

A B S T R A C T B O O K

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Congress Abstracts · Lectures, Talks and Flash Talks

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Cell Biology 1

Smoking Derived Trace Elements and Cardiovascular Diseases - Biological and Clinical Implications

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Background It is well established that excessive uptake of non-essential trace elements is associated with development and progression of cardiovascular diseases. Via binding to oxygen, nitrogen and sulphhydryl groups of different proteins, heavy metals can lead to alterations in enzymatic activities and thus interfere in physiological processes. Moreover, epigenetic changes and epitranscriptomic dysregulation initiated by methylation events have been described. Specifically, smoking derived cadmium (Cd), lead (Pb), and aluminium (Al) have been shown to be associated with cardiovascular pathologies as atherosclerosis, abdominal aortic aneurysms, coronary heart disease and heart failure. Tobacco plants take up large quantities of trace elements, especially Cd, which is then ingested during smoke inhalation. Likewise, electronic cigarette users accumulate Cd and Pb in their tissues, albeit the reported concentrations are lower compared to users of conventional cigarettes.

Materials and Methods Cell culture experiments with isolated human umbilical vein endothelial and smooth muscle cells as well as cardiomyocytes and human colorectal adenocarcinoma cells were performed to analyse effects of incubation with the different metals. Moreover, organ culture models using human saphenous veins and its isolated primary smooth muscle cells were used to analyse the effect of smoke extract (conventional cigarettes, electronic cigarettes). Analyses include investigation of cellular uptake, proliferation and migration properties, and induced signalling pathways as for example cell death mode. For translational purposes, analyses regarding metal contents and effects included animal experiments (vascular and cardiac effects), a human cadaver study (metal content) as well as a human study on female youngsters (intima-media-thickness of carotid arteries).

Results Incubation with Cd leads to inhibited endothelial proliferation and induction of cell death, whereby different cell death modes are activated all finally leading to necrosis. In the mouse model, Cd intake enhanced atherosclerosis with increased expression of vascular proinflammatory markers and more advanced plaques. Cd was associated with early atherosclerotic changes in women, which were abrogated by increased zinc levels. The human cadaver study revealed adipose and muscle tissue as novel Cd pools with low concentrations but relevant total volume. Aside from vascular, the cardiac effects of elevated Cd levels were investigated showing inflammatory signals, cell death as well as increased cardiac fibrosis in mice when combined with a high fat diet. Pb was shown to promote atherosclerosis via activation of endothelial cells, subsequent production of IL-8 rather than cell death induction. Aside from tobacco plants, Al enriched in food is accumulated in endothelial as well as small intestinal cells, leading to increased proliferation and altered gene expression. Even electronic-cigarette aerosols contain toxic

heavy metals, which effects are not analysed in detail. Whole smoke extract induces low level cell death but DNA damage can occur even at low concentrations.

Conclusion

In conclusion, smoking derived heavy and toxic as well also non-toxic metals are associated with different cardiovascular pathologies, shown by in vitro data, in vivo animal models, as well as human studies. Regarding metals derived from electronic cigarettes, there is not yet enough detailed data available, but it is possible that metals contribute to increased DNA damage in this context.

Manganese homeostasis in the nervous system

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Background Manganese (Mn) is an essential metal, but elevated cellular levels are toxic and may lead to the development of an irreversible parkinsonian-like syndrome that has no treatment. Mn-induced parkinsonism generally occurs as a result of exposure to elevated Mn levels in occupational or environmental settings. Additionally, patients with compromised liver function attributable to diseases, such as cirrhosis, fail to excrete Mn and may develop Mn-induced parkinsonism in the absence of exposure to elevated Mn.

Materials and Methods We used a combination of mechanistic and functional studies in cell culture, *Caenorhabditis elegans*, rats, and primary midbrain neurons to study various transport mechanisms in the brain.

Results We showed interaction between Mn and iron (Fe) transport in the uptake of Mn, and established that SLC30A10 is a cell surface-localized Mn efflux transporter that reduces cellular Mn levels and protects against Mn-induced toxicity. Furthermore, we showed that mutations in SLC30A10 that cause familial parkinsonism blocked the ability of the transporter to traffic to the cell surface and to mediate Mn efflux.

Conclusion Our results provide novel insights into the mechanisms involved in Mn import and export and point to potential therapeutic strategies for maintaining brain Mn homeostasis.

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Cadmium Triggers Lysosomal Exocytosis via the Endo/Lysosomal Ion Channel TRPML1 in Renal Pathophysiology

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Background Exposure to cadmium (Cd), is associated with numerous human diseases. Driven by ongoing industrialization and its global consequences, the impact of Cd on the human body remains a subject of continuous relevance. In particular, contaminated soil, water and resulting food, are the main sources of intestinal Cd uptake. Within the human body, Cd is partially excreted but accumulates in the kidneys over decades, where it contributes to nephrotoxicity and cancer development. In renal proximal tubule cells, endocytic and exocytic processes play a crucial role due to the intensive turnover of macromolecules and ions. The endo-lysosomal cation channel mucolipin-1 (TRPML1; gene *MCOLN1*) is a key regulator of exocytosis; however, the influence of Cd on TRPML1 activity remains poorly understood. Therefore, this study aims to determine whether Cd activates TRPML1, potentially enhancing lysosomal exocytosis as an early adaptive stress response.

Materials and Methods To elucidate potential Cd-triggered effects on renal exocytosis, *in vitro* human proximal convoluted tubule (HPCT) cells were treated with CdCl₂ under serum-free conditions, while TRPML1-overexpression (OE) was established by transient transfection with HA/YFP-tagged human *MCOLN1* in pcDNA3.1. Effects of Cd and TRPML1-OE on lysosomal localization were visualized via immunofluorescence staining and microscopy. Tracking of vesicle localization in the resulting images were carried out using artificial intelligence-based deep learning software. Additionally, plasma membranes (PM) were isolated by biotinylation and examined by immunoblotting. HPCT stress response was analyzed using cell viability assays (MTT, trypan blue) and protein detection via immunoblots.

Results Live-cell-microscopy of Cd-treated HPCTs revealed lysosomal scattering and significantly increased lysosomal distance from the nucleus ($13.5 \pm 7.2\mu\text{m}$ in Cd vs. $12.0 \pm 6.8\mu\text{m}$ in controls). Protein expression of lysosomal biogenesis master regulator transcription factor EB (TFEB) was transiently elevated after 5 minutes of 5 μM Cd exposure. In parallel, LysoTracker intensity was increased by Cd. In Cd-treated TRPML1-OE, lysosome mobilization and spreading were also augmented. Co-expression of TRPML1 and lysosome-associated membrane glycoprotein 1 (LAMP1) on PM was significantly elevated following Cd exposure (45.7%) versus controls (33.6%), peaking after 5 minutes and returning to control-like distribution by 1 hour. Complementary biotinylation assay depicted higher TRPML1/LAMP1 abundance on PM, similarly to the TRPML1 agonist ML-SA5, supporting Cd-triggered enhancement of lysosomal exocytosis. Reduction in Cd-induced number of dead cells in TRPML1-OE (2.25×10^4 vs. 3.60×10^4) compared to HPCT vector controls (2.80×10^4 vs. 5.05×10^4) was observed. Considering TRPML1 can be activated by reactive oxygen species (ROS) and Cd is known to elicit oxidative stress, we investigated the impact of the superoxide dismutase (SOD) mimetic Tempol (0.5 mM). Additional trypan blue studies revealed apparent increase in Cd-induced number of dead cells after 24 hours in the presence of Tempol, even in TRPML1-OE, thereby suggesting a modulatory role of ROS in the lysosomal response to Cd.

Conclusion Collectively, these results suggest a transient upregulation of lysosomal biogenesis and exocytosis as an early adaptive mechanism in the renal response to Cd stress. We hypothesize that Cd-triggered TRPML1 activation drives this process, which might represent a crucial role in the progression of Cd-induced nephrotoxicity.

The extreme C-terminus of PMS2 licenses MutL α mismatch repair activity and suppresses microsatellite instability

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Background Lynch syndrome is a hereditary cancer predisposition caused by defects in DNA mismatch repair (MMR), most commonly involving MLH1, MSH2, MSH6, or PMS2. PMS2 encodes the catalytic endonuclease subunit of MutL α , but how its C-terminus licenses repair remains incompletely understood. Furthermore, clinical interpretation of PMS2 missense variants is challenging, with many classified as variants of uncertain significance. In parallel, cadmium exposure has been linked to disruption of endonuclease function, but its mechanism of MMR inhibition remains unclear. Here, we integrate functional studies of PMS2 C-terminal variants with molecular dynamics simulations to define how MutL α repair activity is regulated and disrupted.

Materials and Methods We investigated four PMS2 missense variants at the extreme C-terminus that result in the following mutations within the PMS2 protein: p.His845Gln, p.His845Arg, p.Arg847Ser, and p.Arg847Lys. Variant proteins were examined in a human cell-based complementation system for expression and nuclear localization. MMR activity was assessed using *in vitro* repair assays with mismatched DNA substrates, and microsatellite instability was measured using a longitudinal fluorescent reporter assay. Quantum-based calculations and molecular dynamics simulations evaluated zinc-to-cadmium substitution within the two C-terminal metal-binding sites of MutL α and the role of the conserved MLH1 FERC domain in regulating MutL α conformation.

Results All four PMS2 variants were stably expressed, localized to the nucleus, and stabilized MLH1, indicating intact MutL α assembly. However, none supported mismatch repair. Nuclear extracts containing each variant failed to repair mismatched DNA substrates. Thus, MutL α assembly is necessary but not sufficient for repair. Quantum-based calculations further suggest that cadmium replaces zinc in both C-terminal metal-binding sites while preserving tetrahedral geometry. However, cadmium coordination produced longer bond distances, approximately 0.2-0.3 Å greater than zinc, consistent with altered local structure. Preliminary simulations also lead us to hypothesize a model in which a conserved arginine in the MLH1 FERC domain stabilizes a closed MutL α conformation, likely restricting the conformational transition required for PMS2-dependent endonuclease activity.

Conclusion These findings identify the PMS2 extreme C-terminus and MLH1 FERC domain as regulatory elements that license MutL α repair activity downstream of complex assembly. Functionally, all four PMS2 variants behave as null alleles, supporting reclassification as likely pathogenic. More broadly, our data suggest that cadmium might inhibit MMR by substituting for zinc within MutL α metal-binding sites and promoting a closed, catalytically inactive conformation. This work links inherited PMS2 variation, environmental metal exposure, and microsatellite instability through a shared mechanism of impaired MutL α activation.

Environment & Food 1

Trace Elements and Challenges to Global Public Health

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Trace elements are micronutrients or toxic metals that exist in the body at extremely low levels. They present a dichotomous global public health challenge: both chronic deficiencies and toxic over-exposure disrupt physiological homeostasis, triggering metabolic, cardiovascular, and neurological disorders. Minute quantities of essential trace elements are indispensable for enzymatic cofactors, redox equilibrium, and immune regulation. When the body lacks essential trace elements, it causes widespread undernutrition and chronic diseases, often referred to as "hidden hunger". Moreover, trace elements monitoring poses some technical difficulties and require solid knowledge and expertise to recognize the right form to search (speciation) and the proper detection method. Iodine (I) deficiency remains a leading cause of preventable thyroid disorders and intellectual disabilities worldwide. Iron (Fe) deficiency is the most common nutritional disorder and primary cause of anemia globally, also severely affecting cognitive development and maternal health, impairing oxygen delivery. Widely deficient in global populations, mostly in populations relying on zinc deficient cereal grains, zinc (Zn) is vital for immune function, child growth, and reducing the severity of infectious diseases. Selenium (Se) imbalances compromise antioxidant defenses, and its deficiency affecting up to an estimated 1 billion people globally, leading to localized health crises like Keshan disease. Conversely, the involuntary ingestion or inhalation of non-essential, potentially toxic elements represents a pressing global eco-environmental and public health crisis. Non-essential trace elements and heavy metals pose massive public health threats through air, water, and food chain contamination. Lead (Pb) is a potent neurotoxin affecting neurological development in children and cardiovascular health in adults. Arsenic (As) primarily is a global groundwater contaminant, causing skin lesions and various cancers. Its contamination in drinking water remains a massive public health threat in many regions. Cadmium (Cd) and Mercury (Hg) ubiquitous in contaminated soil, industrial runoff, and polluted seafood, bioaccumulate in food chains and are recognized as severe endocrine-disrupting compounds. Cd contaminates heavily impacting kidney function and bone integrity. Hg bioaccumulates in aquatic food webs and the exposure damages the nervous and immune systems. In the actual situation of rapid technical transformation, world crisis and Climate Change increasing we have some systemic and global health challenges such as the presence in the environment of new trace elements (Pt, Pd, Rh and rare earths). Shifts in weather patterns and intensive agriculture have degraded soil micronutrient levels, directly decreasing the nutritional quality of staple crops. Rapid industrialization, mining, and electronic waste release toxic trace elements into local ecosystems, where they persist and bioaccumulate in the food chain. While low-income regions often suffer from severe, overt deficiencies, middle- and high-income nations increasingly face the dual threats of subclinical nutrient deficits and chronic, low-level exposure to toxic metals from processed diets and environmental pollution. Addressing trace element issues is hindered by complex clinical and environmental barriers. The difference between an optimal dose for physiological function and a toxic level is often microscopically small (e.g., Se or Cu). Exposure to complex mixtures of different metals and

minerals can result in synergistic toxicities that complicate straightforward preventive and clinical solutions.

Identifying Drivers of Skeletal Deformities in Fish from Iron Ore Mine-Impacted Waters in Northern Sweden

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Background

Skeletal deformities in fish are widely used as biomarkers of environmental stress, particularly in aquatic systems receiving industrial effluents. Elevated vertebral mineralization in the caudal region of European perch (*Perca fluviatilis*) has recently been documented in northern Sweden in lakes receiving discharge from two iron ore mines. Follow-up egg-hatching experiments confirmed reduced hatching success and increased larval deformities in perch from effluent-impacted lakes. To identify the drivers of these effects, a semi-static laboratory study using zebrafish (*Danio rerio*) was designed to evaluate individual and combined risk factors under controlled conditions.

Materials and Methods

A 28-day early life-stage exposure protocol was developed by integrating OECD Test Guidelines 210 and 215. Zebrafish were monitored from fertilization through hatching, and larvae were maintained until 28 days post-fertilization. Assessed endpoints included hatching success, fry survival, and skeletal deformity frequency.

At termination, soft tissues were removed by alkaline digestion and trypsin treatment. Skeletons were stained with alizarin red, cleared in potassium hydroxide and glycerol, and photographed under a microscope for image analysis of vertebral abnormalities (Fig. 1).

Fish were exposed to a dilution series of mine excess water as well as water collected upstream and downstream of discharge points to establish dose–response relationships and compare them with field observations. In parallel, zebrafish were exposed to environmentally relevant concentrations of selected trace elements elevated in the actual mine effluents—selenium (Se), strontium (Sr), uranium (U), nickel (Ni), and cobalt (Co)—tested individually and in selected combinations. Observed response patterns were evaluated against regional water chemistry data to identify potential causal drivers.

Results

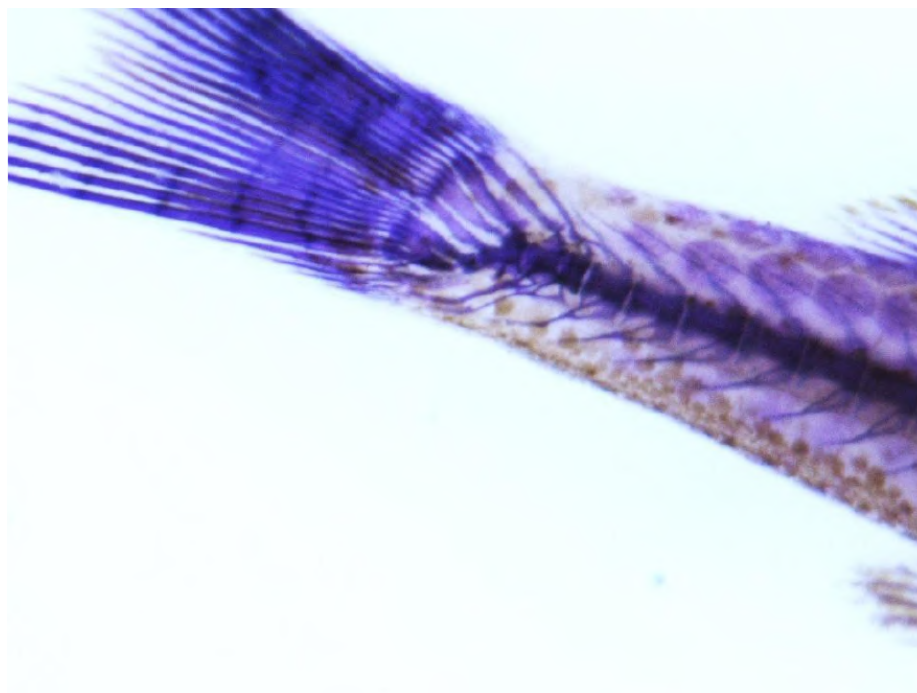
Exposure to mine excess water and downstream recipient waters reproduced the deformation pattern observed in wild perch, notably an increased frequency of deformities in the caudal vertebral region. This indicates that the experimental design successfully simulated environmentally relevant exposure conditions.

Among the individual trace elements tested, strontium exhibited a clear dose–response relationship with skeletal deformities. Although other elements elicited measurable responses, only the effect levels observed for strontium corresponded to concentrations detected in all the impacted water bodies. These findings suggest that strontium is a plausible contributor to the field-observed deformation pattern.

Conclusion

The developed 28-day zebrafish assay, combined with whole-skeleton staining, proved effective for detecting vertebral deformities in juvenile fish. Laboratory exposure to mine effluent and recipient waters successfully replicated field-observed skeletal abnormalities. The results indicate that strontium—potentially through substitution for calcium during early bone mineralization—is a plausible causal factor underlying the skeletal deviations observed in fish inhabiting the studied mine-impacted waters.

Figure 1. Photograph of the caudal peduncle region in zebrafish (*Danio rerio*) following strontium exposure, illustrating vertebral compression in the posterior vertebral column. 100× magnification.



Tracing Elements in Chick Gulls: Trophic and Spatial Patterns of Contamination

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Background

Human activities have profoundly altered the natural distribution, cycling, and bioavailability of trace elements in the environment. As a consequence, the background concentrations of many elements have increased considerably, contributing to widespread and persistent global contamination. In this context, seabirds are widely recognized as important bioindicators of environmental pollution due to their position in marine food webs and their sensitivity to contaminant exposure. Among them, seabird chicks are particularly useful indicators of contamination, as their contaminant burdens largely reflect local environmental conditions and pollution sources in the vicinity of breeding colonies. The present study investigates the ecological determinants influencing trace element concentrations in the blood of chicks belonging to three sympatric gull species inhabiting the French coast of the English Channel.

Materials and Methods

Between 2015 and 2017, a total of 174 birds were sampled for blood and feather collection in two contrasting coastal environments in northern France. Sampling was conducted in the highly industrialised Seine Estuary, including the city of Le Havre and Ratier Island, one of the most contaminated river systems in Europe. In parallel, birds were also sampled in the remote Normand-Breton Gulf on the Chausey Islands, located approximately 200 km further west, which served as a reference site with limited anthropogenic contaminant inputs. Element concentrations were determined using ICP-MS and CV-AAS. The trophic ecology and feeding status of the chicks were assessed using stable isotope analysis based on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$. Statistical analyses were mainly based on General Linear Models and Principal Component Analysis to explore relationships among ecological variables and contaminant burdens.

Results

Ag and V concentrations remained below the quantification limit in all analysed samples, whereas the proportion of non-quantified measurements exceeded 30% for Cd, Cr, and Ni. Among the elements successfully quantified, cobalt (Co) occurred at the lowest concentrations, while Fe was consistently present at the highest levels, resulting in the following concentration gradient: $\text{Co} < \text{Cd} < \text{Ni} < \text{Mn} \leq \text{Pb} < \text{Cr} < \text{Hg} < \text{Cu} < \text{Se} < \text{As} < \text{Zn} < \text{Fe}$. No uniform pattern of elemental concentrations was observed across the studied species and sampling sites, indicating substantial ecological and environmental variability.

Conclusion Individuals occupying higher trophic positions consistently exhibited elevated Hg concentrations, confirming the important role of trophic ecology in mercury exposure. The results also suggested biomagnification processes for As, whereas Pb showed evidence of biodilution along the trophic gradient. In addition, Pb concentrations were particularly elevated at the industrialised site located in the city of Le Havre. Although a substantial fraction of Cd, Cr, and Ni measurements remained below the quantification threshold, higher concentrations of these elements were still generally associated with the marine environment of the Chausey Islands. The concentrations of several trace elements clearly reflected habitat-related differences between study areas. Furthermore, in some individuals the calculated Se:Hg molar ratio was below 4, which is considered a threshold potentially insufficient for reducing the toxic effects of Hg through selenium-mediated protection.

Natural environment and health: how strong is the link?

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Background Several natural substances and materials are now known to have a more or less direct impact on health. For example, asbestos is implicated in the onset of mesothelioma, red earth in the development of podoconiosis, high fluoride levels in water responsible for more or less severe forms of fluorosis, or arsenic, linked to bladder and lung cancer. The list is far from complete, and the main problem concerns understanding the characteristics of natural materials and their complexity (variability over time and space). Essentially, biological matrices are shaped by their interaction with soil, atmospheric particulate matter (solid and spray), and water, which can cause genetic but mostly epigenetic alterations.

Materials and Methods Initially, the geo-environmental and health data, their characteristics, distribution, and spatial density, were the subject of in-depth discussions. Generally, and in the preliminary phase, when the quantity and dispersion of the data allowed, the usefulness of simple correlation was verified. This allows the identification the existence of a correlation between environmental data and health data based on their spatial distribution. To protect privacy, it was decided to establish the municipal territory as the geographical unit of investigation.

Results The results, initially presented at national and international specialist conferences and subsequently published in journals, have attracted considerable interest, prompting criticism and suggestions. Studies conducted to date have yielded some indications, some of which have been validated by meta-analyses. The pathologies considered so far, with a range that is expanding daily, range from autoimmune diseases to skin diseases, from suicide to infertility, cancer and amyotrophic lateral sclerosis.

Conclusion Natural media interact seamlessly. Given the complexity and dynamics of the natural environment, continuous cross-fertilization of knowledge is essential, supported today by technological development. All research conducted to date can be defined as generating hypotheses, not definitive results. Indeed, these studies have had the merit of suggesting whether and which elements, substances, or factors may be involved, thus usefully guiding further investigations.

Cadmium and Heavy Metal Exposure: Integrating Biomonitoring and Reproductive Toxicity in Young Adults

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Background

Exposure to cadmium (Cd) and other trace elements represents a relevant clinical concern due to their persistence, bioaccumulation, and endocrine-disrupting effects. Increasing evidence indicates that even low-level exposure may impair reproductive health through oxidative stress, hormonal dysregulation, and cellular damage. In this context, clinical interpretation of biomonitoring data requires reliable reference intervals derived from healthy populations.

Materials and Methods

This study integrates a clinical biomonitoring investigation with a translational analysis of heavy metal toxicity. Blood concentrations of selected trace elements (Cd, Pb, Hg, Cr, Zn) were measured in 154 healthy young adults using ICP-MS and ICP-OES, and reference intervals were established according to CLSI guidelines. These data were interpreted in light of current evidence on molecular and endocrine mechanisms of reproductive toxicity.

Results

Most individuals showed low or undetectable levels of cadmium and other toxic metals, consistent with non-occupational exposure. However, detectable levels of mercury and chromium indicated ongoing environmental or dietary exposure. Translational interpretation of these findings suggests that even subclinical exposure to Cd and trace elements may contribute to reproductive dysfunction via oxidative stress, apoptosis, ionic mimicry, and endocrine interference. These mechanisms are associated with impaired gametogenesis, altered hormonal balance, and adverse pregnancy outcomes.

Conclusion

From a clinical and laboratory medicine perspective, the integration of biomonitoring data with mechanistic evidence supports the relevance of low-dose exposure to cadmium and trace elements as a potential risk factor for reproductive health. The definition of population-specific reference intervals is essential for accurate clinical interpretation and risk stratification. These findings highlight the role of laboratory medicine in bridging environmental exposure and clinical outcomes,

supporting preventive and personalized approaches in reproductive health.

Cancer & Other Diseases 1

Heavy metal accumulation in biological matrices of cancer patients

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Heavy metals are widespread environmental contaminants. Many of them have well-established genotoxic and carcinogenic properties. In fact, most are suspected to be involved in a number of pathologies and to play a role in tumour development. The International Agency for Research on Cancer (IARC) classifies five of them as Group 1 carcinogens: arsenic, beryllium, cadmium, chromium, and nickel [1]. The carcinogenic potential of these heavy elements is intrinsically linked to their oxidation state, coordination geometry, ligand affinity, and redox activity. Once absorbed, these metals partition differentially across biological matrices – blood, plasma, serum, hair, saliva, urine, nails – depending on metalloprotein binding, ionic mimicry, and pH-dependent solubility [2]. For this reason, their direct quantification in human biological samples from cancer patients has emerged as a critical area of translational research. Across multiple independent studies, significant elevations of specific heavy metals were consistently observed in cancer patients. For example, elevated cadmium in blood and breast cancer [3], or chromium in the nails of lung cancer patients [4]. Saliva has also proven useful: significantly higher levels of lead, cadmium, and copper were found in the saliva of oral squamous cell carcinoma (OSCC) patients compared to healthy controls [5]. However, some studies report conflicting results – differences may stem from analytical methods, sample size, or confounding variables. Nevertheless, the evidence robustly indicates that higher burdens of certain toxic heavy metals are detectable in multiple biological samples of tumour patients [6]. These findings support the potential role of heavy metals as risk factors or co-factors in carcinogenesis and highlight the value of non-invasive matrices (hair, saliva, nails) for future biomonitoring and early risk assessment [7,8]. However, causality and the direction of association remain to be clarified through prospective studies that also measure metal speciation, not just its total concentration.

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Long-term cadmium exposure and fractures, cardiovascular disease, and mortality in a prospective cohort of women

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Background Current health-based guidance values for cadmium (Cd) are based on renal toxicity. Emerging evidence suggests that bone and the cardiovascular system might be more sensitive to Cd exposure. The aim was to assess the association of urinary Cd (U-Cd) with incidence of fractures, myocardial infarction, heart failure, ischemic stroke and mortality in a prospective population-based study among postmenopausal women. The findings will be compared to the available literature.

Methods We used data from 4024 women (56–85 years) in the Swedish Mammography Cohort. U-Cd was measured by ICP-MS at baseline (2004–2009) and categorized into tertiles. Incident cases of the outcomes were ascertained via register linkage through 2019. Multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression.

Results The median U-Cd at baseline was 0.33 µg/g creatinine (cr) (5–95 percentiles 0.15– 0.77). We ascertained: 903 first fracture of any type 149 myocardial infarction, 174 heart failure, 162 ischemic stroke and 545 total deaths during the approximately 11 years of follow-up. U-Cd was dose-dependently associated with risk of any fracture (HR: 1.20, 95% CI: 1.01 to 1.43, p-trend: 0.04) and all-cause mortality (HR: 1.38, 95% CI: 1.10 to 1.74, p-trend: <0.01) when comparing the highest tertile of U-Cd (median 0.54 µg/g cr) with the lowest (median 0.20 µg/g cr). No clear associations were observed for myocardial infarction, heart failure or stroke.

Conclusion Long-term Cd exposure was associated with risk of fractures and all-cause mortality at lower levels than previously suggested.

Hair zinc, copper, and Zn/Cu ratio in children with autism spectrum disorder: an exploratory case-control study

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Background

Zinc (Zn) and copper (Cu) are involved in neurobiological pathways relevant to Autism Spectrum Disorder (ASD), including oxidative stress, neuroinflammation, synaptic function, and enzymatic regulation. Hair-based studies have investigated trace element profiles in children with ASD, but findings remain inconsistent. Some case-control studies reported altered hair Zn and Cu levels in ASD, while more recent investigations have confirmed ongoing interest in hair mineral profiles. However, other studies found no significant differences in hair Zn or Cu between ASD cases and controls. In this context, hair may be considered a biomarker of chronic exposure or tissue deposition rather than a direct measure of systemic nutritional status. This study aimed to compare hair Zn and Cu levels, and the Zn/Cu ratio, between ASD patients and controls, taking sex and age at diagnosis into account.

Materials and Methods

An exploratory case-control study was conducted in ASD patients and controls. ASD diagnosis was established according to DSM V criteria. Hair samples, approximately 1.5-2 cm in length, were collected using clean stainless-steel scissors, and about 50 mg of hair were analyzed. Zn and Cu concentrations were quantified by inductively coupled plasma mass spectrometry (ICP-MS), and the Zn/Cu ratio was calculated. Descriptive analyses were performed to compare hair Zn, Cu, and Zn/Cu ratio distributions between cases and controls and across age classes, sex, and year of diagnosis.

