EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF Exploratory Workshop on

Application Of Non-Traditional Stable-Isotope Systems To The Study Of Sources, Fate And Impact Of Metals In The Terrestrial Environment

Toulouse (France), 10-12 May 2010

Convened by:
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① EcoLab (Campus Ensat, Castanet-Tolosan)

SCIENTIFIC REPORT

1. Executive Summary

Anthropic emissions of metals into the environment pose major risks to ecosystems and human health. Sources, pathways and fate need to be fully understood in order to give appropriate remediation answers and/or maintain sustainable levels.

In many cases, multiple anthropic sources for one or more metals disrupt natural biogeochemical cycles. There is therefore a need to identify, “fingerprint” the sources.

The potential of isotope abundance analyses for the study of metal pollutants has been demonstrated in the case of Pb. Isotope-ratio measurements successfully distinguished sources of Pb in atmospheric particulates, terrestrial and aquatic ecosystems and human body. Unique relative Pb isotope abundances for sources are produced by the radioactive decay of the parent nuclides, depending on the initial parent-to-product elemental ratios and time. Unfortunately, apart from Pb, none of the abovementioned elements has a single isotope that undergoes such a radiogenic ingrowth from a radioactive parent nuclide.

Until recently, detection of variations for elements with masses >40 amu was difficult, if not impossible. However, since the development of multicollector inductively coupled plasma mass spectrometry (MC-ICPMS) in the mid-1990s, numerous groups have identified significant isotopic variability and fractionation for elements up to U, thereby supporting theoretical predictions. These studies, which often addressed problems in earth science, such as the development of proxies for early life or changes in oxygenation in ancient oceans, offered new insights into the biogeochemistry of these non-traditional stable-isotope systems (**NTSI**).

In parallel, one of the main present tasks for ecologists and ecotoxicologists is ***to investigate multiple pollutants impact*** on organisms and ecosystems. In order to assess toxicity of metal cocktails, there is a need of new tools to trace the sources and effects of each xenobiotic.

As matter stands and ***to accelerate research in metal isotope biogeochemistry***, there is an obvious need of interdisciplinary dialogue between isotope geochemists and environmental biologists. If there are already environmental compartments investigated together by geochemists and biologists with Pb isotopes, some compartments (i.e. terrestrial invertebrates) are more or less ignored. In addition, new isotope systems (Cu, Zn, Sb...) are presently ignored, despite the fact that metal isotope fingerprinting consists surely of a major breakthrough in improving our understanding of mechanisms of metal transfer and toxicity to living organisms.

The objectives of the workshop was to identify the different gaps in our knowledge on how metals affect living organisms and terrestrial ecosystems and how can non-traditional metal isotope biogeochemistry help to resolve them.

The workshop was held in Toulouse (France) over two full days. Participation numbered 19 people from eight countries, excluding the French convenor and the ESF representative. By chance, the Iceland volcano maked a pause at this time and every participant could have participated.
If the workshop itself was held at the ENSAT campus, dinners were given downtown in 2 typical French restaurants.

In this multidisciplinary workshop, in the first day and the second day morning, three speakers from three different fields (isotope geochemistry, ecotoxicology and soil ecology and policy) were invited to present these 3 disciplines. After each "keynote" lecture, short talks (<15min) in the discipline were given by the interested researchers and discussion (specific questions, problems) followed.

The last afternoon was dedicated to discussion on further researches, proposals and the writing of a draft for a synthesis article to explain how non-traditional stable isotopes can help soil ecologists and ecotoxicologists.

2. Executive Summary

Researchers working on three main scientific fields were represented in this workshop:

1. isotope geochemistry,
2. ecotoxicology,
3. soil and environmental science.

If most of the researchers were familiar with one field (soil and environmental science), non-traditional stable isotope (**NTSI**) geochemistry is a recent research field and few researchers attending the workshop were aware about this research field. Three specialists investigating **NTSI** in the field of environmental geochemistry therefore dedicated the first session to a general presentation of non-traditional stable isotope geochemistry.

Dominik Weiss gave a keynote lecture on **NTSI** showing their interests in different aspects of Earth and Environmental Sciences. Specifically, Dr. Weiss showed that **NTSI** can be used to fingerprint metal emissions in the environment but also trace some biological processes. An interesting and exemplary isotope system for the field of environmental sciences is the Zn system (*Z = 30, Ar = 65.409, stable isotopes at 64, 66, 67, 68, and 70 amu*).

