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1. PROJECT EXECUTION



website: www.fenk.wau.nl/ecodis

1.1 Project objectives

ECODIS developed sensor technologies for monitoring the physicochemical reactivity and biological impact of inorganic and organic pollutant species in aquatic systems. ECODIS also applied these technologies to the study of the short and long term chemical and biological status of aquatic ecosystems following a pollution disaster. Exposure conditions experienced by organisms are defined by the temporal profiles of concentration and speciation of pollutants. These profiles are quantitatively linked to biological effects via an innovative dynamic approach based on the flux of pollutant species as a key parameter in effective ecosystem quality. The dynamic features of pollutant species distributions over biotic and abiotic components are basic components of a new generic dynamic approach for any macroscopic aquatic ecosystem impacted by a pollution disaster event. This involves the integration of the dynamic features of pollutants with their macroscale transport resulting from diffusion and flows in the water body. One of the major goals of ECODIS is to arrive at a model that includes predicted pollutant species distributions, and ensuing biological risks, in all compartments of the aquatic ecosystem as a function of time and space. Especially in disaster situations, the pollutant sink/source functioning of ecosystems under extreme load forms a key factor in the rate of spread of the disaster impact. ECODIS couples the sink/source function with the transport modelling and derives the ensuing immediate and long term impact of a given disaster case. ECODIS also paves the way to define sophisticated strategies for dynamic risk assessment and ensuing disaster management policies. One of the ultimate targets in ECODIS's action plan was the formulation of a comprehensive set of guidelines for monitoring, data management, and interpretation of pollution disasters.

Key innovative objectives of ECODIS are:

- (i) Development^a of sensors and *in situ* probes^b, covering a range of response times and spatial scales, for measuring (changes in) pollutant species concentrations, and to provide signals that are quantitatively linked to responses from the most appropriate biota over a wide range of concentrations.
- (ii) Integration of short term conversion and transport processes (diffusion, changes in speciation (complexation, adsorption, biotransformation)) with macroscale long term transport/ hydrological models to achieve quantitative knowledge on pollutant spread upon disaster impact.
- (iii) Analysis and modelling of the dynamic speciation and ensuing toxicity of pollutants with respect to their acute and chronic effects following a disaster event, i.e. taking into account the recognised importance of *fluxes* of compounds and their pertaining time scales for different ecosystem interfaces and distance scales. A range of trophic levels will be studied to give insight into potential biodiversity impacts.
- (iv) Creation of a new approach to ecotoxicological risk assessment that links dynamic pollutant speciation (exposure) with toxicology in line with leading scientific understanding. The outcome of this approach will be compared with current practice, based on total concentrations, body burdens, bioassays, and field inventories. This involves the following tasks:

^a the term 'development' refers to (i) expansion of the knowledge on the theoretical principles and methodology of physical/chemical methods in order to make them applicable to dynamic chemical speciation, and (ii) adaptation, dedication and optimisation of the relevant experimental techniques to allow for their *in situ* application under pollution disaster conditions.

^b the term 'probe' is used to denote the entire measurement device that incorporates the analytical sensor, data transmission technology, associated pumps, etc..

- a. identification of the key parameters furnished by biomonitoring and sensor data for ecotoxicological risk assessment, appropriate for regulatory purposes;
 - b. provision of the tools, i.e. sensors and models, for monitoring and quantifying the links between the time evolution of chemical, biological, and physical parameters in disaster impacted aquatic ecosystems. Incorporation of the resulting data into dynamic risk assessment protocols;
 - c. biophysicochemical understanding of combined toxicological effects of pollutant mixtures (organics and metals), at different trophic levels, as a basis for modelling, management and remedial action strategy formulation. The role of dynamic speciation in toxicity is a key knowledge gap for mixtures of metals and organics, while being of great ecological significance, especially under conditions of a disaster event.
- (v) Production of a user friendly guide for crisis managers that outlines strategies for monitoring physicochemical and biological parameters on the site of a pollution disaster, handling and reporting of the ensuing data, and tools for ecological impact predictions for the impacted water body.

1.2 Contractors involved

Partic. Role	Partic. no.	Participant name	Participant short name	Country	Lead scientists
CO	1	Wageningen Universiteit	WU	NL	Herman P. van Leeuwen
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CR	3	Université de Genève	CABE	CH	Jacques Buffle, Marylou Tercier-Waeber
CR	4	Commission of the European Communities – Directorate General Joint Research Centre	JRC-IHCP	IT	Dimosthenis Sarigiannis
CR	5	Universiteit Utrecht	IRAS-TOX	NL	Joop Hermens
CR	6	Masarykova Univerzita v Brno	MU	CZ	Ivan Holoubek
CR	7	Max Planck Gesellschaft zur Foerderung der Wissenschaften E.V.	MPIMM	DE	Dirk de Beer
CR	8	AquaSense B.V.	AquaSense	NL	Anja Derksen
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1.3 Work performed and end results

ECODIS tackled its objectives by an integrated approach comprised of the following four closely interlinked and interdependent sections of work:

- Section I. Development and dedication of dynamic sensor technologies for pollutant speciation analysis and ecotoxicological effects.
- Section II. Determination of the dynamic bioavailability and toxicological effects of pollutant species.
- Section III. Modelling of the temporal spread of pollutants and risks in aquatic ecosystems.
- Section IV. Measurement and management of disaster-site data and development of dynamic risk assessment.

The ultimate biological impact of a given pollutant in the water body is a consequence of a chain of features/events that may involve (i) mobility (physical transport in the bulk medium and across interfaces) and reactivity of the pollutant species* in the medium (encompassing sorption on particles, surfaces and biofilms, and chemical reactivity, including e.g. complex formation, hydrolysis, oxidation/reduction), (ii) chemical conversion of pollutant species into bioactive or inactive species in the local environment external to the organism, e.g. dissociation of lipophobic complexes into the free metal, biodegradation, etc., (iii) transfer of the bioactive species across the biomembrane, and (iv) bioreactivity of the pollutant within the organism. A quantitative understanding of the overall process involves quantification of all the steps involved at the relevant spatial scales and timescales, both external and internal to the organism. All sensors used to probe chemical speciation and reactivity also have their own methodological spatial and timescales.

Comprehensive dynamic physicochemical understanding of the environmental and biological factors that affect bioavailability, biouptake, bioaccumulation and bioreactivity/toxicity of pollutants, and sensor functioning, allows rational interpretation of sensor signals in terms of environmental and biological processes and impacts.

The relationship between the various spatial scales and times scales of sensors and environmental processes is shown schematically in Fig. 1.

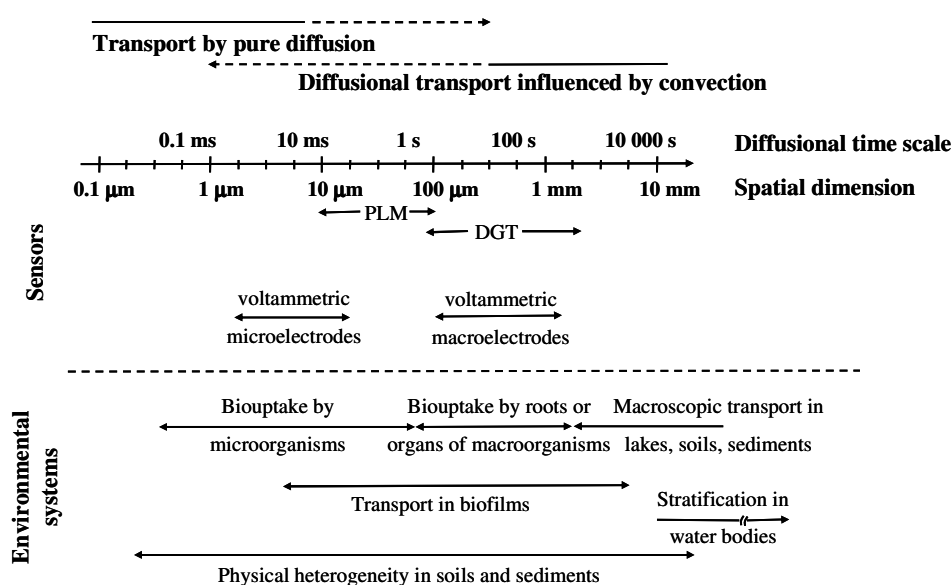


Figure 1. Schematic representation of diffusional timescales and spatial dimensions for a range of environmental processes and analytical sensors. PLM = permeation liquid membrane, DGT = diffusive gradients in thin film.

