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A KNOWLEDGE-BASED APPROACH TO ENVIRONMENTAL BIOMONITORING

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Abstract. This paper presents the design, development and implementation of an integrated GIS-controlled knowledge-based system for environmental monitoring applications, utilizing indigenous flora for assessing quality. The system gathers and combines geographical, ecological, and physicochemical data of organisms' response to pollution within an intelligent computer program that (a) recognises groups of indigenous species suitable for long-term monitoring of a specific pollutant or a combination of pollutants, (b) estimates the ambient concentration of pollutant(s) from the population of the species comprising the bioindicator group and (c) provides biomonitoring capacity indices at national and international/transboundary levels. Significantly, a novel system in the form of a rational framework at the conceptual design level has been developed, that actually contributes towards achieving a cost-effective long-term biomonitoring program, with the flexibility to counter on-course any (anticipated or not) variations/modifications of the surveillance environment: the scheme assumes a robust dynamic cooperation between instrumental and biomonitoring systems, with a view to minimise uncertainty and monitoring costs and increase reliability of pollution control and abatement, aiming eventually at the shifting, partially or totally, from instrumental to natural monitoring. The proposed approach is presently implemented at pilot-scale for establishing a biomonitoring network at a large industrial area in Greece. The results obtained indicate that a cost-effective program can be only attained and maintained under a suitable financial/organizational scheme at the macro level, whereas the micro level viability strongly depends upon careful management of human resources and fixed assets.

Keywords: environmental monitoring, decision support system, bioindicators, ontology, biosurveillance

1. Introduction

The degree and extent of environmental change over the last decades has given a new urgency and relevance to the detection and understanding of environmental change. Concerns over issues such as biodiversity loss, atmospheric pollution, changes in water quality and quantity, land use change, sustainable development and climate change and its impacts have highlighted the need for high quality, long-term monitoring programmes to interpret environmental trends and inform policy making (Urquhart *et al.*, 1998; Oldfield and Dearing, 2003; Simcik, 2005). In practice, controlling (anthropogenic) pollutants is a very complex problem: sources and emissions have to be identified, analytical methods have to be evaluated, loads and levels have to be computed or measured, risks have to be assessed, critical emissions have to be controlled, and economical aspects have to be integrated (Caughlan and Oakley, 2001), especially in large, not easily accessible and remote areas. Successful monitoring programs must be ecologically relevant, enabling predictions to be made on ecological risk in the receiving environment, from abiotic parameters to lower levels of functional complexity and further to impacts at higher organisms including man (Wu *et al.*, 2005; Nicholson and Lam, 2005), statistically credible, broadly dispersed, and cost-effective (Hinds, 1984; Bateman and Walbeck, 2004); programs that neglect any one of these critical areas will face problems and likely fail.

Atmospheric surveillance can be based in dispersion modelling (source-orientation, *a priori* known emission sources) and field measurements of the emission (receptor/effect-orientation). Modelling is used as a useful tool in air quality management to supplement technical field measurements, the extent of which is limited due to high costs and prolonged analysis time (Overton *et al.*, 1995; Argent, 2004), although they are indispensable in validating dispersion models and indicating the presence of sources not known or registered (Wolterbeek, 2002; Uhlenbrook and Sieber, 2005). Although the field of environmental instrumental analysis has reached a stage of development that is challenging and promising, owing to the advances in extraction procedures, microfluidics, chip design, detection, engineering, and software, the continuous, long-term field monitoring of a broad range of chemicals simultaneously has not been yet realised, due to the lack of sufficiently sensitive and inexpensive devices (Guiochon and Beaver, 2004). It is here that biomonitoring comes in.

Biological monitors or biomonitors, ie., organisms (plants, animals, fungi, bacteria, etc.) which show an integrated response to air pollution and other environmental factors, can be used as a complementary system to monitor the effects of pollutants and to provide reliable indications on the quality and the characteristics of the environment (Hinds, 1984; Bargagli, 1998; Beeby, 2001; Conti and Ceccheti, 2001). The relative ease of sampling, the absence of any need for complicated and expensive technical equipment, and the accumulative and time-integrative behaviour of the organisms make them the less costly means of environmental surveillance; although biomonitors are often referred to as sub-organismal responses (Mayer *et al.*, 1992), in a broader context they may also include endpoints at all levels of biological organization, ranging from molecular and biochemical responses to behavioural and population/community changes (Bargagli, 1998; Kong *et al.*, 1999; Beeby, 2001; Conti and Cecchetti, 2001; Wolterbeek, 2002).

Biomonitoring programs can be considered as valuable supplements to standard pollution monitoring and are expected to contribute vastly to (a) increase the between-grid reliability of measurements when interpolation is performed, especially in the case of local peak valleys that are not identified by smooth interpolation (Aboal *et al.*, 2005; Simcik, 2005), and (b) assessing the bio-impact of pollution (Bargagli, 1998; Gaio-Oliveira *et al.*, 2004; Irving and Moncrieff, 2004; Martín-Díaz *et al.*, 2005), which is quite significant for ecosystem and human health. The use of living organisms as environmental gauzes also bear other advantages that, although minor for information retrieval, they are valuable otherwise, as public sensitisation to environmental conservation, realized by the implementation of volunteers either at the local level or through the national/international ecology networks. Volunteer-based projects offer further a strong financial advantage, making the long-term surveillance of large and inaccessible areas possible.

Accounts of such applications have been extensively published, but all too frequently the information is widely scattered, often in obscure or inaccessible literature sources, and lacks synthesis (Seaward, 1995). In theory, the techniques developed could be employed for low technology environmental monitoring where comparable on-site instrumentation would be expensive to install and maintain. However, the relation between response and stress concentration has not been yet elucidated at the extent and depth required to impart 'measurement' with a sufficient degree of certainty.

Lichen sensitivity to sulphur dioxide is well-established, whereas species susceptibility, determined by the maximum levels of SO_2 that the species tolerate, ranges from toxiphobous (extremely sensitive species) to toxitolerant (species that are able to adapt to pollution). During the last 15 years, lichen biomonitoring studies showed the reappearance of lichens in areas previously devoid of these organisms ('lichen desert') and the improvement of lichen biodiversity in many urban and industrial areas (Hawksworth and McManus, 1989; Seaward and Letrouit-Galinou, 1991; Loppi *et al.*, 1998; Loppi *et al.*, 2004); lichen colonization has been attributed to declining SO_2 concentrations, while the actual impoverishment in lichen communities to the constantly high levels of NO_x (Loppi *et al.*, 2004). Nevertheless, SO_2 air pollution assessment can be only attempted retrospectively, since the systemic action of the toxic substance, inducing alterations of the species metabolic activity (Kong *et al.*, 1999), is subjective to the underlying abiotic and biotic parameters: physiology, antagonistic/synagonistic relations and microenvironmental conditions (eg. bark pH and nitrogen availability) (Van Dobben and Ter Braak, 1998; Conti and Cecchetti, 2001; Bates, *et al.*, 2001; Herk *et al.*, 2002; Santitoro *et al.*, 2004). For example, the decline of *Lecanora conizaeoides* in urban areas, the only species proved to be favoured by SO_2 (Bates *et al.*, 2001), can be attributed to the decrease of SO_2. Although the species was expected to be found in abundance around oil refineries, this was not true when the refineries were located within intensively used agricultural areas. In the latter case, the ambient concentration of NH_3 is high (i.e., eutrophicated environment), resulting in an increase of bark pH, which is an extremely unfavourable condition for an acidophytic species (growing on acid barks) as *Lecanora conizaeoides* (Bates, *et al.*, 2001; Herk *et al.*, 2002). The decrease of SO_2 and the increase of NH_3 observed the last 15 years has allowed the remarkable spread of nitrophytic species (growing on nitrogen-rich barks with neutral to basic pH), but not in build-up areas, where NO_x concentrations are high, because the resulting ambient pH acidifies the bark (Van Dobben and Ter Braak, 1998; Bates, *et al*., 2001; Herk *et al*., 2002); this is also the case for *Xanthoria parietina*, which although nitrophytic, it can grow on nitrogen-poor barks provided that the pH is high (Gaio-Oliveira *et al*., 2004). Moreover, the susceptibility of SO_2 in neutrophytes (growing on barks with neutral pH) or some mild acidophytes is increased at acidic conditions, as in the case of *Lecidella elaeochroma*, which although tolerates SO_2 up to 80 ppb, at acidic conditions (eg. when the ambient NO_x levels are high), tolerability drops two-fold (Herk *et al*., 2002).

In view of the above, the employment of lichens to environmental surveillance is not feasible when the spatial patterns and temporal trends are not known *a priori*. Furthermore, the lack of validation, calibration and standardisation according to widely accepted standards, limits biomonitoring to research-based eco-regional programs, encompassing the qualitative or semi-quantitative nature of measurement. The major disadvantage of utilising indigenous vegetation in monitoring lies in the fact that all processes and all sources act at the same time and there is no possibility of separating them or looking for a particular one.

The integration of ecological health data and assessment of environmental pollution status can be proven a complex task owing to the multidisciplinary nature of the domain. Both require an in-depth understanding of ecosystem processes and functions, and knowledge of the manner and extent to which chemical substances are deposited, dispersed and react with it. Even though collection and compilation of existing data requires limited technical expertise, evaluation of the data and subsequent assessment require the input of experts in the fields of environmental chemistry, ecology and physiology. The development of a decision support model specific to the assessment of pollution impact on indicator species and associated data will allow the required expertise to be compiled into a structured knowledge base. This will permit the application of the knowledge-based reasoning to evaluate the current bioindicator capacity of an area and to prioritise management actions and further data collection activities in order to realize a long-term surveillance program.