1/*Attempts to trace Zn pollutant sources* have been promising. Selected rainwater samples from two adjacent areas in Southern France corresponded to the isotopic signature of a Zn-containing chemical widely used in that area. Similarly, Zn isotope ratios in lichens around an ore processing and mining site in Russia were similar to those in ore-bearing granites but not to those in host rocks, suggesting that the Zn in the environment was derived from the mining and mineral processing rather than from local soil dust.

F. Africano on a mining site in Portugal also showed this in the short presentation.

2/ Uptake of free Zn2+ by plants and algae species favours the light isotope, probably because of the kinetic diffusion across cell membranes. Adsorption induces small fractionation: organic tissue preferentially adsorbs the heavy isotope from solution, as experiments with diatoms and plant roots suggest, and small positive and negative fractionations were found during adsorption onto iron oxides, depending on pH and crystal structure. Small fractionation by diffusion (~0.3 ‰) has been confirmed in aqueous solutions with faster transport of the lighter isotope.

Thus, Biological processes induce smaller, but distinct, variations for Zn, but also for Cd, Fe, which opens up the possibility of *quantifying and elucidating nutrient acquisition and translocation processes in microorganisms and plants* (table 1).

Table 1 from Weiss et al. 2008[1]

Direction and magnitude of isotope fractionation during different biogeochemical processes.

Entries are expressed as $\Delta^{i/j}X_{a-b} = \delta^{i/j}X_a - \delta^{i/j}X_b$. Small refers to fractionation <1‰/amu; large refers to >1‰/amu. This table should be taken only as a guide, with respect to direction and to magnitude.

Process	Type	Comment	Fractionation pair		Δ_{A-B}	Extent of fractionation
			A	B	(‰/amu)	
Chemical	Complexation	Weak ligand	Free metal	Complexed metal	negative	Small
		Strong ligand	Free metal	Complexed metal	negative	Large
	dissolution	Congruent, proton-promoted	Solution	Solid	none	
		Incongruent, proton-promoted	Solution	Solid	none, negative	Small
		Incongruent, ligand-promoted	Solution	Solid	negative	Small
		Incongruent, microbe-promoted	Solution	Solid	negative	Small
	Precipitation	abiotic, equilibrium	Solution	Solid	Positive	Small
		abiotic, kinetic	Solution	Solid	Positive	Small to large
	adsorption	on organic surface	Solution	Solid	negative	Small
		on inorganic surface	Solution	Solid	Positive, negative	Small to large
	redox reaction	biological and nonbiological	oxidized	reduced	Positive	Large
	Ion exchange	on ion-exchange resin	Free metal	resin-bound metal	Positive to negative	Large
Biological	nutrient uptake		Solution	Plants and algae	Positive	Small
	Protein–metal		Solution	Protein	Positive	Small to large
	Precipitation	biologically mediated	Solution	Solid	negative, positive	Small to large
Physical	Evaporation		residue	vapor	Positive	Small to large
	diffusion	aqueous	Solution	Source	negative	Small

[1] Weiss DJ, et al, Application of non-traditional stable-isotope systems to the study of sources and fate of metals in the environment., Environ Sci Technol, 2008, V.42, p.655-664

The second part of this morning was dedicated to techniques used to measure **NTSI** with a short lecture given by Nadine Mattielli and Jérôme Viers followed by a discussion. N. Mattielli and J. Viers introduced Multicollection Mass Spectrometry but also resin-exchange chromatography in order to separate elements before isotope analyse. Main discussion points with the assembly were *the detection limit* and the necessary quantity of the analysed element to get *precise and accurate isotopic measurement.* In the case of **NTSI** measurements in living organisms, this is clearly a limiting factor.

After the lunch, D. Weiss finished his presentation with his thoughts on the main interests of **NTSI** geochemistry for environmental sciences (see part 3).
One interest of this way to work was that, after a robust presentation of **NTSI** and their advantages and limitations, the discussions following each presentations of works by ecotoxicologists and soil scientists were all orientated on how **NTSI** can solve their present scientific problems.

The afternoon was dedicated to the ecotoxicology field with a keynote lecture by Kees Van Gestel on "Uptake and effects of metals in soil invertebrates". It was followed by a large session on ecotoxicology in order to gain a common vocabulary.
K. van Gestel pointed out the main environmental parameters influencing bioavailibility of metals to invertebrates in soil like pH or organic matter.
I. Lamy also pointed out organic matter as a key parameter for studies of terrestrial ecotoxicology in her short presentation.
K. van Gestel summarized the purpose of toxikinetics experiments: 1/ estimate bioavailibility, 2/ find out the time that organisms should be exposed to reach equilibrium and to observe potential toxic effects, 3/ predict the physiological fate of chemicals in living organisms, way of detoxification, 4/ kinetics and way of sequestration, which may explain toxicity.
Different uptake models can be constructed to explain uptake and detoxification of the different metals based for example on soil parameters. However these models are unsatisfactory. For example they are specific only to one component. Clear differences of uptake by invertebrate species between different metals (Mo,

Cd, Zn…) were shown. For example, whereas Cd uptake is increasing slowly in *Eisenia andrei*, Zn is regulated after an initial peak.