* throughout the text the term “species” is used almost exclusively to denote chemical species, i.e. different physicochemical forms of a given element or compound, e.g. $\text{Pb}(\text{H}_2\text{O})_6^{2+}$, PbCl_4^{2-} , and Pb-humic complexes. Whenever its use is unavoidable in the biological context, “biological species” is written to prevent confusion.

A given pollution disaster will have its characteristic time constants (τ) for the ecosystem to relax to (new) steady-state conditions; Fig. 2. These relaxation times are a function of transport parameters, speciation, chemical reaction rate parameters, and biological conversion features. For the long term propagation of the pollution 'wave' in the aquatic system of interest, the coupling of hydrological parameters and molecular retention parameters is crucial.

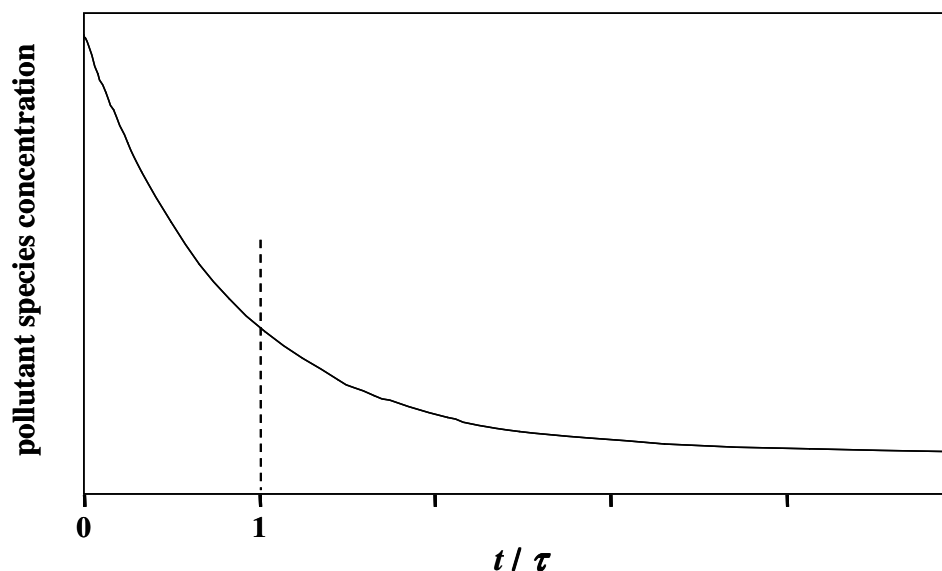


Figure 2. Schematic figure of the evolution of the concentration of a pollutant species with time, t , after an instantaneous pollutant input at $t = 0$ at the site of the disaster; τ is the disaster relaxation time constant which depends on the nature of the pollutant involved (e.g. its speciation, reactivity) and of the receiving water body (e.g. volume, hydrodynamic flows, reactive surfaces).

ECODIS characterised and quantified the factors described above. The results are incorporated into a technical guidance protocol for pollution disaster monitoring and ecological impact prediction that is targeted at disaster response authorities. Recommendations are given as to the appropriate chemical and biological measurements to be made, i.e. what parameters should be measured with which sensors over certain spatial and temporal scales, and how to incorporate the on-site data into a dynamic risk assessment of the ecological impact.

The results of each of the main sections of work are detailed below.

1.3.1 Dynamic sensor technologies

1.3.1.1 Speciation analysis

Dynamic sensor technologies were characterised and dedicated to speciation analysis of metal and organic pollutants under disaster conditions. A dynamic analytical sensor is characterised by its (i) response time, which is analogous to the effective time scale for *bioavailability*, and (ii) accumulation time, t_{acc} , i.e. the length of time over which pollutant species are accumulated in (loaded onto) the sensor prior to quantification. The signal resulting from the accumulation step represents an integration over all exposure variations in the test medium during this time period, t_{acc} , analogous to the concept of *bioaccumulation*. The principles of a dynamic sensor as compared to the process of biouptake are shown schematically in Fig. 3.

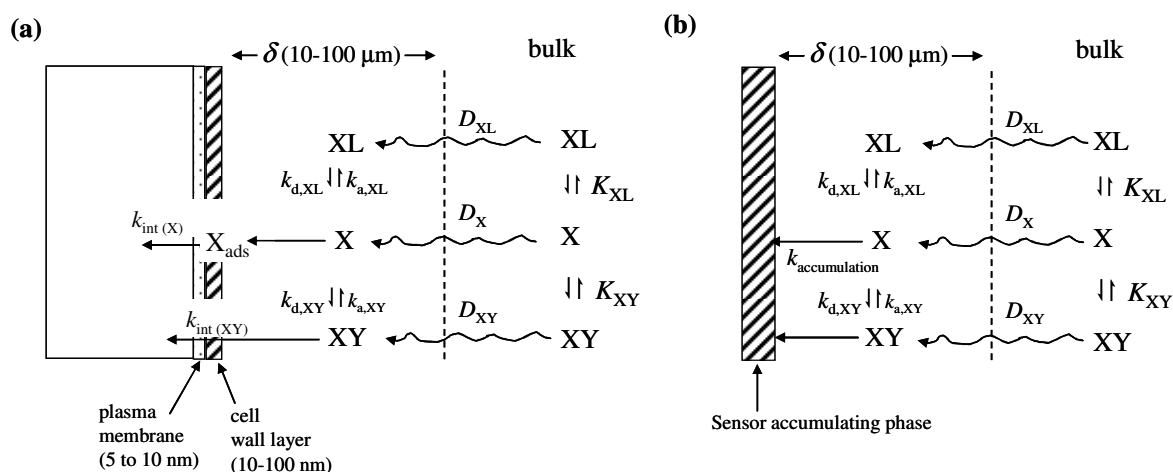


Figure 3. Schematic diagram of accumulation of a pollutant, X, at (a) a microorganism as compared to (b) a generic dynamic sensor. X denotes a metal species or an organic compound, and L and Y denote associated entities, e.g. complexants, particles etc. XY represents a lipophilic entity which may be transported intact through microorganism membranes. XL represents species that can only contribute to accumulation in a sensor or biouptake via prior dissociation into X. Symbols: k_a , association rate constant ($\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$); k_d , dissociation rate constant (s^{-1}); K , equilibrium constant ($\text{dm}^3 \text{mol}^{-1}$); δ , diffusion layer thickness (m); D , diffusion coefficient ($\text{m}^2 \text{s}^{-1}$); k_{int} , internalization rate constant (s^{-1}).

Fundamental knowledge on the functioning of a suite of sensors was extended to strengthen the quantitative link between sensor signals, speciation in the medium, and appropriate biological parameters (e.g. biouptake, toxicity).

Metals

The suite of speciation sensors for metals comprised gel-integrated voltammetric microelectrodes (GIME), stripping chronopotentiometry at scanned deposition potential (SSCP), adsorptive stripping chronopotentiometry (AdSCP), diffusive gradients in thin film (DGT), permeation liquid membrane (PLM), and Donnan membrane technique (DMT).

Notable results for metal speciation sensors include characterisation of the penetration of colloidal metal species into sensor gel layers, incorporation of dynamic metal speciation into the theory of permeation liquid membrane (PLM), characterising the fundamental features of adsorptive stripping chronopotentiometry, validating voltammetric complex lability measurements, as well as advances in quantitative interpretation of metal speciation in multiligand, multimetal systems.

