SCIENTIFIC PROGRAMME

Saturday 4th March 2017

17.00 Registration and Poster Assembly

19.30 Welcome to Meeting / Welcome Buffet

Sunday 5th March 2017

The Conference is Open!

Session 1
The Environment

*Denotes presentation by a student.

Chair: *Xabier Lopez* (Euskal Herriko University, Donostia, Spain)

08.55 Introduction by the Chair

9.00 Platform 1
The unique inorganic chemistry of hydroxyaluminosilicates
Christopher Exley (Keele University, United Kingdom)

9.20 Discussion

9.30 Platform 2
Aluminium buffering in soil solutions
Igor Povar (Institute of Chemistry of the Academy of Sciences of Moldova)

9.50 Discussion

10.00 Platform 3
Aluminium effects on marine phytoplankton: Implications for a revised iron hypothesis – aluminium hypothesis
Linbin Zhou (Chinese Academy of Sciences, Guangzhou, China)

10.20 Discussion

10.30 **COFFEE**

10.50 Platform 4
Temporal changes in the acidification status of mountainous forest soils with an emphasis on aluminium
Ondřej Drábek (Czech University of Life Sciences Prague, Czech Republic)

11.10 Discussion

11.20 Oral Poster 1*
Behaviour of aluminium in forest soils with respect to content and speciation of low molecular mass organic acids (LMMOA)
Petra Hubová (Czech University of Life Sciences Prague, Czech Republic)

11.25 Discussion

11.30 Platform 5
Interaction of acidophilic microbial cells with dissolved aluminium and silica under anoxic conditions: A geochemical perspective with emphasis on biomineralisation
Javier Sánchez-Espaňa (Spanish Geological Survey, Madrid, Spain)

11.50 Discussion

12.00 Platform 6
Evaluation of aluminium mobility using fungal exometabolites and the application of this method to soils
Martin Urík (Comenius University in Bratislava, Slovakia)

12.20 Discussion

12.30 Oral Poster 2*
Evaluation of aluminium leaching from various natural and synthetic phases as affected by the presence of various species of *Aspergillus*
Filip Polák (Comenius University in Bratislava, Slovakia)

12.35 Discussion

12.40 Platform 7*
Exotic bamboo affects mineral dissolution in an acid soil of the savannas domain in Brazil?
Cristiane D. Sarmento (Federal University of Minas Gerais, Belo Horizonte, Brazil)

13.00 Discussion

13.10 **LUNCH**

Session 2
Plants

*Denotes presentation by a student.

[#] Denotes a 15+5 minute Platform Presentation

Chair: *Gea Guerriero* (Luxembourg Institute of Science and Technology, Luxembourg)

14.25 Introduction by the Chair

14.30 Platform 8*
Using two different fluors to identify the location of aluminium in plant cells
Jacqueline Cerdas-Solano (CICY, Mérida, México)

14.50 Discussion

15.00 Platform 9
The effects of aluminium toxicity on caffeine production and signal transduction mechanisms in cell suspensions of *Coffee arabica*
Teresa Hernández-Sotomayor (CICY, Mérida, México)

15.20 Discussion

15.30 Oral Poster 3*
Relationship between aluminium stress and caffeine biosynthesis in cell suspensions of *Coffee Arabica* L.
Roberto Pech-Kú (CICY, Mérida, México)

15.35 Discussion

15.40 Oral Poster 4*
Role of superoxide dismutase activity in aluminium tolerance in suspension cells of *Coffee Arabica* L.
Laura Esquivel-Hernández (CICY, Mérida, México)

15.45 Discussion

15.50 Platform 10*
Measuring extracellular trapping of metals by plant root border cells
David Huskey (University of Arizona, Tucson, USA)

16.10 Discussion

16.20 **TEA**

16.50 Platform 11
Proteomic changes in roots of *Urochloa decumbens* during the activation phase of aluminium tolerance
Charlotte Poschenrieder (Autonomous University of Barcelona, Spain)

17.10 Discussion

17.20 Platform 12#
A chimeric ALMT-type malate transporter shows enhanced response to aluminium and lanthanide ions
Takayuki Sasaki (Okayama University, Japan)

17.35 Discussion

17.40 Platform 13#
Avoidance mechanism involving ROS production in plant cells under aluminium stress
Yoko Yamamoto (Okayama University, Japan)

17.55 Discussion

18.00 Oral Poster 5*
A new mechanism of aluminium-induced cell death involving the vacuolar processing enzyme in both cultured-cell and root systems of tobacco
Koki Karia (Okayama University, Japan)

18.05 Discussion

18.10 Oral Poster 6
A positive relationship between aluminium in chloroplasts and the photosynthetic rate of an aluminium hyper-accumulator
Leide de Andrade (Embrapa Cerrados, Planaltina, Brazil)

18.15 Discussion

18.20 **END OF FIRST DAY**

20.15 **DINNER**

21.15 **Social Event**

Monday 6th March 2017

Session 3
Silicon

*Denotes presentation by a student.

Chair: *Ondřej Drábek* (Czech University of Life Sciences Prague, Czech Republic)

08.25 Introduction by the Chair

08.30 Platform 14
Silicon and plants: more than just a "tonic"
Gea Guerriero (Luxembourg Institute of Science and Technology, Luxembourg)

08.50 Discussion

09.00 Platform 15
Silica deposition in giant horsetail from the Peruvian Amazon
Christopher Exley (Keele University, United Kingdom)

09.20 Discussion

09.30 Platform 16
SISAFE® platform: an innovative nanoparticle drug delivery technology
Flavia Sutera (SiSaf Ltd. Belfast, United Kingdom)

09.50 Discussion

10.00 **COFFEE**

Session 4
Cell and Animal Models

*Denotes presentation by a student.

Chair: *Antonio BS Poleo* (Affiliation)

10.30 Platform 17
Tissue aluminium accumulation in the presence of silicon
Denise Bohrer (Federal University of Santa Maria, Brazil)

10.50 Discussion

11.00 Platform 18
Adjuvant effects from aluminum-containing vaccines are controversial enough: chronic dietary aluminum ingestion produces adverse adjuvant autoimmune inflammatory effects in the small intestine
Judie Walton (University of New South Wales, Australia)

11.20 Discussion

11.30 Platform 19*
Aluminum exposure for 60 days impairs spermatogenesis and sperm quality in rats
Caroline Martinez (Federal University of Pampa, Uruguaiana, Brazil)

11.50 Discussion

12.00 Oral Poster 7*
Cell-specific response to particulate matter: Potential role of metals such as aluminum, copper, and iron
Jaaziel Castro (Western University of Health Sciences, California, USA)

12.05 Discussion

12.10 Oral Poster 8*
***Ilex paraguariensis*: a potential antioxidant addressing aluminum toxicity in an experimental model of Alzheimer's disease**
Carla Alves (Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS), Sertão, Brazil)

12.15 Discussion

12.20 Platform 20
Progressive inflammatory pathology in the brain and retina of aluminium-fed 5xFAD transgenic mice
Yuhai Zhao (LSU Health Sciences Center, New Orelans, USA)

12.40 Discussion

12.50 Oral Poster 9*
Genome wide transcriptome analysis in the hippocampus of aluminium-treated rats
Yirong Xu (Shanxi Medical University, Taiyuan, China)

12.55 Discussion

13.00 Platform 21
A proposed approach to the assessment of aluminum burden in the central nervous system
John Savory (University of Virginia, Charlottesville, USA)

13.20 Discussion

13.30 **LUNCH**

FREE AFTERNOON/Excursion

20.00 **DINNER**

Tuesday 7th March 2017

Session 5
Human Exposure

*Denotes presentation by a student.

# Denotes a 15+5 minute Platform Presentation

Chair: *Teresa Hernández-Sotomayor* (CICY, Mérida, México)

8.25 Introduction by the Chair

8.30 Platform 22
Aluminium and breast cancer: an overview of the current status
Philippa Darbre (University of Reading, United Kingdom)

08.50 Discussion

09.00 Platform 23*
Breast cancer and the use of underarm hygiene products with aluminium-salts: A case control study
Caroline Linhart (Medical University of Innsbruck, Austria)

09.20 Discussion

09.30 Platform 24#
Physician versus scholar: the British controversy about aluminium in the 1930's
Florence Hachez-Leroy (Centre de recherches historiques (UMR 8558, EHESS/CNRS), Paris, France)

09.45 Discussion

09.50 Platform 25#
Magic bullet or snake oil? Aluminium dust and the prevention of silicosis in Western Australia, 1948-1963
Criena Fitzgerald (University of Western Australia, Perth, Australia)

10.05 Discussion

10.10 Platform 26#
The McIntyre powder project: A retrospective study of the health effects of respirable aluminium dust in a cohort of Ontario miners
Janice Martell (OHCOW, Ontario, Canada)

10.25 Discussion

10.30 **COFFEE**

11.00 Platform 27
In vivo measurement of aluminium in bone; recent experience and current capabilities
Fiona McNeill (McMaster University, Hamilton, Canada)

11.20 Discussion

11.30 Platform 28
Classical fluorescent molecular probes for the identification of aluminium and related neuropathologies in familial Alzheimer's disease (fAD) brain tissue
Matthew Mold (Keele University, United Kingdom)

11.50 Discussion

12.00 Platform 29
Association between H3K4me3/BDNF and cognitive function in workers occupationally exposed to aluminium
Qiao Niu (Shanxi Medical University, Taiyuan, China)

12.20 Discussion

12.30 Oral Poster 10*
Impact of occupational aluminium exposure on cognitive function and lymphocyte glutamate receptor protein
Pei Ren (Shanxi Medical University, Taiyuan, China)

12.35 Discussion

12.40 Platform 30#
Thomas M Riddick, an unsung pioneer of the aluminium age
Esko Meloni (Enopop,Tmi, Finland)

12.55 Discussion

13.00 **LUNCH**

Session 6
Human Exposure cont.

*Denotes presentation by a student.

All Platform Presentations in this final session are 15+5 minute talks.

Chair: *Philippa Darbre* (University of Reading, United Kingdom)

14.15 Introduction by the Chair

14.20 Platform 31
Mapping the affinity of aluminum to biomolecules, using a computational approach.
Xabier Lopez (Euskal Herriko University, Donostia, Spain)

14.35 Discussion

14.40 Platform 32
Assessing the solubility of aluminium adjuvants in the lysosomal compartment and the consequent impact upon the viability of phagocytic immune populations
Emma Shardlow (Keele University, United Kingdom)

14.55 Discussion

15.00 Platform 33*
Cognitive and behavioural studies in sheep over immunized with aluminium-containing vaccines
Javier Asín (University of Zaragoza, Spain)

15.15 Discussion

15.20 Platform 34
Transcriptomic analysis of aluminum effects on intestinal tissues from control and Crohn's disease patients.
Mathilde Body-Malapel (University of Lille, France)

15.35 Discussion

15.40 Platform 35
Aluminium, mercury and microRNA (miRNA) signalling in autism spectrum disorder (ASD)
Walter Lukiw (LSU Health Sciences Center, New Orleans, USA)

15.55 Discussion

16.00 Platform 36
Alum adjuvant neurotoxicity and its biodisposition assessment in mice following intramuscular injections using nanodiamond technology
Housam Eidi (UBC, Vancouver, Canada)

16.15 Discussion

16.20 Platform 37
Macrophagic myofasciits-associated cognitive dysfunction: A reappraisal of neuropsychological profile
Jérôme Authier (Paris Est-Creteil University, Creteil, France)

16.35 Discussion

16.40 **TEA**

Final Session Chair: *Christopher Exley* (Keele University, United Kingdom)

JD Birchall Lecture

17.25 **Introduction by Chair**

17.30 **Macrophagic myofasciitis: Aluminium hydroxide and susceptibility genes**
Romain Gherardi (Paris Est-Creteil University, Creteil, France)

18.20 Discussion

18.30 **CONCLUSION OF MEETING**

20.00 Conference Dinner

List of Additional Posters

*Denotes presentation by a student.