Results

The study included 61 ASD patients (72.1% males) and 93 controls (30.1% males). Median (IQR) hair Zn concentrations were higher in cases [129.0 (80.5-161.0)] than in controls [75.8 (52.2-118.0) ug/g,] while hair Cu concentrations were similar between groups [8.8 (7.1-11.9) vs 8.8 (6.5-12.8) ug/g]. The Zn/Cu ratio was higher in cases than in controls [13.7 (6.6-17.8) vs 8.1 (4.6-13.3)]. The Zn/Cu ratio was generally shifted toward higher values in cases across age classes and tended to increase with age in both groups, with a

steeper pattern and wider dispersion in the oldest age class among cases. In controls, male and female distributions were largely overlapping, whereas in cases greater variability was observed, particularly among

females in the oldest age class. Among cases, hair Cu distributions appeared relatively stable across years of diagnosis, while hair Zn and the Zn/Cu ratio showed greater variability and a possible decreasing pattern in more recent diagnosis years, especially among females.

Conclusion

Given the exploratory design and the lack of universally accepted clinical reference values for hair Zn and Cu in children, the observed patterns should be considered hypothesis-generating. The quantification of hair

Zn, Cu, and the Zn/Cu ratio may help characterize long-term trace element profiles in children with ASD, as

biomarkers of chronic exposure or tissue deposition, and may support hypothesis generation for future analytically standardized studies.

Arsenic-induced atherosclerosis in a humanized Arsenic (3) Methyltransferase hyperlipidemic mouse model.

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Background Arsenic (As) contamination in drinking water poses a major global health risk, with chronic exposure linked to cardiovascular diseases (CVD). Atherosclerosis is an inflammatory response in the large arteries leading to gradual occlusion of the vessel with fibrofatty plaque. It is a key driver of cardiovascular morbidity and is exacerbated by As exposure. Previous studies using apolipoprotein E–knockout (ApoE^{-/-}) mice, a well-established hyperlipidemic model, demonstrated a dose-dependent increase in atherosclerotic plaque size after chronic exposure to 10–200 ppb inorganic As in drinking water for 13 weeks. Inorganic As is metabolized by arsenic (+3 oxidation state) methyltransferase (As3MT) through sequential methylation

reactions. Mice exhibit much higher methylation efficiency than humans, excreting nearly all arsenic as dimethylarsinic acid (DMA), the less toxic metabolite, whereas humans retain higher proportions of inorganic As (iAs) and monomethylarsonic acid (MAs), the more toxic intermediates. Consequently, mice are less susceptible to As-induced toxicity, limiting the translational value of traditional rodent model. However, a mouse expressing the humanized version of As3MT has been generated and has human-like arsenic metabolism. We hypothesized that humanized mice would be more susceptible to As-induced atherosclerosis.

Materials and Methods We generated an ApoE^{-/-} mouse carrying the humanized arsenic (+3 oxidation state) methyltransferase that recapitulates human-like As metabolism on a hyperlipidemic background. Male and female mice of the humanized and ApoE^{-/-} model were exposed to tap water or 10, 200 or 400 ppb sodium arsenite in drinking water for 4 or 13 weeks. We measured urine arsenic metabolites by inductively coupled plasma mass spectrometry (ICP-MS). Atherosclerotic plaque was assessed by oil red O staining and

collagen, macrophage, and smooth muscle content within the plaque was measured by immunohistochemistry or immunofluorescence.

Results Our model retained human-like urinary arsenic methylation profiles, characterized by higher proportions of iAs and DMAs and lower levels of MAs, confirming that ApoE deletion does not alter arsenic metabolism. Further, we show sex-specific differences in arsenic-induced atherosclerosis in the humanized mice, and an increased sensitivity to arsenic.

Conclusion Together, our findings highlight the combined influence of sex and species-specific As metabolism on atherosclerotic plaque development.

Toxicology 1

The safe range of selenium intake: lessons learned from experimental studies

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Selenium is a trace element of both nutritional and toxicological interest that is ubiquitous in the environment. Uncertainties still surround its relation to human health, given the large potential for bias in most studies, particularly exposure misclassification and unmeasured confounding. Given this context, experimental studies in humans, especially randomized controlled trials using a double-blind study design, are the best source of evidence to establish causal relationships. Over the past three decades, results from RCTs on selenium supplementation and a range of outcomes—including cancer, cardiovascular disease, and overall mortality—have generally led to the dismissal of the claims about the need of selenium supplementation in Western populations to prevent chronic disease. These RCTs have also raised new concerns about potential toxicity of this element. Such experimental results will be presented and discussed.

From Safe Doses to Risky Mixtures: The Toxicity of Real-Life Metal Mixtures

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Background Chronic non-occupational exposure to cadmium and other toxic elements occurs primarily through complex environmental mixtures, whereas risk assessment still largely relies on single-substance approaches. The aim of this integrative work was to combine human biomonitoring data with complementary experimental studies in order to evaluate whether real-life, low-dose mixtures containing Cd, As, Hg, Ni, Cr, and Pb can disrupt endocrine, metabolic, and redox homeostasis, and whether the benchmark dose (BMD) approach may serve as a translational bridge between human and experimental toxicology.

Materials and Methods The investigation integrated several complementary studies. In the human component, associations between blood metal concentrations and hormonal/metabolic outcomes were analyzed in 435 adults for insulin levels, reproductive hormones, and thyroid function. Metals were quantified using GF-AAS and/or ICP-MS methods, while hormones were measured using standard immunochemical procedures. Dose–response relationships were modelled using PROAST software with a benchmark response of 10%. The experimental component involved orally administered mixtures based on human biomonitoring-derived doses, including BMDL values, median concentrations, and 95th percentile exposure levels. Wistar rats were exposed for 28 and/or 90 days, followed by assessment of histopathology, hormone levels, redox biomarkers, Nrf2/HO-1 signalling, aromatase activity, and in silico mechanistic analyses.

Results The human studies revealed marked sex-specific effects. In women, arsenic and mercury were positively associated with serum insulin levels, whereas nickel showed a negative association; similar trends were confirmed in the overall population. Among the 207 men, dose–response relationships were confirmed between Cd, As, Hg, Cr, and Ni and serum testosterone, LH, and FSH levels, with the narrowest intervals observed for Cd–testosterone (0.273–3.62 µg/L) and Hg–LH (4.08–55.6 µg/L).

Experimental studies demonstrated that real-life metal mixtures induced histological and functional alterations in all investigated organs, often already at BMDL- or median-based doses. Female rats exhibited greater sensitivity in the thyroid dysfunction model, showing increased TSH, fT3, and T3 levels after 28 days, accompanied by histological abnormalities in all treated groups. In ovaries, both prooxidative and antioxidative parameters were altered after 28 days, whereas after 90 days antioxidant depletion predominated. The strongest dose–response relationships were observed for LH and FSH after 28 days and for SH groups, IMA, and Nrf2 after 90 days. In liver and kidney tissues, impaired Nrf2/HO-1 signalling, depletion of GSH in males, increased renal MDA levels in both sexes, and elevated hepatic IMA levels in males were observed. In testes, Cd and As accumulation, decreased LH levels, and reduced aromatase activity were associated with morphological lesions, with LH emerging as the most sensitive hormonal marker.

Conclusion

Taken together, cadmium should not be viewed as an isolated toxicant, but rather as part of biologically active mixtures capable of disrupting metabolism, thyroid function, reproductive health, and hepatorenal homeostasis even at environmentally relevant doses. These findings support a transition from evaluating “safe” individual doses toward a mixture-oriented, sex-specific, and biomonitoring-driven approach to risk assessment.

Manganese: A neurotoxic compound with a ‘soft’ health-based guidance value

Thorhallur Ingi Halldorsson

The neurotoxicity of manganese is well established, based on studies in experimental animals administered high oral doses and evidence from occupational exposures. Case reports also suggest that adverse neurological effects may occur, albeit rarely, in the general population. However, low and variable absorption from food and drinking water across life stages, together with limitations in assessing neurotoxicity in rodent models, have complicated the derivation of a health-based guidance value.

In 2023, the European Food Safety Authority (EFSA) conducted a systematic review of the evidence on manganese neurotoxicity. After screening more than 14,000 publications, 31 human studies and 63 rodent studies were included in the evaluation. While EFSA concluded that the available evidence “supports neurotoxicity as a critical effect”, it was not possible to establish a dose–response relationship or derive a tolerable upper intake level. Instead, a ‘safe level of intake’ of 8 mg/day for adults was proposed. This value is not anchored in a defined threshold for adverse effects but is instead based on the upper range of observed intakes in the European population, without a demonstrated dose–response relationship.

This presentation will briefly review the current evidence on manganese neurotoxicity in humans and animals and examine the rationale underlying the EFSA ‘safe level of intake’. It will be argued that the resulting ‘soft’ health-based guidance value partly reflects conservative approaches to evidence synthesis, which prioritize robust dose–response information from individual studies over the integration of evidence across studies through weight-of-evidence approaches or dose–response meta-analyses.

Toxicological Risk Assessment of Elemental Impurities in Pharmaceutical Products

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Background During the manufacturing and storage of pharmaceuticals, components such as packaging materials, delivery systems, or production equipment can release small amounts of chemicals into the final product. Elements can be an important component of these chemicals as an impurity. Since elemental impurities can cause some kind of toxicity endpoints, their levels in pharmaceutical products should be assessed and considered for acceptable limits which are defined in international guidelines such as ICHQ3D.

Methods To assess elemental impurities in pharmaceutical products, extractable and leachable studies should be conducted. In leachable study, elements migrate into a drug or device product under normal manufacturing, storage, and use conditions. In contrast, in experimental study, they can be intentionally drawn out under controlled laboratory conditions using solvents of varying polarity, such as acidic, alkaline, or nonpolar media. Generally, extractables represent potential leachables.

Results Upon detecting of elemental impurity levels of products, risk assessment is started using the toxicity data for every elements detected and Permitted Daily Exposure (PDE) for each element of toxicological concern.

Because these elements can pose health concerns during the therapeutical application, manufacturers and marketing authorization holders are required to evaluate them. A toxicological risk assessment must be conducted to ensure that any potential exposure to elements is within acceptable safe limits.

Conclusion In this presentation, it will be outlined the fundamental principles of toxicological risk assessment for extractable and/or leachable elements and share practical examples of how these assessments are applied in real-world scenarios.

Cell Biology 2

Dependency of metal toxicity on its speciation: lessons from cadmium and hexavalent chromium in the kidney

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Background

Environmental pollution by cadmium (Cd) and chromium (Cr) is increasing worldwide due to industrial activities and their non-biodegradable nature. In fact, ~15% of the world's cropland is contaminated with toxic metals (TMs). Following uptake of TMs from contaminated foodstuffs or air and absorption through the intestine and/or lungs, they initially bind to plasma proteins as well as to erythrocytes in the blood. The behaviour and toxicity of TMs is strongly dependent on the valency, complexation, physical-chemical properties, and particle characteristics. In the proximal tubule (PT) of the kidney, filtered Cd-bound thiol-containing proteins are endocytosed by the receptor complex, megalin:cubilin and ionic Cd is released from lysosomes to elicit oxidative distress and cell stress. In contrast, dichromate ($\text{Cr}_2\text{O}_7^{2-}$) is taken up by anion transporters, reduced to Cr^{III} , causing DNA interference, and triggering apoptosis or mutations. In this study, internalisation of relevant Cd-protein complexes at physiological concentrations and candidate anion transporters for $\text{Cr}_2\text{O}_7^{2-}$ will be presented and discussed.

Materials and Methods

An immortalised renal PT cell line (WKPT), which expresses megalin:cubilin and LcRn endocytotic receptors in addition to organic anion transporters (OATs) and organic cation transporter (OCTs), was exposed to CrO_3 (Cr^{VI})/ $\text{Cr}_2\text{O}_7^{2-}$ (Cr^{VI}) or Cd complexed with metallothionein (MT), albumin (Alb), transferrin (Tf), β 2-microglobulin (B2M) or α 1-microglobulin (A1M) for <24 h and MTT cell viability assay was performed. In $\text{Cr}_2\text{O}_7^{2-}$ uptake studies, inhibitors of anion exchange (DIDS, 10 μM) and OATs (probenecid, 0.5 mM) were used and HBSS incubation buffer was modified as follows: pH (6.5-8.5), CO_2 saturation, chloride omission, NaSO_4 supplementation. Uptake of FITC-Alb or immunostaining of B2M (100 nmol/l) in serum-free medium was monitored by fluorescence microscopy after 24 h.

Results

To mimic the Cd-protein concentrations measured in the filtrate, 100 nmol/l of Cd-protein

complexes were used. In megalin:cubilin expressing WKPT cells, Cd-Alb and Cd-B2M were internalised and significantly decreased cell viability by ~40% whereas Cd-MT, Cd-Tf and Cd-A1M had no toxic effects. When megalin:cubilin were lost as a result of high cell passage, uptake and toxicity by Cd-Alb and Cd-B2M were abolished. Thus, renal PT cells do not succumb to Cd toxicity by Cd-MT uptake. Rather, megalin:cubilin endocytosis of other Cd-complexed ligands, namely Cd-B2M and Cd-Alb damages the PT.

Toxicity by CrO_3 and $\text{Cr}_2\text{O}_7^{2-}$ was DIDS-sensitive and probenecid-insensitive, evidencing involvement of an anion transporter and ruling out OATs. Acidification increased toxicity by $\text{Cr}_2\text{O}_7^{2-}$ more than by CrO_3 (difference between pH 6.5 and pH 8.5: 4.2-fold for $\text{Cr}_2\text{O}_7^{2-}$ and 1.5-fold for CrO_3), which was confirmed in 20% CO_2 studies (~3.2-fold change for $\text{Cr}_2\text{O}_7^{2-}$), and could be explained by protonation of $\text{Cr}_2\text{O}_7^{2-}$ to HCr_2O_7^- for more efficient transport into cells. Omission of

chloride augmented toxicity whereas NaSO_4 supplementation reduced chromium cytotoxic effects.

These observations point to SLC26 anion transporter family as a strong candidate for mediating cellular Cr^{VI} uptake and renal PT cell damage.

Conclusion

In summary, complexation of Cd to distinct protein ligands and differences in toxicological profiles of Cr^{VI} chemical forms highlight the important of TM speciation in dictating bioaccumulation and potential harm.

The cadmium-induced renal toxicity regulated by the transcriptional pathway

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Cadmium (Cd) induces severe toxicity in various tissues including the kidney. In particular, long-term exposure to Cd causes chronic renal toxicity with damage to proximal tubular cells. Our previous study demonstrated that Cd induces apoptosis by changing the expression of apoptosis-related genes. These findings strongly suggest that Cd exerts its cytotoxicity by disrupting gene expression. Our recent studies showed that Cd alters the activities of large numbers of transcription factors in rat and human proximal tubular cells.

We focused on the transcription factors whose activities were inhibited by Cd treatment. YY1 (Yin-Yang 1) and FOXF1 (forkhead box F1) were among the transcription factors whose activities were inhibited

by Cd. YY1 specifically regulated the expression of *UBE2D2* (ubiquitin-conjugating enzyme E2 D2),

whereas FOXF1 was involved in the regulation of *UBE2D4* expression. The double knockdown of *UBE2D2* and *UBE2D4* resulted in elevated levels of intracellular protein level of p53 (apoptosis inducer), which is critically involved in Cd-induced apoptosis. Moreover, it was found that the inhibited the transcriptional activity of ARNT (aryl hydrocarbon receptor nuclear translocator) by Cd induced cell toxicity. The inhibited activity of ARNT suppressed the expression of BIRC3 (apoptosis inhibitor), which in turn induced apoptosis

in human proximal tubular cells. These results suggest that the inhibition of YY1, FOXF1 and ARNT transcriptional activity is directly involved in the expression of Cd toxicity. On the other hand, PPAR δ (peroxisome proliferator-activated receptor δ) plays a critical role in the modification of susceptibility to Cd

renal toxicity and that the apoptosis pathway may be involved in PPAR δ -modified Cd sensitivity. Addition to the apoptosis-related pathways, the energy metabolism disruption is found to be important to Cd renal toxicity. Cd inhibited the activity of the transcription factor, myocyte enhancer factor 2A (MEF2A), in

human proximal tubular cells. MEF2A binds to the promoter region of *SLC2A4*, the GLUT4 (glucose transporter)-encoding gene, to regulate its expression. Knockdown of *SLC2A4* eliminated both cell viability and Cd toxicity. In addition, Cd-induced suppression of *SLC2A4* expression decreases cellular ATP levels, partly by reducing glucose levels.

Therefore, Cd-induced cytotoxicity is mediated through multiple transcription factors and their downstream targets, ultimately leading to apoptosis and metabolic disruption. This presentation will highlight novel molecular pathways underlying the Cd toxicity.

Investigating the Effects of Cadmium Exposure on Prostate Cancer Cell Behaviour and Metastatic Signalling

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Background

Cadmium is an environmental heavy metal classified as a human carcinogen and has been associated with prostate cancer (PCa) progression and metastasis. Emerging evidence suggests that cadmium exposure may influence epithelial –mesenchymal transition (EMT), migration, and extracellular matrix remodelling through dysregulation of oncogenic signalling pathways. microRNA - 21 (miR -21), a well - established oncogenic microRNA in PCa, has been implicated in tumour progression and metastatic behaviour. This study aimed to investigate the effects of cadmium exposure on PCa cell viability, migration, clonogenicity, and EMT -associated signalling using wild - type and miR -21 knockout PCa models.

Materials and Methods

PC3 wild -type (WT), PC3 miR -21 knockout (KO), and LNCaP PCa cell lines were treated with cadmium chloride under varying experimental conditions. MTT assays were performed to determine cell viability and establish LC 50 profiles in both 1% and 10% foetal bovine serum (FBS) conditions. Long -term cadmium exposure studies were conducted over 14 days using 5 µM and 10 µM cadmium concentrations, with viability assessed at days 0, 7, and 14. Functional changes in migration and clonogenic potential were evaluated using wound healing and colony formation assays, respectively. Quantitative RT -PCR analysis was performed to assess the expression of EMT -associated genes, while secreted MMP9 levels were quantified from conditioned media supernatants using ELISA.

Results

Preliminary findings demonstrated indicated no significant difference in cadmium LC₅₀ profiles between 1% and 10% FBS conditions. Exposure to 10 µM cadmium reduced PCa cell viability, however, cells remained viable throughout the 14 -day treatment period , suggesting adaptation to chronic cadmium - induced stress . Wound healing migration assay demonstrated reduced migratory capacity in miR -21 KO cells following cadmium exposure compared to WT controls, supporting a role for miR -21 in cadmium - associated migratory signalling .. Colony formation assays demonstrated displayed reduced clonogenic behaviour following cadmium exposure in both WT and miR -21 KO cells, with a greater reduction observed in KO cells . Cadmium exposure was associated with altered expression of EMT -associated genes and modulation of secreted MMP9 levels.

Conclusion

Our findings suggest that chronic cadmium exposure induces phenotypic changes associated with metastatic plasticity in PCa cells. Despite reduced viability at higher cadmium concentrations, PCa cells remained viable throughout prolonged exposure, suggesting adaptation to chronic cadmium - induced stress. Furthermore, differential migratory and clonogenic responses observed between WT and miR -21 KO cells support a role for miR-21 in cadmium -mediated regulation of metastatic - associated signalling pathways. Ongoing studies are focused on validating EMT -associated molecular changes and further characterising the mechanisms underlying cadmium -driven PCa progression.

Modulation of Gemcitabine Sensitivity by Chronic Cadmium Exposure in Pancreatic Cancer Cells

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Background Cadmium (Cd) is a toxic heavy metal released into the environment mainly through industrial activities. The International Agency for Research on Cancer (IARC) classifies Cd as a carcinogen by inhalation, while evidence for carcinogenicity via oral ingestion is limited. Epidemiological studies associate Cd with several cancers, including pancreatic cancer (PC), a particularly aggressive tumor with <10% 5-year survival. Although Cd accumulates in pancreatic tumor tissues, its link to PC remains unclear. Several studies have demonstrated that the exposure to heavy metals, such as Cd, influence the response to the treatment in different tumors. Gemcitabine (GCB) is the first-line treatment for PC, but only 30% of patients respond, and resistance develops quickly. We hypothesized that Cd may influence GCB efficacy.

Materials and Methods BxPC-3 cell line derived from primary PC is characterized by wild-type RAS and by p53 missense mutations. AsPC-1 cell lines derived from metastatic PC carried KRAS mutation and p53 frameshift mutations. Cells were cultured in RPMI-1640 + 10% FBS + 1% penicillin/streptomycin at 37 °C, 5% CO₂. The cells were treated with different concentrations of cadmium (Cd) and Gemcitabine (GCB) for 48, 72, and 96 h, to determine the non-toxic concentration of Cd and the effective concentration of GCB on both cell lines. Then, to simulate a chronic exposure to Cd, the cells were cultured with 2.5–5 µM for 28 days and 10 µM for 14 days of Cd. Cd-treated cells were exposed to 5 µM for 72 h of GCB. The cytotoxic effect and proliferation capacity was investigated using MTT assay, Flow cytometry and Western blot.

The effect of treatment was analyzed using one-way ANOVA for MTT and Western blot analysis. Apoptosis and cell cycle data were analyzed by two-way ANOVA (dose x treatment) followed by Tukey's post-hoc test. Statistical analysis was performed with GraphPad Prism 10 software (Boston, MA, USA). The results are the means of at least three independent experiments, performed at least in quadruplicate. Data are expressed as mean ± SD and differences were statistically significant at p<0.05.

Results MTT and apoptosis assays showed increased viability in BxPC-3 cells treated with Cd+GCB vs GCB alone (p<0.01), suggesting reduced sensitivity. Cell cycle distribution showed a reduction of cells in sub-G1 phase. Conversely, AsPC-1 cells treated with Cd+GCB showed reduced viability versus GCB alone (p<0.01), suggesting a synergic effect. Cell cycle analysis revealed an increased number of cells in the sub-G1. The increase in PARP cleavage reinforced these results

Conclusion our results suggest that combined Cd+GCB treatment could exerts a potential protective effect against GCB alone in primary PC derived- cells (BxPC-3) and reinforces the cytotoxicity of GCB in metastatic-PC-derived cells (AsPC-1). In conclusion, the chronic environmental exposure to Cd can differentially modulate chemosensitivity depending on the genetic, molecular and aggressiveness of the tumor. From a clinical perspective, this observation underscores the potential consequences for patients with PC who are exposed to high environmental levels of Cd. Therefore, further in vivo studies will be needed to validate these findings.

Female Reproductive Toxicology of Cadmium

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Background

Reproductive organs are essential not only for the life of an individual but also for the survival and development of the species. The response of reproductive organs to toxic substances differs from that of other target organs, and they may serve as an ideal “barometer” for the deleterious effects of environmental pollution on animal and human health.

Materials and Methods

The aim of this study was to determine effects of Cd on the structure of rabbit ovary, oviduct and uterus after an experimental administration. Animals were divided into three groups according to cadmium administration – short term group (A, Cd i.p., 48 h), long term (C, Cd p.o., 5 month) and control (K).

Results

Decreased relative volume of growing follicles and increased stroma after Cd administration were detected. The number of atretic follicles was significantly higher after administration of Cd. The most frequent ultrastructural alterations observed were undulation of external nuclear membrane, dilatation of perinuclear cistern and endoplasmic reticulum. In all studied types of cells mitochondria with altered structure were found. In the oviduct the highest amount of epithelium in the group with long-term Cd administration was found. Microscopic analysis showed oedematization of the oviduct tissue, caused by disintegration of the capillary wall. An electron microscopic analysis showed dilatation of perinuclear cistern. The intercellular spaces were enlarged and junctions between cells were affected. Mainly after a long-term cadmium administration nuclear chromatin disintegration was present. In the uterus a significant change was determined in the relative volume of glandular epithelium. Increase of stroma was a sign of uterus oedematization caused by damage in the wall of blood vessels and subsequent diapedesis. After Cd administration alteration in uterus were less expressed, in comparison with ovary and oviduct. Alteration of nuclear chromatin contain following Cd administration suggests degenerative functional changes.

Conclusion

The results of our study elucidate the effects of cadmium on gonadal function and should also serve to increase the level of awareness of its effects on human and animal health.

Acknowledgement

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Flash Talk 1, Cell Biology, Cancer & Other Diseases

Tor1 Modulates Adaptive Responses to Cadmium: Enhanced Metabolic Plasticity Following Sub-Toxic Pre-Exposure

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Background

Cadmium (Cd) is a toxic heavy metal with no known physiological function in living organisms. It exerts embryotoxic, mutagenic, and carcinogenic effects, particularly under long-term exposure. Interestingly, sub-toxic concentrations of Cd have been reported to stimulate cell growth and DNA synthesis. Understanding how cells adapt to such stressful conditions remains a significant challenge. The fission yeast *Schizosaccharomyces pombe* serves as an excellent model for studying cytotoxicity and mechanisms of cellular resistance and adaptation.

Materials and Methods

Wild-type and Tor1-deficient *S. pombe* cells were cultured either in the absence or presence of a sub-toxic Cd concentration (1 μ M) for 10 days. Cell lifespan and doubling time were monitored to assess the effects of prolonged low-dose exposure. Following pre-treatment, cultures were standardized to OD₆₀₀ = 0.5 and subsequently exposed to a high Cd concentration (50 μ M). Cell growth was evaluated spectrophotometrically. The activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), was measured spectrophotometrically to assess cellular oxidative stress responses.

Results

Long-term exposure to 1 μ M Cd did not significantly affect the lifespan or doubling time of *S. pombe* cells. However, pre-treatment with sub-toxic Cd enhanced the growth of wild-type cells upon subsequent exposure to 50 μ M Cd, compared to non-pre-treated controls. Notably, Tor1-deficient cells exhibited higher growth intensity than wild-type cells under high Cd conditions, and pre-treatment further increased their growth. Pre-treatment with 1 μ M Cd resulted in a significant decrease in SOD and CAT activities in wildtype cells. In contrast, Tor1-deficient cells displayed elevated antioxidant enzyme activities relative to wild-type cells. These findings suggest that sub-toxic Cd exposure induces adaptive responses that enhance cellular tolerance to subsequent high-dose stress.

Conclusion

Sub-toxic Cd pre-treatment promotes adaptive responses that improve cellular resistance to high Cd concentrations in *S. pombe*. This adaptive effect is associated with the modulation of antioxidant defense mechanisms. Importantly, the enhanced tolerance observed in Tor1-deficient cells indicates that Tor1 negatively regulates the cellular response to Cd stress. These findings highlight the role of TOR signaling

in mediating adaptation to heavy metal exposure and contribute to a better understanding of stress-response mechanisms in eukaryotic cells.

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Human iPSC-Based Cardiac Platforms for Translational Cardiotoxicity Testing

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Background Cardiotoxicity is a major concern in drug development and in the risk assessment of environmental and industrial chemicals. Human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) have emerged as a physiologically relevant in vitro model for cardiotoxicity testing, enabling detection of ion channel dysfunction, arrhythmogenesis, cell death, and structural as well as mechanical impairment. Both two-dimensional (2D) monolayers and three-dimensional (3D) engineered tissues are used depending on the research question. While 2D systems offer high throughput, efficiency, and reproducibility, 3D models more accurately recapitulate sarcomeric organization and contractile function. Overall, the clinical relevance, diverse readouts, and adaptable culture formats make hiPSC-CMs a powerful platform for assessing cardiotoxic effects of cadmium and other trace metals.

Materials and Methods hiPSC-CMs were differentiated in-house and characterized by flow cytometry (cTnT⁺), sarcomeric immunofluorescence, and functional assays. Electrophysiology was assessed using video-based calcium and voltage imaging, with computational analysis extracting beat rate, action potential duration, arrhythmic events, and conduction velocity. Microelectrode arrays (MEA) enabled continuous monitoring and electrical pacing. Cells were cultured in 2D monolayers and 3D formats, including engineered cardiac microtissues in microfluidic perfusion systems and custom 3D-printed silicone chambers. Acute (minutes–24 h) and chronic (up to 14 days) exposure paradigms were applied.

Results We established a platform ranging from high-throughput 2D screening (up to 384-well format) to low-throughput, high-content 3D microphysiological systems. Compared to 2D cultures, 3D microtissues showed improved cellular alignment, enhanced electrical coupling, and more physiological contractile behavior. hiPSC-CM systems demonstrated high sensitivity to functional perturbations and suitability for long-term studies. Applications included prediction of cardiac liability of repurposed COVID-19 therapeutics, proarrhythmic risk stratification, and in vitro assessment of drug polytherapy. Integration with computational modeling enabled quantification of ion channel block, electromechanical coupling, active tension, and spatial electrophysiological properties. Furthermore, coupling with other organ-on-chip systems (e.g., liver) allowed investigation of systemic effects.

Conclusion hiPSC-CM–based biochips and engineered cardiac microtissues provide a robust, human-relevant platform for assessing cardiotoxicity under acute and chronic conditions. The combination of advanced electrophysiology, controlled pacing, and physiologically relevant 3D architecture enables detailed mechanistic insights into cardiac dysfunction. These models support translational research and are well suited to investigate cardiotoxic effects of cadmium and other trace metals.

