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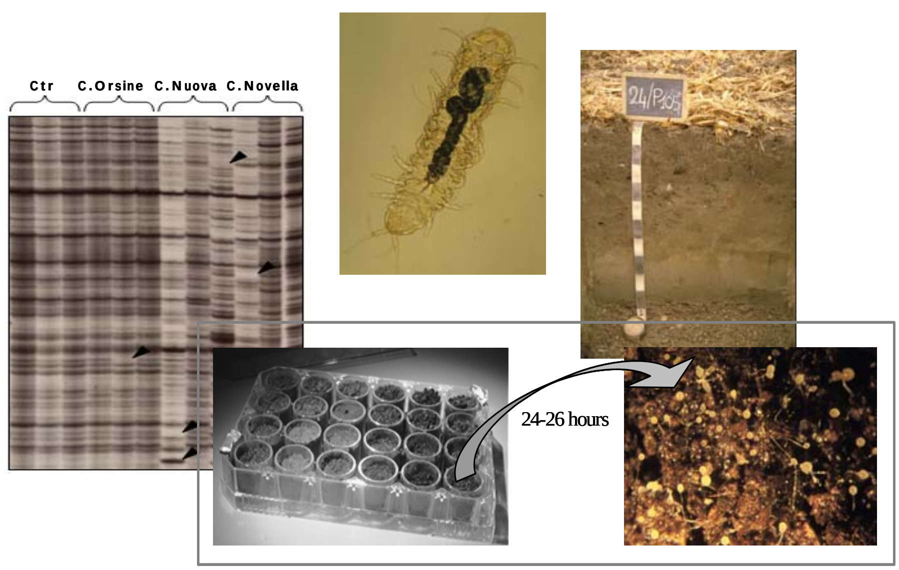
Holistic approach to biodiversity and bioindication in soil

R. M. Cenci and R. J. A. Jones (eds)



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Contact information
Address: Professor R. M. Cenci - TP 280 - Land Management and Natural Hazards Unit
Joint Research Centre, Via E. Fermi n. 2749
21027 ISPRA (VA) Italy

E-mail: roberto.cenci@jrc.ec.europa.eu
Tel.: +39 0332 789771
Fax: +39 0332 786394

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Edited by

R.M. Cenci[1] and R.J.A.Jones[2]

[1] Institute for Environment and Sustainability
Land Management and Natural Hazards Unit
Joint Research Centre (JRC)
TP280 Via E. Fermi n. 2749
21027 ISPRA (VA) Italy

[2] Natural Resources Department
National Soil Resources Institute (NSRI)
Cranfield University, Cranfield
MK43 0AL, England UK

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Preface

After the Rio Conference in 1992, Biodiversity became synonymous with protecting the environment. Bioindication has emerged as useful process for environmental evaluation particularly of the soil, which is a complex entity able to perform a multitude of key functions, vital for life, such as breathing, assimilating nutrients like carbon and nitrogen, transforming and mineralizing organic materials like vegetables and animals, storing substances in reserve in the form of humus. Direct or indirect contamination of the soil, by inorganic elements and/or organic compounds, can significantly change the activity and the composition of the organisms living in soil (soil biodiversity) and irreversibly prevent the soil fulfilling its key functions to support the planet's ecosystems. For example, decline in organic matter content is closely linked to the loss of soil biodiversity. Recognising that soils contain as much biodiversity as the above ground habitats is the catalyst needed to protect this precious resource from further degradation (Montanarella, 2006).