Poster 1
Distribution of aluminium soluble and insoluble, organic and inorganic chemical species in natural aqueous systems
Igor Povar (Institute of Chemistry of the Academy of Sciences of Moldova)

Poster 2
Relationship among the nitrogen source and aluminium toxicity in suspension cells of *Coffea arabica* L.
Muñoz-Sánchez J. Armando (CICY, Mérida, México) E-mail: arms@cicy.mx

Poster 3
NMDAR- ERK signal pathway mediates expression of H3K9ac, H3K9me2 in the hippocampus of chronically aluminium treated rats
Qiao Niu (Shanxi Medical University, Taiyuan ,China)

Poster 4*
Long-term studies in subcutaneous reactions following inoculation with aluminium-containing products in sheep
Javier Asín (University of Zaragoza, Spain)

Poster 5*
Human exposure to aluminium: How much aluminium is in our everyday diet?
Raquel Rodriguez (University of Toledo, Spain)

Poster 6
Non-linear dose-response of aluminium hydroxide adjuvant particles: selective low dose neurotoxicity
Housam Eidi (UBC, Vancouver, Canada)

Poster 7*
Characterization of substituent effects and binding features of different Al(III)-chelator complexes
Gabriele Dalla Torre (Euskal Herriko University, Donostia, Spain)

Poster 8
Predictive value of cerebral FDG-PET for diagnosing aluminium hydroxide-induced Macrophagic Myofasciitis (MMF)
Jérôme Authier (Paris Est-Creteil University, Creteil, France)

Poster 9
Aluminium exposure and markers of iron homeostasis in human breast cells *in vitro*
Philippa Darbre (University of Reading, United Kingdom)

Poster 10*
Aluminum exposure promotes vascular dysfunction and increases blood pressure in rats: a concerted action of NAD(P)H oxidase and COX-2
Caroline Martinez (Federal University of Pampa, Uruguaiana, Brazil)

Poster 11
A technique for *in vivo* bone aluminium measurement: present performance and prospects for a transportable system
Fiona McNeill (McMaster University, Hamilton, Canada)

Poster 12
Evaluation of aluminium levels in Alzheimer's Disease subjects involved in a ketogenic diet study
Angela Juby (University of Alberta, Edmonton, Canada)

Poster 13*
An in vitro model of the gastrointestinal absorption of aluminium in human infants.
Isabel Rodriguez Nunez-Milara (Keele University, United Kingdom)

PLATFORM 1

The unique inorganic chemistry of hydroxyaluminosilicates

Christopher Exley

The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, United Kingdom c.exley@keele.ac.uk

In 2017 at Keele12 I am celebrating 30 years since my personal discovery of the unique inorganic chemistry of hydroxyaluminosilicates. In marking this event I will present a rapid-fire history of their discovery in my laboratory and the various seminal moments which have slowly lead to our understanding of their structure, formation and significance. The latter may prove to be the antidote to living in the aluminium age!

Birchall JD, Exley C, Chappell JS & Phillips MJ (1989) Acute toxicity of aluminium to fish eliminated in silicon-rich acid waters. Nature, 338, 146-148.
Exley C & Birchall JD (1992) Hydroxyaluminosilicate formation in solutions of low total aluminium concentration. Polyhedron, 11, 1901-1907.
Exley C & Birchall JD (1993) A mechanism of hydroxyaluminosilicate formation. Polyhedron, 12, 1007-1017.
Exley C, Pinnegar JK & Taylor H (1997) Hydroxyaluminosilicates and acute aluminium toxicity in fish. Journal of Theoretical Biology 189, 133-139.
Doucet FJ, Schneider C, Bones SJ, Kretchmer A, Moss I, Tekely P & Exley C (2001) The formation of hydroxyaluminosilicates of geochemical and biological significance. Geochimica Cosmochimica Acta 65, 2461-2467.
Schneider C, Doucet F, Strekopytov S & Exley C (2004) The solubility of an hydroxy-aluminosilicate. Polyhedron 23, 3185-3191.
Strekopytov S & Exley C (2005) The formation, precipitation and structural characterisation of hydroxyaluminosilicates formed in the presence of fluoride and phosphate. Polyhedron 24, 1585-1592.
Strekopytov S & Exley C (2006) Thermal analyses of aluminium hydroxide and hydroxyaluminosilicates. Polyhedron 25, 1707-1713.
Exley C, Korchazhkina O, Job D, Strekopytov S, Polwart A & Crome P (2006) Non-invasive therapy to reduce the body burden of aluminium in Alzheimer's disease. Journal of Alzheimer's Disease 10, 17-24.
Strekopytov S, Jarry E & Exley C (2006) Further insight into the mechanism of formation of hydroxyaluminosilicates. Polyhedron 25, 3399-3404.
Davenward S, Bentham P, Wright J, Crome P, Job, D, Polwart A and Exley C (2013) Silicon-rich mineral water as a non-invasive test of the 'aluminium hypothesis' in Alzheimer's disease. Journal of Alzheimer's Disease 33, 423-430.
Beardmore J, Lopez X, Mujika JI and Exley C (2016) What is the mechanism of formation of hydroxyaluminosilicates? Scientific Reports 6:30913.

PLATFORM 2

Aluminium buffering in soil solutions

Igor Povar

Institute of Chemistry of the Academy of Sciences of Moldova, 3 Academiei str., MD 2028 Chisinau, Republic of Moldova, e-mail: ipovar@yahoo.ca

We established that the analyzed heterogeneous systems, involving *Al* minerals, manifest buffer actions towards all the soluble and insoluble components, including protons and aluminium ion. The analytical expressions for buffer capacities of all components have been derived. It is proved that the derived buffer capacities are mutually proportional and in the presence of solid phases (*Al* minerals) depend on their stoichiometric composition. We found that the presence of aluminium in soil solutions significantly increases the value of the buffer capacity towards proton. This essential conclusion has to be considered at the assessment of the soil buffer action, which increases in the presence of Al^{3+}, capable to form even in insignificant quantities the complex species. The derived expressions may be successfully used to estimate long-term chemical changes in soil solutions as a response of the soil environment to changes in atmospheric deposition and at technogenic loads increasing.

References:

1. Povar I., Spinu O. *Solv. Extr. Ion Exch.*, 2015, *33 (2)*, 196-209.
2. Povar I., Spinu O. *Ecol. Process.*, 2015, 4(1), 1-10.

PLATFORM 3

Aluminum effects on marine phytoplankton: implications for a revised Iron Hypothesis—Aluminum Hypothesis

Linbin Zhou[1,2,*] *, Yehui Tan*[1,2] *and Liangmin Huang*[1,2]

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[2] Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

(E-mail: zhoulb@scsio.ac.cn /zhoulinbin2004@163.com)

In contrast to substantial studies and established knowledge of aluminum (Al) effects (mainly toxicity) on freshwater organisms and terrestrial plants, and even on human health, only a few studies of Al effects on marine organisms have been reported, and our understanding of the role of Al in marine biogeochemistry is limited. In this presentation, we review the results of both field and laboratory experiments on the effects of Al on marine organisms, including Al toxicity to marine phytoplankton and the beneficial effects of Al on marine phytoplankton growth, and we discuss possible links between the Al effects and the efficiency of the biological carbon pump in the ocean. We propose a revised Iron Hypothesis, i.e., the Aluminum Hypothesis that Al as well as iron play an important role in the glacial-interglacial change in atmospheric CO_2 concentrations; Al could not only stimulate marine phytoplankton growth under conditions of phosphorus limitation, but it may also reduce the solubility of biogenic particles and make dissolved organic carbon more difficult to decompose. Further mesoscale iron and Al fertilization experiments to reveal the role of Al are suggested.

Acknowledgements

We thank Prof. Peter G.C. Campbell for discussing the whole idea of the presentation, and Prof. Peter G.C. Campbell and Prof. Claude Fortin for their support for my (Linbin Zhou) stay as a visiting scholar at the INRS-ETE. This work is supported by the National Key Basic Research Program of China (973 Program, 2015CB452904 and 2015CB452903), the National Natural Science Foundation of China (41130855; 41276162; 41506150), and the Natural Science Foundation of Guangdong Province, China (2015A030310169).

PLATFORM 4

Temporal changes of acidification status of mountainous forest soil with emphasis on aluminium

Sabina Chovancová[1], Václav Tejnecký[1], Monika Hradilová[1,2], Karel Němeček[1], Zuzana Michalová[3], Luboš Borůvka[1], Ondřej Drábek[1]

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[2] Agrovýzkum Rapotín Ltd., Výzkumníků 267, Vikýřovice, Czech Republic
[3] Department of Forest Ecology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague, Czech Republic

The aim of this contribution is to describe temporal chemical changes in the forest mountainous soils under three different vegetation covers between 2008 and 2013. Overall, soils were assessed as strongly acid and Al was the prevailing exchangeable cation. Organic horizons exhibited lower pH than mineral horizons indicating that they are more sensitive to external factors such as vegetation cover and atmospheric deposition.

The soil sorption capacity decreased with increasing depth. Mineral horizons were unsaturated by bases. In general, soils under beech forest and in clear-cut areas exhibited more favourable soil chemical characteristics compared to spruce forest soil, which showed the lowest soil pH, lowest base saturation and lowest Ca:Al ratio, and thus possesses the highest exchangeable acidity. Changes between the investigated years show an upward temporal trend in Al content and base saturation, and decreasing exchangeable acidity for each stand in organic horizons. On the contrary, mineral horizons showed ongoing soil acidification and leaching of base cations.

ORAL POSTER 1

Behaviour of aluminum in forest soils with respect to content and speciation of low-molecular-mass organic acids (LMMOA)

Petra Hubová[a], Václav Tejnecký[a], Michaela Češková[b], Ondřej Drábek[a], Luboš Borůvka[a],

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[b] Department of Forest Ecology, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6 - Suchdol, Czech Republic

The aim of this study was to determine the content, distribution and relationship between Al and LMMOA in soils under beech forest, and to assess the effect of herb vegetation on it.

Different soil horizons were sampled separately: organic L, F, H, organo-mineral A, and mineral B horizons. Al forms: available (Al_{H2O}; water extractable) and exchangeable (Al_{ex}; 0.1 M $BaCl_2$) and speciation of LMMOA in water extract were determined. Principal soil properties - pH, major cations and anions, dissolved organic carbon etc. were also determined.

The exchangeable soil reaction was the lowest in H horizon, there was also the highest amount of Al_{ex}. The highest content of LMMOA (lactate, oxalate) was found in all organic horizons. We can hypothesize that LMMOA mobilize Al from mineral fraction and this Al consequently occupies soil sorption complex. Generally we can conclude that LMMOA and thus soil vegetation cover play important role in Al soil cycle.

Acknowledgements

The research was supported by the Czech University of Life Sciences Prague (internal project No. CIGA 20152011) and internal grant provided by FAFNR, Czech University of Life Sciences Prague (No. SGS 21130/1313/3133).