Of Whales and Men, Translating How Chromosome Instability Drives Environmental Lung Carcinogenesis across Species and Biological Levels of Organization

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Background

Lung cancer is the top cause of cancer death worldwide, with substantial amounts of the disease caused by environmental chemicals other than tobacco. Chromosome instability is a driving force in lung cancer, with tumors characterized by altered chromosome number and structure. How chemicals induce chromosome instability to cause lung cancer is poorly understood. Hexavalent chromium [Cr(VI)], a chemical with widespread environmental exposure, causes human lung cancer. We use Cr(VI) to define mechanisms for how environmental chemicals cause structural chromosome instability and are finding the mechanism involves the induction of DNA double strand breaks and the loss of high-fidelity break repair.

Materials and Methods

To assess the effects of Cr(VI) on RAD51, we exposed human lung cells (WTHBF-6) and primary bowhead lung cells to various zinc chromate concentrations for acute (24 h) and prolonged (120 h) exposures. RAD51 was assessed at various levels including: 1) function measured by its ability to form immunofluorescent repair foci, 2) localization analyzed with immunofluorescence, 3) protein levels through western blot and 4) mRNA with RT-qPCR and RNAseq. We translated this mechanism to oropharyngeally exposed rat lung tissues and tumor tissues from chromate workers using immunofluorescence methods.

Results

We discovered Cr(VI) induces DNA double strand breaks and inhibits their repair by homologous recombination (HR) in human lung cells. Acute Cr(VI) exposure induced the canonical HR repair response, but prolonged Cr(VI) exposure disrupted RAD51 nucleoprotein filament formation. In fact, prolonged Cr(VI) exposure had a profound effect on RAD51: a) inhibiting its function, b) reducing the amount and quality of filaments formed, c) altering its subcellular localization and d) reducing protein and mRNA levels. Further, the loss of repair and RAD51 response were permanent, heritable phenotypic changes. We translated these outcomes from human lung cells to rodent cells to rat lung tissue and to human lung tumors. However, we also found, in whale cells, Cr(VI) induced the same amount of DNA double strand breaks but did not inhibit HR repair.

Conclusion

These outcomes robustly support the conclusion that the mechanism of Cr(VI)-induced lung cancer involves chromosome instability, DNA double strand breaks and the loss of high-fidelity DNA repair, but also indicate whale cells may have evolved protective mechanisms. Altogether the findings have important implications for Cr(VI)-induced lung cancer. This work was supported by National Institute of Environmental Health Sciences grants R01ES016893 & R35ES032876 (to JPWSr).

Results and Troubleshooting in Intracellular Heavy Metal-Targeting of a Fluorescent Hydroxy Naphthyl-Methylbenzoxazole Based Dye

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Background

The increasing concern regarding heavy metal contamination has turned their selective detection into a critical branch of environmental and biomedical research. Among various pollutants, Cadmium (Cd^{2+}) is particularly hazardous; classified as a Class I carcinogen, it accumulates through the food chain, leading to severe organ damage and ecotoxicological impacts. Conventional instrumental techniques, such as atomic absorption spectrometry, cannot analyze trace metal ions in still living cells. To bridge this gap, fluorescent small molecules have emerged as ideal tools for real-time, non-destructive monitoring of intracellular metal

fluctuations.

Materials and Methods

The efficiency of a recently developed 2-(2-hydroxynaphthyl)benzoxazole-based probe (HNBO-DEN, where DEN represents the diethylenetriamine chelating unit) was evaluated. Testing was performed on human

colorectal adenocarcinoma cells (HT-29) and hemocytes from the marine bioindicator *Mytilus galloprovincialis*. Flow Cytometry and Confocal Microscopy were utilized to assess probe internalization, Cd^{2+} detection efficiency, and cell viability through 7-AAD staining.

Results

In biological models, both systems without significant cytotoxicity at working concentrations, identified cadmium. HNBO-DEN appeared to have good sensitivity to detect cytoplasmic Cd^{2+} . In the HT-29 cells, a significant increase in mean fluorescence intensity was observed following 24 h of exposure to 150 μM CdCl_2 . In mussel hemocytes, granulocytes proved to be the most sensitive subpopulation for tracing environmental Cd^{2+} concentrations (from 1 nM to 1 mM), showing a proportionality between metal concentration and fluorescence intensity. The study also addressed troubleshooting related to dye specificity, cell autofluorescence and the accurate distinction between viable and damaged cells, crucial for environmental assessments.

Conclusion The free fluorescent probe provides an effective diagnostic tool for heavy metal detection, mapping the

presence of the metal in the cytosol. Further investigations will focus on the detection and actions of Cadmium as a metallo-estrogen in breast cancer cell models, such as MCF7.

The Effect of ZnO Nanoparticles on H295R Cells in Vitro

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Zinc oxide (ZnO) nanoparticles have attracted considerable attention in recent years owing to their unique physicochemical properties and broad spectrum of potential applications in biomedicine, energy, and environmental sciences. Nanoparticles exhibit several remarkable characteristics, including photocatalytic and antimicrobial activities, as well as promising capabilities in imaging and drug delivery systems. The cellular uptake of ZnO nanoparticles is influenced by multiple factors, such as particle size, morphology, and surface charge. ZnO nanoparticles may interact with various cellular structures, including proteins, DNA, and intracellular organelles. Nevertheless, further research is required to fully elucidate the mechanisms underlying their biological effects and to optimize their properties for specific applications. The aim of the present *in vitro* study was to evaluate the potential effects of ZnO nanoparticles (0.1–25 mg/mL) on mitochondrial activity, cell membrane integrity, and reactive oxygen species (ROS) production in H295R cells following 24 h exposure. Mitochondrial activity was assessed using the MTT assay, cell membrane integrity was evaluated by the CFDA-AM method, and ROS generation was determined using the NBT assay. The obtained results demonstrated a significant ($p < 0.001$) reduction in mitochondrial activity and impairment of cell membrane integrity in all experimental groups after 24 h exposure. Furthermore, the NBT assay confirmed a significant ($p < 0.001$) increase in ROS production at the highest applied concentration (25 mg/mL). The findings of this study confirm the ability of ZnO nanoparticles to induce significant alterations in multiple cellular parameters. However, further detailed investigations are necessary to better understand the complexity of the underlying molecular mechanisms.

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Trace metal exposure and childhood leukemia risk: an Italian case-control study

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Background Childhood leukemia is the most common form of cancer in children. Although therapeutic advances have significantly improved prognosis, the etiology remains largely unknown. Recent evidence suggests the involvement of heavy metals and metalloids in the onset of tumors. In this study we investigate the association between the concentrations of some trace metals and the risk of childhood leukemia.

Methods Between 2020-2023 in collaboration with the Pediatric Oncohematology Units of the University Hospitals of Modena, Padua and Catania, we recruited 75 children, 58 newly diagnosed leukemia cases and 17 controls. We collected a blood sample for each child to quantify plasma levels of the following trace elements: cadmium (Cd), iron (Fe), manganese (Mn), copper (Cu), selenium (Se) and zinc (Zn). We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression model to assess leukemia risk according increasing plasma metal tertiles, using the lowest category as a reference. Analyses were adjusted for age, sex and passive smoking by quantifying urinary cotinine.

Results: The mean age of the population was 6 years (interquartile range 3-10 years). Cu, Mn, and Se concentrations were higher in children with leukemia than in controls, while Cd and Zn concentrations were slightly higher in controls. Fe concentrations showed no difference in the two groups. We found a higher risk of leukemia in relation to all the metals examined, with the exception of Zn. The highest risk was found for Cu, with OR 36.94 (95% CI 3.77 - 362.40). For all metals, a dose-response relationship was found except for Mn where ORs of 5.27 (95% CI 1.21 - 22.91) and 2.72 (95% CI 0.67 - 11.05) were found for the second and third tertiles, respectively. For Se, an association with leukemia risk was found only in the third tertile OR 1.33 (95% CI 0.57 - 3.10).

Conclusions Our results indicate a positive correlation between blood levels of Cd, Cu, Fe, Mn, and Se and risk of childhood leukemia, with Cu showing the strongest association, suggesting their potential role in this disease. However, risk of reverse causation and residual confounding could not be ruled out as possible explanations for our findings.

Post Mortem Cerebrospinal Fluid for Trace Metal Analysis: A Pilot Study on Analytical Potential and Interpretative Pitfalls

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Background Postmortem cerebrospinal fluid (CSF) represents a potentially valuable but still poorly investigated matrix for trace metal analysis in forensic toxicology. Compared with blood and solid tissues, CSF may provide complementary information regarding central nervous system exposure and toxicokinetics, potentially being less affected by some of the postmortem redistribution phenomena that may influence conventional specimens. However, the very low concentrations of trace metals typically found in CSF make this matrix particularly susceptible to pre-analytical influences. Contamination during sample collection and handling may significantly affect analytical results, creating important challenges for data interpretation. This study was designed to evaluate both the analytical potential and the major interpretative pitfalls associated with trace metal determination in postmortem CSF.

Methods Postmortem CSF samples were collected during judicial autopsies using a contamination-controlled sampling protocol. Attention was paid to minimizing blood contamination and avoiding contact with metallic instruments. Samples were transferred into polypropylene tubes and stored at -20 °C until analysis. After thawing, specimens were diluted 1:10 with ultrapure demineralized water and analyzed by inductively coupled plasma mass spectrometry. Vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), arsenic (As), tin (Sn), antimony (Sb), and mercury (Hg) were quantified.

Results Twenty postmortem CSF samples were collected during the study period. More than half of the specimens showed evidence of blood contamination and were excluded from further evaluation, underscoring the technical challenges associated with obtaining suitable CSF samples for trace metal analysis. The remaining samples were successfully analyzed by ICP-MS. All investigated elements were detectable, demonstrating the analytical feasibility of multi-element determination in post mortem CSF. Trace metal concentrations showed considerable inter-individual variability. While this variability may partially reflect differences in environmental exposure and individual toxicokinetics, the influence of pre-analytical factors cannot be excluded. The absence of established reference values for post mortem CSF further complicates the result interpretation.

Conclusions Post mortem CSF appears to be a promising matrix for trace metal analysis, providing information to that obtained from conventional biological specimens. This pilot study demonstrates the analytical feasibility of ICP-MS multi-element determination in post mortem CSF. However, sample collection emerged as the major critical issue. The high rate of specimen exclusion due to blood contamination highlights the vulnerability of this matrix to pre-analytical bias and underscores the need for rigorous contamination-control procedures during sampling and handling. The forensic applicability of post mortem CSF for trace metal investigations will depend on the development of standardized collection protocols and reliable interpretative frameworks.

Plasma Metallome Profiling by ICP-MS/MS in Bipolar Disorder and Major Depressive Disorder: A Chemometric Cross-Sectional Study

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Trace elements act as obligate cofactors for enzymes controlling mitochondrial bioenergetics, antioxidant defence, monoaminergic neurotransmitter synthesis, and neuroendocrine regulation.^[1,2] Their peripheral concentrations are altered in mood disorders, yet prior work has relied on univariate comparisons of single elements without accounting for multicollinearity or integrating coordinated metallomic patterns.^[3–5] The differential diagnosis between Major Depressive Disorder (MDD) and Bipolar Disorder (BD) during depressive episodes carries misclassification rates of 20–40% and treatment delays of up to a decade,^[13] underscoring the unmet need for analytically rigorous peripheral biomarker approaches. Mn — as the obligate cofactor of mitochondrial MnSOD and glutamine synthetase — offers a peripheral surrogate of the mitochondrial redox stress documented in BD;^[7,8] Cr potentiates insulin receptor kinase signalling via the chromodulin oligopeptide;^[9] Mo drives sulfur amino acid catabolism and purine degradation through three molybdoenzymes, with XOR-derived ROS as a potential oxidative burden contributor.^[10]

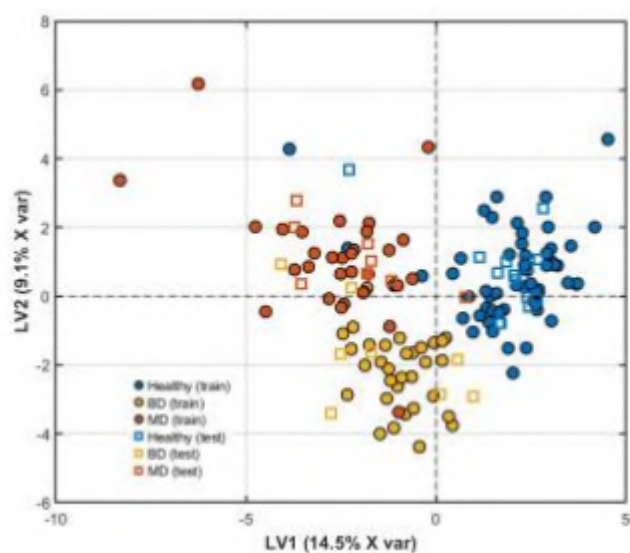
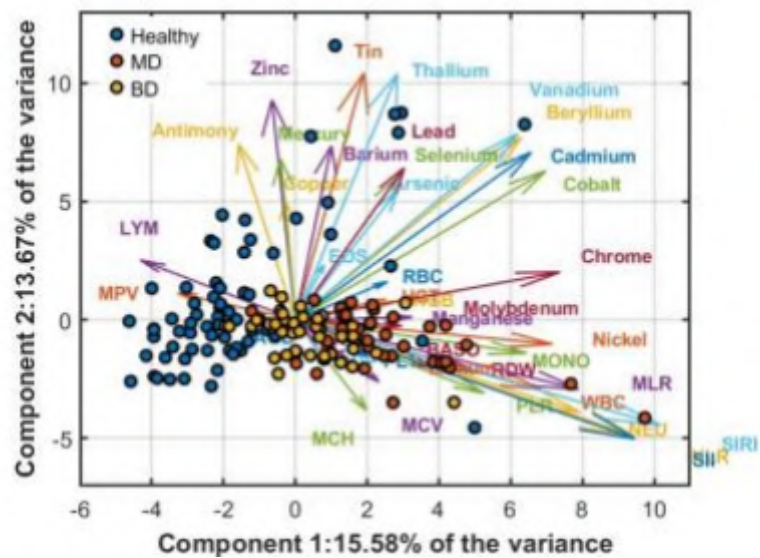
In this cross-sectional observational study, 151 adults were allocated to MDD (n=41), BD type I/II (n=40, euthymic), and healthy controls (HC, n=70). Diagnoses: SCID-5/DSM-5-TR; severity: HDRS-17 and YMRS. Total plasma concentrations of 22 elements were measured by triple-quadrupole ICP-MS/MS (Agilent 8800 QQQ; LOQ 0.001 ng/mL) under ISO 17025/2017 accreditation, with MS/MS-mode polyatomic interference elimination and ClinChek® CRM/OELM quality control. Five hematological inflammatory indices (NLR, MLR, PLR, SIRI, SII) were derived from CBC, yielding a 40-variable dataset (BDNF and NLRP3 excluded by design). After autoscaling, data were split into training (n=121) and hold-out (n=30) sets and analysed by PCA, t-SNE, and PLS-DA (RMSECV-guided, stratified 5-fold CV, 100 repetitions, 999-permutation testing).

Fig. 1. PCA biplot (PC1–PC2). HC (blue) at positive Fig. 2. PLS-DA score plot (LV1/LV2). HC separates PC1; MDD (orange) toward Cr/NLR/SIRI; BD (yellow) along LV1; BD/MDD partially separated along LV2. intermediate.

ISO 17025/2017-accredited ICP-MS/MS plasma metallomics, combined with hematological inflammatory indices in a permutation-validated chemometric framework, reveals non-random biological structure distinguishing MDD, BD, and HC. Under the point of view of the metal profile, manganese, chromium and molybdenum are highly involved in the biochemistry of these disorders and seem to be able to discriminate between the two classes of patients.

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Trace Element Exposure and Risk of Progression from Mild Cognitive Impairment to Dementia

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Background

Human exposure to trace elements and minerals is widespread, arising from environmental, occupational, and dietary sources. Most elements can cross the blood–brain barrier and accumulate in the central nervous system, where they may disrupt oxidative balance, mitochondrial function, and protein homeostasis. Some trace elements are required for neuronal function, but their dysregulation may promote neurotoxicity and neuroinflammation. Similarly, toxic metals and metalloids have been implicated in neurodegeneration through mechanisms such as oxidative stress. Mild cognitive impairment (MCI) represents a heterogeneous and transitional condition between normal aging and dementia. This stage offers a critical window to investigate environmental and metabolic determinants of clinically overt decline. Despite growing experimental evidence, prospective human studies linking trace element exposure to progression from MCI to dementia remain limited. We therefore evaluated whether exposure to macro and trace elements are associated with MCI progression to dementia.

Methods

We enrolled 56 individuals newly diagnosed with amnesic or non-amnesic MCI at the Memory Clinic of Modena University Hospital (Northern Italy) during the years 2008–2014. At the time of diagnosis we collected serum for 46 participants and we quantified baseline concentrations of minerals (magnesium, phosphorus, sulphur, calcium) and trace elements (iron, copper, zinc, selenium, manganese, cobalt, molybdenum, arsenic, antimony, vanadium, silver, cadmium, mercury, lead) using inductively coupled plasma mass spectrometry. Participants were reassessed every six months until 2021 to evaluate possible conversion to dementia. We used multi-adjusted regression and Cox proportional hazards models with restricted cubic splines to evaluate non-linear associations between element concentrations and conversion risk, adjusting for age, sex, education, and Mini-Mental state examination score (MMSE).

Results

Our cohort comprised 30 males and 26 females, with a median age of 66.2 years (IQR: 61.8–72.3).

Over a median follow-up of 54 months, 36 participants converted to dementia, in 27 cases due to Alzheimer's disease. Converters showed slightly lower baseline copper and manganese median concentration, though still within physiological ranges. Arsenic, cadmium, and lead were slightly higher in dementia converters compared with stable MCI participants. In spline regression analyses, baseline levels of zinc, selenium, and manganese were positively associated with risk of conversion to dementia.

Conclusion

Overall, our results suggest that altered homeostasis and imbalances of minerals and trace elements may contribute to progression to dementia in MCI patients.

Amyotrophic Lateral Sclerosis and Trace Elements: A Preliminary Case-Control Study in Sardinia, Italy

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Background

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease whose etiology remains largely unknown; however, growing evidence suggests a potential role for environmental factors, including exposure to heavy metals. Sardinia, an Italian island, represents a genetic outlier within Europe, as numerous genes display allele frequencies that markedly deviate from European population averages. Population-based investigations have reported that approximately 23% of ALS patients in Sardinia have a documented family history of the disease, compared with about 5% observed in other European ALS populations. The present study aimed to investigate a possible correlation between heavy metals exposure, antioxidants and the onset of ALS in the Sulcis-Iglesiente area, a region characterized by a long history of mining activities and a relatively high incidence of the disease.

Methods

In this context, concentrations of Cd, Co Cr, Cu, Hg, Mn, Ni, Pb, Se, V, and Zn were determined in blood and urine samples of 14 subjects with ALS and 15 controls using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique. In parallel, several biomarkers of oxidative stress and antioxidant defense were assessed, including IMA, TBARS (MDA), PON-1, p-SH, and MPO.

Results

The results showed significantly higher Pb concentrations in ALS patients compared to healthy controls. In particular, blood and urinary Pb levels were, on average, approximately two-fold higher in patients than in the control group. Overall, the findings indicate that Pb exposure may represent a relevant environmental risk factor in the development of ALS in the studied area. Although the limited sample size warrants further investigation in larger populations, the

results support the hypothesis that Pb may play a significant role in disease pathogenesis and highlight the importance of environmental and biological monitoring of heavy metals in at-risk areas.

Conclusions

This case-control study supports a role of environmental lead exposure in amyotrophic lateral sclerosis (ALS). ALS patients showed significantly higher lead levels in blood and urine than controls from the same mining-impacted area, suggesting increased systemic burden or individual susceptibility. No significant differences were observed in oxidative stress biomarkers, highlighting the complexity of ALS-related redox alterations. These findings support a multifactorial model in which environmental neurotoxic exposure interacts with genetic and metabolic susceptibility factors in ALS pathogenesis.

Levels of Toxic Metals in the Meconium of Newborns

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Background Meconium represents a valuable biological sample for assessing long-term fetal exposure to toxic metals during prenatal development. The presence of toxic metals such as cadmium (Cd) and lead (Pb) during this critical period may disrupt organogenesis, affect metabolic and endocrine pathways, and increase the risk of chronic diseases later in life. Since meconium begins to accumulate from the 12th–16th week of gestation and continues until birth, it serves as a cumulative record of exposure throughout most of the fetal period. Evaluating toxic metal concentrations in meconium allows for a direct assessment of maternal-fetal transfer and provides insight into potential environmental or dietary sources of exposure.

Methods A total of 36 meconium samples were collected in 2025 at the Clinic for Gynecology and Obstetrics, University Clinical Center of Serbia, with approval from the relevant ethics committees, and stored at –20 °C until analysis. After drying, samples (0.2–3 g) were subjected to microwave digestion using HNO₃ and H₂O₂, and the resulting solutions were quantitatively diluted with deionized water. Cadmium (Cd) and lead (Pb) concentrations were determined by graphite furnace atomic absorption spectrometry (GF-AAS). Statistical analysis was performed using GraphPad Prism 8, applying descriptive statistics, and results are presented as medians with minimum and maximum values.

Results Cd concentrations ranged from 0.748 µg/g to 4.286 µg/g, with a median of 1.452 µg/g (0.0129 µmol/g) and a mean value of 1.664 µg/g (0.0148 µmol/g). Pb concentrations ranged from 0.03 µg/g to 1.659 µg/g, with a median of 0.1365 µg/g (0.000658 µmol/g) and a mean value of 0.3293 µg/g (0.00159 µmol/g). Considerable variability was observed among individual samples.

Conclusion This study demonstrated the presence of cadmium (Cd) and lead (Pb) in all analyzed meconium samples, with higher Cd levels compared to Pb, indicating different exposure patterns and sources during pregnancy. The pronounced inter-individual variability suggests a strong influence of environmental factors and maternal lifestyle on fetal exposure. Compared with published data, the observed concentrations were lower than those reported in highly industrialized regions but still indicate ongoing prenatal exposure. These findings confirm meconium as a reliable biomarker of long-term fetal exposure to toxic metals.

Maternal Copper Levels in Early Pregnancy and Risk of Miscarriage

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Background

Miscarriage is a common obstetric complication defined as the spontaneous loss of pregnancy before fetal viability. It may occur as a complete miscarriage, involving total expulsion of the conceptus, or as an incomplete miscarriage, when only part of the conceptus is expelled from the uterine cavity [1]. Copper (Cu) is an essential trace element and a structural component of numerous enzymes, including superoxide dismutase (SOD), which plays a pivotal role in oxidative stress regulation, cellular respiration, and neurotransmitter synthesis. Physiologically, maternal copper concentrations increase during pregnancy. However, copper status is influenced by multiple factors, including dietary intake, supplementation, lifestyle, and environmental exposure. Disturbances in copper homeostasis have been reported in various pregnancy-related pathologies [2,3].

The aim of this study was to evaluate whole blood copper concentrations in women in early pregnancy and in women who experienced miscarriage.

Materials and Methods

The study material comprised whole blood samples, placental tissue fragments, and questionnaire data collected from women in the first trimester of uncomplicated pregnancy (control group) and from women diagnosed with miscarriage (study group). Whole blood copper concentration was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). The presence of copper deposits (Cu²⁺) in placental tissue was identified using the Rhodanine Copper Detection Staining Kit. Dietary copper intake, including supplementation, was evaluated using a 24-hour dietary recall model.

Results

Women who experienced miscarriage demonstrated significantly lower whole blood copper concentrations compared with women with ongoing healthy pregnancies. Copper deposits (Cu²⁺) were not observed in the placental tissue of either group, or if they were present, they would be in quantities that are not detectable under optical microscopy. The mean dietary copper intake among women after miscarriage was below the Estimated Average Requirement (EAR) and was approximately fourfold lower than that observed in pregnant women in the control group [4].

Conclusions

Reduced whole blood copper concentration and insufficient dietary copper intake may be associated with an increased risk of miscarriage. Monitoring copper status prior to conception and during pregnancy, particularly in women with potential disturbances in copper metabolism, may contribute to early identification of abnormal copper levels and improved prevention strategies. Establishing trimester-specific reference intervals for whole blood copper in pregnant women is warranted to define clinically relevant deficiency and excess thresholds.

Routine assessment of total copper concentration in whole blood, combined with dietary evaluation, should be considered as part of standard perinatal care to facilitate early detection of pregnancy-related complications, including miscarriage.

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Concentration of metals in follicular fluid of women undergoing assisted reproductive technology

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Background The World Health Organization defines infertility as a disease of the male or female reproductive system, characterized by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. The assisted reproductive technology has been widely used to treat infertility over the past 30 years. In this context, the ovary contains several metals that play a crucial role in oogenesis, oocyte maturation, ovulation, and normal oocyte function. However, imbalances in these metal levels—either deficiencies or excess—may impair oogenesis, adversely affect oocyte quality, and contribute to infertility. For this reason, metals in follicular fluid, which can effectively reflect the environmental exposure levels of oocytes and embryo development, were investigated.

Materials and methods The follicular fluid was collected from 31 women for the quantification of Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn. After water dilution, samples were determined by the inductively coupled plasma mass spectrometry technique, and the association between metal concentrations and clinical outcomes of assisted reproductive technology was evaluated.

Results The results demonstrated a positive association between Cu and Zn concentrations and the number of retrieved oocytes, as well as between Zn and oocyte count. Conversely, Cu, V, and Zn showed negative associations with endometrial thickness. Furthermore, Cu levels decreased as the fertilization rate increased.

Conclusion These findings highlight the critical importance of considering metal exposure in relation to female fertility.

Cadmium and Lead in Placentas: Evidence from Croatia

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Background Monitoring placental levels of Cd and Pb is important for toxicology and public health because adverse pregnancy outcomes linked to these metals are well established. Cd exposure has been associated with intrauterine growth restriction and lower birth weight through impaired placental function, oxidative stress, and disrupted transfer of essential nutrients, while Pb exposure has been linked to impaired neurodevelopment and an increased risk of preterm birth. The **aim** of this study was to assess temporal trends in placental Cd and Pb levels over the past three decades.

Materials and Methods This study included pregnant women from Zagreb and nearby areas who gave birth in Zagreb maternity hospitals and had no occupational exposure to Cd and Pb. The final dataset comprised manually extracted summary data from previously published studies that reported placental Cd and Pb levels across three periods: the 1990s, 2008–2010, and 2017–2019. Data were analysed using R (version 4.2.2, and RStudio 2025.09.2; R Foundation for Statistical Computing, Vienna, Austria).

Results Women who gave birth in the 1990s had the highest placental metal burdens, with overall mean concentrations of 18.9 ± 8.0 ng/g wet weight (w.w.) for Cd and 30.7 ± 17.6 ng/g w.w. for Pb. Concentrations decreased markedly by 2008–2010 (Cd: 8.9 ± 1.9 ng/g w.w.; Pb: 5.7 ± 0.49 ng/g w.w.). The lowest levels were observed among women recruited in 2017–2019, with overall mean values of 6.8 ± 1.2 ng/g w.w. for Cd and 2.2 ± 0.57 ng/g w.w. for Pb.

Conclusion Placental Cd and Pb levels decreased substantially over the past three decades (1990s–2019) in women from the Zagreb area.

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Cancer & Other Diseases 2

Trace elements as biomarkers in Oral Carcinogenesis: Where we stand in India!

Sunali Khanna

One of the most serious conditions affecting the oral tissues in oral cancer; this is specifically a problem in the Indian Subcontinent, where it has been found to be the most common form of cancer mainly due to excessive consumption of smokeless tobacco.

In India, oral precancer and cancer is spreading like an epidemic targeting the young generations. Significant reduction in cancer related deaths can be achieved by advances in early detection, prevention and implementation of treatment leading to improvement in survivorship.

Recent advances associated with detection of immunological and biochemical markers have marked an end of an era of groping in the dark for early detection of oral precancer and cancer. Proactive intervention in the diagnosis of oral cancer helps tackling the disease at an early stage which improves the prognosis of the patients.

This has shifted the emphasis from palliative treatment of cancer patients to curative and chemopreventive management protocols

Evaluation of Circulating immune complexes and trace elements (copper, iron, selenium and molybdenum) in serum of patients with oral Leukoplakia, oral submucous fibrosis and oral squamous carcinoma patients are immunological and biochemical markers in the early detection and management of these lesions.

The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesions. Histologically, over 95% of oral cancers are squamous cell carcinomas. It has been suggested that a vast majority of oral squamous cell carcinomas in India arise from pre-existing Leukoplakia. In context of current economic growth, access to public health is vital. However, since 1990's public health system is collapsing in India and private health sector has flourished at the cost of public health sector. Decline of public investment in health sector has weakened it further. Thus, strongly affecting the vulnerable and marginalized sections of society. Despite significant improvements in the oral health populations across the world, it has been reported that lower socio-economic groups compared to higher socio-economic groups have poorer oral health. Therefore concerted efforts are required at proactive intervention to facilitate prevention strategies.

Keywords Areca Nut, Betel Quid, Oral Submucous Fibrosis, Leukoplakia, Oral Cancer, Dental Public Health.

