Biodiversity indices for the assessment of air, water and soil quality of the "Biodiversity Friend" certification in temperate areas

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ABSTRACT
"Biodiversity Friend" is a standard certification developed in 2010 by World Biodiversity Association to evaluate the biodiversity and promote its conservation in agriculture. The procedure to obtain the certification considers the environmental impacts of the agricultural activities on the agrosystem and the biodiversity and suggests operational strategies to improve the environmental quality of the agriculture areas. The evaluation is referred to 12 actions related to low-impact methods of pest and weed control, reconstitution of soil fertility, rational management of water resources, diffusion of hedges, woodlands and nectariferous plants, conservation of agricultural biodiversity, soil, air and freshwater quality through Biodiversity Indices, use of renewable sources for energy supply, lower CO_2 production and CO_2 storage and other actions that may have beneficial effects on biodiversity.
The environmental conditions of the agrosystem are evaluated by biomonitoring of air, water and soil. The biodiversity of soil and aquatic macroinvertebrates and the biodiversity of epiphytic lichen communities decrease very quickly when the soil, water and air conditions are altered by different causes such as pollution, synthetic and organic pesticides, bad land use practices, etc. The protocol of the three indices of the standard certification "Biodiversity Friend": Lichen Biodiversity Index (LBI-bf), Freshwater Biodiversity Index (FBI-bf), and Soil Biodiversity Index (SBI-bf) are here presented in detail.

KEY WORDS biodiversity; bioindicators; pollution; certification; agrosystem.

Received 28.02.2014; accepted 14.03.2014; printed 30.03.2014

INTRODUCTION

Up to date, on the Earth about two million species have been recorded (Fontaine et al., 2012), but the naturalists estimate that the total number of species is at least 8.7 million (Mora et al., 2011), three-quarters of them concentrated in the tropical rain-forests. So, we know only about one fourth of plant and animal species on our planet. Zoologists and botanists describe about 17,000 new species every year (Fontaine et al., 2012), but the destruction of tropical rainforests at a rate of several ten thousands sq km a year (Skole & Tucker, 1993; Katzman & Cale, 1990) determines the extinction of thousands

of species annually; therefore, the loss of biodiversity is one of the most important environmental emergencies today.

The recognition of such an emergency has led 150 countries to sign, at the Rio de Janeiro Earth Summit in 1992, the "Convention on Biological Diversity". With the aim of promoting sustainable development, the Convention recognizes that the protection of biodiversity is not concerned only to living organisms and their ecosystems, but it involves and affects the whole human community and its basic needs (the right to food, health, air, water and soil quality). Despite the Convention's member countries have met regularly to establish actions and strategies, the rate of biodiversity loss increased continuously. The minimum target set in the 6th Conference in Johannesburg on 2002, has been fixed in a meaningful reduction of the current rate of biodiversity loss at global, regional and national levels, within 2010 (Decision 6/26). Unfortunately, unsustainable patterns of production and consumption, lack of education and awareness about this problem at any level did not allow to get significant results: the rate of biodiversity loss has not been reduced; on the contrary, the destruction of rainforests is proceeding very quickly every day.

From a long time, the European Community recognized the conservation of biodiversity as a key objective of the strategy for sustainable development (Convention on Biological Diversity, 1992). The preservation of biodiversity is closely connected with other environmental emergencies, such as climate change and resources' availability, about which in the coming decades the fate of the entire human community will be played.

Biodiversity as a resource. Most people have a romantic vision of biological diversity, mainly linked to emotional and aesthetic criteria. Even though few people recognize its value, biodiversity is the most important resource of natural systems in the Earth. Therefore, its conservation is functional to real preservation of ecosystems, from which depend, directly or indirectly, all human activities. In essence, we can say that every living species is a potential resource, an option for the future, on the contrary every extinct species is a missed opportunity.

Today, at global level, the destruction and fragmentation of habitats, pollution, climate change, irrational exploitation of resources, human popula-

tion growth and spread of alien species are the main threats to biodiversity (Convention on Biological Diversity, 1992).

Biodiversity is a fundamental resource for human beings, such as energy and water resources. The maintenance of high biodiversity in the environment must be an overriding objective for production activities, especially in the primary sector. The agrosystem can be considered as a man-controlled environment in which the coexistence of vegetal and animal species is not characterized by stable relationships between them; therefore it can not be considered a true ecosystem. However, it represents the best possible solution to assure environmental quality and food production. A modern farmer has to face the problem of how to encourage biodiversity in its farm and to manage the effects of a possible reduction since it was established the close relationship between the biological quality of the environment and the quality of products. The use of "good agricultural practices" to ensure conservation of soil fertility, correct water management, weed and pest control through environmentally friendly methods contribute to the maintenance of biodiversity in the agrosystems. Other actions such as the increase of hedgerows, wolds, wooded areas and nectar species, the leaving of necromasses and the use of multi-year rotations, increase biodiversity in the agrosystems, at the same time improving the quality of air, water and soil (Lowrance et al., 1986).

Supporting biodiversity in agrosystems. In this changing world, we are facing a strategic challenge for the future of the planet: to ensure, in terms of sustainability, the productivity of economic systems and the preservation of natural resources.

World Biodiversity Association, a non-profit organization since its foundation October 4, 2004 at the Museo Civico di Storia Naturale di Verona, has been engaged in studying and conserving biodiversity hot spots, in Italy and worldwide.

In the matter of environmental responsibility, World Biodiversity Association is moving for a long time to promote among the companies a greater consciousness of their role into the field of conservation and the sensitization of their clients to sustainability.

With the support of a team of naturalists, agronomists, foresters, and its International Scientific

Committee, WBA developed in 2010, a certification that, starting from the assumption of reducing the biodiversity losses in the cultivated areas, encourages farmers to increase biological complexity of the agrosystem, towards a real sustainability and quality of the crops. The new certification, named "Biodiversity Friend" (BF) is not merely confined to certify the engagement of the farm to a significant reduction of the biodiversity loss, but represents an incentive for the farm towards a progressive increase of biological diversity, that ultimately coincides with an improvement of the health and quality of the products. BF certifies that the production processes do not involve loss of biodiversity, and the certified company is constantly committed to improve the quality of the environment in which it operates.

The Biodiversity Friend standard. The Biodiversity Friend (BF) protocol considers the environmental impacts of the agricultural activities on the ecosystem quality and biodiversity. BF has the objective of defining a complete picture of the interactions of a product or service with the biological diversity of the territory. Moreover, the new protocol suggests operational strategies to improve the environmental quality, with the aim to reduce the impacts of the agricultural activities on agrosystems and their biodiversity.

Operative strategies are defined in 12 actions which are related to:

1) low-impact methods of pest and weed control (organic or integrated production)
2) low-impact methods for the reconstitution of soil fertility
3) rational management of water resources
4) presence of hedges, woodlands and dry stone walls/terraces
5) abundance of nectariferous plants
6) conservation of agricultural biodiversity
7) soil quality through the Soil Biodiversity Index
8) freshwater quality through the Freshwater Biodiversity Index
9) air quality through the Lichen Biodiversity Index
10) use of renewable sources for energy supply
11) moderate CO_2 production, CO_2 storage and low-impact manufacturing techniques
12) other actions that may have beneficial effects on biodiversity.