Knowledge-based systems aim at eliciting explicit and tacit knowledge and reasoning strategies from experts so that a computer model of such expertise could be developed that, by incorporating a suitable inference engine, become a valuable tool for offering assistance/consultancy to end-users in a variety of tasks, especially in decision-making processes. A knowledge base is a logical representation of a problem in terms of relevant entities in the problem domain and logical relations among them (Debenham, 1998). Interpretation of data by a knowledge base engine provides an assessment of system states and processes represented in the knowledge base as topical entities. The introduction of knowledge-based techniques into databases has typically taken the form of expert systems, particularly useful in modelling complex domains (Cohen and Shoshany, 2002; Saunders *et al*., 2004; Irving and Moncrieff, 2004); in nature management activities, this technology is increasingly being used for supporting in ecological assessments and decision making (Avouris, 1995; Seder *et al*., 2000). Knowledge-based Geographical Information Systems

(GISs) play an important role in spatial and temporal analysis of pollution distribution, especially in inspecting and visualizing field data covering large areas and identifying changed sites. Thereby, GIS can be considered as a virtual sensor containing ecogeometric information together with its explicitly given semantics (Fischer, 1994; Cohen and Shoshany, 2002; Cohen and Shoshany, 2005).

This paper presents the design, development and implementation of an integrated GIS-controlled knowledge-based system for environmental biomonitoring applications. The system gathers and combines geographical, ecological, and physicochemical data of organisms' response to pollution within an intelligent computer program that (a) recognises groups of indigenous species suitable for long-term monitoring of a specific pollutant or a combination of pollutants and (b) estimates the ambient concentration of pollutant(s) from the population of the species comprising the bioindicator group. The program supports a novel biosurveillance scheme, designed, developed and implemented by the authors in the form of a rational framework, especially applicable for the surveillance of large, decentralized areas, such as forests; the scheme assumes a robust dynamic cooperation between instrumental and biomonitoring systems, with a view to minimise uncertainty and monitoring costs and increase reliability of pollution control and abatement, aiming eventually at the shifting, partially or totally, from instrumental to natural monitoring. The proposed network initially consists of both, field monitoring equipment and bioindicators, as part of a validation phase, which, by removing most of the former devices, gives rise to a hybrid monitoring scheme, engaging mostly bioindicators and a few equipment (Figure 1). By means of bioindicator recalibration with periodic revisiting of the field equipment the scheme progressively reaches a steady-state, thus ensuring reliability and robustness.

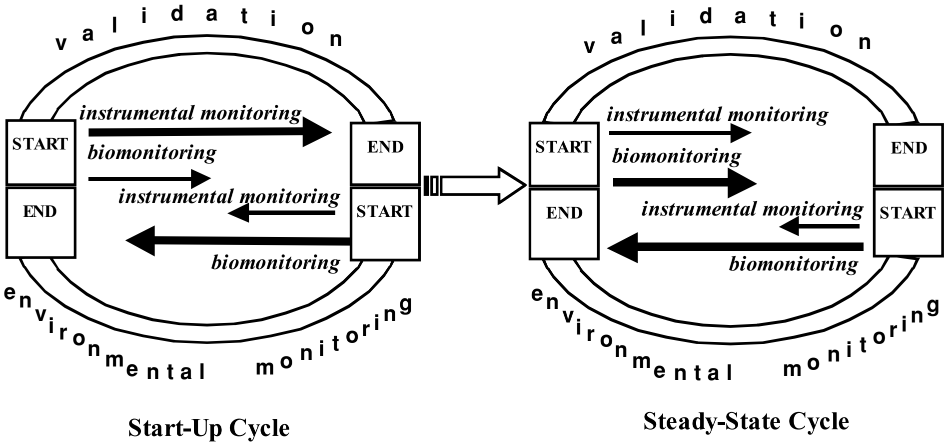


Figure 1. The cyclic operation of the cooperative/synergistic system presented herein; the size of the arrows indicates the relative importance between biosensing and biomonitoring.

The proposed approach is presently implemented at pilot-scale for establishing a biomonitoring network at a large industrial area in Greece. The results obtained thus far indicate that a cost-effective program can be only attained and maintained under a suitable financial/organizational scheme at the macro level, whereas the micro level viability strongly depends upon careful management of human resources and fixed assets. The advantages of this novel and pioneering approach will further offer a cost-effective dynamic system utilised in (a) environmental impact studies and risk assessment (positive/analytic approach), (b) decision-making in the short-run (normative/tactic approach), and (c) policymaking in the long-run (normative/strategic approach). To further reduce costs, the proposed biosurveillance system takes advantage of the local forestry monitoring schemes regarding (i) information required for the geological, edaphic and hydrological conditions through space and time and (ii) laboratory facilities. Owing to the characteristically high rate of spatio-temporal ecological and geographical heterogeneity of many regions, as for example the Mediterranean region or the Balkan areas, the implementation of the proposed schema could be proven valuable since large scale instrumental surveillance based on networks can be only considered in the short-term, as the costs involved in the maintenance of the field equipment and the associated network are high.

2. Bioindicator Ontology in Response to Environmental Exposure

Seen from an ontological point of view, the relation between exposure and response (phenomenological at surface level and physicochemical at deep level) should be elucidated. Consequently, the construction of an ontology, linking multi-directionally the effect of the pollutant (regulated or suspected) species, alone and in combination, upon the monitor organisms is essential. In knowledge management, ‘ontology’ is an explicit formal specification of how to represent the object, concepts and other entities that are assumed to exist in sum area of interest and the relationships that hold among them. Ontology resembles faceted taxonomy but use richer semantic relationships among terms and attributes, as well as strict rules about how to identify/specify/analyse/synthesize terms and relationships (Neches, *et al.*, 1991; Saunders *et al.*, 2004). Since ontologies do more than just control a vocabulary, they are thought of as knowledge representation, suitable to provide information for (a) decision making in complex interdisciplinary domains (like surveillance technology and ecosystems) and (b) problem solving within these domains (Avouris, 1995; Seder *et al.*, 2000; Cohen and Shoshany, 2002; Saunders *et al.*, 2004; Irving and Moncrieff, 2004). Exploiting the alteration of the physicochemical/metabolic pathways of the bioindicators due to each pollutant species and their possible combinations, the gap between external characteristics, bioaccumulation and bioavailability will be bridged.

Although some of these relations have been studied *in vitro* and *in vivo* even to gene level with respect to single exposure (one pollutant), the available information is scattered and highly unorganised (Seaward, 1995; Bargagli, 1998; Conti and Cecchetti, 2001; Wolterbeek, 2002). Among the mostly studied, in number and extent, is the exposure of lichens to sulphur dioxide or nitrogen oxides, providing dose-response relations, sensitivity indices, bioavailability correlations, bioaccumulation levels, and metabolic/biochemical pathways of infestation (Seaward, 1995; Loppi *et al.*, 1998; Beeby, 2001; Conti and Cecchetti, 2001; Wolterbeek, 2002; Zschau *et al.*, 2003; Szczepaniak and Biziuk, 2003); however, the effect of the two pollutants in combination has been only studied at a phenomenological level (morphological characteristics). Various other information can be retrieved from species classification studies, as for example the constraints affecting the association of the bacteria with the fungi at the cellular level (Boissiere *et al.*, 1987; Seaward and Letrouit-Galinou, 1991), or behaviour studies (Loppi *et al.*, 1998; Conti and Cecchetti, 2001; Wolterbeek, 2002; Bargagli *et al.*, 2002; Loppi *et al.*, 2004). Also, toxicological studies offer the genetic variations imposed by long-term exposure (Bargagli, 1989; Heij *et al.*, 1991; Bargagli, 1998; Szczepaniak and Biziuk, 2003).

The collection of the available information and the corresponding classification into taxonomic and partonomic relations provides a database linking pollutants with stress responses, sensitivity indices, bioavailability correlations, bioaccumulation levels, metabolic impact, and approximate inhibition patterns (antagonistic/synagonistic relations), all considered with respect to seasonal variation, species variability, and ecosystem parameters (climatic conditions, nutrient availability, background pollution level, geomorphologic characteristics). The authors have constructed such an ontology using a relational database management system (Figure 2) for mining the data gathered and generate the relations. The domains of investigation have been linked to all levels of constraints through rules-based reasoning in order to provide the species sensitivity indices. The first domain studies pollution impact at a surface/experiential level, registering the degree and extent of morphological changes (e.g. colour, shape) and behavioural adjustments (eg. species diversity and abundance) at varying levels of a pollutant (individual chemical substance) within a given pollution background (co-existing chemicals). Although one pollutant may be prevailing in the area, the combinatorial effects of other substances present cannot be ruled out as they can induce and/or modify stress responses (Van Dobben and Ter Braak, 1998; Bates, *et al.*, 2001; Herk *et al.*, 2002); in order to prevent adoption of incorrect or unnecessary assumptions, the additional use of atmospheric pollution indices has been proven suitable for elucidating, at a later stage, species specific responses. All information is related to geographic zones and seasons (GIS-mapping), considering lichen taxonomy (species, families and classes) and ecosystem characteristics (antagonistic/synagonistic relations and ecosystem biodifferentiation). The systemic impact is investigated in the second domain, hosting data derived from the lab analysis of the indicator species (respiration rate, plasmolysis, chlorophyll content) in order to elucidate the toxicological profiles of the pollutants (eg. non-toxic, subtoxic and hypertoxic ambient levels), considering locale parameters