An interesting tool, close to future use of **NTSI**, is to investigate uptake kinetic, with the use of radioactive isotope like ^{109}Cd or ^{65}Zn. It can be also used to identify location of metal in the organism and type of uptake.

Main conclusions by K. Van Gestel were:

1.Bioaccumulation of metals in soil invertebrates is related to availability in soil, but relationships are not always clear,

2.Large scatter and unexplained peak in uptake phase,

3.Kinetics may help unravelling peculiarities of routes of exposure and uptake in soil invertebrates,

4.Toxicity is not directly related to total body concentration,

5.Flux of metal through body of relevance for toxicity and so is internal distribution/sequestration of metals.

K. Van Gestel from his own experience and colleagues identified some combined use of isotopes and improved ICP-MS, which may help addressing issues of metal bioavailability, uptake, internal sequestration and toxicity, such as:

1. Identifying sources of uptake and relative contribution of each exposure pathway to uptake,

2. Assessing vitality of test organisms (or toxic responses) in relation to body compartmentalization of metals;

3. Assessing induction of metal detoxicification mechanisms.

One key-parameter not really questioned in terrestrial studies but already well investigated in aquatic and sediment studies is *the role of speciation* in uptake and toxicity. Jos Vink highlighted this in the short presentation.

W. Peijnenburg and E. Joner emphasized the role of the different interfaces, both chemical and biological. W. Peijnenburg tries to evaluate the mechanisms for plant-ion interactions and their effectiveness on rhizotoxicity. In his short presentation, he showed how electrostatic interactions between cations at the external cell surface can affect metal uptake. E. Joner pointed out the role of mycorrhiza in plant ecotoxicology and the differences between pot and field experiment in this case.

Eric Pinelli, in his short presentation of the use of micronucleus approach to investigate Pb toxicity to *Vicia faba* drew attention to the scale differences between the different approaches already encountered in this meeting. Whereas lethal and reproduction studies are investigating acute and chronic toxicity, genotoxicity studies like micronucleus and comet studies are need to investigate long term toxicity (figure 1)

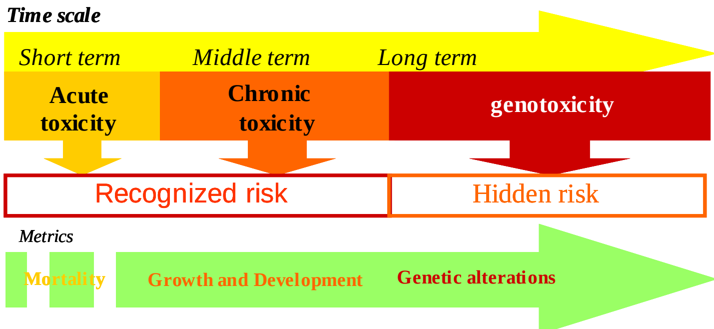

Figure 1: Genotoxicity in an integrated assessment of risks to ecosystems

Erik Smolder on Friday morning presented us a broader keynote lecture on the implementation of bioavailability research in defining environmental regulations for trace metals and metalloids in soil.

He separated three possible ways to define the thresholds:

a/ Standard laboratory toxicity tests, b/ field experiments and c/ gradient studies in contaminated fields (figure 2).

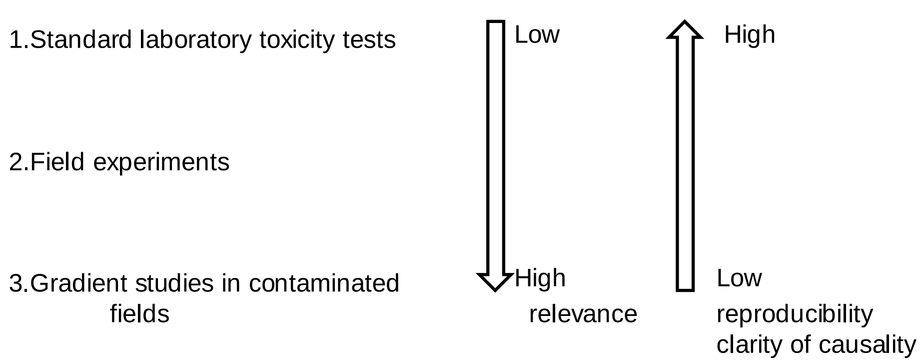

Figure 2: lab tests vs. field tests

One main advantage of gradient studies is to avoid artificial effect from spiking with a contaminant.
He called attention to the fact that there is no indication that 'fixed' (non labile) metal contributes to toxicity. Thus *a definition of soil limits should be based on 'labile' or 'adsorbed' metal rather than total metal.*