Each action corresponds with a score. The commissioner must obtain a minimum score of 60 out of 100 to be certified. To maintain the certification the commissioner must increase the biodiversity every year through effective actions that can be suggested by the evaluators and verified in the annual controls. When the farm get a score of 80 out of 100, no other improvement is requested (Caoduro & Giachino, 2012).

Since 2010 to the present day about 50 organic and integrated production farms have been certified "Biodiversity Friend". Many of them already placed on the market their products with the brand "Biodiversity Friend", to show the consumers their engagement in biodiversity conservation. In 2010 "Biodiversity Friend" obtained the patronage of the Ministry of Agricultural, Food and Forestry Policies of Italy. The brand "Biodiversity Friend" is exclusive property of the WBA and has been registered as an international trademark in Italy, European Union, China and U.S.A.

The Biodiversity Friend environmental quality assessment

The actions related to the environmental conditions of the agrosystem have a very high importance for the BF certification. They concern the assessment of the quality of the air, water and soil by using synthetic biomonitoring procedures based on methods recognized by scientific community. In the years 2009 and 2010 a group of WBA naturalists coordinated by Dr. Gianfranco Caoduro, under the supervision of the WBA Scientific Committee, developed different procedures for evaluating the complexity, in terms of biodiversity, of the soil and freshwater communities of temperate agricultural areas. In the same way, the Lichen Biodiversity Index (LBI), the most frequently used procedure to assess atmospheric pollution using bioindicators, has been modified to allow an easier application of the method. The operation allowed to identify three different procedures of the "Biodiversity Friend" protocol for the assessment of the quality of air, water and soil based on biodiversity indices. The biodiversity of soil and aquatic macroinvertebrates and the biodiversity of epiphytic lichen communities decrease very quickly when the soil, water and air conditions are altered by natural or anthropic causes such as pollution, synthetic and organic pesticides, bad land use practices, etc.

MATERIAL AND METHODS

The three indices of the standard certification "Biodiversity Friend" for temperate areas of North Hemisphere are represented by: Lichen Biodiversity Index (LBI-bf), Freshwater Biodiversity Index (FBI-bf), and Soil Biodiversity Index (SBI-bf).

THE LICHEN BIODIVERSITY INDEX OF BIODIVERSITY FRIEND (LBI-BF)

Lichens and air pollution in agriculture. Frequently air pollution is considered a problem related to industrialized and urban areas. However, in the last decades the impacts of agriculture on air quality has been recognized. Air pollutants like pesticides and ammonia substances can have negative effects also on freshwater, groundwater and soil (National Research Council, 2009). Many authors showed that air pollutants produced by agricultural activities have a reliable impact on epiphytic lichens (Alstrup, 1991; Brown, 1992; Loppi, 2003; Carrera & Carreras, 2011). Lichens are generally considered to be good indicators of air quality: altered composition of atmospheric gases is reflected in changes in epiphytic lichen communities. The sensitivity of lichens is particularly relevant to fungicides, but herbicides and insecticides also have an important impact on them. In particular, lichen species richness was demonstrated to be negatively influenced by the frequency of pesticide treatments (Bartok, 1999).

Lichen as bioindicators. Lichens are organisms formed by a symbiosis between a fungus and an alga. To date, more than 14,000 species of lichens have been described by lichenologists. Lichens can give excellent indications on the level of environmental alteration because their metabolism depends strictly by the air quality. The characteristics that make lichens excellent bioindicators of the air quality, both in urban and in rural areas, are: a) high capacity of absorption and accumulation of substances absorbed from the atmosphere; b) resistance to environmental stress; c) impossibility to get rid of the polluted parts; d) longevity and slow growth; e) high sensitivity to the pollutants.

In the evaluation of the air quality lichens can be used as bioindicators and bioaccumulators.

Frequently, a decrease in the number of lichen species is recorded together with a reduction of the number of specimens of each species. While morphological and physiological alterations are difficult to evaluate, the ecological variations allow to convert the lichen reactions into numeric values, related to different levels of air pollution. Generally, nearing the pollution sources, there is a progressive deterioration in lichen's health condition.

The first studies on lichen sensitivity to air pollution date back to the XIX century, but only since some decades they are used in large-scale biomonitoring. Recently many methods based on appropriate interpretation levels have been proposed. The most used procedure calculates the Lichen Biodiversity Index (LBI) based on the state of the lichen diversity in standard conditions, after a long exposition to atmospheric pollution and/or other kinds of environmental stress; the lichens considered for the index calculation are, essentially, the epiphytic ones. Specific indications on the sampling system and survey procedures of the lichen biodiversity are available on the Manual for the application of the index, published by ANPA (ANPA, 2001).

A synthetic method to evaluate the air quality of the rural areas is the use of the lichens as biosensors of phytotoxic gases (Nimis, 1999). The epiphytic lichen biodiversity is an excellent indicator of the pollution produced by air pollutants. By means of this approach it is possible to correlate different levels of environmental alteration to variations of the external aspect of the covering and floristic richness of the lichen communities. A phytotoxic agent, at determined concentrations, can cause the death of the lichens sensitive to it. As the sensitivity to the pollutants is related to the morphology of the lichen tallus, to its ecological, physiological and structural characteristics, the disappearance of the lichens from a polluted area is not simultaneous, but deferred in time: first the more sensitive species die and then the more resistant ones. Therefore, the floristic composition becomes an indirect measure of the concentration of pollutants in a certain place.

Lichens answer with a relative velocity to alterations of the air quality, but they can recolonize in few years industrial and urban environments if air quality conditions improve, as many European countries revealed. The studies of air quality through lichens found a large diffusion in Italy starting from the eighty years, at the same time with the resumption of the interests for the lichenological studies.

Many investigations were realized both in urban and in rural areas, in natural protected areas and in areas where the human activities are particularly intense.

The methodology adopted in Italy starting from the beginning of the 2000 years is indicated as "ANPA Method" (ANPA, 2001). This approach minimizes the subjective elements of the guide lines previously proposed in Italy and Germany, giving specific attention to the selection of the sampling sites, of the trees to be monitored and the position of the sampling grid.

This method estimates the state of the lichen biodiversity in standard conditions after a long exposition to air pollutants and/or other kinds of environmental stresses. It is important to specify that lichens considered in evaluation of biodiversity are essentially the epiphytic ones; this allows to limit the variability of the ecological parameters unrelated with pollution, such as base content or water capacity, very changeable in the lithic substrates.

