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A new scheme for biomonitoring heavy metal concentrations in semi-natural wetlands

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Abstract

This work introduces a semi-natural wetland biomonitoring framework for heavy metal concentrations based on a robust dynamic integration between biological assemblages and relevant biosensors. The cooperative/synergistic scheme developed minimizes uncertainty and monitoring costs and increases reliability of pollution control and abatement. Attention is given to establishing a fully functioning and reliable network approach for monitoring inflows and achieving dose–response relations and calibration of biomonitoring species. The biomonitoring network initially consists of both, biosensors and species, as a validation phase in each wetland of the surveillance area; once the species monitoring efficiency is verified by the biosensors, the biosensor network moves to the next wetland and so on, following a circular pattern until all area wetlands have a fully functional natural monitoring scheme. By means of species recalibration with periodic revisiting of the biosensors, the scheme progressively reaches a quasi steady-state (including seasonality), thus ensuring reliability and robustness. This framework, currently pilot-tested in Voiotia, Greece, for assessing chromium levels, has been built to cover short-, medium- and long-term monitoring requirements. The results gathered so far, support the employment of the proposed scheme in heavy metal monitoring, and, further, arise the need for volunteer involvement to achieve long-term viability.

Keywords: Decision support system; Fuzzy multicriteria analysis; Heavy metals biomonitoring; Semi-natural wetland

1. Introduction

Wetland biomonitoring evaluates the health of the waterbody by directly measuring the condition of one or more of its taxonomic assemblages (e.g., macroinvertebrates, plants) and supporting chemical and physical attributes [1]. A major premise of biomonitoring is that the community of plants, animals and lower organisms will reflect the underlying status of the wetland in which they live. When a natural wetland is damaged by human activities or a semi-natural one is receiving industrial inflows, biological attributes such as taxonomic richness, community structure, trophic structure, and health of individual organisms will change. Continuous or intermittent discharges in semi-natural wetlands create shock loadings to a water body, and the ecological effects depend on many variables and complex interactions (Fig. 1). For example, in disturbed systems the number of intolerant taxa typically decreases and the proportion of tolerant individuals typically increases [2]. Moreover, many runoff pollutants become attached to sediment particles and settle quickly, exerting detrimental effects over a long period. The high peak flow rates and volumes of urban runoff degrade habitat (e.g., channel and bank erosion) and elevate sediment deposition, the effects of which are not detected by chemical monitoring.

Several approaches have been implemented to exploit biological stress response for evaluating environmental conditions in complex ecosystems as aquatic [3,4], semi-aquatic and semi-terrestrial habitats [5–8], aiming at analyzing species environmental relationships for the development of models that could be used to predict impacts on biodiversity under altered hydrogeochemical regimes. However, only a few of them have been systematically expanded into standardized and tested approaches [6,8–10], leading to valuable ecologically relevant classification schemes, biological metrics and indicators. Assessments using plant functional types in community descriptions and biogeography show great potential, although, with limited exceptions [8,11,12], linkages remain to be made

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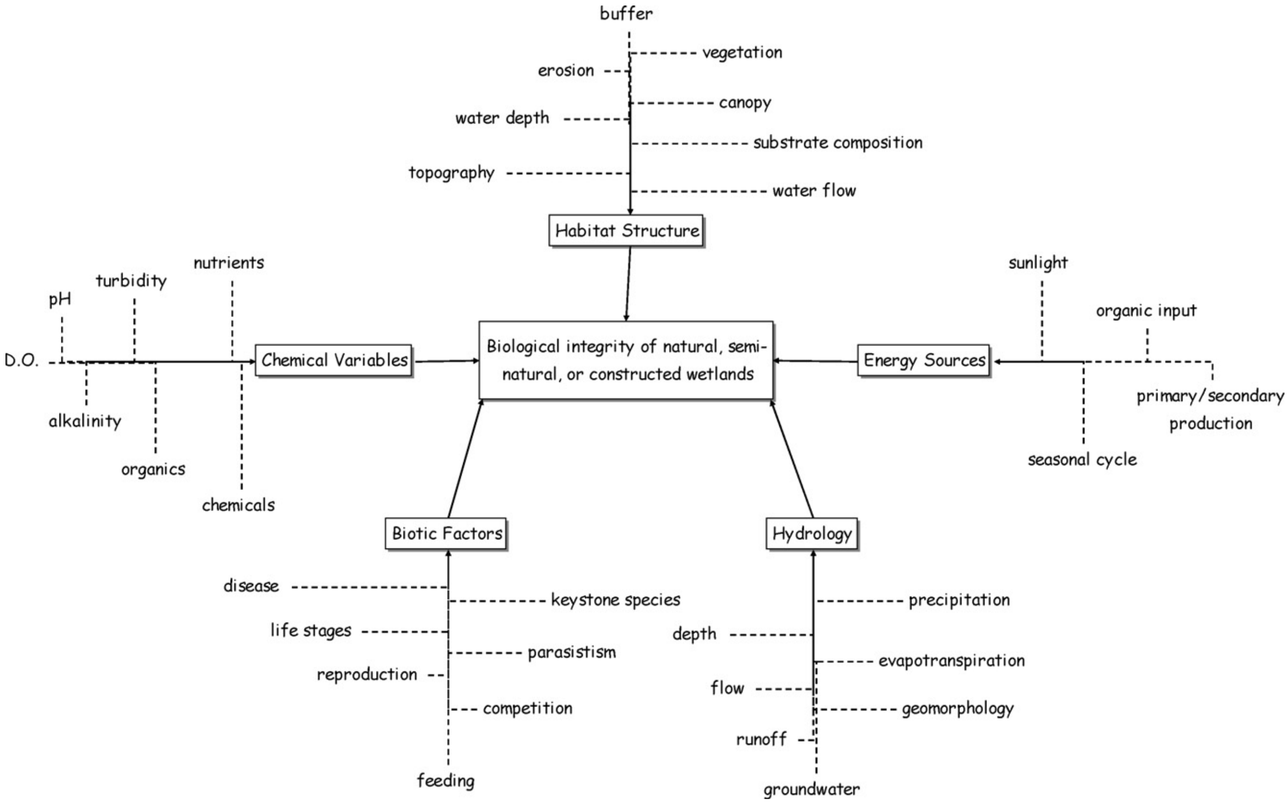


Fig. 1. Ecosystem influences on biological integrity.

between phenotypic responses and specific functions such as nutrient uptake capacity of wetland vegetation and heavy metal systemic accumulation facilitated by soil microbial processes. Notwithstanding the progress made in mapping the environmental status and creating methods and models for predicting ecosystem change using biological organisms, biomonitoring has not yet been seen as an actual measuring system that could be used for quantifying comprehensively pollution loads.

Undoubtedly, the most effective way to evaluate wetland pollution burden is to directly monitor the most representative biological component of wetlands and to support that information with chemical and physical data. Considering, however, the high cost of instrumental long-term monitoring [13,14], the shifting to completely natural biomonitoring seems inevitable if long-term and large-scale biosurveillance is required and/or sought for [15,16]. Such a scheme would necessitate the use of biologically relevant instrumentation aiming at ‘calibrating’ the biological signals via suitable correlations over a period of time, gradually translating the biological responses to measurements; thus by lessening the associated uncertainty, the scheme will finally lead to natural biomonitoring, only periodically tested/re-calibrated in field by biosensor revisiting. A similar approach has been successfully implemented by the authors in the case of air pollution monitoring of remote areas based on lichen groups and biosensors developed on the lichens’ biochemical systems affected by the stressors [15].

The selection of the biological-relevant systems for biosensor development clearly depends primarily upon the selection of the flora/fauna species. Thus the emphasis of any decision-making procedure should be given to the appropriate choice of a group of organisms that fulfill a set of environmental, scientific and practical criteria among a large number of roughly studied flora/fauna candidates, each of which bears its own strengths and limitations. Multicriteria decision aiding is well suited for environmental planning issues [17,18]. It may provide deep insight into the structuring of the problem and it can treat the uncertainty of the required information through probabilistic distributions, fuzzy sets and threshold values inclusion to yield the best solution from a set of composite alternatives that can be used for addressing the problem. These alternatives represent either object-oriented options, i.e., serial sub-systems differing in one link and the aim is to select the best link for enhancing the performance of the system [19], or more subjective solutions, based on (i) the availability of their precursors (sources-oriented) [15,16] and/or (ii) their intended use (target-oriented) [20,21]. The problem that is structured herein falls in the sources-oriented sub-category, where the best alternative for monitoring a given heavy metal should not only approximate the way that the human mind expresses and synthesizes preferences but also the way the environment expresses itself.

The aim of the present work is to introduce a wetland biomonitoring framework for heavy metals based on a robust dynamic integration between biological assemblages (including plants, animals, invertebrates, phytoplankton, zooplankton, etc.) and relevant biosensor systems. This cooperative/synergistic scheme will minimize uncertainty and monitoring costs and increase reliability of pollution control and abatement. Attention is given to establishing a fully functioning and reliable network approach for monitoring inflows of special concern, such as chromium, and achieving dose–response relations and calibration of species, also taking into account the biological remediation activities and potential of the area. The proposed scheme comprises of four main features, interrelated and integrated sufficiently through an activity and managerial framework that will ensure its long-term and large-scale biosurveillance capacity, feasibility and viability. These features are: (a) the biomonitoring potential of the area wetlands, i.e., the inhabiting species that can be used as reliable indicators or scavengers of the heavy metal(s) selected for monitoring; (b) a network of biosensors, developed on the basis of the biochemical/cellular systems of the species (preferably as expressed in their stress response mechanisms and characteristics for allowing direct signal correlations); (c) a local biochemical laboratory unit for research/development/control/maintenance of biosensors, that could be hosted at local universities or institutes; (d) a local flora/fauna nursery, caring for the biological assemblages (cultivation, seeding, growing, examination, etc.). The group of species is selected through multicriteria ranking using fuzzy reasoning to count for uncertainty. The biomonitoring network initially consists of both, biosensors and species, as part of a validation phase in each wetland of the surveillance area; once the biological monitoring efficiency is verified by the biosensors, the biosensor network moves to the next wetland and so on, following a circular pattern in the surveillance area, until all area wetlands have a fully functional natural monitoring scheme. By means of species recalibration with periodic revisiting of the biosensors, the scheme progressively reaches a quasi steady-state (including seasonality), thus ensuring reliability and robustness. The framework presented and currently pilot-tested in Voiotia, Greece, for assessing chromium levels, has been built to cover short-term (alarm), medium- and long-term requirements for voluntary wetland biomonitoring.

2. Methodology

The proposed scheme, is effectuated through a knowledge base (KB) that accommodates pollution (stressor) information and dispersion models, wetland characteristics (geographical, hydrogeomorphic, and habitat-based) and historical data (if available), biological response to stressors (from community to biochemical level), as well as biosensor construction details and metrological data. The information/data entering the KB and processed through a multi-layer platform, follow a hierarchical taxonomy based on functional component and operational structure decomposition and enables structured data management, both on the surface-knowledge (phenomenological) and the deep-knowledge level [16]. For example, each potential monitoring species is decomposed according to mechanism and the type of response to stress, from initial uptake to metabolism and release or accumulation, i.e., community, systemic, cellular, sub-cellular, and biochemical effect. Cross-domain combination of parts (partonomy) is based on the intra- and inter-relations of all components involved, i.e., species *available* in a certain wetland type, *expressing* a stress mechanism *on* a given