Organics

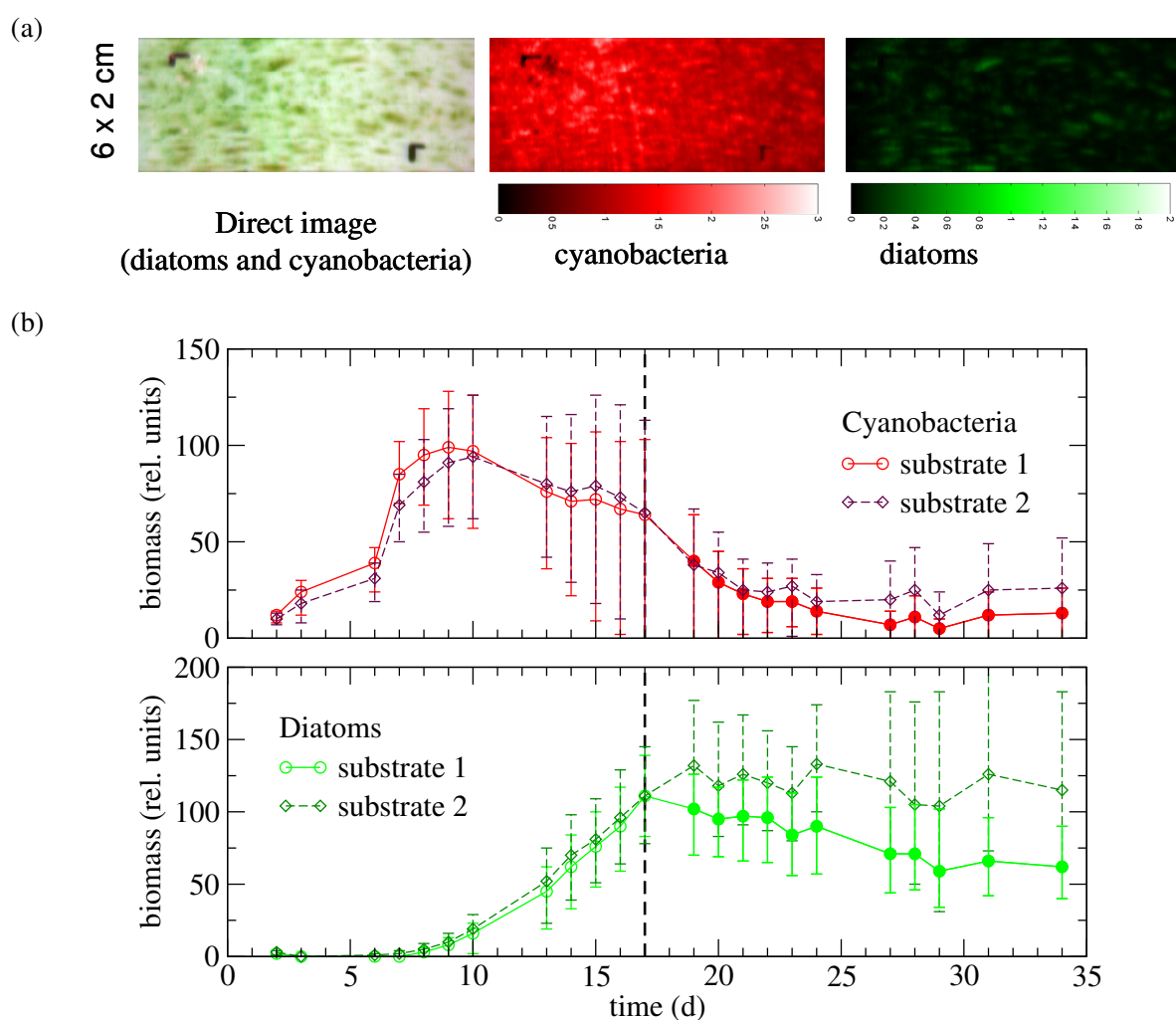
The basis for dynamic analysis of organic compounds by solid-phase microextraction fibers and PLM was developed. A dynamic framework, analogous to that developed for metals, was elaborated for organic compounds. This represents a major advance in tools for understanding organic pollutant speciation in aquatic ecosystems. Various dynamic features of organic compound speciation were characterised. For example,

dissolved natural organic matter impacts on the exchange rate of hydrophobic compounds. Pollutants sorbed to humic substances and latex nanoparticles were found to contribute to the accumulation flux of the sensor. Consequently, organic pollutants associated with such entities are potentially bioavailable. This hypothesis was confirmed by measurements of biouptake of pyrene and benzo[b]fluoranthene by an aquatic worm (*Lumbriculus variegatus*) in the presence of humic substances. That is, the rate of release of organic pollutants sorbed to NOM and nanoparticles is fast on the effective timescale of accumulation by sensors and organisms. A lability criterion was derived to quantify this condition.

1.3.1.2 Ecotoxicological effects

A multi-faceted approach was adopted for detection of ecotoxicological effects, namely hyperspectral imaging, a panel of bioassays, and genomic methods.

Hyperspectral imaging emerged as a useful new technology for identifying ecotoxicological effects via detection of shifts in microbial community composition. Examples of the images obtained and the population dynamics of mixed cyanobacterial and diatom biofilm are shown in Fig. 4.



A *panel of bioassays* was developed for disaster conditions, including a new *flash bioassay* for rapid screening of acute toxicity. A set of validated toxicological assays, and their advantages and limitations as well as direct applications for the ecological risk assessment are available online in the form of the interactive web portal DATEST 2.0 (<http://projects.cba.muni.cz/datest/>). ECODIS developed novel ecotoxicological tools suitable for complex environmental toxicity testing. The developed procedure involves both rapid acute bioassays such as kinetic bioluminescence inhibition test as well as more complex tools evaluating chronic toxicity (including endocrine disruptive effects or reproduction toxicity).

The approach was applied to analysis of toxicity of organic pollutants within the contaminated Zlin region (CZ). This area is subject to frequent floods, and the distribution of organic contaminants varied among regions and seasonally. Most of the specific bioassays led to statistically significant regional and seasonal differences in profiles and enabled clear separation of upstream and downstream regions. The outcomes of these bioassays indicated an association with concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) as master variables. There were significant interrelations among dioxin-like activity, antiandrogenicity and content of organic carbon, clay and concentration of PAHs and PCBs (Fig. 5), which documents significance of abiotic factors in accumulation of pollutants. The study demonstrates the strength of the specific bioassays in indication of the changes in contamination and emphasizes the crucial role of both spatial and temporal dynamics for the correct interpretation of information for predictions of risk.