PLATFORM 5

Interaction of acidophilic microbial cells with dissolved aluminum and silica under anoxic conditions: A geochemical perspective with emphasis on biomineralization

Javier Sánchez-España

Spanish Geological Survey (IGME), Calera, 1, Tres Cantos, 28760, Madrid, Spain

In low pH environments (e.g., volcanic crater lakes, acid pit lakes, acid mine waters), dissolved aluminium and silica may be present at very high concentrations. Under anoxic conditions, Al^{3+} contents can be high enough to be poisonous for many microorganisms, so its solubility and geomicrobial interactions becomes of major importance. The microscopic examination (FESEM-EDS, S/TEM-EELS, cryoTEM) of acidophilic microorganisms inhabiting deep anoxic environments of acid lakes reveals an important aluminium and silica incorporation to microbes by surface catalyzed precipitation and/or biosorption. The cells become encrusted by a nanometric layer of Al-Si amorphous material which is in turn rapidly coated by an external sphere of adsorbed ferrous iron. These Al bio-mineraloids may also incorporate Mg and important trace metals (e.g., Se, V, Cr, U). Whether this biomineralization is merely a passive process derived by cell properties or represents an adaptive mechanism of detoxification or nutrition is still to be explored.

PLATFORM 6

Evaluation of aluminium mobility using fungal exometabolites and application of this method on soils

Martin Urík[1,2], *Katarína Boriová*[2], *Marek Bujdoš*[1,2], *Peter Matúš*[1,2]
[1]Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 84215 Bratislava, Slovakia
[2]Slovak Spectroscopic Society, member of the Association of Slovak Scientific and Technological Societies, Mlynská dolina, 84215 Bratislava, Slovakia

Microorganisms in soil strongly affect mobility of metals. This fact is often excluded when mobile metal fraction in soil is studied using extraction procedures. Thus, we evaluated *Aspergillus niger*'s exometabolites contribution on aluminium mobilization. Fungal oxalic, citric and gluconic acids proved to be the most efficient agents in aluminium extraction from alumina. Furthermore, we simplified acquisition of equally efficient extracting mixture by chemically mimicking composition of main organic acid components of fungal exudates. This mixture was then successfully applied for aluminium extraction from soil samples and compared to standard single step extraction techniques. This showed there is at least 2.9 times higher content of mobile aluminium fraction in soils than it was previously detected, if contribution of microbial metabolites is considered in extraction procedures.

Acknowledgements:

The financial support was provided by grants VEGA 1/0203/14 and 1/0836/15, and Slovak Spectroscopic Society, member of the Association of Slovak Scientific and Technological Societies with following logo:

ORAL POSTER 2

Evaluation of aluminium leaching from various natural and synthetic phases as affected by presence of various *Aspergillus* species

Filip Polák[1,2]*, *Martin Urík*[1,2], *Marek Bujdoš*[1,2]

[1]Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 84215 Bratislava, Slovakia
[2]Slovak Spectroscopic Society, member of the Association of Slovak Scientific and Technological Societies, Mlynská dolina, 84215 Bratislava, Slovakia

This contribution investigates aluminium bioleaching efficiency from various resources, including alumina, gibbsite and other aluminium containing materials using fungal strains of *Aspergillus niger* and *A. clavatus*. Character of exudates produced by these species differed significantly. Thus, the concentration of aluminium in culture medium didn't overcome 3.3 $mg.L^{-1}$ in *A. clavatus* treatments which produced more alkaline metabolites, while *A. niger* increased aluminium concentration over 100 $mg.L^{-1}$. Bioleaching from gibbsite was lesser than 0.09%, because of its more rigid mineral structure compared to alumina. Application of various strains of *A. niger* showed that leaching efficiency from red-mud is relatively uniform and superior to *A. clavatus*. Hence, the aluminium bioleaching from studied materials proved efficient for *A. niger* and viable for application in bio-hydrometallurgy or waste management.

The financial support was provided by VEGA 1/0203/14 and 1/0836/15, and Slovak Spectroscopic Society, member of the Association of Slovak Scientific and Technological Societies with following logo:

PLATFORM 7

Exotic Bamboo Affects Mineral Dissolution in an Acid Soil of the Savannas Domain in Brazil?

Cristiane D. Sarmento[1], *Clésia C. Nascentes*[2], *Marcel G.C. França*[1]

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[2] Exact Science Institute, Chemistry Department, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil

The long-term establishment of the exotic *Phyllostachys aurea* in an acid soil in the Brazilian savanna domain results in modified assemblages of phytoliths as well as increases organic carbon stores and microbial biomass and activity. Soil pH is raised from 5.0-5.3 to 5.5-5.7 and this reduces the biological availability of aluminium to the bamboo. To try to understand the processes which underlie these effects, the soil was separated into fractions including labile organic carbon, fulvic and humic acids, humins and minerals and aluminium in each fraction was measured, using an S2 Picofox (Bruker). The majority of aluminium was found in the mineral fraction. Since this catchment area is composed of minerals such as feldspar, mica and quartz we hypothesized that microbial increases in CO_2 affected mineral dissolution[1] and favoured the formation of hydroxyaluminosilicates by the reaction of silicic acid with aluminium hydroxide[1,2].

Key-words: Brazilian Cerrado, soil physicochemical and biologic traits

References:

[1] **Exley C, Schneider C, Doucet FJ**. **2002**. The reaction of aluminium with silicic acid in acidic solution: An important mechanism in controlling the biological availability of aluminium? *Coordination Chemistry Reviews* **228**: 127–135.

[2] **Exley C**. **2007**. The Solubility of Hydroxyaluminosilicates and the Biological Availability of Aluminium. In: Letcher TM, ed. *Thermodynamics, Solubility and Environmental Issues*. Amsterdam: Elsevier B.V., 315–325.

Research funded by FAPEMIG (Foundation of Support to the Research of the State of Minas Gerais).

PLATFORM 8

The use of two different fluorochromes to study the localization of aluminium in plant cells

Cerdas-Solano Jacqueline[a], *Exley Christopher*[b] *and Hernández-Sotomayor S. M. Teresa*[a]

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Aluminium is the most abundant metal in the earth's crust and it's chemical form dependent on pH, where Al^{3+} is the most toxic form at pH<5.5 in acidic soils, affecting the optimal development of crops under these conditions and accumulated in root apices cells.

One of the methods to determine the presence of Al is using fluorescence. Morin (2´3´4´5´7´pentahydroxyflavone) and lumogallion [4-chloro-3-(2,4dihydroxyphenylazo)-2-hydroxybenzene-1-sulphonic acid] are fluorochromes and have a high affinity for Al^{3+}, identifying intracellular aluminium.

We showed the specifity signal in suspension cells of plants, with pH 4 and 7, in treatments with 100 and 500 µM of Al in current time, stained with both fluorochromes and comparisson between them. We demonstrated that the signalling to evaluate morin produces intense autofluorescence signals and can cause false positives, but with lumogallion demonstrated low signalling in autofluorescence, and in current time, the signalling increased with time and concentration of Al exposure.

Project funded by CONACYT (219893) to SMTH-S, and the scholarship number 89292 to JC-S.

PLATFORM 9

Study of the effect of Aluminium toxicity on caffeine production and signal transduction mechanisms in cell suspensions of *Coffea arabica*

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Aluminium (Al) is the most abundant metal on Earth. It represents 7% of the all elements. The toxicity produced by this metal is widely documented in tropical acid mineral soils. It is the major factor limiting the productivity of crop species. Coffee is one of the most important crops economically worldwide mainly due to the production of the secondary metabolite caffeine. This crop grows on acid soils where the existence of Al is greater. Therefore, coffee yield is limited by the toxic effects of this element. We have developed a biological model in which suspension cells of *Coffea arabica* have been used. We found that aluminium toxicity affected caffeine's production and signal transduction trough the production of different signalling molecules associated to the phosphoinositide signalling pathway. An overview of the latest results will be presented.

Research founded by CONACYT (219893).

ORAL POSTER 3

Relationship between aluminum stress and caffeine biosynthesis in cell suspensions of *Coffea arabica* L.

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Toxicity by aluminum is a growth-limiting factor in plants cultivated in acidic soils. This metal also promotes signal transduction pathways leading to biosynthesis of defense compounds, including secondary metabolites. Coffee trees are grown in acidic soil which frequently has aluminum toxicity. Little is known about the effects of aluminum on secondary metabolites biosynthesis. Therefore, the aim of this project was to study the relationship between $AlCl_3$ stress and caffeine biosynthesis, using as a model *in vitro* cell suspension of *C. arabica* L. Results suggest that exposure to cell of $AlCl_3$ 500 µM resulted in increased caffeine amounts, recovered both in cell (62%) and in spent medium (30%). This augmentation coincided with an increase in enzyme activity of caffeine synthase (CS) as well as the levels of transcripts of the gene encoding this enzyme (*CCS1).*

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ORAL POSTER 4

Role of superoxide dismutase activity in aluminum tolerance in suspension cells of *Coffea arabica* L.

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Aluminum toxicity modifies a wide range of physiological, cellular and molecular processes. Under normal conditions, cells generate reactive oxygen species (ROS), when these conditions change to induce an environmental stress, ROS production increases. In our laboratory, we have generated two *C. arabica* cell lines to study aluminum toxicity: a tolerant line (LAMt) and a sensitive one (L2). The mechanisms for aluminum tolerance in the LAMt line are yet not understood. The goal of this work was to evaluate whether the enzymatic activities of the different superoxide dismutase (SOD) isoenzymes modified specifically in response to aluminum stress. Accumulation of hydrogen peroxide (H_2O_2) in different intracellular compartments was also evaluated. A significant difference was found in total SOD activity levels in the cell lines and different SOD isoenzymes were identified in L2 and LAMt cells, after treatment with 100 μM $AlCl_3$. Furthermore, the intracellular H_2O_2 accumulation in response to treatment with $AlCl_3$ in L2 cells was higher compared to treated LAMt cells.

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PLATFORM 10

Extracellular trapping of metals by plant root border cells

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Most plant species produce specialized 'border cell' populations programmed to disperse from the root tip into the external environment.[1] Like neutrophil extracellular traps (NETs) in animals, [2,3] an extracellular DNA-based matrix produced by border cells traps pathogens and toxic metals to prevent infection and injury. In 2001, border cells of pea and snapbean were found to trap aluminum rapidly and thereby prevent uptake into the growing root.[4] Subsequent studies also have documented border cell extracellular trapping of arsenic, cadmium, lead, and other contaminants.[5] No reports, to date, have determined the amount of metal that border cells can trap. In preliminary tests, border cells from a single cotton or corn root were found to remove up to 85% of lead from a 1-ml sample (1.0 mM) during a 1-hour period of incubation. Defining the mechanism of trapping in plants as a model system may facilitate applications in medicine and agriculture.