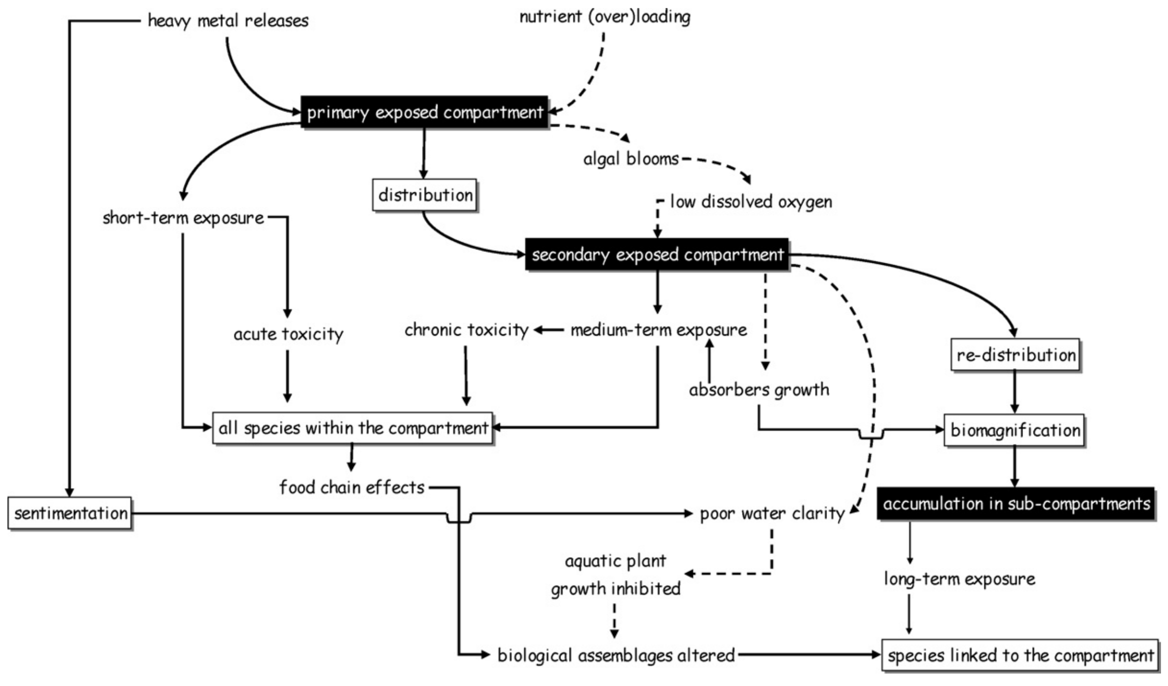


Fig. 2. Heavy metal biological distribution within a semi-natural wetland. The surface species near the release point (mostly algal blooms and insects) are the first to be affected (primary exposed compartment) to various degrees and extent (depending upon species tolerability). The distribution to the secondary and the long-term sub-compartments involves both chemical dispersion (resulting in sedimentation) and biochemical distribution (through the food chains). The species including/affected in each compartment are candidates for short-, medium- and long-term bioassessment/monitoring, respectively. Their type and magnitude of response, however, is strongly affected by nutrient availability: nutrient overloading, critical for the rapid growth of the phytoremediation species (absorbers or converters), enhances the food chain effect, as well as the dissolved oxygen and sunlight availability, modifying accordingly the cellular functions of the biological assemblages in the secondary exposed compartment and altering the rates of biomagnification.

heavy metal *based on* biochemical effect, that *can be utilized in* the construction *of an* enzyme-based biosensor (phrases in italics denote ontological semantics [15,16]). The development of the current KB has been focused on representational issues and detailed analysis to support retrieval and composition, rather than minimizing space requirements and improving efficiency. The KB is continually enriched by the outputs/results of the ongoing project as well as from external databases by the use of an intelligent agent (IA), according to Ref. [22].

This hierarchical structure supports the development of efficient domain-specific query mechanisms, providing among others (a) the site/wetland type-available biological assemblages, i.e., groups of species (plants, animals, insects, annelida, mollusks, echinoderms, phytoplankton, zooplankton) that can be used for heavy metal monitoring, as based on their stress response (accumulation, mortality, behavioral changes, intoxication, biochemical responses, etc.), and (b) the related biosensors. The biological assemblages finally selected for employment in the area of interest (through a fuzzy multicriteria process, specially adopted to cover the current needs) are split into three categories (Fig. 2), depending on the phase of the program that are called to serve: species with hours to days response are used for alarm monitoring, species with stressor tolerance that lasts over or expressed after a few weeks cover the medium-term impact assessment, whereas species, basically scavengers, that can withhold heavy metals for a long period of time (months) or have high tolerance and only after a certain threshold level manifest threatening or lethal deformities, serve the long-term phase. Depending on the needs of the area, these three phases can run consecutively or even independently; for example, if relevant information alarms for a threatening situation, the first phase is activated, whereas if the remediation activities have to be monitored, the medium-phase is established. In case the status of the wetlands needs evaluation as regards previous loads (if such historical data is available from the KB), the last phase can provide a quick assessment.

On the basis of the biological assemblages chosen and the available resources (financial and technical), biosensors are developed [15] in order to provide meaningful translations of

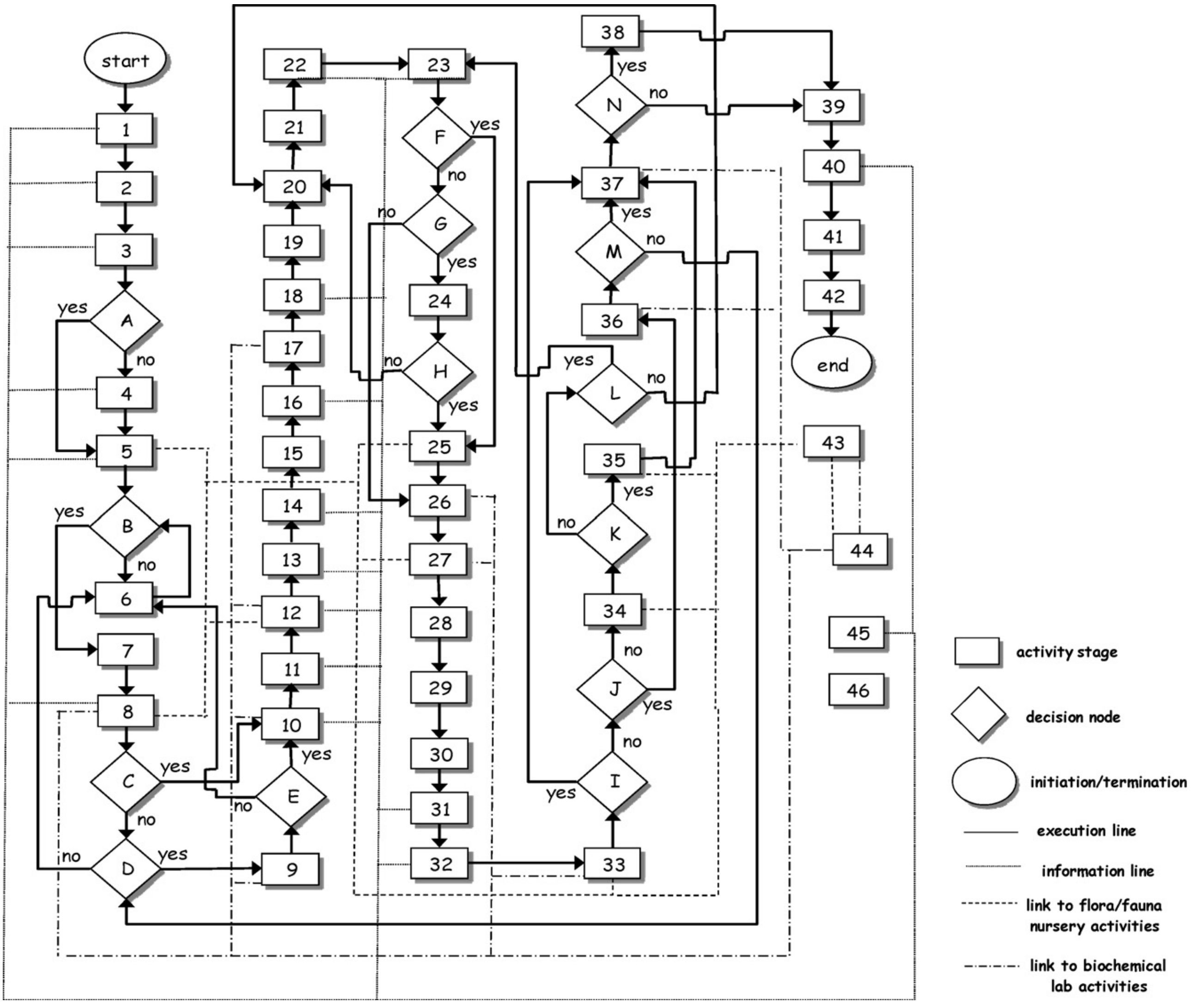


Fig. 3. The methodological framework, under the form of an algorithmic procedure, developed for biomonitoring heavy metals in semi-natural wetlands.

the biological responses. The selection of the biological system to be utilized for biosensor construction, depends on the type of response of the organism, including enzymatic (e.g., nitrogenase), biochemical (e.g., metallothionein complex), cellular (e.g., photosystem P_{450}) or whole cells (e.g., algae); alternatively, small organisms (e.g., diatoms) can be readily identified by nucleic acid biosensors, a practice commonly applicable in the case of species showing acute toxicity and remain the best indicators of heavy metal inflows [23–25].

2.1. The methodological framework

The methodological framework, is split into three parts, namely, preliminary, preparatory, and project, designed/developed by the authors in the form of an algorithmic procedure, containing 46 activity stages and 13 decision nodes, as described here below; for their interconnection see Fig. 3.

Activity stages

(1) Collection of information regarding the heavy metal releases from sources in the area (or/and from similar sources elsewhere) and on their reactivity/fate after release.
(2) Collection of land use, habitat activities, and wetland characteristics, including historical data (if available).
(3) Selection of reference (minimally impaired) and severely damaged sites as well as some in between to establish/represent the gradient of human disturbance.
(4) Utilization of reference data available from similar wetlands in neighboring areas or worldwide for creating a predictive model of minimal impairment.
(5) Creation of a KB for local flora and fauna (genus level) and their classification according to their response to stressors and their potentiality for use in short-, medium- and long-term biomonitoring.
(6) Search for suitable species (as per monitoring potentiality and habitat similarity) or from relevant data from similar installations and technical literature.
(7) GIS mapping of the biosurveillance area with layers covering (a) geomorphological characteristics, (b) environmental characteristics that determine the aquatic ecosystem status, and (c) the biomonitoring potential of the area.
(8) Pre-selection of species that exhibit stress responses suitable for biosensor development: (a) biochemical response intended for the development of enzyme-based biosensors; (b) systematic response intended for the development of whole cell biosensors; (c) species exhibiting acute toxicity and can be identified by nucleic acid biosensors.
(9) Preliminary lab studies intended for verifying the correlation of the biosensor system to the species response.
(10) Pre-selection of transducer technology for biosensor development on the basis of local resource and technical know-how/support availability.
(11) Selection of species groups with high indicatory relevance for ecological changes in the bank, according to Ref. [6].
(12) Selection of abiotic parameters (e.g., pH, nutrient content, protein content, etc.) to be used as control for determining (a) species health and growth status, and (b) environmental conditions related to biosensor signal generation.
(13) Selection of environmental variables for bank assessment (soil variables, vegetation, groundwater chemistry, hydrological variables, etc.).
(14) Selection of chemical substance(s) or condition(s) to be monitored.
(15) GIS griding of the biosurveillance area and determination of (a) the sampling points and (b) the biosensor measuring sites.
(16) Stratification of bank on GIS layers in order to capture the full range of hydrological conditions and soil types, according to Refs. [6,8].
(17) In field measurements to determine/verify heavy metal(s) concentration ranges.
(18) Aggregation of bank soil and substrate types, according to Ref. [8].
(19) Computer-aided clustering, as per the effect-based model, the complimentarity model, or the statistical model.
(20) Evaluation/modification of the clusters from a mixed panel consisting of experts and volunteer representatives to form a set of n biological assemblages alternatives A_j ($j = 1,2,\ldots,n$), to be subsequently ranked by multicriteria analysis. Selection criteria: well-established sensitivity to the heavy metal(s) to be monitored; relative specificity to the heavy metal(s) to be monitored; ability to be sampled throughout the seasonal cycles; taxonomic richness; relative knowledge of taxa life history requirements; tolerances; habits; ease of identification; convenience of sampling.
(21) Selection of experts to constitute a representative sample for evaluating the preference matrix and its adjacent weights vector, according to a modified Delphi method.
(22) Defining the criteria for the multicriteria analysis and assignment of grades to the elements of the weights vector and the preference matrix by the experts (in fuzzy form to count for uncertainty); running of the fuzzy multicriteria ranking algorithm.
(23) Performance of sensitivity analysis, especially as regards the ranked ‘first’ against ‘second-best’ alternative.
(24) Selection from the KB of suitable species for reinforcing the ‘first’ biological assemblage.
(25) Final decision on the biological assemblages to be used.
(26) Development/validation/testing of biosensors for (a) alarm confirmation/quantitation of heavy metal(s) loading, (b) assessing/confirming medium-term impacts, and (c) assessing/confirming long-term effects, according to Refs. [15,16], depending on the required phase of the biomonitoring program.
(27) Calibration of species response via biosensors, according to Refs. [15,16].
(28) Study design, including the development of metrics for the biological assemblages, biosensor signal processing (according to Ref. [26]), data treatment (according to Ref.