The Lichen Biodiversity Index of "Biodiversity Friend"

According to the complexity of the ANPA method, which can be performed only by an expert lichenologist, Biodiversity Friend uses a simplified application of it, allowing to use the procedure also by non specialists. In the application of the "Biodiversity Friend" method the taxonomic identification of the lichen species is not necessary; the operator is required only to distinguish the major morphological differences among the species of the lichen community. The operator, therefore, identifies the "Species A", from the "Species B", from the "Species C" and so on. All other operations correspond exactly to the ones used by the ANPA Method. The use of the traditional sampling grid allows the calculation of a numerical index based on lichen diversity and on the frequency of the various species, through which it is possible to define the alteration level of the lichen community. The density of the sampling sites is calculated in relation to the extension of the total farm surface, as described in Table 1.

Each sample is formed by three trees (phorophyta) with the characteristics required by the protocol. The site must be located inside the farm lands, preferably in the central area. The operator must choose the three trees nearest to the farm center. If in the farm there are not trees suitable to be

Total Farm Surface	Number of samples
≤ 20 ha	One sample
20-200 ha	1 + (total surface – 50)/50 The result must be rounded to the inferior integer number
≥ 200 ha	3 + (total surface – 200)/100 The result must be rounded to the inferior integer number

Table 1. Number of air quality sampling sites in relation to farm surface.

sampled the operator must search other trees in the peripheral zones. The geographic coordinates of the site must be reported on the sample form, together with a synthetic map with the location of the trees to make their finding easier in the following surveys. If the total farm surface is larger than 20 hectares and it is necessary to locate more than one site, these must be located at least at 150 m of distance among them. About the selection of the tree species, two groups can be distinguished according to the pH of the bark, as in Table 2.

Species with subneutral bark	Species with acid bark (to be preferred)
Acer pseudoplatanus	*Prunus domestica*
Acer platanoides	*Olea europaea*
Ceratonia siliqua	*Quercus petraea*
Ficus sp.	*Alnus glutinosa*
Fraxinus excelsior	*Castanea sativa*
Fraxinus ornus	*Quercus pubescens*
Juglans sp.	*Quercus cerris*
Populus x *canadensis*	*Betula pendula*
Sambucus nigra	*Prunus avium*
Ulmus sp.	*Tilia* sp.

Table 2. Tree species that can be used in biomonitoring of air quality by the LBI-bf.

For the biomonitoring the trees with a bark easily exfoliable (e.g. *Aesculus*, *Platanus*) must be excluded; the use of *Sambucus* and *Robinia* is not recommended for the high water tolerance of their bark. *Celtis australis* and *Populus alba* are not recommended because they maintain for a long time a smooth bark, poorly colonizable by lichens; *Fagus* is suggested only in mountain areas. Samples based on trees of different groups are not directly comparable. Only one tree species is to be used. When this is not possible, it is best to use another species of the same group. It is preferable to use species with acid bark, in particular, trees of the genus *Tilia* (Table 2). The sample trees must have the following characteristics: 1) the inclination of the trunk must not exceed 10° to avoid effects due to the excessive eutrophication of inclined surfaces; 2) circumference larger than 60 cm to avoid situations with pioneer lichens; 3) absence on the bark of evident factors of disturbance or pathologies.

The presence and frequency of the lichen species on the bark are detected by means of a sampling grid formed by a vertical ladder of 10x50 cm, divided in five subunities of 10x10 cm; the ladder must be applied to each of the four cardinal points, with the base at about 100 cm from the ground level. To exclude from the sample any unfit part of the trunk, a rotation up to 20° clockwise can be allowed.

Even if the lichen cover is high, the positioning of the grid in each cardinal point must avoid: decorticated or damaged portions of the trunk, portions with evident knots, portions corresponding to rainwater tracks, portions covered with more than 25% by bryophytes (however, also muscicolous lichens must be considered in the calculation, if they are present).

To allow the repetition of the survey, for every tree in the survey form must be noted: a) the exact location of the tree, using a geo-referenced system or a detailed map; b) the exact exposure (in degree) of each grid position; c) the height, from the ground level, of the grid base; d) circumference of the trunk in the middle of the grid.

All the lichen species present in each subunit must be recorded together with their frequency, calculated as number of squares in which each species is present (the frequency values of each species, therefore, vary from 0 to 5); if the same specimen of a certain species is present in more than one square, its frequency is equivalent to the number of squares in which it is present. The removal and damage of the lichens inside the grid area must be avoided to permit the repetition of the sample. Considering that the identification at specific level of each species can be difficult for a non-lichenologist operator, on the survey form is sufficient to determine the diversity of epiphytic lichens present on the tree specimen, by noting on the form: "Species A", "Species B", "Species C", etc., making sure that they are not damaged or underdeveloped specimens of species already present in the grid. In case of doubts in identifying a species, the operator can use the magnifying glass to confront at microscopic level the different morphologies and the camera for macro photography for a following identification. The value of lichen biodiversity of each sampled tree is obtained summarizing the frequencies recorded in each unit.

Calculation of the Biodiversity Lichen Index

The Biodiversity Lichen Index of the site is statistically determined on the basis of the values collected during the survey. The first step is to summarize the frequencies of the species recorded on each tree. As it is predictable a substantial growth difference among the sides of the trunk, the frequencies must be noted separately for each cardinal point. In this way, for each tree will be obtained four sums of frequencies (BLjN, BLjE, BLjS, BLjW). In each site the following operations must be realized:

1) for each tree the frequencies of all the lichen species detected are summed (in this way we have the biodiversity related to the single phorophyta);

2) all the frequencies gathered on each tree are summed and the total is divided by three (the number of phorophyta). In this way we obtain the Lichen Biodiversity Index of the site (LBI);

The Lichen Biodiversity Index of the site must be superior or equal to 45. In case of surveys to make in more sites (farms with total surface larger than 40 hectares), the total Lichen Biodiversity Index emerges by the sum of the indices of all sites, divided by the total number of sites. The ratio must be 45 or more, for an acceptable air quality.

Classes of lichen biodiversity

Generally, seven classes of Lichen Biodiversity are used, corresponding to the same number of air

quality levels. The reference scale under reported is the one calibrated for the Padan-Adriatic biogeographical area. For different areas a re-calibration of the classes is necessary.

- *Value of L.B. equal to 0*: corresponds to the so called "lichen desert", and therefore to a situation of very high alteration of the lichen community, corresponding to the worst level of air quality (very poor air quality).

- *Values of L.B. between 1 and 15*: are referred to zones with a high level of alteration of the lichen community. These zones have a very scarce air quality.

- *Values of L.B. between 15 and 30*: correspond to situations of medium alteration of the lichen communities. These zones have a scarce air quality.

- *Values of L.B. between 30 and 45*: are referred to zones with a low alteration level of the lichen communities and a low air quality.

- *Values of L.B. between 45 and 60*: are referred to zones with a medium level of naturalness of the lichen communities. In these areas the air quality is moderately good.

- *Values of L.B. between 60 and 75*: in these zones the lichen communities have a high level of naturalness. The air quality in these areas is good.

- *Value of L.B. more than 75*: in these zones the lichen communities have a very high level of naturalness. The air quality in these areas is very good.