Adipose tissue cadmium as a biomarker of long-term cadmium exposure and its cardiometabolic implications in the GraMo cohort

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Background Cadmium (Cd) is a persistent environmental toxicant with potential metabolic, cardiovascular and socioeconomic implications. Adipose tissue has been scarcely explored as a biological matrix for cadmium exposure assessment, despite its active involvement in multiple metabolic and endocrine processes and its potential to provide complementary information on long-term internal burden. This communication summarizes the main findings from a research line conducted within the GraMo cohort (Southern Spain),

aim ed at evaluating adipose tissue Cd concentrations as a biomarker of chronic exposure and exploring their potential implications for cardiometabolic health.

Materials and Methods The GraMo cohort includes adults recruited in 2003-2004 in Southern Spain. Adipose tissue samples were collected intraoperatively and Cd concentrations were quantified by high-resolution inductively coupled plasma mass spectrometry. Sociodemographic, lifestyle, dietary, residential and occupational information was collected through validated questionnaires. The research line included cross-sectional analyses of exposure determinants and pollutant mixtures, longitudinal analyses of 16-year type 2 diabetes mellitus (T2DM) incidence, analyses of glucose homeostasis biomarkers, assessment of serum PON1 activity and complementary analyses of healthcare expenditure.

Results Cd was detected in all adipose tissue samples, with a mean concentration of 12.66 ± 18.91 µg/kg. Higher Cd concentrations were associated with smoking, age, egg consumption and self-perceived exposure to paints, whereas male sex, higher body mass index and meat consumption were inversely associated with Cd levels. In mixture analyses, Cd clustered with polychlorinated biphenyls and other metals, supporting the relevance of co-exposure assessment. Higher adipose tissue Cd concentrations were suggestively associated with increased T2DM risk, particularly among current smokers, and with higher insulin resistance and insulin levels in complementary analyses. Cd concentrations were also positively associated with healthcare expenditure, suggesting potential implications for healthcare resource use. Finally, higher adipose tissue Cd was associated with reduced serum PON1 activity, with susceptibility differing by functional PON1Q192R phenotype and increasing in the order QQ < QR < RR.

Conclusion Findings from the GraMo cohort support adipose tissue Cd as a promising biomarker of chronic exposure. We also evidenced both cross-sectional and longitudinal cardiometabolic implications related to Cd. Further prospective and mechanistic studies integrating metals, pollutant mixtures and cardiometabolic outcomes are warranted.

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Plasma and Urinary Heavy Metal Profiles as Biomarkers for Prostate Disease Progression: A Multi-Metal ICP-MS Study

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BACKGROUND Prostate cancer (PC) is the second most common malignancy in men globally, with incidence projected to reach 2.9 million cases by 2040. Environmental exposure to heavy metals has been implicated in chronic inflammation and carcinogenesis through multiple biological pathways, including oxidative stress, immune dysregulation, endocrine disruption, and epigenetic modifications. However, their specific role across the *clinical continuum* from benign prostatic hyperplasia (BPH) to precancerous lesions (PL) and overt PC remains poorly characterised. This study aimed to quantify a broad panel of trace elements in plasma and urine of patients spanning the full prostate disease spectrum, and to evaluate their potential as diagnostic and prognostic biomarkers.

METHODS A total of 154 male subjects from Sardinia, Italy, were enrolled between September 2021 and December 2022 at the Urology Clinic, University Hospital of Sassari: 61 BPH (controls), 15 PL, and 78 histopathologically confirmed PC cases. Patient classification was based on ultrasound-guided prostate biopsy with histopathological assessment. Concentrations of 14 heavy metals — manganese (Mn), cobalt (Co), vanadium (V), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), arsenic (As), molybdenum (Mo), cadmium (Cd), antimony (Sb), barium (Ba), mercury (Hg), and lead (Pb) — were determined in both plasma and urine by Inductively Coupled Plasma Mass Spectrometry (ICP-MS/MS, Agilent 8800 QQQ) following US EPA Method 6020B, accredited to UNI EN ISO 17025:2017. Statistical associations between metal concentrations and disease group were assessed by Kruskal–Wallis test, Mann–Whitney U test, and *probit regression analysis*, adjusted for age, total prostate-specific antigen (PSA), and haematological/inflammatory parameters including white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), neutrophils, lymphocytes, monocytes, and monocyte-to-lymphocyte ratio (MLR).

RESULTS Significant intergroup differences were identified for plasma antimony (Sb) ($p = 0.034$) and plasma manganese (Mn) ($p = 0.049$). Plasma Sb concentrations were significantly lower in PC patients and remained independently associated with PC risk across all adjusted regression models (OR -0.25 ; 95% CI -0.43 to -0.08 ; $p < 0.001$), including after correction for age, PSA, and inflammatory indices (MLR, SIRI). Plasma vanadium (V) was significantly elevated in the PL group and showed a robust association with precancerous lesions after adjustment for RBC (OR 13.10 ; 95% CI 1.39 – 24.81 ; $p = 0.03$) and age (OR 12.35 ; 95% CI 0.99 – 23.70 ; $p = 0.03$), suggesting a role in *early neoplastic transformation*. Among urinary metals, urinary lead (Pb) concentration was significantly associated with PC (OR 0.28 ; 95% CI 0.02 – 0.54 ; $p = 0.03$), an association that persisted and was strengthened after combined adjustment for age and PSA (OR 0.33 ; 95% CI 0.04 – 0.61 ; $p = 0.02$), consistent with long-term environmental bioaccumulation. A trend toward significance was observed for urinary Cu and PC ($p = 0.06$). No significant intergroup differences emerged for the remaining urinary metals. The monocyte-to-lymphocyte ratio was significantly elevated in PL and PC patients ($p = 0.03$), supporting a heavy metal-mediated inflammatory mechanism in prostate carcinogenesis. To our knowledge, this is the first study to simultaneously quantify 14 metals in both plasma and urine across the full BPH–PL–PC clinical continuum.

CONCLUSIONS This study provides novel evidence that plasma vanadium and antimony, together with urinary lead, are significantly associated with prostate disease progression from benign to precancerous and cancerous states. These metals emerge as candidate *non-invasive biomarkers* for prostate cancer risk stratification. Mechanistic pathways likely involve reactive oxygen species (ROS)-induced oxidative stress, androgen/oestrogen receptor interference, and immune dysregulation. The complementary roles of

plasma (reflecting ongoing systemic exposure) and urine (reflecting long-term accumulation) highlight the value of a dual-matrix approach. Future studies should integrate multi-matrix sampling — including plasma, urine, prostate tissue, and hair — and nutritional profiling to validate these biomarkers in larger multicentre cohorts and inform prevention and management strategies for prostate disease.

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It's Complicated: How Sex, Diet and Metals Interact to Drive Liver Disease

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Background

Chronic liver disease kills over 2 million people each year. Recently, the most rapidly growing contributor to liver mortality and morbidity has shifted from alcohol-induced liver disease to metabolic dysfunction-associated steatotic liver disease (MASLD). Despite advances at the bench and in the clinic, MASLD prevalence has doubled over the past 20 years and continues to rise. While obesity is a major risk factor, it cannot account for all cases, indicating other risk factors, such as, sex and environmental exposures, increase susceptibility to MASLD. Exposures to cadmium and hexavalent chromium [Cr(VI)], both drinking water contaminants of major public health concern, are implicated in the development and progression of liver disease. However, how exposures to these metals contribute to liver disease is not well known. In addition, there is increasing evidence that the adverse health effects associated with exposures to these toxic metals manifest differently in women and men.

Materials and Methods

In vivo models were designed to study the effects of exposure to cadmium or Cr(VI) on high fat diet (HFD)-induced MASLD, considering outcomes in both males and females. For the cadmium study, adult C57BL/6J mice fed normal diets (ND) were exposed to 0, 0.5 or 5 ppm cadmium-containing drinking water for 1 week before breeding. Pregnant dams and offspring were continuously exposed to the same Cd concentration. At weaning, offspring were fed ND or HFD and then sacrificed after 24 weeks. For the Cr(VI) study, 3-month-old rats were exposed to Cr(VI) in drinking water (0, 0.05, or 0.1 ppm) for 90 days.

Results

Whole-life exposure to cadmium exacerbated HFD-induced liver injury in male mice only, possibly through an altered metallothionein or ER stress response. Exposure to low levels of Cr(VI) in drinking water for 90 days resulted in sex-dependent alterations of diet-induced lipid profiles, markers of liver injury, alpha-fetoprotein gene expression and DNA damage.

Conclusion

Results from these studies confirm the multi-hit nature of MASLD, show metal exposures alter

HFD-induced MASLD, and highlight the importance of sex as a risk factor in disease development. This work was supported by the Jewish Heritage Fund for Excellence Faculty Recruitment Grant Program at the University of Louisville, School of Medicine (JLW) and the National Institute of Environmental Health Sciences [R35ES02837 (MCC), R21ES033327 (JPWJr) and R35ES032876 (JPWSr)].

Environment & Food 2

New insights into human biomonitoring of cadmium exposure

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Background . Human biomonitoring is a powerful tool for assessing personal exposure to hazardous chemicals. Biomonitoring of exposure to and accumulation of cadmium (Cd) is typically performed by measuring Cd concentrations in blood and urine. The aim of this work is to review the analytical

methods used for the determination of Cd in biofluids, the levels reported in human biomonitoring studies, and the values currently applied for result interpretation and health risk assessment.

Materials and Methods . A review of analytical methods and human biomonitoring studies investigating Cd in urine and blood in environmental and occupational settings was performed. Occupational exposure limit values, as well as biological limit and reference values proposed or established by major international agencies and regulatory bodies for the interpretation of Cd biomonitoring data, were also reviewed.

Results . Current levels of Cd in human biofluids can be measured with high sensitivity using inductively coupled plasma mass spectrometry (ICP-MS), and the accuracy of analytical results can be ensured through participation in international quality-control programs. Over the last decades, Cd concentrations in blood and urine have shown a decreasing trend worldwide, both in occupationally exposed groups and in the general population. A recent EU directive has proposed a lower occupational exposure limit for Cd, which will enter into force in 2027. The directive also highlights biological monitoring as a preventive measure in the workplace, indicating a biological limit value of 5 µg/g creatinine for urinary Cd. Within the framework of the EU human biomonitoring initiative (HBM4EU), human biomonitoring guidance values (HBM-GVs) for cadmium and its compounds have been established, recommending urinary Cd levels of 1 µg/g creatinine for the general population and 2 µg/g creatinine for workers.

Conclusions . Recent studies consistently show a decreasing trend in Cd exposure worldwide. Several biomonitoring-based limit and reference values have been proposed in recent years to support human health protection; these are largely based on the measurement of cadmium in urine, which is the preferred biomarker for long-term Cd exposure assessment.

Cadmium Burden and Disrupted Metal Homeostasis in Multiple Sclerosis Patients from a Volcanic Area: A Case–Control Study

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Background

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease influenced by both genetic and environmental factors. Among environmental exposures, toxic metals have been increasingly implicated in neuroinflammation, oxidative stress and immune dysregulation^{1,2}. Cadmium (Cd), a well-known neurotoxic metal, may contribute to neuronal damage through oxidative pathways. Its role may be particularly relevant in volcanic areas, where environmental metal dispersion is increased.

Materials and Methods

We conducted a case–control study including 86 MS patients and 47 healthy controls from the province of Catania (Italy). Serum Cd concentrations were measured after acid digestion using inductively coupled plasma mass spectrometry (ICP-MS). Data were expressed as median and interquartile range (IQR), and comparisons between groups were performed using the Mann–Whitney U test. Cadmium-to-essential metal ratios were also calculated to evaluate potential imbalances in metal homeostasis

Results

MS patients showed significantly higher blood Cd levels compared to controls (median [IQR]: 0.03 [0.03–0.96] vs 0.03 [0.03–0.03] µg/L; $p < 0.001$). Although median Cd concentrations were similar, the distribution in MS patients was markedly broader, with higher upper quartiles and extreme values, suggesting the presence of a subgroup with increased Cd burden. To further investigate Cd-related metal imbalance, cadmium-to-essential metal ratios were assessed. MS patients showed higher Cd/Cu, Cd/Fe, Cd/Mg and Cd/Zn ratios than controls. Overall, all four ratios were shifted upward in MS patients, indicating a more pronounced disequilibrium between cadmium burden and essential metal homeostasis. The distributions were strongly right-skewed in the MS group, with several extreme values, especially for Cd/Mg and Cd/Fe, supporting the presence of individuals with disproportionate Cd accumulation relative to physiological metal regulation. These findings are consistent with previous studies reporting increased Cd levels in MS patients across different populations^{3–5}, although variability in absolute concentrations has been described according to environmental exposure and analytical methodology.

Conclusion

MS patients living in a volcanic area exhibit an altered Cd profile characterized by increased variability and the presence of a high-exposure subgroup. The significant increase in cadmium-to-essential metal ratios suggests a disruption of metal homeostasis, potentially contributing to disease-related mechanisms. Cadmium-related dyshomeostasis may represent a relevant environmental factor in MS pathophysiology, supporting the need for integrated studies on environmental exposure and metal metabolism in this disease.

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Density-Dependent Efficiency: The Role of Isidia and Algal Cells on Mercury Accumulation in Lichens

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Background

Mercury (Hg) is a toxic element that poses a serious environmental risk due to its strong potential for bioaccumulation, biomagnification, and long-range atmospheric transport⁽¹⁾. Lichens are as excellent Hg bioaccumulators and are widely used as biomonitors of Hg pollution⁽²⁾, due to the absence of cuticle and root system, and their direct dependence on atmospheric deposition for nutrient uptake⁽³⁾. Despite growing interest in lichen-based Hg biomonitoring, critical knowledge gaps persist regarding the influence of lichen morphotypes on Hg bioaccumulation, the subcellular sites of Hg sequestration, the contributions of each symbiont to Hg uptake, and the ultrastructural consequences of Hg exposure. The present study aimed to address these knowledge gaps.

Materials and Methods

Lichen transplant experiments were conducted using *Pseudevernia furfuracea* (L.) Ach, a species widely used in both active and passive biomonitoring^(4–7) and characterized by variable isidia density. These finger-like, algal-rich vegetative propagules can double the thallus surface area, increasing gas exchange and the entrapment of particulate matter⁽⁸⁾. Highly isidiate (HI) and poorly isidiate (PI) thalli were collected from a pristine area (Casera Razzo, BL, NE Italy) and transplanted to three areas affected by Hg pollution of different origin: a waste incinerator (Spilimbergo, PN), a decommissioned chlor-alkali plant (Torviscosa, UD), and a decommissioned cinnabar mining and Hg production site (Abbadia San Salvatore, SI). After two- and four-month exposure periods, Hg content was quantified using a Direct Mercury Analyzer (DMA-80) in HI and PI pre- and post-exposure samples. Further measurements were also performed on samples of isolated isidia, isidia-deprived thalli, and thalli without the upper cortex. Ultrastructural alterations and subcellular Hg localization were investigated by Scanning Electron Microscopy and Transmission Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy applied to unexposed, field-exposed, and artificially Hg-enriched thalli obtained from HI thalli immersed in 1, 10, and 500 μM HgCl_2 solutions for 24 hours.

Results

Significant Hg accumulation was observed at the chlor-alkali plant and the mercury mining site, but not near the waste incinerator. At both these polluted sites, Hg content increased with exposure duration. HI thalli consistently accumulated more Hg than PI thalli, and isolated isidia exhibited the highest Hg content among all sample types. This pattern was consistent across all exposed samples and was also confirmed in the pre-exposure samples. These findings clearly indicate that isidia density influences Hg bioaccumulation. At the ultrastructural level, algal cells from Hg-rich thalli displayed consistent alterations, including an increased number and size of granule-shaped deposits within chloroplasts.

Conclusion

This study provides a comprehensive framework for understanding Hg accumulation in *P. furfuracea*. It confirms that this lichen is an excellent biomonitor of Hg pollution, and highlights the importance of a specific morphological character, previously overlooked, as well as the particular role of the photobiont

cells: Isidia not only increase the exposed surface area but also become preferential sites of Hg accumulation because they are very rich in photobiont cells. Biomonitoring guidelines should take these aspects into account, suggesting the use of samples uniform in isidia density.

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Importance of chemical speciation in understanding the cycling of mercury in the environment

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Mercury (Hg) is a persistent, toxic, and bioaccumulative pollutant that exists in multiple physical states and chemical forms depending on environmental conditions. The atmosphere is the primary pathway for mercury transport from emission sources to various environmental compartments. The three most common atmospheric mercury species are gaseous elemental mercury (GEM, Hg^0), gaseous oxidized mercury (GOM), also referred to as reactive gaseous mercury (RGM), and particle-bound mercury (Hg_p , total particulate mercury [TPM]). In this presentation, analytical techniques for the identification and quantification of atmospheric mercury species will be briefly reviewed, and associated challenges will be discussed. Applications of these techniques have led to the discovery of springtime polar atmospheric mercury transformation and deposition events, which reveal an important transport pathway of mercury to polar regions and help explain historical trends in mercury concentrations in the hard tissues of various Arctic biota.

Flash Talk 2, Environments, Agriculture & Veterinary

Elevated Temperature Potentiates Cadmium Toxicity during In Vitro Maturation of Oocytes and Early Embryonic Development in the ovine model

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Background Climate change and environmental pollution are increasingly recognized as major threats to reproductive efficiency in both humans and animals. Cadmium (Cd), a widespread environmental contaminant, is one of the most toxic heavy metals and has been shown to impair oocyte quality, oxidative balance, and embryo development even at low concentrations. Heat stress (HS), resulting from rising global temperatures, also negatively affects oocyte maturation and developmental competence by inducing oxidative stress and apoptosis. Although the individual effects of Cd and HS on female gametes have been widely investigated, little is known about their combined impact. This study aimed to determine whether elevated temperature can enhance Cd-induced toxicity during in vitro maturation (IVM) of ovine oocytes and subsequent embryonic development.

Materials and Methods Cumulus–oocyte complexes (COCs) were recovered from ovaries of prepubertal Sarda lambs collected at local abattoirs and matured in vitro for 24 h under four experimental conditions: control (38.5°C), Cd exposure (100 nM CdCl₂ at 38.5°C), heat stress (41.5°C), and combined heat stress plus Cd exposure (HS-Cd). After IVM, cumulus cell (CC) expansion and apoptosis were evaluated by morphological assessment and TUNEL assay, respectively. Oocyte nuclear maturation was assessed by Hoechst staining, while early apoptosis was analysed by Annexin-V assay. Intracellular reactive oxygen species (ROS) and glutathione (GSH) levels were measured by fluorescence staining. Expression of stress- and apoptosis-related genes in CCs was analysed by RT-qPCR. Mature oocytes underwent parthenogenetic activation followed by in vitro culture to evaluate cleavage and blastocyst development rates.

Results Heat stress significantly increased the toxic effects of Cd during oocyte maturation. The HS-Cd group showed the lowest percentage of fully expanded CCs (11.4%) compared to the CTR (53.5%), Cd (44.4%), and HS (22.3%) groups. Nuclear maturation rates significantly decreased in HS (79.8%) and HS-Cd (71.4%) oocytes compared with controls (91.2%). Following parthenogenetic activation, cleavage rates were markedly reduced in the HS-Cd group (24.4%) compared with the CTR (65.3%), Cd (47.2%), and HS (42.5%) groups, and blastocyst development was also significantly impaired. Combined exposure significantly increased Annexin-V-positive oocytes and intracellular ROS levels, indicating enhanced oxidative stress and apoptosis. In contrast, Cd alone at physiological temperature produced limited effects on embryo development. Gene expression analysis revealed a significant reduction in CIRBP expression under heat stress conditions, whereas BCL2 expression increased in Cd-exposed groups and

persisted during combined exposure. HSPA1A expression was significantly upregulated only under heat stress conditions.

Conclusion The present study demonstrates that elevated temperature potentiates Cd-induced reproductive toxicity during ovine oocyte IVM. The combination of HS and Cd exposure severely compromised cumulus cell function, oocyte maturation, oxidative balance, and embryonic developmental competence compared with either stressor alone. These findings suggest that global warming may exacerbate the detrimental reproductive effects of environmental contaminants, representing an additional risk factor for female fertility in livestock species.

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Comparative Analysis of Cadmium Profiles in Liver, Kidney, and Muscle of Bovines, Equines, and Cervids

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Background

Understanding interspecies physiological variability is crucial in veterinary diagnostics, food safety, wildlife management and comparative biology. Organs such as liver and kidney have well-documented species-specific functions: the equine liver exhibits distinctive metabolic and enzymatic responsiveness to stress and hepatopathies, while cervid kidneys display notable anatomical and functional diversity relative to other ungulates. Despite this, quantitative multivariate comparisons across bovines, equines and cervids remain limited. This study reports an analytical comparison of liver, kidney and muscle cadmium concentration in these species by Principal Component Analysis (PCA).

Materials and Methods

Cadmium measurements were performed on liver, kidney, and muscle samples from bovines, equines, and cervids in northwestern Italy (N=30 per group), from both official surveillance and research-based monitoring. Samples were weighted and subjected to acid mineralization using a microwave digestion system (Ultra WAVE, Milestone), based on Single Reaction Chamber (SRC) technology. After digestion, the solutions were diluted to appropriate volumes, spiked with internal standard (Rh), and analysed by inductively coupled plasma mass spectrometry (ICP-MS Agilent 7800). Limit of quantitation (LOQ) was 0,020 mg/kg. Data were standardized and subjected to PCA. Species-specific clusters were visualized using 95% confidence ellipses based on covariance matrices. Loadings were interpreted to determine organ contributions to interspecies separation

Results

Descriptive statistics is shown in Table 1 and PCA in Figure 1.

The PCA explained 89.7% of total variance (PC1 = 57.1%; PC2 = 32.6%), with liver and kidney showing the largest contributions to PC1 (loadings 0.692 and 0.699), while muscle largely defined PC2 (loading 0.982). A clear and robust separation of equine samples was observed, driven by markedly higher liver and kidney values. This is consistent with published evidence showing that the equine liver exhibits pronounced metabolic sensitivity and distinct biochemical responses under metabolic stress or altered hepatobiliary function. Equine samples formed a distinct cluster, consistent with known species-specific hepato-physiology and heightened metabolic sensitivity

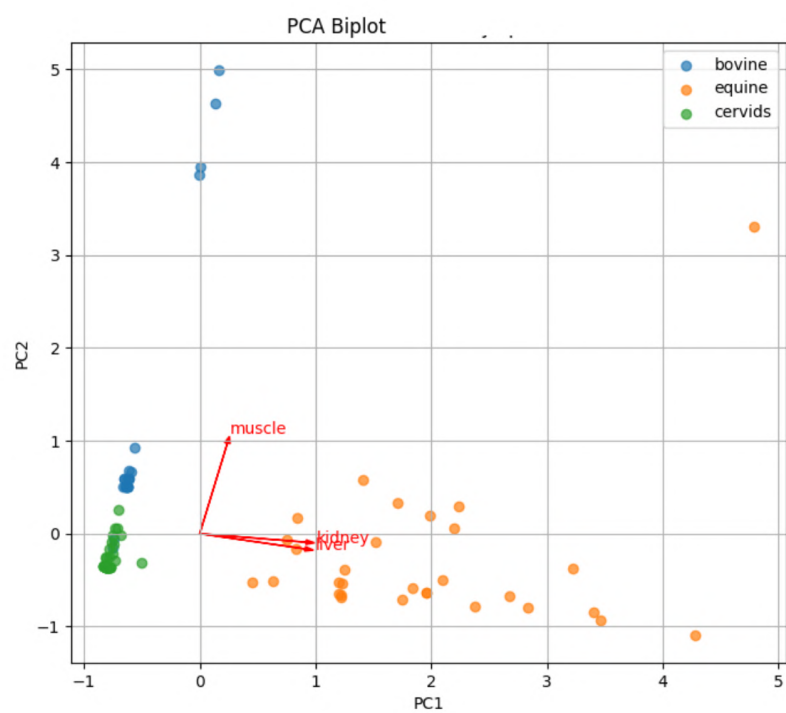
reported in equine liver literature. Cervids displayed broad variability, particularly in renal-associated values, aligning with comparative renal physiology studies showing structural and functional heterogeneity among cervid kidneys. Bovines clustered tightly, indicating a more homogeneous organ profile. Muscle contributed minimally to between-species separation.

Conclusion

PCA demonstrates that liver and kidney profiles are robust discriminators of species identity, while muscle offers limited discriminatory power. This multivariate study confirms strong species-specific organ signatures among ungulates. Equines display a distinct hepatic and renal profile, cervids show marked physiological heterogeneity, and bovines remain comparatively homogeneous. These findings highlight liver and kidney as reliable interspecies biomarkers and support their integration into comparative veterinary physiology and diagnostic frameworks. Figure 1 PCA biplot of Cd concentrations in muscle, liver, and kidney tissues in bovine, equine, and cervid specie

Table 1. Cadmium concentration in animal tissues (mg/kg)

Tissue	Species	Mean	Median	Min	Max
LIVER	bovine	0,14	0,19	0,033	0,21
LIVER	cervids	0,14	0,076	0,020	2,0
LIVER	equine	10	11	7,0	14
KIDNEY	bovine	0,60	0,86	0,03	1,0
KIDNEY	cervids	0,72	0,64	0,043	2,2
KIDNEY	equine	53	45	10	140
MUSCLE	bovine	0,18	0,12	0,010	0,63
MUSCLE	cervids	0,017	0,010	0,010	0,081
MUSCLE	equine	0,078	0,046	0,022	0,53



Differential Association of Trace Elements with Homocysteine and Oxidative Stress Markers in Freshwater Fish in Situ

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Anthropogenic activities in environmental contamination of aquatic ecosystems can cause the accumulation of pollutants in fish tissues and induce oxidative stress. Stress markers such as malondialdehyde and protein carbonyls are widely used in ecotoxicological monitoring. Homocysteine is closely associated with oxidative stress and can be increased by exposure to potentially toxic metals. We evaluated how multiple selected trace elements are differentially associated with HCY and oxidative stress markers under in situ conditions in freshwater fish. Blood was collected from grass carp (*Ctenopharyngodon idella*) captured in spring, summer and autumn at a university (SUA in Nitra) experimental fishpond. Concentrations of Al, Ba, Be, Co, Cu, Fe, Ni, Sr and Zn, HCY, reactive oxygen species (ROS), malondialdehyde (MDA) and protein carbonyls (PC), were quantified using ICP-OES, biochemistry analyser and spectro-fluoro luminometer. To assess relationships among biomarkers, metal levels and fish morphometrics across seasons, we applied non-parametric statistics (Kruskal–Wallis test with Dunn’s post hoc test), Spearman correlation analysis, principal component analysis (PCA) and generalized linear models (GLM). Several elements showed pronounced seasonal variability, with higher Co, Fe, Zn in autumn and elevated Al, Ba, Ni, Sr in spring season. The concentrations of Cu and Be did not vary significantly among seasons. Oxidative stress markers were strongly and positively inter-correlated with each other and with HCY across seasons. After adjustment for standard length and body mass, the GLM matrix shows significant negative associations of ROS, PC, and MDA with Ni and MDA with Be, and significant positive association of HCY with Zn, while other metals exhibit no independent effects on these biomarkers. Linear regression indicated that HCY increased with rising Al, whereas ROS decreased with Fe, MDA decreased with Ni, and PC was inversely related to Cu. The PCA biplot shows that the first principal component (PC1) separates samples mainly based on metal concentrations such as Al, Ba and Be, while the second component (PC2) reflects the degree of oxidative stress defined by ROS and MDA parameters. The plot also shows partial seasonal clustering, where spring samples show a higher affinity for metal accumulation, while summer samples are characterized by a different biomarker profile. Our in situ data demonstrate element-specific and season-dependent linkages between trace elements, HCY and oxidative stress in freshwater fish, highlighting Zn, Al, Cu, Ni, Be, Ba, Co and Sr as sensitive components of a multi-biomarker framework for field ecotoxicological monitoring. This study was supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic under project VEGA 1/0571/23 and by the Slovak Research and Development Agency under contract APVV-21-0168.

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Heavy Metals in Blue Crab (*Callinectes sapidus*) from Sardinia: Preliminary Food Safety Assessment and Regulatory Considerations

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BACKGROUND

The blue crab (*Callinectes sapidus*) is an invasive species that has recently expanded throughout several Mediterranean coastal ecosystems, including Sardinia. Although the species represents a growing ecological concern, its commercial exploitation for human consumption may also provide an opportunity for fishery valorisation. In this context, the evaluation of chemical contaminants in edible tissues is essential to assess food safety and compliance with European regulations. Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) are of particular interest because of their persistence, bioaccumulation potential and toxicological relevance. This study aimed to evaluate the concentrations of heavy metals in blue crab samples collected in Sardinia and to assess their compliance with the maximum levels established by Regulation (EU) No. 2023/915.

METHODS

A total of 58 blue crab samples (*Callinectes sapidus*) were analysed for heavy metal determination. Metal concentrations were determined by Inductively Coupled Plasma Tandem Mass Spectrometry (ICP-MS/MS; Agilent 8800 Triple Quad), following accredited analytical procedures based on US EPA 3052 for microwave-assisted acid digestion and US EPA 6020B for instrumental analysis. The analytical method included quality assurance measures through the use of certified reference materials (Mussel Tissue SRM 2976 and Fish Muscle ERM 422), process blanks and recovery testing. Limits of detection (LOD) and quantification (LOQ) were calculated according to Commission Regulation (EU) No. 333/2007. All results were expressed on a wet weight basis. In accordance with Commission Regulation (EU) No. 420/2011, which specifies that the maximum levels for metals in crabs apply exclusively to appendages, all analyses were performed only on crab appendices.