[27]), and frequency/duration of sampling/biosensoring (to account for seasonal cycles).

(29) Project design, including the coverage of financial needs and the establishment of a volunteer-based network (recruitment, training, field assessment).

(30) Selection of a representative wetland of the biosurveillance area (as per threatening level, or testing suitability, etc.) for pilot running for a short period of time the biomonitoring program, including both species and biosensors, thus initiating the program establishment period.

(31) Bank soil and related herbage sampling and analysis, according to Ref. [7].

(32) Adoption of suitable deterministic ordination models to describe species environmental relations in soil/water interfaces, according to Ref. [6]; evaluation of stage-31 results.

(33) Parallel species and biosensor measurements and evaluation of the results.

(34) Lab testing of species to define the causes of diversification.

(35) Selection and establishment of more controls that will consider the current situation and rectify the signal correlation.

(36) Lab testing of biosensors to define the causes of failure, according to Ref. [26].

(37) Moving of the biosensor network to the next wetland, rerunning stages 33–36, while biomonitoring in the previous wetland continues with the biological assemblages, and so on, until all wetlands are covered.

(38) Interruption of the establishment period and moving the biosensor network to the threatening area for signal verification.

(39) Completion of the establishment period when all wetlands in the surveillance area have satisfactory biomonitoring efficiency.

(40) Adjustments/refining of the program (if necessary).

(41) Estimation of time periods (duration and frequency) that biosensors should execute a full program of measurements together with the biological assemblages acting on a permanent basis.

(42) Full-scale application of the program.

(43) Flora/fauna nursery (connected to all relevant stages).

(44) Biochemical lab (connected to all relevant stages).

(45) Creation/operation/enrichment/updating of a KB for the needs of the wetland management.

(46) Searching within external bases by means of an IA, according to Ref. [22].

Decision nodes

(A) Do established conditions/historical data availability allows for such selection?

(B) Is the biomonitoring capacity of the area sufficient to cover its needs?

(C) Is the specific stress response mechanism easily isolated?

(D) Is the utilization of a more general or related biochemical system suitable?

(E) Is such correlation adequate for (a) species calibration and (b) required monitoring specificity?

(F) Is the solution robust as regards the ranked ‘first’ against ‘second-best’ alternative?

(G) Is it feasible to reinforce the ranked ‘first’ alternative with the introduction of species that would increase its grade and therefore the robustness against the ‘second-best’?

(H) Is the consideration of using a combination of the first two alternatives feasible and could this solution be robust against the rest of the alternatives?

(I) Is the correlation of biosensors to species satisfactory?

(J) Does the species response follow the anticipated pattern?

(K) Are the diversification causes accounted for reasons other than unanticipated systemic activities?

(L) Are there any other alternatives from stage 22 suitable for biomonitoring purposes?

(M) Is it feasible to rectify the faults?

(N) Is biomonitoring from a wetland signals threatening levels of heavy metal(s) during the program establishment period?

2.1.1. Preliminary part

The first part, involves the stages 1–10 related to information and data gathering (from the surveillance area and from similar works elsewhere; similarity is defined as per the geomorphologic and biological community characteristics) and their classification according to a knowledge-based scheme (stage 5) that will enable their utilization for the intended use. This part is expected to yield the local potential and the possibilities that indigenous flora and fauna provide for employing the proposed (or any) biomonitoring scheme, under the prevailing area conditions.

Geographical information system (GIS) mapping (stage 7) also forms the core of the program, providing information not only on the geomorphologic picture, but also on the aquatic ecosystem and the biomonitoring potential/capacity of the surveillance area. Such an activity is critical to the management of large, often remote, areas [6,8], and further is expected to provide the means of an integrated wetland classification system establishing correct similarity indices that would allow (a) comparison of the current area to similar areas, (b) the transfer of species from similar areas in case the biomonitoring capacity of the surveillance area is poor, (c) the transfer of know-how and equipment to similar areas for establishing an analogous program, (d) the increase of comparability of results in parallel-running biomonitoring programs in similar areas, and (e) the extension of the local network to regional, national, transboundary and international level.

This part includes, also, the feasibility studies on biosensor development based on local resources and technology (stages 9 and 10). The identification and isolation of biological elements from the available species would provide tailor-made biosensors that could respond analogously to the natural systems [16], thus allowing for a more ecologically relevant assessment of the environment. Such an activity, however, poses its own restrictions to biomonitoring, even short-term, since the possibility and progress in biosensor development limits, inevitably, the selection of species (decision nodes C and D).

2.1.2. *Preparatory part*

The preliminary outcome is tested in the preparatory part (stages 11–27), where more detailed information with the required granularity and depth will give conclusive patterns, with special emphasis on biosensor development relevant to species.

2.1.2.1. Selection of biological assemblages. The selection of biological assemblages involves the selection of species that can be utilized in the three project phases: short-term/alarm monitoring, medium-term monitoring, long-term/impact monitoring. Alarm monitoring for assessing early poisoning/inflow using acute toxicity species (e.g., mortality of stressor intolerant species and/or appearance of stressor tolerant species). Medium-term monitoring for (a) selecting remediation procedure (if the system could itself adsorb and manage pollution, no remediation is required or the remediation activities should be in-line with species anti-stress mechanisms, etc.), (b) monitoring remediation effect (will require intensification of biosensor distribution and will result in establishing a correct gradient of human disturbance), (c) better assessing/managing toxicity if alarm is inconclusive. Long-term/impact for bioaccumulation/biomagnification, distribution, monitoring decreasing levels (following remediation activity), etc.

The KB provides the site-specific (or site-similar) species from various taxa with known/studied or (reasonably) suspected (and verified by laboratory analysis in stage 9) response towards the target environmental stressor, e.g., a heavy metal. All organisms or groups of organisms (e.g., algae versus invertebrates) do not respond the same to environmental stress [11]. Depending on various disturbance characteristics (intensity, frequency, etc.), each group may respond at different rates and provide different information [2,7,11,12,14,23]. For example, if the objectives are to detect potential environmental change attributed to increases in phosphorus or nitrogen, microbes or plants may best signal early changes and potential degradation [8,11,25]. Changes in the abundance of small, rapidly reproducing species with wide dispersal capabilities are among the earliest responses to stress [11]. Longer-lived organisms that are slower to recover (macrophytes, some invertebrates, and many fish) may indicate the impact of pulsed stressors that occur only periodically. Many studies have utilized specific taxa for monitoring aquatic health, deriving relevant metrics and indices [2–4,9,10,14]; however, if the objectives are to determine the impact of an exotic invasion, interacting species (prey and competitors) will be the best indicators. Clearly, it is necessary to include several groups in the biological assemblage in order to indicate overall environmental health and integrity given the potential for multiple, difficult to measure, non-point-source pollutants.

The presented approach to biomonitoring defines an array of measurements, each of which represents a measurable characteristic of the biological assemblage that changes in a predictable way with increased or decreased environmental stressor. This measurable characteristic can be based on either (a) the effect that the stressor has upon the species (physiological, biochemical, behavioral, etc.), or (b) complimentarity, based on the local trophic structure and dynamics, or (c) statistical metric models for abundance, diversity, etc. Computer-aided clustering (stage 19) groups the available species as per pre-set measurable characteristics, providing candidate groups of organisms, each comprised of various taxa having common (or similar) response to stressors (in effect-based clustering), or represent the propagation of the stressor to the top of the trophic chain (in complimentarity-based clustering), or can be assessed through registered changes over sets of metrics in response to perturbation by certain stressors (in metric-based clustering).

These candidate biological assemblages are reviewed by a mixed panel (stage 20), consisting of three biomonitoring experts and two volunteer representatives, by means of an ad hoc modified group decision-making procedure (Fig. 4); the participation of volunteers in the final structuring of the alternatives has been found necessary from a practical point of view, since, based on the authors' experience from similar works [15,16], the incorporation of their field experience and level of knowledge (which for certain issues, as their knowledge of the surveillance area, is valuable) will reduce substantially field errors and uncertainty. The mixed panel finalizes the species participating in each candidate biological assemblage, providing the alternatives, which are ranked through a multicriteria process, based on fuzzy reasoning to account for uncertainty.

The criteria for the multicriteria ranking, applicable to assemblage selection for all phases (stage 22), are the following: f_1-ability to discern individual health (malformations, deformities, lesions) from exposure to wetland; f_2-laboratory analysis (time, cost, lab equipment); f_3-reflection of wetland conditions; f_4-short time lag of response to stressor (for the alarm phase) or integrate effects over time (for medium-/long-term); f_5-sensitivity to nutrient enrichment; f_6-sensitivity to habitat/hydroperiod alteration; f_7-difficulty of sampling protocols (time, effort, etc.); f_8-ease of identification (number of species, relative skill required to identify genus or species).

The objective function of the multicriteria problem under consideration is $\max\{f_1(a),\ldots, f_K(a) \mid a \in A\}$ where A is the set of T alternatives and f_k, $k = 1,\ldots,K$, are the K criteria used for evaluation of each alternative. The computational procedure consists of two main steps: (i) the formulation of the preference matrix ($K \times T$), where each element x_{kt} is the evaluation of alternative A_t according to criterion f_k (ii) the ranking of the alternatives, as a result of applying to the rules of the selected MCA method. PROMETHEE [28] has been used as an outranking method, in its fuzzy version to count for uncertainty [20,21], allowing for incomparability ($a\mathrm{R}b$) and weak preference ($a\mathrm{Q}b$) between the alternatives a, b, in addition to the strict preference ($a\mathrm{P}b$) and indifference ($a\mathrm{I}b$) that the 'classical' methods are based on. The notion of a generalized criterion is used to construct an outranking relation by defining the preference index $\prod(a,\ b) = \sum w_i P_i(a,\ b)/\sum w_i$ as the weighted average of the preference functions P_i, that quantifies the preference of the decision maker of alternative a over b, taking into consideration all the criteria. In terms of topology, the preference index values can be represented as a valued outranking graph, the nodes of which are the alternatives. By summing the column elements in each row of the outranking relation matrix, the flow leaving each node is obtained, which shows its outranking character, while by summing the row elements in each column,

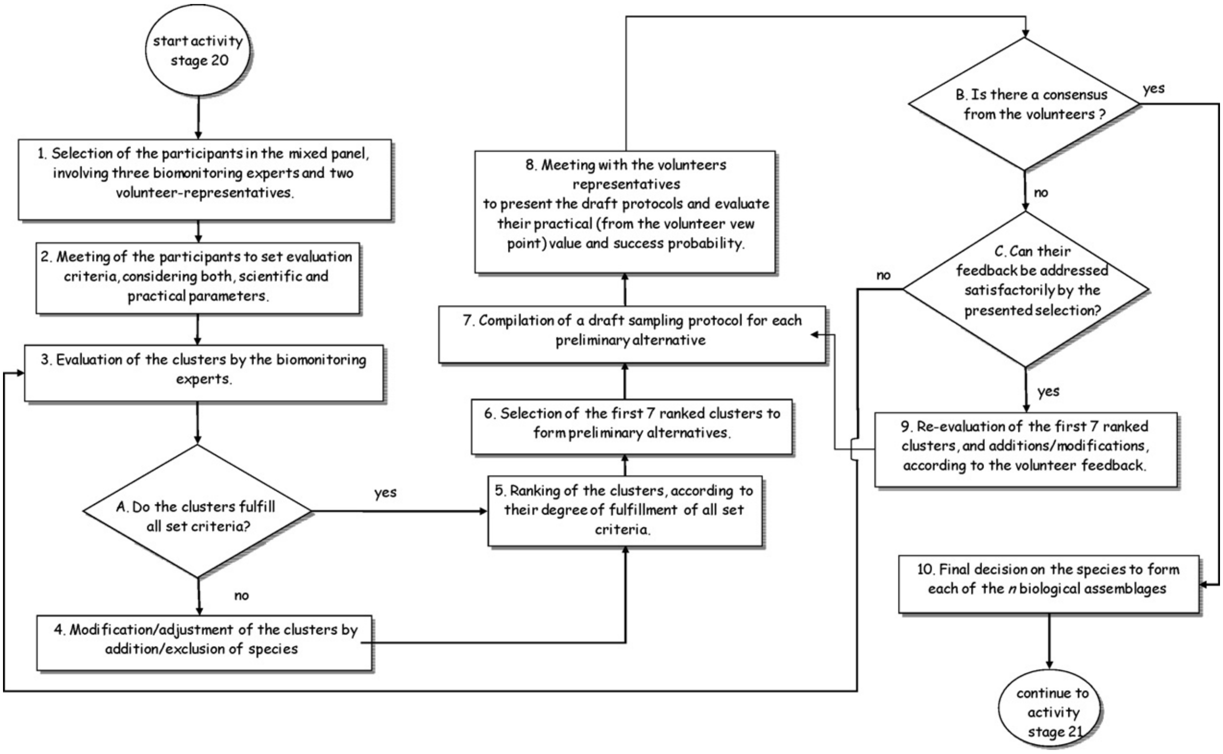


Fig. 4. The evaluation/modification of the clusters produced in stage 19 is performed from a mixed panel consisting of experts and volunteer representatives, through a 10-stage algorithmic procedure, that finally yields the set of n biological assemblages alternatives.