According to the "Biodiversity Friend" procedure, the conformity to the action is reached by a value of L.B. equal or greater than 45, corresponding to an air quality quite good, good or very good, on the basis of the calibrated scale of the Padan-Adriatic biogeographic area (ANPA, 2001).

The survey can be performed during all the year.

Before starting the survey, the operator must have the following material:

- handbooks with epiphytic lichens identification keys
- survey form for LBI-bf
- Global Positioning System
- magnifying-glass (at least 10x)
- digital camera for macro-photos
- sampling grid formed by a vertical grid of 10x50 cm
- compass
- measuring tape (at least 3 m)

THE FRESHWATER BIODIVERSITY INDEX OF BIODIVERSITY FRIEND (FBI-BF)

There are several ways to make an environmental quality analysis of the freshwater and each of them can point out different aspects and critical points. It is possible to divide these methodologies in two main groups: the direct approaches, related to the physical-chemical analyses, and the indirect ones, represented by the biotic indices. Generally, the physical-chemical monitoring can be very detailed but it is related to simple problems and reveal single criticalities in a punctiform way. Chemical analysis targets only specific substances and it may miss intermittent or periodic pollutants, or substances outside the range of the analysis.

To analyze complex systems as the ecological net of a river or a stream, the biotic indices can be more suitable. The biomonitoring of the organisms living in waterways can reveal the effects of pollutants not detected by chemical analysis, as well recorded in modern literature since the proposal of the Beck's biotic index (Beck, 1955). The strategy of the biotic indices is based on the identification of macroinvertebrates, the sensitivity of which to water quality is well known; for this reason they are defined bioindicators. The community of the benthic macroinvertebrates in a water body is particularly adapted to be used as a source of bioindicators because it is easy to investigate, it is abundant and generally always available and it has moderate seasonal variations. Monitoring the animals that live in water bodies can reveal the effects of pollution not detected by chemical monitoring. For this reason the biotic indices had a very important role in the wide-ranging environmental analysis of the last half of the last century, till their recognition and standardization in the national and European regulations related to the monitoring and classification of the water bodies (Directive 2000/60/EC).

However, in the last years the standardized model of the Extended Biotic Index (Woodiwiss, 1964; 1978) has been improved by adding significant contributions. The E.B.I. works well if it is applied in well known areas, where the tolerance parameters of the single species are known, and if the investigation has a high detail from a taxonomic point of view, with the involvement of specialists in macroinvertebrates and hydrobiology. To bypass this methodological limit, and at the

same time to increase the systemic complexity of the analysis to the study of bioindicators, some authors suggested to reduce the taxonomic resolution, rewarding it with a more accurate description of the ecosystem and its functionality, using the "River Continuum Concept" (Vannote et al., 1980; Siligardi et al., 2007; or the Italian SEL in the D.M.391/2003).

As a further evolution of the biotic index methodologies, the Freshwater Biodiversity Index of "Biodiversity Friend" is proposed to evaluate the suitability of a water environment to host a rich biodiversity. This protocol adapts common used assessing methods to evaluate biodiversity in freshwater environments, detecting the diversification and stability of the biotic communities (Klemm et al., 1990; Rosenberg et al., 1997), relating them to the river continuum and to the functional parts of the hydromorphology.

Determination of the FBI-bf. An environment suitable to host many kinds of organisms should be primarily heterogeneous, with different survival strategies. Therefore, a general classification of the entire ecosystem functional to the water course, conditioning its dynamics, is necessary. The operator has to fill out a survey form in which different morphological and ecological parameters are listed. If the water body presents significantly diversified ecological conditions, the operator must fill out a different form for each riparian zone; the final score will be obtained as the mean of the final values obtained for each zone considered.

Hydro-morphological Assessment

Width. The width of a water body is very important considering that the most food sources of the refuge and reproduction sites of the aquatic fauna are located near the banks. The width of the bed must be evaluated in normal water conditions; the bed of the stream includes the part occupied by the water and a riparian strip lacking of vegetation, trees and shrubs that can not survive in conditions of frequent submersion and erosion of the substrate caused by high floods.

During periods of low floods, a part of the bed can be colonized by pioneer herbaceous vegetation. Therefore, the operator must observe carefully the banks to locate the real width of the water body. The width will be evaluated transversally, from the extreme margins of the bed, in normal water conditions.

If the banks and the bed are completely overbuilt or if the flows are regulated, involving the drainage of the water body for more than three months in a year, or the bed is dredged more than twice a year, the water body must be considered "artificial".

Fluvial morphology. Dikes and canalization and flood-relief works artificially modify the water bodies to have as less impact as possible on the human activities, to prevent overflows and bank erosion. In many agricultural areas is very difficult to keep rivers in their natural conditions, especially in Europe where anthropization and urbanization are widely spread (U.N., 2012). On the contrary, a strong artificial management leads to an homogenization of the fluvial structure, reducing the capacity of the water bodies to support complex biotic communities. A compromise is, however, possible.

A straight channel, completely artificial, with overbuilt banks offers very few food sources and refuge sites; it will be colonized, in the best case, only by few and very resistant organisms. A more sinuous and irregular course with natural banks, at least in some reaches, on the contrary, can transform radically a little agricultural channel in a wetland of great interest for the freshwater flora and fauna.

Hydrological regime. Water flow variations are natural and related to seasonality; they can support the alternation of different host species and increase aquatic biodiversity. A constant natural flow, determined by a well-structured hydrological network, on the other hand, even guaranteeing more stability and continuity to some species, can reduce the possible strategies.

Alterations to the natural hydrological regime such as water withdrawals for agricultural, hydroelectric or civilian uses can influence significantly the functionality of the water body, causing temporary shallows incompatible with the life cycles of many organisms; also the artificial irrigation ditches can be considered in this category (Bunn & Arthington, 2002; Ferrington & Sealock, 2005).

The flow variations must not be evaluated by the size of the wetting bed at the moment of the survey, but they must be deduced from the extension and complexity of the perifluvial vegetation and, even-

tually, by information given by other sources of monitoring (e.g. Literature, recording stations, etc.).

Riparian vegetation. The perifluvial vegetation, besides conditioning the position and extension of the shaded areas, influences the riparian morphology by creating niches and sites adapted to host the aquatic fauna and produces the most of its food sources. If, in absence of riparian vegetation, only few particularly resistant species can survive, every increase in terms of diversity and complexity of the riparian communities will be followed by an increase of the aquatic animal species. Compiling the survey form, different categories can be added together, if they are present (e.g. trees, shrubs and herbs). Only hygrophilic and riparian species can be considered in the survey; exotic species and not riparian herbaceous vegetation must not be considered.

Taxonomic diversity and pollution tolerance

After the hydro-morphological assessment of the water body has been surveyed, the operator evaluates the diversity of the aquatic biocenosis by a direct sampling. The "Biodiversity Friend" procedure does not consider the species as in the classic taxonomy but as morphotypes, as a compromise between a simple evaluation suitable for non-taxononomists and an accurate quantitative evaluation of species diversity.

