

**Biotests and Biosensors in the Analysis of (eco)toxicological Risk of Soils Highly Polluted by Cadmium, Lead and Zinc: Bioavailable Fractions cause Toxic Hazard**

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**Introduction.** Current practice in contaminated land risk assessment starts with a chemical characterization of the site whereas the protocols of state agencies used for legislative decision-makers rely on analytical methods that involve vigorous extraction of soils and sediments either with organic solvents (e.g., to extract PAHs) or concentrated acids (to extract heavy metals). Then, the biological effects and risks are assessed interpreting the chemical data in biological or toxicological terms, although the **bioavailable** concentrations potentially hazardous to living organisms are completely different. Due to that, there is a growing awareness among environmental toxicologists, risk assessors and regulatory agencies that the total concentration of a toxicant in a contaminated environment frequently overestimates the risk of pollutants to humans, animals and plants (Alexander et al., 2000). It has been agreed that for the meaningful environmental hazard assessment the chemical and toxicological methods should be used together. Contamination of soils by **heavy metals** is a worldwide problem. The bioavailability (and thus toxic risk) of heavy metals depends on the exposed organism, exposure pathway and soil type. Traditionally, the environmental risk caused by heavy metal pollution in solid matrices is determined by quantification of total metals after digestion with strong acids by chemical analysis. However, these methods are not able to distinguish between available (potentially hazardous) and non-available (potentially non-hazardous) fractions of metals to biological systems.

**Material and Methods.** As a result of a collaborative project between NICPB (Estonia) and ISA (France) an agrochemical and ecotoxicological mapping of the territory around two zinc and lead smelters in the North of France has started. The territory (over 100 km<sup>2</sup>) is heavily polluted with heavy metals, affecting nearly 15,000 inhabitants: the concentration of Zn reaching ~2,000 mg/kg, Pb ~1,000 mg/kg and Cd ~20 mg/kg in the most polluted samples, exceeding the natural background values up to 37 times. According to the EU directive 86/272/EEC, heavy metal levels in soil exceeding 1-3 mg/kg for Cd, 50-300 mg/kg for Pb & 150-300 mg/kg for Zn pose a hazard to the environment.

The **soil-water path (eco)toxicological risk** of these highly polluted soils was analyzed using a battery of aquatic toxicity tests (algae *Selenastrum capricornutum*, photobacteria *Vibrio fischeri*, protozoa *Tetrahymena thermophila*, crustaceans *Thamnocephalus platyurus*) applied to water leachates of these soils. A novel photobacterial ToxScreen assay was applied for the **evaluation of the prevailing nature of water-extractable toxicity**. The ToxScreen assay using *Photobacterium leiognathii* as a test organism and two special buffer sets (*pro-organic* and *pro-metal* buffer) enables differentiation the toxicity caused by the organic and cationic metal pollutants (Ulitzur et al., 2002).

**Particle-bound bioavailable toxicity** was evaluated by Solid-Phase Flash-Assay (a new kinetic toxicity test that uses *Vibrio fischeri* as a test organism; Lappalainen et al., 1999). In addition, the recombinant luminescent bacterial sensors were used for the **specific quantification of the bioavailable concentrations** of cadmium (Cd-sensor) and lead and cadmium (Cd-Pb sensor)(Tauriainen et al., 1998) in the cases of aqueous and contact exposure. The recombinant luminescent bacterial sensors for heavy metals are unique and powerful tools for sensitive and specific quantification of bioavailable fractions of heavy metals in the case of different exposure types (Ivask et al., 2002).

**Results and discussion.** The results obtained on the first 50 soil samples analyzed showed that in the aqueous extracts of the soils (1+9; soil:water) no toxicity was detected with algae, protozoa,

crustaceans and photobacteria, even in the most polluted samples. The absence of the water-extractable toxicity could be explained by very strong sorption of heavy metals to the soil particles: the concentrations of heavy metals in the aqueous extracts of the soils were very low (up to 0.02 mg Cd/L, up to 0.7 mg Pb/L and up to 1.9 mg Zn/L). Thus, only 0.5% of Cd, 0.4% of Pb and 0.9% of Zn (median values) proved water-extractable.

The ToxScreen assay also did not show the metal-caused water-extractable toxicity of these soils but surprisingly registered the organic-pollutant caused toxicity. Although the sensitivity of the ToxScreen assay for the heavy metals is high (the EC50 value for Zn being 0.6 mg/L, for Cd 0.06 mg/L and for Pb 0.1 mg/L; Ulitzur et al., 2002) the water-extractable bioavailable levels of the heavy metals were still too low (the nominal concentrations up to 1.9 mg Zn/L, 0.02 mg Cd/L and up to 0.7 mg Pb/L) to cause the toxic signal. Also, it could be supposed that even in the aqueous extracts of the soils the heavy metals sorb to humus particles and become less bioavailable. For the identification of the source of the organic toxicity registered by ToxScreen assay further analysis will be applied. Thus, although the soils were heavily contaminated with heavy metals, no apparent toxicity was detected via the soil-water path, except organic pollutants causing toxicity in the ToxScreen test.

However, the recombinant luminescent Cd-sensor (sensitivity limit 0.3 ppb Cd) showed in the case of **contact exposure** (if the test organisms are in the direct contact with soil particles) remarkably higher bioavailability and potential hazard. Namely, the bioavailability of Cd in these soils reached 43% (being ~150 times higher than respective water-extractable fraction of Cd). The Solid-Phase Flash Assay did not register particle-bound toxicity that could be explained by the low sensitivity of *Vibrio fischeri* towards heavy metals.

The ecotoxicological data and bioavailability of Zn, Cd & Pb in these soils will be compared in terms of their chemical mobility in water and acetic acid. Altogether 200 soil samples will be analyzed for the comparative agrochemical and (eco) toxicological mapping.

### References

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