the entering flow is obtained for each alternative, which shows its outranked character. By considering the leaving and entering flows, as well as the fact that the higher the leaving flow and the lower the entering flow the better the alternative, the partial preorder (PROMETHEE I) is obtained. Although the partial preorder carries more realistic information, sometimes the total preorder (PROMETHEE II) is requested to avoid any incomparabilities; this preorder is induced by the net flows, i.e., the difference between the leaving and the entering flows. The generalized criterion used is a piecewise linear preference function $P = H(d) \in [0,1]$, where d is the difference of the evaluation of two alternatives a, b. The parameters of $H(d)$ are an indifference threshold q, the greatest value of d below which there is indifference, and a preference threshold p, the lowest value of d above which there is strict preference—the interval between q and p can be considered as the weak preference region.

To conclude a partial or complete preorder from the resulting fuzzy sets, the Tseng and Klein [29] method was used which makes pairwise comparison of the alternatives by calculating the (crisp) dominating areas in each pair consisted of triangular fuzzy sets (partial preorder); subsequently, the summation of the elements of each row (alternative) of the domination matrix gives a measure of the strength of each alternative that leads to the total preorder.

2.1.2.2. Biosensor calibration of species. The construction of biosensors (stage 26) and the calibration of the species (stage 27) are performed following the algorithmic procedure described in Refs. [15,16]. The organism's response to a given heavy metal can be thus studied in depth by decomposing the organism and examining, qualitatively and quantitatively, its effect upon each of the isolated biochemical systems or sub-systems. It is feasible to use the enzymes or the systems participating in stress-response as bioelements for developing biosensors; thus, the heavy metal effect can be qualified and quantified for each step of its way through the organism on the basis of the biosensors' response.

Developing biosensors for concentration-dependent response can be proven useful in identifying biochemical systems that are not affected, mostly affected or threshold-triggered by the heavy metal(s), whereas tolerance limits and dose–response relations can be estimated [15]. By investigating the mechanism of signal generation of the biosensors, the impact of the stressor upon the metabolism of the organism will be clarified. By means of interference and matrix effect studies, the effect of other substances present upon the adsorption of the target heavy metal(s) by the organism (effect upon the signal of the device, competition for the bioelement binding sites) can be qualified. These dose–response relations can be linked to morphological alterations, whereas sensitivity indices can be derived from the comparison of the performance of the biosensors developed from different species [15]. Establishing and maintaining a study scheme as such, the investigation of seasonal variations, species variability, pollution background, combination of pollutants, spot variables, etc., upon species performance becomes realistic.

2.1.2.3. Bank monitoring and management. Banks are semi-terrestrial soils with aquatic or epiaquatic moisture regimes [8] and they usually reveal higher concentrations of pollutants due to organic and mineral particles sedimentation [7]. Due to the complex water regimes existing in these soil/water interfaces [12], affecting vegetation correspondingly, the monitoring/management of this part of any natural or semi-natural wetland necessitates a more intensive approach in selecting both, abiotic and biotic key elements, in order to (a) determine and explore species environmental relations, (b) produce conclusive results for the impacts of heavy metal pollution on biodiversity, and (c) integrate the derived models in the system's remediation efficiency [5–8]. Under the assumption of stable conditions of land use and wetland inflow, water variables are assumed to be the most important environmental factors determining species occurrence and abundance (stage 13). In most cases they are also most difficult and time-consuming to measure, making them a most important potential focus for biomonitoring [6,12]. The species that have the ability to reflect and adequately represent these geochemical, hydrological and ecological characteristics of the bank strata (stage 16), are mostly vascular plants, molluscs, carabid beetles and hoverflies (stage 11) [5,6]. Besides their abundance and high indicatory relevance for ecological changes, these species cover a wide gradient of mobility [6], further to their ability to withstand changes in water levels [6,7] and nutrient input [8,11].

2.1.3. *Project part*

The final part (stages 28–42) involves mostly the managerial and administrative framework of the program, in its pilot testing and the establishment of the network. Pilot testing follows a circular pattern where one wetland is monitored by both, biosensors and biological assemblages (stage 33), and the results are used to verify the correlation model of stage 27. The biosensor network moves then to the next wetland, and so on, until all the final adjustments/refinements/corrections are made (stage 40) and area biomonitoring is reliable enough to be established for a long period of time. Bank soil and vegetation samples (stage 31) are analyzed according to Ref. [12], whereas results are evaluated with appropriate models (stage 32) [6]. Bank-specific species are evaluated as per their monitoring/remedial efficiency and if appropriate modified/adopted or, if xenophytes should be used, acclimatized in the nursery (stage 43).

The framework can be utilized/adjusted to any environmental system for multi-elemental monitoring in both phases, the gas and the liquid (since biomonitoring species are direct absorbers or influenced by air loads), encompassing a regional framework around a pollution source in the form of a LAN, with on-line data collection and mining, as regards biosensoring. Natural monitoring requires periodic field observations, the frequency of which

Fig. 5. Map of the survey area. The site contains the lakes 'Iliki' and 'Paralimni' and the springs 'Piges ton Chariton' connected by the river 'Voiotikos Kifissos' that runs into 'Yliki'. The mountains around the lakes are basically bare. The rest of the surrounding area is part of the plain of the now drained lake 'Kopais' and is cultivated land. 'Kifissos' has been canalized where it drains the plain. Around 'Paralimni' there are some small vineyards. Lake 'Yliki' can be described by the habitat type 3120 of Annex I as an oligotrophic lake (although it does not have the characteristic flora of this habitat type as it is described by Corine codes 22.11 × 22.34).

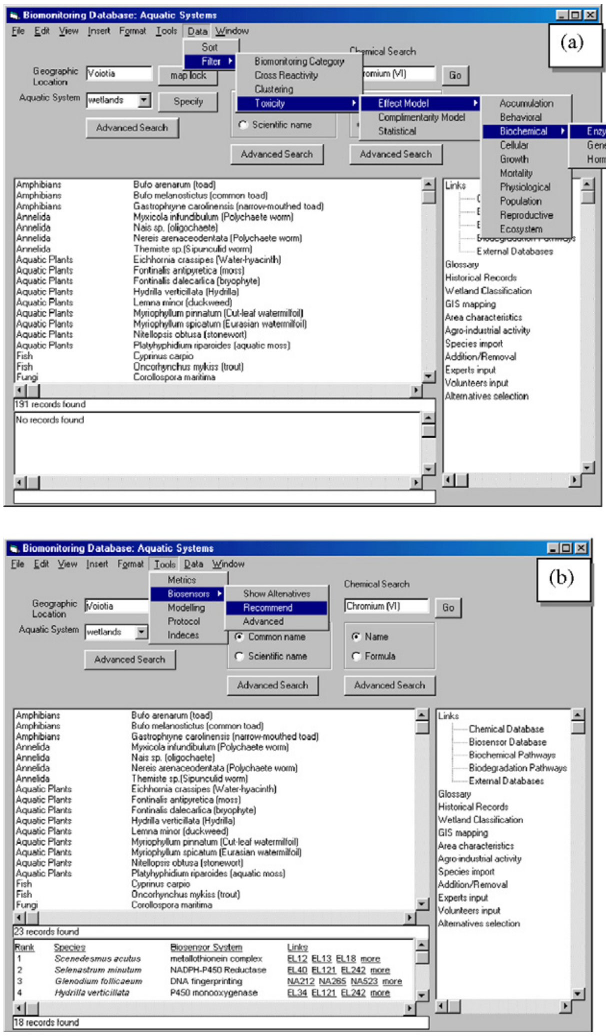
can be modified according to the signal retrieved by the species; for example, an alarming situation revealed by the biological assemblages (increasing trend of heavy metal(s) load) would prompt an in situ investigation/verification with the biosensor network and the priming, if necessary, of the appropriate countermeasures (stage 38). The correlation of biosensor signal to species response can be also used to confirm the validity of the correlation model (stage 27), especially in cases of abrupt load increases (peaks) where the physiological response of the organisms could differ significantly from that observed in simulated environment.

3. Implementation

The proposed framework has been implemented in the case of Voiotia prefecture, central Greece (Fig. 5), which recently has been alarmed by the presence of total chromium in levels above the limit of 50 μg/l and chromium(VI) at 69 μg/l. Voiotia has a population of 10,000, a few engaged in agricultural activities, while most man the 450 industrial units located in the area, which are further supported by 11,000 people from neighboring areas. Kifissos river is running through the prefecture work ending in Attiki, thus transferring its load to Athens, threatening to pollute the surface waters of densely populated regions. Eleven wetlands are located in the area of interest, 2 of which are protected by the Ramsar convention (Lakes Iliki and Paralimni); 3 are semi-natural wetlands, designed to receive the effluents of 10 industrial units next to numerous small works and an agriculturally used landscape.

Wetland inflows contain, apart from chromium other heavy metals as well, such as cadmium, arsenic and copper (stage 1) [30,31]. The levels of total chromium show significant variability across the area wetlands, ranging from 10 to 200 μg/l. The preliminary search in the KB for indigenous species sensitive to chromium(VI) (stage 5) showed that the capacity of the area for biomonitoring purposes is adequate (Fig. 6a), although there are only few species specific to chromium(VI), necessitating the program design to heavily rely upon the biochemical analysis of the species for the determination of the level of chromium accumulation [22,24]; however, field assessments of visible malformations have not been presently ignored, as these are critical in determining the bioremediation efficiency and bioaccumulation levels [1–4,9,11], especially for the heavily toxic chromium(VI).

The alternatives finally selected for each project phase (stage 20) are presented in Tables 1–3. Each assemblage has its own strengths and limitations for developing wetland biomonitoring methods, as assessed by the mixed panel. The biomonitoring experts agreed to an effect-based clustering model (Fig. 6b), although it might not be adequate for the long-term phase to satisfactorily register biomagnification (where the complimentarity model is thought of as most appropriate [32]), on the rationale that heavy metal biomonitoring reliability is almost exclusively provided by laboratory analysis, hence the effect-based grouping is more convenient for protocol development and laboratory scheduling. Furthermore, such clustering simplifies the quality control, as inevitably it is reduced to individual species identification and the misidentification of one species will not affect the rest of the field data; in the complimentarity model, however, the correct identification of the complete chain is essential for justifying the survey results.