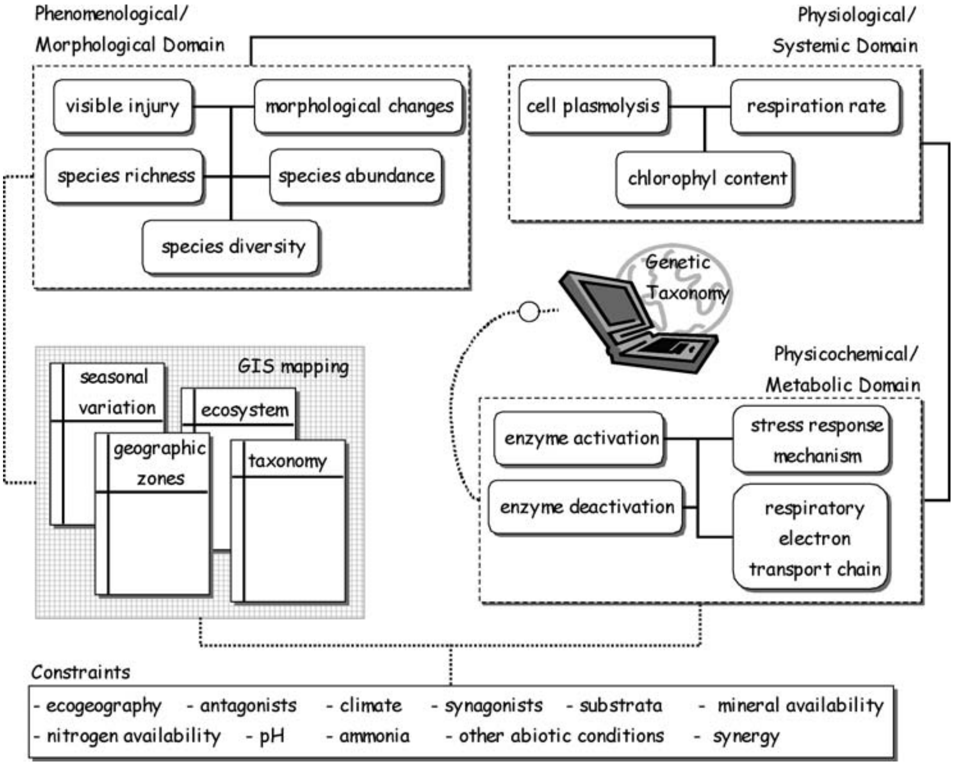


Figure 2. Infrastructure of the bioindicator ontology in response to environmental exposure, designed for optimal decision making in environmental surveillance.

(eg. climatic conditions and soil properties) and non-pollution related health status (e.g. diseases); based on the results, a maximum tolerance value (the maximum pollutant concentration that a species can sustain) is assigned to each organism, which is used for estimating the sensitivity indices. The third domain links metrology and visible injury to the biochemical effect of a single pollutant (a) on the phytobiont (primary attack point), (b) on the transportation of materials to the fungus (secondary attack point), and (c) on the fungus (tertiary attack point), taking into account spot characteristics (eg. bark pH and nutrient availability). The combinatorial biochemical effect of several pollutants derives from dedicated *in vivo* or *in vitro* studies, in the absence of which possible pathways are provided by models with suitable approximations, either existing or *ad hoc* produced. The third domain is connected to the existing lichens gene libraries in order to provide more in-depth correlations/associations.

As the domain referring to the biochemical/cellular basis of pollution impact, the semantic links are mainly of the *is-a* or *has-a* type, while in the domains dealing with systemic responses the semantic links *is-part-of-a* and *means-of-a* (*is-expressed*/*examined-by*) dominate. The ontology can be used to retrieve species specific for one or several pollutants, ranked by their sensitivity indices and their availability in the area of interest, or otherwise indicate suitable species for transplantation. It also provides information on the dose-response relations, type of response (visual, lab determination) and variation, expected biochemical mechanisms, validation parameters, and frequency of sampling; when no available data can be referred to, this information can be predicted when an acceptable similarity with other species is established at the systemic and metabolic level.

Although highly promising, the bioindicator ontology is currently condemned to limited use owing to lack of necessary information. The morphological and/or genetic differences between different species in the same locale or the same species in different locales affect significantly the response towards the same pollutant, however most studies have a local character. Furthermore, any significant impact on the behaviour of the biomonitor organism may lead to changes in the way the organism responds to pollution, thus disturbing the assumed relationships. However, species tolerability through genetic diversity have not considered at a level suitable for biomonitoring capacity evaluation; existing information is highly heterogeneous and unrelated, rendering its introduction to knowledge-based systems difficult. Furthermore, extensive biomonitoring data comparisons are not available and biochemical mechanisms are still ‘not well understood’, seasonal variation and species variability are not considered in depth, not to mention that harmonized monitoring and validation protocols do not exist. Further research is required, focused mainly in the cellular and subcellular response to stress, especially due to combination of pollutants, and guidelines should be agreed upon so that comparisons and temporal/spatial analyses could be possible.

3. Biomonitoring with Lichens

3.1. Selection of grouped patterns for biomonitoring

The information stored in/derived from the Lichen Ontology have been used as the basis for designing/developing an *ad hoc* computer program that can (a) provide grouped patterns of lichens suitable to cover the desired pollutant(s) concentration range in the area under surveillance: a number of organisms with various levels of tolerance to the pollutant(s) of interest are selected so that the expected pollutant concentration range is fully accounted for, under known, uncertain or unknown underlying conditions, and (b) estimate the pollution ambient level by the observed population and colony spread of each of the species of the selected group, i.e., the absence of certain species and the presence (and abundance) of other species.

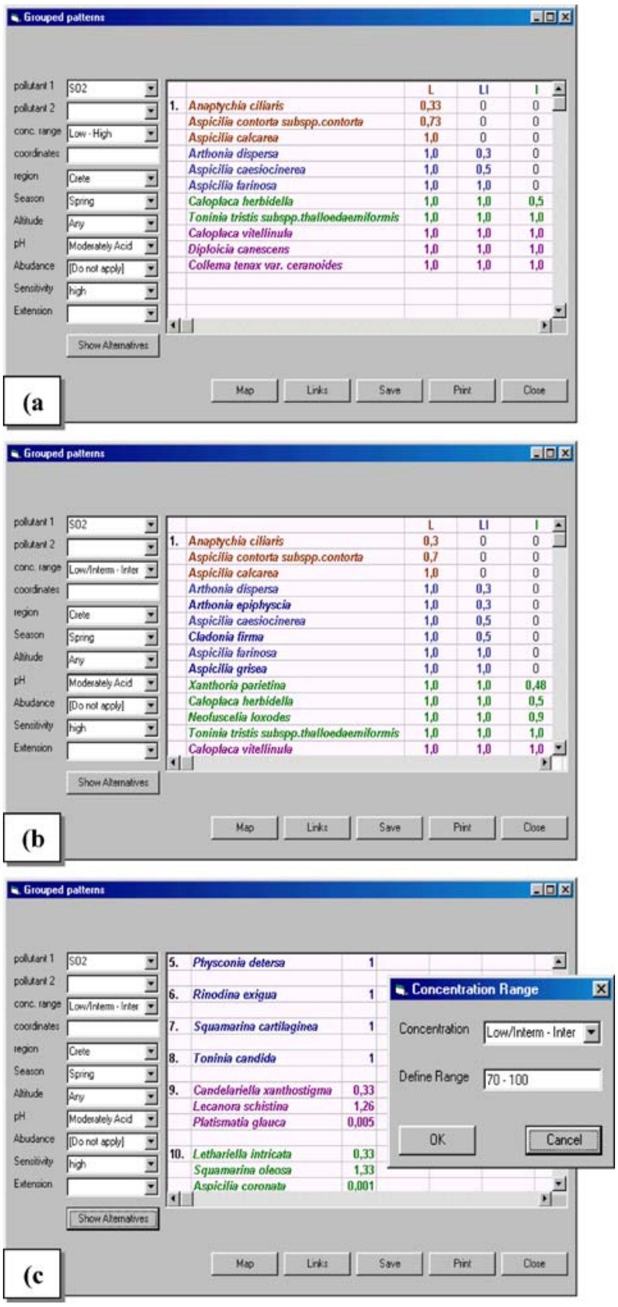
The program searches the database for lichens indigenous to a specific geographic area, selected either form the pre-determined region options (that covers countries, districts, prefectures, towns, etc.,) or through point-source co-ordinates. The user defines the pollutant or the combination of pollutants and the required concentration range. For each pollutant, concentration levels follow the international norms for atmospheric pollution: *normal*, *low*, *low*-to-*intermediate intermediate*, and *high*; this partitioning however is not effective since the range defined in each case is quite wide (eg. low SO_2 level is 3–40 ppb), limiting considerable the precision of the result and hindering the assignment of lichen species in that region. It was thus necessary to assume a reasonable overlapping and to further subdivide the intervals; for example, the basic divisions of sulphur dioxide are: *normal* (N): 0.2–5 ppb; *low* (L): 3–43 ppb; *low to intermediate* (LI): 35–105 ppb; *intermediate* (I): 100–210 ppb; *high* (H): >200 ppb, each divided into 3 subintervals.

Following the screening of the database for the available sensitive native species, the program assigns each species under a concentration subinterval, based on its maximum tolerance values and then ranks the species in each subinterval in descending order according to the closeness of their tolerability to the upper limit of the subinterval; this closeness is defined as the *degree of coverage* (adequacy), a, given by $a = T - L/U - L$, where T is the lichen max. tolerance value, whereas L and U are the lower and upper concentrations of the subinterval, respectively (see Appendix). In case the determining factor is not the upper limit but the lower, i.e., the species growth is actually favoured by the presence of the pollutant (as in the case of nitrophytic lichens favoured by high ammonia levels), the equation for estimating a is adjusted as appropriate. In each subinterval, full coverage is defined above a threshold value, set by the user; a 0.8 threshold has been proven to be most suitable, and indicates that the species selected in that interval or subinterval can account for at least the 80% of the relevant concentration range.

