et al. 1986). The same authors also reported nephrocalcinosis case in a mare following high zinc and cadmium exposure. Moreover, the interaction of cadmium with zinc and copper as well as iron and calcium absorption and metabolism (López-Alonso, 2012) could have a major impact on sport horses health and performance. This study will focus on the review of the literature on cadmium concentrations in different tissues and the possible effects of cadmium exposure and accumulation in sport horses.

ICS 2015

Poster Session

P-1 _ SOURCES OF CADMIUM EXPOSURE IN AN ITALIAN POPULATION: A CROSS-SECTIONAL STUDY

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Main sources of Cadmium (Cd) exposure in the human are food and cigarette smoking as, but also outdoor and indoor air pollution can be important, mainly from industrial emissions, fossil fuel combustion and solid waste incineration. The aim of this study was to assess the influence of outdoor air pollution on serum Cd levels in fifty residents randomly selected from the municipal population of Modena, Northern Italy. We geocoded the current residence of these subjects and modeled their outdoor ambient air concentration of particulate matter ≤10μm (PM10), with the CAlifornia LINE Source Dispersion Model version 4 (CALINE-4), as a proxy of environmental air Cd. Information on smoking habits and Cd dietary intake were also collected, to assess these two additional sources of exposure. We used both crude and multivariate linear regression models to determine the influence of outdoor PM10 levels, smoking and dietary Cd intake on serum Cd. Median values (25th–75th) for serum and dietary Cd were 40.85 ng/l (30.05-53.50) and 13.36 μg/die (10.45-16.63). Crude β-coefficients were 0.617 (95% CI -0.194–1.428, P=0.133), 0.026 (-0.827–0.829, P=0.952) and 6.962 (-0.022–13.945, P=0.051) for PM10, diet and smoking, respectively. Corresponding adjusted values were 0.463 (-0.365–1.292, P=0.266), -0.036 (-0.866–0.793, P=0.930) and 6.057 (-1.175–13.289, P=0.099), respectively. In our population, the most important factor influencing Cd serum content thus appears to be cigarette smoking, followed by outdoor air pollution and lastly by diet.

P-2 _ CADMIUM IN FOOD CHAIN: POTENTIAL HAZARD TO HUMAN HEALTH AND EXPERIMENTAL MODELS

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Cadmium is a heavy metal widely distributed in the environment due to natural and anthropogenic activities. The metal often disrupts the cellular antioxidant status, leading to increased lipid peroxidation rates and endogenous antioxidant system impairment. Thus, we wished to evaluate whether oral exposure to cadmium (Cd) in an environmentally relevant dose could both increase its concentration in Wistar rat testis and disrupt its cellular oxidant status. Ten male Wistar rats, aged 75 days, were divided into group I (n=5) (pure drinking water) and group II (drinking water with 75 mg Cd/L H2O). After treatment, testes were processed for biochemical analysis and atomic absorption spectrometry. A significant increase in testicular lipid peroxidation was found in the cadmium exposed group (110.96 □ 42.01) when compared to controls (40.92 □ 10.23). Also, a significant increase in testicular cadmium concentration was assessed for group II (0.32 □1) 0.16 μg/g) in relation to the control (0.14 □1) 0.04 μg/g). These results not only show that increased lipid peroxidation rates are proportional to cadmium accumulation, but also that control animals unexpectedly showed detectable levels of it. This was very likely absorbed from commercial rat feed, mainly made from corn. These data expose a concerning environmental scenario in which cadmium is being introduced into crops grown for human consumption, posing a threat to human health and eventually affecting experimental models used to investigate reactive oxygen species and cellular signaling.

P-3 _ CADMIUM DIETARY INTAKE IN A NORTHERN ITALY POPULATION

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Cadmium (Cd) is an ubiquitous toxic heavy metal and recent epidemiological studies have shown a direct association between cadmium exposure and risk of cancer at several sites (lung, endometrium, bladder and breast). General population, except for smokers and occupationally-exposed individuals, is exposed to Cd mainly through diet. The highest Cd concentrations are generally found in products such as seaweed, fish and seafood, chocolate, mushrooms, oilseeds and edible offal, while food groups that mainly contribute to dietary Cd exposure, as a result of high consumption, are cereals and cereal products, vegetables, nuts, potatoes and meat products. We investigated Cd dietary intake in 719 residents of five provinces of Emilia Romagna region in northern Italy, using a validated food frequency questionnaire specifically developed as a part of the European Prospective Investigation into Cancer and Nutrition (EPIC) study for the Northern Italy population. We found that annual average Cd intake was 1.39 µg/kg body weight/week, with a higher value in females than in males (1.44 and 1.33 µg/kg body weight/week respectively, P=0.07). Food categories which gave the major contribution were mushrooms (30.6%), rice (16.9%), pasta (7.6%), bread (6.1%), leafy vegetables (5.3%) and shellfish (4.2%). A higher Cd exposure characterized usual consumers of mushrooms, whose intake was 3.1 µg/kg body weight/week. Values of Cd intake characterizing our study population were lower than those reported for the European population (2.5 µg/kg body weight/week), though a high consumption of mushrooms and shellfish can considerably increase levels of intake.