Geographic Location: Voiotia

Aquatic System: wetlands

romium (VI)

Amphibians	Bufo arenarum (toad)
Amphibians	Bufo melanostictus (common toad)
Amphibians	Gastrophryne carolinensis (narrow-mouthed toad)
Annelida	Myxicola infundibulum (Polychaete worm)
Annelida	Nais sp. (oligochaete)
Annelida	Nereis arenaceodentata (Polychaete worm)
Annelida	Themiste sp.(Sipunculid worm)
Aquatic Plants	Eichhornia crassipes (Water-hyacinth)
Aquatic Plants	Fontinalis antipyretica (moss)
Aquatic Plants	Fontinalis dalecarlica (bryophyte)
Aquatic Plants	Hydrilla verticillata (Hydrilla)
Aquatic Plants	Lemna minor (duckweed)
Aquatic Plants	Myriophyllum pinnatum (Cut-leaf watermilfoil)
Aquatic Plants	Myriophyllum spicatum (Eurasian watermilfoil)
Aquatic Plants	Nitellopsis obtusa (stonewort)
Aquatic Plants	Platyhyphidium riparoides (aquatic moss)
Fish	Cyprinus carpio
Fish	Oncorhynchus mykiss (trout)
Fungi	Corollospora maritima

191 records found

No records found

Geographic Location: Voiotia

Aquatic System: wetlands

Chromium (VI)

Amphibians	Bufo arenarum (toad)
Amphibians	Bufo melanostictus (common toad)
Amphibians	Gastrophryne carolinensis (narrow-mouthed toad)
Annelida	Myxicola infundibulum (Polychaete worm)
Annelida	Nais sp. (oligochaete)
Annelida	Nereis arenaceodentata (Polychaete worm)
Annelida	Themiste sp.(Sipunculid worm)
Aquatic Plants	Eichhornia crassipes (Water-hyacinth)
Aquatic Plants	Fontinalis antipyretica (moss)
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Aquatic Plants	Myriophyllum spicatum (Eurasian watermilfoil)
Aquatic Plants	Nitellopsis obtusa (stonewort)
Aquatic Plants	Platyhyphidium riparoides (aquatic moss)
Fish	Cyprinus carpio
Fish	Oncorhynchus mykiss (trout)
Fungi	Corollospora maritima

23 records found

Rank	Species	Biosensor System	Links
1	*Scenedesmus acutus*	metallothionein complex	EL12 EL13 EL18 more
2	*Selenastrum minutum*	NADPH-P450 Reductase	EL40 EL121 EL242 more
3	*Glenodium follicaeum*	DNA fingerprinting	NA212 NA265 NA523 more
4	*Hydrilla verticillata*	P450 monooxygenase	EL34 EL121 EL242 more

18 records found

Fig. 6. Sample screenshots from the computer program designed/developed by the authors for aiding the species retrieval and selection procedure. (a) The search for aquatic species indigenous to the area of Voiotia that are responsive to chromium(VI) retrieved 191 records. The data can be further refined through the 'filter' option in the 'data' menu, as, for example, in effect-model clustering for species expressing biochemical response. (b) The clustering reduced the number of species to 23. The program can perform several other tasks through the 'tools' option, as, for example, producing a ranked list of biosensors that can be developed based on the selected cluster; the ranking process considers, amongst others, the reported (successful) use of the biological material in biosensor construction, as well as its exploitation for the detection of the heavy metal selected.

Convenience and time in sampling were the key factors that the volunteer representatives set in selecting a biological assemblage (stage 20); area representativeness, reliability of metrics and cost were the factors set by the scientists in order to assess an assemblage's practical usefulness and ability to reflect real changes in wetland condition. Plants and macroinvertebrates are the most commonly used assemblages in wetland bioassessments [1–9,11]. Vegetation is a convenient assemblage because

Table 1
The set of alternatives of biological assemblages for short-term monitoring chromium(VI) in the survey area

Short-term monitoring alternatives	
A1	Amphibians: *Bufo bufo* (common toad); *Gastrophryne olivacea* (narrow-mouthed toad) Annelida: *Nais* sp. (oligochaete) Insects: *Chironomus* sp.; Trochoptera (Caddisfly order); Zygoptera (Damselfly order) Phytoplankton: *Aulosira fertilissima* (blue-green algae); *Dunaliella tertiolecta* (green-algae); *Glenodium folliaceum* (dinoflagellate); *Hydrodictyon reticulatum* (green algae); *Scenedesmus acutus* (green algae); *Selenastrum minutum* (green algae) Zooplankton: *Acartia clausi* (Calanoid copepod)
A2	Annelida: *Myxicola infundibulum* (polychaete worm) Aquatic plants: *Lemna minor* (duckweed) Nematoides and flatworms: *Dugesia* sp. Phytoplankton: *Anabaena oryzae* (blue-green algae); *Chlorella pyrenoidosa* (green algae); *Gymnodium splendens* (dinoflagellate); *Isochrysis galbana* (haptophyte); *Macrocystis pyrifera* (giant kelp); *Nitzchia palea* (diatom); *Nostoc muscorum* (blue-green algae); *Phaeodactylum tricornutum* (diatom); *Selenastrum minutum* (green algae); *Thalassiosira guillardi* (diatom)
A3	Aquatic plants: *Eichhornia crassipes* (water hyacinth); *Hydrilla verticillata* (hydrilla) Fungi: *Corollospora maritima* Insects: *Chironomus* sp. Phytoplankton: *Glenodium folliaceum* (dinoflagellate); *Scenedesmus acutus* (green algae); *Selenastrum minutum* (green algae) Zooplankton: *Daphnia ambiqua* (water flea); *Daphnia galeata* (water flea)
A4	Annelida: *Myxicola infundibulum* (polychaete worm) Fungi: *Corollospora maritima* Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Glenodium folliaceum* (dinoflagellate); *Hydrodictyon reticulatum* (green algae); *Macrocystis pyrifera* (giant kelp); *Phaeodactylum tricornutum* (diatom) Zooplankton: *Acartia clausi* (Calanoid copepod); *Daphnia galeata* (water flea)
A5	Amphibians: *Bufo bufo* (common toad); *Gastrophryne olivacea* (narrow-mouthed toad) Annelida: *Myxicola infundibulum* (polychaete worm) Aquatic plants: *Eichhornia crassipes* (water hyacinth) Fungi: *Corollospora maritima* Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Chlorella pyrenoidosa* (green algae); *Glenodium folliaceum* (dinoflagellate); *Gymnodium splendens* (dinoflagellate); *Hydrodictyon reticulatum* (green algae); *Macrocystis pyrifera* (giant kelp); *Phaeodactylum tricornutum* (diatom) Zooplankton: *Acartia clausi* (Calanoid copepod); *Daphnia galeata* (water flea)
A6	Amphibians: *Bufo bufo* (common toad); *Gastrophryne olivacea* (narrow-mouthed toad) Annelida: *Myxicola infundibulum* (polychaete worm); *Nais* sp. (oligochaete) Aquatic plants: *Eichhornia crassipes* (water hyacinth) Fungi: *Corollospora maritima* Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Chlorella pyrenoidosa* (green algae); *Glenodium folliaceum* (dinoflagellate); *Gymnodium splendens* (dinoflagellate); *Hydrodictyon reticulatum* (green algae); *Isochrysis galbana* (haptophyte); *Macrocystis pyrifera* (giant kelp); *Nitzchia palea* (diatom); *Phaeodactylum tricornutum* (diatom) Zooplankton: *Acartia clausi* (Calanoid copepod); *Daphnia ambiqua* (water flea); *Daphnia galeata* (water flea)

it occurs in most wetland types and there are well-established sampling protocols [6–10]; however, identifying metrics can be challenging [7]. Macroinvertebrates have been widely used in stream biomonitoring and show a lot of promise for wetlands [1,2], but current sampling methods focus on wetlands with standing water. Algae have been used to a limited degree [15] but offer an inexpensive and effective alternative for some wetland types. Amphibians offer many advantages but have insufficient taxonomic diversity in some regions for traditional assessment methods [10]. The mobility of birds makes them well suited for landscape-level assessments [4], but quite unsuitable for metal-specific wetland monitoring, thus none have been included in the current study. On the other hand, hoverflies, despite their high mobility, respond strongly to small-scale ecological changes in soil/water habitats, but no satisfactory correlation to biological traits could be made [6]. Fish have many advantages that have been demonstrated in other waterbodies [1,2], and although their identification by untrained personnel is often difficult, some species have been included for the medium- and long-term phase.

Short-phase alternatives are mostly those that are acutely, even lethally, affected by chromium(VI), as the toad eggs of *Bufo bufo* at low levels or some diatoms at high levels. Medium- and long-phase alternatives include those species with late-triggered response or accumulation effects. Note that, the number of species available for biomonitoring are decreased from phase to phase, owing to the low tolerability of the indigenous species. The last phase includes mostly nematoides and flatworms, with known tolerance and/or accumulation potential. Also, the stress response may change with the time or level of exposure. Insects, phytoplankton or zooplankton may initially demonstrate biochemical effects (suitable for the alarming phase), followed by community changes or visual malformation after a few days (allowing for medium-phase assessment) and finally expressing more threatening symptoms, even mortality (justifying their inclusion in the long-term assessment).

Scoring (stage 22) has been performed by another experts panel, including 2 biologists, 2 biochemists, and 1 ecologist on the range 2–8, the highest value assigned to the better alternative. The results (stage 23) for the alarm phase are shown in

Table 2
The set of alternatives of biological assemblages for medium-term monitoring chromium(VI) in the survey area

Medium-term monitoring alternatives	
A1	Amphibians: *Gastrophryne olivacea* (narrow-mouthed toad Annelida: *Nereis* sp. (polychaete worm) Fish: *Cyprinus carpio*; *Oncorhynchus mykiss* (trout) Insects: *Ephemerella subvaria* (mayfly) Nematoides and flatworms: *Dugesia tigrina*; *Schistosoma haematobium* (Trematode parasite) Phytoplankton: *Aulosira fertilissima* (blue-green algae); *Skeletonema costatum* (diatom); *Chlorella protothecoides* (green algae) Zooplankton: *Tisbe holothuriae* (Harpacticoid copepod); *Artemia salina* (Brine shrimp); *Brachionus calyciflorus* (rotifer)
A2	Aquatic plants: *Myriophyllum spicatum* (Eurasian watermilfoil) Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Gymnodinium splendens* (diniflagellate); *Amphiprora* sp. (diatom); *Anacystis flosaquae* (blue-green algae) Zooplankton: *Daphnia magna* (water flea); *Gammarus* sp. (scud, amphipod); *Colpidium campylum* (ciliate)
A3	Amphibians: *Pleurodeles waltl* (Iberian ribbed newt) Aquatic plants: *Hydrilla verticillata* (Hydrilla) Fungi: *Corollospora maritima* Phytoplankton: algae (algal mat); *Anabaena cylindrica* (blue-green algae); *Anabaena variabilis* (blue-green algae); *Nostoc muscorum* (blue-green algae); *Synechocystis aquatilis* (blue-green algae)
A4	Amphibians: *Pleurodeles waltl* (Iberian ribbed newt) Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Macrocystis pyrifera* (giant kelp); *Skeletonema costatum* (diatom); *Amphiprora* sp. (diatom); *Anacystis flosaquae* (blue-green algae); *Chlorella protothecoides* (green algae) Zooplankton: *Tisbe holothuriae* (Harpacticoid copepod); *Artemia salina* (Brine shrimp)
A5	Amphibians: *Pleurodeles waltl* (Iberian ribbed newt) Aquatic plants: *Hydrilla verticillata* (Hydrilla) Nematoides and flatworms: *Dugesia tigrina* Phytoplankton: *Anabaena oryzae* (blue-green algae); *Anabaena variabilis* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Macrocystis pyrifera* (giant kelp); *Nostoc muscorum* (blue-green algae); *Skeletonema costatum* (diatom) Zooplankton: *Daphnia magna* (water flea)

Table 3
The set of alternatives of biological assemblages for long-term monitoring chromium(VI) in the survey area

Long-term monitoring alternatives	
A1	Annelida: *Nereis arenaceodentata* (Polychaete worm); *Nereis arenaceodentata* (Polychaete worm); *Themiste* sp. (Sipunculid worm) Phytoplankton: *Anabaena cylindrica* (blue-green algae); *Anabaena torulosa* (cyanobacteria); *Scenedesmus acutus* (green algae); *Achnanthes brevipes* (diatom); *Alexandrium catenella* (dinoflagellate) Zooplankton: *Tisbe holothuriae* (Harpacticoid copepod); *Acanthamoeba* sp. (amoeba)
A2	Aquatic plants: *Eichhornia crassipes* (water-hyacinth); *Hydrilla verticillata* (Hydrilla); *Azolla* sp. (water fern); *Callitriche* sp. (macrophyte) Phytoplankton: *Anabaena cylindrica* (blue-green algae); *Anabaena torulosa* (cyanobacteria); *Anabaena variabilis* (blue-green algae); *Nostoc muscorum* (blue-green algae); *Scenedesmus acutus* (green algae)
A3	Aquatic plants: *Carex diandra* (sedge) Phytoplankton: *Anabaena oryzae* (blue-green algae); *Aulosira fertilissima* (blue-green algae); *Chlorella pyrenoidosa* (green algae); *Isochrysis galbana* (haptophyte); *Macrocystis pyrifera* (giant kelp); *Nitzschia palea* (diatom); *Nostoc muscorum* (blue-green algae); *Selenastrum capricornutum* (green algae)
A4	Nematoides and flatworms: *Acrobeles* sp. (roundworm); *Rotylenchus* sp. (reniform nematode); *Polycelis* sp. (planarian) Phytoplankton: *Aulosira fertilissima* (blue-green algae); *Dunaliella tertiolecta* (green algae); *Glenodinium halli* (dinoflagellate); *Hydrodictyon reticulatum* (green algae); *Scenedesmus acutus* (green algae); *Selenastrum capricornutum* (green algae); *Skeletonema costatum* (diatom)
A5	Annelida: *Nereis arenaceodentata* (Polychaete worm); *Themiste* sp. (Sipunculid worm) Crustaceans: *Amphilocus* sp. (amphipod); *Anonyx* sp. (amphipod) Nematoides and flatworms: *Dugesia dorotocephala*; *Acrobeles* sp. (roundworm); *Rotylenchus* sp. (reniform nematode); *Polycelis* sp. (planarian)
A6	Aquatic plants: *Hydrilla verticillata* (Hydrilla) Fungi: *Corollospora maritima* Insects: *Chironomus* sp. (Midge) Phytoplankton: algae (algal mat); *Anabaena cylindrica* (blue-green algae); *Dunaliella tertiolecta* (green algae); *Scenedesmus acutus* (green algae); *Selenastrum capricornutum* (green algae)