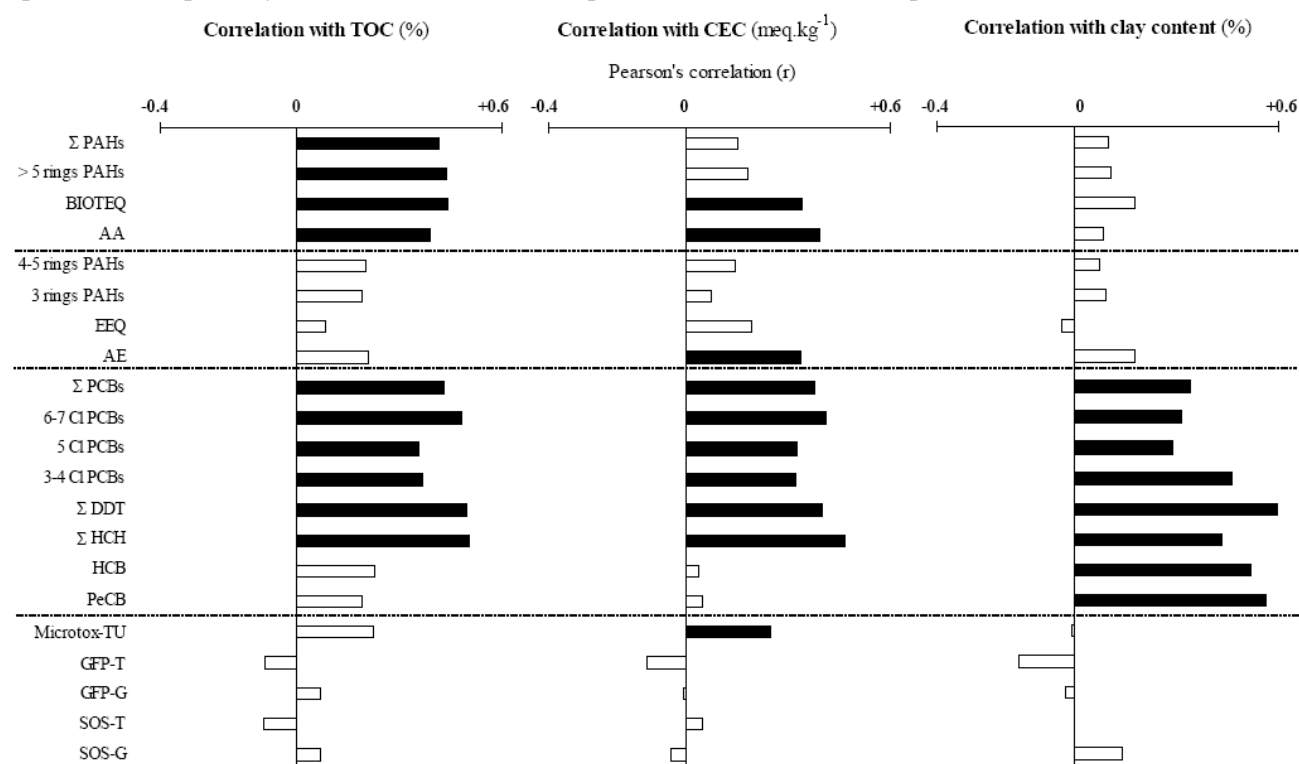


Figure 5. Rank plots of Pearson correlation coefficients between POPs, biotest measures and abiotic parameters of sediments in the Zlin region (CZ). Chemical and biotest parameters are grouped according to their mutual correlation; solid bars: statistically significant correlation; $p < 0.05$. EEQ = estradiol-equivalent, BIOTEQ = TCDD-equivalent, AE/AA = antiestrogenic/antiandrogenic index, SOS-T and SOS-G = toxicity and genotoxicity from SOS chromotest, GFP-T and GFP-G = toxicity and genotoxicity from GFP test, PAH TEQ – dioxin equivalent calculated from chemical analysis, CEC = cation exchange capacity, TOC = total organic carbon, PAHs = polycyclic aromatic hydrocarbons, PCBs = polychlorinated biphenyls, HCHs = hexachlorocyclohexanes, HCB = hexachlorobenzene; PeCB = pentachlorobenzene.

Genomic approaches were also employed in parallel with complementary measurements. Real time quantitative polymerase chain reaction (real time qPCR) was performed to quantify induction of cadmium and nickel specific genes induced in *Chlamydomonas reinhardtii*; phytochelatin concentrations were determined by liquid chromatography using mass spectroscopy detection (LC-MS); photosynthesis efficiency of algae was determined following exposures; and cell viability based on the production of formazan by living cells was quantified. The most sensitive indicators were the genomic ones. Several genes were significantly upregulated at a contaminated field site with respect to both control cells taken prior to exposure and cells exposed to the control site. It is recommended that several genes, each with different detection windows, are monitored to fully elucidate sublethal toxicological responses to environmental Cd.

1.3.2 Dynamic bioavailability and toxicological effects of pollutant species

1.3.2.1 Bioaccumulation

Studies on biouptake encompassed characterizing the role of biogels in pollutant accumulation, determination of uptake fluxes by algae from trace metal mixtures and the accumulation of PAHs by an aquatic worm.

Biogels such as alginate and biofilms themselves are found to have a high capacity for sorption of Cd, Pb, and Cu. Most of the sorbed metal is bound to the biogel matrix and is nonlabile on the microelectrode timescale. The amount of metal sorbed is dependent on both the pH and the ionic strength of the exposure medium.

For the algae *Chlamydomonas reinhardtii*, bioaccumulation for two model contaminants, Cd(II) and Ni(II), in the presence or absence of competing metals, Cu(II), Pb(II), Ni(II) (or Cd(II)) was characterized. This work enabled: (i) demonstration of the applicability of dynamic models in metal mixtures, and (ii) determination of the applicability of steady-state constants for single metals in mixtures. The bioaccumulation results supported the applicability of a steady-state model employing equilibrium constants that are determined for the metal mixtures. Dynamics were shown to play an important role in bioaccumulation, especially following the initial contaminant pulse. Complementary toxicity and gene profiling experiments showed that the organisms are able to modify contaminant internalisation fluxes.

For organic pollutants, bioaccumulation rates of PAHs by an aquatic worm (*Lumbriculus variegatus*) were found to be enhanced in the presence of dissolved organic matter. This result concurs with the dynamic speciation analysis described in section 1.3.1.

Particular attention was paid to linking the temporal evolution of the pollutant speciation in the exposure medium to the dynamics of biouptake, including the ability of the organism's physiology to process the accumulated pollutant. Physiology-based biokinetic models were developed that allow estimation of the bioaccumulation potential in different types of biological tissue. An example case of the temporal dynamics of Cd bioaccumulation in fish following transient exposure to a pulse of this metal ion is shown in Fig. 6.

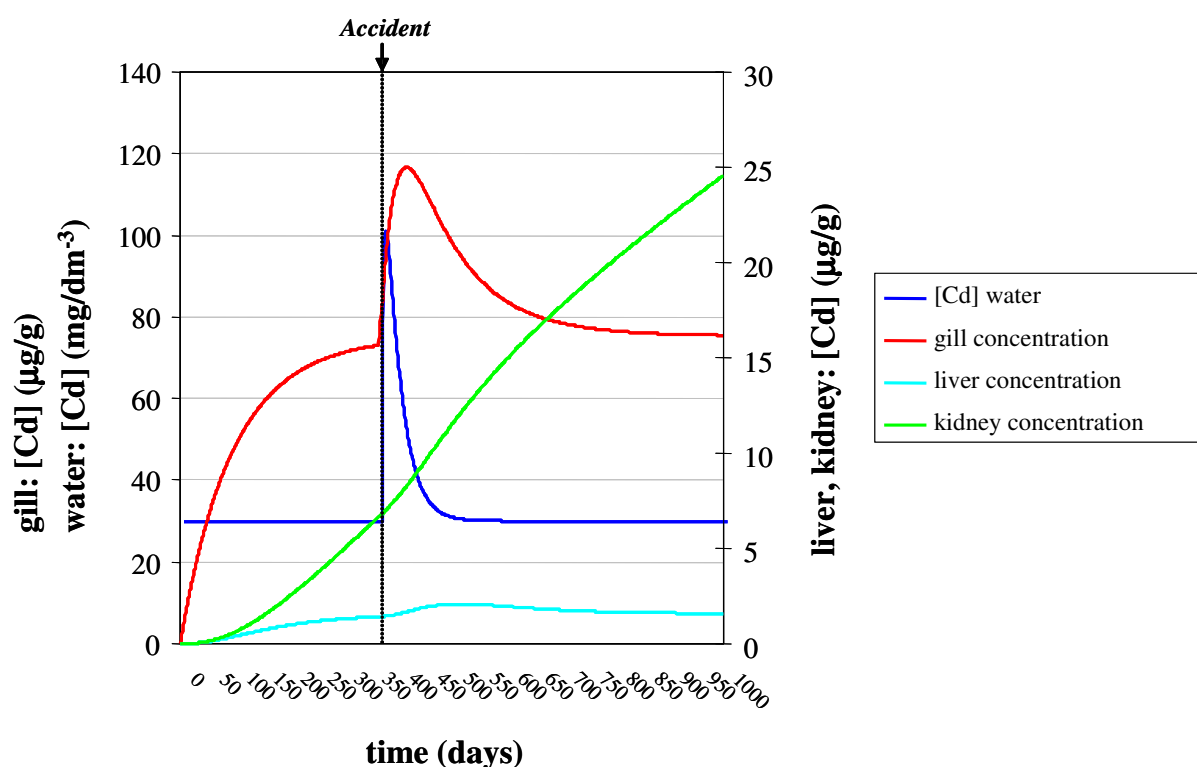


Figure 6. Modeled temporal evolution of Cd(II) concentrations in target organs of a fish following a transient release of Cd(II) into the water body

1.3.2.2 Toxicological effects

Development of sensors for detection of toxicological effects are described in section 1.3.1. Models for dose-response relationships were developed that target different physiological endpoints. In particular, for pollution disaster situations it is useful to discriminate between (i) long-term effects and (ii) short-term responses to increased exposure concentrations. This differentiated approach involved consideration of two types of dose-response relationships:

- relationship between mortality and morbidity indices (e.g. LC_{50} , EC_{50}) and the total concentration of the toxicant in the aquatic organism (total biologically effective dose, taking into account the parent substance and its metabolites, especially when the toxic potency of the metabolites is different than that of the parent substance), and
- relationship between mortality and morbidity indices and the biologically effective dose (BED) of the toxicant (including its metabolites) at the target tissue, which expresses the respective physiological response.