P-4 _ CORRELATION BETWEEN CADMIUM AND SELENIUM BLOOD LEVELS IN AN ITALIAN POPULATION

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Cadmium (Cd) is established as a human carcinogens while selenium (Se) is a metalloid showing an intriguing relation with human health, particularly with cancer. Se is usually present both in the environment and in living organisms in various inorganic and organic forms, having considerable variations in both their toxicological and physiological properties. The aim of this study was to explore the relation of these two elements in a sample of Modena municipality residents. Linear unadjusted and adjusted regression analyses were performed, including in the models age, body mass index, smoking habits, energy intake (Kcal/die), time from sample collection, and dietary Cd intake. Median (25th-75th) serum levels were 40.85 (30.05-53.50) ng/l and 118.5 (109-136) µg/l for Cd and Se, respectively. Crude regression β coefficients were -0.320 (95% CI -0.550,-0.089; P=0.008), -0.195 (-0.448,0.058; P= 0.128), 0.240 (-0.124,0.605; P=0.191) and -0.019 (-0.191,0.152, P=0.821) for total, inorganic and organic Se species and for glutathione peroxidase-linked Se. In multivariates analysis, adjusted β values were -0.219 (-0.471,0.032; P=0.086), -0.072 (-0.345,0.201; P= 0.598), -0.129 (-0.478,0.221; P=0.461) and -0.141 (-0.330,0.047; P=0.138), respectively. These results suggest an inverse correlation of Cd with total Se in human blood, mainly due to an inverse relation with inorganic Se species and with glutathione peroxidase-Se, with potential implications for the toxic effects of both Cd and Se, as well as for the nutritional availability of Se.

P-5 _ SELENIUM COUNTERACTS CADMIUM TOXICITY IN HUMAN NEURONS

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Cadmium has been shown to cause severe damage to a variety of organs; also the nervous system appears to be a sensitive target for Cadmium toxicity. Its high blood-brain barrier permeability, and/or its uptake from the nasal mucosa via olfactory pathways represent direct access routes to the nervous system. Proven effects of Cadmium neurotoxicity include generation of reactive oxygen, disturbances in calcium/zinc-dependent processes, dysregulation of cell repair systems, epigenetic modifications and estrogen-mimicking effects. However the detailed mechanisms for cadmium neurotoxicity remains unclear.

Our previous in vitro results showed that Zinc counteracts the toxic effects of Cadmium on human neurons. The present study evaluates the effects of Selenium in preventing and/or counteracting the effects of exposure to Cadmium in human neurons.

SH-SY5Y human neurons were treated with different sub-toxic concentrations of cadmium for 24 h after a 24 h pre-treatment with Selenium. Morphological modifications, cell viability and protein expression of specific markers of neuronal plasticity and apoptosis (Gap43, caspase 3) were studied.

Our results suggest a role for Selenium compounds in protecting neurons and rebuilding the neuronal network connections.

P-6 _ THE INTERACTIONS OF INORGANIC CADMIUM WITH BIOMIMETIC MEMBRANES AND ITS EFFECT ON MEMBRANE FLUIDITY

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On Earth, Cadmium is present as a non-essential metal and a highly toxic environmental pollutant. When Cadmium enters the body from contaminated food, water or air, it is absorbed through the gastrointestinal tract into the blood where its first potential targets are proteins in the blood or the red blood cells (RBCs) themselves. While the interactions between cadmium and proteins in the blood including serum albumins and small peptides like glutathione are well studied, the interactions of Cadmium with the lipid bilayer surrounding RBCs is much less studied. This is relevant as it is estimated that the lipid bilayer occupies approximately 75% of the RBC surface area making this a large potential target. The goal of this study was to elucidate the interactions between inorganic cadmium (Cd) and the lipids present in the RBC membrane. This was achieved using fluorescence spectroscopy with Laurdan Generalized Polarization in order to study the effects of Cd on membrane fluidity. This was done in combination with Dynamic Light Scattering (DLS) which was used to study how Cd affects membrane fluidity by monitoring changes in the membranes phase transition. Thermodynamics of Cd-lipid interactions were then studied using Isothermal titration Calorimetry (ITC). Our results show that Cd selectively binds and rigidifies the negatively charged lipid phosphatidylserine while not binding or affecting the fluidity of the neutrally charged lipid phosphatidylcholine. As Cd is predicted to form predominantly positively charged species under physiological pH and chloride salt conditions, this interaction is hypothesized to be electrostatically driven. These studies will increase our understanding of Cd(II) induced red blood cell toxicity.

P-7 _ EFFECT OF LOW LEVEL CD EXPOSURE ON GLUCOSE HOMEOSTASIS AND B CELLS FUNCTION

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Several epidemiological and in vivo studies suggest a positive association between cadmium (Cd) exposure and incidence and severity of diabetogenic symptoms, but the association remains controversial. Besides, the animal studies have mostly been carried out with high levels of Cd, over short periods of time and with artificial routes of administration. Furthermore, relatively few studies are available on cell models maintained in the presence of Cd for a long time. Our goal is to study the impact of Cd on the endocrine function of the pancreas in environmentally relevant conditions using animal and cell lines as biological models.

In vivo study

Groups of adult Wistar rats were exposed for three months to 0, 5, 50 or 500µg/kg/j of CdCl2 in drinking water. Cd was found to dose dependently accumulate in liver and kidney without inducing kidney damage (assessed by measurement of Kim-1) or any variation in oxidative status parameters and glucose and insulin tolerance tests. However, at the highest dose, basal plasmatic insulin levels were significantly increased by 66% for female rats and histological studies showed a trend toward increased β cells mass in the pancreas.