RESULTS

No food safety concerns associated with heavy metal contamination emerged from the analysed samples. Concentrations of Pb, Cd and Hg were below the maximum levels established by Regulation (EU) No. 2023/915 in all examined specimens. Table 1 summarises the descriptive statistics for cadmium, mercury, lead and nickel concentrations determined in blue crab appendages.

Table 1. Descriptive statistics of selected trace elements in blue crab appendages (µg/kg wet weight).
Element Mean ± SD Median Min Max Cadmium (Cd) 2.47 ± 4.82 1.17 0.06 28.35 Mercury (Hg) 40.73 ± 18.02 40.93 9.94 106.22 Lead (Pb) 13.23 ± 17.52 7.36 1.90 119.09 Nickel (Ni) 51.98 ± 36.55 40.97 15.46 188.89

Seasonal variations were observed for some of the analysed elements. Cadmium concentrations showed the highest mean values during summer months, particularly in July, while mercury concentrations tended to increase during autumn months, with higher mean values recorded in October and November. Lead concentrations remained generally low throughout the sampling period, although isolated higher values were detected in May. Nickel showed greater variability, with the highest concentrations recorded in late winter and autumn samples.

Table 2. Seasonal trends of selected trace elements in blue crab appendages. Element Main Seasonal Trend Observation Cadmium (Cd) Summer increase Highest mean concentrations observed in July

Mercury (Hg) Autumn increase Higher average values detected in October–November Lead (Pb) Stable with isolated peaks Generally low concentrations with sporadic higher values Nickel (Ni) Variable seasonal pattern Greater variability observed in winter and autumn samples

The analytical method showed satisfactory performance characteristics, including limits of quantification below 10 µg/kg, recoveries higher than 95%, and measurement uncertainty generally below 20% for toxicologically relevant elements. Although no exceedances of regulatory thresholds were observed, the determination of additional trace elements provided preliminary information useful for future comparative assessments. However, currently no specific regulatory maximum levels are available for several of these elements in blue crab tissues.

CONCLUSIONS The results obtained in this preliminary survey indicate that the analysed blue crab samples did not present critical issues related to heavy metal contamination and were compliant with current European food safety regulations for Pb, Cd and Hg. Considering the increasing commercial interest in *C. sapidus* in Mediterranean countries, these findings support the potential use of this species for human consumption under monitored conditions. Nevertheless, further investigations would be valuable to compare the concentrations of additional trace elements with data reported in the scientific literature for other crab species and marine crustaceans.

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Analysis of Mercury Concentration in Selected Types of Loose-Leaf Teas

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Background Tea is among the most popular non-alcoholic beverages worldwide and in Poland.

With growing interest in natural medicine, increasing numbers of people are turning to tea leaves.

Despite extensive scientific evidence for the health-promoting effects of infusions, their consumption carries a risk of adverse effects. Rising environmental pollution contributes to the accumulation of toxic compounds, such as mercury, in plant leaves. Drinking large quantities of tea contaminated with heavy metals may pose a threat to health, and even to life.

The aim of the study was to determine the concentration of mercury (Hg) in various types of loose-leaf teas available on the Polish market. The study sought to establish whether the Hg content in tea leaves exceeds permissible European limits and whether it poses a risk to consumer health, as well as to identify the type of tea with the highest and lowest Hg content.

Materials and methods The study material comprised 85 samples of different loose-leaf teas, divided into six types (green, black, red, blue-oolong, white, yellow). Samples were analysed using atomic absorption spectrometry (AAS) with the amalgamation technique on an AMA 254 analyser. This approach determines total Hg content regardless of its chemical form, enabled by pyrolytic mineralisation within the spectrometer.

Results Mercury was detected in all examined loose-leaf teas. The mean Hg concentration across all samples was 5.97 µg/kg, and the median was similar - 5.05 µg/kg. The minimum Hg content was 1.96 µg/kg and the maximum 24.29 µg/kg. Statistically significant differences in Hg content were found between tea types ($p < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). The highest Hg content was recorded in oolong teas (10.97 µg/kg), and the lowest in black teas (4.12 µg/kg).

Conclusions Hg concentrations in most of the tea samples tested were within the permissible limits specified in Commission Regulation (EU) 2018/73 (0.02 mg/kg). Only one sample (oolong tea) had a Hg content slightly exceeding the limit. The order of teas by increasing Hg content was: black, yellow, white, red, green, oolong. The study indicates that loose-leaf teas do not pose a risk to consumer health with respect to Hg content. It is recommended that future research be expanded to include teas originating from different regions of the world. Given ongoing environmental contamination with heavy metals, Hg levels in plant-based products should be continuously monitored to ensure consumer safety.

Influence of drip irrigation on the mobility and accumulation of Arsenic (As), Cadmium (Cd), and Nickel (Ni) in a commercial hybrid rice variety

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Background

Traditional paddy rice cultivation through flooding is typically associated with high water consumption and specific dynamics regarding the mobility of potentially toxic elements (PTE) in soil, e.g. higher As mobility and reduced Cd availability. Transitioning from flooding to intermittent **methods**, such as drip irrigation, significantly lowers water demand, reduces greenhouse gas emissions, and minimizes the soil-to-kernel translocation of many PTEs. Also, the use of hybrid rice genotypes is becoming increasingly widespread to maximize nutrient uptake and support high yields. Within this context, the Micro4Life project evaluated drip irrigation as a sustainable alternative to improve crop resilience and limit the accumulation of PTE in rice.

Materials and Methods

The trial was conducted in 2024 in Zeddiani (OR, Sardinia, Italy) on a non-contaminated agricultural silty-clay soil. Two systems were compared: continuous flooding (CF; total water consumption ~14,721 m³/ha) and aerobic conditions using drip irrigation (DI) based on two evapotranspiration restoration levels (0.8 ET_o and 1.2 ET_o, with total water consumption between 5,025 and 7,738 m³/ha respectively). A commercial rice hybrid (RTH74 FullPage®) was used, sown in dry soil. Chemical analyses monitored the total amounts of As, Cd, and Ni in both the soil and the grain.

Results

As: In the flooded system, rice showed As concentrations of 0.294 mg/kg, likely exceeding the legal limits for human consumption (0.1-0.15 mg/kg of iAs). Conversely, in the aerobic systems (0.8 and 1.2 levels), As concentration was always below the limit of quantification, confirming that an oxidizing environment drastically limits the bioavailability of this metalloid. Cd: Although the Cd concentration measured for rice samples obtained for each irrigation method remained always well within EU limits (0.15 mg/kg), no statistically significant difference was found among the average amounts measured for each irrigation thesis.

Ni: The rice analyses highlighted critical issues for Ni, especially in the aerobic system. While the value in the flooded system (2.2 mg/kg) exceeded by ~45% the limit of EU Regulation 2024/1987 (1.5 mg/kg), levels in aerobic conditions rose to 4.5-5.6 mg/kg, significantly exceeding (by ~300-

400%) safety thresholds. All reported data for Ni are significantly higher than those obtained in previous studies using the same soil-water system and several dozen non-hybrid genotypes (e.g., 0.03–0.18 mg/kg for flooded irrigation and 0.17–1.2 mg/kg for aerobic irrigation).

Conclusion

Shifting the cultivation of the RTH74 hybrid rice genotype from CF to DI allowed for water savings of over 50% while producing higher grain yields (~11.5 vs 9.6 t/ha in DI and CF respectively; $P < 0.05$). Although this change caused the minimization of the As bioaccumulation in kernels, and no statistically meaningful variation of the Cd level, it exacerbated the accumulation of PTE, such as Ni, compared to previous literature findings. These results underscore the need for integrated management strategies that balance high-yielding hybrid genotypes, water conservation and food safety, considering the varying mobility of metals within the soil profile.



Role of Humic Acids in Shaping Trace Element Content in Maize Cultivated on Iron Supplemented Soil

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Background

The effects of soil contamination with trace elements often appear with a time delay, leading to ecological disruption. One of them is iron. This study aimed to evaluate the potential of *Zea mays* L. as a phytostabilising species in soils with high iron concentrations, focusing on the role of humic acids (HAs) in modifying metal availability in the soil environment. The accumulation of trace elements in the above-ground parts of plants was analysed, treating their content as an indicator of the physiological response of plants to metal stress.

Materials and Methods

The study was conducted as a pot experiment under controlled conditions with two experimental factors: soil contamination with iron at concentrations of 0, 250, 500 and 750 mg kg⁻¹ of soil and the addition of humic acids (HAs) at rates of 0, 0.3, 0.6 and 0.9 g kg⁻¹ of soil.

Results

In systems without HAs, the highest dose of Fe used caused profound disturbances in growth processes, resulting in a decrease in maize biomass yield by up to 93%. The application of humic acids had a clearly positive effect on plant growth, leading to an increase in biomass production by a maximum of 53%. This relationship was almost linear with the increase in the dose of HAs, which confirms their ability to modify the dynamics of iron uptake and stabilize its forms in the substrate. Similar trends were also observed for the dry matter content. The introduction of iron into the soil resulted in a significant three times increase in its accumulation in maize biomass. At the same time, a decrease in Cd content by 44% and an increase in Pb concentration by 75% were demonstrated. The addition of humic acids limited both the total Fe content in the biomass and the dynamics of its accumulation under contaminated conditions, which confirms their function as natural metal chelators. A similar effect of HAs was noted with respect to the remaining analysed microelements - their concentration in maize biomass was reduced in the presence of HAs, with the exception of Pb, for which no reduction was found.

Conclusion The obtained results confirm that humic acids may play a key role in reducing the toxicity of iron in the soil by stabilizing its chemical forms and modulating the processes of metal uptake by plants. At the same time, they show the potential to improve the growth and condition of plants under metal stress, which increases the effectiveness of maize as a phytostabilising species.

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Cadmium Distribution in Soils and Iron Oxyhydroxides: Implications for Environmental Mobility

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Background Environmental concerns regarding cadmium (Cd) are increasing, as it is considered one of the most ecotoxic metals due to its severe negative impacts on soil biological activity, plant metabolism, and the health of humans and animals. Our contribution focuses on the concentration of cadmium in soil as well as in iron oxyhydroxides, which influence its mobility in the environment.

Materials and Methods Available data on soils from across Slovakia were compared with cadmium concentrations in soils affected by mining activity. Nine anthropogenically impacted sites located in western, central, and eastern Slovakia were selected, including historical mining heaps and tailings ponds differing in geology, mineralization type, and pH conditions. In addition to soil samples, Fe oxyhydroxides were also collected at the study sites. To enable a more detailed interpretation of the data, the sample set was supplemented with synthetic ferrihydrite and goethite with adsorbed cadmium. After preparation, all solid samples were analyzed using an ARL QUANT'X EDXRF instrument in the laboratory of Slovak National Museum. Samples of Fe oxyhydroxides were also analyzed using a DXR3xi Raman Imaging Microscope (Thermo Fisher Scientific) equipped with a 785 nm laser over the 0–3400 cm⁻¹ spectral range.

Results Cadmium concentrations in the humus horizons of Slovak soils range from 0.1 to 9.2 mg·kg⁻¹, with a characteristic range of 0.2–0.4 mg·kg⁻¹ and a median Cd concentration of 0.3 mg·kg⁻¹.

High degree of direct correlation was observed in Fluvisols between Cd and Zn ($r = 0.9$), while a high degree of correlation between Cd and Pb was identified in Fluvisols and Leptosols ($r = 0.87$) and in Pararendzinas ($r = 0.71$) (Čurlík and Šefčík, 1999). Cadmium concentrations in soils from the investigated mining areas reached up to 1.9 mg·kg⁻¹, while concentrations in the associated Fe oxyhydroxide samples reached as high as 33.8 mg·kg⁻¹, with an average value of 8.30 mg·kg⁻¹. The observed affinity of cadmium for Fe oxyhydroxides was further investigated using synthetically prepared samples. The potential application of this sorption process for remediation purposes was evaluated through a simple batch experiment (with positive results).

Conclusion The spatial distribution and variability of cadmium concentrations in the humus horizon indicate an enrichment of Cd in the surface soil horizons. This enrichment is highly uneven and is mainly associated with forest soils of high mountain regions. On the other hand, elevated Cd concentrations are also characteristic of certain ore-bearing zones and areas with a history of ore processing. A positive correlation between Fe and Cd concentrations in soils is commonly associated with the adsorption and accumulation of Cd on Fe oxyhydroxides, which represent important geochemical carriers controlling Cd mobility and bioavailability in soils.

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Legacy Cadmium in Abandoned Mining Waste Deposition Areas

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Background Cadmium (Cd) is a highly toxic, non-essential trace element widely present in the environment from natural and anthropogenic sources. Its persistence, mobility, and bioaccumulation make it a priority emerging pollutant, especially in aquatic systems. Cd is also an endocrine disruptor capable of interfering with hormonal regulation at low concentrations, posing risks to ecosystems and human health. Environmental risk assessment is challenged by complex matrices, mixture effects, and episodic mobilization events. Abandoned mining areas are long-term sources of potentially toxic elements (PTE). In Portugal, the legacy of coal mining in the Douro Coalfield (18th century–1990s) is materialized today by the existence of dozens of waste piles, notably São Pedro da Cova and Fojo (Peirão area), both affected by self-burning after wildfires, intensifying contamination impact. The W-Sn Regoufe mine, located in Central Portugal and closed since the 1970s, was intensively exploited for tungsten concentrates, producing mine wastes that are still exposed to weathering. This study integrates data from 2017–2022 across three abandoned mining areas in northern and central Portugal to assess contamination and environmental risks in post-mining landscapes.

Materials and Methods Sampling sites were selected to minimize non-mining influences. Water samples were collected following ISO 5667-3:2018 and analyzed for pH, electrical conductivity, major ions, and trace metals. Soil leachates were obtained using the USGS Field Leach Test (USGS-FLT) to simulate rainfall infiltration and evaluate PTE mobility (Cd,Cr,Ni,Cu,As,Pb). At São Pedro da Cova, groundwater from two mine galleries (G1,G2) and upstream(SS-U) and downstream surface water(SS-D) were sampled over nine campaigns (2017–2019). At Fojo, 29 soil samples were collected across different hydrogeological zones —Uphill Soil(US), Unburned Coal Waste(UCW), Burned Coal Waste(BCW), Mixed Burned Waste(MBW), and Downhill Soil(DS)—together with interflow water during heavy rainfall events (March and November 2022). At Regoufe, three water points were sampled in February 2022: groundwater from a main drainage gallery(P1), surface water downstream(P2), and groundwater from an isolated gallery(P3).

Results Clear geochemical contrasts were observed. Fojo exhibited the most dynamic conditions due to self-burning. Coal waste zones showed high susceptibility to contaminant leaching, while uphill soils had lower mineralization and PTE concentrations. Interflow waters after rainfall were highly acidic, sulfate-rich, and showed the highest PTE mobilization (notably Cd and Ni, with 16 and 626 µg/L mean values, respectively), exceeding soil leachates by over an order of magnitude and surpassing Directive 2008/105/EC limits.¹ At São Pedro da Cova, groundwater was relatively stable and near neutral, with high EC, SO₄, Al, Fe, Mn, As, and Ni, but Cd was low or undetectable. Downstream waters showed partial dilution but retained mine drainage signatures.² At Regoufe, groundwater (P1) had significant As and Cd concentrations, contaminating surface water and confirming arsenopyrite-rich wastes as persistent PTE sources. Overall, abandoned mine wastes significantly contribute to water contamination.³

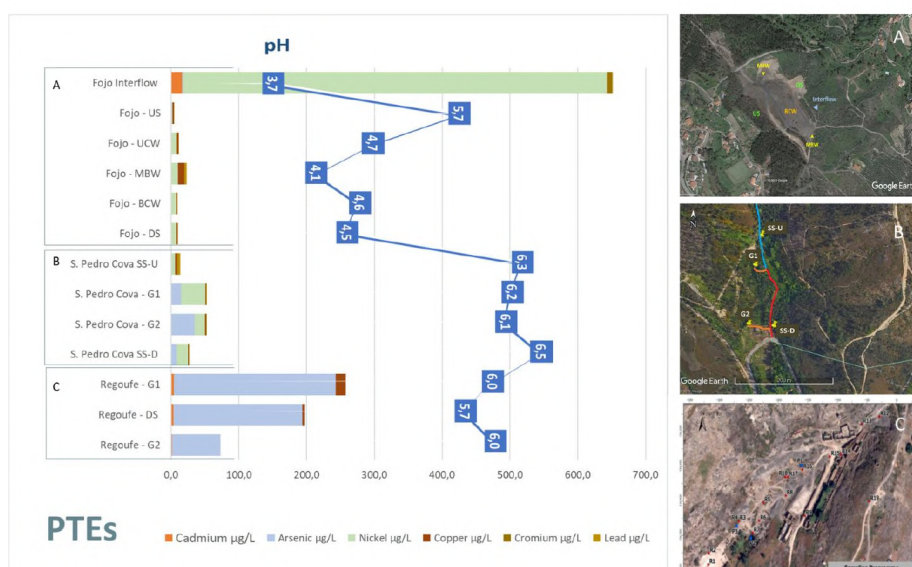
Conclusions Cd behavior in post-mining environments is controlled by site-specific geochemical and hydrological processes. Fojo is a critical case, where self-burning enhances Cd mobility through mineral transformations, acidic conditions, and rainfall-driven transport. São Pedro da Cova shows minimal Cd despite broader contamination, while Regoufe presents persistent but lower-intensity Cd release with potential long-term risks. These findings support site-specific remediation strategies, particularly in

thermally altered systems like Fojo, combining conventional treatments with sustainable approaches (e.g., phytoremediation and nature-based solutions). Integrated hydrogeological and hydropedological approaches are essential to manage contamination and protect ecosystems and human health.

Figure 1. Mean contents of PTEs and pH values of samples collected at Fojo (A), S. Pedro da Cova (B) and Regoufe (C) mine areas.

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Cadmium accumulation in plants and fungi growing on historically contaminated mining sites

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Background

Historical mining activities in Slovakia (Central Europe) have resulted in extensive environmental contamination by potentially toxic elements, including cadmium (Cd), originating from abandoned mine galleries, heaps, and tailings. Cadmium is highly toxic, bioaccumulative and poses serious risks to ecosystems and human health. Plants and fungi inhabiting mining-impacted areas may accumulate Cd to varying degrees, making them suitable indicators of contamination and potential agents for biological remediation. This study aimed to evaluate Cd accumulation and distribution in selected plant and fungal species from contaminated mining environments in Slovakia and to identify taxa with potential applications in biomonitoring and phytoremediation.

Materials and Methods

Nine anthropogenically impacted sites located in western, central, and eastern Slovakia were selected: Bratislava, Vrakuňa (BA); Čučma (CU); Dúbrava (DU); Kuchyňa (KU); Medzibrod (MD); Mlynky (ML); Pezinok (PK), Rákoš (RA); Špania Dolina (SD), including historical mining heaps and tailings ponds differing in geology, mineralization type, and pH conditions. Plant and fungal samples were collected from established study plots, with emphasis on dominant species and traditionally used medicinal plants. Aboveground plant parts and fungal fruiting bodies were dried, homogenized, and analyzed for potentially toxic elements using ICP-MS and CP-OES methods at Bureau Veritas Commodities Canada Ltd. Species composition and Cd concentrations were subsequently compared among taxa, families, and localities.

Results

Cadmium concentrations varied considerably among species and localities. Most plant species exhibited low Cd concentrations ($<0.05 \text{ mg}\cdot\text{kg}^{-1}$), whereas a smaller number accumulated substantially higher levels, reaching up to approximately $0.85 \text{ mg}\cdot\text{kg}^{-1}$. The strongest accumulation was observed mainly in representatives of the Brassicaceae and Plantaginaceae families, while Ranunculaceae and Urticaceae showed consistently low Cd concentrations. Species-specific variability appeared to be more pronounced than locality-related differences. Fungal species demonstrated significantly higher Cd accumulation compared to plants. The highest concentrations were detected in representatives of the genus *Agaricus*, particularly *A. bohusii* ($40.93 \text{ mg}\cdot\text{kg}^{-1}$), *A.*

arvensis (38.87 mg·kg⁻¹), *A. bitorquis* (29.91 mg·kg⁻¹), and *A. sylvicola* (24.37 mg·kg⁻¹). Elevated Cd levels were also found in *Amanita muscaria*, *Tricholoma saponaceum*, and *Hydnellum conicum*. In contrast, *Boletus edulis*, *Macrolepiota procera*, and *Leccinum scabrum* accumulated comparatively low Cd concentrations. The most contaminated localities were BA, PK, and DU, although species identity exerted a stronger influence on Cd accumulation than locality itself.

Conclusion

The study confirmed that historical mining areas in Slovakia remain important sources of cadmium contamination. Cadmium accumulation in both plants and fungi was found to be primarily species-dependent, although local environmental conditions may enhance accumulation processes. Several plant and fungal taxa exhibited pronounced Cd accumulation potential and may therefore serve as suitable bioindicators or candidates for phytoremediation and bioremediation strategies. In particular, species of the genus *Agaricus* showed exceptional bioaccumulation capacity, highlighting potential ecological and public health risks associated with the consumption of wild mushrooms from mining-impacted environments.

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D-3-Hydroxybutyrate in Fish: Seasonal Dynamics and Associations with Selected Trace Elements in Situ

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D-3-hydroxybutyrate (D3-HB) is an important intermediate of energy metabolism and a potential biomarker of stress in fish. Its importance lies in its ability to indicate responds sensitively to physiological and environmental stress and may indicate disturbances in mitochondrial processes and oxidative metabolism. Therefore, the concentration of D3 HB reflects the organism's ability to adapt to adverse environmental conditions. The aim of this study was to evaluate the extent to which seasonal changes in the concentrations of selected metals and physicochemical parameters of water influence D3-HB levels in the blood of freshwater fish. In three seasons (spring, summer, autumn), we monitored D3-HB concentrations in blood and, in parallel, concentrations of Co, Cu, Ni, Sr and Zn in water together with temperature, dissolved oxygen, pH and conductivity. Blood was collected from grass carp (*Ctenopharyngodon idella*) captured in spring, summer and autumn at a university (SUA in Nitra) experimental fishpond. Concentrations of selected trace elements were quantified using ICP-OES, and D-3-hydroxybutyrate was analysed using a RX Monaco (Randox) biochemistry analyser. Overall correlation analysis showed only weak monotonic relationships between D3 HB and individual metals or physical parameters, with RS values ranging approximately from -0.2 to 0.3. These results indicate the absence of a simple linear effect of individual factors. However, seasonal correlation matrices revealed more pronounced relationships during specific periods of the year. In summer, D3 HB showed stronger associations with Cu, Zn, and partly Ni. In spring, correlations were observed mainly with Co and dissolved oxygen. In autumn, negative relationships were identified between D3 HB and both conductivity and oxygen. Seasonal environmental dynamics had a major impact on the distribution of samples, as confirmed by principal component analysis (PCA), where the first two components explained a total of 52.6% of the data variability (PC1: 34%; PC2: 18.6%). The PCA biplot clearly separated autumn samples, which showed a strong positive association with dissolved oxygen, conductivity and cobalt and zinc levels. In contrast, spring and summer samples were defined by higher water temperature and increased nickel and strontium concentrations. Our results suggest that, in fish populations, D3-HB reflects the combined influence of seasonally changing environmental and contamination factors rather than the effect of a single metal, and its ecotoxicological interpretation therefore requires consideration of the multivariate environmental context. This study was supported by the Ministry of Education, Research, Development and Youth of the Slovak Republic under projects VEGA 1/0571/23, KEGA 010SPU-4/2026 and by the Slovak Research and Development Agency under contract APVV-21-0168.

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A Sustainable Strategy for Metal Remediation: Sequestration and Speciation of trans-Aconitic Acid with Divalent Cations

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Background trans-Aconitic acid (tAA) is a naturally occurring, biodegradable tricarboxylic acid identified as a sustainable and green alternative to persistent chelating agents such as EDTA for metal remediation and scale prevention.

Materials and Methods The acid–base behavior and chelating properties of tAA toward Ca^{2+} , Mg^{2+} , Cu^{2+} , Co^{2+} , and Zn^{2+} were investigated in aqueous KCl solutions at 298.15 K. An integrated experimental and computational approach combining potentiometry, UV–Vis spectroscopy, and $^1\text{H}/^{13}\text{C}$ NMR spectroscopy with Density Functional Theory (DFT) calculations was employed to evaluate thermodynamic stability and molecular geometries [1].

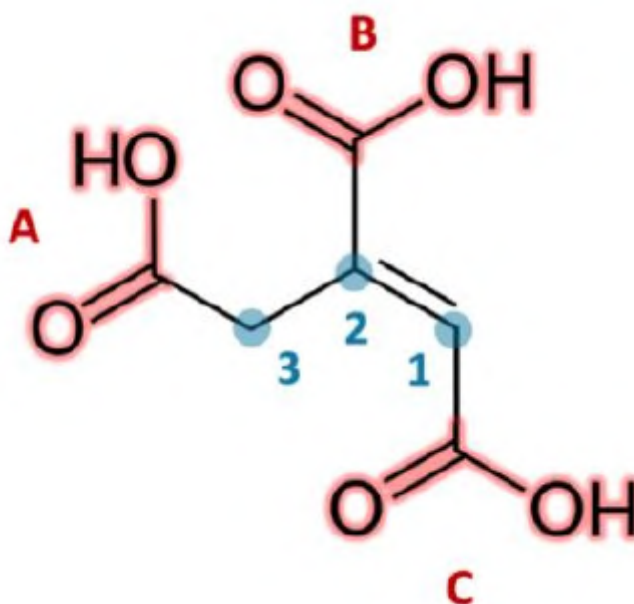
Results tAA formed several complex species, including MH_2L^+ , MHL^0 , and ML^- , displaying particularly high sequestration affinity toward Cu^{2+} and Mg^{2+} . Spectroscopic and computational analyses established a site-specific deprotonation sequence of $\text{C} \rightarrow \text{B} \rightarrow \text{A}$ and revealed distinct coordination modes for alkaline earth metals arising from differences in ionic radii [1].

Conclusion trans-Aconitic acid is a versatile and eco-friendly ligand whose moderate complex stability provides an effective balance between metal binding and reversibility. These findings support its application as an efficient and biodegradable agent for environmental and industrial water treatment [1].

Trans-Aconitic Acid (tAA) Structure

References

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Review of Heavy Metals Associated with Airborne Particulate Matter in Africa

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Airborne toxic metals contribute to respiratory and cardiovascular diseases. This paper synthesizes the studies reporting heavy metal concentrations in particulate matter (PM_{2.5}, PM₁₀, and total suspended particles) across African regions between 2010 and 2025.

Results show that research is concentrated in Morocco, Mali, Nigeria, Tanzania, Uganda, and South Africa, while Central Africa and the Horn of Africa remain largely unrepresented. Most studies applied acid digestion to the total and size-segregated particulate matter collected on filter papers, followed by quantification using ICP-MS or ICP-OES. Heavy metal concentrations range from 18 $\mu\text{g m}^{-3}$ in rural areas to 548 $\mu\text{g m}^{-3}$ in urban regions, but metal speciation in the particulate matter remains limited. Elemental profiles reflect both natural (crustal) and anthropogenic sources, including traffic, industry, biomass burning, and indoor fuel use.

Key research gaps include uneven geographic coverage, limited long-term datasets, and insufficient chemical characterization. Addressing these shortcomings will require coordinated multi-site monitoring networks, standardized chemical speciation protocols, and longitudinal studies that link exposure data with health outcomes. Establishing baseline concentrations of toxic metals in particulate matter—particularly in countries where no such data currently exist - is essential for improving exposure assessment and support evidence-based air quality policies across Africa.

Municipal Waste Burning – An Important Source of Atmospheric Cadmium

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Background

The environmental risks of municipal waste burning are present worldwide. Practices, however, are different. Especially in the developing regions of the world, it might be a common disposal practice while it is prohibited in certain countries. Depending on the composition of the waste burned, serious emissions of particulate matter (PM) have been experienced. Particles bind potentially toxic chemicals, including cadmium. The objective of the study was to provide information on the composition and potential ecotoxicity of PM emissions generated during controlled burning of domestic waste samples.

Materials and Methods

In the first experiment, controlled combustion of different waste types such as polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PU), oriented strand board (OSB) and rag (RAG) was performed in a domestic cast-iron residential stove (type: *Servant S114*, rated at: 5 kW). PM₁₀ samples were collected on quartz filters (Ø15cm) with Kalman high-volume aerosol sampler (flow rate 32m³h⁻¹). Aqueous extracts were prepared from the filters which were further used for analytical measurements and ecotoxicological testing. Test battery involved the *Vibrio fischeri* bioluminescence inhibition assay, *Daphnia magna* immobility test and the seedling emergence assay.

In the second experiment, mixed domestic waste was combusted under the conditions described above, processing of the filters was also completed similarly. In this experiment, phytotoxicity was assessed on common ornamental plants widely used in Hungarian gardens such as *Gaillardia aristata*, *Calendula officinalis*, *Alcea rosea annua*, *Ipomoea purpurea*, *Mirabilis jalapa* and *Cucurbita pepo*. In this case, the protocol of the No. 227 OECD Guideline for the Testing of Chemicals: Terrestrial Plant Test was followed, phytotoxicity was assessed based on growth inhibition, photosynthetic pigments and peroxidase enzyme levels.