Linking the BED at a specific tissue at a certain time (shortly after the accident occurs) to dynamic estimates of lethal and sublethal impacts on the affect biological community provides valuable information for the development of an integrated strategy for dynamic risk assessment of pollution disasters. Figure 7 gives a graphic example of the implementation of these dynamic and tissue-specific dose-response functions in the case of accidental release of Cd in the water as regards fish mortality.

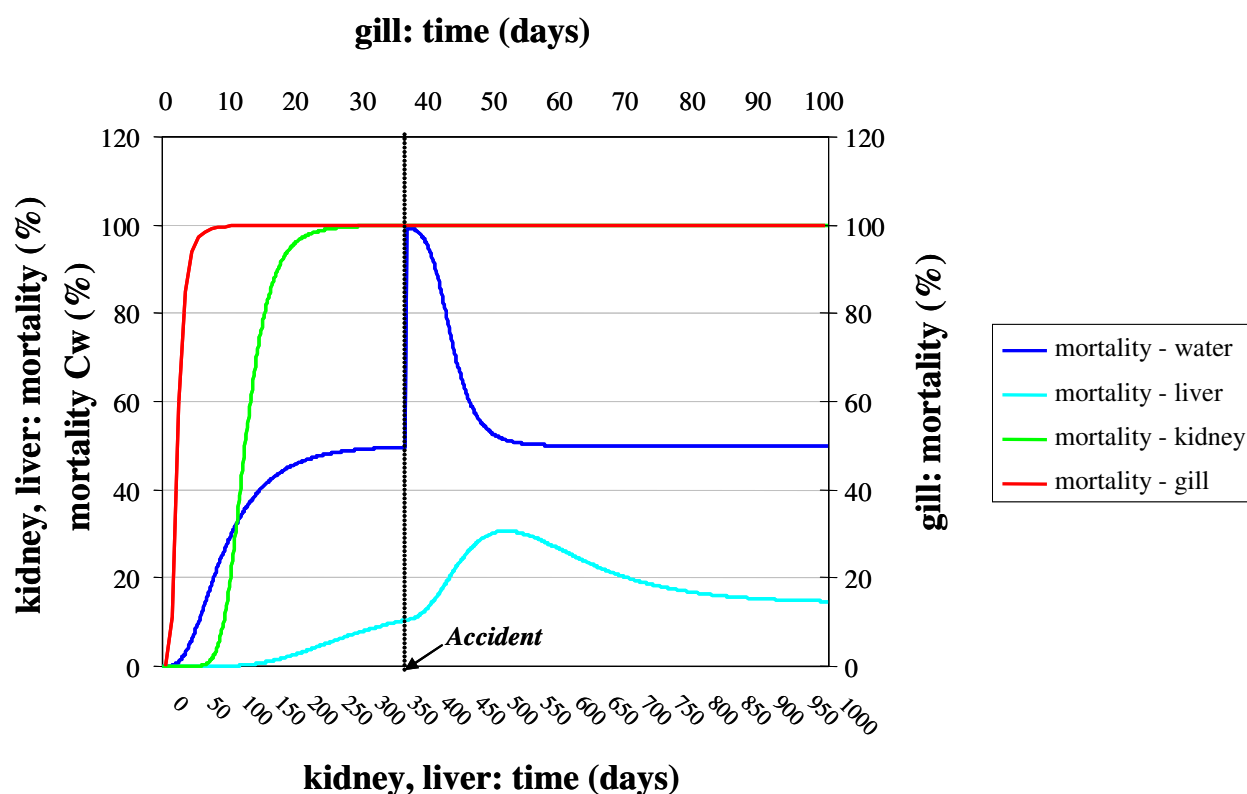


Figure 7. Estimated temporal mortality of fish as a function of the biologically effective dose of Cd(II) in selected organs following a transient release of Cd(II) into the water body

1.3.3 Modelling of the temporal spread of pollutants and risks in aquatic ecosystems

ECODIS generated new knowledge in understanding and predicting pollutant chemodynamics at the microscale, and in the coupling of these processes with macroscale flows in the water body. Sink/source functioning of sediments and biofilms is a process of key importance in this regard. The overall outcome is a model for the temporal spread of pollutants and risks in aquatic ecosystems at the catchment scale.

At the microscale, the effect of protonation of ligand and metal species on metal complexation kinetics was quantified. An important identified feature is that the species which is kinetically leading, may be only a minor component of the equilibrium composition of the exposure medium. Dissociation kinetics of complexes was found to be modified in mixtures of ligands; both enhancements and suppressions of the individual fluxes can occur depending on the composition of the mixture.

Computer codes for computation of fluxes arises from microscale processes, e.g. accumulation at a dynamic sensor or by an organism, were developed. These codes are freely available via the internet at: <http://www.unige.ch/cabe/dynamic/>.

In terms of sink/source functioning, a generic dynamic framework was established for the kinetics of metal release from various types of ligands. The results are summarised in Table 1.

Table 1. Dynamics of metal ion release in aquatic systems

Type of inner-sphere complex	Characteristics	Dissociation kinetics ^a
Dissolved	Small inorganic/organic species Macromolecular species (humic acids, polysaccharides, etc.)	Eigen (k_w, K^{os}) $k_d = k_w K^{os} / K$
Hard colloid / surface bound	Colloidal/surface species Confined ligand distribution	surface Eigen (k_w, K_s^{os}, ψ^s) $k_d^* = k_d \exp(\psi^s) \left\{ 1 + \frac{k_a \bar{c}_s}{4\pi a D_M \rho_p} \right\}^{-1}$ (sphere, low M/L)
Soft colloid bound	Confined ligand distribution Donnan potential	Donnan-Eigen (k_w, K^{os}, ψ^D) $k_d^* = k_d \exp(\psi^D) \left\{ 1 + \frac{k_a \bar{c}_s}{4\pi a D_M \rho_p} \right\}^{-1}$ (sphere, low M/L)
Biofilm bound	Diffusive exchange with medium Efflux, lysis	k_d, k_d^* k_{eff} } coupled with $k_{diff} O(D/d^2)$

^a Symbols: k_d = dissociation rate constant for a homogeneous complex, k_a = formation rate constant for a homogeneous complex, k_w = rate constant for water substitution in the inner hydration shell, k_d^* = apparent dissociation rate constant for a colloidal ML complex, K^{os} = stability constant for the surface outer-sphere complex, K = stability constant for the inner-sphere complex, ψ^D = Donnan potential, D = diffusion coefficient.

Advection was found to impact significantly on metal exchange, retention, and release, at the sediment/water interface. Significant accumulation is found in the surface layer, pointing to the importance of biofilms for metal transport in permeable sediments.

Integrated micro- and macroscale models were developed by coupling modules for microscale processes with macroscale distributed hydrological and transport models. In case of a disaster, it is crucial to make a transport model of the receiving catchment at short notice. ECODIS developed an approach based on scaling and self-organization which enables rapid generation of a catchment model up to a certain order, after which a simple 1D transport and associated dynamic chemical model can be used to predict the spread and fate of pollutants. An illustrative example that includes a 1D transport model which accounts for convection, dispersion and Langmuir sorption is shown in Fig 8. This example shows the predicted fate of a pollutant for two scenarios: (a) one without and (b) one with lateral inflow to illustrate the effects of dilution. It is evident that lateral inflow has a profound impact on the concentration profile of the pollutant.