The morphotypes are here considered as groups of organisms which at macroscopic level are characterized by similar shapes. It is not important to define the taxonomic level: e.g. a sample of two species of Plecoptera, one species of Amphipoda and three different genera of Mollusca corresponds to six morphotypes.

However, the identification of a morphotype needs a good knowledge of the aquatic fauna, considering that many individuals of different species can look identical to an untrained eye. An adequate training, even if not at specialistic level, will be necessary to recognize differences in the number of appendixes, the different form or position of bristles or hooks and so on.

The number of the morphotypes gives a direct evaluation of the biodiversity richness and complexity of the communities. The dominance of few morphs indicates a scarce species richness, the heterogeneity of the morphs, indicates good species richness. If an healthy aquatic environment can host a rich variety of organisms, the presence of a pollutant can limit this condition. Each species, according to scientific literature (e.g. Mandaville, 2002; see Table 3), has a certain tolerance to pollution, but it is possible to identify a predisposition to tolerance also at a higher taxonomic level, obviously arriving at some detail compromises which are considered acceptable by many authors (Olsgard et al., 1997). If it is not infrequent to find tolerant invertebrates in low polluted sites, the opposite is not true.

Therefore, the presence of at least two bioindicators belonging to groups particularly sensitive to pollution is here considered as a significant indication to evaluate the minimum quality of the aquatic environment. In the survey form the two lower values are identified to define the pollution tolerance, corresponding to the rounded down mean of these two values.

Survey: materials and methods

Before the biological survey, the monitoring procedure of the FBI-bf provides also the analysis of the main physico-chemical parameters of the freshwater measured by portable instruments. In particular must be surveyed and reported on the FBI-bf form the following parameters: temperature, pH, electric conductivity and dissolved oxygen. These additional information can be useful to understand the reason for eventual discrepancies between an apparently good environment and a rich variety of organisms and suggest the commissioner effective action to reduce the pollution.

Sampling of water macroinvertebrates is performed with a collecting-net for aquatic invertebrates (grid 500 μm), according to the procedure proposed by the British Standards Institute (ISO 10870: 2012). In some circumstances the identification of aquatic invertebrates is possible also from the bank, investigating the lower surface of rocks and rubbles. Before sampling with the collecting-net, the operator must verify the activity of surface insects, collect by hands the stones and submerged wood of the bottom for at least two minutes. All the groups of macroinvertebrates observed during these surveys will be reported on the FBI-bf form. The sample with the collecting-net must begin from the most downstream point of the water body, proceed-

MACROGROUPS	TROPHIC GROUP	POLLUTION TOLERANCE
Plecoptera	Shredders/Grazers/Predators	2
Ephemeroptera	Collectors	3
Tricoptera	Collectors/Grazers/Shredders	4
Megaloptera	Predators	4
Platyhelminthes	Collectors	4
Coleoptera (larvae)	Predators/Grazers/Shredders/Collectors	4
Heteroptera	Predators	5
Odonata Anisoptera	Predators	5
Odonata Zygoptera	Predators	8
Arachnida Hydracarina	Predators	6
Diptera (larvae)	Collectors/Grazers/Predators/Shredders	6
Crustacea Amphipoda	Collectors	5
Crustacea Decapoda	Collectors/Grazers	6
Crustacea Isopoda	Collectors	8
Mollusca	Collectors/Grazers	7
Oligochaeta	Predators	7
Hirudinea	Predators/Collectors	9
Nematoda/Nematomorpha	Predators	8

Table 3. Trophic characteristics and synthetic index of pollution tolerance (from Mandaville, 2002 modified) of the most common types of freshwater macroinvertebrates.

ing upstream; in this way the aquatic environment is not disturbed before the sampling. The collecting-net must be placed against the flow; the operator's feet and contemporarily the aquatic net can be used in deeper water bodies to move the ground debris and drive out burrowers and climbers. In these conditions the net must be held vertically, in opposition to the water flow, downstream the operator's feet.

After 3-4 minutes of sampling, the material collected by the net is put into a little white tank and the operator will begin the identification of the invertebrates morphotypes, with the aid of a magnifying glass. In case of uncertain identification, small size invertebrates can be collected by means of en-

tomological pincers or little brush and put in a test-tubes with ethyl alcohol 70% to be identified later.

After having finished the sample the Freshwater Biodiversity Index of the site can be easily calculated by summing all the scores obtained in each section of the form: hydromorphology, taxonomic diversity and pollution tolerance. To have acceptable conditions of biodiversity the result must be 30 or more.

The survey must be done in low or normal water conditions coming from decreasing flows, from spring to autumn. Most benthic invertebrate populations are subjected to seasonal life cycles and this should be considered in the results. The sampling

can give results not reliable in the following situations:

- during or immediately after flood events (it is recommended to wait at least two weeks to allow the recolonization of the substrates);
- during or immediately after periods of drought (it is recommended to wait at least four weeks);
- impediments caused by environmental factors such as the high turbidity of water.

The samples must be done in a congruous number, also in relation with the extension of the superficial water grid of the farm or in near areas, on the base of the Table 4.

Total Farm Surface	Number of samples
≤ 20 ha	Two samples
20-200 ha	2 + (total surface – 40)/50 The result must be rounded to the inferior integer number
≥ 200 ha	5 + (total surface – 200)/100 The result must be rounded to the inferior integer number

Table 4. Number of water quality sampling sites in relation to farm surface.

Completed the samples, in relation to the extension of the farm surface, the general Freshwater Biodiversity Index of the farm can be easily calculated by summing the scores obtained in each survey form. The mean of the results must be 30 or more, for acceptable condition for biodiversity.

Before starting the survey, the operator must have the following material:

- handbooks with aquatic macroinvertebrates identification keys
- survey form for FBI-bf
- digital portable thermometer
- digital portable pH meter
- digital portable EC meter
- dissolved oxygen test kit
- aquatic net (ISO 10870:2012)
- magnifying glass 10x
- little white tank 30x40 cm
- lattice gloves
- entomological pincers
- test-tubes with ethyl alcohol 70%
- digital camera for macro photos
- Global Positioning System

THE SOIL BIODIVERSITY INDEX OF BIODIVERSITY FRIEND (SBI-BF)

The soil can be considered an ecosystem formed by a complex mixture of mineral particles, water, air, organic matter and living organisms; being the basic factor of the agricultural production, it is one of the most valuable natural resources on the Earth. A large part of Europe's land is affected by soil deterioration due to erosion, compaction, contamination, loss of organic content and change in land use (Jones et al., 2012). To be sustainable, agriculture in the future must adopt a careful soil management.

The utilization of the soils for the purpose of producing food needs a very high level of maintenance of the resource. The soil quality is traditionally evaluated by means of physical, chemical and microbiological indicators. Some methods based on the use of soil microarthropods in evaluating the soil quality were proposed in the past by different authors. In fact, many endogean animals show high sensitivity to land management practices and can be easily related to the soil ecosystem functions (Black, 1965; Menta, 2008).