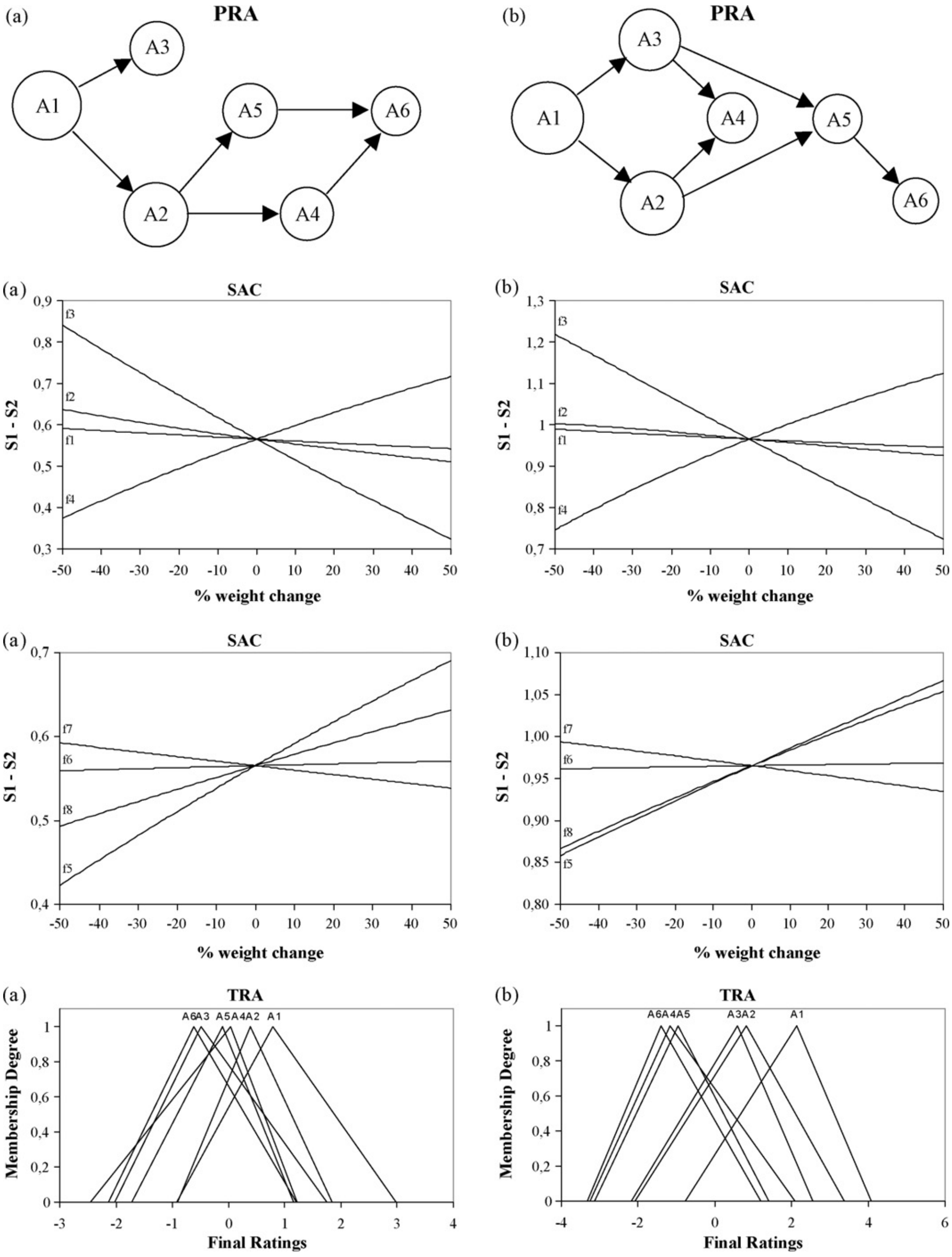

Fig. 7. Partial ranking of short-phase alternatives (PRA), sensitivity analysis of each criterion (SAC) and total ranking of alternatives (TRA), at (a) low preferability resolution with medium q, p values and (b) high preferability resolution with low q, p values; the arrow '→' means 'better than'. At both resolution levels, the alternative A1 prevails for short-term monitoring, while the SAC graphs indicate that this is a robust solution.

Fig. 7 at (a) low preferability resolution with medium parameter q, p values ($q = 1.5$, $p = 3.0$), and (b) high preferability resolution, with low parameter q, p values ($q = 0.5$, $p = 1.0$). In Fig. 7, (i) the partial ranking of alternatives (PRA) output is shown as a set of circles with areas proportional to the crisp number, S_t, which indicates the corresponding relative value of the alternative A_t in the ranking vector, and (ii) the TRA output is shown as a set of triadic fuzzy numbers in the usual forms of triangles to reveal the common parts which constitute a measure of overlapping along the relative scale of pre-order (i.e., the horizontal axis), especially for lower membership function values.

The total ranking of alternatives (TRA) is A1 > A3> A2 > A5 > A4 > A6 and A1 > A2 > A3 > A4 > A5 > A6 at low and high preferability resolution, respectively; the symbol '>' stands for 'better than'. This is a significant indication that the solution suggesting the biological assemblage A1, is robust, without any indication of incomparability between the 'best' and the suggested as 'second-best', although their final results are close. Robustness is confirmed by mono-parametric sensitivity anal-

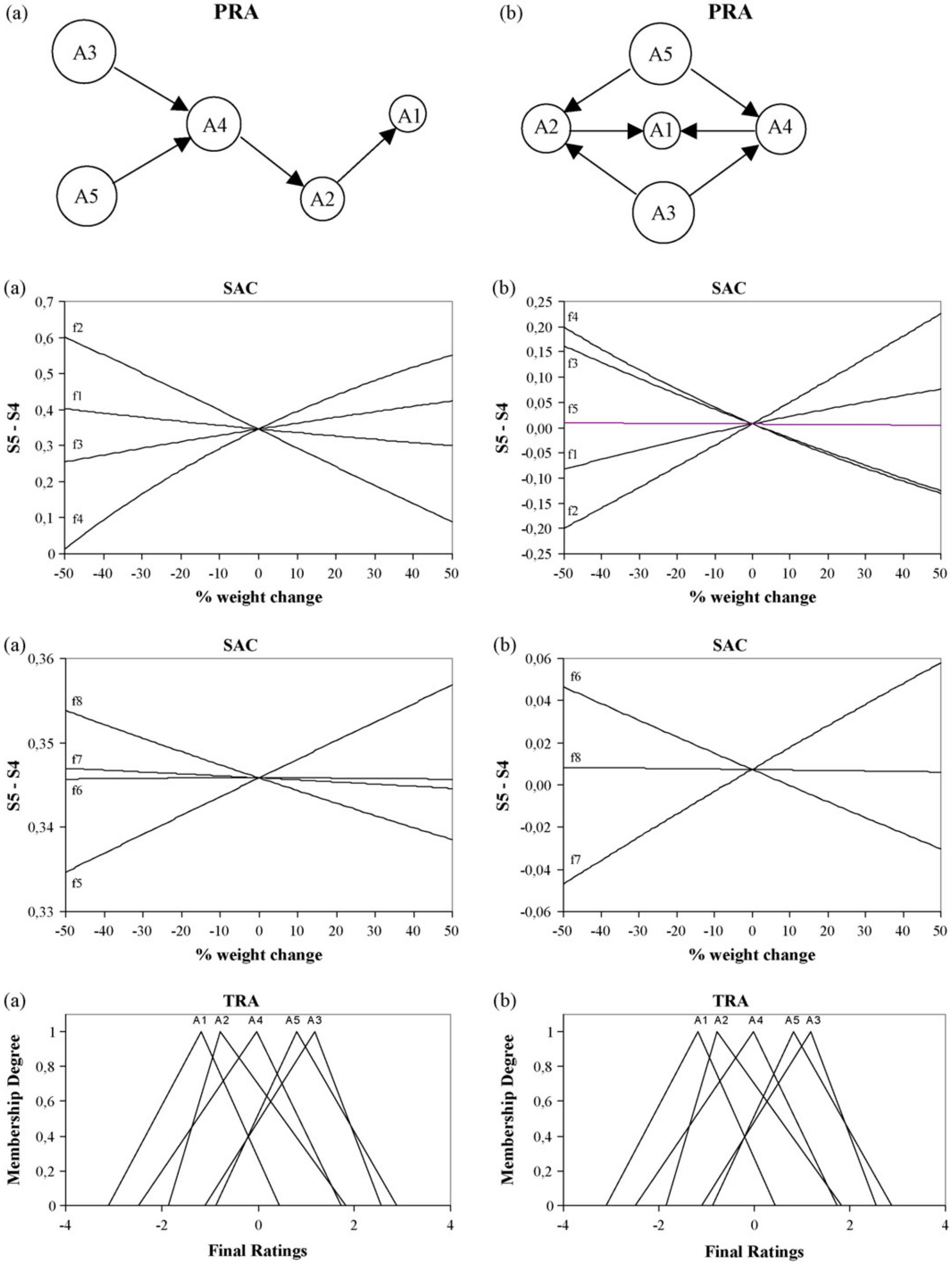

Fig. 8. Partial ranking of medium-phase alternatives (PRA), sensitivity analysis of each criterion (SAC) and total ranking of alternatives (TRA), at (a) low preferability resolution with medium q, p values and (b) high preferability resolution with low q, p values; the arrow '→' means 'better than'. At the low-resolution level, the alternative A3 prevails for medium-term monitoring with 'second-best' the A5 assemblage; the order reverses at the high-resolution level. The SAC graphs indicate that the solution is not robust.

ysis over the wide range ± 50% round the central defuzzified value of each criterion (SAC): the difference S1 − S2 is always positive, being lower only for quite decreased f_5-values. The 'best' assemblage contains species representative of the wetland condition and able to show within hours of exposure the inflow of threatening chromium levels, without necessitating costly laboratory analysis, which in this case is run only as confirmatory to field assessment.

As regards the medium-phase results (Fig. 8), however, sensitivity analysis reveals a strong incomparability between the 'best' (A3) and 'second-best' (A5) alternative at both preferability levels. The main reason lies probably within the uncertainty of medium-term toxicity, expressed primarily by growth, behavioral and community changes, which are generally considered as stress indicators and used mostly for heavy metal inflow study after a stress level is established rather than actually measuring its bioavailability and distribution. In this case, the proposed final decision (stage 25) includes both alternatives, on the rationale that the introduction of the medium-term accumulators of the A5 assemblage will certainly increase reliability as they can be used as internal controls of the monitoring scheme.

Long-phase results (Fig. 9) present a significant indication that the suggested 'best' A6 is robust against the 'second-best' A5, although two-parameter sensitivity analysis is alarming for combined very low f_4- and f_3-values (not presented in Fig. 9, where only the mono-parameter sensitivity analysis is shown). In order to increase reliability, it is necessary to reinforce the 'best' solution as per this criterion by introducing species with low or none laboratory cost, as the zooplankton group in the A1 alternative.