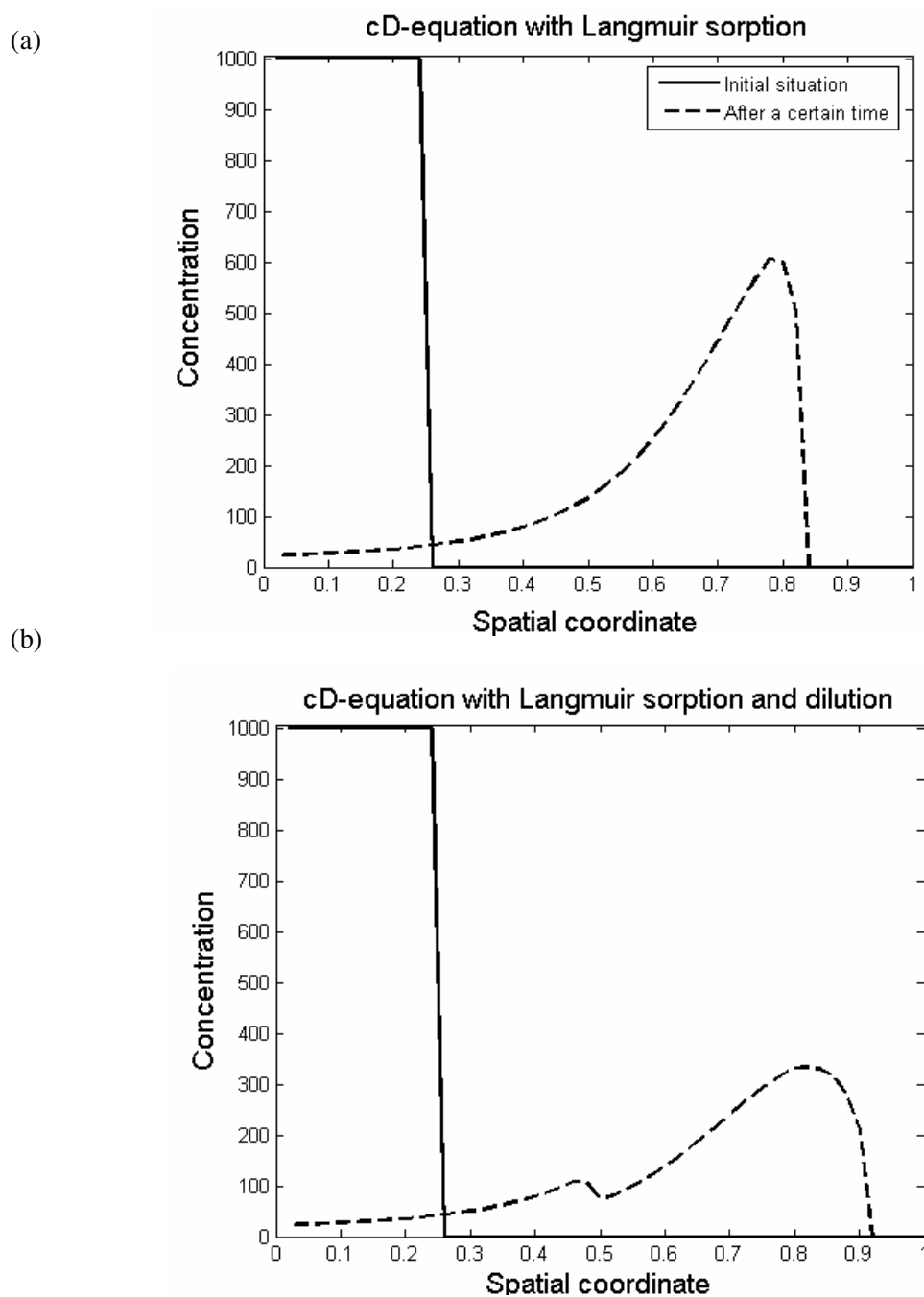


Figure 8. Evolution of the spreading of a pollutant computed via a 1D transport model coupled with Langmuir sorption. (a) no lateral inflow, (b) with lateral inflow at $x = 0.5$

1.3.4 Measurement and management of disaster-site data and development of dynamic risk assessment

1.3.4.1 Monitoring of pollution disaster impacted aquatic ecosystems

Field campaigns have been conducted during which simultaneous chemical speciation, biouptake, and ecotoxicological measurements were made *in situ*. The results highlight the utility of the multidisciplinary approach adopted in ECODIS for monitoring and interpretation of temporal and spatial pollutant chemodynamics coupled with biological impacts. An illustrative example is shown in Fig. 9 for a time series of Cd(II) speciation together with master variables (O_2 and pH) that were controlled by the activity of phototrophic benthic biofilms in this case. This result shows that substantive variations in the exposure conditions can occur on the diel timescale, thus underscoring the need for continuous real-time monitoring to adequately characterise the impact of pollutants on aquatic ecosystems.

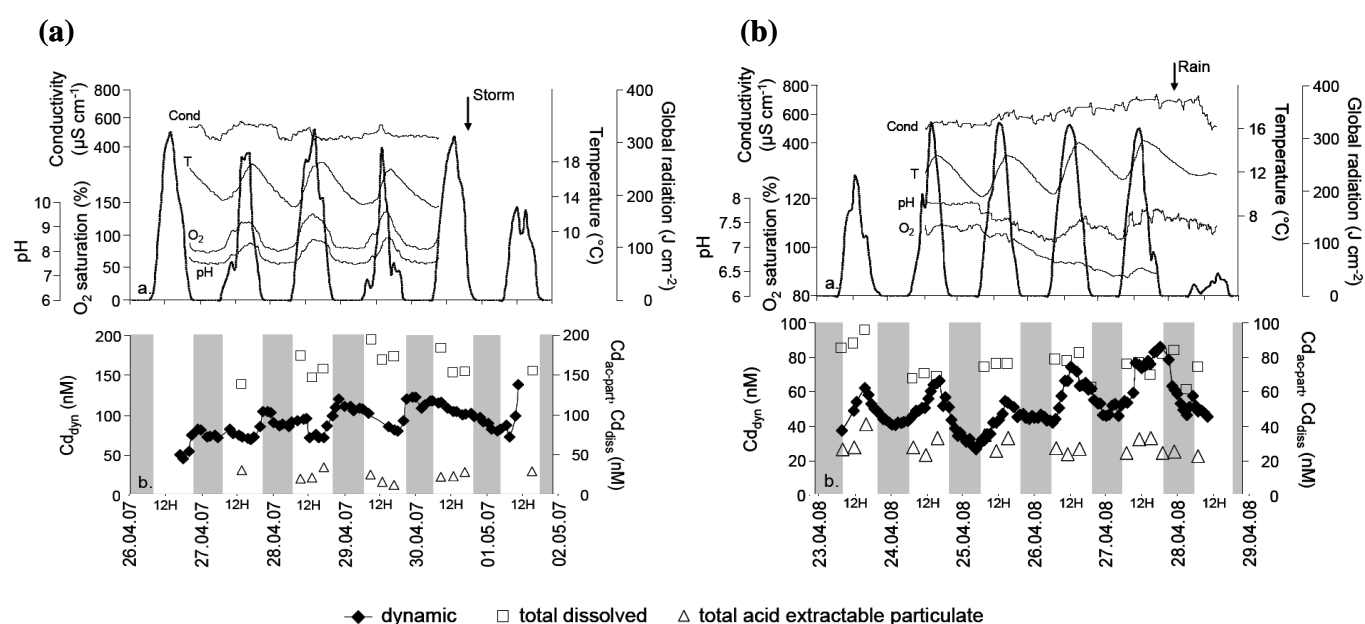


Figure 9. *In situ* continuous voltammetric measurements of dynamic Cd species and master variables (conductivity, temperature, pH, and oxygen saturation) recorded simultaneously at the Riou-Mort Joanis site during the (a) 2007 and (b) 2008 ECODIS Riou-Mort/Lot watershed field campaigns coupled to hourly global radiation data and Cd total dissolved and total acid extractable particulate fractions measured in samples collected three times per day (09:00, 13:00, 17:00).

1.3.4.2 Dynamic risk assessment

The damage to the aquatic environment following an accidental spill of dangerous substances is determined by the intrinsic properties of the substance itself (aquatic toxicity, persistence, bioaccumulation, solubility in water, etc.), the fate of the substance in the aquatic environment (evaporation, sedimentation, dilution, chemical reactions, degradation, etc.), the physical conditions of the aquatic environment (flow rate, dimensions, physicochemical properties of the water, prior pollution, etc.), and the population and sensitivity of aquatic habitats. Exposure conditions experienced by organisms and consequently the related ecological risk are defined by the temporal profiles of concentration and speciation of pollutants. These profiles need to be quantitatively linked to biological effects and finally, estimates of ecotoxicological risk. One of the major ECODIS goals was to develop a modelling framework that includes predicted pollutant species distributions, and ensuing biological risks, in all compartments of the aquatic ecosystem as a function of time and space.

