

Work Package 9: Heavy Metal Detection

Heavy-metals (HM) have been included in the BioCop project because they are highly toxic to both plants and animals and have well-documented neurotoxic (lead, mercury), haematotoxic (lead) and nephrotoxic (lead, cadmium, mercury) effects on humans. It is also known that organo-metallic complexes such as methyl mercury can be even more toxic than the simple metallic form, primarily due to their high affinity for -SH donors, thus facilitating their accumulation in tissues. Unlike carbon-based contaminants that can be completely degraded to form relatively harmless products, metal ions can be transformed in only a limited number of ways by biological or chemical remediation processes. In recent years the presence of mercury species in natural waters, soil and seafood has been recognised as an issue of major food safety concern (figure 1).

The current repertoire of standard techniques for trace heavy-metal analysis includes Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma-Mass Spectrometry (ICPMS). However, these methods require expensive equipment, which cannot be used in the field, and also produce gaseous effluents that are difficult to treat and dispose of. Moreover, virtually all of the methods involve complicated and time-consuming sample treatment and pre-concentration steps that can be carried out only by trained professionals. This prohibits screening for heavy metals at various stages of food production and hinders the objective of preventing heavy metal contamination as early as possible in the production chain. Electrochemical methods are seen as complementary to the aforementioned techniques, and are especially attractive because they allow the possibility of creating inexpensive and portable instrumentation.

BioCop is developing cost-effective, validated methods and systems to meet the needs of a range of end-users: analytical laboratories and food companies, for which contamination of raw materials by heavy metals causes a significant problem. New technologies are required that enable robust, rapid, accurate and cost-effective screening of raw materials.

The screening methods to be developed, while based on established electrochemical techniques, will make use of novel disposable sensors that are easy to produce (using thick film technology) and which can be used with portable and cost-effective, user-friendly instrumentation. The

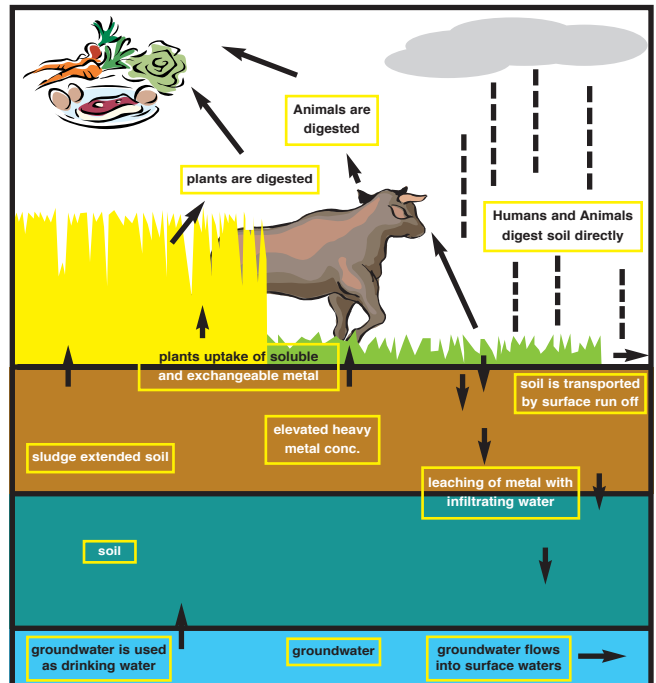


Figure 1: The heavy metal cycle, with heavy metals moving from the environment (pollution) to the human body through the food chain.

electrochemical procedures for heavy metals will be based on the most advanced biosensor and immunosensor technologies, particularly "advanced sampling methods". For solid samples, the most rapid methods of extraction will be employed. For liquid samples or extracts, the approach will be based on the use of magnetic particles, modified with mercury chelating polypeptides, and immunodetection of chelated mercury. This combination allows extraction and concentration in one step and will be linked to a direct electrochemical assay system that can be highly selective.

In the case of lead, methods based on electrochemical pre-concentration steps will be developed to improve detection at low concentrations with minimal sample preparation. In the case of certain liquid matrices, such as milk and beverages, the use of adsorptive electrochemical techniques can reduce sample preparation to a minimum.

Finally, the robust, rapid, and cost-effective detection devices that are foreseen as a result of WP9 can serve as prototypes for designing electrochemical sensors and immunosensors for the detection of other contaminants, covered by other work packages.



Lead (Pb) Detection

Screen printed electrodes have been designed and produced after careful testing and selection of different inks and silver pastes. Ink composition has been optimised for printing and the best combinations selected to yield the best signal:noise ratio.

Different pre-treatment procedures required for lead determination in milk, by anodic stripping voltammetry, were preliminarily investigated using carbon paste electrodes modified with thin films of mercury. Square wave voltammetry was used to monitor the oxidation step. On the basis of calibration curves, it was demonstrated that lead could be detected in the 1 to 100 parts per billion range.

For the pre-treatment of milk samples, a new method involving acid precipitation of proteins was compared with the procedures specified for the official AOAC method, which have some serious disadvantages (complexity, duration and high temperatures). In the new method, acid precipitation is followed by centrifugation and filtering to produce a clear filtrate for analysis. The suitability of this pre-treatment step was studied by determination of the recovery using standard addition methods.

Printed electrodes have also been modified by the deposition of a bismuth film on the surface of the working electrode (in place of mercury). Ongoing work involves characterisation of the electrodes in terms of linear range and detection limits for Pb in buffer as well as in milk extract, to evaluate the matrix effect.

Mercury (Hg) detection

The main aim of the early work has been to synthesise mercury chelating groups, amenable to cross-linking with proteins to produce antigens and also to cross-link to magnetic microbeads. Two ligands for mercury have been chosen: 1,2-bis(o-aminophenylthio) ethane was synthesised by following the established procedure, from the literature; a new derivative of 1,3-benzenediamidoethanethiol ligand has also been synthesised.

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