Results

In the first experiment, analytical measurements showed that Cd content of the PVC emission sample was extremely high, 22.4 µg/L, resulting in high toxicity in the *Vibrio* and *Daphnia* assays. Mutagenic responses were also detected. In the second experiment, emission generated during the combustion of the mixed domestic waste was also characterised by high Cd content, 15.3 µg/L. It should be noted that the mixed waste also contained PVC. In the tested species, growth impairment and elevated levels of the antioxidant enzyme peroxidase were the typical symptoms.

Cadmium has been reported to produce reactive oxygen species (ROS) in the living body, triggering oxidative stress. Oxidative stress is a major driving factor for toxic responses.

Conclusion

With the increase of plastic products, their share in the domestic waste will most possibly also increase. Unfortunately, as burning of domestic waste cannot be avoided, emission of potentially toxic compounds, including heavy metals such as Cd, as well as persistent organic compounds poses a serious hazard to the environment. This hazard continues to threaten both humans and the non-human biota.

Raman spectroscopy of cadmium oxalate: Implication for bioremediation

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Background Among the secondary phases capable of immobilizing cadmium, metal oxalates are particularly significant because oxalate is both a powerful chelating ligand and a metabolic product of plants, fungi, lichens, and bacteria. Oxalic acid and oxalate are central to biomineralization processes, where they mediate the precipitation of stable metal-bearing phases, potentially reducing metal toxicity while contributing to long-term sequestration. Although cadmium oxalate has been recognized in synthetic and biological systems, its Raman spectroscopic characterization remains limited despite the increasing relevance of Raman methods in environmental mineralogy and biomineral discovery. This study presents the Raman spectroscopic characterization of cadmium oxalate dihydrate ($\text{CdC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) and evaluates its implications for identifying cadmium biominerals in natural and engineered remediation contexts.

Material and methods Cadmium oxalate dihydrate crystals were synthesized by reacting oxalic acid (3 g / 60 mL) with cadmium sulfate octahydrate (1 g; CAS 7790-84-3) under heated conditions (100 °C) to enhance reactant dissolution and crystal precipitation. The resulting white precipitate was dried and analyzed using a DXR3xi Raman Imaging Microscope (Thermo Fisher Scientific) equipped with a 532 nm laser over the 0–1800 cm^{-1} spectral range. Ten single crystals were measured to account for spectral variability, and spectra were baseline-corrected, normalized, and merged using OMNIC spectral processing tools to obtain a representative high-definition spectrum.

Results The Raman spectrum of cadmium oxalate dihydrate revealed characteristic bands at 71, 99vs, 141, 194, 238s, 512s, 528, 577, 857, 922m, 1448sh, 1476vs, 1602, 1649, and 1713 cm^{-1} . Consistent with previous vibrational assignments of other metal oxalates [1], the dominant 1476 cm^{-1} band could be attributed to asymmetric C=O stretching, while the 1448 cm^{-1} shoulder and 922 cm^{-1} region could correspond to symmetric C–O stretching coupled with O–C=O deformation. The strong 512 cm^{-1} band likely reflects Cd–O and C–C vibrational contributions, providing an important marker for cadmium coordination. This Raman spectrum reference for cadmium oxalate dihydrate may facilitate detection of cadmium-bearing biominerals in environmental samples, with direct relevance to bioremediation, as Cd immobilization through oxalate biomineralization has been documented in metal-tolerant plants such as *Gomphrena claussenii* [2], oxalogenic fungi including *Beauveria caledonica* [3], and potentially lichenized symbioses capable of mineral transformation [4]. By improving spectral recognition of cadmium oxalates, this work supports future studies in phytoremediation, mycoremediation [5], and biomonitoring, while also contributing to the expanding field of biomineral discovery.

Conclusion This spectroscopic investigation established a refined Raman reference for cadmium oxalate dihydrate that may facilitate detection of cadmium-bearing biominerals in environmental samples. Improved spectral recognition of cadmium oxalates supports future studies in phytoremediation, mycoremediation, biomonitoring, and biomineral discovery. Raman spectrum can expand the analytical toolkit for tracing cadmium stabilization in biologically influenced environments, where oxalate-mediated mineralization may represent an underrecognized detoxification pathway. Overall, this study demonstrates the value of integrating Raman spectroscopy with environmental and biological research to better characterize pathways that convert toxic cadmium into more stable, potentially less bioavailable mineral phases.

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Toxicology 2

Cadmium and cardio-metabolic risk: epidemiological evidence

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Background Cadmium is a toxic heavy metal. Main exposure source of exposure is tobacco smoke, but also outdoor air pollution and dietary intake from contaminated foods may be relevant, especially in non-smoking populations. Therefore, concerns about its chronic long-term exposure have been investigated. Cadmium is a known carcinogen, classified in Group I by International Agency for Research in Cancer. More recently, several studies indicated its potential detrimental role of non-carcinogenic chronic diseases, in particular it has been linked to elevated cardio-metabolic risk.

Methods We carried out an online literature search to assess epidemiological evidence about the relation between cadmium exposure and cardio-metabolic risk, including lipid profile, diabetes, hypertension, kidney and cardiovascular diseases using keywords and MeSH terms tailored at the identification of both experimental and non-experimental studies, assessing whenever possible dose-response relation.

Results and Conclusions: The lecture will present the most recent epidemiological findings showing available evidence about the increased risk of type 2 diabetes, hypertension, cardiovascular and kidney diseases associated with Cd exposure, focusing on both general populations and selected subgroups. Finally, evidence from trials aimed at decreasing cadmium concentrations and subsequently the risk of cardio-metabolic conditions will be also presented.

Early-Life Cadmium Exposure and Child Health and Development: Two Decades of Insights

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Background Current risk assessments of cadmium (Cd) are based on adverse health effects following long-term exposure in adults, while emerging studies indicate that exposure during sensitive periods of development may be of concern for child health and development.

Materials and Methods Studies on the health effects of early-life Cd exposure have mainly been conducted in two mother-child cohorts in two different geographical regions; the MINIMat cohort (Maternal and Infant Nutrition Interventions in Matlab) in rural Bangladesh (nested subcohort of about 1,500 mother-child pairs) and the NICE cohort (Nutritional impact on

Immunological maturation during Childhood in relation to the Environment) in northern Sweden (n=655 mother-child pairs). In MINIMat, the mothers' environmental exposures were assessed repeatedly during pregnancy and thereafter in the children, who were followed from birth to young adulthood. In NICE, mothers' environmental exposures were assessed in early third trimester, and their children were assessed on several occasions from birth to 6 years of age. Cd and other metals were measured in several biological media (blood, urine, hair, placenta, breast milk) along with various effect biomarkers. Clinical assessments included anthropometry, neurodevelopment, puberty, allergy, and lung function and questionnaires were used to collect detailed information on potential confounders.

Results In our studies, the diet has been the main source of Cd exposure. Maternal Cd levels often increased during pregnancy due to increased gastrointestinal absorption. Unlike many other toxic metals, Cd accumulated in the placenta, with low levels being transferred to the fetus. Similarly, excretion to breast milk was low. Still, gestational Cd exposure was associated with decreased fetal growth and size at birth, and girls appeared more susceptible than boys. Both gestational and childhood Cd exposure were associated with impaired child growth and cognitive abilities up to pre-pubertal age, as well as decreased lung function in mid-adolescence. Children's Cd exposure was also associated with later menarche. Underlying modes of action include hampered placental function, DNA methylation changes that linger from birth to prepubertal age, hormone interactions, and altered bone formation.

Conclusion Low-level dietary Cd exposure during pregnancy and early childhood was associated with several adverse health outcomes in childhood and adolescence. Hence, health risk assessment of early life Cd exposure with establishment of health-based guidance values are urgently needed.

Environmental Mixtures with Cadmium: A Multi-Contaminant Perspective

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Heavy metal pollution refers to the significant increase in environmental levels of metals due to human activities, an outcome that leads to ecological deterioration and poses risks to human health. The most investigated Cd mixtures are with metals such as mercury (Hg) and lead (Pb) or with other environmental contaminants such as different kinds of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), decabromodiphenyl ether (decaBDE), perfluorooctanoic acid (PFOA), and PFOA-related compounds, phthalates, pesticides, etc.

Chemical risk assessment is commonly conducted with isolated compounds without considering possible joint effects. The mixture effects are largely unknown. These effects can occur even when each compound is present at concentrations below the no-observed-adverse-effect-level (NOAEL). From a mechanistic point of view, complex effects elicited by a mixture of toxic substances may be linked not only to the molecular mechanisms of individual components but also to distinct molecular pathways that underlie mixture toxicity. The combinations may significantly enhance oxidative stress, reduce antioxidant defences, and alter biochemical pathways in various tissues, often leading to greater dysfunction than Cd alone.

The ability to accurately predict the adverse effects of chemicals in mixtures is restricted by major challenges, such as multi-species considerations and a lack of consideration of interactions. An integrated approach to risk assessment addresses situations of multichemical, multimedia, multiroute, and multispecies exposures. An improved understanding of the mixture toxicity can greatly improve risk estimates and reduce uncertainties.

Single-cell mass cytometry for immune profiling and trace-element detection: from nanomaterials to cadmium at the cellular level

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The immune system governs every aspect of our health. The quantification and tracing of cadmium presence on immune cells is a fundamental aspect to advance knowledge on the cadmium-related biological cascade of events. High-dimensional single-cell technologies provide a unique opportunity to capture this complexity, revealing cell-type-specific responses that would remain hidden in bulk assays. We proposed single-cell mass cytometry and imaging mass cytometry to detect and trace emerging 2D nanomaterials, including transition metal carbides/carbonitrides, known as MXenes. These approaches enabled the simultaneous assessment of immune-cell phenotypes, activation states, and material-associated elemental signals at single-cell resolution. We showed how single-cell mass cytometry enables detection of material-cell associations across a broad range of primary immune-cell subsets. More recently, we reported the immune modulation induced by selected MXenes and demonstrated their label-free detection. Moreover, I will present results on a wider panel of materials, including MoS₂, WS₂, and bismuthene. More recently, we have shown the detection of nanoplastics palladium doped on a wide variety of immune cells. Together, these data show that single-cell mass cytometry can serve not only as an immunophenotyping platform, but also as a powerful elemental-tracing technology. This is particularly relevant for trace elements and environmental contaminants such as cadmium, whose biological effects depend strongly on cellular uptake, distribution, and cell-type-specific susceptibility. By integrating metal isotope detection with high-dimensional immune profiling, mass cytometry has the potential to quantify cadmium-associated signals at the single-cell level while simultaneously identifying the affected immune-cell populations and their functional states.

Background and Materials and Methods

Single-cell mass cytometry is a high-dimensional analytical platform that combines flow cytometry with elemental mass spectrometry, enabling simultaneous immune profiling and detection of metal-associated signals in individual cells. This technology is particularly valuable for studying complex biological responses to nanomaterials and emerging pollutants because it preserves cellular heterogeneity and identifies which immune-cell subsets interact with specific elements. In our experiments, peripheral blood mononuclear cells were isolated from blood of healthy donors and exposed to multiple nanomaterials, nanoplastics, and cadmium-based barcoding tags. This approach allowed us to detect elemental signatures at the single-cell level while linking exposure to immune-cell identity and phenotype.

Results

Detection of nanomaterials, nanoplastics and cadmium-based tags will be shared with the audience. The following published results will be shared together with unpublished results.

Nanoplastics: Immune Impact, Detection, and Internalization after Human Blood Exposure by Single-Cell Mass Cytometry. Fusco L, et al. and Delogu LG.

Adv Mater. 2025 Mar;37(12):e2413413. doi: 10.1002/adma.202413413. Epub 2024 Oct 24.

Immune Profiling and Multiplexed Label-Free Detection of 2D MXenes by Mass Cytometry and High-Dimensional Imaging. Fusco L, et al. and Delogu LG. Adv Mater. 2022 Nov;34(45):e2205154. doi: 10.1002/adma.202205154. Epub 2022 Oct 7.

Conclusion

Our combined analytical strategy based on high-dimensional assays designed for single cell mass cytometry bridge nanotoxicology, immunology, and trace-element biology. It provides a framework to study how engineered nanomaterials and emerging pollutants interact with complex cellular systems, and how elements such as cadmium can be traced directly within heterogeneous immune-cell populations. Overall, our results support the use of single-cell mass cytometry as a high-content platform for mechanistic studies of trace-element toxicity.

Agriculture & Veterinary 1

In Vitro Assessment of Heavy Metal-Induced Toxicity on Mammalian Female Gametes

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Heavy metals represent one of the most hazardous classes of environmental contaminants affecting female reproductive health in both humans and animals. Among them, cadmium has attracted particular attention due to its widespread environmental distribution, long biological half-life, persistence, and marked bioaccumulation within reproductive tissues. Increasing evidence demonstrates that heavy metal exposure can impair ovarian function and compromise oocyte developmental competence by inducing oxidative stress, mitochondrial dysfunction, DNA damage, cytoskeletal alterations, and endocrine disruption. Since the ovarian follicular reserve is finite and non-renewable, female gametes are especially vulnerable to toxic insults, even at low exposure levels. In this context, in vitro reproductive models have become essential tools for investigating the mechanisms underlying heavy metal reproductive toxicity. In vitro maturation systems and cumulus– oocyte complex models allow the controlled assessment of toxicant exposure on oocyte maturation, fertilization, and early developmental competence. Moreover, these approaches are highly relevant within the framework of the 3Rs principle (Replacement, Reduction, and Refinement), as they reduce the need for experimental animal use by employing biological materials recovered from slaughterhouses or routine veterinary procedures. In particular, large animal models such as sheep provide important translational value for human reproductive biology due to the physiological similarities in folliculogenesis and oocyte maturation.

This lecture will provide an overview of the current advances in the in vitro assessment of heavy metal toxicity on female gametes, with particular focus on cadmium-induced reproductive damage. Experimental findings obtained in ovine models will be discussed, highlighting the detrimental effects of cadmium on oocyte meiotic maturation, fertilization ability, mitochondrial activity, reactive oxygen species accumulation, meiotic spindle organization, cortical granule distribution, and developmental competence. In addition, transcriptomic analyses of cumulus cells exposed to nanomolar cadmium concentrations revealed significant dysregulation of genes involved in steroidogenesis, oxidative stress response, cell signaling, cumulus expansion, and oocyte competence, identifying potential non-invasive biomarkers of oocyte quality.

The lecture will also address the protective role of antioxidant compounds, such as resveratrol, which has shown promising effects in counteracting cadmium-induced oxidative stress, preserving mitochondrial function, and improving oocyte quality and fertilization outcomes during in vitro maturation.

Finally, particular attention will be dedicated to the importance of companion animals, especially cats and dogs, as sentinel species for monitoring environmental contamination within a One Health perspective. Since small animals share the same environment and exposure routes as humans, the detection of heavy metal accumulation in their ovarian tissues may provide valuable information on environmental reproductive hazards and human exposure risks. Overall, in vitro reproductive toxicology

models combined with molecular approaches represent powerful strategies for understanding the impact of heavy metals on female fertility and for developing preventive and therapeutic interventions against environmental reproductive toxicity.

Mitigating cadmium in multi-contaminated mining soils: longterm field evidence for biochar-assisted phytostabilization

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Background

Soils contaminated with potentially toxic elements (PTEs) are a major environmental concern, especially in abandoned mining areas where long-term accumulation of arsenic (As), antimony (Sb), lead (Pb), zinc (Zn), and cadmium (Cd) poses severe ecological and human health risks. Among these contaminants, Cd is of special concern due to its high mobility in soil-plant systems, strong toxicity at low concentrations, and tendency to enter food chains. Assisted phytoremediation, which combines tolerant plant species with soil amendments, has emerged as a promising strategy. However, long-term field evidence on Cd dynamics in a multi-contaminated soil remains limited. This study aimed to assess the role of biochar in reducing Cd mobility and uptake, in a multi-contaminated mining soil, while evaluating vegetation establishment and soil functioning over 28 months.

Materials and Methods

A long-term field experiment was conducted at the abandoned Argentiera mining site (40° 44' 11"N, 8° 08' 52"E, Sardinia, Italy). The experimental design included four treatments: untreated control soil (CTRL), biochar-amended soil (BC, 3% w/w), mineral fertilization (NPK), and soil receiving both biochar and mineral fertilization (BC + NPK). Soil physicochemical and biochemical properties were analysed for all treatments. The mobility of Cd and other PTEs was assessed through sequential extraction procedures. Vegetation surveys quantified species composition and structure, on this basis *Lotus cytisoides* L. was selected as a model species for detailed analysis of biomass production and Cd uptake in roots and shoots.

Results

Biochar significantly altered Cd dynamics in soil, leading to a marked reduction in its mobility and bioavailability. In particular, labile Cd fractions decreased by approximately 40%, while the most stable residual fractions increased, indicating strong immobilization within the soil matrix. These changes were associated with increased soil pH, organic carbon, and cation exchange capacity. In plants, Cd uptake showed a clear treatment-dependent pattern: BC reduced Cd concentrations in both shoots and roots of *L. cytisoides*, whereas NPK fertilization increased root Cd uptake due to enhanced plant growth and nutrient-driven metabolic activity. Importantly, translocation factors for Cd were consistently below 1 in BC-amended soils, demonstrating strong retention of Cd in roots and limiting its movement to aerial parts. Mineralomass and stabilization indices confirmed that Cd was predominantly stabilized in the root compartment under biochar treatments, highlighting effective phytostabilization. In contrast, control and fertilized soils showed higher Cd mobility and greater risk of translocation.

Conclusion

The results demonstrate that biochar is particularly effective in controlling cadmium behaviour in a multi-contaminated mining soil, reducing its mobility, plant uptake, and potential environmental risk. The strong immobilization of Cd in the root zone, combined with limited translocation to shoots, confirms the

effectiveness of biochar-assisted phytostabilization. *L. cytisoides* proved to be a resilient species capable of surviving in highly contaminated conditions while contributing to Cd stabilization in the rhizosphere. Although some reductions in microbial activity were observed, the overall system benefits in terms of Cd risk mitigation and soil restoration were substantial. These findings highlight biochar-assisted phytoremediation as a promising long-term strategy for managing Cd-contaminated environments, particularly in degraded mining areas.

Concentrations of Biogenic and Risk Elements in Semen of Various Animals and Their Correlations

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Background Many recent studies have indicated an increasing prevalence of various abnormalities of reproductive system in males. Possible factors explaining this phenomenon include stress, lifestyle, and a variety of endocrine-altering chemicals in the environment, that can be linked to decreased male reproductive capacity, as indicated mainly by the results of experimental animal studies.

Materials and Methods Semen samples (bulls, rams, boars, stallions and foxes) were obtained, digested and analyzed by means of the atomic absorption spectrophotometer. Data were analyzed statistically with the Student's t-test and Scheffe's test using PC programs SAS and Excel.

Results The seminal copper concentration was significantly higher in ram (2.49 ± 0.18 mg/kg) and fox (2.16 ± 0.53 mg/kg) than that in bull (1.64 ± 0.21 mg/kg), boar (1.64 ± 0.28 mg/kg) and stallion (0.86 mg/kg). In boar a significantly higher seminal zinc concentration (171.74 ± 65.72 mg/kg) was found in comparison with stallion (86.20 ± 45.88 mg/kg), bull (83.15 ± 61.61 mg/kg), ram (60.46 ± 35.37 mg/kg) and fox (13.09 ± 5.22 mg/kg). The iron concentration in semen was significantly higher in ram [(40.32 ± 10.81) mg/kg], bull [(38.04 ± 22.07) mg/kg] and fox [(33.16 ± 24.36) mg/kg] than that in boar (16.14 ± 10.35 mg/kg) and stallion (12.68 mg/kg). The seminal cadmium concentration was relatively low ($0.05 \sim 0.12$ mg/kg) in all studied species. The seminal lead concentration was the highest in ram (0.35 ± 0.68 mg/kg), which was higher than in fox (0.08 ± 0.06 mg/kg), bull (0.06 ± 0.04 mg/kg), stallion (0.05 ± 0.05 mg/kg) and boar (0.02 ± 0.03 mg/kg). The level of seminal nickel was significantly higher in fox (0.35 ± 0.24 mg/kg) and ram (0.31 ± 0.19 mg/kg) in comparison with bull (0.12 ± 0.07 mg/kg) and boar (0.06 ± 0.08 mg/kg). The concentration of nickel in the semen of stallion was (0.20 ± 0.24 mg/kg). There was a high positive correlation between seminal iron and zinc in bull ($r=0.723$) and stallion ($r=0.723$), between cadmium and lead in ram ($r=0.976$) and boar ($r=0.973$) and between iron and cadmium ($r=0.783$) and iron and lead ($r=0.791$) in boar. A high negative correlation between seminal nickel and copper in ram ($r=-0.709$) and between seminal copper and lead in fox ($r=-0.854$) was found.

Conclusion There are significant differences in the concentrations of seminal elements in different animals. There is a high concentration of seminal copper in ram and fox, a high zinc level in boar, a high iron level in bull, ram and fox and a high nickel level in ram and fox.

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Nickel-Triggered Toxicity Disrupts Antioxidant Capacity and Cell Cycle Regulation in Fission Yeast Through Altered Tor and MAPK Signaling

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Background

Nickel (Ni) is an essential trace element; however, at elevated concentrations, it becomes toxic and may contribute to carcinogenesis. Anthropogenic activities are the primary source of Ni accumulation in soils and aquatic systems, increasing its entry into terrestrial ecosystems and the food chain, thereby posing risks to food safety and quality.

Materials and Methods

Ni toxicity was investigated using *Schizosaccharomyces pombe* as a model organism. Cell growth and respiratory activity were assessed spectrophotometrically. Spectrophotometric and fluorometric approaches were used to evaluate oxidative stress by measuring superoxide dismutase (SOD) and catalase (CAT) activities, as well as levels of malondialdehyde (MDA), reduced glutathione (GSH), and reactive oxygen species (ROS). Fluorescence microscopy was used to analyze cell morphology and detect apoptosis and necrosis. Molecular mechanisms underlying Ni toxicity were examined using quantitative RT-PCR and western blot analysis.

Results

Exposure to NiSO₄ (200 and 500 µM) reduced cell growth in a dose-dependent manner and increased cell death, with ~12% and ~20% of cells undergoing apoptosis or necrosis, respectively. Ni exposure elevated ROS and MDA levels while reducing GSH, indicating pronounced oxidative stress. This was accompanied by increased SOD and CAT activities. Gene expression analysis revealed upregulation of antioxidant defense genes (*sod1*, *sod2*, *ctt1*, *gpx1*, *grx1*, and *pgr1*), supporting activation of oxidative stress responses. Ni-induced growth inhibition was associated with altered expression of key cell cycle regulators (*cdc2*, *cdc13*, and *cdc25*). Furthermore, dysregulation of *tor1*, *sin1*, *ste20*, and *wat1* suggests impairment of TORC2 signaling. Furthermore, TOR signaling involvement in Ni-induced toxicity was indicated by western blot analyses showing changes in Rps6 phosphorylation. Changes in *sty1* expression indicate involvement of the MAPK signaling pathway in the cellular response to Ni stress.

Conclusion

Excess Ni induces oxidative stress, disrupts antioxidant homeostasis, and alters cell cycle regulation in *S. pombe*. Importantly, our findings identify TOR and MAPK signaling pathways as key contributors to Ni toxicity. These results provide new insights into the molecular mechanisms of metal-induced stress and highlight the interplay between environmental exposure and cellular signaling pathways.

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Circulating elements in the bloodstream of growing foals: focus on heavy metals and bone remodeling across growth stages.

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Bone tissue undergoes continuous remodeling during growth, regulated by hormonal, biomechanical, and metabolic factors. This study investigated longitudinal changes in circulating minerals and heavy metals in growing foals from weaning to 24 months of age. A total of 40 serum samples (8 foals × 5 timepoints, T0–T4) were analyzed using ICP-MS according to the US EPA 6020B method. Cadmium (Cd) was the only element showing significant temporal variation, with concentrations increasing between T0 and T1 ($p = 0.005$) and decreasing between T1 and T2 ($p = 0.020$). Essential trace elements remained stable throughout growth, indicating preserved mineral homeostasis. These findings suggest that the post-weaning period may represent a sensitive developmental stage for cadmium dynamics, highlighting the importance of monitoring heavy metals even under low-exposure conditions.

Background Bone growth in young horses depends on regulated mineralization and remodeling processes that may be influenced by essential trace elements and toxic heavy metals. Cadmium (Cd) has been associated with impaired bone metabolism, but little is known about its temporal dynamics during equine growth. This study evaluated longitudinal changes in circulating trace elements and heavy metals in growing foals from weaning to 24 months of age.

Methods Eight healthy Anglo-Arab foals were monitored across five developmental stages (T0–T4), generating 40 serum samples. Animals were maintained under standardized feeding and management conditions. Trace elements and heavy metals were quantified using inductively coupled plasma mass spectrometry (ICP-MS/MS) according to the US EPA 6020B method. Statistical analyses included repeated-measures ANOVA or Friedman tests with corrected post-hoc comparisons. Principal Component Analysis (PCA) evaluated multivariate relationships.

Results Among 20 quantified analytes, cadmium was the only element showing significant temporal variation during growth ($p = 0.015$). Circulating Cd concentrations increased significantly between T0 and T1 ($p = 0.005$), followed by a decline between T1 and T2 ($p = 0.020$). No further differences were observed. Essential trace elements, including iron, zinc, copper, selenium, and manganese, remained stable throughout development, indicating preserved mineral homeostasis. PCA showed substantial overlap among developmental stages, confirming stability of the multielement profile.

Conclusions Growing foals maintained stable circulating concentrations of most trace elements throughout development. Cadmium was the sole analyte exhibiting a transient fluctuation during the post-weaning period, suggesting that this stage may represent a sensitive window for heavy metal dynamics. These findings support the importance of monitoring environmental toxicants during skeletal development, even under low-exposure conditions.

Cell Biology 3

Cadmium-Induced Non-Coding RNA Reprogramming and Phenotypic Plasticity in Aggressive Epithelial Cancers

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Background Cadmium is an environmental carcinogen increasingly implicated in tumour progression, metastatic plasticity, and non-coding RNA dysregulation across multiple epithelial malignancies. Recent evidence suggests that chronic cadmium exposure contributes to aggressive tumour behaviour through molecular reprogramming rather than through direct effects on proliferation alone. Previous studies in pancreatic ductal adenocarcinoma (PDAC) demonstrated cadmium-associated dysregulation of oncogenic microRNAs (miRs/miRNA) and epithelial-mesenchymal transition (EMT)-related markers, including reduced E-cadherin and increased Snail and Zeb1 expression, supporting a role for cadmium in cancer cell plasticity and progression. miR-21 is a well-established oncogenic miRNA linked to invasion, phenotypic plasticity, and metastatic signalling in prostate cancer. In this study, we investigated whether chronic cadmium exposure promotes invasive and EMT-associated changes in prostate cancer cells and whether these effects are influenced by miR-21. To address this, metastatic PC3 cells, weakly metastatic epithelial-like LNCaP cells, and a CRISPR/Cas9-generated PC3-miR21 knockout (KO) cells were used.

Materials and Methods PC3, PC3-miR21 KO, and LNCaP prostate cancer cells were exposed to 10 μ M CdCl₂ for 14 days. Cell proliferation was assessed using the MTT assay, while invasive capacity was evaluated using matrigel invasion assays. Molecular changes associated with extracellular matrix remodelling were assessed through analysis of MMP2 and MMP9 expression, while EMT-associated changes were examined through E-cadherin, Snail, and vimentin mRNA expression following treatment. Comparative analyses were performed between untreated and cadmium-treated groups within each cell line and between PC3 WT and PC3-miR21 KO cells to determine whether miR-21 loss moderates the response to chronic cadmium exposure.

Results Chronic cadmium exposure did not significantly alter short-term proliferation in prostate cancer cells, suggesting that the principal effects of cadmium extend beyond direct cytotoxicity. In contrast, prolonged cadmium exposure promoted invasive behaviour and induced molecular changes consistent with EMT-associated phenotypic plasticity, including dysregulation of MMP2, MMP9, E-cadherin, Snail, and vimentin. Differential responses observed between PC3 WT and PC3-miR21 KO cells further supported a mechanistic role for miR-21 in cadmium-associated invasion and cellular reprogramming. Cadmium-associated molecular and phenotypic alterations were also observed in LNCaP cells, indicating that these effects are not restricted to only PC3 cells. Previously, cadmium-associated EMT and miRNA changes have also been identified in PDAC cells, suggesting that environmentally driven ncRNA-mediated plasticity may represent a broader mechanism across aggressive epithelial cancers.

Conclusion

Together, our data indicates that cadmium exposure promotes an invasive, plastic phenotype characterised by increased matrix-remodelling activity and dysregulation of EMT-associated markers. The

reduced cellular invasion in PC3-miR21 KO cells compared with PC3 WT cells suggests that miR-21 contributes to cadmium-mediated regulation of invasion-associated pathways. Integration of findings from prostate and pancreatic cancer in vitro further indicates that cadmium exposure may engage non-coding RNA and EMT-associated signalling pathways across aggressive epithelial malignancies. These studies provide a mechanistic rationale for investigating environmentally induced ncRNA signatures as biomarkers of tumour progression and as potential targets for therapeutic intervention.