Biosensor development in underway (stage 26), initially started on the basis of the following design/construction parameters: (a) for short-phase, the metallothionein complex of *Scenedesmus acutus* (green algae) and the P_{450} system of the *Selenastrum minutum* (green algae) are employed to produce enzyme biosensors on field effect transistor (FET) technology

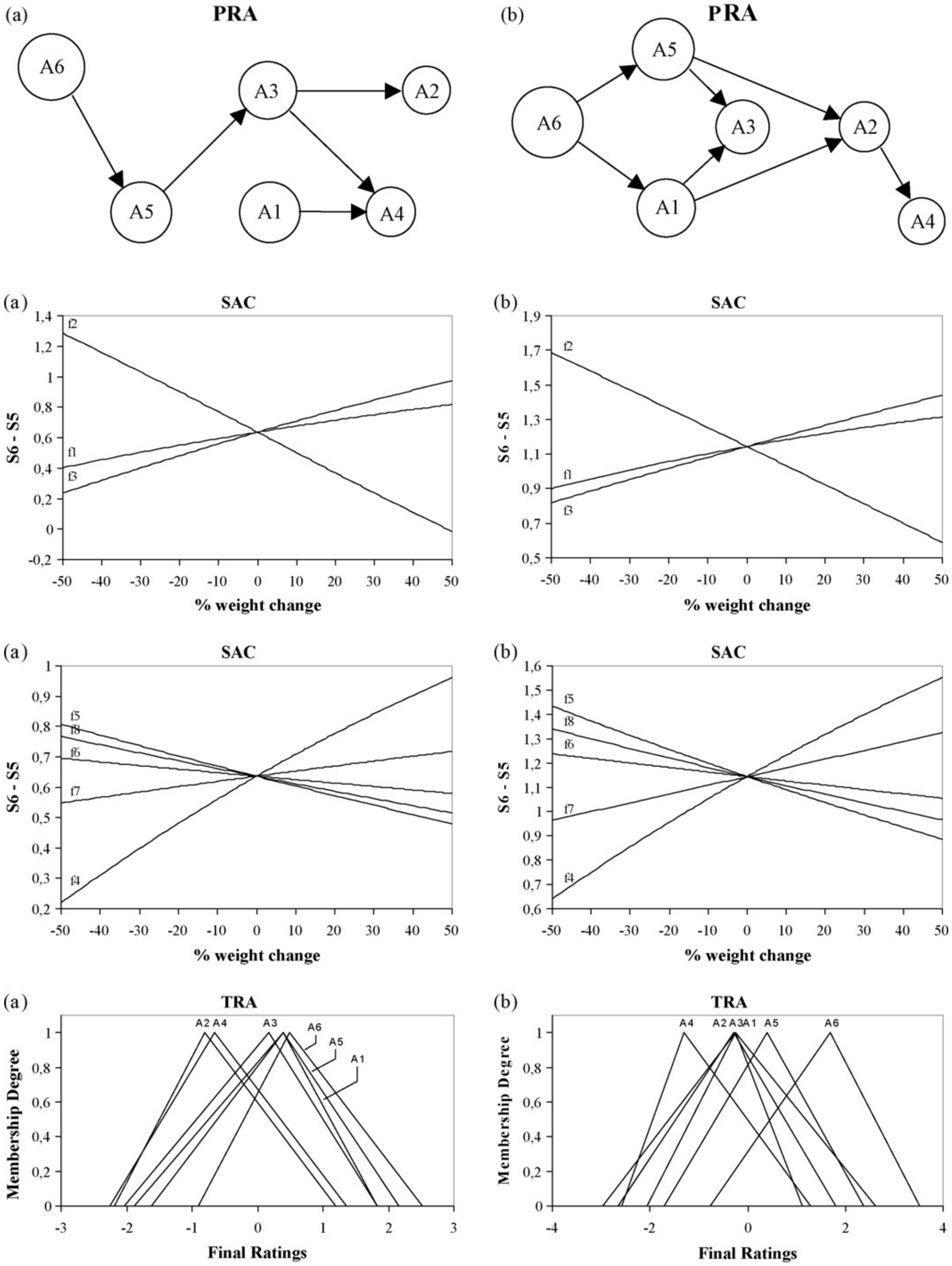

Fig. 9. Partial ranking of long-phase alternatives (PRA), sensitivity analysis of each criterion (SAC) and total ranking of alternatives (TRA), at (a) low preferability resolution with medium q, p values and (b) high preferability resolution with low q, p values; the arrow '→' means 'better than'. At both resolution levels, the alternative A6 prevails for long-term monitoring. The SAC graphs indicate that this solution is robust.

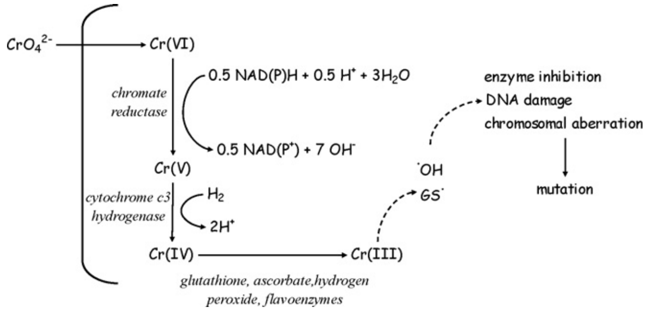

Fig. 10. The cellular reduction of chromium(VI) in *Gastrophryne olivacea*. The heavy metal enters the cell by the sulphate transport system; once inside the cell, it is readily reduced to the less toxic Cr(III) through the unstable Cr(V) intermediate; the mechanism is catalyzed by two enzymes, also involving in the last steps glutathione, ascorbate, hydrogen peroxide and flavoenzymes. This step may result in the production of free radicals, which attack DNA and induce mutations. Cr(III) species can also interact directly with DNA–chromatin to form monoadducts, DNA interstrand, intrastrand and DNA–protein cross-links, and other forms of DNA damage; these intermediates may also activate or repress specific transcription factors either directly or indirectly through signaling pathways.

(Fig. 6b), whereas the chromium(VI) cellular biodegradation of the narrow-mouthed toad is under investigation (Fig. 10) [24]; a DNA biosensor is also developed for *Glenodium folliaceum* (dinoflagellate), that will detect declining levels of the species, reminiscent of chromium(VI) levels above 15 μg/l; (b) for medium-phase monitoring, *Anabaena cylidrica* and *Nostoc muscorum* (blue-green algae) provide for nitrogenase biosensors, whereas the respiratory activity of *Synechocystis aquatilis* (blue-green algae) is used to construct a microbial biosensor; also, β-galactosidase, trypsin, and esterase of *Daphnia magna* (water flea) are considered, along with nitrate reductase of Hydrilla for electrochemical biosensors, and *Skeletonema costatum* (diatom) for heavy metal residue measurements with an SPR biosensor; (c) long-term biosensoring is based on scavenger biochemistry, with the construction of electrochemical biosensors for measuring the glycerol and chlorophyll content of *Dunalliela* spp. (green algae) and the chlorophyll content of the algal mat; although the latter is still controversial (as per design, reliability and application) is very promising for monitoring long-term exposure effects of tolerant species, especially when the results are verified by *Corollospora maritima* (fungi) analysis.

4. Discussion

The heavy metal monitoring framework presented herein, introduces an ecologically relevant character and dynamics in instrumental monitoring with the use of biosensors, which will not only provide the means for in depth study and clarification of aquatic toxicity processes, owing to the inherent biomimicking basis of tailor-made biosensoring, but will also eventually lead to the shifting to natural monitoring. The preliminary results from the current project and from previous similar projects [15,16] indicate that, on the premises of biosensor utilization, such a shifting is feasible, at least for small-scale and well-controlled implementation areas. However, more implementations are required to substantiate the value and credibility of such a scheme in full-scale application. The construction of an environmental impact database on an ontological platform has been proven a useful tool for the design/development/implementation of a network for the monitoring of a variety of stressors over time and space and the assessment of environmental quality. The collection of the available information and their classification into taxonomic and partonomic relations provided a database linking multi-functional, phenomenological and in-depth, pollutants with response, with respect to ecological parameters, relations and hydrogeomorphology. Such an ontology affords organism responses to become understandable, links biomonitoring to policymaking and health impact assessment and displays a significant utility to cost-benefit surveillance.

The problems arising involve mostly the limited knowledge of organisms' systematic and sub-systematic response to stressors, especially when many substances are acting simultaneously or synergistically or competitively upon macro- or micro-life, not to mention the protective and anti-stress mechanisms that can be employed at threshold levels which can modify substantially (and unpredictably) any toxicity model. For example, it is not uncommon for macrophytes to engage avoidance mechanisms [33], as root re-direction, after a period of exposure, or for accumulative cyanobacteria to modify slightly their membrane biochemistry and increase release kinetics [11]. The use of biosensors, as controls/calibrators in the later parts of the proposed scheme, can promptly warrant for such activities, since the signals produced are not representative of the ambient (water) concentrations but rather related to bioavailable levels and thus play a significant role in aquatic modeling.

When deciding what to monitor to adequately represent any system, the purpose or objective of the modeling and the depth and extend of the system knowledge are the limiting factors. The proposed framework is addressed to semi-natural wetlands, structured/exploited for managing industrial wastewater or abandoned extraction pits in mines. The latter form small catchments at local level, where rain and mine drainage are accumulated and start to establish an ecosystem; managers aim primarily at area restoration, especially if human settlements are nearby. The authors are presently study such a case in a lignite extraction field in Halkida (Greece). When a wetland is used for wastewater treatment, any previously established ecosystem is significantly altered due to the industrial inflow, which reduces the population of intolerant species, leaving tolerant species to spread out; the main concern in this case is treatment efficiency, without disregarding ecology. In both cases, the major inflows are known or suspected. Dealing with artificial wetlands implies the creation of an ecosystem from scratch, whereas natural wetlands pose limitations to species introduction due to restrictions in altering the established habitat, further to the need of extensive abiotic and biotic sampling and analysis for identifying pollutants, especially when historical data is not available. Moreover, economic issues are seen differently in these cases: in artificial wetlands the cost is the prime concern, whereas in natural wetlands, ecology is the top priority.

The current biomonitoring scheme aims at the short-, medium- and long-term charting of the state of the semi-natural wetland ecology, as a result of different management strategies and continuous exogenous interventions. The investment required to establish such a scheme is expected to be subsidized by national (through the local authorities) and community funds up to 55%, according to the Greek environmental legislation. Furthermore, the payback period will be minimized since the designed/developed wetland will be used as a know-how-transfer vehicle, i.e., as a pilot, for decreasing restoration cost of creating/improving similar waterbodies in the vicinity, taking also advantage of the already established flora/fauna nursery and the rest infrastructure. The use of biosensors instead of conventional instrumentation can encourage the local production of ready-to-market/use devices, utilizing indigenous materials and taken advantage of scale economies due to large production [15]. It is worthwhile noting that in the time-course, the expected monitoring cost will decrease further as a result of (a) human experience accumulation and (b) incorporating know-how within the system itself. This is a common characteristic in systems approach to multi- and inter-disciplinary issues, as it has been stressed by several authors engaged with solving problems in different disciplines, e.g., Senge [34] and Bellamy et al. [35] argue about some kind of 'learning organization' that by means of a systemic view leads to knowledge enrichment, implying progressive improvement of the system itself. This is only counter by a possible self-organizing of the ecosystem that may develop resistant species; in that case, new biological assemblages (stage 24 of the algorithmic procedure shown in Fig. 3) are required, as well as more extensive revalidation in comparison with the periodic one.

This, in fact, is seen in many cases, where the indicators believed to be most scientifically defensible, are those for which insufficient data exists to allow any sort of predictive modeling [3,5]. Estimates of dose–response relationships, nutrient and biological conditions in reference and degraded systems with the use of biosensors decrease significantly the need of large sampling from the region, increasing precision considerably. The biomonitoring programs reported so far are obliged to determine the variation associated with one-time assessments from single samples by re-sampling a specific number of wetlands during the survey; measurement variation among replicate samples can then be used to establish the expected variation for onetime assessment of single samples. Re-sampling does not establish, however, the precision of the assessment process but rather identifies the precision of an individual measurement [36]. Thus, the employment of a relevant measuring system that correlates to biological signals, such as biosensors, adds considerably to the quality control of the survey.