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PLATFORM 11

Proteomic changes in roots of *Urochloa decumbens* during the activation phase of aluminium tolerance

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Expression of Al resistance in *Urochloa decumbens* requires a lag time and is preceded by an alarm phase characterized by growth inhibition [1, 2]. Comparative proteomics performed after 0, 24 and 96 h exposure to 200 µM Al revealed abundance differences in only 11 proteins; 6 were identified. During the alarm phase (24 h) phenylalanine ammonium liase (PAL), methionine synthase (MS), and deoxymugineic acid synthase (DMAS) decreased, while acid phosphatase (APase) increased. With recovered growth (96 h), PAL and MS returned to initial levels. Carbonic anhydrase (CA) and adenylate kinase (AK) were much more abundant. Changes during the alarm phase indicate enhanced phosphorus mobilization and downregulation of iron acquisition and phenolic biosynthesis. After recovering, biosynthesis of phenolics and methionine, but not Fe mobilization were restablished. Enhanced dark fixation of CO_2 and higher AK abundance indicate increased organic acid formation and better provision of ADP and Mg^{2+} to ATP synthase, respectively.

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PLATFORM 12

A chimeric ALMT-type malate transporter shows enhanced response to aluminium and lanthanide ions.

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Aluminium (Al)-activated malate transporters of wheat and *Arabidopsis* (TaALMT1 and AtALMT1) confer Al tolerance by releasing malate from roots. Our recent electrophysiological study showed that Al could activate TaALMT1 function in *Xenopus* oocytes, but not AtALMT1 function. In the study, the chimeric proteins, Ta::At and At::Ta, were also created by swapping N- and C-terminal half domain regions of TaALMT1 and AtALMT1, and only Ta::At chimera exhibited Al activation. In this report, we further analyzed the transgenic cultured-tobacco cells expressing the Ta::At chimera and native ALMT proteins, and focused on the sensitivity and specificity of their activation by trivalent cations. The Al-activated malate efflux was two-fold greater in the chimera than native proteins. The chimera also exhibited higher malate efflux activated by several lanthanides than natives. These results suggest that the Ta::At chimera protein acquires enhanced response to a range of trivalent cations compared to the native proteins in plant cells.

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PLATFORM 13

Avoidance mechanism of ROS production under aluminum stress in plant cells

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Aluminum (Al) ion is a major constraint on crop productivity in acidic soils, where Al ion attacks elongating cells specifically at root apical meristem of seedlings, leading to elongation inhibition and cell death, accompanying the production of reactive oxygen species (ROS). In order to elucidate cellular and molecular mechanisms of the Al toxicity at root apex, we have investigated cultured tobacco cell lines at logarithmic phase of growth as a model system of the meristematic cells. Compared to SL (wild-type cell line), ALT301, Al-tolerant cell line isolated from SL, rarely produces ROS under Al stress, and also exhibits tolerance to hydrogen peroxide and ferrous iron[1-3]. Several analyses including metabolomic profiling and microarray suggest that the avoidance mechanism of ROS production in ALT301 seems to be based on two mechanisms, an enhancement of antioxidant systems and a suppression of the energy metabolism in mitochondria which produces ROS during the oxidative phosphorylation.

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ORAL POSTER 5

A new mechanism of aluminium-induced cell death involving vacuolar processing enzyme in both cultured-cell and root systems of tobacco

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Free aluminium (Al) ion is a main factor which causes plant growth inhibition in acidic soils. Vacuolar processing enzyme (VPE) localized in vacuole is reported to be necessary to induce hypersensitive cell death in tobacco (*Nicotiana tabacum L.*) after infection of tobacco mosaic virus[1]. VPE is a protease which exhibits the same substrate specificity as animal caspase-1, but is structurally unrelated. The enhancement of VPE activity is due to an induction of gene expression, instead of modification of the VPE protein itself. In this study, we examined the Al-induced cell death process in cultured tobacco cell line BY-2 and in seedlings of tobacco (cv. Bright Yellow), focusing on VPE. The results indicate that enhancement of *VPE* gene expression occurs before a start of cell death in cultured cells[2] or simultaneously with cell death in roots of tobacco, suggesting that VPE could be an execution factor of cell death under Al stress.

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ORAL POSTER 6

A positive relationship of Al in chloroplast with the photosynthetic rate of an Al-hyperaccumulator Cerrado native plant species

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Plants differ in their ability to tolerate high concentrations of metals in the environment, as aluminium (Al). The Brazilian native species *Qualea grandiflora* (Vochysiaceae) (Qg) presents levels above 0.1% Al kg^{-1} dry matter. We investigated the effects of Al in photosynthetic rate in leaves of Qg, in which Al accumulates in chloroplasts, after 160 days of growth in complete nutrient solution with 0 µM or 150 µM Al, pH 4.5. In seedlings subjected to Al in solution, the photosynthetic rate was 4.24 ± 0.95 µmol CO_2 m^{-2} s^{-1}, significantly higher than in plants without Al, 1.41 ± 0.38 µmol CO_2 m^{-2} s^{-1}. In these conditions, the presence of Al in the chloroplast did not affect the plant shoot growth or the performance of photosynthesis in Qg plants. A higher photosynthetic rate in seedlings grown with Al in solution suggests a physiological role of Al in the chloroplast metabolism of Qg.

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PLATFORM 14

Silicon and plants: more than just a "tonic"

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Silicon (Si) is non-essential for plants, however, when supplied, it improves plant vigour and stress response. The beneficial role of Si for plants is extensively reported in the literature (e.g. Fauteux et al. 2006; Detmann et al. 2013). Its protective role is proposed to be due to the effects on the cell wall, both direct and indirect (reviewed by Guerriero et al. 2016). Cell wall macromolecules template biosilicification (Law and Exley 2011), but Si can also indirectly act on the metabolic branch(es) involved in cell wall biosynthesis (e.g. Fleck et al. 2011; Yamamoto et al. 2012). These metabolic changes trigger modifications in the cell wall structure/composition which ultimately contribute to the improved resistance to plant stresses. In this presentation the current knowledge concerning the relationship between Si and cell wall components/metabolism will be discussed. Future possible lines of research will also be proposed.

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PLATFORM 15

Silica deposition in giant horsetail from the Peruvian Amazon

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There is a horsetail, *Equisetum myriochaetum*, which can grow up to 7m high. Anthropologist, Mike Paul, who studies the indigenous peoples of the Peruvian Amazon, told me about the giant horsetail and I was intrigued to find out if, like its diminutive British counterpart, *Equisetum arvense*, it also deposited silica in its tissues. Mike brought me back some fronds and stems of the giant horsetail from Peru and we used microwave-assisted acid digestion to separate the silica from these tissues. We very quickly learnt that these giants of the horsetail family were silica rich and we used both PDMPO fluorescence and SEM to reveal the incredible nature of this heavily silicified plant.

PLATFORM 16

SISAFE® platform: an innovative nanoparticle drug delivery technology

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The acne market has huge unmet needs in terms of efficacy and safety. Presently marketed drugs are not capable of curing the disease completely - they only reduce lesions by approximately 50% and do not suppress their re-occurrence. Adverse reactions associated with both oral and topical treatments for acne skin disorder indicate that none of the available products offers a completely safe profile.
SiSafe® is a drug delivery technology based on bio-adaptive nano-silicon that combines biocompatibility and biodegradability [1] with the ability to deliver actives to the hair follicles.
An anti-acne formulation based on SiSafe® was tested on Grade II and Grade III acne patients over a period of 30 days. The results showed a reduction of up to 95% of facial acne vulgaris. In safety and toxicity tests, the formulation also achieved the best possible results across all human safety markers [2-5].

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PLATFORM 17

Tissue aluminum accumulation in the presence of silicon

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The bioavailability of Al in the presence of Si was investigated using a model animal. Male adult Wistar rats (8-week old) were divided in 4 groups: control, Al, Si, and Al+Si, with 10 individuals each. Solutions, including saline used in the controls had their pH adjusted to 7. The doses, consisting of 0.5 mg/kg/day Al and 2 mg/kg/day Si, were intraperitoneally administered for 3 months. Tissues were analyzed not only for their Al and Si content but also for lipidic peroxidation and δ-ALA-D activity. Tissue digestion was optimized for the determination of Al and Si in the same sample by Graphite Furnace Atomic Absorption Spectrometry. The presence of Si reduced in 73% the Al present in the liver, 45% in the kidneys, 16% in the bones, and 30% in the blood, suggesting that it may act as a protector against Al toxicity, by either reducing Al absorption or increasing its excretion.

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PLATFORM 18

Adjuvant effects from aluminum-containing vaccines are controversial enough: chronic dietary aluminum ingestion produces adverse adjuvant autoimmune inflammatory effects in the small intestine

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A study, based on rats that developed Alzheimer's disease-equivalent dementia (ADED) in old age, and age-matched controls, examined tight junction proteins, in enterocytes that mostly form the small intestine barrier, for possible causes of enhanced aluminum absorption that could account for elevated aluminum levels occurring in plasma/serum, brain and cerebrospinal fluid of AD patients. Abnormal localization of immunostained small intestine tight junction proteins (zonula occludens-1, claustrin-3 and occludin) coincides with gaps between small intestine enterocytes. Chronic aluminum ingestion, mainly in the form of additives to food and alum-treated water, disregulates small intestine iron metabolism, showing inflammation, edema, elevated levels of cytotoxic T-lymphocytes, and several modes of cell death; effectively evidence of aluminum adjuvancy in small intestine. This study indicates increased small intestine leakiness constitutes an early event in AD and that Alzheimer's disease is systemic. Comparable changes in elderly humans may also explain some gastrointestinal disorders of hitherto unknown cause.

PLATFORM 19

Aluminum exposure for 60 days impairs spermatogenesis and sperm quality in rats

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Human exposure to Aluminum (Al) is inevitable, and its real consequence perhaps unknown. While a good deal of research has been conducted on the acute reproductive effects of Al, little is known about the effects of longer-term exposure. Moreover, due to the non-linear dose-response effect of Al, is urgent to investigate the effects of Al exposure at a human dietary level and then to compare with Al effects at high levels. Herein we investigated the effects of Al exposure at three different doses: two low doses representing human Al exposure by diet and, one model of exposure at high level known to produce toxicity, and then we have compared these results. Three-month-old male *Wistar* rats were divided into two major groups: 1) low aluminum levels, and 2) a high aluminum level. Group 1 rats were treated orally by drinking water for 60 days as follows: a) control – received ultrapure drinking water; b) aluminum at 1.5 mg/kg b.w. and c) aluminum at 8.3 mg/kg b.w. Group 2 rats were treated through oral gavages for 42 days as follows: a) control – received ultrapure water; b) aluminum at 100 mg/kg b.w. We analyzed sperm parameters (daily sperm production per testis, sperm number, transit time in epididymis, morphology and motility), biomarkers of oxidative stress in testis, epididymis and prostate, testis and epididymis histology and immunohistochemistry and, Al content in reproductive tissues. Al treatment even at low doses impaired spermatogenesis and sperm quality, increased reactive oxygen species and lipid peroxidation, altered the antioxidant capacity and induced an inflammation pattern testicular with an increase on macrophage activation. Our data demonstrate that 60-day subchronic exposure to low doses of Al from feed and added to the water, which reflect human dietary Al intake, reaches a threshold sufficient to promote male reproductive dysfunction. Based on the pro-oxidant actions of Al, we decided to investigate the effects of egg white protein hydrolysate (EWH), obtained after enzymatic hydrolysis with Pepsin and with known antioxidant properties, on reproductive effects caused by subchronic Al exposure. For this, Al-exposed rats were co-treated with 1 g/kg/day of EWH during the same period of Al exposure. Surprisingly, EWH prevented the male reproductive dysfunction as well as reduced the Al content in testis and epididymis showing an ability to counteract the toxic effects of the Al.

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ORAL POSTER 7

Cell-Specific Response to Particulate Matter: Potential Role of Metals Such as Aluminum, Copper, and Iron.