Erik Smolder pointed out also the role of metal mixture. This was also one main point of P. Bauda in her talk. The terrestrial Biotic Ligand Model (t-BLM) is one way to model ecotoxicity of metals in soils. However a better description and explanation of antagonisms and synergisms in the t-BLM concept, i.e. competition effects on soil chemistry and on the biotic ligand, is expected in the future.

Finally, Erik Smolder concluded on the concept of bioavailibility in terms of EU regulations:
1/ In EU, the bioavailability concept has been adopted in regulations;
2/ The free ion in solution is likely the most available and toxic form of a metal, however the free metal ion activity does not explain toxicity between soils due to competition effects,
3/ The 'available dose' is the metal concentration on the biotic ligand (a theoretical concentration); an approximation to this is the fractional occupancy of the metal in the eCEC for cationic metals.

Finally, the workshop ended with a general discussion in the form of a round table moderated by the convenor G. Le Roux to define future research priorities (see next part).

3. Assessment of the results, contribution to the future direction of the field, outcome

The last afternoon was dedicated to debate on the following actions:

1/ an informal or formal network linking ecotoxicologists, soil ecologists and **NTSI** geochemists?

Up to now, **NTSI** geochemistry is a rather immature scientific field. Small collaborative actions should be preferred (risk-cost balance issues). Student exchange (Marie Cure Network for example) should be preferred to establish stronger bridges between the three disciplines. However some General questions – Guidelines for a European Proposal were identified:

- What elements? Why?
- Study environmental processes or element cycles?
- Technical issues (inter-calibration, laser ablation, double spike)

Applications and Mechanisms (how fundamental should we be?)

- Plant Soil:
  - /Transfers, Translocations in plants (and humans?) – Biochemical tool?
  - /Theoretical calculations of fractionations (see work with Mg)
  - /Mechanisms of e.g., nutrient efficiency, phyto-tolerance
- Biogeochemical cycle:
  - /Fractionation along the food chain and ecosystem cycle (aquatic and terrestrial) – do we develop a proxy better than others?
  - /Study of individual processes (e.g., complexations, uptake)
- Pollution:
  - /Local vs. regional vs. global scale (contribution from air vs. particle deposition?)
  - /Identification and sources
  - /Airborne pollution, legacy contamination
- Uptake of metal in organisms
  - /Biomagnification (trophic chains)
  - /The fractionation in organisms and in tissues
  - /Distribution of metals
  - /What are the different concentration effects on biology (multiple pollutions)?

2/ a summarizing article explaining why using **NTSI** in ecotoxicology and soil ecology will be proposed to a scientific journal (i.e. The Science of The Total Environment or Journal of Environmental Monitoring). It will identify some problems as discussed in the workshop how **NTSI** can help to solve them:

1.Sources identification

? Anthropogenic vs. Natural background (ex. contribution %)

? Atmosphere vs. Soil

2. Trophic transfer understanding

? Biomagnification (invertebrate granula, plant translocation etc...)

3. Speciation and bioavailibility (in solid phase, in soil solution)

At changing conductions

? fluxes, ageing

? redox

4. Distribution uptake, transport and distribution inside the organisms, organs (internal speciation)

We clearly also identify the need to recognize some technical problems, and develop common practices. There are clearly need of standardization, the development of reference biological and background soil materials. To facilitate future projects, in-house facilities in each institute were listed.