Ideally, water quality monitoring programs produce long-term data sets compiled over multiple years to capture the natural, seasonal, and year-to-year variations in biological communities and waterbody constituent concentrations [37]. Multiple-year data sets can be analyzed statistically to identify the effects of seasonality and variable hydrology. Once the pattern of natural variation has been described, the data can be analyzed to determine the ecological state of the waterbody. In spite of the documented value of long-term data sets, there is a tendency to intensively study a waterbody for 1 year before and 1 year after exposure/treatment. A more cost-effective approach, supported by the present framework is the periodical biosensoring of the species or indices most directly related to the stressor of interest, i.e., those parameters or species that reflect the status of the wetland medium- and long-term. Comparisons over time between reference and at-risk or degraded systems can help describe biological response and annual patterns in the presence of changing climatic conditions.

An obvious challenge facing wetland scientists is to distinguish changes in biological communities caused by human disturbances from natural variations. This challenge is complicated by the natural variation found among the variety of wetland types [38]. One way to simplify the evaluation is to classify the wetlands and only compare wetlands with others within the same class. Most surveys conclude that some kind of classification is necessary before proceedings with biomonitoring [5–8]. The use of the classification system proposed herein provides a suitable framework for allowing inter- and intra-wetland comparisons, critical for developing metrics and indices in biomonitoring. For purposes of developing a monitoring scheme applicable to many case studies, the goal is to establish classes of wetlands that have similar biological communities that respond similarly to human disturbances. Although many classifications systems have been reported and some are effectively in use, relying on an established system without certain adaptations to count for regional peculiarities may lead to erroneous results. Researchers often start with one or more systems and then lump or split classes as needed to end up with an appropriate number of groups of biologically distinct wetlands. For example, when the Montana Department of Environmental Quality (MT DEQ) developed its bioassessment project, it used ecoregions as a first tier and then further separated wetlands by landscape position and other characteristics (e.g., acidity and salinity). MT DEQ later determined that it could lump the wetlands of two ecoregions because their macroinvertebrate communities were similar and responded similarly to anthropogenic stressors. While establishing classes, examine other natural factors that may affect wetland communities (e.g., size, successional stage, age of the wetland, salinity) to determine if they should be included in the classification system.

The authors have currently tried to develop wetland classes that are broad enough to allow comparisons of several wetlands, yet narrow enough to provide site-specific biologically meaningful comparison. The KB constructed by the authors that hosts a system as such permits the input and retrieval of deep-level knowledge information, delving deeper into biochemistry in order to help identify differences between wetlands and develop more subclasses. For each wetland class that is identified, however, a new set of wetlands must be sampled to calibrate analytical methods. The endpoint therefore represents a balance of (a) a need to have broad inclusive classes that will facilitate the comparisons of many wetland types, (b) a desire to have a narrow classification that includes detailed large-scale data, and (c) constraints on financial and staff resources. Physical and chemical information can be very useful while classifying wetlands, interpreting biological data, and identifying potential stressors. Periodical or incidental changes, as flooding or dredging, should be also taken into consideration since they significantly affect soil/water regimes [5–8].

Volunteer involvement is inevitable in order to achieve the financial viability of the proposed scheme long-term is discussed. Although, our experience from similar works and from the ongoing project shows that recruitment and enthusiasm is not a problem, long-term commitment and field efficiency are actually difficult to assure. Every volunteer-based program is very likely to underrate or overrate the ecological condition of the environment and fail to differentiate impaired species. Well-meaning, dedicated volunteers do not necessarily produce valid scientific results by carefully adhering to a biomonitoring protocol that has been promulgated for their use. The decrease of willingness to participate in subsequent field surveys results in major losses in field-trained staff (with known or accounted for uncertainty) and the incoming of new untrained participants. Without rigorous validation studies, scientists will always be skeptical, and justifiably so, about volunteer gathered data. The authors have developed a volunteer training software program, as part of a similar work with lichens, aiming at assessing their efficiency and familiarize them with the required needs. Preliminary results have proven its usefulness, but still a more integral and comprehensive approach is required before training and survey protocol development will lead to data credibility. The inclusion of volunteer representatives in the final selection of the monitoring organisms, as presented herein, establishes a promising channel for eliciting the opinion of volunteers, which, further to the practical gains, imparts meaningful volunteer recognition while demonstrating the value of community participation in environmental projects.

5. Conclusions

The proposed biological monitoring scheme provides a framework for improving semi-natural wetland management in a cost-effective and ecologically relevant way. Biosensoring provides information relevant to the biological observations, thus offering a platform for converting stress responses to real-time measurements, and preparing the shifting to natural monitoring. The presented framework is based upon the utilization of local resources, offering, long-term, the possibility of area self-monitoring, as based on the biomonitoring capacity given by indigenous species. The structuring of the monitoring biological assemblage is finalized through a computer-aided procedure, amended by experts and refined by volunteers, thereby ensuring the scientific and practical functionality of the proposed scheme.

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References

[1] J.R. Karr, Biological integrity: a long-neglected aspect of water resource management, Ecol. Appl. 1 (1) (1991) 66–84.

[2] J.R. Karr, Health, integrity, and biological assessment: the importance of whole things, in: D. Pimentel, L. Westra, R.F. Noss (Eds.), Ecological Integrity: Integrating Environment, Conservation, and Health, Island Press, Washington, DC, 2000, pp. 209–226.

[3] D.L. Courtemanch, S.P. Davies, E.B. Laverty, Incorporation of biological information in water quality planning, J. Environ. Manage. 13 (1) (1989) 35–41.

[4] W.S. Davis, T.P. Simon, Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making, Lewis Publishers, Boca Raton, FL, 1995.

[5] F. Dziock, K. Henle, F. Foeckler, K. Follner, M. Scholz, Biological indicator systems in floodplains—a review, Int. Rev. Hydrobiol. 91 (4) (2006) 271–291.

[6] K. Henle, F. Dziock, F. Foeckler, K. Follner, V. Hüsing, A. Hettrich, M. Rink, S. Stab, M. Scholz, Study design for assessing species environmental relationships and developing indicator systems for ecological changes in floodplains—the approach of the RIVA project, Int. Rev. Hydrobiol. 91 (4) (2006) 292–313.

[7] M. Overesch, J. Rinklebe, G. Broll, H.-U. Neue, Metals and arsenic in soils and corresponding vegetation at Central Elbe river floodplains (Germany), Environ. Pollut. 145 (2007) 800–812.

[8] J. Rinklebe, C. Franke, H.-U. Neue, Aggregation of floodplain soils based classification principles to predict concentrations of nutrients and pollutants, Geoderma 141 (2007) 210–223.

[9] J.R. Karr, D.R. Dudley, Ecological perspective on water quality goals, J. Environ. Manage. 5 (1981) 55–68.

[10] U.S. EPA, Lake and Reservoir Bioassessment and Biocriteria: Technical Guidance Document, Office of Water, Washington, DC, 1998 (EPA 841-B-98-007).

[11] M.M. Lasat, Phytoextraction of toxic metals: a review of biological mechanisms, J. Environ. Qual. 31 (2002) 109–120.

[12] R. Iqbal, H. Tachibana, Water chemistry in Sarobetsu Mire and their relations to vegetation composition, Arch. Acker. Pfl. Boden (Arch. Agron. Soil Sci.) 53 (1) (2007) 13–31.

[13] F.R. Thibodeau, B.D. Ostro, An economic analysis of wetlands protection, J. Environ. Manage. 12 (1981) 19–30.

[14] N.E. Roth, J.D. Allan, D.L. Erickson, Landscape influences on stream biotic integrity assessed at multiple scales, Landscape Ecol. 11 (3) (1996) 141–156.

[15] F. Batzias, C.G. Siontorou, A novel system for environmental monitoring through a cooperative/synergistic scheme between bioindicators and biosensors, J. Environ. Manage. 82 (2) (2007) 221–239.

[16] F.A. Batzias, C.G. Siontorou, A knowledge-based approach to environmental biomonitoring, Environ. Monit. Assess. 123 (1–3) (2006) 167–197.

[17] B. Fischer, Fuzzy environmental decision-making: applications to air pollution, Atmos. Environ. 37 (2003) 1865–1877.

[18] S. Dahiya, B. Singh, S. Gaur, V.K. Garg, H.S. Kushwaha, Analysis of groundwater quality using fuzzy synthetic evaluation, J. Hazard. Mater. 147 (3) (2007) 938–946.

[19] I. Mergias, K. Moustakas, A. Papadopoulos, M. Loizidou, Multi-criteria decision aid approach for the selection of the best compromise management scheme for ELVs: the case of Cyprus, J. Hazard. Mater. 147 (2007) 706–717.

[20] A.F. Batzias, F.A. Batzias, Fuzzy multicriteria choice of instrumental methods for measuring physical quantities—application in the case of dielectric aluminium anodic oxide films, in: Proceedings of IEEE Instrumentation and Measurement Technology (IMTC), vol. 3, 2004, pp. 2217–2222.

[21] F.A. Batzias, Computer aided multicriteria determination of solar energy storage material, in: Proceedings of the World Renewable Energy Congress (WREC IX), 2006.

[22] F.A. Batzias, E.C. Marcoulaki, Restructuring the keywords interface to enhance CAPE knowledge via an intelligent agent, Comput. Aided Chem. Eng. 10 (2002) 829–834.

[23] K.R. Krishna, L. Philip, Bioremediation of Cr(VI) in contaminated soils, J. Hazard. Mater. B121 (2005) 109–117.

[24] C. Mant, S. Costa, J. Williams, E. Tambourgi, Phytoremediation of chromium by model constructed wetland, Bioresour. Technol. 97 (2006) 1767–1772.

[25] W.H. Blake, R.P.D. Walsh, J.M. Reed, M.J. Barnsley, J. Smith, Impacts of landscape remediation on the heavy metal pollution dynamics of a lake surrounded by non-ferrous smelter waste, Environ. Pollut. 148 (1) (2007) 268–280.

[26] F.A. Batzias, C.G. Siontorou, Investigating the causes of biosensor SNR decrease by means of fault tree analysis, IEEE Trans. Instrum. Meas. 54 (4) (2005) 1395–1406.

[27] F.A. Batzias, Incorporating an artificial neural network into an air pollution measuring equipment to overcome the biased reference function employed, in: Proceedings of the CEM 2006 Seventh International Conference on Emissions Monitoring, 2006, pp. 215–224.

[28] J.P. Brans, P. Vincke, B. Mareschal, How to select and how to rank projects: the PROMETHEE method, Eur. J. Oper. Res. 24 (1986) 228–238.

[29] T.Y. Tseng, C.M. Klein, New algorithm for the ranking procedure in fuzzy decisionmaking, IEEE Trans. Syst. Man Cyb. 19 (1989) 1289–1296.

[30] N. Yassoglou, C. Kosmas, J. Asimakopoulos, C. Kallianou, Heavy metal contamination of roadside soils in the Greater Athens area, Environ. Pollut. 47 (4) (1987) 293–304.

[31] T. Sawidis, J. Stratis, G. Zachariadis, Distribution of heavy metals in sediments and aquatic plants of the river Pinios (Central Greece), Sci. Total Environ. 102 (1991) 261–266.

[32] L. Palma, P. Beja, P.C. Tavares, L.R. Monteiro, Spatial variation of mercury levels in nesting Bonelli's eagles from Southwest Portugal: effects of diet composition and prey contamination, Environ. Pollut. 134 (3) (2005) 549–557.

[33] O. Falik, P.A. Reides, M. Gersani, A. Novoplansky, Root navigation by self inhibition, Plant Cell Environ. 28 (2005) 562–569.

[34] P.M. Senge, The Fifth Dimension: The Art and Practice of the Learning Organisation, Random House Australia, Milsons Point, NSW, 1992.

[35] J.A. Bellamy, D.H. Walker, G.T. McDonald, G.J. Syme, A systems approach to the evaluation of natural resource management initiatives, J. Environ. Manage. 63 (2001) 407–423.

[36] M.E. Kentula, R.P. Brooks, S.E. Gwin, C.C. Holland, A.D. Sherman, J.C. Sifneos, An Approach to Improving Decision Making in Wetland Restoration and Creation, CK Smoley, Boca Raton, FL, 1993.

[37] C.M. Tate, Patterns and controls of nitrogen in tallgrass prairie streams, Ecology 71 (1990) 2007–2018.

[38] L.M. Cowardin, V. Carter, F.C. Golet, E.T. LaRoe, Classification of Wetlands and Deepwater Habitats of the United States, U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC, 1979 (FWS/OBS-79/31).