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Exposure to ambient particulate matter (PM) has been linked to cardiovascular and neurological diseases. These disorders are associated with enhanced oxidative stress and inflammatory events. Hypothesis: based on cell type and metal content, particles will differentially impact cells. Three cell types: human monocytes, astrocytes, and neurons were exposed to particles to evaluate cell-specific effects. PM composition included several metals. The highest levels were in aluminum, copper, iron, and lead. Cell viability, reactive oxygen species (ROS) formation, and tumor necrosis factor alpha (TNF α) were measured. After exposure to PM, ROS production was unchanged, but TNF-α levels significantly increased, particularly in human monocytes. Particles activated inflammatory parameters in immune-competent cells independent of metal content. Because of the association between heightened inflammatory responses and disease processes, it may be advisable to minimize exposure to particles by avoiding rigorous outdoor activities at peak pollution times.

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ORAL POSTER 8

Ilex paraguariensis: potential antioxidant on aluminum toxicity, in an experimental model of Alzheimer's disease

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Ilex paraguariensis, a native plant to Brazil, has importance in culture and economy. It known has antioxidant potential; this could help reduce risk of developing neurodegenerative diseases. Other hand, the plant has high Aluminium levels that recognized potential neurotoxic and promotes neurological disorders, such AD. In this study was evaluated the potential of *I. paraguariensis* in prevention of AD, Al concentration and their effect in AD, through the analysis of behavioural parameters of nematode *Caenorhabditis elegans*. After long-term exposure to Al and plant extract, results indicated that altered behavior of *C. elegans*. 5,5 mg/L of Al concentration inhibited the behavioural parameters. Moreover, the extract obtained at 65ºC promoted negative result; however, the extract obtained at 75ºC presented neuroprotector role to *C. elegans*. Thus, suggesting potential effect protector of 75ºC extract, but, Al exposure and the presence of metals in plant extract obtained at 65ºC shows capacity to promote AD progression.

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PLATFORM 20

Progressive inflammatory pathology in the brain and retina of aluminum-fed 5xFAD transgenic mice

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Approximately 60 murine transgenic models for Alzheimer's disease (Tg-AD) have been developed that overexpress the 42 amino acid amyloid-beta (Aβ42) peptide in the central nervous system (CNS). These 'humanized murine Tg-AD models' have significantly expanded our understanding of the contribution of Aβ42 peptide-mediated pro-inflammatory and amyloidogenic neuropathology to the AD process. Several laboratories using different amyloid-overexpressing Tg-AD models have independently reported that the supplementation of murine Tg-AD diets and/or drinking water with aluminum (sulfate) significantly enhances Aβ42 peptide-mediated inflammatory pathology, amyloidogenesis and AD-type cognitive change compared to age-matched controls. In humans AD-type neuropathology appears to originate in the limbic system and progressively spreads into primary processing and sensory regions such as the primary visual cortex and the retina. For the first time, here we assess the propagation of Aβ42 peptide-mediated amyloidogenesis and pro-inflammatory gene expression (at the level of miRNA, mRNA and protein) in the neocortical-thalamic-retinal visual pathway of 5xFAD Tg-AD amyloid-overexpressing mice whose diets were supplemented with aluminum (sulfate). Methods: 5xFAD Tg-AD murine models, RNA sequencing, GeneChip (microRNA and mRNA), RT-PCR, LED-Northern, Western, ELISA and bioinformatics analysis. The three most significant findings were **(i)** in aluminum-supplemented animals, markers for inflammatory neuropathology appeared in both the brain and the retina as evidenced by an evolving presence of Aβ42 peptides; **(ii)** increases in Aβ42 abundance were accompanied by the up-regulation of several pro-inflammatory markers including cyclooxygenase-2 (COX-2) and C-reactive protein (CRP); and **(iii)** that as similarly reported in other Tg-AD murine models, there was a significantly accelerated development of Aβ42-mediated inflammatory neuropathology in 5xFAD Tg-AD mice fed aluminum. Taken together the results indicate that in the 5xFAD Tg-AD model aluminum not only enhances an Aβ42-mediated inflammatory neurodegeneration in the brain but also significantly induces AD-type neuropathology in anatomically-linked primary sensory areas that involve the acquisition and processing of visual signals.

Acknowledgements
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ORAL POSTER 9

Genome wide transcriptome analysis in Hippocampus of Aluminum-treated Rats

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Aluminum is a widely exposed neurotoxicant. However, the molecular mechanism underlying Aluminum toxicity remains to elucidate. In order to find the key transcription changes in Aluminum-induced neurotoxicity. RNA-seq in hippocampus of Aluminum-treated rats was performed using Illumina HiSeq platform. cDNA libraries were constructed from six rats' hippocampus (3 in control group and 3 in aluminum-exposed group). 96 up-regulated and 652 down-regulated Differentially expressed genes (DEGs) were identified. GO analysis results showed that multiple functional genes are most significantly affected by aluminum exposure, including glial cell differentiation, neural transmission and vesicle trafficking. Furthermore, KEGG pathway analysis results revealed that these DEGs were clustered in several signal pathway, including ECM-receptor interaction; PI3K-Akt signaling pathway; Focal adhesion and cAMP signaling pathway. Taken together, expression profiling identified that the genes involved in glial cell differentiation, cell adhesion and vesicle trafficking were candidate genes to be used in the research on aluminum induced neural toxicity.

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PLATFORM 21

A proposed approach to the assessment of aluminum burden in the central nervous system

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Assessment of body burden of trace metals has proved to be a difficult task. Direct measurement of the metal in body fluids has been helpful in some instances such as lead poisoning, but often blood or urine analysis has been of little value. Zinc status is a prime example where plasma zinc is essentially worthless but a more meaningful measurement has been the protein, metallothionein, which in zinc deficiency is reduced in plasma and urine. I hypothesize that aluminum overload, which has been shown in experimental animals to mimic most of the neuropathological and biochemical changes seen in Alzheimer's disease, also leads to many specific protein changes. Such a response could have been developed during evolution to protect the central nervous system against this highly neurotoxic element which is ubiquitous in the environment. New experiments could involve injecting minute amounts of aluminum maltolate into rabbit brain, coupled with the application of time of flight mass spectrometry for proteomic studies, and the measurement of RNA biomarkers in brain tissue and body fluids such as CSF and blood.

PLATFORM 22

Aluminium and Breast Cancer: an overview of the current status

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Application of aluminium-based antiperspirant salts has been embraced globally as a way of reducing sweating in the underarm region but without any prior knowledge of the long-term consequences. Coincidentally, the upper outer quadrant of the breast has become the site of highest incidence of both benign and malignant breast diseases. Measurements in human breast tissue, breast cyst fluid and nipple aspirate fluid have demonstrated the presence of aluminium in the human breast. Studies in cell culture demonstrate that aluminium can enable development of several of the hallmarks of cancer, including anchorage-independent growth, genomic instability, cell migration and invasion. More recently published studies demonstrate that exposure of non-transformed breast epithelial cells to aluminium in cell culture leads to enhanced tumour growth and metastasis when the cells are injected into mice. This lecture will provide an overview of research findings, and will identify data gaps and regulatory needs.

PLATFORM 23

Breast cancer and the use of underarm hygiene products with aluminium-salts: A case control study.

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Previous epidemiologic studies concerning breast cancer and antiperspirant use have shown conflicting results. Therefore, we designed a hospital based case-control study including interview data and bio-samples.
Antiperspirant use and clinical records were compared between 209 women suffering from breast cancer and 209 age-matched healthy controls. Aluminium concentrations in bio-samples were measured in a subgroup of 100 cases and 52 controls.
Case-control comparisons confirmed established risk factors for breast cancer such as positive family anamnesis. A univariate significant relationship between antiperspirant application and breast cancer was identified for an intensive use under the age of 30, doubling the risk for breast cancer (p=0.045). Median (interquartile) aluminium concentration in breast tissue was 5.8 nmol/g (2.3-13.1) in cases and was significantly lower in controls (3.8 nmol/g, 2.5-5.9, p=0.0013).
First results show some indications of differences in self-reported underarm cosmetic application and aluminium concentration of tissue samples between cases and controls. These results are preliminary and await thorough multivariate analysis.

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We like to thank all medical students involved in the project: Johanna Kowalski, Silke Regensburger, Dominik Panosch, Carolin Buddensick, and Franziska Weidenbeck. Special thanks also to the secretary of the breast ambulance, Alfred Wieser.

PLATFORM 24

Physician versus Scholar: the British Controversy about the Aluminium in the 1930's

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In the 1930's in Great Britain, a scientific controversy about aluminium took place. The origin of the crisis was the publication of a book, entitled "The Danger of Contamination by Aluminium". The author was not an academic researcher, but a physician. His methodology was nevertheless thoughtful: he started with the observation of his patients, and performed experiments with the help of a scientist from a laboratory in London. His book caused many negative reactions in the community of scholars but received also positive feedbacks, particularly from other physicians. This book had a huge influence on the debate about aluminium in Great Britain.

We will first examine the nature of the players involved in the controversy. We will then analyse the arguments and references used by each one, and particularly a reference book published with the support of the aluminium companies. Finally, we will compare with a similar case in United-States concerning the aluminum baking powder.

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PLATFORM 25

Magic bullet or snake oil? Aluminium dust and the prevention of silicosis in Western Australia, 1948-1963

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In 1948, the Western Australian government amended the Mining Act to legislate for aluminium dust to be dispersed to underground miners to prevent silicosis. Although all mining companies understood that the suppression of quartz dust and good ventilation underground prevented silicosis, the industry favoured a 'magic bullet', to reduce the costs of mining and improve the productivity of their workforce and increase profits. Aluminium therapy was one such 'magic bullet', that would, they hoped, reduce their production costs and the costs of compensation for occupational diseases. Public health doctors viewed the treatment as 'snake oil', and as effective, but until the establishment of the Occupational Health Department, they had no professional leverage over the Mines Department regarding silicosis prevention. This paper examines the introduction of aluminium dust into Western Australian mines, the response of workers and the attempts by physicians to 'enlighten' the Mines Department and industry about its efficacy.

PLATFORM 26

The McIntyre Powder Project: A retrospective study of the health effects of respirable aluminum dust in a cohort of Ontario miners

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1. Occupational Health Clinics for Ontario Workers, Inc.

Between 1943 and 1980, at least 20,000 miners were treated prophylactically with McIntyre Powder – a finely ground, respirable dust comprised of 85% aluminum oxide and 15% elemental aluminum. No other group has been exposed to aluminum in this form, intensity, duration, or by similar route of administration (an inhalable, airborne suspension). The only two clinical studies ever conducted on this specific group of workers both supported putative neurologic effects of McIntyre Powder exposure. Over an 18-month period, an informal voluntary registry of 322 exposed workers was compiled by the daughter of a McIntyre Powder-exposed miner – 65% of exposed workers had respiratory diagnoses or symptoms, and 33% had neurological disorders or symptoms. Based on these preliminary findings, a database of exposed mine workers is being compiled by the Occupational Health Clinics for Ontario Workers to investigate the causal relationship between McIntyre Powder exposure and adverse health outcomes.

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PLATFORM 27

In vivo measurement of aluminium in bone: recent experience and current capabilities

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Recently, we demonstrated that aluminium can be measured in bone painlessly and non-invasively in people, using *in vivo* neutron activation analysis on McMaster's Tandetron accelerator. We measure aluminium and calcium in the bones of a person's hand. This is convenient for the person being measured, results in a low radiation dose and allows the measurement to be reported in micrograms aluminium per gram of calcium.