In vitro study

The rat β cell line model INS1 was exposed during g72 hours to Cd doses below the onset of cell death. Mitochondrial function was assessed as a key step in insulin secretion. At sub-lethal doses, Cd had no impact on mitochondrial respiration, but it decreased the ATP/ADP ratio at 2.5µM Cd. Insulin secretion is being studied.

Conclusion

In these conditions, no major impact of Cd was observed in glucose homeostasis in vivo although Cd accumulated in organs. In INS1 cells, Cd impacted the ATP/ADP ratio at the highest studied dose without altering mitochondrial respiration.

P-8 _ CADMIUM TOXICITY, BIOMARKERS, AND RELATED TESTS

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Background

Multi-level approach for evaluating cadmium toxicity in humans is suggested. These biologic markers may improve the early detection of toxic effects of metal cadmium overexposure.

Methods

We review combined old and new biomarkers and related testing of cadmium toxicity in humans.

Results

Measurements of whole blood cadmium (limit, <1.7 micrograms per liter) - along with urinary cadmium on 24-hours specimens (limit, <1.3 micrograms per liter) - may be useful index of exposure to cadmium. Serum ferritin concentration can be used in women who have a higher absorption rate of cadmium. Serum neuron-specific enolase (NSE) may be measured considering cadmium-mediated lung toxicity in humans. Serum aptoglobin may be suppressed in cadmium overexposure. Serum and/or plasma zinc (and copper) status should be performed. To establish the degree of renal involvement, serum creatinine studies may help. As nephrotoxicity testing, urine levels of alfa-1 microglobulin, N-acetyl-β-glucosaminidase (NAG), retinol binding protein (RBP), beta-2-microglobulin, immunoglobulin light chains, and albumin have also been proposed. Cadmium is also considered to be immunotoxicant, by immunophenotyping, CD69, CD23, CD28 elevations of these markers were observed in patients. Plasma renin should be carefully monitored because cadmium may interfere with the function of endocrine system, inducing hyperreninemia.

Conclusion

This review considers current approaches to laboratory testing for cadmium toxicity in human.

P-9 _ CADMIUM AND OTHER NEUROTOXIC METALS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): A POPULATION-BASED CASE-CONTROL STUDY

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In this work, a population-based case-control study on possible associations between human exposure to toxic metals and sporadic amyotrophic lateral sclerosis (ALS) was conducted. Following informed consensus, ALS patients were enrolled from different Care Centers of Sardinia, an Italian region characterized by elevated rates of ALS cases and significant history of mining activities. Aluminium, Cd, Hg, Mn and Pb concentrations were determined in blood, hair and urine by ICP-MS in 34 ALS patients (mean age, 62 ± 10 years, male/female ratio 1.6) and 30 controls (mean age, 65 ± 11 years; male/female ratio 1.5). Taking into account blood samples, concentrations of Al (p=0.045) and Pb (p=0.026) were higher in ALS patients than in control. In hair of ALS patients, a significant depletion of Al (p=0.006) and Mn (p=0.032) respect to controls was found. In contrast, there were not significant differences between cases and controls in urine samples. As well, cases and controls showed no differences in Hg patterns; however, ALS patients showed strong correlations among Hg levels in blood, urine and hair, while controls did not. In addition, metal-metal correlations were investigated, showing that Cd, Hg and Pb display synergic pattern, differently from Al and Mn (antagonistic pattern). In conclusion, even if a direct ALS-metals causative relationship cannot be established, this study suggests that some metals could be involved in the ALS pathology depending on environmental exposures to toxicants, as well as on internal degenerative process and metal-gene interactions.

P-10 _ ASSESSMENT OF CADMIUM LEVELS IN SERUM, TOENAILS AND DIET: A CROSS SECTIONAL STUDY IN MODENA, NORTHERN ITALY

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Cadmium (Cd) is a heavy metal that poses serious environmental health hazards to humans. Cigarette smoking and some occupations are major sources of exposure, while for non-smokers and subjects unexposed in the workplace, ingestion through food is the most important source, mainly due to vegetables and cereals, but also to fish, offal, wild mushrooms and chocolate. Blood Cd concentration represents both short and long-term exposures, while toenails Cd reflects medium-term exposure. The aim of the study was to assess Cd exposure and its determinants in fifty adults randomly drawn from the municipal population of Modena, by determining Cd levels in plasma and toenails, as well as its dietary intake using a semi-quantitative food frequency questionnaire. Median (25th-75th) values were 40.85 (30.05-53.50) ng/l, 5.66 (0.50-11.39) ng/g and 13.36 (10.45-16.63) µg/die in serum, toenail and diet, respectively. In stratified analyses for gender, age and smoking habits, males shown higher serum Cd content than females, as did current smokers versus never-smokers, while age shown an inverse correlation. Pearson's correlations were 0.028 (95%CI -0.252, 0.304; P=0.845) between serum and dietary Cd, 0.001 (-0.277, 0.280; P=0.993) between toenail and dietary Cd, and -0.075 (-0.346, 0.208; P=0.606) between serum and toenail Cd, with little gender-related differences. When we excluded current smokers from analysis, only the correlation between serum and dietary Cd changed, slightly increasing (r=0.068, 95% CI -0.245,0.367; P=0.675

P-11 _ LIPID PROFILE, GLYCAEMIC CONTROL AND CORRELATION WITH HEAVY METALS IN PATIENTS WITH TYPE 1 DIABETES MELLITUS IN NORTHERN SARDINIA, ITALY