4. Programme

Monday 10 May 2010

Afternoon	Arrival
20.00	Get-together - Informal (fountain, Place Wilson) - Dinner

Tuesday 11 May 2010

08.15	Departure from the hotel to ENSAT (subway travel)
08.45-9.00	Registration
09.00-09.20	**Welcome by Convenor** **Dr. Gaël Le Roux** (EcoLab, Toulouse, France)
09.20-09.40	**Presentation of the European Science Foundation (ESF)** **Dr. Sonja Lojen** (Jozef Stefan Institute, Ljubljana) Standing Committee for Life, Earth and Environmental Sciences (LESC)
09.40-12.30	**Morning Session: Non-traditionnal Stable isotopes**
09.40-10.40	**Keynote Lecture: Application of Non-traditional Stable-Isotope Systems to the Study of Sources, Fate and Impact of Metals** **Dr. Dominik Weiss, Imperial College** (Imperial College , London, UK)
10.40-11.00	*Coffee / Tea Break*
11.00-12.00	**"What is currently done in Non-traditional Stable-Isotope BioGeochemistry?":** **Short presentations (5-10 min) on different topics by "the isotope geochemists" + Discussion**
12.00-12.30	**Discussion: On the limits and possibilities of Non-traditional Stable-Isotope analytical Geochemistry: what can be done?** **Discussion Leaders Pr. N. Matielli** (IPE, Bruxelles, Belgique) **& Pr. J. Viers** (LMTG, Toulouse, France)
12.30-14.00	*Lunch*
14.00-18.15	**Afternoon Session: Metal Ecotoxicology**
14.00-15.00	**Keynote Lecture: Uptake and effects of metals in soil invertebrates** **Dr. Kees Van Gestel** (VU Amsterdam, the Netherlands)
15.00-16.00	**"What is currently done in metal terrestrial ecotoxicology?"** **Short presentations (5-10 min) on different topics by soil ecologists and ecotoxicologists + Discussion**
16.00-16.30	*Coffee / tea break*

16.30-17.30	**"What is currently done in metal terrestrial ecotoxicology?" Short presentations (5-10 min) on different topics by soil ecologists and ecotoxicologists + Discussion**
	Jos Vink: *The origin of speciation: Consequences for metal uptake and toxicity*
	Willie Peijnenburg: *Evaluating Mechanisms for Plant-Ion Interactions and their Effectiveness on Rhizotoxicity - Cell Membrane Surface Potential and Ion Uptake and Toxicity*
	Agnieszka Bednarska: *Problems in studying metal toxicokinetics in invertebrates*
	Irena Grzes: *Metal pollution and wild populations of ants*
	Martina G. Vijver: *Quantifying metal-induced ecological effects in the field*
	Pascale Bauda: *Toxicological impacts of metal cocktails on living organisms ?*
	Eric Pinelli: *Role of Lead speciation in genotoxicity*
17.30-18.15	**Discussion: ¨Main open questions about toxicological impacts of metal cocktails on living organisms?"** **Discussion Leader Pr. Eric Pinelli** (EcoLab, Toulouse, France)
19.30	*Dinner*

Wednesday 12-05-2010

08.30	Departure from the hotel to ENSAT (subway travel)
09.00-12.30	**Morning Session: Combining geochemistry, ecology and ecotoxicology**
09.00-10.00	**The implementation of bioavailability research in defining environmental regulations for trace metals and metalloids in soil** **Dr. Erik Smolders** (KU Leuven, Belgium)
10.00-11.00	**"Environmental Geochemistry and ecotoxicology"**
	Short presentations (5-10 min)
	Erik Joner: *Overview of relevant activities at Bioforsk Soil & Environment & 2 words on Mycorrhiza -A major rhizosphere interaction*
	Francisco Martín Peinado: *Soil degradation and toxicity after a pyrite tailing spill & Toxicity assessment of heavy metals in soils as an environmental management tool*
	Isabelle Lamy: *Do the different fractions of soil organic matter have the same role as sink of pollutants*
	Fatima Africano: *Zn isotopic study of atmospheric deposition and soil contamination at the abandoned mining area of S. Domingos, south Portugal*
11.00-11.30	*Coffee / Tea Break*

11.30-12.30	**Round-table: Use of non-traditional stable isotopes in laboratory ecotoxicology** **Discussion Leaders: E. Pinelli and G. Le Roux**
12.30-14.00	*Lunch*
14.00-17.00	**Afternoon Session: Perspectives using non-traditional stable isotopes**
14.00-15.00	**Round-table: Use of non-traditional stable isotopes in terrestrial ecology** **Discussion Leaders: D. Weiss and I. Lamy**
15.00-17.00	**Discussion on follow-up activities / networking / collaboration**
17.00	*End of Workshop, Departure*

5. List of Participants

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6. Statistical information on participants

- 19 participants, one convener and 1 ESF representative,
- 4/20 young scientists,
- 7/20 female scientists,
- 8 Countries: Belgium, France, the Netherlands, Norway, Poland, Portugal, Spain, UK.