We recently completed a pilot study which compared the aluminium to calcium ratio in a group of people suffering from Alzheimer's disease to that in a control group. We found a statistically significant difference in Al/Ca bone content between the groups, even though neither group showed raised levels compared to published literature values.

We will review the technique and methodology, the radiation dose in comparison to other clinical techniques and discuss the pilot study outcomes. We describe the potential of this technique to assess aluminium accumulation in different exposure situations.

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PLATFORM 28

Classical fluorescent molecular probes for the identification of aluminium and related neuropathologies in familial Alzheimer's disease (fAD) brain tissue

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Autosomal dominant or familial Alzheimer's disease (fAD), most often occurs through genetic mutations in the amyloid protein precursor (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2), genes [1]. Such genetic predispositions give rise to a rare and aggressive form of the disease, characterised through an earlier age of onset (< 65) [2] and pathologically defined via Congophilic amyloid angiopathy (CAA), intracellular neuritic tangles and extracellular senile plaque deposition in human brain tissue. Aluminium has been implicated as an aggressor driving disease aetiologies in neurodegenerative disorders, including Alzheimer's disease. The concomitant presence of aluminium in tissues of patients diagnosed with fAD has only recently been investigated [2], in which the quantitative technique of graphite furnace atomic absorption spectroscopy (GFAAS), revealed some of the highest aluminium contents ever observed (36.56 μg/g dry wt.) in comparison to a previous study of 60 human brains (*ca* 1.00 μg/g dry wt.) [3]. Using lumogallion as a complementary and unequivocal fluorescent molecular probe for aluminium [4,5] the study demonstrated focal deposits of aluminium in all lobes of every brain investigated [2].

It remains unknown however, as to how aluminium may contribute towards the pathogenesis of fAD. A lack of data demonstrating the concomitant and unequivocal presence of the metal ion with neuropathologies *in vivo* may explain the absence of defined mechanisms driving disease progression. Herein, classical fluorescent molecular probes aim to identify aluminium and related neuropathologies including senile plaque deposition in fAD brain tissue. Autofluorescence from brain tissue in the absence of additional stains or fluorophores frequently hampers the detection of true fluorescent signals of the moieties of interest. In order to improve the sensitivity of the staining methods employed, quenching agents have been utilised to improve the detection of aluminium and associated neuropathologies, above background. Such improvements may aid to increase the signal to noise ratio, providing clear distinctions between aluminium and biological injury, *in vivo*.

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PLATFORM 29

Association between H3K4me3/BDNF and the cognitive function of workers occupationally exposed to Aluminum

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BDNF is involved in synaptic plasticity and may be modified by H3K4me3, thus affecting learning and memory. 235 aluminum-exposed male workers were recruited. Cognitive tests were performed. Plasma aluminum concentration was tested by GFAAS. The subjects were divided into three groups by the 25, 50 and 75 percentile of the blood aluminum concentration. The lymphocyte H3K4me3 contents and plasma BDNF were determined by ELISA. The plasma aluminum levels were 100.19, 134.36 and 178.96μg/L respectively. The cognitive indices MMSE, DSFT, DST scores of high blood aluminum concentration group were lower than those of other two groups ($P<0.05$), and expression of H3K4me3 and BDNF of it were lower than those of the low($P<0.05$) and middle blood aluminum groups ($P<0.05$). Multiple correlation analysis showed that blood aluminum concentration was negatively correlated to H3K4me3, BDNF, MMSE, DSFT, DST ($P<0.05$). **Conclusion:** aluminum impairs cognitive function, decreases of lymphocyte H3K4me3 level and plasma BDNF expression.

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ORAL POSTER 10

Impact of occupational aluminum exposure on cognitive function and lymphocyte glutamate receptor protein

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A number of researches had revealed that occupational aluminum exposure would impair workers' cognitive function, Glutamate receptors are role points in learning and memory function. 121 potroom workers, 231 no-aluminium exposed workers were included in this study. Using Mini-Mental State Examination, Clock-Drawing Test, Digit-Span Test, Verbal Fluence Test, Fuld Object-Memory test to evaluate cognitive function. Plasma aluminum measured by GFAAS. Using ELISA to assay receptors protein. All objects were divided into control, low-, medium-, high-exposure group. The scores of short time memory in MMSE,DST and VFT were shown significantly decreasing. With the increasing of plasma aluminum, NR1, NR2A showed decreasing trend, but mGluR1 showed increasing trend. The correlation coefficient indicated that NR1,NR2A had positive correlation with cognitive tests. Above all, NR1 and NR2A protein in lymphocytes could research as surrogate biomarkers that indicate impairment of cognitive functions of occupational aluminum exposure.

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PLATFORM 30

Thomas M Riddick, an unsung pioneer of the aluminium age

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In 1968 Thomas M. Riddick, an eminent water chemist from New York, after studying colloids for more than 10 years, wrote and published a book on colloid stability (1). The book has a long "closing chapter" on cardiovascular disease. Riddick himself had a long history of heart problems. He was applying his expertise on blood.
He developed an instrument, the sclerascope, and a method for assessing the state of a person's blood by observing the arterioles and venules of his/her eyes. With a magnification of 20 – 60 times the flow and state of intravascular coagulation (IVC) of individual blood cells becomes visible. The degree of IVC of the blood is evaluated on a scale of 0 – 6 (0: free flow, 6: pronounced stasis and clumping).

Applying the Schulze-Hardy rule on coagulation and dispersion of colloids, he was looking for a multivalent anion for reducing the blood's tendency to coagulate. Riddick ended up with potassium citrate, and he developed a "Regimen" for the heart consisting of potassium citrate and potassium bicarbonate dissolved in distilled water. It was to be consumed 1 – 1.5 litres daily.

He established a test group consisting of a dozen persons. They consumed the heart water for two years under the control of doctors and chemists. The conditions of the participants' blood and heart were improved without exception. The IVCs improved 1 – 2 points on the 0 – 6 scale, arrhythmia of the heart was diminished, as well as false heartbeats. Riddick himself, with a ten-year history of one attack of paroxysmal tachycardia per month, was completely "cured" except for two episodes.

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PLATFORM 31

Mapping the affinity of aluminum to biomolecules, using a computational approach.

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The increased availability of aluminum in biological environments, due to human intervention in the last century, raises concerns on the effects that this so far "excluded from biology" metal might have on living organisms. Consequently, the bioinorganic chemistry of aluminum has emerged as a very active field of research. However, the experimental determination of structure and affinities of Aluminum-Bioligand complexes is not without difficulties and theoretical methods have emerged as a fundamental tool to unveil aluminum biochemistry. In the present talk I will review some of the recent advances made by our group on this field. In particular we will show how computational methods (DFT, QM/MM and classical molecular dynamic simulations) can determine the relative affinity of aluminium towards potential biological chelators, and shed light on the type of biological compounds prompt to interaction with aluminium.

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PLATFORM 32

Assessing the solubility of aluminium adjuvants in the lysosomal compartment and the consequent impact upon the viability of phagocytic immune populations.

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Aluminium salts are routinely included within clinical vaccinations containing recombinant antigens and act to potentiate the immunological response to these weakly immunogenic components. While previous studies have shown stark differences in the cytotoxicity induced by two commercial aluminium adjuvants,[1] the precise mechanism responsible for these events is still unclear. Adjuvant preparations were exposed to an artificial lysosomal fluid medium and their relative solubility determined by an established filtration-GFAAS methodology. Amorphous adjuvants generated a higher amount of biologically reactive aluminium over the period of study in comparison to their crystalline counterparts. Furthermore, links were established between the solubility of aluminium salts within the lysosomal compartment and the viability of phagocytes *in vitro*. The relative benignancy of crystalline materials, at least in terms of acute cytotoxicity, could serve to explain their heightened biopersistence and systemic translocation.

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PLATFORM 33

Cognitive and behavioral studies in sheep intensively immunized with aluminium-containing vaccines

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The use of aluminium-containing vaccines has been associated with adverse reactions in sheep including acute nervous signs related to meningoencephalitis and chronic weight loss with neurodegenerative changes, all these changes being part of the ovine Autoimmune/inflammatory Syndrome induced by Adjuvants (ASIA syndrome). Moreover, ASIA-affected animals show an array of typical behavioural changes although they have not been fully proved or characterized. Three groups (n=7 each) of breed and sex-matched three-month-old lambs were established and maintained in identical conditions of housing, management and diet along 12 months. Group A received a total of 16 doses of commercial aluminium-adjuvant containing vaccines; group B received the adjuvant alone with the same amount of Al^{3+} and group C received PBS. Cognitive and behavioural tests (T-maze, open field, novel object, recordings for behavioural observations) were performed together with welfare blood panels. Vaccinated and adjuvant-inoculated groups showed i) a significant increase in aggressive behaviours and stereotypies, ii) a significant decrease in affiliations, especially in winter time and iii) higher levels of stress biomarkers in winter. These results highlight worse welfare indicators both in over-vaccinated and aluminium-adjuvant inoculated animals and they can explain the behavioural changes seen in ASIA-affected animals.

PLATFORM 34

Transcriptomic analysis of aluminum effects on intestinal tissues from control and Crohn's disease patients.

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Aluminum occurs naturally in the environment and is also released due to anthropogenic activities such as mining and industrial uses in the production of aluminum metal and other aluminum compounds. A variety of aluminum compounds are produced and used for different purposes, such as in water treatment, papermaking, fire retardant, fillers, food additives, colors and pharmaceuticals. Aluminum metal, mainly in the form of alloys with other metals, has many uses including in consumer appliances, food packaging and cookware. Therefore, ingestion of aluminum from both the diet and drinking water is a common form of human exposure. The health-based guidance value established by the European Food and Safety authority (EFSA) of 1mg/kg bw/week is exceeded by 0,2% of adults and 1,6% of children in France, in a non-negligible part of the population in Canada and European countries and in almost all the population in China, considering here food as the sole source of exposure to aluminum.
Inflammatory bowel diseases (IBDs), which include Crohn's disease and ulcerative colitis, are chronic diseases characterized by an excessive uncontrolled intestinal inflammation resulting from an abnormal immune response to commensal microbiota in a susceptible host. The role of aluminum as an environmental risk factor for IBD has been revealed. Indeed, the intestinal epithelium is the first physiological barrier that aluminum meets after ingestion. Oral bioavailability, namely, the part of aluminum which is absorbed through the gut and reached the systemic circulation, is very low and considered to be between 0.1 and 1%. Therefore, the majority of ingested aluminum remains associated with the gut. Moreover, oral administration of low dose of aluminum in mice worsens intestinal inflammation and delays mucosal repair in experimental models of colitis, favoring intestinal barrier dysfunction and granuloma formation. The aim of this study was to assess the transcriptomic response of Crohn's disease and control colon biopsies to aluminum.

Methods:
Human colon samples from Crohn's disease patients and control patients have been collected. They have been treated during 3h with aluminum citrate (100 µg/ml). Total RNA has been extracted. The RNA quality and concentration have been determined by measuring the absorbance ratios 260/280 nm and 260/230 nm using a Nanodrop ND-1000 (NanoDrop Technologies) and a 2100 bioanalyser (Agilent Technologies).
Human Whole Genome Agilent 44K 60-mer oligonucleotide Microarray has been performed according to the Two-Color Microarray-Based Gene Expression protocol (Agilent Technologies). Microarrays have been scanned using the Agilent scanner G2505C and Feature Extraction software (v10.5). Data have been processed with the GeneSpring (v10) for normalization, filtering, and statistical analysis. The genes upregulated or downregulated with a ≥ 1.5 fold-change and with statistical significance ($P < 0.05$) were sorted using asymptotic P value computation and Benjamini Hochberg false discovery rate multiple testing corrections.