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Sardinia is an Italian region with a high incidence of type 1 diabetes mellitus. This study aimed to determine the associations of trace elements with lipid profiles and glycaemic control in patients with T1DM. A total of 192 patients with T1DM who attended the Unit of Diabetology and Metabolic Diseases in Sassari, Italy, were enrolled. Heavy metals as chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), cadmium (Cd) and zinc (Zn) were measured in whole blood by sector field inductively coupled plasma mass spectrometry. The correlations between metabolic variables and the levels of trace elements were determined. Zinc was positively correlated with total cholesterol (P= 0.023), low-density lipoprotein (P= 0.0015), and triglycerides (P= 0.027). Iron as significantly correlated with TC (P=0.0189), LDL (P= 0.0121), and high-density lipoprotein (HDL)(P=0.0466). Cadmium was positively correlated with

low-density lipoprotein (P= 0.0222) and with HbA1c % (P= 0.0292);. In males, Cd was positively correlated with HbA1c % (P = 0.0296) and in females was correlated with LDL(P= 0.0002). Chromium was positively correlated with HDL (P = 0.0079) and Se, in females was correlated with TG(P= 0.0113). Overall, 63.5% of the patients had moderate HbA1c (7%–9%,). Copper was significantly correlated with HbA1c % in males (P= 0.0155). In conclusion, the results of this study indicate that heavy elements show different associations with lipid levels and glycaemic control in T1DM. Zinc, Fe, and Se were associated with lipid levels whereas Cu and Cr were associated with HbA1c %. Cadmium was correlated with LDL- protein.

P-12 _ INFLUENCE OF METALS IN RINOSINUSAL POLYPOSIS, ULTRASTRUCTURAL STUDIES

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The metals have a strong toxic effect on human health, and can act as immunoregulatory.

The purpose of our study is to determine whether there is a correlation between the concentration of metals in the clinical course of nasal polyposis (NP).

We investigated the concentration of 10 metals (Zn, Mn, Se, Fe, Cr, Ni, Pb, Al, Cd, Cu) on NP from 58 patients, with 29 healthy controls of the nasal mucosa. We examined by electron microscopy ultrastructural changes between NP and controls.

Patients were clinically staged according with Stammberger. The concentration of metals was determined using a mass spectrometer (ICP-SF-MS). Ultrastructural analysis of the samples was performed using transmission electron microscopy (TEM) and scanning (SEM).

The average of the concentrations of all metals tested were significantly lower in NP than in controls ($p < 0.001$). For the statistical analysis was performed Student's t test. As regards the differences between different clinical stages has been shown that clinical stages I and II showed a metal concentration higher than in stages III and IV, but without statistical significance. TEM and SEM demonstrated that the structure of the NP changes in the passage from polyposis isolated (stages I, II) to those massive (III, IV) with progressive fibrosis, devascularization and inflammation.

Concentration of metals in nasal polyps is lower compared to control, and this fact is even more evident in the massive polyposis. The structure of the polyp could contribute to a lower supply of metals by exposing the tissue to increased oxidative stress.

P-13 _ IMMUNOTOXIC EFFECTS INDUCED BY INTRA-ORAL CADMIUM

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Background

Dental patients who carry metal-based restorations have continuous and prolonged exposure to transition metal ions and heavy metals released from metal-based dental alloys. Cadmium is thought to be an allergen implicated in oral pathology and/or systemic allergic contact dermatitis. We determine the prevalence of allergic sensitization cadmium in a cohort of 77 adult patients.

Methods

In a cohort study of 77 patients, we assessed the prevalence of immunologic sensitization to metallic cadmium by using lymphocyte proliferation assay (also termed lymphocyte blastization test - LTT).

Results

The prevalence of immunologic sensitization to cadmium was 33.48 percent with a mean age of 45.4±12.8, and 79.2 percent were women. Clinical manifestations of immunotoxic reactions to cadmium included 22 patients with burning mouth syndrome (BMS), 10 had other neurological disorders, 9 patients had multiple chemical sensitivity (MCS), which is a neurogenic inflammation. 10 had other neurological disorders. We believe that metallic cadmium may be consider a cotoxicant to mercury and both are released from mercury-containing dental amalgam fillings. Long-term effects of coexposure to cadmium and mercury are associated with notable adverse health effects. Both are neurotoxicants and nephrotoxicants.

Conclusion

Cadmium – as intra-oral toxicant metal – is able to induce an immunotoxic response from the immune system in humans.

P-14 _ SALIVARY CADMIUM LEVELS AND DENTAL ALLOYS MATERIALS

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Evidence that cadmium is present in saliva specimens of patients with dental alloy restorations is lacking. We measured saliva cadmium concentrations in subjects with clinically apparent adverse events related to metal-based dental alloys.

Chewing gum-stimulated saliva specimens were collected from 63 patients (at the study visit) with clinical and laboratory confirmation of adverse event to dental alloys. Concentrations of cadmium were assessed by inductively coupled plasma mass spectrometry (ICP-MS) method. Threshold limit value for salivary cadmium in saliva is <1.1 micrograms per liter.

In a group of 63 patients with oral and/or systemic adverse health effects from alloy-based dental restorations, we quantified the concentrations of cadmium in saliva samples and the mean (SD±) cadmium levels was 2.11±0.58 micrograms per liters. In 63 patients, cadmium in saliva concentrations ranged from 0 to 3 micrograms per liters. The mean age of patients was 50.3±12.4 years, and 68.2 percent were women. In the cohort of 63 persons in whom salivary cadmium was measured, 7.9 percent (5 of 63 persons) had elevated concentrations in saliva specimens. Among patients with high levels of cadmium in saliva samples, one female patient had oral lichen planus, one female patient had tinnitus, one female patient had burning mouth syndrome (BMS), interestingly, 2 male patients had oral and facial angioedema. Dental amalgam fillings may be the primary source of cadmium, the content of metallic cadmium in dental amalgam fillings is 4.5 micrograms per gram.