If a species falls below the threshold value, the program combines species from the same or adjacent subintervals in order to provide a measure by comparison. For example, *Lethariella intricata* and *Caloplaca carphinea*, tolerating SO_2 up to 80 ppb and 75 ppb, respectively, are ranked under the LI3 interval (70–105 ppb); providing a degree of coverage at 0.28 and 0.14 respectively, they are both excluded from stand-alone selections and a combination has to be made. Combing only the two species, the uncertainty at the upper border level of the specific subinterval would be high; incorporating, however, *Collema nigrescens*, with a max. tolerance value of 110 ppb, the uncertainty of the measurement is significantly decreased, as SO_2 levels near 80 ppb would be designated by the presence of the latter species in the absence of the former.

The program provides the first in line grouped pattern for biomonitoring; the second, third, etc., alternatives play the role of the pool, providing suitable replacements in case the biomonitors have not functioned as anticipated. If a grouped pattern cannot be formed due to species shortage, the program retrieves foreign species growing at similar ecogeochemical environment for transplanting.

An example is illustrated in Figure 3. The monitoring of SO_2 in Crete, during summer and at moderately acid conditions (pH: 4.7–5.8), can be realised using the



pollutant 1: SO2
pollutant 2:
conc. range: Low - High
coordinates:
region: Crete
Season: Spring
Altitude: Any
pH: Moderately Acid
Abudance: [Do not apply]
Sensitivity: high
Extension:

		L	LI	I
1.	*Anaptychia ciliaris*	0,33	0	0
	Aspicilia contorta subspp.contorta	0,73	0	0
	Aspicilia calcarea	1,0	0	0
	Arthonia dispersa	1,0	0,3	0
	Aspicilia caesiocinerea	1,0	0,5	0
	Aspicilia farinosa	1,0	1,0	0
	Caloplaca herbidella	1,0	1,0	0,5
	Toninia tristis subspp.thalloedaemiformis	1,0	1,0	1,0
	Caloplaca vitellinula	1,0	1,0	1,0
	Diploicia canescens	1,0	1,0	1,0
	Collema tenax var. ceranoides	1,0	1,0	1,0

pollutant 1: SO2
pollutant 2:
conc. range: Low/Interm - Inter
coordinates:
region: Crete
Season: Spring
Altitude: Any
pH: Moderately Acid
Abudance: [Do not apply]
Sensitivity: high
Extension:

		L	LI	I
1.	*Anaptychia ciliaris*	0,3	0	0
	Aspicilia contorta subspp.contorta	0,7	0	0
	Aspicilia calcarea	1,0	0	0
	Arthonia dispersa	1,0	0,3	0
	Arthonia epiphyscia	1,0	0,3	0
	Aspicilia caesiocinerea	1,0	0,5	0
	Cladonia firma	1,0	0,5	0
	Aspicilia farinosa	1,0	1,0	0
	Aspicilia grisea	1,0	1,0	0
	Xanthoria parietina	1,0	1,0	0,48
	Caloplaca herbidella	1,0	1,0	0,5
	Neofuscelia loxodes	1,0	1,0	0,9
	Toninia tristis subspp.thalloedaemiformis	1,0	1,0	1,0
	Caloplaca vitellinula	1,0	1,0	1,0

pollutant 1: SO2
pollutant 2:
conc. range: Low/Interm - Inter
coordinates:
region: Crete
Season: Spring
Altitude: Any
pH: Moderately Acid
Abudance: [Do not apply]
Sensitivity: high
Extension:

5.	*Physconia detersa*	1
6.	*Rinodina exigua*	1
7.	*Squamarina cartilaginea*	1
8.	*Toninia candida*	1
9.	*Candelariella xanthostigma*	0,33
	Lecanora schistina	1,26
	Platismatia glauca	0,005
10.	*Lethariella intricata*	0,33
	Squamarina oleosa	1,33
	Aspicilia coronata	0,001

Concentration: Low/Interm - Inter
Define Range: 70 - 100

Figure 3. Sample screenshots of the developed computer program for selecting grouped patterns to monitor SO_2 in the island of Crete (Greece): (a) best grouped pattern for covering the *low* to *high* concentration ranges, (b) best grouped pattern to account for *low*-to-*intermediate* to *intermediate* intervals; some species are added in order to increase sensitivity and reliability of measurements, and (c) the available alternative species for the 70–100 ppb sub-interval.

first grouped pattern shown in Figure 3a, comprised from species indigenous to the area, retrieved by the program as most suitable to cover the concentration range 5–250 ppb (*low* to *high*). The numbers in the right columns represent the coverage of the species response towards the concentration level, as fractions of 1, shown in the first row; for example, *Anaptychia ciliaris* ($T = 20$ ppb), *Aspicilia contorta* subspp *contorta* ($T = 31$ ppb) and *Aspicilia calcarea* ($T = 40$ ppb) cover 33.3%, 73% and 100%, respectively of the *low* level (5–40 ppb): the absence of *Anaptychia ciliaris* but the presence of *Aspicilia contorta* subspp *contorta* indicate a SO_2 level of 20–30 ppb; the absence of the latter in the presence of *Aspicilia calcarea* will raise the level to 40 ppb.

If further information regarding the pollution level of the area is known, as for instance that the expected range would most likely be between 35–200 ppb (*low intermediate* to *intermediate*), the program performs ‘fine tuning’ (Figure 3b), i.e., the anticipated range is reinforced with more species in order to increase measurement reliability: the *low intermediate* interval has been enriched with three more species and the *intermediate* interval with two. In this way, uncertainty is decreased significantly, especially towards the boundaries of the pollution levels. When a species is not functioning as anticipated, the program will correct its ranking, whereas the alternatives pool will provide a suitable replacement (Figure 3c).

In case the available information on the indigenous vegetation is not adequate, the ‘extension’ button will activate a new query for locating species inhabiting neighbouring areas with similar climatic, geomorphologic, and geochemical parameters, with established response towards the pollutant of interest, in order to enrich knowledge. The same can apply if indigenous species with adequate sensitivity towards the pollutant of interest are scarce; in that case, transplants should be considered, whereas the use of moss bags could be useful as a pre-screening activity, in order to evaluate level of contamination with respect to availability parameters.

The lichen selection program developed aims at providing on site monitors, specific to the pollutant(s) to be monitored. Considering that the program usefulness is mainly seen in large area surveillance, the major issues arisen concern representativity of the monitoring sites and the monitors, as well as adequate frequency of occurrence of the selected species. Depending on the pollutant in question, a site may be more or less influenced by local sources, and thus, unless sampling at the exact spot, local sources may blur the picture significantly. Extensive and frequent sampling can ensure representativity, both readily allowed by the nature of the gauzes, cost-wise. However, this implies primarily adequate abundance of the selected grouped pattern species and, secondarily, adequate information available regarding the correlation between stress response of the species and degree of environmental damage. Regarding the former, the end-user can modify the selected grouped patterns and their ranking by activating the relevant button (Figure 3) to meet the criterion. The satisfaction of the latter, however, requires detailed knowledge on the relation between ambient, bioavailable and bioaccumulated levels, an area which is still studied and expanding. The tool presented herein can be used for directing R&D activities towards such knowledge gaps which should be bridged in order for a full-scale biomonitoring program to be realized.

3.2. BIOMONITORING CAPACITY INDICES

The construction of a lichen database with respect to environmental pollution has led to the estimation of useful biomonitoring indices for each country. Although ecological indices have been widely used to assess environmental quality on the basis of biotic data, the gauge capabilities of the ecosystem have not been considered. However, the need for the development of cost-effective and ecological relevant long-term biosurveillance networks clearly points to biotic gauges; such initiatives could be only considered when the potential of the area under consideration is known in order to support decision-making. The biomonitoring capability indices can be calculated for any region or country, with respect to a pollutant or combination of pollutants, applicable to point and non-point sources, provided that the indigenous species are fully accounted for and registered.

Considering seven countries, Afghanistan, Albania, Armenia, Cyprus, Greece, and Romania, their registered indigenous lichen species responsive to sulphur dioxide number to 208, 143, 201, 187, and 176, respectively. The assignment of these species to the concentration intervals *normal* to *high* (0.5–300 ppb) is shown in Table I, along with the distribution index of the species throughout the *normal-high* concentration range, i.e., the ratio of the number of species in each interval to the total number of responsive species.

As expected, the number of species tolerant to high levels of the pollutant are limited to 2–6% for >200 ppb and 4–14% for 100–200 pbb. As also shown in Table I, the majority of the species are classified under the *low* and *low*-to-*intermediate* interval, whereas the number of species indicating a 'clean environment' are below

TABLE I

Country biomonitoring capacity for SO_2 monitoring, as sensitive species diversity (absolute numbers) and distribution index in the *normal* to *high* range: N is 0.2–5 ppb, L is 3–43 ppb, LI is 35–105 ppb, I is 100–210 ppb, and H is >200 ppb

	No. of sensitive species					Distribution index				
Country	N	L	LI	I	H	N	L	LI	I	H
Afghanistan	30	97	68	8	5	0.14	0.47	0.33	0.04	0.02
Albania	24	68	32	13	6	0.17	0.48	0.22	0.09	0.04
Armenia	30	92	61	13	5	0.15	0.46	0.30	0.06	0.02
Cyprus	25	60	63	27	12	0.13	0.32	0.34	0.14	0.06
Greece	29	80	50	23	11	0.15	0.41	0.26	0.12	0.06
Romania	27	85	38	17	9	0.15	0.48	0.22	0.10	0.05

TABLE II

Country biomonitoring capacity for SO_2 monitoring, given as numbers of grouped patterns that can be formed (grouped pattern index) for individual concentration intervals and for wider ranges: N is 0.2–5 ppb, L is 3–43 ppb, LI is 35–105 ppb, I is 100–210 ppb, and H is >200 ppb

Country	N	L	LI	I	H	N-H	L-LI	L-I
Afghanistan	30	23	2	1	1	1	2	1
Albania	24	14	7	6	3	3	7	6
Armenia	30	22	15	5	2	2	15	5
Cyprus	25	14	13	7	4	4	13	7
Greece	29	17	10	8	5	5	10	8
Romania	27	22	3	5	4	3	3	3

20%, owning to the sustained low pollution level in these countries. It should be noted however, that a detailed register of the lichen and lichenocolous species in each country does not exist and the data presented derives from relevant publications and personal collections. When such registers are constructed and the tolerability of the species is investigated, the number of sensitive species as well as their distribution index are expected to increase.