PLATFORM 35

Aluminum, mercury and microRNA (miRNA) signaling in autism spectrum disorder (ASD)

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Extensive use of advanced LED-Northern assays, miRNA- and messenger RNA (mRNA)-based expression arrays, reverse transcription polymerase chain reaction (RT-PCR), short post-mortem interval human brain tissues, luciferase reporter vector-transfection assays, Western-, ELISA-, electrothermal atomic absorption spectroscopy (ETAAS) and bioinformatics analysis has enabled us to identify a small group of up-regulated miRNAs and their mRNA targets that are significantly down-regulated in autism spectrum disorder (ASD). Two of these up-regulated, inducible and pro-inflammatory miRNAs, miRNA-34a and miRNA-146a appear to target and down-regulate SHANK3, a multi-domain synapse and scaffolding protein whose deficits are strongly linked to the acquisition of the ASD phenotype. Interestingly, in contrast to several other known neurotoxins, two environmentally-abundant neurotoxic metals, aluminum (Al) and mercury (Hg) were found to drive miRNA-34a and miRNA-146a upregulation and down-regulate SHANK3 expression in human brain cell cultures and/or C57BL/6J murine models. The data suggest that aluminum and mercury, either alone or together, are two environmental factors capable of driving the altered miRNA-regulated expression of pro-inflammatory, immune and synaptic genes such as SHANK3 resulting in the acquisition of the ASD phenotype. Here for the first time we: **(i)** have analyzed the molecular-genetics and gene expression patterns at the level of miRNA, mRNA and expressed protein in human ASD, in human primary brain cells treated with Al and/or mercury, and in control C57BL/6J mice and mice transgenic for ASD (Tg-ASD); **(ii)** have studied these factors in human ASD brain anatomical regions targeted by the ASD process and in human primary neuronal-glial cell co-culture models for ASD; and **(iii)** have analyzed the contribution of these neurotoxic factors in C57BL/6J control and Tg-ASD murine models and in animals exposed to Al and/or Hg in their food and drinking water. These studies have specifically accentuated the role of miRNA-34a and miRNA-146a in regulating SHANK3 and SHANK-related signaling. These studies have not only expanded our mechanistic understanding of ASD but have also advanced and developed improved murine models to further elucidate the molecular-genetics of this incapacitating human neuropsychiatric disorder.

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PLATFORM 36

Alum adjuvant neurotoxicity and its biodisposition assessment in mice following intramuscular injections using nanodiamond technology

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Fluorescent nanodiamonds (fNDs) have a specific and perfectly photostable fluorescence allowing their microspectrometric detection in tissues at low levels. In this study, the biodisposition of aluminum oxyhydroxide (alum) was tracked by fNDs. Specificities of alum/fNDs (AluDia) complexes were comparable to the whole reference vaccine (anti-hepatitis B vaccine) in terms of particle size and zeta potential.
Our data show that alum biodisposition and neurotoxicity depend on mouse strain, alum concentration and its administration route. An easy and early detection of AluDia in deferent organs including the brain was observed in male C57BL/6J mice i.m injected by 400 µg/kg of alum. In contrast, i.m injection of 400 µg/kg of alum in female CD1 mice showed for the first time a markedly delayed translocation of alum without any increase in Al brain content and behavioral changes. Additionally, when these mice subcutaneously injected by a lower dose of alum (200 µg/kg) showed an early brain translocation and neurotoxic effects.
Careful attention should be paid to those parameters in future studies focusing on the potential toxic effects of aluminium-based adjuvants.

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PLATFORM 37

Macrophagic myofasciits-associated cognitive dysfunction: A reappraisal of neuropsychological profile

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Patients with long-lasting aluminium hydroxide-induced macrophagic myofasciitis (MMF) present with diffuse arthromyalgias, chronic fatigue, and cognitive disorder. One hundred five unselected consecutive MMF patients were subjected to neuropsychological evaluation and brain MR imaging (MRI). From descriptive analysis, 64/105 (61%) patients had fronto-subcortical cognitive deficits with pathological results at executive functions and selective attention tests (performances below -1.65 SD). Among them, 24 had pathological results in storage and consolidation functions for episodic verbal memory, and 9 left ear extinction at dichotic listening test, in addition du fronto-subcortical impairment. Remaining 41 patients (39%) displayed performances above pathological threshold in all tests. However, inter-test analysis showed that these patients performed significantly worse to executive functions and attention tests compared to others. None of the patients had instrumental dysfunction. Brain MRI analysis did not show specific features associated with cognitive impairment.

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JD BIRCHALL LECTURE

Macrophagic myofasciitis: Al hydroxide and susceptibility genes

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Vaccines have allowed containment or eradication of several life-threatening infectious diseases during the past century but are also increasingly suspected to induce overt and hidden immunological and neurological adverse effects. Aluminium oxyhydroxide (Alhydrogel®), and aluminium hydroxyphosphate (Adjuphos®), are nanomaterials, with a crystalline and platy appearance, respectively, widely used as immunologic adjuvants of human and animal vaccines. Series of patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) temporally linked to multiple Al-containing vaccine administrations have been reported in at least 7 different countries. In France and Portugal, where muscle biopsy is routinely performed in the deltoid muscle, such patients were found to bear a specific muscle lesions, called macrophagic myofasciitis (MMF), assessing longstanding persistency of aluminium hydroxide agglomerates within innate immune cells at site of previous immunization. The clinical condition has been called "autoimmune/inflammatory syndrome induced by adjuvants"(ASIA) by Yehuda Shoenfeld in 2011. It mainly manifests by arthro-myalgia, marked chronic asthenia, and cognitive dysfunction, including attention and memory impairment, sleep disturbances and a stereotyped pattern of cerebral hypometabolism at neuroimaging (see FJ Authier presentation).

Reassuring claims on aluminium oxyhydroxide innocuity appear to be mainly based on fundamental misconception of its biodisposition and pharmacokinetics and short-term clinical studies. In mouse models, the compound, as other poorly biodegradable particles, is promptly phagocytozed in the injected muscle and disseminates within phagocytic cells to lymphoid organs and then throughout the body, and eventually accumulates in the brain long after injection. Dissemination of the adjuvant is driven by the CCL2/MCP1 (Monocyte chemoattractant protein-1) signalling. An intriguing result recently came from a dose-response study showing that low doses of the compound, uniquely forming easy-to-capture small bacteria-size agglomerates, selectively induce cerebral Al accumulation and long-term neurotoxicity in mouse (see H. Eidi presentation). These results suggest that capture and long-term aluminium oxyhydroxide biopersistency within phagocytic cells may be a prerequisite for its neuromigration and neurotoxicity.

Xeno/autophagy is instrumental in intracellular solubilisation of mineral particles and detoxification. It is involved in many crucial functions in the immune and central

nervous systems potentially relevant to both desirable and adverse effects of aluminium adjuvants. Promising preliminary data have been obtained by DNA screening (in collaboration with Baharia Mograbi, IRCAN, Nice University, France): analysis of 104 single nucleotide polymorphisms (SNPs) in the 34 genes governing xeno/autophagy yielded 7 SNPs located in 6 genes ($P < 0.0001$ to 0.04) that were significantly associated with MMF-ASIA (n=365 patients) compared to normal controls of the "1000 genome project". There appeared to be a cumulative effect, more than 1 SNP being found in 93% of patients versus 14% of healthy subjects. These results may be used to set up genetic tests to predict an increased risk to develop adjuvant intolerance, and offer a nearly unique opportunity to decipher the "genes x environment interactions" paradigm underpinning ME/CFS, a serious disease currently affecting 1 to 4 millions of people in USA.

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POSTER 1

Distribution of aluminium soluble and insoluble, organic and inorganic chemical species in natural aqueous systems

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In order to better understand the effects of acid precipitation on soil solutions and natural waters (named here as natural aqueous systems, **NAS**) and predict the toxic concentration of soluble aluminium species, it is essential to have an efficient tool to forecast how various species of *Al* will respond to changes in the **NAS** chemical composition. In this paper, a thermodynamic approach for the complex chemical equilibria analysis has been developed and applied to heterogeneous systems, containing aluminium minerals (as solid species) in **NAS**. This approach uses thermodynamic expressions joined to unusual mass balance equations, where all the components of both phases are explicitly expressed. The distribution of aluminium soluble and insoluble, organic and inorganic chemical species, based on the constructed diagrams of heterogeneous chemical equilibria, as functions of chemical composition and temperature of analyzed natural systems, are found to be in a good agreement with the current experimental evidence [1-4].

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POSTER 2

Relationship among the nitrogen source and aluminium toxicity in suspension cells of *Coffea arabica* L.

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The presence of aluminum toxicity in the form of Al^{3+} is affecting the production of many crops growing in acid soils. Additionally the availability of minerals decreases in the solution soil when pH is less than 5.5. Nitrogen is a mediating factor in agricultural crops' productivity, where the predominant form of Nitrogen is nitrate. However, in acid soils with high organic matter, the ammonia could be the most Nitrogen source. Then, it is important to determine the Al-Nitrogen source relationship and its effect on the development of crops with high economic impact, such as *Coffea arabica* crops. The aim of this project was to evaluate how different Nitrogen sources in the culture medium affects cell growth in the presence of Al^{3+} in suspension cells of *C. arabica*. Results will be presented regarding the protein profile and the activity of the nitrate reductase a key enzyme in the nitrogen cycle.

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POSTER 3

NMDAR- ERK signal pathway mediates Expression of H3K9ac, H3K9me2 in the hippocampus of chronically aluminum treated rats

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Histone modification of NMDAR, Phospho-Erk, H3K9ac, H3K9me2 and related regulation of HP1, BDNF affect learning and memory. 24 healthy SPF grade SD male rats were randomly divided into four groups by weight, which drank water containing different doses of aluminum chloride($AlCl_3$) (0, 2, 12, and 72mg/kg Al^{3+}) for 120d, Expressions of NMDAR,P-ERK,H3K9ac,H3K9me2,HP1, and BNDNF were detected with western blot. The expressions of NMDAR in the Al-exposed groups were significantly lower than that of the control group ($P<0.05$), and P-ERK, H3K9ac, and BDNF in them were significantly lower than those of the control group too ($P<0.01$), but the expressions of H3K9me2 and HP1 in them were both significantly higher than those in the control group ($P<0.05$). [Conclusion] Chronic aluminum exposure may change the histone modification via inhibiting the signal pathway of NMDAR-ERK, and therefore impair the ability of learning and memory in rats.