Cadmium in saliva may be elevated in humans carrying metal-based dental alloys.

P-15 _ CHELATION THERAPY: EVALUATION OF THE COORDINATION ABILITY OF BIS-KOJIC AND HYDROXYPYRIDINONE DERIVATIVE LIGANDS TOWARDS Cd(II) IONS VIA NMR SPECTROSCOPY

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The soft acid Cd(II) is an accumulative toxic metal ion which is able to substitute for the essential borderline acid Zn(II) ion in many zinc enzymes. Nuclear magnetic resonance (NMR) spectroscopy was used to assess the coordination ability of two ligands, an amine-bearing bis-kojic acid: 6'-(2-(diethylamino)ethylazanediyl)bis(methylene)bis (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) [1,2] and a hydroxypiridinone derivative: 5-hydroxy-2-(hydroxymethyl)pyridin-4(1H)-one [3], for use as potential chelating molecules towards Cd(II) ions in detoxification treatment.

A combination of 1D, 2D total correlation spectroscopy (TOCSY), heteronuclear single quantum coherence spectroscopy (HSQC) and rotating-frame Overhauser effect spectroscopy (ROESY) experiments was used to assign the signals of both free and metal-bound ligands, as previously reported for similar systems [4-6]. The metal-ligand system was studied at physiological pH and different temperature values. Competition experiments with essential Zn(II) ions were also performed.

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P-16 _ EFFECT OF ACANTHOPANAX SENTICOSUS ON THE ACCUMULATION OF CADMIUM AND ON THE IMMUNE RESPONSE OF SPLEEN CELLS

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The aim of the study was to evaluate the effects of an AS extract on Cd accumulation and changes in the presence of splenic immune cells in hosts during a chronic metal exposure. Chronic Cd exposure of BALB/c mice was induced by providing them solutions containing different levels of CdCl2 (25 or 250 mg/L) in double-distilled water, with/without a concurrent presence of AS root extract (approximately 151 g material/L), for 8 wk. At the study end, Cd levels in spleen were measured. Cd concentration in spleen specimens was determined by atomic absorption spectrophotometer Perkin-Elmer Zeeman 3030. Levels of key splenic immune cells, including macrophages, T-lymphocytes, and B-lymphocytes, were determined by immunohistochemistry using, respectively, CD68, CD3, and CD20 antibodies. Preparation of extract from roots of ES was made in the factory “Valentis” (Lithuania).

The combined administration of high-dose $CdCl_2$ with AS significantly decreased Cd^{2+} levels in spleen approximately 39-fold relative to concentrations seen when high-dose $CdCl_2$ was given alone.

Administration of AS alone or AS together with low or high doses of $CdCl_2$ significantly increased the amount of macrophages compared to levels noted in organs of control mice. Combined administration of low and high doses $CdCl_2$ with AS solution significantly elevated the amount of splenic T-lymphocytes compared to controls, Cd25 only, and Cd250 only. The numbers of B lymphocytes were significantly higher in mice that were given AS solution relative to concentrations noted in controls. The added presence of AS solution also exerted a significant effect on levels of B-lymphocytes in mice given high doses of metal. The combined administration of high-dose $CdCl_2$ with AS significantly elevated B-lymphocyte levels in spleen compared to high-dose $CdCl_2$ alone.

In conclusion, studies showed that the AS influenced cellular and humoral immune responses in Cd-intoxicated hosts.

P-17 _ CONSUMPTION OF GRAPE JUICE CONCENTRATE (G8000®) PROTECTS THE LIVER AGAINST CADMIUM INTOXICATION IN THE MALE RAT; AN ULTRASTRUCTURAL ANALYSIS

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Liver alterations can be avoided with quality food intake such as grape juice consumption, whereby a greater amount of polyphenols can improve hormone levels and general metabolic characteristics. Liver is the first site for metabolism and is very susceptible to metal accumulation. This relation guided us to observe the protective potential of grape juice concentrate (G8000®) against cadmium intoxication. 24 male Wistar rats (50 days old) were organized into groups: C(water); Cd(water and $CdCl_2$); J(G8000®); CdJ(G8000® and $CdCl_2$). The dose 2g/Kg/G8000® was offered daily by gavage for 86 days and $CdCl_2$ was injected intraperitoneally in a single 1.2mg/Kg dose when the rats were adults (80 days old). After 56 days in adult life (136 days old) animals were euthanized, liver fragments were collected according to routine procedures for Transmission Electron Microscopy. We found evidence of damage due to intoxication in the Cd group, such as increased frequency of mitochondria, rough endoplasmic reticulum and glycogen accumulation. Positive G8000® action was observed comparing the similar pattern between CdJ and C, indicating recovery or a protective process. No alterations were found in J. This ultrastructural analysis suggests that G8000® consumption has a protective effect against cadmium intoxication in the adult rat liver.