The evaluation of the state of natural integrity, or alteration, of the edaphic ecosystem can be effectively realized through the study of the soil fauna. The edaphic or subterranean animals living in the soil have a close series of relationships among them and interact continuously with the physical environment. Any alteration of this environment is "registered" by the soil community which, therefore, can be used as indicator of the variation of the natural conditions (Giachino & Vailati, 2005; 2010).

Considering the complexity of soil communities, in qualitative investigations are usually examined some groups of animals that have species with fundamental requirements to be considered good biological indicators: to be assessable, to be easily determined and to be sufficiently known from an ecological and biogeographic point of view.

Coleoptera Carabidae and Staphylinidae, Opilionida, Lumbricidae and Enchytreidae were the groups more frequently used in the past for investigations of this kind (Brandmayr et al., 2005). But the application of these procedures were often limited by the difficulty of classification at species level, that requires the work of specialists in zoology.

The method of evaluation of the biological soil quality in relation to the presence of edaphic microarthropods, was proposed by Parisi in 2001 (QBS-ar, Qualità Biologica del Suolo-Arthropoda), initially with the aim to develop a procedure able to characterize the maturity of woodland soils. Using the ecological concept of Biological Form (or ecotype), similar to Sistematic Unit in the Extended Biotic Index, and analyzing the morphological and functional convergence among the soil microarthropods, Parisi (2001) assigned a different importance to each group characterizing the structure of the soil community, defining the so called ecomorphological indices (EMI).

The method of the standard "Biodiversity Friend" is based on the analysis of soil samples in which the presence of the animal taxa (Table 5) is detected to determine the Soil Biodiversity Index (SBI-bf); the presence of each group is recorded with a score in the proposed form. In comparison with the QBS-ar method, in addition to the Arthropoda, Mollusca and Annelida have been considered. These groups have a fundamental role in the dynamics of the edaphic ecosystem (Liu et al., 2012).

PHYLUM	CLASSES	ORDERS (or families)	SCORE
Mollusca	Gastropoda	Pulmonata and terrestrial Prosobranchia	10
Annelida	Oligochaeta	Enchytraeidae	10
		Lumbricidae	25
Arthropoda	Aracnida	Pseudoscorpionida	20
		Palpigrada	20
		Araneae	5
		Opilionida	10
		Acaroidea	25
	Crustacea	Isopoda	10
	Myriapoda	Diplopoda	15
	Insecta	Chilopoda	15
		Pauropoda	20
		Symphyla	20
		Collembola	25
		Protura	20
		Diplura	20
		Thysanura	10
		Orthoptera (Gryllotalpidae and Gryllidae)	10
		Dermaptera	5
		Blattodea	5
		Embioptera	15
		Psocoptera	5
		Coleoptera	10
		Hymenoptera (Formicidae)	5
	Larvae of Holometabola	Diptera	10
		Coleoptera	10
		Other Holometabola	5

Table 5. Table for the determination of the Soil Biodiversity Index of "Biodiversity Friend" (SBI-bf)

BIODIVERSITY FRIEND CHECKLIST
SURVEY FORM OF THE LICHEN BIODIVERSITY INDEX (FORM LBI-bf)

Farm _____ Locality _____ Province _____

Date _____ Operator _____

Site UTM Coordinates: _____ Altitude m a.s.l. _____

Lichen Biodiversity Index-bf: _____

List of the species	Code of the phorophyta and exposition												Frequency
	1				2				3				
	N	E	S	W	N	E	S	W	N	E	S	W	
TOTAL													

LBI			

LBI TOTAL	

NOTES:

Figure 1. Survey form of the Lichen Biodiversity Index (Form LBI-bf).

BIODIVERSITY FRIEND CHECKLIST

SURVEY FORM OF THE FRESHWATER BIODIVERSITY INDEX (FORM FBI-bf)

Farm _____ Locality _____ Province _____

Date _____ Operator _____

Site UTM Coordinates: _____ Altitude m a.s.l. _____

_____

Length of the considered reach: _____ m   **Freshwater**   **Biodiversity**   **Index-bf**: _____

H$_2$O physico-chemical parameters: **t** ___ °C   **pH** ___   **Elect. cond.** ___ µS/cm   **dissolved O$_2$** _____ mg/l

1) HYDROMORPHOLOGY					
Category	**Score: 5**	**Score: 3**	**Score: 2**	**Score: 0**	**Total**
Width	>6 m	2-6 m	<2 m	artificial	
Riparian vegetation	hygrophilous herbaceous	arbustive riparian	arboreous riparian	absent or not functional	
Hydrolog. Regime	seasonal natural	constant natural	seasonal altered	Artificial	
Fluvial morphology	heterogeneous	irregular	simple	canalized	
				Final score (1)	

BIOINDICATORS GROUPS	NUMBER OF MORPHOTYPES		POLLUTION TOLERANCE
Plecoptera			2
Ephemeroptera			3
Trichoptera			4
Megaloptera (Sialidae)			4
Platelmintes (planarian flatworms)			4
Coleoptera			4
Hemiptera			5
Odonata: Anisoptera			5
Odonata: Zygoptera			8
Hydracarina			6
Diptera			6
Amphipoda			5
Decapoda			6
Isopoda (Asellidae)			8
Bivalvia/Gasteropoda			7
Oligochaeta			7
Hirudinea			9
Nematoda/Nematomorpha			8
TOTAL MORPHOTYPES	(*)	**MEAN OF THE TWO LOWER VALUES OF TOLERANCE**	(**)

2) TAXONOMIC DIVERSITY					
Category	**Score: 25**	**Score: 15**	**Score: 5**	**Score: 0**	**Final score (2)**
N. morphotypes (*)	heterogeneous distribution (>20)	light dominance (9-20)	heavy dominance (4-8)	dominance/complete absence (0-3)	

3) POLLUTION TOLERANCE					
Category	**Score: 25**	**Score: 15**	**Score: 5**	**Score: 0**	**Final score (3)**
Mean tolerance (**)	0-2	3-4	5-7	8-9	

Index FBI-bf (1+2+3)		Scarce 0-29	Acceptable 30-44	Good 45-64	Excellent >65

NOTES:

Figure 2. Survey form of the Freshwater Biodiversity Index (Form FBI-bf).