Cadmium complexation with glutathione: new insights into cellular uptake mechanisms

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Background Cadmium (Cd) accumulates in renal proximal tubule cells (PTCs) and drives chronic nephrotoxicity. In blood, Cd readily complexes with glutathione (GSH), a key tripeptide antioxidant. Uptake of Cd-GSH is unclarified. Proximal-tubule γ -glutamyltransferase (GGT1/5) is enriched at the apical brush border and initiates extracellular GSH breakdown by γ -glutamyl cleavage to glutamate + cysteinylglycine, followed by dipeptidase hydrolysis to cysteine + glycine. We hypothesize this processing yields transport-competent low-molecular-weight Cd-thiol species (Gly-Cys-Cd-Cys-Gly and Cys-Cd-Cys), which better match the substrate size/chemistry constraints of established amino-acid transport systems than the intact, highly polar Cd-GSH tripeptide complex. Depending on net charge and amino-acid mimicry, candidate uptake systems include xCT/SLC7A11 (cystine/glutamate antiport; stress-inducible), EAAT3/SLC1A1 (apical cysteine/glutamate transport), B⁰AT1/B⁰AT3 (SLC6A19/18; apical neutral amino acids), and LAT1/LAT2 (SLC7A5/7A8; neutral amino-acid exchange).

Aim Test GGT dependence and identify which candidate transport systems contribute to Cd-GSH-derived metabolite uptake and toxicity.

Materials and Methods Renal PTC lines (including rat WKPT) were exposed to ionic Cd or freshly preformed Cd-GSH (1:2 stoichiometry). Acute toxicity was quantified by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction) viability assays (24 h) and oxidative stress was assessed using dihydrorhodamine 123 (DHR123) oxidation to rhodamine 123⁺ (Rh⁺) fluorescence (60-180 min). Expression of GGT1/5 was quantified by qPCR. Functional relevance was tested by GGT inhibition with acivicin (5 μ M) and by cystine co-treatment (0-2 mM) as a competitive probe for cystine-sensitive amino-acid transport pathways. To assess direct membrane effects, membrane phase properties were accessed in extruded plasma membrane-mimetic nanoliposomes with laurdan generalized polarization (GP; interfacial/polar region) and 1,6-diphenyl-1,3,5-hexatriene (DPH) anisotropy (hydrophobic core) in nanoliposomes mimicking plasma membrane lipid composition. Cd-GSH coordination geometry was evaluated by NMR.

Results GSH complexation attenuated Cd cytotoxic potency after 24 h by MTT, right-shifting EC₅₀ 11 μ M for Cd²⁺ by six-fold across four PTC lines. In rat WKPT PTCs, Cd²⁺ (50 μ M) increased reactive oxygen species (ROS) to 270% and 177% above control after 60 and 180 min, respectively, whereas 50 μ M Cd-GSH caused a weaker response (109-115%). GGT1 and GGT5 mRNA expression was confirmed in all tested lines. Pharmacologic inhibition of GGT activity with acivicin and application of cystine, a substrate of several amino-acid membrane transporters, significantly reversed Cd-GSH toxicity, but not toxicity induced by ionic Cd²⁺, consistent with a requirement for extracellular Cd-GSH processing prior to cellular uptake. In plasma membrane nanoliposome mimics, Cd-GSH selectively altered interfacial lipid packing (decrease in Laurdan GP, indicating fluidization) without measurable effects on the hydrophobic core (unchanged DPH anisotropy), supporting a surface-proximal interaction rather than bulk permeation through the bilayer. NMR analyses predict Cd-GSH predominantly forms linear Gly-Cys-Cd-Cys-Gly and Cys-Cd-Cys species.

Conclusion Despite markedly attenuating Cd-driven ROS generation, GSH ligation does not completely eliminate Cd cytotoxicity in renal PTCs. The partial rescue by acivicin and cystine is consistent with a GGT-

linked extracellular processing component that generates transport-competent, low-molecular-weight Cd-thiol species, with cystine-sensitive amino-acid transport representing a contributing entry route.

Co-Exposure to Arsenic and Cadmium Enhances DNA Damage and Apoptosis in 3T3 Fibroblast Cells

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Background

Arsenic (As) and cadmium (Cd) are widespread environmental heavy metals known to exert serious toxic effects on living organisms. Increasing simultaneous exposure to these metals has highlighted the importance of investigating their combined toxicity. The present study aimed to evaluate the cytotoxic, oxidative, and genotoxic effects induced by individual and combined As/Cd exposure in 3T3 fibroblast cells.

Materials and Methods

Cells were exposed to different concentrations of As and Cd, individually or in combination, for 24 h. Cell viability was assessed using the MTT assay, while intracellular reactive oxygen species (ROS) levels were analyzed as indicators of oxidative stress. Genotoxic effects were evaluated using comet and micronucleus assays. In addition, apoptosis formation was determined by double fluorescence staining.

Results

The obtained results demonstrated that both individual and combined As/Cd treatments significantly decreased cell viability in a concentration-dependent manner in 3T3 fibroblast cells. Combination index (CI) analysis revealed that co-exposure to As and Cd induced a synergistic toxic effect. Increased intracellular ROS levels further supported the critical role of oxidative stress in the toxicity mechanism. Moreover, findings from comet and micronucleus assays showed that combined exposure significantly increased DNA strand breaks and chromosomal damage. Double fluorescence staining results also demonstrated a marked increase in apoptotic cell numbers, indicating that As and Cd enhanced their toxic effects through the activation of cell death mechanisms.

Conclusion

In conclusion, combined exposure to As and Cd induced significant toxic effects in 3T3 fibroblast cells through increased oxidative stress, DNA damage, and activation of apoptotic pathways. These findings contribute to a better understanding of synergistic toxicity mechanisms associated with combined heavy metal exposure and provide important insights for environmental toxicity and public health risk assessment.

Kupffer Cells as Key Targets of Hepatic Cadmium Handling: Putative Uptake Mechanisms of Free and Protein-Bound Cadmium

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Background Cadmium (Cd) is a toxic heavy metal, which is released from the Earth's crust mostly by industrial activities, contaminating air, water and foodstuffs. After intestinal uptake, Cadmium is transported via the portal vein to the liver. In the portal circulation, Cadmium can form complexes with several serum proteins such as albumin (Alb), metallothionein (MT) and transferrin (Tf). In the liver, hepatocytes, endothelial cells, hepatic stellate cells, and resident Kupffer cell (KC) macrophages form a functional network, the hepatic cellular niche. According to the current dogma, ionic Cadmium induces the expression of MT in the liver, which is released into the bloodstream as CdMT through lytic cell death of hepatocytes. However, no direct evidence exists. In fact, Cadmium was detected mainly in endothelial and KCs compared with parenchymal cells¹. Moreover, after 15 minutes of a single intravenous injection of CdMT in rats, MT was immunolocalized exclusively in intracellular organelles in KCs, whereas MT staining in hepatocytes was unchanged². Furthermore, KC inactivation by GdCl₃ prevents hepatotoxicity³. So far, a detailed investigation of KCs as the primary key target for Cadmium handling in the liver has been neglected. The aim of this project is to investigate KC and hepatocyte cell uptake and responses to Cadmium-protein complexes and free Cadmium.

Materials and Methods Primary rat KCs were isolated by liver perfusion followed by Percoll density gradient centrifugation and characterized by Western blot and qPCR. Immortalized Kupffer KUP5 and the hepatocyte AML-12 cell lines were treated with CdCl₂, CdTf, CdAlb, and CdMT for 24 hours, and metabolic activity using MTT and cell viability using Trypan Blue were determined. Uptake of Tf and Alb was monitored by fluorescence live cell imaging. Furthermore, qPCR and Western blot were used to investigate the expression of MT, putative uptake candidates, including the transferrin receptor 1 (TFR1), lipocalin-2 receptor SLC22A17, endocytic receptor megalin and metal transporters, such as the divalent metal transporter 1 (DMT1) and the zinc transporters ZIP8 and ZIP14.

Results Compared to hepatocytes, KUP5 were more sensitive to Cadmium effects (CdMT < CdTf < CdAlb < CdCl₂), determined by MTT. While ionic Cadmium caused ~70% cell death, Tf- and Alb-bound Cadmium only altered cellular metabolism and KC morphology with no significant changes in total cell number or trypan blue-positive cells. Within 15 min in KUP5 cells, fluorescently-labeled Tf

was peripherally distributed, suggesting recycling, whereas perinuclear Alb indicated lysosomal trafficking. TFR1, SLC22A17, DMT1, ZIP8 and ZIP14 are more abundant in KCs compared to hepatocytes whereas megalin was absent in primary or immortalized KCs. CdCl₂ (0.5-5 µM) treatment did not affect TFR1 and SLC22A17 protein expression but increased MT after 1 h up to 24 h.

Conclusion Our data demonstrate KUP5 internalize multiple species of Cadmium and causing, depending on the speciation (ionic or complex), different cell reactions. Furthermore, increased expression of receptors and transporters indicate potentially increased capacity for Cadmium uptake in KCs. Possible fates of Cadmium handling in the hepatic cell niche will be discussed.

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Flash Talk 3, Toxicology

Cadmium Contamination in Seafood: RASFF Trends and ICP-MS Monitoring Results

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Background

Cadmium (Cd) is a highly toxic heavy metal that poses significant risks to marine ecosystems and human health through the consumption of contaminated seafood. From 2020 to 2025, 88 RASFF notifications concerned cadmium in seafood, predominantly in cephalopods, crustaceans, and bivalve molluscs. Most alerts and border rejections involved cephalopods (squid, octopus, and cuttlefish) originating mainly from non-UE countries (India, Vietnam, Thailand, Morocco, Oman, Senegal, and Chile) while Italy, France, Spain, Germany, the Netherlands, and Switzerland represent the main alert notifying countries. This study evaluates the bioaccumulation patterns of Cd across three distinct marine taxa, bivalves, crustaceans and molluscs to identify species-specific accumulation trends and potential food safety risks.

Materials and Methods

Seafood samples (N=200) from official controls and research monitoring were homogenized and weighted then underwent acid mineralization using a mixture of HNO₃ and H₂O₂. Cd concentrations were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7800); results are expressed in mg/kg on a wet weight basis. The entire analytical process was validated in accordance with internal Quality Assurance and Quality Control (QA/QC) standards UNI EN ISO/IEC 17025). A combination of descriptive statistics, One-Way ANOVA, and Principal Component Analysis (PCA) was employed to analyze the dataset.

Results

Descriptive analysis revealed that bivalves exhibited the highest average concentration and frequency of accumulation, while crustaceans displayed the highest individual peak concentration (Table 1). The boxplot (Figure 1) illustrated Cd concentrations across the three categories of marine organisms. Bivalve have shown the highest median concentration, slightly below 0,2 mg/kg; however, there are several outliers (the open circles) well above 1,0 mg/kg, suggesting that while most samples are within a lower range, a notable portion of the population could be exposed to high levels of contamination. Crustaceans have the lowest median concentration, centered around 0,1 mg/kg; this group contains the most extreme outlier in the entire dataset, reaching toward 5,0 mg/kg. Mollusk distribution is like bivalves but generally slightly lower.

ANOVA results confirmed significant differences in Cd levels between the three groups ($p = 0,042$). The PCA indicated that the first two principal components explained approximately 90% of the total variance. PC1 was strongly associated with the overall contamination load, primarily driven by bivalve samples, while PC2 highlighted the high-intensity accumulation pattern in crustaceans (Figure 2). The consistency

between RASFF notifications and ICP-MS analytical results confirms that cadmium contamination is a persistent issue in international seafood supply chains.

Conclusion

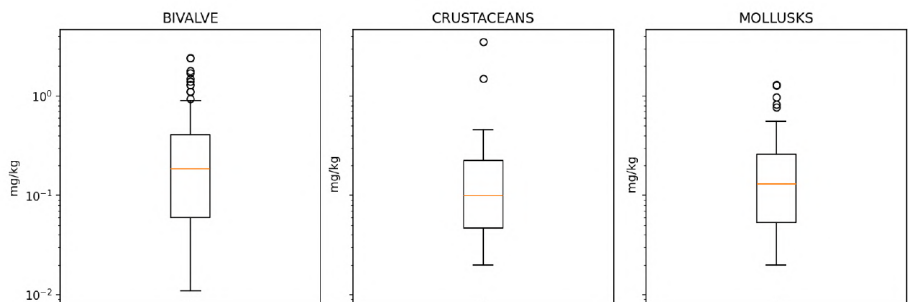
The findings suggest that bivalves serve as the most reliable bio-indicators for chronic environmental monitoring due to their consistent filtration-driven accumulation. In contrast, crustaceans present a higher risk of acute toxicological spikes. These results underscore the necessity for taxon-specific monitoring strategies to ensure food safety and to manage anthropogenic impacts in coastal environments. Cadmium contamination in seafood remains a critical issue that warrants continuous and systematic monitoring. Targeted surveillance should prioritize specific sentinels and high-risk categories. Furthermore, special attention must be directed toward origins with recurrent non-compliance, to ensure consumer safety and compliance with international standards.

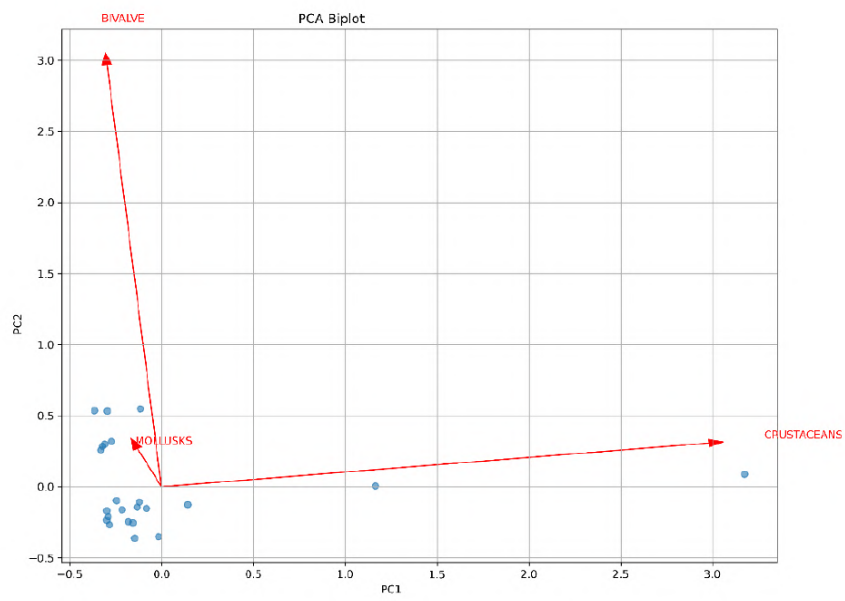
Figure 1 Cadmium Bioaccumulation Profiles in Bivalves, Crustaceans, and Molluscs

logarithmic scale on the y-axis, orange line represents median values Figure 2 Principal Component Analysis (PCA) biplot illustrating and variance among marine taxa.

Table 1. Cadmium in seafood (mg/kg)

Category	Mean	Median	Std. Dev.	Max
Bivalve	0,34	0,23	0,44	2,4
Crustaceans	0,33	0,13	0,73	3,5
Mollusks	0,24	0,14	0,31	1,3





Blood arsenic, mercury, and selenium as biomarkers of seafood intake – association with children’s cognitive abilities

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Background Studies on the neurodevelopmental benefits of seafood consumption are inconclusive. The objective of the present study was to assess the use of biomarkers for evaluating the impact of early-life seafood intake on children’s cognitive abilities. **Methods:** We studied 472 pregnant women and their 4-year-old children in the NICE birth cohort in northern Sweden. Women’s seafood intake (lean fish, fatty fish, shellfish) was assessed by web-based semi-quantitative food frequency questionnaire (FFQ) at gestational week 34, reflecting intake during the previous month. Children’s usual seafood consumption at age 4 was assessed using parental-reported FFQ. Potential seafood-related biomarker concentrations in blood (erythrocyte fraction) at gestational week 29, birth, and 4 years included arsenic, mercury (mainly methylmercury), and selenium. Children’s cognitive abilities at 4 years were assessed by Wechsler Preschool and Primary Scale of Intelligence-Fourth Edition (WPPSI-IV). Associations were explored using multivariable-adjusted linear or spline regression.

Results Estimated maternal seafood intake (median: 184 g/week) correlated significantly with erythrocyte mercury (strongest, $\rho=0.487$), arsenic ($\rho=0.336$; shown to be mainly arsenobetaine), and weakly with erythrocyte selenium ($\rho=0.150$), but was not associated with child IQ. Still, gestational erythrocyte arsenic and mercury concentrations were positively associated with children’s verbal abilities. Likewise, children’s erythrocyte arsenic (also mainly arsenobetaine) and mercury were cross-sectionally associated with verbal abilities, arsenic also with total IQ scores. Gestational erythrocyte selenium (above 97 $\mu\text{g/kg}$) and cord erythrocyte selenium (linearly) showed strong associations with children’s cognitive abilities (both verbal and total scores).

Conclusions Seafood intake biomarkers, particularly arsenobetaine, originating from marine seafood, confirmed seafood benefits for neurodevelopment, while assessment of seafood intake failed to do so. The positive associations of methylmercury, originating from both marine and freshwater fish, indicate that nutrients in seafood compensated for the toxic effects of methylmercury. Prenatal and newborn selenium, originating only partly from gestational seafood intake, appears essential for child development.

Potential Protective Molecular Mechanisms of Magnesium Against Cadmium Toxicity: A Toxicogenomic Analysis

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Background

Cadmium (Cd) is a highly toxic heavy metal widely present in the environment, and human exposure occurs mainly through contaminated food, tobacco smoke, water, and industrial emissions. It induces severe adverse health effects, including renal, hepatic, and cardiovascular damage, primarily through oxidative stress, inflammation, and disruption of essential metal homeostasis. Although magnesium (Mg) has been suggested to mitigate heavy metal toxicity and both elements may interact at the molecular level, the underlying mechanisms of their interaction remain insufficiently understood. The aim of this study was to investigate the molecular mechanisms underlying the potential protective role of Mg against Cd-induced toxicity, with a focus on key regulatory genes and signaling pathways, using an *in silico* toxicogenomic approach.

Materials and Methods

First, gene sets associated with Cd and Mg exposure were retrieved from the Comparative Toxicogenomics Database (CTD; <https://ctdbase.org/>), and common genes were identified using the MyVenn tool. Cytoscape (3.10.4.) with the CytoHubba plugin was used to identify key hub genes among the common genes. The effects of Cd and Mg on the expression of selected hub genes were further evaluated using CTD. Functional enrichment analysis was performed using the ToppGene Suite ToppFun function (<https://toppgene.cchmc.org/enrichment.jsp>) to identify associated biological processes, molecular functions, signaling pathways, and diseases.

Results A total of 9,693 genes associated with Cd exposure and 230 genes associated with Mg exposure were retrieved from the CTD database, with 195 common genes identified between the two sets. Functional enrichment analysis revealed that these genes are mainly involved in receptor-mediated signaling and protein interaction networks, as well as cellular responses to endogenous and oxidative stress stimulus. Pathway analysis highlighted significant involvement in immune and inflammatory signaling cascades, particularly cytokine- and interleukin-mediated pathways. Disease association analysis further linked the common gene set to cardiovascular and metabolic disorders, including myocardial ischemia, hypertension, and diabetes mellitus. CytoHubba analysis identified *IL6*, *TNF*, *INS*, *AKT1*, and *TP53* as the top hub genes within the shared molecular network. CTD analysis showed that Cd predominantly increased *TNF*, *IL6*, and *TP53* expression, while reducing *AKT1* and *INS* protein expression, indicating activation of inflammatory, oxidative stress-related, and apoptotic pathways, along with impairment of metabolic and cell survival signaling. In contrast, Mg was associated with decreased *TNF* and *TP53* protein expression, suggesting its potential role in attenuation of Cd-induced inflammatory and stress-related molecular responses.

Conclusion

These findings suggest that Mg may exert protective effects against Cd-induced toxicity through modulation of key hub genes involved in inflammation, oxidative stress, apoptosis, and metabolic dysfunction. The identified molecular interactions provide further insight into the potential mechanisms

underlying the antagonistic relationship between Mg and Cd. (Nos. 451-03-33/2026-03/200161 and 451-03-34/2026-03/200161)

E-Cigarette Aerosols as a Source of Inhaled Metal Exposure and Pulmonary Metal Deposition

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Background

E-cigarette use is rapidly increasing, particularly among adolescents and young adults, yet the health risks associated with vaping remain poorly understood. E-cigarette liquids typically contain a vehicle composed of propylene glycol and vegetable glycerin (PG/VG), nicotine salts, and flavouring chemicals. Although e-cigarettes are often perceived as a safer alternative to combustible cigarettes, heating and cooling of the e-liquid, metal coils, and device components may promote the release of metals, including arsenic, cadmium, lead, nickel, and chromium. During vaping, these metals can be transferred into the aerosol and inhaled into the respiratory tract. However, the presence of these metals in aerosols and their deposition in lung tissue remain poorly understood. This study aimed to characterize metal concentrations in e-liquids and aerosols and determine whether these metals are detectable in lung tissue following inhalation exposure. *We hypothesized that heating e-cigarette products increases the release of metal nanoparticles and their subsequent deposition in the lung.*

Materials and Methods

E-liquids and aerosol condensates were collected from three popular pod-based devices (JUUL, Vuse, and STLTH) used in Canada. Metal nanoparticles in these pods were quantified using single-particle inductively coupled plasma time-of-flight mass spectrometry. To assess pulmonary metal deposition, mice were exposed twice daily for three days to air, PG/VG, or tobacco-flavoured aerosols from these devices using our established exposure protocol of 4 puffs/min for 20 minutes. Lungs were harvested one hour after the last exposure, formalin-fixed, paraffin-embedded, and sectioned at 20 µm. Metal deposition in lung tissue was assessed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), allowing spatial quantification while preserving anatomical context.

Results

Chromium, nickel, arsenic, cadmium, and lead were detected at ppm levels in e-liquids and aerosol condensates across all three devices, with concentrations increasing following the addition of nicotine salts and flavourings. Metal nanoparticle number ranged from 61 to 4,154 nanoparticles in heated e-liquids and numbers varied by device type.

Using LA-ICP-MS, we quantified metal concentrations within the lung tissue of exposed mice and localized metal deposition within the pulmonary architecture. Following inhalation exposure, cadmium, titanium, arsenic, lead, nickel, and chromium were detected in the lungs of exposed mice. Lung metal concentrations increased with the addition of flavouring and nicotine salts across the devices tested.

Conclusion

Our findings demonstrate that e-cigarette aerosols contain detectable levels of metals and that these metals can deposit in the lung following short-term inhalation exposure. These results suggest that vaping represents a source of inhaled metal exposure and highlight the need to better understand the respiratory consequences of metal inhalation from e-cigarette devices.

Risk assessment of titanium dioxide (E171) intake in an Italian population before 2021 ban by the European Union

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Background Titanium dioxide (TiO₂ – E171) is widely used as a food additive, however, in 2021 the European Food Safety Authority (EFSA) raised concerns over genotoxicity of TiO₂ and could not establish any safe level for daily intake. In 2022 it was banned by the European Commission as a food additive, although it still remains authorized in the UK. Limited information exists about on TiO₂ concentrations in foods and an estimation of dietary intake can be useful for safety risk assessments.

Materials and Methods We measured titanium content in 908 food samples, and, taking a conservative approach, we assumed that all titanium detected was in the form TiO₂ with a conversion factor of 1.67. We then estimated TiO₂ intake using a validated semi-quantitative food frequency questionnaire administered to a representative sample of Northern Italy population.

Results We found higher than average TiO₂ concentrations in dry fruits/seeds (2042 µg/kg), milk and dairy products (1839 µg/kg), sweets (1573 µg/kg), and legumes (1320 µg/kg). In particular, we detected the highest TiO₂ levels in chocolate products and candy bars (5573 µg/kg), nuts and seeds (2672 µg/kg), and cheeses (2414 µg/kg). The mean dietary intake of TiO₂ in the study population was 21.11 µg/kg body weight (bw) per day. Overall, estimated dietary intakes were substantially lower than health-based guidance upper value of 10 mg/kg bw/day derived from animal models.

Conclusion Our findings provide updated estimates of TiO₂ concentrations in foods and dietary TiO₂ intake in an Italian population indicating very low intake in a period prior to the 2022 EU ban. Although, there is a need for a more accurate assessment of TiO₂ exposure through improvements of comprehensive TiO₂ food composition databases, and further evaluation of the relation between TiO₂ intake and adverse health outcomes in humans is recommended, intake levels are expected to go down even further.

Analysis of mercury concentration in various honeys available on the Polish market

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Background Honey is a natural food product valued for its flavour as well as its nutritional and health promoting properties. It is produced by honeybees from plant nectar or honeydew. Environmental contaminants, including heavy metals such as mercury (Hg), can accumulate in melliferous plants and subsequently enter honey. Consumption of contaminated food may negatively affect human health. Mercury is a toxic element that damages cellular structures and can impair multiple organ systems, particularly the nervous system and kidneys. Monitoring Hg levels in honey is therefore important for consumer safety. The aim of this study was to determine the concentration of Hg in various types of honey available on the Polish market and assessment of the health risks associated with their consumption.

Materials and Methods A total of 51 honey products purchased from retail stores and directly from apiaries in Poland were analysed. All samples were produced in Poland. The study included nectar honeys (acacia, buckwheat, linden, multifloral, dandelion, rapeseed, and heather) and honeydew honeys (deciduous and coniferous). Samples included both pure honeys and those with added plant ingredients such as fruits or seeds. Hg concentration was determined using atomic absorption spectrometry (AAS) with the AMA 254 mercury analyzer (Altec, Czech Republic). The method quantified total Hg content without distinguishing between organic and inorganic forms.

Results The mean Hg concentration across all samples was 0.84 µg/kg, with values ranging from 0.18 µg/kg to 1.45 µg/kg. Nectar honeys showed slightly higher Hg levels (0.85 µg/kg) compared with honeydew honeys (0.79 µg/kg). Among the different types, the highest average concentration of Hg was observed in heather honey (0.94 µg/kg), while buckwheat honey exhibited the lowest level (0.67 µg/kg). Honeys without additives contained lower mean Hg concentration (0.82 µg/kg) than those with plant additives (1.07 µg/kg). The differences in Hg concentration between honey types were not statistically significant ($p > 0.05$).

Conclusion The Hg concentration in all honey samples available on the Polish market was well below the permissible limit established by the European Union (10 µg/kg; Commission Regulation (EU) 2018/73). The values of Estimated Weekly Intake (EDI) and Provisional Tolerable Weekly Intake (%PTWI) indicates that consuming a standard portion of 19 g of the tested honeys per week is safe for both adults and children. Nevertheless, it should be noted that Hg exposure may also occur through other foods and environmental sources. Continuous monitoring of Hg levels in various food products remains essential for maintaining consumer safety.

Cadmium Levels in Complete Feed: A 14-Year Monitoring Study in Northwestern Italy

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Background

Cadmium (Cd) is a highly toxic heavy metal that poses significant risks to animal health. The kidney is the primary target organ; Cd accumulates in the renal tubules, leading to chronic dysfunction and impairing the animal's ability to filter waste. Because it is chemically similar to essential elements like zinc and calcium, Cd "mimics" these minerals and can displace calcium in the skeletal structure, leading to osteomalacia and skeletal deformities, which are especially critical in developing fish. Moreover, since animals excrete this element very slowly, it can accumulate in edible tissues (muscle, liver) posing a potential risk to consumers. Then, cadmium levels in feed are strictly regulated by Directive 2002/32/EC. This study analyzes the concentration of Cd within the "Complete feed" category, reflecting monitoring activities performed in Northwest Italy within the framework of the National Official Feed Control Plan, issued by the Italian Ministry of Health.

Materials and Methods

Complete feed samples (N = 285) were collected from 2011 to 2025. The dataset included feed for several species, primarily fish, with smaller contributions from poultry, swine, and other animals. Samples were homogenized and subjected to acid mineralization using a mixture of HNO₃ and H₂O₂. Cd concentrations were then determined by Atomic Absorption Spectrometry (Analytic Jena ZEEnit 700P); results were expressed in mg/kg at a standardized moisture content of 12%. The limit of quantification (LOQ) was 0.25 mg/kg. The analytical process was validated and accredited in accordance with internal Quality Assurance and Quality Control (QA/QC) standards and UNI EN ISO/IEC 17025.

Results

Fifty out of 285 complete feed samples have shown quantifiable Cd concentrations (>LOQ). Most originated from Northern Italy, particularly from the province of Turin (TO), Cuneo (CN), and Vercelli (VC). Between 2011 and 2018, Cd levels remained stable, ranging between 0,25 and 0,45 mg/kg (Figure 1). In 2016 and 2019, isolated outliers of 0,80 mg/kg were recorded, representing the highest values in the dataset. To assess whether Cd concentrations differed across provinces or species, post-hoc Tukey HSD tests were performed (Table 1). The analysis revealed no significant pairwise differences among provinces or species, with all adjusted p-values well above the 0,05 threshold. Overall, the

results indicate that Cd levels are relatively homogeneous across the sampled provinces and species, and that apparent differences likely reflect sampling variability rather than true underlying disparities.