P-18 _ ASSOCIATION OF CADMIUM AND ILEX PARAGUARIENSIS (ST. HILL) INFUSION: EFFECT ON WISTAR RAT TESTIS

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Cadmium (Cd) is a toxic metal that is hazardous to health; it is induces oxidative stress in different degrees to various organs and tissues. The Brazilian flora is diverse and many of these plants can be used as natural tonics and remedies, however few plant species have been studied with experimentation. Among them, Ilex paraguariensis, known as yerba mate, has proved to be useful as an effective anti-oxidant for cardiovascular, respiratory, muscular and gastrointestinal systems. This study was undertaken to evaluate the effect of I. paraguariensis infusion on the testis of Wistar rats intoxicated with cadmium, using morphological analysis. The volume and volume ratio of testicular parenchyma did not show significant differences after treatment, measuring tubular diameter and total length of the seminiferous tubule. However the epithelial height showed a significant difference in the group that received both cadmium and I. paraguariensis infusion. Histopatologycal analysis clearly showed cadmium induced damage and suggests a protective role for I. paraguariensis since there was substantial improvement of data regarding testicular dynamics and integrity.

P-19 _ CADMIUM INTOXICATION ON THE 21ST POST NATAL DAY: RAT TESTIS AND EPIDIDYMIS ULTRASTRUCTURAL ANALYSIS IN ADULTHOOD

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Cadmium (Cd) is one of the most toxic metals found in the environment. The general population is exposed to Cd via contaminants found in drinking water, food and tobacco smoke, while occupational exposure to Cd usually takes place during mining or manufacturing of batteries and pigments. The study aimed to evaluate the effect of cadmium chloride single dose (1.2 mg/kg body weight) injected intraperitoneally on the 21st postnatal day and analyzed after 70 days). The animals were fixed by whole body perfusion with glutaraldehyde 2.5% and paraformaldehyde 4% in 0.1 M sodium phosphate buffer, with pH 7.2 for 25-30 minutes and then post fixed in the same solution for 24 hours. The tissues were post-fixed with 1% osmium tetroxide in the same buffer, dehydrated in acetone and embedded in epoxy resin for transmission electron microscope analysis. The control group showed a normal organization for the testis and epididymis. In the testis of the cadmium group, Sertoli cell vacuolization and separation between these cells and the germinative cells were observed, as well as poorly condensed chromatin in sperm heads. The epididymis was less strongly affected, but lipid globules and vacuoles accumulated in the principal cell cytoplasm. A thickened basement membrane with the presence of leukocytes was also noted.

P-20 _ COPPER IN LIVER AND KIDNEY OF RATS EXPOSED TO CADMIUM - BENCHMARK APPROACH

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Cadmium (Cd) exposure has been proven to affect homeostasis of copper (Cu), essential trace element with multiple biological roles. Literature data indicate that Cu levels are dependent on the dose of Cd, duration of intoxication and animal species, and the precise dose-response curve has not yet been proposed.

The aim of this study was to estimate Benchmark dose of 5% (BMD5) for the effect of different doses of Cd on Cu levels in liver and kidney.

Investigation was carried out on male Wistar rats treated orally with 2.5, 7.5 or 15 mg Cd/kg/day for 28 days. Copper and Cd were measured in liver and kidney samples by AAS after microwave digestion with HNO_3 and H_2O_2. Dose-response relationship was evaluated for the effects of Cd on Cu content and BMD5 and lower confidence limit (BMDL5) were calculated: BMD5 was 2.1 mg Cd/kg/day with BMDL5 1.542 mg Cd/kg/day for liver and BMD5 was 9.46 mg Cd/kg/day with BMDL5 5.678 mg Cd/kg/day for kidney.

The present study confirms Cd ability to cause disturbances in Cu levels observed for liver and kidney tissue suggesting Cd-Cu interactions as one of the possible mechanisms of Cd toxicity. Furthermore, the obtained BMDL5 values implicate that, under investigated experimental conditions, liver is more sensitive to Cd-induced changes in Cu levels than kidney.

P-21 _ CHRONIC CADMIUM EXPOSURE LEADS TO INHIBITION OF SERUM AND HEPATIC ALKALINE PHOSPHATASE ACTIVITY IN WISTAR RATS

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Alkaline phosphatase (ALP) activity in the serum and liver from rats administered with cadmium (Cd) in drinking water was studied. After metal administration, Cd showed a time-dependent accumulation in liver, meanwhile metallothionein had a maximum increase at 1-month, remaining in this level until the end of the study. On the other hand, serum and liver ALP activity was decreased after 3-months exposure. To determine if Cd produced an inhibition on enzyme, apo-ALP prepared from both non-exposed and exposed rats was reactivated with Zn, showing 60% more activity as compared with the enzyme isolated from non-exposed rats. In vitro assays shown that Cd-ALP was partially reactivated with Zn; however, in presence of cadmium, Zn-ALP was completely inhibited. Kinetic studies indicate a non-competitive inhibition by Cd that can involve substitution of Zn from the enzyme and/or an interaction with nucleophilic ligands essential for the enzymatic activity.

P-22 _ MORPHOLOGICAL ASPECTS OF THE RENAL DAMAGE INDUCED BY CADMIUM IN EXPERIMENTAL ANIMALS

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The toxicity of Cadmium depends on several factors, including administration mode, dose, species and other environmental or nutritional factors. In animals, including humans, the kidney is particularly susceptible to toxins as a results of its high blood supply and ability to concentrate metabolities. Cadmium induced toxicity, interferes with the intracellular signaling network and gene regulation (3) through a variety of mechanisms. It can be associated with the ROS production, the alteration of cell-cell junctions and with a carcinogenic activity (2).