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POSTER 4

Long-term studies in subcutaneous reactions following inoculation with aluminium-containing products in sheep

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In sheep, aluminium in vaccines is related to local inflammatory reactions whose evolution is generally monitored for just a few months to assess vaccine safety. In order to study these reactions and their long-term evolution three groups (n=28 each) of three-month-old lambs were used. Group A was subcutaneously inoculated with aluminium hydroxide-containing vaccines, Group B received the adjuvant alone with equal amount of Al^{3+} and Group C received PBS. A total of 19 inoculations were performed in 15 months in each group. Local reactions were periodically assessed in vivo by palpation. Post mortem studies included gross and microscopic pathology, microbiology, scanning transmission electron microscopy (STEM) and energy-dispersive X-ray (EDX) spectroscopy. Reactions consisted in sterile foreign body granulomas, they appeared in vaccinated (100%) and aluminium-inoculated (85.7%) animals and they were more severe and persistent in vaccinated animals. Reactive macrophages in granulomas contained aggregates of a spiculated material that was identified as aluminium. In sheep aluminium-induced granulomas are persistent and accumulate to previous vaccinations. These persistent granulomas might be related to some of the previously-described adverse events included in the ovine ASIA syndrome.

POSTER 5

Non-linear dose-response of aluminium hydroxide adjuvant particles: selective low dose neurotoxicity.

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Concerns about the safety of aluminium-based adjuvant (ABA) Alhydrogel® emerged following recognition of unexpectedly long-lasting biopersistence of their agglomerates within immune cells. ABA nanofibers spontaneously aggregate and exhibit a slow transportation from injected muscle to the brain in mice. The present study was aimed at evaluating mouse brain function and aluminium (Al) concentration long after intramuscular injections of various doses of ABA (200 to 800 µg Al/kg of body weight). An unusual neuro-toxicological pattern limited to the lowest dose of Alhydrogel® was observed at 200 µg Al/kg long-term Al cerebral accumulation and neurotoxic effects were observed. Limited particle size seems to be critical for these neurotoxic effects, presumably by favouring capture and transportation of bacteria-sized agglomerates by monocyte-lineage cells. These data suggest that *in vivo* neurotoxicity of ABA obeys the specific rules of particle toxicology rather than those of classical toxicology, making over simplistic the reassuring contention "the dose makes the poison" in this field.

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POSTER 6

Characterization of Substituent Effects and Binding Features of Different Al(III)-Chelator Complexes

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Here we present a theoretical approach based on *state-of-the-art* Density Functional Theory calculations and Bader's Quantum Theory of Atoms In Molecules (QTAIM) analyses. The protocol was applied to the investigation of three families of bidentate chelating agents that show high affinity toward aluminum[1]: catechols, salicylic acids and hydroxy-pyridine-carboxylic acids (HPCs)[2,3].

The aim of the work is to carefully characterize the effect of different substituents, such as Electron Donating Groups (EDGs, CH_3 and OCH_3) and Electron Withdrawing Groups (EWGs, NO_2), toward the modulation of the Al-ligand binding affinity. Depending on the stoichiometry of the complexes, we observed *intra*- or *inter*-ligand substituent effects.

Besides, by means of the Bader's QTAIM, we found that there is a small degree of covalency in these mainly electrostatic closed-shell interactions, which can be modulated by the opposite *intra*-ligand effect of EDGs and EWGs.

The present findings would provide a valuable help in the design of new, suitable Al(III) chelating agents.

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POSTER 7

Predictive value of cerebral FDG-PET for diagnosing aluminium hydroxide-induced Macrophagic Myofasciitis (MMF)

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Positron Emission Tomography with ^{18}F-fluorodeoxyglucose (FDG-PET) appears to be efficient to identify a cerebral substrate in patients with known MMF. We present here the case of a patient referred for diffuse arthromyalgias and cognitive impairment, which occurred few years after aluminium hydroxide-adjuvanted vaccine injections. While first deltoid muscle biopsy was unremarkable, cerebral FDG-PET revealed the typical spatial pattern of a cerebral glucose hypometabolism involving occipital cortex, medial temporal areas and cerebellum. Given the clinical suspicion of MMF and FDG-PET findings, a second deltoid muscle biopsy was performed and confirmed the diagnosis of MMF with lesions assessing abnormal long-term persistence of aluminium hydroxide within macrophages at the site of previous immunization. This case highlights the predictive value of cerebral FDG-PET as a potential non-invasive tool for MMF diagnosis, even when muscle biopsy result comes back negative. Further studies are warranted to confirm our findings in a large prospective design.

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POSTER 8

Aluminium exposure and markers of iron homeostasis in human breast cells *in vitro*

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Aluminium (Al) has been previously measured at increased concentrations in nipple aspirate fluids taken from women with breast cancer compared to those without, and a positive correlation was reported between the levels of Al and the iron-binding protein ferritin in the breast cancer-affected nipple aspirate fluids. In order to further investigate the nature of this relationship, the effects of Al exposure on ferritin levels have been investigated using cultured human breast cells. Results will be reported of the effects of Al exposure times from 1 week up to 30 weeks using human breast cancer cells (oestrogen responsive MCF-7, oestrogen unresponsive MDA-MB-231), immortalised non-transformed human breast epithelial cells (MCF12A) and human mammary fibroblast cells (HMF-3A). Effects on levels of NDRG-1 (N-myc Downstream Regulated Gene-1) which acts as an iron-regulated metastasis suppressor will also be reported. The results show that exposure to Al can alter levels of ferritin in breast epithelial cells and suggest that the previous observations *in vivo* in nipple aspirate fluids may have a mechanistic basis.

POSTER 9

Aluminum exposure promotes vascular dysfunction and increases blood pressure in rats: a concert action of NAD(P)H oxidase and COX-2

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Aluminum (Al) is a significant environmental contaminant. This non-essential metal has been related with several diseases, mainly age-related neurological changes which oxidative and inflammatory disorders are the postulated toxicity mechanisms. At cardiovascular system, there is not enough evidence of Al induced toxicity. We aimed to investigate the effects of a 60-day Al exposure at doses similar to human dietary levels on cardiovascular system. 20 three-month-old male *Wistar* rats (± 300 g) were divided into two groups and received for 60 days in drinking water: a) Control - ultrapure water; b) $AlCl_3$ - aluminum chloride at a dose of 8.3 mg/kg bw. Systolic blood pressure (SBP) was measured by plethysmography. Vascular function was studied in aortic and mesenteric resistance arteries (MRA) in isolated organ bath. Concentration-response curves to acetylcholine (ACh) and sodium nitroprusside were performed. Vasoconstrictor response to phenylephrine (PHE) in presence and absence of endothelium and in presence of NOS inhibitor (L-NAME), potassium channels blocker (TEA), NAD(P)H oxidase inhibitor (apocynin), superoxide dismutase (SOD), non-selective COX inhibitor (indomethacin), selective COX-2 inhibitor (NS 398), and AT_1 selective receptor blocker (losartan), were analyzed. Systemic and vascular reactive oxygen species (ROS), lipid peroxidation and total antioxidant capacity, were measured. The mRNA expression analysis of eNOS, NAD(P)H oxidase 1, SOD 1, COX-2 and thromboxane A2 (TXA-2) receptor were investigated. Results were expressed as mean and SEM, compared by t–test and ANOVA followed by Bonferroni test (*$P<0.05$). Ethics Committee Approval 028/2014 - Unipampa. $AlCl_3$-exposure at low doses increased SBP (Ct: 120.3 ± 1.17 *vs* Al:126.9 ± 1*mmHg, n=8), decreased ACh induced concentration-dependent relaxation, increased vasoconstrictor response to PHE (MRA - Rmax % to KCl Ct: 111.7 ± 5.4 % *vs* $AlCl_3$: 126.1 ± 11.4 %*, Aorta - Ct: 89.7 ± 17.4 % *vs* $AlCl_3$: 111 ± 22.4 %* n=10), decreased the endothelium vasoconstrictor – modulation, nitric oxide (NO) bioavailability, potassium channels involvement, as well as increased ROS production from NAD(P)H oxidase and contractile prostanoids mainly from COX-2. Al exposure at low levels increased plasmatic and vascular ROS production and lipid peroxidation as well as altered the antioxidant status in plasma, aorta and MRA. Al decreased mRNA expression of eNOS and SOD 1 and stimulated the NAD(P)H oxidase 1, COX-2 and TXA-2 expressions. Our results point to the excess of ROS mainly from NAD(P)H oxidase after aluminum exposure and the increased vascular prostanoids from COX-2 acting in concert to decrease NO bioavailability, thus inducing vascular dysfunction and increased blood pressure. Therefore, the 60-day chronic exposure to $AlCl_3$, which reflect common human dietary Al intake, appears to poses a risk for cardiovascular system.

POSTER 10

A Technique for *in vivo* bone aluminium measurement: present performance and prospects for a transportable system

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At McMaster University, the technique of neutron activation analysis is used to measure aluminium in the bones of a person's hand non-invasively and painlessly. The present system uses the $^{7}Li(p,n)^{7}Be$ nuclear reaction on a Tandetron accelerator as a neutron source. After a short irradiation and transfer, the activity in the person's hand is counted in a highly sensitive array of NaI(Tl) detectors. The current detection limit is 7.6 μg Al per g Ca. A limitation on the widespread use of this technique is that a person has to visit our accelerator laboratory.

An alternative neutron source would be to use the $^{2}H(^{2}H,n)^{3}He$ reaction with a D-D neutron generator. Such a generator is relatively compact and nearly an order of magnitude cheaper than the Tandetron. However, a question that must be addressed is whether neutrons from a D-D generator are energetic enough to give rise to an interfering reaction with phosphorus in the bone, $^{31}P(n,\alpha)^{28}Al$. This interfering reaction is theoretically possible, but the current literature is not clear regarding its potential significance.

This presentation will contrast and compare the both neutron source techniques and present a discussion of the potential for interfering reactions through a review of available data. We will identify both further data needs and possible mitigation strategies.

POSTER 11

Evaluation of aluminium levels in Alzheimer's Disease subjects involved in a ketogenic diet study

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Background
Alzheimer's Disease (AD) is a common neurodegenerative disease, and the prevalence is increasing with the ageing demographic. The aetiology of AD is unknown, but some known risk factors include vascular disease and heavy metal toxicity (including mercury and aluminium).

Methods
Subjects enrolled in an AD study evaluating the impact of MCT oil ingestion, had baseline aluminium evaluation. Each has the chance to consume a silicon-rich water for the 6 month open label part of the study.

Results
The study is ongoing. Of the 20 participants, 11 are men. Age ranges from 54-85 years. Baseline MMSE ranged from 10-29, and MoCA ranged from 4-27. Baseline aluminium levels averaged 44nmol/mmol creatinine, but ranged from 8.4-208.

Conclusion
There is a large diversity in baseline aluminium levels in these AD patients. Ongoing evaluation will determine the impact of intake of silicon-rich water on cognitive function, especially in those with high baseline levels.

POSTER 12

An *in vitro* model of the gastrointestinal absorption of aluminium in human infants.

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Humans are exposed to aluminium on a daily basis via several routes, all of which can contribute to the body burden of aluminium. One of the most important of these routes in the infant is the diet [2]. Aluminium is found throughout the human body, in every cell and in all body fluids. This observation alone demonstrates that aluminium crosses epithelia/endothelial barriers including the gastrointestinal tract [3].

It is highly likely that infants are additionally susceptible to absorption of aluminium from their diet [4]. For example, clinical studies have shown that the intestine of new-born infants and especially preterm infants has a higher capacity for absorption of macromolecules compared to the mature adult intestine [1,6].

The aim of this study is to develop a quantitative in vitro model of the aluminium absorption across the infant gut and to establish dietary factors which may influence aluminium uptake with particular reference to infant formulas. Preliminary results indicate that the optimal time is 3 hours and the acid pH is the best conditions for the barrier crossing.

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