ECODIS developed strategies to adapt current risk assessment practice, based on PEC/PNEC ratios, to dynamic disaster conditions. The approach focused on obtaining a better estimate of toxicity that considers the details of

the disaster-impacted exposure conditions, i.e. the temporal profile of pollutant speciation in the external medium and the ability of the organism's physiology to handle the internal dose within a given timeframe. This represents a novel approach to addressing ecotoxicological risk in the case of ecological disasters, introducing biology-based concepts for assessing dose-response relationships between environmental concentrations of toxicants in the aquatic environment and health effects on aquatic organisms such as muscles, shellfish and other fishes. Dose-response-time estimates of toxicity were generated, which extend the use of conventional "static" risk metrics. The dynamic toxicity model estimates target organ concentrations and physiological response as a function of time, thus improving our understanding of the dynamic relationships between concentration exposure and hazard to aquatic species. Furthermore, appropriate biological tissues that may serve as biomarkers sensitive to acute and transient exposure to waterborne contaminants such as heavy metals and persistent organic pollutants (POPs) are identified. The implementation of the methodology was shown on the mortality of tilapia (a tropical fish species) and of rainbow trout due to arsenic and cadmium contamination respectively. These results helped identify the biologically effective dose of the respective toxicants in tissue such as the gill, the liver and kidney as a key predictive indicator of short and long-term toxicity.

According to this approach, a time-integrated biologically effective dose of the toxicant, which considers not simply the area under the dose-response curve, but rather the complex volume delimited under the three-dimensional dose-response-time surface can be calculated and linked to observed pathologies. The integrated dynamic risk assessment methodology outlined above was implemented in the case of the WP9 campaign results from the Riou Mort (FR) for the case of cadmium contamination. Results for Cd accumulation in target tissues of trout and the ensuing mortality are shown in Figs 10 and 11, respectively.

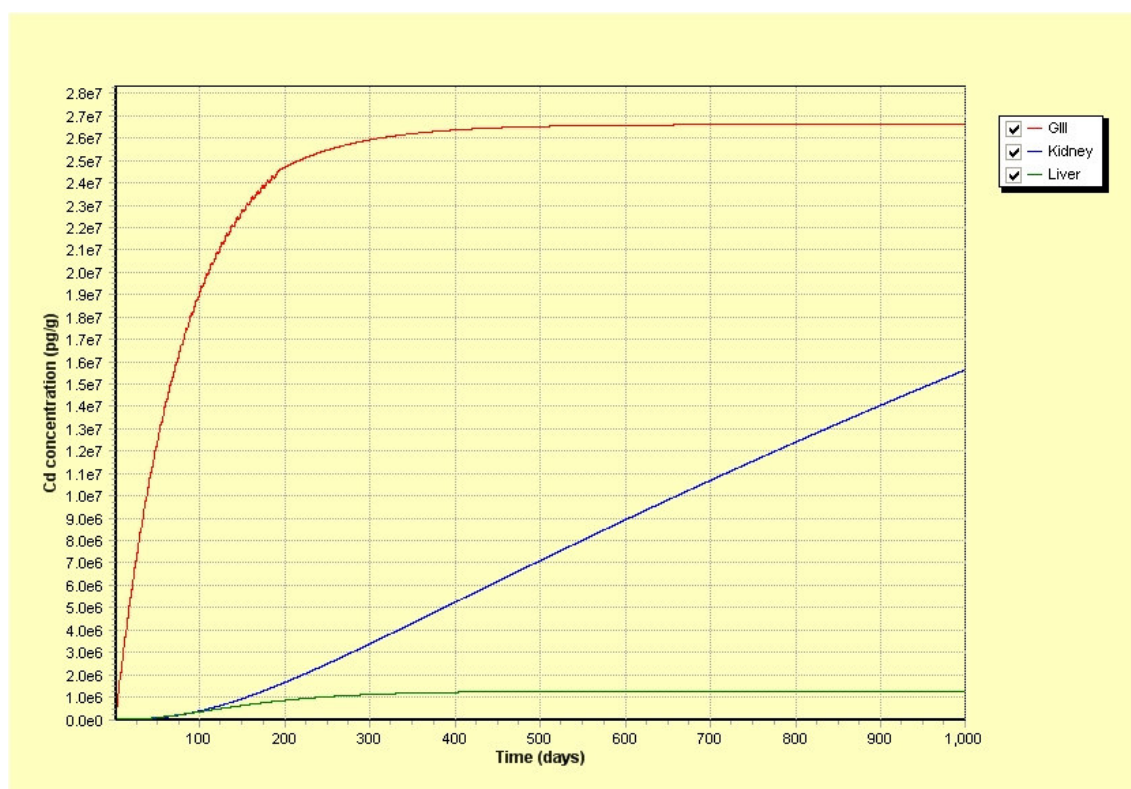


Figure 10. Internal concentration of Cd in target tissues of trout modelled for conditions prevailing during the Riou-Mort 2007 field campaign

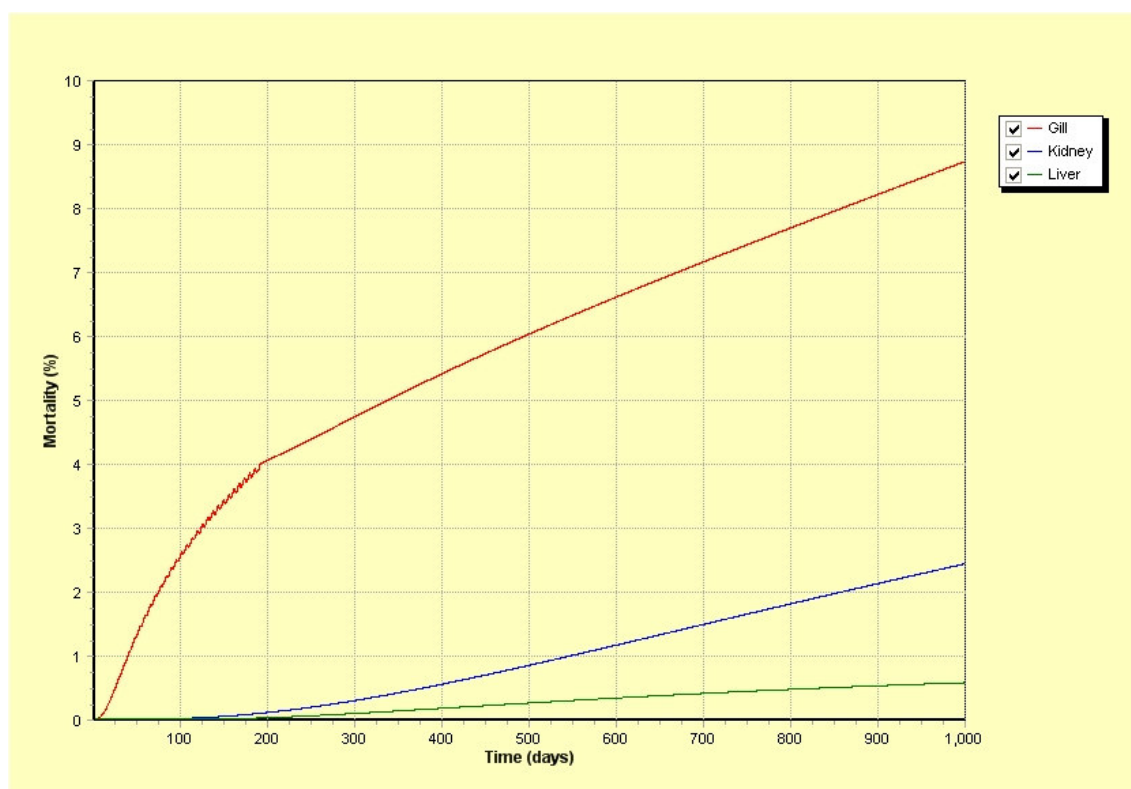


Figure 11. Trout mortality based on biological effective dose (BED) of Cd in target organs modelled for conditions prevailing during the Riou-Mort 2007 field campaign

The results indicate that a biologically-based risk assessment for metal exposure improves our understanding of the dynamic relationships between concentration exposure and hazard to selected aquatic species. In this context, the concentration of contaminant in some target organs (e.g. gill) may serve as a surrogate biomarker sensitive to acute exposure to waterborne contaminant (e.g. As, Cd), while concentrations in other organs (e.g. liver and kidney) can be used as biomarkers that are sensitive to chronic exposure.