BIODIVERSITY FRIEND CHECKLIST

SURVEY FORM OF THE SOIL BIODIVERSITY INDEX (FORM SBI-bf)

Farm _____ Locality _____ Province _____

Date _____ Operator _____

Site UTM Coordinates: _____ Altitude m a.s.l. _____

Meteo conditions: ▢ clean ▢ moderately cloudy ▢ cloudy   t = ___ °C   **Soil Biodiversity Index-bf:** ___

Soil (texiture): ▢ clay   ▢ silty-clay   ▢ silt-loam   ▢ loam   ▢ sandy-loam   ▢ sandy   skeleton % ___

PHYLUM	CLASSES	ORDERS (or families)	Score	Presence
Mollusca	Gasteropoda	Pulmonata and Prosobranchia	10	
Annelida	Oligochaeta	Enchytraeidae	10	
		Lumbricidae	25	
Arthropoda	Arachnida	Pseudoscorpionida	20	
		Palpigrada	20	
		Araneae	5	
		Opilionida	10	
		Acaroidea	25	
	Crustacea	Isopoda	10	
	Miriapoda	Chilopoda	15	
		Pauropoda	20	
		Synphyla	20	
		Diplopoda	15	
	Insecta	Collembola	25	
		Protura	20	
		Diplura	20	
		Thysanura	10	
		Orthoptera (Gryllotalpidae and Gryllidae)	10	
		Dermaptera	5	
		Blattodea	5	
		Embioptera	15	
		Psocoptera	5	
		Coleoptera	10	
		Hymenoptera (Formicidae)	5	
	Larvae of Holometabola	Diptera	10	
		Coleoptera	10	
		Other Holometabola	5	
			Final score SBI-bf	

NOTES:

Figure 3. Survey form of the Soil Biodiversity Index (Form SBI-bf).

Survey methodology of the SBI-bf

One of the most common methods of collecting soil macroinvertebrates is through the "free hunting" (with or without aspirator). During this operation the exploration of the muscicolous, saproxylic and lapidicolous enviroments must be done. In the "Biodiversity Friend" survey the collecting of the specimens is not required; the simple observation of the animals will be recorded on the survey form. By describing carefully the content of the samplings is possible to evaluate the Soil Biodiversity Index and, therefore, the variety of the soil community of a certain soil. The synthetic value obtained is used in the "Biodiversity Friend" checklist to evaluate the conditions of the cultivation substrate.

According to the "Biodiversity Friend" standard, the technique used for the soil survey is based on the use of the entomological litter reducer. The survey is made by digging with a spade a volume of soil of about three square decimetres. The hole must have a depth of about 25-30 cm. The soil is collected and put into an entomological litter reducer with a sieve having meshes of 10 mm. The material obtained is sieved again through another sieve with 4 mm mesh. The particles of soil must be sieved on a white square piece of cloth (1x1 m large). The large soil particles collected in the sieve are put in a corner of the cloth.

At this point, the operator begins the identification of the invertebrates, directly or with the help of a magnifying glass. Little by little the different taxa of invertebrates are found and identified; their presence is noted on the survey form. In case of uncertain identification, for large size organisms (more than 5 mm) a camera can be used, while small size organisms can be collected by means of entomological pincers or little brush and put in a test-tubes with ethyl alcohol 70% to be identified successively.

Before starting the survey, the operator must have the following material:

- handbooks with invertebrate identification keys
- survey form for SBI-bf
- Global Positioning System
- entomological litter reducer
- work gloves
- portable spade
- sieve with 4 mm mesh
- magnifying glass 10x
- white cloth 1x1 m
- entomological pincers
- aspirator
- little brush with soft bristles
- test-tubes with ethyl alcohol 70%
- digital camera for macro-photos

The samples must be collected in workable (in "tempera") soil; too dry or too rainy periods must be avoided. The most favourable seasons are spring and early autumn. However, surveys must be realized with sunny and warm conditions (more than 18° C), to stimulate the soil fauna to move after sieving.

If the surveys are made during a droughty spring or autumn, with dryness of the superficial soil layers, the samples can be taken sieving the soil collected from around the roots of cultivated or spontaneous plants of the crop. The most advisable thing is to collect the whole plant and insert it with all its roots and soil clod in the litter reducer. In the driest periods the pedofauna looks for moisture in the deepest layer of the soil or near the root apparatus of cultivated or spontaneous plants.

In the same way, further investigations by hand-collecting can be made under stones deeply buried in the soil, if they are present in the crop.

At the end of the survey, the operator sums all the scores registered on the form SBI-bf. According to the Soil Biodiversity Index a biologically active soil must reach a total score of 100 or more. The surveys must be done in an adequate number of samples in relation to the extension of the farm surface. The number of samples on each more representative crop of the farm must be proportionally related to the extension of the farm (Table 6).

After having finished all the samples, in relation to the extension of the farm surface, the Soil Biodiversity Index can be easily calculated by summing the scores of each samples, divided by the total number of samples. The ratio must be 100 or more, for a farm with soils of acceptable quality. Besides the surface extension, the definition of main or more representative crops considers also the criticality in terms of the use of resources. The woodlands must be considered as crops if they are managed using various silvicultural systems.

Total Farm Surface	Number of samples
≤ 20 ha	Three samples distributed on the main or more representative crops
20-200 ha	3 + (total surface – 20)/40 The result must be rounded to the inferior integer number. The samples must be distributed in the 4 main or more representative crops
≥ 200 ha	7 + (total surface – 100)/100 The result must be rounded to the inferior integer number. The samples must be distributed in the 5 main or more representative crops

Table 6. Number of soil quality sampling sites in relation to farm surface

DISCUSSION AND CONCLUSIONS

The three indices here presented are (for survey forms see figures 1-3) original contribution based on existing and largely used method of assessing biodiversity and the quality of different environments adapted to the operative methodology of the certification protocols. The procedures here proposed are the result of a rational compromise between a detailed and complete anal-ysis and the need of fast assessing protocols for non-specialist operators. To reduce the potential errors and approximations due to a high level of taxonomical identification of the samples a multidisciplinary approach has been used. The different fields of investigation and kind of source of information allow a comparison of different trends that can lead to a single solid conclusion, reducing the aberration possible in a mono-thematic approach. The open structure of the surveys and all the collateral information obtained, with every step forward a more detailed analysis beyond the final score, allow the operator to get also an idea on the single issues that may threat or alter the analysed environment, and propose resolutions.

ACKNOWLEDGEMENTS

REFERENCES

Alstrup V., 1991. Effects of pesticides on lichens. Bryonora, 9: 2–4.

ANPA, 2001. I.B.L. Indice di Biodiversità Lichenica. Manuale ANPA 2. ANPA, Roma, 85 pp.

Bartók K., 1999. Pesticide usage and epiphytic lichen diversity in Romanian orchards. The Lichenologist, 31: 21–25.

Beck W.M. Jr., 1955. Suggested method for reporting biotic data. Sewage and Industrial Wastes, 27: 1193–1197.

Black C.A. (Ed.), 1965. Method of Soil Analysis, Part 2, Chemical and Microbiological Properties, American Society of Agronomy, Inc, Publisher, Madison, Wisconsin USA, 1159 pp.Brandmayr P., Zetto T. & Pizzolotto R., 2005. I Coleotteri Carabidi per la valutazione ambientale e la conservazione della biodiversità. Manuale operativo. APAT, Manuali e Linee Guida, 34: 240 pp.

Brown D.H., 1992. Impact of agriculture on bryophytes and lichens. In: Bates J.W. & Farmer A.M., 1992. Bryophytes and Lichens in a Changing Environment, Oxford, Clarendon Press, pp. 259–283.

Bunn S.E. & Arthington A.H., 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management, 30: 492-507.