Seen from the managerial point of view, the establishment of a full-scale long-time biomonitoring program in a country or a region, realised through the knowledge-based approach presented herein, is depending upon the number of grouped patterns that can be formed at the desired concentration range. Table II presents the grouped pattern index for each concentration interval and for the three most common ranges, *normal* to *high* (N-H), *low* to *intermediate* (L-I) and *low* to *low*-to-*intermediate* (L-LI). The grouped pattern index is low when number of grouped patterns that can be formed to an interval contained within the concentration range is limited. In most cases, the limiting factor lies with the *high* interval grouping, with the exception of Romania, where the limiting factor is found within the *low*-to-*intermediate* interval.

The correlation between the distribution index and the grouped pattern index is usually but not necessarily positive. For example, although Afghanistan has a distribution index of 0.33 and adequate number of species covering the *low*-to-*intermediate* interval (Table I), the corresponding grouped index is 2 (Table II). This is because the indigenous lichen species assigned under the 35–105 ppb interval have in their majority maximum tolerability for SO_2 up to 50 ppb, i.e, most of the species in this interval are actually assigned under the LI1 sub-interval (Table III).

The average number of species per grouped pattern (Table IV), produced by dividing the number of species in each interval by the respective number of grouped patterns, is indicative of the biomonitoring reliability of a country, since the higher the number the more combinations are needed to cover the interval in concern, thus the higher the costs for sampling and analyses. The best index value is 1.00, indicating that only one species is required to provide the pre-set percentage coverage

TABLE III

Country biomonitoring capacity for SO_2 monitoring, given as numbers of grouped patterns that can be formed under each sub-interval (sub-interval index): L1 is 3–16 ppb, L2 is 14–35 ppb, L3 is 30–43 ppb, LI1 is 35–50 ppb, LI2 is 46–74 ppb, LI3 is 70–105 ppb, I1 is 100–160 ppb, and I2 is 150–210 ppb

Country	L1	L2	L3	LI1	LI2	LI3	I1	I2
Afghanistan	26	24	23	16	6	2	2	1
Albania	23	17	14	11	7	11	7	6
Armenia	24	24	22	19	17	15	5	6
Cyprus	19	14	16	24	15	13	14	7
Greece	24	20	17	20	10	11	9	8
Romania	24	24	22	19	5	3	5	6

TABLE IV

Country biomonitoring reliability for SO_2 monitoring, given as average number of species forminmg a group pattern in each interval: N is 0.2–5 ppb, L is 3–43 ppb, LI is 35–105 ppb, I is 100–210 ppb, and H is >200 ppb

Country	L	LI	I	H
Afghanistan	4.22	34.00	8.00	5.00
Albania	4.86	4.57	2.17	2.00
Armenia	4.18	4.07	2.60	2.50
Cyprus	4.29	4.85	3.86	3.00
Greece	4.71	5.00	2.88	2.20
Romania	3.86	12.67	3.40	2.25

of the interval in concern. In most cases, combinations are formed after the first 3 or 4 ranks, starting from binary and moving to ternal combinations after the 7 ranks. This pattern is represented by an index of ca. 4 or less; at index values higher than 5, the binary and ternal combinations start at much higher ranks.

A relative comparison of the biomonitoring capacity of the countries can be proven useful when viable transplants are considered or even information are required regarding similar species. Geographic, climatic and geochemical similarities would indicate possible sources; for instance, northern Greece should turn to Albania or Romania, whereas southern parts of the country would look at Cyprus. Considering Greece as control (index 1.00), the relative biomonitoring capacity indices (ie., the ratio of the country grouped patterns over the respective grouped patterns of Greece) are presented in Table V. Index values greater than 1 indicate a greater capacity compared to Greece; likewise lower capacity is dictated by values less than 1.

Looking at the indices of Table V, the lichens if Cyprus and Romania can be proven useful alternatives at 35–160 ppb and 3–43 ppb SO_2 range, respectively. If transplants are sought after, the chance of finding in these countries species of

TABLE V

Relative country biomonitoring capacity for SO_2 monitoring with respect to Greece: L1 is 3–16 ppb, L2 is 14–35 ppb, L3 is 30–43 ppb, LI1 is 35–50 ppb, LI2 is 46–74 ppb, LI3 is 70–105 ppb, I1 is 100–160 ppb, and I2 is 150–210 ppb

Country	N	L1	L2	L3	LI1	LI2	LI3	I1	I2	H1	H2
Afganistan	1.03	1.08	1.20	1.35	0.80	0.60	0.18	0.22	0.13	0.20	0.20
Albania	0.83	0.96	0.85	0.82	0.55	0.70	1.00	0.78	0.75	0.60	0.60
Armenia	1.03	1.00	1.20	1.29	0.95	1.70	1.36	0.56	0.75	0.60	0.40
Cyprus	0.86	0.79	0.70	0.94	1.20	1.50	1.18	1.56	0.88	1.40	0.80
Romania	0.93	1.00	1.20	1.29	0.95	0.50	0.27	0.56	0.75	0.80	0.80

related genera to Greek community is high. The indices of Albania however, are less than 1 throughout the range; this is highly unlikely due to the geomorphological, ecological and climatic similarity between the two countries and could be attributed to the very limited national lichen registry.

4. Framework for Implementation

Biomonitoring, considered as a long-term quality management tool, should be thoroughly designed, tested and validated, in order to overcome the knowledge deficit and assign credibility to the system without compromising on cost-effectiveness. The authors argue that such a system can only serve its purpose when significant reliability is imparted through the cooperation with instrumental monitoring, a well-established and broadly-used system, playing the role of the benchmark.

The design, development and establishment of a cooperative/synergistic monitoring system engaging natural and instrumental gauges, considering the diversity and the complexity of the former and the performance problems of the latter, especially in long-term and large-scale field measurements, could be quite problematic. Depending on one's role, there may be subtle or distinctly different priorities for outcomes and even on criteria for measuring whether the outcomes have been achieved. These differences may be necessary for the proper functioning of the system as a whole but they make development and management difficult. Any system as such should (a) establish practical, valid and equitable evaluation criteria by which qualitative and quantitative changes of pollutants can be monitored and assessed, and (b) involve methodological pluralism (including both qualitative and quantitative methods) to ensure rigour and comprehensiveness in assessment. Both these requirements entail high costs that would eventually render any program inapplicable. The cost of collecting consistent data for a large number of local pollution sources has made it impossible, until recently, to test the relative importance of particular variables on the status of the environment, and thus to move from the concept of surveillance to integrated monitoring. The issue remains pressing for policymakers, since such natural resources are often critical to the national economies and the conservation of biodiversity around the world. It is therefore imperative to establish a surveillance programme which will assimilate to existing frameworks for information exchange and utilize local resources, in a manner ensuring the development of a continually growing in knowledge and experience scheme, easily adaptable to any local, national or international need.

The methodological framework designed and developed by the authors to address the problems of the herein proposed cooperative/synergistic biomonitoring system (Figure 4), consists of nine phases. Phase 1 is concerned with gathering information for the area to be monitored and the relevant parameters. Apart from studying the pollution distribution patterns, setting the surveillance boundaries and developing/designing the GIS layers for mapping, a survey is performed for determining comparable (similar) sub-regions, aiming at retrieving information on relevant parameter-values, investing on the experience acquired by similar projects and keep alternatives in store, in case controls or replacements are required.

Phase 2, ie., the process of lichen species selection, is presented in Figure 5. The computer program, presented in Section 3.1, provides the lichen species that could participate in the monitoring network, allowing for response confirmation and/or transplantation by the extension capability that the program offers; the biomonitoring capability indices presented in Section 3.2, can serve as time-saving guides to network design and development. Following analysis of the species to verify response and selection of the potential reliable gauges, the anticipated concentration range of the pollutant is divided into appropriate sub-intervals; each species is assigned to a sub-interval and ranked according to the preset degree of coverage and a grouped pattern is finally selected to monitor the quality of the environment. The supplementary groups provided by the program, can replace or reinforce the existing pattern, if required.

Phase 3 runs in parallel, concluding in the selection of field monitoring equipment, suitable for the intended application as regards cost and sensitivity. The utilization of local resources in designing and developing *ad hoc* monitors can be considered in this phase, an option which would decrease monitoring cost and contribute to sustainable development of decentralised areas. When the equipment engaged is based on well-known or easily accessible technology, as chemical sensors, which are widely used for *in situ* (indoors or outdoors) surveillance, local development is possible. When deciding upon living and instrumental gauzes, standardisation is achieved by means of comparing the two types of monitoring and determining *a priori* a quantitative correspondence (Phase 4). The correlation between stress response and instrumental measurement can be very helpful in determining bioavailability factors and examine representativity.