1.3.4.3 Technical Guidance

A Technical Guidance document was produced that integrates the ECODIS results in the framework of recommendations for measurements to be undertaken in the event of a pollution disaster. Details are given as to how to interpret monitoring data for predictions of the temporal evolution and spread of pollutants and their biological impacts within the receiving water catchment. The key components of the guidance document are shown in Fig. 12.

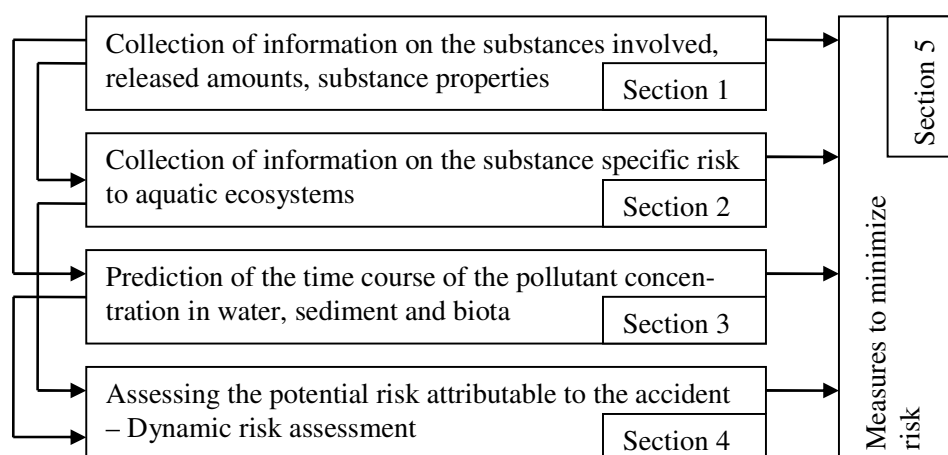


Figure 12. Components of disaster management following the accidental release of pollutant(s) to freshwater ecosystems and structure of the technical guidance document (arrows indicate a flow of information).

The Technical Guidance document includes annexes that provide (i) a case study for Cd(II) speciation and fate, (ii) an overview of closely related EU projects, and (iii) a review of past industrial accidents that have had significant adverse repercussions on the aquatic environment across the EU.

1.4 Summary of end results, and intentions for use and impact

The ECODIS end results are broad in scope, encompassing fundamental scientific knowledge, technology for *in situ* monitoring, and a user-friendly guide for crisis managers. Specific results comprise:

- A suite of sensors and *in situ* probes, covering a range of response times and spatial scales, that measure the (changes in) pollutant species concentrations under disaster conditions. The speciation sensors are complemented by ecotoxicological sensors for generic and specific biological responses, as well as an *in situ* probe for measuring algal bioaccumulation fluxes.
- Theoretical concepts that quantitatively link analytical signals from the speciation sensors to responses from the most appropriate biota over a wide range of pollutant species concentrations.
- Models, coupling micro- and macroscale processes, that quantitatively describe the pollutant spread in a water body upon disaster impact
- An innovative approach to ecotoxicological risk assessment that links dynamic pollutant exposure profiles with toxicology, resulting in sophisticated strategies for disaster management policies.
- A set of user-friendly guidelines for crisis managers that outlines strategies for monitoring, data management, and interpretation of pollution disasters

Impact on its industry and research sectors

Directly, the ECODIS results will be used by crisis managers on site of pollution disasters, both to direct actions in the immediate wake of the event, and to develop optimal responses based on their predicted spread and ecological impact. The scientific knowledge generated contributes to furthering multidisciplinary understanding of ecosystem functioning and response to pollutants. The sensors and models can be applied to characterization of the physicochemical features of pollutants of concern to provide a more comprehensive database of properties that are relevant for dynamic risk assessment.

Within the broader scope of societal and policy objectives, the results of ECODIS contribute in a wide range of contexts. For example, the better understanding of the spatial and temporal impacts of pollution disasters that we develop will help define the most suitable geographical locations for future establishment of industries. The sound basis we establish for quantifying the actual and potential damage to aquatic ecosystems impacted by a pollution disaster will provide a more comprehensive foundation for addressing the issues of liability and compensation. The quantitative understanding of key impact parameters identified in ECODIS will contribute to the design of optimal strategies for disaster clean-up, thus minimizing expense.

The ECODIS results also contribute to development and implementation of the Commission's policies. Specifically, the Water Framework directive calls for measures to reduce the impact of accidental pollution incidents and systems to detect or give warning of such events. The sensors and models developed by ECODIS address these issues. Other policies for which the ECODIS results are of relevance include the Integrated Pollution Prevention and Control Directive, and the Seveso II Directive on the control of major accident hazards.

2. DISSEMINATION AND USE

The majority of the sensors dedicated for pollution disaster monitoring within ECODIS were already in the public domain at the outset of the project. Consequently the scientific publications arising from the new knowledge on their functioning serve as the best means for dissemination and promotion of use. The publishable results of the final plan for using and disseminating the knowledge are given below.

2.1. Stirred Underwater Biouptake System, SUBS

Result description: SUBS is a device for *in situ* measurement of bioaccumulation by microorganisms in aquatic systems. It is comprised of two compartments: one houses the electrical components, and the other maintains a continually stirred suspension of algae surrounded by a semi-permeable membrane, through which ions can diffuse.

Possible market application: application in research and in water quality monitoring.

Stage of development: prototype device

Collaboration sought: testing of prototype in diverse aquatic environments.

Collaborator details: academic, water quality monitoring institutes.

Contact details: Dr. K. J. Wilkinson, Department of Chemistry, Université de Montreal, C.P. 6128, Succursale Centre-Ville, Montreal, H3C 3J7 Canada. Email: kj.wilkinson@umontreal.ca

2.2 EcoRa-test

Result description: web portal for ecological risk assessment methods. The EcoRa-test Portal features a public on-line database of methods used in ecological risk assessment practice. The portal can be used to quickly find relevant tests for given situation and thus support the decision making process. The portal includes collation of a wide range of ecotoxicological methods with a user-friendly interface and quick search capabilities. Each method is described in detail, including citations to relevant literature and links to other information sources on the internet. The database is freely accessible at: <http://projects.cba.muni.cz/ecoratest/index.php?part=welcome>

Possible market application: application in research and in ecological risk assessment practice.

Stage of development: accessible web portal with currently ca. 100 records. Database is actively updated.

Collaboration sought: addition of further tests to the database and evaluation of their applicability.

Collaborator details: academic, industrial, regulatory bodies, etc.

Contact details: Prof. I. Holoubek, RECETOX, Masarykova Univerzita v Brno, Kamenice 126/3, 62500 Brno, Czech Republic. Email: holoubek@recetox.muni.cz

2.3 Computer codes for computation of dynamic physicochemical processes: FLUXY and MHEDYN

Result description: Downloadable computer codes with user-friendly interface for computation of fluxes towards consuming interfaces (e.g. sensors, organisms). FLUXY is a code for calculation of steady-state metal fluxes at interfaces, in complexing solutions containing simple ligands, fulvics, particulate/aggregate complexants or their mixtures. A database for metal complexes, cations and ligands, with thermodynamic constants, chemical rate constants, diffusion coefficients, and other parameters required for flux computation of metals at interfaces is included. MHEDYN is a code for computation of metal fluxes at consuming interfaces in multiligand solutions. It enables computation of transient and steady-state fluxes as well as concentration profiles at the interface. Both codes, and associated user manuals are freely available from: <http://www.unige.ch/cabe/dynamic/>

Possible market application: application in research and in interpretation of environmental speciation and bioavailability data.

Stage of development: completed functioning code. New features added as new knowledge is obtained.

Collaboration sought: application of computations to range of systems.

Collaborator details: academic, endusers, etc.

Contact details: Prof. J. Buffle, Université de Genève, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland. Email: Jacques.Buffle@unige.ch