Conclusion

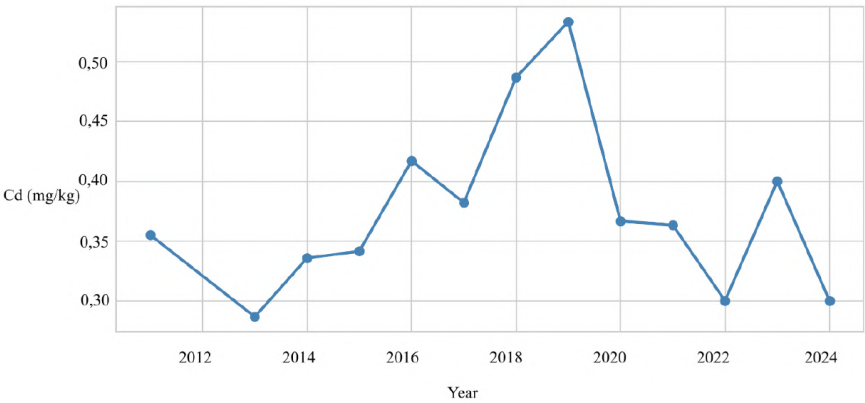
The analysis of cadmium concentrations in complete feed samples collected in Northwest Italy over a fourteen-year period indicates that Cd levels in commercial feed are generally stable and well controlled. Statistical testing not detected significant differences across provinces or species, and post-hoc Tukey HSD comparisons likewise revealed no meaningful pairwise contrasts once groups with insufficient sample size were excluded. These findings suggest that the observed variability is largely attributable to sampling fluctuations rather than systematic geographic or species-related factors. Overall, cadmium

concentrations in complete feed remain within safe ranges, no sample was found exceeding the maximum limit set by Directive 2002/32/EC. Continued monitoring remains essential, but the current dataset supports the conclusion that Cd contamination in feed within the considered regions is effectively managed and does not exhibit concerning temporal or spatial patterns.

Figure 1 Cadmium levels from 2011 to 2025 (mg/kg, mean values)

Table 1. Cadmium in complete feed

Parameter	Value
Total samples	285
Quantifiable samples	50
Cd range (mg/kg)	0,25–0,80
Mean Cd (mg/kg)	0,39
Highest values	0,80 mg/kg (2016, 2019)
Tukey HSD (provinces)	No significant differences ($p > 0.05$)
Tukey HSD (species)	No significant differences ($p > 0.05$)



S-DRIVE Artificial Intelligence for Heavy Metal Screening

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Introduction Heavy metal contamination (such as lead, cadmium, mercury, and arsenic) represents one of the most critical environmental and health threats globally, due to their persistence and tendency to bioaccumulate in the environment and, consequently, along the food chain. Monitoring heavy metals and their environmental impact on human health has traditionally relied on biological matrices analyzed chemically and quantitatively. However, the advent of epigenetics and applied biophysics has highlighted how environmental factors, including heavy metals, alter gene expression without modifying DNA. For many years, the hair follicle and often the hair shaft have been used as biosensors and biological antennas for rapid and precise assessment, capable of detecting the presence of numerous molecules and substances, including heavy metals. A cutting-edge system equipped with Artificial Intelligence, such as the one featured in the S-DRIVE instrument, enables the identification of epigenetic stressors, with particular attention to the detection of environmental toxins, including heavy metals.

Purpose of the study: Sardinia, considered a nearly pristine region for its environment, longevity, healthy food, and community life, offered the opportunity to select Sardinian individuals of varying birthplace, activity, and age to assess the presence/absence of heavy metals in their bodies and their possible interactions with their metabolic processes.

Comparison between these individuals offered the opportunity to test how the presence/absence of heavy metals can impact an individual's metabolism and health. This analysis characterized cellular activity that can be altered by heavy metals, initially epigenetic and therefore still modifiable, up to genetic alterations specific to degenerative and/or tumor-related diseases.

Materials and Methods Hair samples with intact hair follicles were collected from:

Long-lived from Perdasdefogu (Ogliastra)

Walking athletes

Random individuals (males and females between 20 and 40 years old)

were immediately placed on the S-Drive device. The system digitized the longitudinal waves and resonances emitted by the hair root, sending the information streams to a central server for automated algorithmic decoding. The presence or absence of heavy metals was categorized into low, medium, and high presence.

Results Biophysical mapping allowed for the highly sensitive identification of dissonance profiles associated with the latent presence of heavy metals (such as lead, cadmium, and aluminum) at the cellular level, simultaneously highlighting secondary imbalances in the homeostasis of essential minerals (zinc, selenium), vitamins, amino acids, and fatty acids, and the resulting impact on intestinal absorption—all factors that impact the individual's overall health.

The presence of heavy metals was detected in relation to their impact on overall metabolism, evident in all selected samples, but the long-lived individuals from Ogliastra demonstrated a greater capacity for resistance to the negative effects that heavy metals can have on the body's overall metabolism.

Conclusions S-Drive technology proves to be a non-invasive, predictive, and extremely effective screening tool for integrative medicine, capable of detecting the presence of heavy metals at low, medium, or high levels, potentially harmful to an individual's health, reflecting the environmental

conditions in which they live and work. S-DRIVE does not specify the heavy metal present in the body, but generates an alert result that, if positive, can be further evaluated with further clinical tests, should the individual's medical history deem it necessary.

Synthetic Mn-Peptide Antioxidant Systems Inspired by *Deinococcus radiodurans*: Applications in Radioprotection and Biotechnology

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Background

Deinococcus radiodurans withstands extreme ionizing radiation (IR) and oxidative stress through a synergy of efficient DNA repair and robust proteome protection. Central to this resilience is a high intracellular manganese/iron (Mn/Fe) ratio, which prevents iron-dependent Fenton reactions while promoting the formation of small-molecule Mn(II)-antioxidant complexes. Unlike most organisms, *Deinococcus* protects its proteins from oxidative damage, ensuring that DNA repair enzymes remain functional even after massive IR exposure [1,2].

Materials and Methods

Rationally designed synthetic decapeptides, mirroring the most abundant amino acids in *Deinococcus* cell-free extracts, were complexed with Mn(II) and characterized using integrated spectroscopic and thermodynamic analyses to define their binding affinities and molecular arrangements in solution. Antioxidant activity and protein preservation were evaluated under extreme oxidative stress and high-dose gamma irradiation [2–4].

Results

The study identified a highly efficient ternary complex (Mn-peptide-phosphate, MDP) acting as a powerful non-enzymatic antioxidant. This system effectively protects proteins under extreme irradiation and oxidative conditions. Beyond radioprotection, this MDP complex demonstrates significant potential in the development of radiation-inactivated whole-cell vaccines by preserving critical surface epitopes [5].

Conclusion

Deinococcus-derived Mn-peptide systems represent a transformative resource for biotechnology, offering stable and effective solutions for medical radioprotection and vaccine platforms.

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The Impact of Metal Mixtures on Red Blood Cell and Thrombocytes Parameters in Male and Female Wistar Rats

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Background Heavy metal exposure is known to impair hematological profiles. This study investigated thrombocytes count, red blood cell (RBC) indices and related parameters in Wistar rats subjected to a mixture of toxic metals (Cd, Pb, As, Hg, Cr, Ni) at levels regarded as safe and reflective of typical human exposure.

Materials and Methods A total of 90 albino Wistar rats, including 45 males and 45 females, were included and exposed to the metal mixture for either 28 or 90 days. Animals treated for 28 days were allocated into four experimental groups (M1–M4 / F1–F4) and a control group, while those exposed for 90 days were assigned to three experimental groups (M1–M3 / F1–F3) with a corresponding control. The administered doses were structured as ascending levels derived from human biomonitoring data to reflect real-life exposure, corresponding to Benchmark doses-based values for selected endocrine parameters, population mean concentrations, the 95th percentile, and NOAEL values for each metal reported in the literature. Following 28 and 90 days of exposure total RBC and thrombocytes counts, hemoglobin, hematocrit and RBC indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were determined.

Results After 28 days of exposure, the number of RBC was significantly higher ($p < 0.05$) in M1, M2, and F4 groups compared to an appropriate control group. Subchronic 90-days exposure resulted in the increase in RBC in F3 group of animals in comparison with a control group ($p < 0.05$). The increase in RBC number was followed with the significant increase ($p < 0.05$) in hematocrit in M1 and F4 groups (28-days treatment) and M1, M3, and F3 groups (90-days treatment) compared to an appropriate control group. No significant change was noted regarding hemoglobin levels. As for the RBC indices, after 28 days of exposure, MCV was significantly higher in M4 group ($p < 0.001$), whereas MCHC was significantly lower in the same group ($p < 0.01$) compared to the control group. After 90 days of treatment, MCV was significantly higher in F2 group ($p < 0.001$) compared to the control group, whereas MCHC was significantly lower in F3 group ($p < 0.01$) in comparison with the control group. Exposure for 28 or 90 days did not result in any detectable alterations in MCH parameter. The number of thrombocytes in the F4 female group of animals was significantly increased after 28 days of exposure compared to the control group ($p < 0.001$). After subchronic 90-days treatment, the thrombocyte count was higher in M1 and M3 groups ($p < 0.001$) followed with the increase in all 3 female groups (F1, F2 and F3) ($p < 0.05$) compared to an appropriate control group.

Conclusion Exposure to a realistic mixture of toxic metals, despite being within accepted safety limits, can lead to notable changes in thrombocytes count, red blood cell (RBC) indices and related parameters, highlighting the necessity for a critical reevaluation of current exposure limits to protect overall health.

The Impact of Metal Mixtures on White Blood Cell Differential in Male and Female Wistar Rats

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Background

Toxic heavy metals are known to adversely affect hematological parameters. The present study

aim ed to assess the total and differential white blood cell (WBC) counts in Wistar rats exposed to a mixture of toxic metals (Cd, Pb, As, Hg, Cr, Ni) in doses that are considered safe and reflect the real exposure of the general population.

Materials and Methods

The study included 45 male (M) and 45 female (F) albino Wistar rats treated for 28 or 90 days with a mixture of toxic metals. Animals treated for 28 days were allocated into four experimental groups (M1–M4 / F1–F4) and a control group, while those exposed for 90 days were assigned to three experimental groups (M1–M3 / F1–F3) with a corresponding control. The administered doses were structured as ascending levels derived from human biomonitoring data to reflect real-life exposure, corresponding to Benchmark doses-based values for selected endocrine parameters, population mean concentrations, the 95th percentile, and NOAEL values for each metal reported in the

literature . The percentage and absolute count of the five main types of WBCs: neutrophils, lymphocytes, monocytes, eosinophils, and basophils were measured after subacute (28 days) and subchronic (90 days) exposure.

Results

After subacute exposure, the number of WBC was significantly lower ($p < 0.01$) in M4 group, as well as in F2 ($p < 0.01$), F3 and F4 groups ($p < 0.05$) compared to an appropriate control group. After subchronic exposure, the WBC count wasn't significantly changed in both male and female groups. In terms of the analysis of five main leukocyte subsets, the significantly lower absolute number of lymphocytes ($p < 0.01$), monocytes ($p < 0.05$), neutrophils ($p < 0.05$), and basophils ($p < 0.001$) was found in M4 group compared to the control group after 28 days of treatment. As for the female rats, the absolute number of lymphocytes was lower in F2 ($p < 0.01$), F3 and in F4 groups ($p < 0.05$) and a significantly higher eosinophile number was found in F4 group ($p < 0.01$) compared to an appropriate control group after subacute exposure. After 90 days of exposure, only the number of basophils was significantly changed and the increase in number was found in F1 and F2 groups compared to the control group ($p < 0.05$). Regarding leukocyte differential proportions (%), after 28 days of exposure, the significant change was observed in the percentage of monocytes, eosinophils and basophils. The monocyte and basophil percentage count was lower in the M4 group compared to the control group ($p < 0.05$) while the relative eosinophile count was higher in the same group ($p < 0.05$). As for the female rats, the significantly higher percentage count of eosinophiles was found in F4 ($p < 0.01$) group compared to the control group. After subchronic exposure, a significant decrease in the relative lymphocyte count in the M3 group ($p < 0.05$), and a significant increase in the relative monocyte count in M2 group ($p < 0.05$) was observed compared to the control group. The same exposure time has affected the percentage of monocytes in F2 group and the

percentage of eosinophiles in F1 and F2 groups which were lower compared to the control group ($p<0.05$).

Conclusion

Although applied at levels considered safe, toxic metals in a complex mixture that mimics real-life exposure can markedly affect white blood cell profiles.

The Effect of Mercury on Rat Kidneys in Vivo

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Background Mercury toxicity (poisoning) is a severe condition caused by exposure to different forms of mercury (elemental, inorganic, or organic). It primarily targets the nervous, digestive, and immune systems, as well as the lungs and kidneys.

Materials and Methods In this study effects of mercury administration on the kidney structure of adult rats were evaluated. Rats received mercury (HgCl₂) in single intraperitoneal dose 20 mg HgCl₂ (group A), 10 mg HgCl₂ (group B) and 5 mg HgCl₂ (group C) per kilogram of body weight and were killed after 48 hours following mercury administration. After the preparation of histological samples, the results were compared with control group (K).

Results In kidney decreased diameters of glomeruli and renal corpuscles, damaged tubules with affected quality of tubular cells and infiltration of interstitium were detected. Quantitative analysis demonstrated increased relative volume of tubules and renal corpuscles. Also, the number of nuclei and glomeruli was increased in all experimental groups. The diameter of glomeruli and renal corpuscles decreased.

Conclusion This study reports a negative effect of mercury on the structure and function of kidney.

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Association Between Selenium Species and Brain Volume in Subjects with Mild Cognitive Impairment

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Background Selenium (Se) is a trace element with a twofold nature. It displays both nutritional and toxicological properties, depending on its exposure levels and chemical form. Several studies have suggested how Se may play a role in the etiology of neurodegenerative diseases, including cognitive decline and dementia. Mild cognitive impairment (MCI) implies structural changes in the brain, with an overall shrinkage of its volume, which may therefore be indicative of a cognitive decline. Another factor suggesting increased risk of cognitive decline and dementia forms is the presence of white matter hyperintensities (WMHs). We assessed the association between Se concentrations in serum and cerebrospinal fluid (CSF) and brain volume to investigate the role of Se species in cognitive impairment in subjects with MCI.

Methods We estimated Se and Se species concentrations in serum of 116 subjects and in CSF of 76 subjects, all diagnosed with MCI. Each participant underwent brain magnetic resonance imaging (MRI). From brain MRI, we assessed the volumes of total brain, white matter, grey matter, and WMHs, as the percentage of total intracranial volume, and hippocampal standardized volume. We evaluated the relation between Se species levels and characteristic brain volumes using restricted cubic spline regression analysis, adjusting for potential confounders.

Results We observed a negative inverse association between total Se levels and most Se species and percentage volumes of total brain, white matter and grey matter, as well as for the hippocampus. Exceptions were U-shaped associations between Se-GPX and grey matter and hippocampal volumes, and Se-methionine with hippocampal volume only in CSF. For inorganic forms, we found an inverse U-shaped association with selenate (Se VI) with decreased volumes above 1.0 µg/L, particularly for the hippocampal volume, but not for selenite (Se IV). Analysis on WMHs showed a slight increase of the percentage volume for Se IV, while little association was observed for other Se forms.

Conclusion Our findings suggest a complex relation between selenium species and brain volumes, generally in favor of an inverse association, especially for hippocampus, a key structure involved in cognitive impairment and its progression to dementia.

Beyond the Number: Biological Limits, Cut-Offs and Interpretative Challenges in Trace Metal Exposure

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Background

The interpretation of trace metal concentrations in biological matrices remains a major challenge in occupational medicine, clinical toxicology, and forensic sciences. Biological limit values, reference intervals, and toxicological cut-offs are commonly used as indicators of exposure or risk; however, their interpretation is frequently oversimplified. The growing diffusion of biomonitoring strategies has further increased the need for a critical evaluation of the significance and limitations of these thresholds.

Methods

A narrative interdisciplinary overview was conducted focusing on the most commonly adopted biological limits and reference values for trace metals in occupational, clinical, environmental, and forensic contexts. International recommendations and scientific literature concerning cadmium, lead, mercury, arsenic, and manganese were examined, with particular attention to biomonitoring matrices, exposure assessment, analytical variability, and interpretative pitfalls.

Results

Biological thresholds differ substantially according to their intended purpose, including occupational surveillance, environmental monitoring, surveillance of the general population, clinical diagnosis, and forensic interpretation. A clear distinction emerged between reference values, biological guidance values, biological limit values and toxicological cut-offs, which are frequently used interchangeably despite serving different scientific, clinical and regulatory purposes. Several critical issues emerged, including variability related to biological matrices, timing of sampling, chronic versus acute exposure, interindividual susceptibility, dietary and environmental confounders, laboratory analytical differences and the need to account for the effects of concomitant exposure to multiple toxic agents. Particular attention is devoted to the assessment of chronic low-dose exposures, below established threshold levels, as these may still represent a health risk, especially for more vulnerable population groups such as children and older adults. The availability of large-scale genetic and epigenetic studies offers new opportunities to improve our understanding of sensitivity and susceptibility conditions in specific population groups and even in individual subjects, thereby enabling the refinement of exposure thresholds toward more personalized and protective health-based standards.

Conclusions

Trace metal concentrations should not be interpreted as isolated numerical values detached from clinical, occupational, environmental, and forensic context. A multidisciplinary approach involving occupational physicians, toxicologists, and forensic experts is essential to avoid misinterpretation of biomonitoring data and inappropriate causal inferences. Greater harmonization of biological thresholds and increased awareness of their interpretative limitations are needed in both clinical and medico-legal practice.

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Tackling the effects of chronic human lifetime exposure to toxic metal(loid)s: can results from advanced LC studies contribute to predict disease onset?

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Background

Globally, 14-17% of agricultural soils are contaminated with potentially toxic metal(loid)s including arsenic, cadmium, lead, mercury nickel and copper.¹ The associated production of food crops will therefore inevitably expose certain human populations – including children - to elevated concentrations of these environmentally ubiquitous metal(loid)s over their lifetime. Considering that the adverse health effects and the possible onset of diseases are not well understood, one of the main problems that needs to be tackled in the postgenomic era is to better understand the toxicological chemistry of metal(loid) species that unfold at the bloodstream-organ nexus as they collectively determine disease onset.

Materials and Methods

We design and employ advanced LC approaches to untangle the bioinorganic chemistry of cadmium and other toxic metal(loid)s that unfold in blood plasma, red blood cells, and target organ cells. To this end, we employ different LC separation techniques, such as size-exclusion, anion exchange, and reversed phase chromatography to probe interactions of toxic metal(loid) species with relevant biomolecules, including plasma proteins and small molecular weight ligands using physiological buffers to simulate the conditions in these biological compartments.

Results

In this presentation recent results will be highlighted which provide a better understanding of the intricate bioinorganic chemistry of Cd^{2+} , Hg^{2+} , MeHg^+ and Ni^{2+} that unfold at physiological pH and/or in the bloodstream-organ system.

Conclusion

Tailoring LC approaches to unravel the bioinorganic chemistry of toxic metal(loid)s in the bloodstream-organ system emerges as a fruitful strategy to uncover the biomolecular processes which ultimately define their exposure-response relationships in humans.^{2,3} Results from these

studies represent an important starting point to reconsider current regulatory guidelines for toxic metal(loid) concentrations in baby foods⁴ to reduce the associated disease burden in vulnerable populations.

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Cadmium is not just Cd²⁺: the overlooked role of hydrolysis in environmental speciation and remediation

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Background Cadmium is a persistent environmental pollutant whose toxicity, mobility, and bioavailability are strictly related to its chemical speciation in aqueous media. Nevertheless, in many environmental and toxicological studies Cd²⁺ is often treated as a simple free aquo ion, while the contribution of hydrolytic equilibria is frequently underestimated. Although cadmium exhibits a lower tendency toward hydrolysis compared to highly charged metal ions, the formation of hydrolytic species under environmentally relevant conditions can significantly influence metal reactivity, transport, and interaction with natural or synthetic ligands. A proper understanding of Cd²⁺ speciation is therefore essential for the interpretation of cadmium behavior in aquatic systems and for the development of effective remediation strategies based on metal sequestration.

Materials and Methods The coordination behavior of Cd²⁺ in aqueous solution was investigated through a combined thermodynamic, spectroscopic, and computational approach. Potentiometric measurements were employed to determine protonation and complex formation equilibria, while UV–Vis and NMR spectroscopies were used to characterize metal–ligand interactions in solution. Density Functional Theory (DFT) calculations provided additional structural information on the coordination modes and stability of the formed species. Ligand systems based on kojic acid derivatives containing *O*- and *O/N*-donor groups were selected as representative chelating agents potentially applicable to cadmium sequestration processes.

Results The obtained data highlighted the strong influence of aqueous speciation on Cd²⁺ coordination behavior under environmentally relevant conditions. In particular, hydrolytic equilibria were found to play a non-negligible role in modulating metal availability and ligand binding processes, especially as a function of pH. The investigated kojic acid derivatives showed appreciable affinity toward Cd²⁺, forming stable species in aqueous solution. Spectroscopic and computational results supported the proposed coordination models and confirmed the involvement of donor oxygen and nitrogen atoms in metal binding. The combined experimental evidence emphasized that the effectiveness of chelating systems toward cadmium sequestration cannot be properly discussed without considering the contribution of hydrolytic and competing solution equilibria.

Conclusion The results underline the importance of considering Cd²⁺ aqueous speciation as a key factor in environmental and remediation studies involving cadmium. Even when not explicitly

addressed, hydrolytic equilibria may significantly affect cadmium reactivity, coordination behavior, and interaction with chelating agents. The integration of thermodynamic, spectroscopic, and computational approaches represents a valuable strategy for obtaining a more realistic description of cadmium chemistry in aqueous systems and for supporting the design of effective remediation methodologies.

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Vascular effects of exposure to accidental doses of cadmium in female rats - Protective effect of Red Quinoa Hydrolysate: a plant-based therapeutic alternative

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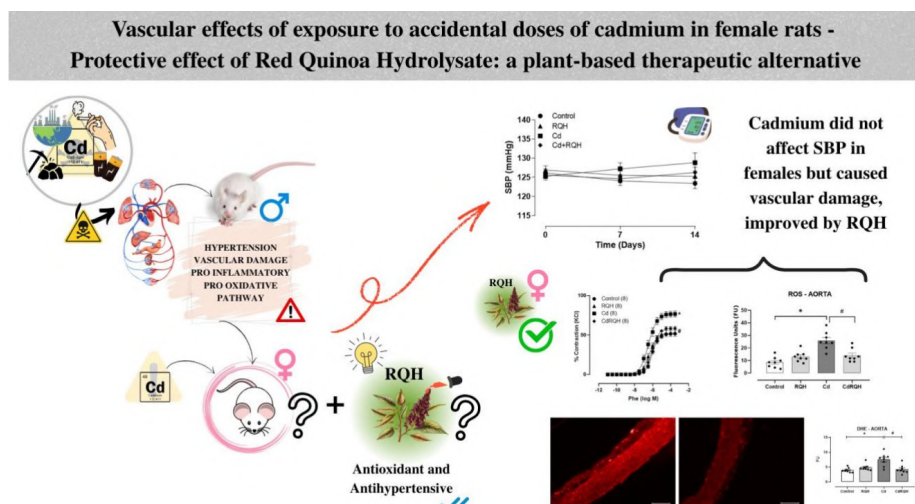
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Background Cadmium (Cd) exposure is associated with vascular dysfunction mediated by oxidative stress, reduced nitric oxide (NO) bioavailability, and increased inflammatory signaling. However, most available evidence derives from male models, and female responses to Cd may differ due to hormonal influences, particularly the modulatory effects of estrogens on vascular tone, redox balance, and inflammatory pathways. These sex specific mechanisms remain insufficiently explored. Plant based therapeutic strategies may represent a promising approach to mitigate Cd induced damage. Red quinoa hydrolysate (RQH), which exhibits antihypertensive and antioxidant properties, could provide vascular protection. This study investigated whether RQH protects the aorta of female rats from Cd induced injury. **Materials and Methods:** Female Wistar rats (12 weeks, approximately 300 g) were treated for 14 days and divided into four groups: Control (Ct): intraperitoneal (i.p.) distilled water plus tap water by gavage; Cadmium (Cd): CdCl₂ 1 mg/kg/day i.p. plus tap water by gavage; RQH: RQH 1 g/kg/day by gavage plus distilled water i.p.; and CdRQH: combined treatments (CEUA/Unipampa: 010/2023). Systolic blood pressure (SBP) was recorded weekly. Plasma estradiol levels were measured using an ELISA kit. Aortic vascular reactivity was evaluated in an isolated organ bath. Immunofluorescence analysis of the aorta was performed for NOX 1, COX 2, and TNF alpha. Data are expressed as mean ± SEM (two-way ANOVA, p < 0.05).

Results Cd exposure did not modify SBP but induced significant vascular alterations. Specifically, RQH co-treatment: a) maintained plasma estradiol levels at control values, preventing the Cd induced increase (E2 levels - Ct: 30.5 ± 2.6; RQH: 34.3 ± 1.9; Cd: 50.2 ± 3.2^{*}; CdRQH: 27.6 ± 1.4[#] in pg/mL, ^{*} vs Ct, [#] vs Cd); b) reduced Cd induced hypercontractility to phenylephrine (Rmax: Ct: 51.4 ± 2.2; RQH: 47.9 ± 2.7; Cd: 71.8 ± 3.0^{*}; CdRQH: 53.5 ± 1.7[#]; % KCl, ^{*} vs Ct, [#] vs Cd); c) prevented endothelial dysfunction and restored NO bioavailability; c) reduced COX 2 pathway contribution to vascular responses; d) preserved GPER mediated modulation of phenylephrine induced contraction, which was impaired by Cd exposure; e) prevented Cd induced redox imbalance; f)

decreased aortic fluorescence intensity of NOX 1, COX 2, and TNF alpha. Conclusion: Cd exposure induces vascular dysfunction in female rats without altering blood pressure and is associated with increased circulating estradiol, impaired GPER signaling, supporting a role for Cd as an endocrine disruptor with metalloestrogenic properties. RQH exerts antioxidant and anti-inflammatory actions, preserves GPER-dependent vascular responses, normalizes estradiol levels, and emerges as a promising plant-based therapeutic strategy against Cd-induced vascular damage in females.



Imaging-based fish cell micronucleus assay for trace metal genotoxicity: toward animal-reduced, high-throughput evaluation

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Background

Genotoxicity assessment is a cornerstone of chemical safety, including for trace metals where chromosomal breakage and mitotic missegregation must be detected reliably. We evaluated an imaging-based *in vitro* micronucleus (MN) assay using adult fish primordial germ cells (AF-PGCs) expressing H2B-eGFP to meet OECD TG 487 requirements while advancing animal-reduction goals. The AF-PGC system provides non-tumour, stable proliferation with low baseline MN rates and is amenable to high-throughput imaging in multiwell formats, supporting standardized, mechanistically informative testing. To contextualize trace metal applications, ammoniummetavanadate was included among positive controls in KCB-GFP experiments.

Materials and Methods

2 AF-PGC lines were seeded in 96-well plates and imaged on a Cytation 7 platform at 26 °C. Test designs followed MN assay principles: exposure to model substances, inclusion of medium/solvent and positive controls, and post-exposure imaging at 24–48 h to capture MN formed after mitosis. Assay validity and cytotoxicity were monitored by the relative increase in cell count (RICC); treatment groups with RICC below 50% were excluded from MN evaluation (threshold applied per well and per group). The positive-control panel comprised 4-nitroquinoline-N-oxide (NQO), griseofulvin and cyclophosphamide. Subsequent assays refined griseofulvin and NQO ranges and included ammoniummetavanadate as a trace metal.

Results

The aneugen griseofulvin produced clear MN induction with a defined non-cytotoxic window; RICC fell below 50% at 5 µg/ml, establishing 4 µg/ml as the highest reliable concentration for MN scoring and supporting a dose–response within acceptable cytotoxicity. For NQO a narrow non-cytotoxic window was detected: Only 0.03 µM yielded MN increases with marginally acceptable RICC, underscoring the risk of cytotoxic interference and a constrained effective range. While initial studies indicate metabolic competence of the AF-PGC lines, the pro-genotoxic compound cyclophosphamide did not significantly induce MN without exogenous enzymes, indicating a need for stimulation of metabolic pathways.

Conclusion

The AF-PGC H2B-eGFP MN assay enables high-content, 96-well imaging to detect aneugenic and clastogenic events with standardized validity criteria, showing strongest performance with aneugens such as griseofulvin. Ammoniummetavanadate was part of the KCB-GFP control battery, demonstrating assay integration of metal compounds within OECD 487-aligned designs. Furthermore, the platform promises advancements in the 3Rs by reducing reliance on animal-derived S9 and employing non-tumour, reproducible cell systems. Given its demonstrated integration of a metal compound control and sensitivity to mitotic disruption, this fish cell MN platform is well-positioned for genotoxicity evaluation of trace metals within OECD-aligned workflows, with future work focused on metabolic competence enhancements and expanded metal testing.