Caoduro G. & Giachino P.M., 2012. "Biodiversity Friend": certificare la conservazione della biodiversità in agricoltura. Regione Piemonte, Annali del Settore Fitosanitario regionale, 2011: 69–73.

Carrera M.F. & Carreras H.A., 2011. Efectos de la aplicación de glifosato sobre parámetros químico-fisiológicos en *Usnea amblyoclada* (Müll. Arg.) Zahlbr. Ecología austral, 21: 353-361.

Ferrington L.C. Jr. & Sealock A.W., 2005. Relationship of benthic macroinverbetrate biodiversity to recent and past ditching practices in Hardwood Creek near Hugo, Minnesota. Interim Report, 11 pp.

Fontaine B., van Achterberg K., Alonso-Zarazaga M.A., Araujo R., Asche M., Aspöck H., Aspöck U., Audisio P., Aukema B., Bailly N., Balsamo M., Bank R.A., Belfiore C., Bogdanowicz W., Boxshall G., Burckhardt D., Chylarecki P., Deharveng L., Dubois A., Enghoff H., Fochetti R., Fontaine C., Gargominy O., Lopez M.S., Goujet D., Harvey M., Heller K.G., van Helsdingen P., Hoch H., De Jong Y., Karsholt O., Los W., Magowski W., Massard J. A., McInnes S.J., Mendes L.F., Mey E., Michelsen V., Minelli A.,

Nafrıa J.M., van Nieukerken E.J., Pape T., De Prins W., Ramos M., Ricci C., Roselaar C., Rota E., Segers H., Timm T., van Tol J. & Bouchet P., 2012. New Species in the Old World: Europe as a Frontier in Biodiversity Exploration, a Test Bed for 21st Century Taxonomy. PLoS ONE.; 7(5):e36881.

Giachino P.M. & Vailati D., 2005. Problemi di protezione dell'ambiente ipogeo e note sull'impatto delle attività di ricerca in ambiente sotterraneo. In: Gili R.R. & Peano G., 2005. L'Ambiente Carsico e l'Uomo. Atti Convegno Nazionale, Bossea (5-8 settembre 2003): 303–314.

Giachino P.M. & Vailati D., 2010. The subterranean environment. Hypogean life, concepts and collecting techniques. WBA Handbooks 3, 130 pp.

ISO 10870:2012, 2012. Water quality. Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters. British Standards Institute, 38 pp.

Jones A., Stolbovoy V., Tarnocai C., Broll G., Spaargaren O., Montanarella L., Anisimov O., Arnalds Õ., Arnoldusen A., Bockheim J., Breuning-Madsen H., Brown J., Desyatkin R., Goryachkin S., Jakobsen B.H., Konyushkov D., Mazhitova G., Mccallum I., Naumov E., Overduin P., Nilsson, Solbakken E., Ping C.L. & Ritz K.S., 2012. The State of Soil in Europe. JRC Reference Reports, European Environment Agency. Report EUR 25186 EN, 71 pp.

Katzman M.T. & Cale W.G. Jr., 1990. Tropical forest preservation using economic incentives. Bioscience, 40: 827–832.

Klemm D.J., Lewis P., Fulk F., & Lazorchak J.M., 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency, Washington, 256 pp.

Liu J., Lu Z., Yang J., Xing M, Yu F. & Guo, M., 2012. Effect of earthworms on the performance and microbial communities of excess sludge treatment process in vermifilter", Bioresources Technology, 117: 214–221.

Loppi S., 2003. Mapping the effects of air pollution, nitrogen deposition, agriculture and dust by the diversity of epiphytic lichens in central Italy. In: Lichens in a changing pollution environment. English Nature Research Reports. Rep. N. 525: 37–41.

Lowrance R., Hendrix F.P. & Odum E.P., 1986. A hierarchical approach to sustainable agriculture. American Journal of Alternative Agriculture, 1: 169–173.

Mandaville S.M., 2002. Benthic Macroinvertebrates in Freshwaters-Taxa Tolerance Values, Metrics, and Protocols. Project H-1, Soil & Water Conservation Society of Metro Halifax, 128 pp.

Menta C., 2008. Guida alla conoscenza della biologia e dell'ecologia del suolo. Funzionalità, diversità biologica, indicatori. Oasi Perdisa Editore, Bologna, 304 pp.

Mora C., Tittensor D.P., Adl S., Simpson A.G.B. & Worm B., 2011. How Many Species Are There on Earth and in the Ocean? PLoS Biology, 9 (8): e1001127.

National Research Council, 2009. Global Sources of Local Pollution: An Assessment of Long-Range Transport of Key Air Pollutants to and from the United States. The National Academies Press, Washington, 248 pp.

Nimis P.L., 1999. Linee guida per la bioindicazione degli effetti dell'inquinamento tramite la biodiversità dei licheni epifiti. In: Piccini C. & Salvati S., 1999. Atti Workshop "Biomonitoraggio della qualità dell'aria sul territorio nazionale". A.N.P.A., Roma, pp. 267–277.

Olsgard F., Somerfield P.J. & Carr M.R., 1997. Relationships between taxonomic resolution and data transformations in analyses of a macrobenthic community along an established pollution gradient. Marine Ecology Progress Series, 149: 173–181.

Parisi V., 2001. La qualità biologica del suolo: un metodo basato sui microartropodi. Acta naturalia de "L'Ateneo Parmense", 37: 97–106.

Rosenberg D.M., Davies I.J., Cobb D.G. & Wiens, A.P. 1997. Ecological Monitoring and Assessment Network (EMAN) Protocols for Measuring Biodiversity: Benthic Macroinvertebrates in Fresh Waters. Dept. of Fisheries & Oceans, Freshwater Institute, Winnipeg, Manitoba. 53, Appendices.

Siligardi M., Avolio F., Baldaccini G., Bernabei S., Bucci M.S., Cappelletti C., Chierici E., Ciutti F., Floris B., Franceschini A., Mancini L., Minciardi M.R., Monauni C., Negri P., Pineschi G., Pozzi S., Rossi G.L., Sansoni G., Spaggiari R., Tamburro C. & Zanetti M., 2007. I.F.F. 2007 Indice di funzionalità fluviale APAT, Ministero dell'Ambiente e della Tutela del Territorio e del Mare, ARPA Trento, 325 pp.

Skole D.L. & Tucker C., 1993. Tropical Deforestation and Habitat Fragmentation in the Amazon: Satellite Data from 1978 to 1988. Science, 260: 1905–1910.

United Nations, Department of Economic and Social Affairs, Population Division, 2012. World Urbanization Prospects: The 2011 Revision. United Nations, New York, 32 pp.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E., 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130–137.

Woodiwiss F.S., 1964. The biological system of stream classification used by the Trent River Board. Chemistry and Industry, 14: 443–447.

Woodiwiss F.S., 1978. Comparative study of biological-ecological water quality assessment methods. Second practical demonstration. Summary Report. Commission of the European Communities. Severn Trent Water Authority. Brussels.