The next phase is the operational planning, which includes the determination of sampling sites, sample size and frequency of sampling according to abundance of the lichen species and the concentration of the pollutant(s) to the sub-regions, utilized in GIS-griding, as well as the design of a network for gathering and analysing

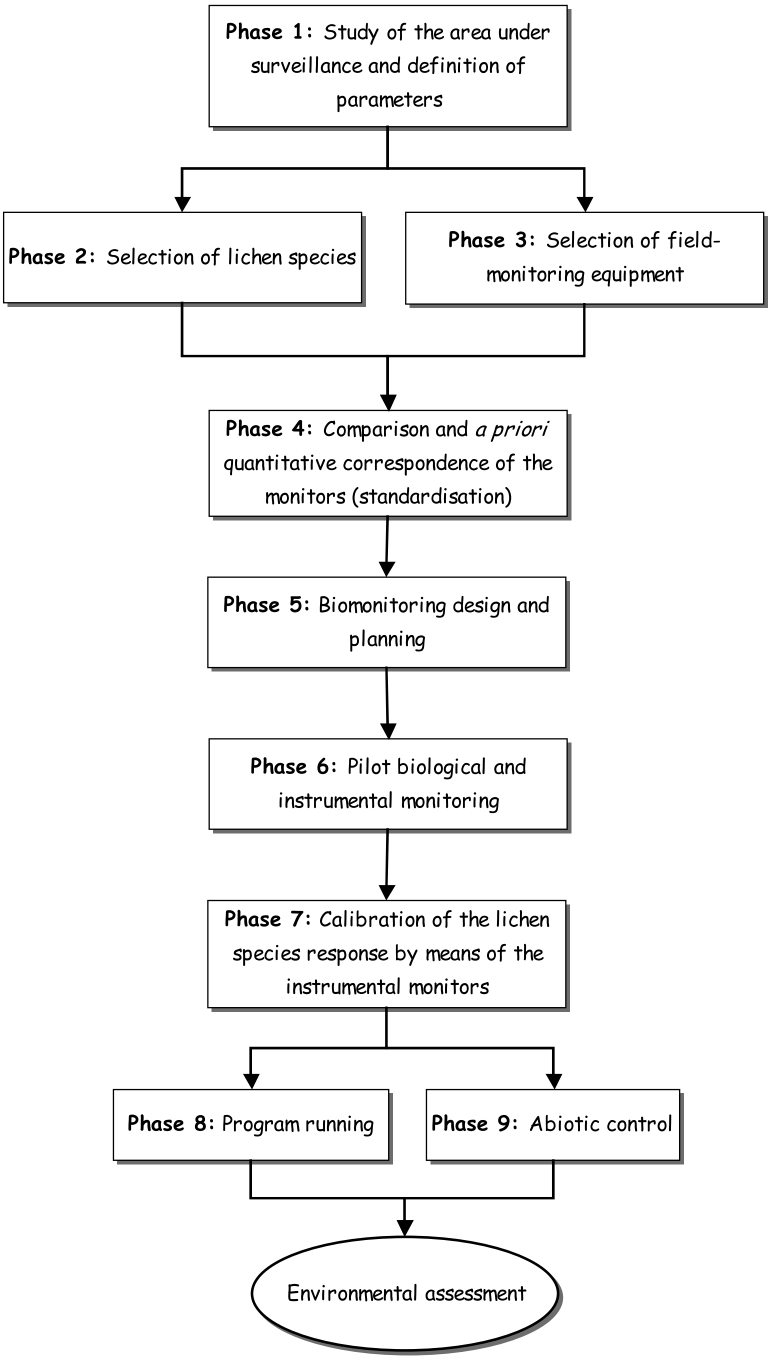


Figure 4. Simplified overview of the methodological framework designed and developed for integrated environmental biomonitoring through the multi-level synergy between bioindicators and field gauges.

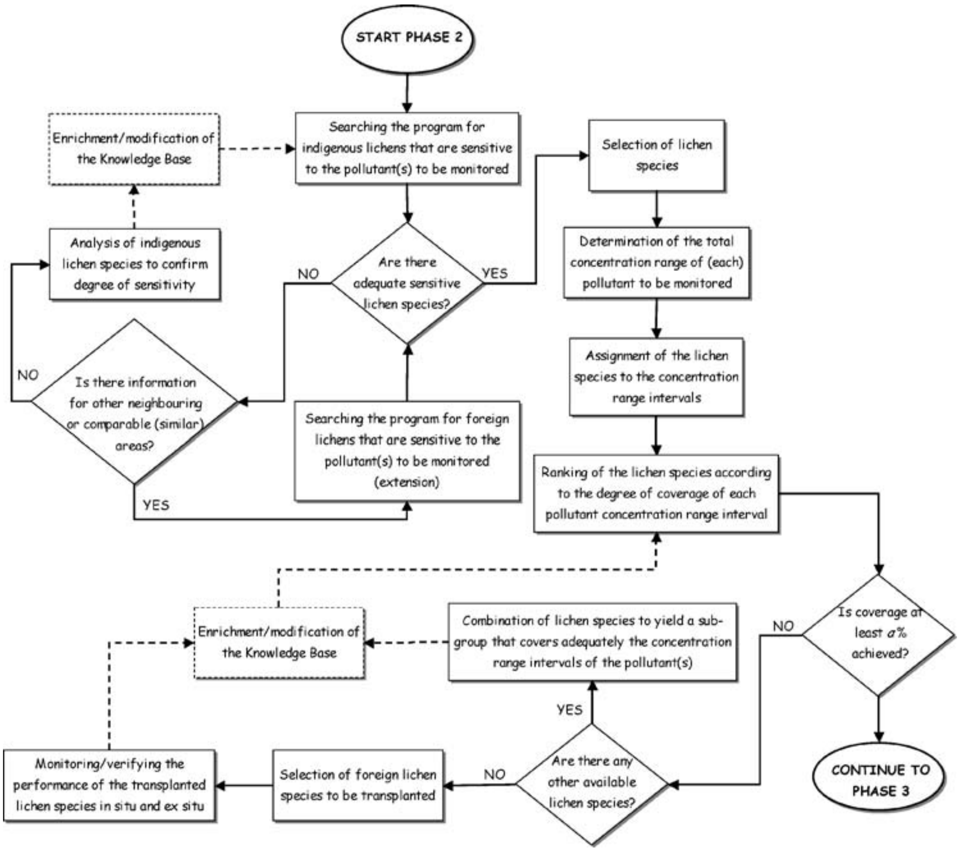


Figure 5. Phase 2 sub-algorithmic procedure: Selection of lichen species.

monitoring equipment data. A pilot implementation of the program (Phase 6) with parallel instrumental and biological monitoring will provide (a) program validation on both strategic efficiency and operational functionality, (b) confirmation on degree and extent of biological stress responses as well as species suitability, and (c) the necessary adjustments and modifications that should be performed for scale-up. Biological monitoring requires periodic field observations, the frequency of which can be modified according to the signal retrieved by the network; for example, an alarming situation revealed by the field equipment (increasing trend of pollution load) would prompt an *in situ* investigation of the lichen response and the priming, if necessary, of the appropriate countermeasures. The verification of the monitoring signal to bioindicator response can be also utilised to the conformation of the correlation model (Phase 4), especially in cases of abrupt pollution load increases (peaks) where the physiological response of the organisms could differ significantly from that observed in a stable environment.

The necessary quantitative assessment of elemental availability asks for well-defined dose-response relationships, and knowledge on disturbances by impacts on

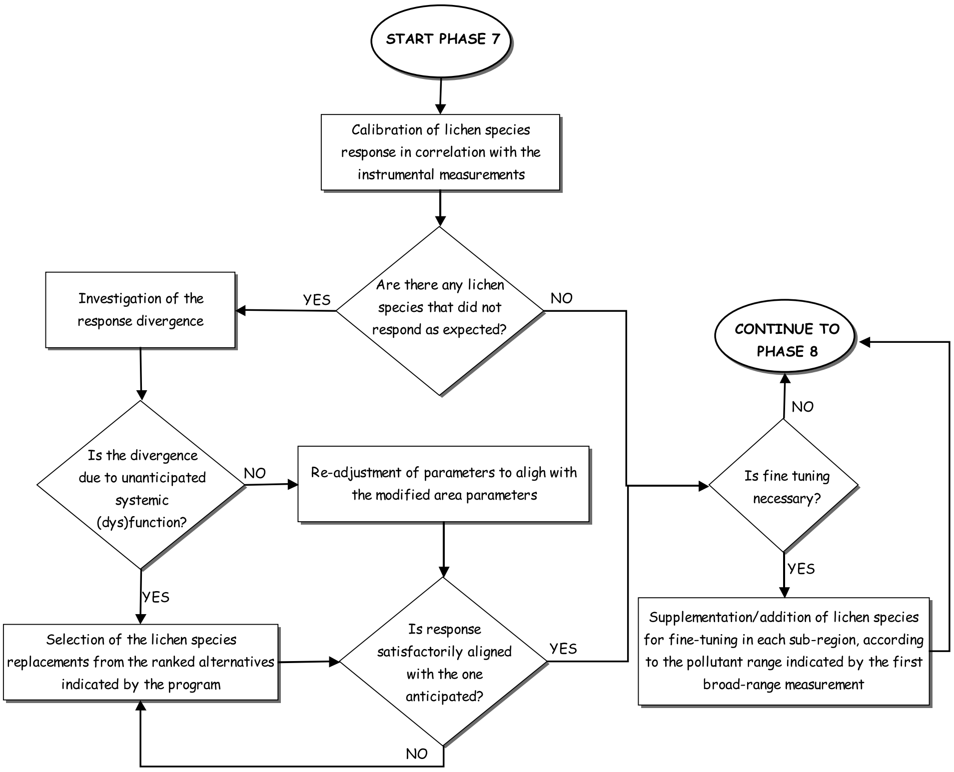


Figure 6. Phase 7 sub-algorithmic procedure: Calibration of the lichen species response by means of the instrumental monitors.

the plant parameters on accumulation, retention and release processes (Beeby, 2001; Wolterbeek, 2002). The calibration of the bioindicators by means of the instrumental monitors (Phase 7) is the central component of the framework rather than an activity external to the program process (Figure 6). It forms the basic platform for the system developing in order to address problems as biomonitoring validation. Since field equipment are used for validating the performance of bioindicators, the correlation between the former and the latter is only possible through inter-calibration and model construction. In that way problems such as misinterpretation of data, incorrect or biased signals, and uncertainty of measurements are diminished. Such a calibration may retain a strict *ad hoc* character and local applicability, it would be however valuable for the interpretation of biological responses and environmental assessment, as well as contribute to uphold representativity by determining suitable correlation factors.