Wistar Rats with low leves of kallikrein were selected to undergo Cadmium administration for six weeks, under ambient conditions controlled: twelve hours of artificial light exposition and 24 +/- 1 °C of temperature; food and water access free ad libitum. Cadmium acetate was administrated in drinking water (60 mg/l-half of letal dose). After six weeks, the rats were sacrificed and kidneys were collected. Appropriated number of rat was used as a control. Kidney parenchyma samples were subjected to light and electron microscopy and morphologic evaluation of tubular system and glomerular barrier was made.

The proximal tubular epithelial cells, from Cd-treated rats, have irregular shape with gaps between the cells and a vacuolated cytoplasm; this general appearance suggests that the cells may have retracted from each other. TEM analysis shows spaces between adjacent cells and in magnified it is possible to see junction systems in the remaining zones of intercellular contact.

This confirms results of previous works, in fact subcronic exposure to Cadmium causes morphologic alterations in kidney structure that involve, in particular, both proximal tubule epithelium and glomerular basal lamina.

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P-23 _ CADMIUM LEVELS IN LIVER AND KIDNEY OF SHEEP KEPT ON PASTURES WITHOUT AND WITH FLOODING

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Flooding of pastures results sometimes in a heavy metal load (lead, cadmium a.s.o.) of the crop that is ingested by grazing animals. Cadmium occurs also on agricultural land as a contaminant of phosphorous fertilizer and in sewage sludge and residues of biogas plants (which is also used for fertilization).

Sheep are kept frequently on ditches for the purpose of retaining soils or as a defense against erosion and also on pastures mentioned above.

Cadmium accumulates mainly in the liver and kidney of them and may then enter the human food chain.

Against this background cadmium levels in liver and kidney of sheep kept on pastures without and with flooding were analysed. Results showed that sheep kidneys regularly exceed the maximum residue limit for cadmium, whereas the levels in liver tissues varied (with only few exceptions) in the permitted range.

P-25 _ THE BEEHIVE MATRICES TO STUDY HEAVY METAL POLLUTION: A CASE OF STUDY IN A POST-MINING AREA (SARDINIA, ITALY)

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Mining activities represent one of the major source of environment contamination. The aim of this study was to evaluate the use of bees and beehive matrices as bioindicators to detect the heavy metal impact in post-mining areas. The biomonitoring programme was conducted over a 3-year period. The experimental design involved three monitoring stations where repeated sampling activities focused on chemical detection of cadmium (Cd), chrome (Cr) and lead (Pb) from different matrices, both from hosted beehives (foraging bees, honey and pollen) and from the surrounding environment (stream water and soil). The heavy metal content detected in stream water from the control station was always below the analytical limit of quantification. In the case of soil, the content of Cd and Pb from the control was lower than that of mining sites. The mean heavy metal concentrations in beehive matrices from mining sites were mainly higher than the control, and as a result of regression and discriminant analysis, forager bee sampling was an efficient environmental pollution bioindicator. Combined biomonitoring with forager bees and beehive matrices represents a reliable tool for heavy metal environmental impact studies.

P-26 _ CADMIUM LEVELS AND EVALUATION OF THE DIETARY INTAKE OF *Mytilus Galloprovincialis* AND *Ostrea Edulis* FROM SOUTHERN AND NORTHERN FARMING AREAS ALONG THE CROATIAN COAST OF THE ADRIATIC SEA

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Due to fact that the Adriatic Sea is a closed water system, toxic metals may arise from industrial and urban waste, anthropogenic sources, rinsing of rocks and atmospheric emissions. Many marine organisms, such as shellfishes used in human nutrition, accumulate high levels of metals from the environment and may be used as biomarkers of toxic metals pollutions in the marine area. From the standpoint of food safety, these are unsuitable for human consumption. The aim of this study was to determine cadmium concentrations in the tissue of mussels (*Mytilus galoprovincialis*) and oysters (*Ostrea edulis*), the most commonly bred species in Croatia, cultivated using traditional floating park technologies. Mussels (n=25) and oysters (n=25) were sampled during 2013 at two farming areas: Mali Ston Bay (south) situated between the mainland and the Pelješac Peninsula near the Neretva River delta on the southern Adriatic coast and the Istrian Peninsula (north) situated on the northern Croatian coast and bordering on Slovenian waters. The results showed mean cadmium concentrations in muscles of oysters (mg/kg): south 1.59, north 1.48. Mean cadmium levels in mussels were (mg/kg): south 0.30, north 0.56. Significantly higher levels were found in mussels from northern than southern locations ($p<0.01$). These concentrations exceeded the maximum permissible concentration set by the European Community (1 mg/kg). For the purpose of risk assessment for cadmium levels measured in shellfishe, the estimated weekly intakes were calculated and compared with the toxicological values of the provisional tolerable weekly intake (PTWI) set at 2.5 µg/kg BW by EFSA. The contributions to the PTWI were for (%): oysters: north 118.4, south 127.2; mussels: south 24, north 44.8. Therefore, the consumption of large quantities of oysters may pose a health risk to the normal consumer regarding high Cd concentrations and high tolerable weekly intake limit.