However, bridging laboratory findings to field results could be proven problematic, unless the detection system of the monitor is similar to the organism's stress response mechanism, so that the mechanistic relations can be properly identified. A potential solution can be presented by the use of biosensors, the 'nature-mimicking' devices, that employ natural biorecognition schemes couples to physicochemical transducers for the qualitative and quantitative analysis of complex mixtures (Rogers, 1995; Siontorou *et al*., 2000; Malhorta *et al*., 2005). For example, the response of lichens to sulphur dioxide stress is triggered by the participation of the sulphate ion in the redox reactions of the cytochrome P system of the phytobiont (Kong *at al*., 1999); a similar mechanism is utilized for the detection of SO_2 from a screen printed gas phase amperometric biosensor (Malhorta *et al*., 2005). It is quite feasible to develop biosensors based on the lichen enzymatic cascades; the bioindicator stress response can be thus directly linked to cellular and subcellular changes based on the biochemical alterations indicated by the biosensors' response, permitting the tracking of the pollutant effect all its way through the organism.

Upon correlation of instrumental and biological responses, deviations could be attributed to modifications of the organisms' growing conditions (e.g. change in soil mineral content or pH) due to reasons other than the monitored pollution. The lab analysis of the species and available abiotic data (Phase 9) should clarify that point, indicating the shift towards another bioindicator grouped pattern in case the selected species show systematic or behavioural deviations that renders them unsuitable for monitoring purposes (by means of selecting a replacement sub-group) or, in the case of unanticipated environmental alterations, a change in the correlation model; the latter involves a detailed investigation of the origins, extent and fluctuation of the alteration, and depending on its severity, would call for re-adjustment of parameters.

Running the program at full-scale for a certain time period (Phase 8) provides information on minor adjustments necessary and establishes a sufficient degree of reliability in biomonitoring. However, the core of the framework is the removal of most equipment from the field (stage 34) and the shift to biomonitoring for saving economic resources, although a number of them should be kept in order to maintain predictability within an acceptable region. These devices removed will be placed again in the field as part of recalibration/revalidation phase of biomonitoring. Taking advantage of the knowledge/experience acquired, they can be also used, including any supporting equipment necessary, for initiating/maintaining a similar surveillance system in another area. This scheme provides adequate quality assurance and control, ensuring the proper function of the program and the reliability of the obtained data.

When the area under surveillance includes woodland, the established local forest monitoring program can provide information regarding the edaphic, hydrological and geological parameters of the area (Phase 9); since lichen activity and stress response is basically determined by the underlying environmental conditions, such information can be provide useful insight regarding species growing conditions and the causes of divergence from the anticipated response. Furthermore, the use of the established laboratory facilities and even of the field observation schedule can further reduce monitoring costs.

The framework presented can be utilised/adjusted to any environmental system for multi-elemental monitoring in both phases, the gas and the liquid, encompassing a regional framework around a pollution source in the form of a LAN, with on-line data collection and mining, as regards instrumental monitoring. The GIS griding that takes place in Phase 5, can be also followed in the case of continuously expanding environment, as expansion of mine tunnels (Batzias and Siontorou, 2004a) or leachates plume (Batzias and Siontorou, 2004b); in the latter case, the required monitoring sensitivity increases as the leachates plume grows up, especially if flow takes place towards a water body. By estimating the groundwater flow velocity, via observation wells, we can determine a grid and use as a GIS background for predicted downstream concentration of leachate species (Phase 1); e.g., for a groundwater flow velocity of 4m/year, which has been adopted as best estimate (van Breukelen *et al.*, 2004), a time-step of 0.5 years can be taken, resulting in a 2 m cell-length of the grid. Taking into account the time period elapsed since the start of landfilling, we can estimate the expected spatial/temporal distribution of polluting species by using a non-linear optimisation program like PEST. In this way, the distribution of the monitoring points is determined (even if certain measurements will take place in the lab) along critical flow paths, according to experimental design techniques. Subsequently, the transportation model is checked and certain parameter-values are revised. If, by performing new computer program runs of the model, a different spatial distribution of species concentration is found, indicating that another flow path is most likely the critical, the initiation of Phase 6 is postponed in order to readjust the grids.

5. Case Study for Implementation

The above-mentioned framework is presently implemented at pilot-scale at Thriasio Pedio, Attiki, Greece (Figure 7a). The study area covers 344 sq. km, 120 sq. km of which is forest, and accommodates 46 industrial units, among which some of the largest industries in the country. Sulphur dioxide has been selected as it is one of the main contributors to the area pollution; current levels fall under the *low* to *intermediate* interval (Figure 7b), showing a slightly increasing trend.

Phase 2 has been concluded and the indigenous lichen species retrieved form the database have been proven adequate to provide three grouped patterns to cover the concentration range L3 to LI2. The grouped pattern selected has a re-enforced LI1 interval (fine tunning has been performed), since measurements are mostly concentrated in this area, and include *Ramalina farinacea, Aspicilia calcarea, Arthonia dispersa, Lecanora conizaeoides* and *Lecidela elaeochroma*. The latter, a nitrotolerant species that grows on neutral barks, has been included as control for the high NO_x levels present in the study area; although it tolerates SO_2 up to 80 ppb, its susceptibility increases considerably at low pH, enduring stress levels well below 50 ppb, whereas nitrogenous environments inhibits colonization at large numbers

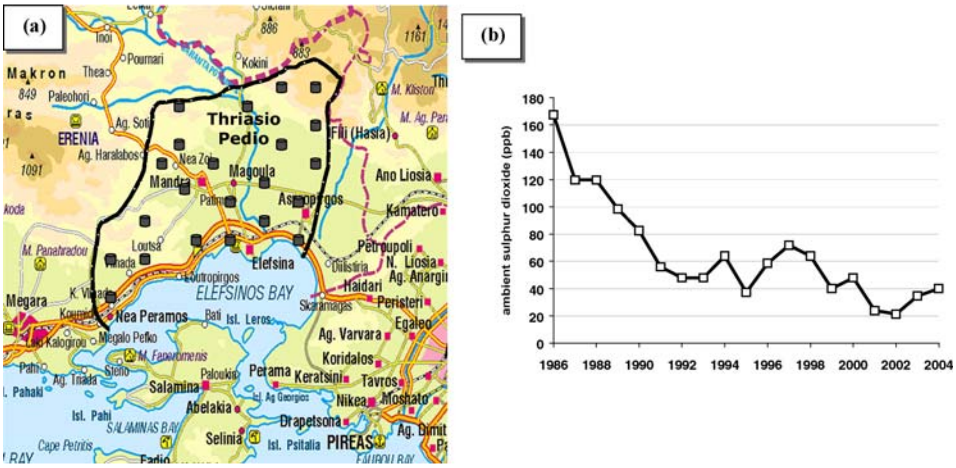


Figure 7. (a) Study area (Thriasio Pedio, Prefecture of Attiki, Greece) with location of monitoring stations (a). The study area covers 344 sq. km, with ca. 120 sq. km of forest, and includes the municipalities of Aspropyrgos, Mandra, Magoula and Nea Peramos with a total population of 77881; in the area 46 industries are operating, among which are 2 oil refineries, 2 steel industries, and 2 cement plants. (b) The course of sulphur dioxide pollution (mean annual levels) for the period 1986–2004; current pollution level falls under the LI1 interval (35–37ppb) with a maximum daily peak of 52 ppb observed in June 2004.

(Van Dobben and Ter Braak, 1998; Herk *et al.*, 2002). Lichen diversity is assessed following standardized procedures, as per sampling design and survey (Asta *et al.*, 2002). The frequency of occurrence of the lichen species comprising the biomonitoring grouped pattern within the surveillance region is used to estimate the degree of environmental stress. The partitioning of the space of frequency of occurrence has been defined by the linguistic terms: *none* (singleton), *rare*, *few*, *moderate*, and *plenty*.

Twenty two monitoring stations are covering the area under surveillance (Figure 7a), nine of which are in the forest area. Measurements are performed with electrochemical and infrared sensors (Phase 3); both types of sensor being roughly equal in popularity. One of the major problems with SO_2 is its extremely corrosive nature and the affinity of sulphur dioxide for water. The presence of any condensate will quickly remove all traces of SO_2 from a measuring system. In addition, there is an absorption line for water very near the line for SO_2; this means that the presence of any water vapour will affect the low readings of sulphur dioxide. The infrared sensor has the advantage of lower cost, but at the expense of greater size. Nevertheless, infrared sensors are one of the preferred methods of measuring SO_2 in the field. The detection method is based on the principle that light of a certain wavelength is absorbed by a particular gas, the amount of absorption proportional to the number of molecules of the gas present in the path of the light. By measurement of this absorption the concentration of the gas can be determined with great accuracy. Currently, the possibility of local production of the sensors is considered and Phase 9 has been initiated.

Another issue that has received considerable attention during the pilot implementation of biomonitoring in Thriasio Pedio, is the cost that such schema entails. The preliminary estimations made for a testing period of two years indicated that (a) the cost associated with lab analyses of the lichens for the verification/quantification of the stress response is significant and (b) the management of the network becomes difficult since field observations require considerable human resources, especially when the monitoring area is not easily accessible, and data/samples transferring for processing should be performed at the shortest possible time. In order to establish a cost-effective program, a suitable management and financial/organization system is necessary; under the scope of the work presented, cost indices have been estimated for the design/development/maintenance of the biomonitoring system per unit surface area of the Thriasio Pedio for the following three financial/organizational schemes: (i) *Hybrid Unit*, subordinate to neighbour local Authorities, University and tertiary education schools, professional chambers and local forest services, (ii) *Independent Organization* dedicated to the management of the specific forest, capable to develop know-how and transfer to similar forestries, and (iii) *Semi-independent node* within a nation-wide network established for the protection/exploitation of forests. As a matter of fact, external economies are descending and scale economies are ascending by moving from (i) to (iii).

Apart from the above, essential to the viability of the program at the micro level is (a) the establishment of field units, in cooperation with the local ranger force, the local users and volunteers and (b) the creation/operation of a laboratory with analytical and bioculture facilities that could respond and cover rapidly the handling, analysis, and manipulation of biological material related to a large number of monitoring areas. Under these conditions, the operation cost of the program, running for the first two years has been estimated to 80% of the cost calculated for conventional metrological systems. As time progresses and experience/knowledge accumulates, costs are expected to drop further to 40–60%.

6. Discussion

It is widely acknowledged that detection, assessment and monitoring of the effects of pollution on the environment require long-term integrated programs that must be ecologically relevant, statistically credible, broadly dispersed, and cost-effective; the viability of such programs relies upon robust design concepts and suitable management frameworks (Bateman and Walbeck, 2004). The methodological framework presented herein considers both evaluation and management tasks supporting decision-making. It permits quantitative and qualitative analyses of the environment in a cost-effective manner, since the main objective is the gradual withdrawal of the analytical instrumentation, and the shifting of the monitoring system to nature.

Most monitoring programs or methodological frameworks reported are *ad hoc* developed, based on the substances to be monitored, the conditions of the area under surveillance, the equipment available and the recourses (Caughlan and Oakley, 2001; Conti and Cecchetti, 2001; Szczepaniak and Biziuk, 2003; Argent, 2004; Guiochon and Beaver, 2004). Limited budget compels most of the times the selection of simplistic approaches as more affordable and acceptable by the authorities that finance the program. The proposed scheme is of a modular structure that will enable decisions to be made regarding costs as instrumental monitoring can be increased/reduced over time according to the performance of the program and the needs arisen and can encourage the local production of ready-to-market/use devices, utilising indigenous materials and taken advantage of scale economies due to large production. Furthermore, the experience obtained and the developed set of field monitors and supporting equipmentation used for engaging and maintaining the monitoring scheme in one area can be used, mostly as is, in starting-up/maintaining a biomonitoring scheme in another area.

It is worthwhile noting that in the time-course, the expected validation cost will decrease 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; eg. Senge (1992) and Bellamy *et al.* (2001), 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 grouped patterns are required, as well as more extensive revalidation in comparison with the periodic one.

Nonetheless, the main objective of the framework is the shift to natural monitoring with minimal human intervention. The design/development of an environmental ontology of the monitoring organisms, as the lichen ontology presented, can provide a useful tool for an environmental network allowing the monitoring of a variety of pollutants over time and space and the assessment of environmental quality. It is the first time in literature, that the scattered, disorganized and inconsistent information on pollution toxicity on lichens is organized, referenced and cross-referenced with *ascomycete* systematics and substrata chemistry, considering lichens as quantitative 'measuring systems' requiring validation, calibration, standardization, and quality assurance. The collection of the available information and the 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 geomorphologic characteristics. Such an ontology affords indicator responses to become understandable, links biomonitoring to policymaking and displays a significant utility to cost-benefit assessments. In that context, biomonitoring can support management decisions or quantify the success of past decisions.

Furthermore, statistical indices have been introduced to account for both, bioindicator-species richness and the evenness with which individuals are distributed along pollutant levels. By doing so, reliable/representative statistic parameters are obtained as quantified criteria for biomonitoring capability, which can play a significant role in the hierarchical criteria choice of the optimal grouped pattern in as much as these criteria may reflect also an impact of pollution on the population of the species comprising the group: in the time course, the value of the parameter may decrease, decreasing at the same time its significance as a criterion, while increasing its importance as part of the suffering ecosystem.

The schema proposed by the authors, based on the development of a knowledge base on environmental exposure, improves the concept of biomonitoring considerably, conferring reliability and quality assurance to biomarkers, as: (1) environmental fate, bioavailability and interactions of contaminants in the environment can be taken into account; (2) a time-integrated estimate of environmental levels of contaminants (or stress) can be provided, thus reducing, in time, the need and cost for instrumental measurements; (3) exposure and adverse effects can be related to contaminants, thus providing a mechanistic understanding of effects and allowing the establishment of causal relationships; and most importantly, (4) the measured biological effects can be more meaningfully linked to environmental consequences so that environmental concerns can be directly addressed.

The linking of the GIS-supported framework to knowledge bases, as the lichen ontology developed by the authors, enhances the semantic interoperability of the latter, since ontologies seem to be an adequate methodology that helps to define a common ground between different information communities (Seder et al., 2000; Irving and Moncrieff, 2004; Saunders *et al.*, 2005). This paper describes an example of biosurveillance and an ontology that serves as the basis for carrying out such schemes. It is focused on the way ontologies can support environmental monitoring and how the provision and (re)use of ontologies can aid decision making and cost-effectiveness. Access through the Internet could enable users to construct ‘common ontologies’ across GIS boundaries; the increased need for mobile interoperable tools makes the ontology-based approach valuable for field GIS.

The success of environmental monitoring programmes significantly depends upon the proper selection of monitors and monitoring sites so that measurements could be considered as representative of the whole area. Representativeness is defined as a typical area (ecogeographical unit) that has the necessary characteristics permitting the assessment of the adopted model. In order to ensure spatial and temporal representativeness of measurements, continuous monitoring on a long-term basis of a large number of sites is required. Conventional methodology is rendered inappropriate due to high instrumental cost. The implementation of biomonitors, however, adequately controlled and validated by parallel abiotic control, allows the operation of such schemes, especially when considered on a volunteer-basis and exploits local resources.

The lichen selection program presented herein is based on two priorities: (a) the identification of species that respond to a given ambient pollutant in a way that qualitative information (eg. frequency of occurrence) would assume a quantitative character, and (b) the assembly of a group of local monitors, the response pattern of which (eg. the presence of one species and the absence of another) would reliably provide the ambient concentration of the pollutant. This approach prerequisites the existence of adequate monitors, regarding species and numbers, in the area, which may not always be possible. The biomonitoring capacity indices presented can provide an estimation of the biomonitoring value of a region determining the availability of the monitors. In the lack of exhaustive biological data this leads inevitably to compromises: manage uncertainty versus bear the high cost of laboratory analyses. It, also, stresses out the notion of representativity, where even a species poor site has a high biomonitoring value if it comprises of species with a varying degree of tolerability so that their overall status at a given time can reliably reflect the level of the pollutant.

Another issue to be addressed when biosurveillance networks are proposed on a long-term basis is the cost that such schema entails (Caughlan and Oakley, 2001). The preliminary estimations made for the small-scale testing presented above indicated that (a) the cost associated with lab analyses of the lichens for the verification/quantification of the stress response is considerable and (b) the management of a decentralized network becomes difficult since field observations require considerable human resources, especially when the monitoring area is not easily accessible, and data/samples transferring for processing should be performed at the shortest possible time.

The concept of integrated biosurveillance to detect indications and warnings for a possible peak event is also covered by the proposed methodology. Current biosurveillance systems fail to provide pre-event predictive information and are stage-piped across multi-sector domains. The integration of multiple data sources (a variety of organisms and a variety of biosensors) into a LAN offers the potential for better predictive ability and greater lead-time warning than that provided by separate stand-alone monitoring systems, not only in the area under consideration but also in neighbouring areas that might be affected from transportation phenomena. The value of the proposed integrated biosurveillance will be realized when lead-time for response planning is expanded by providing pre-event alerts that enable priming of the response system. The LAN, as part of a WAN and through appropriate transportation/dispersion models, can provide the basis of or trigger rectifying/prevention measures that cover a wide range in short time. Comparison of multiple data sources suggestive of a bio-event may also decrease the number of false positive alerts. Holistic integration of the proposed methodology to address impacts on plants, animals and humans across foreign and domestic domains is critical to further develop this approach.

7. Conclusions

With environmental concerns escalating, environmental planning and monitoring programmes are increasingly being promoted globally as means for assessing environmental quality. This paper has sought to overcome some of the significant challenges to validation and evaluation in a complex monitoring system, such as biomonitoring, and to the management of information collected from the sites of intensive monitoring. The bioindicator ontology of environmental exposure presented links pollution to morphological (surface) response and biochemical (mechanismic) alterations, also providing a useful tool for species selection to fit a robust and reliable biomonitoring system. Based on this procedure, a computer program has been designed/developed as a decision support system and has been successfully tested on a representative population of species indigenous to six countries.

Significantly, a novel system (scheme) in the form of a rational framework at the conceptual design level has been developed to guide improvements in such initiatives, that actually contributes towards achieving a cost-effective long-term monitoring program, with the flexibility to counter on-course any (anticipated or not) variations/modifications of the surveillance environment. A pilot-scale implementation has thereby proven that the combination of permanent biomonitors with periodic sensors (which can be produced locally utilising indigenous materials) becomes a strategy for saving resources; further to minimizing capital costs and maintenance costs, this combination creates a local network that apart from contributing to environmental surveillance it can also monitor the growth rates of native vegetation.

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