P-27 _ SURVEY OF CADMIUM LEVELS IN CEPHALOPODS (*Octopus vulgaris, Sepia officinalis, Loligo vulgaris, Todarodes sagittatus*) PURCHASED IN SARDINIA IN THE PERIOD 2008-2014

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A survey of cadmium levels in cephalopods was performed on samples purchased in Sardinia covering the period 2008-2014. Cadmium occurrence was determined in 139 samples, among different species (commons octopus, cuttlefish, common squid and flying squid), collected in local retail outlets. Results were evaluated in order to establish compliance with European Commission Regulation. Cadmium was determined by ICP-MS, with accredited method as required by Commission Regulation 882/EC. Mean concentrations (and percentages in brackets exceeding the maximum level) were 0.12 mg/kg (0%), 0.20 mg/kg (8%), 0.23 mg/kg (6%) and 0.55 mg/kg (11%) in commons octopus, cuttlefish, common squid and flying squid, respectively. The occurrence data were used to assess dietary exposure. The metal intake, related to the consumption of a portion of cephalopods, was calculated to be 39 µg, about 22% of the tolerable weekly intake (TWI) health based value, established by the Scientific Panel on Contaminants in the Food Chain (CONTAM) for an adult person.

P-28 _ MORPHOLOGICAL EFFECTS OF CD ON THE GREEN ALGAE *Chaetomporpha linum*

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Plant growth is markedly affected by environmental conditions. Plant exposure to mild abiotic stresses induces a range of morphogenic responses. In order to compare such responses in “higher” and “lower” plants, the effects of cadmium (as $CdCl_2$) on plant shape were observed in seedlings of *Arabidopsis thaliana* and in the seaweed *Chaetomorpha linum* (Chlorophyta). Arabidopsis seedlings subjected to Cd treatment show the typical features of Stress Induced Morphogenic Response (SIMR), with reduced cell size, root hardening, and relocation of the plant hormone auxin. The filamentous seaweed *C. linum*, on the contrary, shows little or no changes in cell size, whereas an increase in the number of necrotic cells and an unexpected increase in the number of epiphyte diatoms are observed. The higher amount of epiphytes could be linked to the presence of Cd-dependent enzymes in diatoms, and could have interesting applied consequences for bioremediation.

P-29 _ INVESTIGATIONS ON THE CADMIUM CONTENT OF DIFFERENT SEEDS AND FRUITS FROM BUSHES AND TREES IN NUTRITION OF EXOTIC ANIMALS

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Seeds and fruits from bushes and trees are fed to exotic companion animals like parrots or reptiles to simulate the feed offer as it can be found in the wild and to enable a typical feeding behaviour of those animals. On the market, there are products (seeds/fruits) from foreign countries but also of bushes and trees of the indigenous vegetation. Due to potential environmental burdens the heavy-metal concentrations (Cd, Pb and Cu) in these products are of interest, even if these components are sometimes ingested in only small amounts.

Against this background the investigations were focussed on the chemical composition (dry matter content, cadmium level) of the whole fruit or of that part of the fruit (that is really ingested) of Chokeberry, Sallow thorn, Rowan, Acorn, Rose hip, Blackthorn, Sabal, Pine and Maple.

The analysed levels varied between 0.05 and 1.89 mg/kg (88% dry matter); some of them (e.g. Sabal) were higher than legally permitted.

P-30 _ CADMIUM IN SARDINIAN PASTURELAND VEGETATION, FEED GRAINS, ORGANIC AND CONVENTIONAL WHEAT

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Cadmium and other heavy metals concentrations were checked in feed grains, in vegetation and soils of five Sardinian pastureland areas grazed by sheep, selected according to their proximity to potential sources of pollution. The same determinations were made on soil samples and wheat tissues from two areas devoted to organic farming and a conventional farming area. Acid treatment of samples was performed in Teflon® bombs, sample digestion by means of a CEM microwave digester was done according to manufacturer suggestions. Heavy metals were determined by graphite furnace atomic absorption spectroscopy by means of a Shimadzu AA6800.

The highest cadmium concentrations in vegetation and soils of pasturelands were respectively 0.076 and 0.061 ppm and were found close to industrial areas; while in the control pastureland area cadmium concentration was 0.008 in pastureland legume species and 0.022 ppm in soil. The highest Cd concentration found in feed grains was 0.011 ppm. The concentration of cadmium in wheat samples from the organic farms ranged from 0.015 to 0.027 ppm, while in soil samples ranged from 0.030 to 0.078 ppm. This study provides additional data on cadmium concentration in Sardinian pasturelands and can be useful in the assessment of potential risks for grazing sheep.

P-31 _ CADMIUM AFFECTS ROOT FORMATION AND DEVELOPMENT BY ALTERING AUXIN TRANSPORT AND ACCUMULATION

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Cadmium (Cd) is a very toxic element for plants and animals, easily absorbed through the root system and accumulated in plant tissues. This pollutant causes serious damages to the root apparatus of numerous plants, by altering the quiescent center (QC) and the surrounding apical meristem of the primary root (PR) and the differentiation of its tissues. The QC controls apical meristem definition and activities in the PR, and post-embryonic roots (lateral roots, LRs, and adventitious roots, ARs). The QC identity and maintenance are controlled by auxin levels. In this work we investigated Cd effects on Arabidopsis thaliana root apparatus investigating QC maintenance and functioning, and analyzing auxin levels and distribution in LRs and ARs, in particular. To the aim, seeds of the wild type and QC25::GUS, DR5::GUS, PIN1::GUS, LAX3::GUS transgenic lines were sown in the presence and absence of different Cd concentrations. The results show that Cd significantly inhibited the PR and hypocotyl growth, but significantly increased LR and AR formation. Moreover, Cd compromised the regular QC definition and functioning in LRs and ARs, and disturbed the expression patterns of auxin transport genes, and auxin accumulation.

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