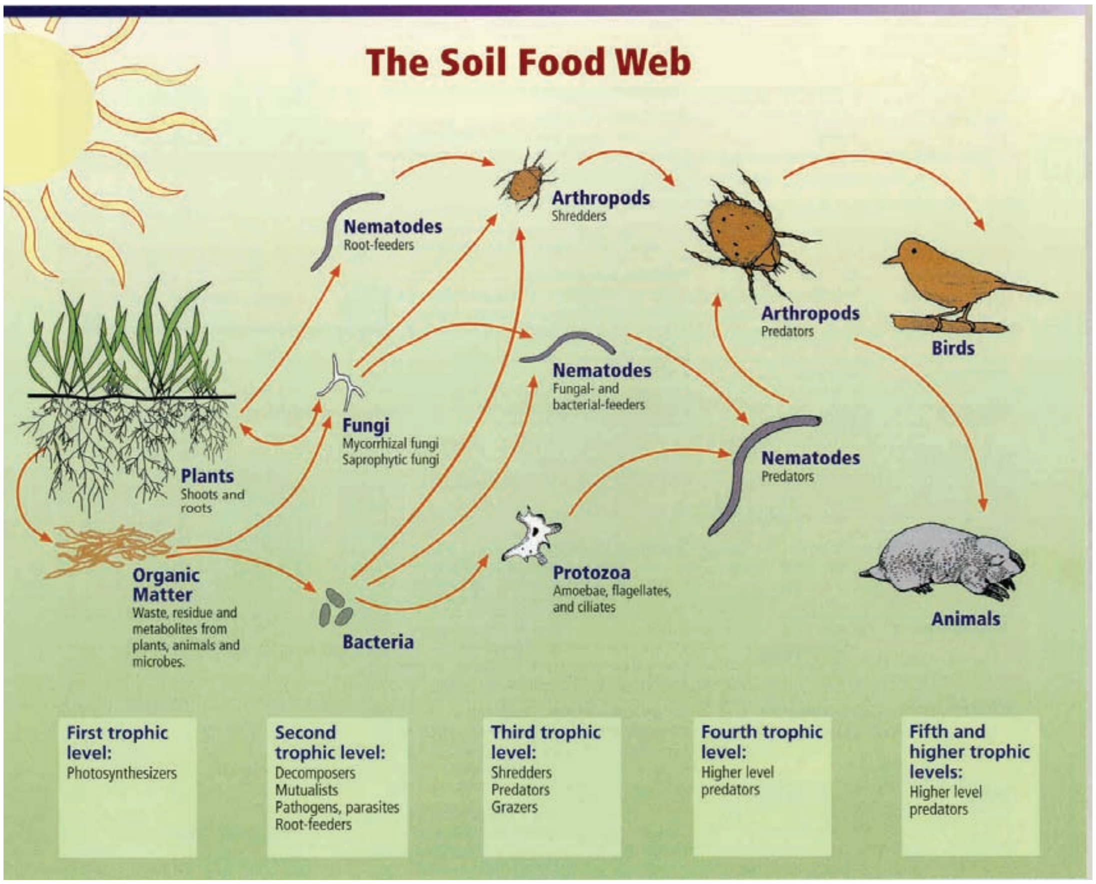


Figure 1. Exemplification of soil food web.
Source: USDA – NRCS Soil Quality Institute

Project BIO-BIO has wanted to give a signal tightly applying an important number of Bioindicators with appearance that cover the field of the Biodiversity for a reading very diversified that cover the three nutritional nets and that in the same time holds in consideration of the temporal appearance with the seasonal influences that can change the replies of the bioindicators, everything supported from a robust chemical-physical soil analyses.

Chemical-physical soil analyses are very important for a complete interpretation of soil quality. The results obtained, sole in their fill, will be able to serve to other experts like example to imitate to appraise, in manner exhaustive and entire, the quality and the health of the soil with special attention to those areas that have an important human pressure.

This report is a summary of the findings of the BIO-BIO Project and contains text, tables and graphs taken from the chapters of the BIO-BIO Project Report 'Biodiversity-Bioindication to evaluate soil health', EUR 22245 EN, edited by Cenci and Sena (2006). The contributors to this report are given due acknowledgement throughout the text.

Introduction

The ‘Pavia Project’ had as its principal objective the evaluation of the quality and health of soil in Pavia Province, Lombardy, in northern Italy (Cenci, 2006). A further objective was to adopt an innovative and multidisciplinary approach. The area under investigation covered 3000 km^2. Taking account of the different uses of soil in Pavia Province, international standard methods were adopted for the identification of sampling points, the collection, treatment and analysis of the samples for heavy metals, macro-elements, dioxins, furans, soil acidity, physical properties (water retention, pore size, geochemical profile, etc.) and biological data (bacteria and terrestrial mosses).

Soil is a complex entity that respires, assimilates carbon and nitrogen, decomposes and mineralises organic compounds of vegetable and animal origin, and stores reserves in the form of organic matter. These functions are enabled by the presence in the soil of organisms that intervene, through their metabolism, in the processes of transformation and regeneration of the soil components. Energy enters in the soil-system mainly through the decomposition of organic residues, whose rate of degradation is regulated mainly by the microbial biomass. Another aspect to consider is the contamination of soil by inorganic elements and/or organic compounds that can significantly change manner the activity of the microbial pool and other indispensable organisms ensuring that soil remains a living ecosystem.

The Pavia Project included a study of biodiversity and bioindication called the BIO-BIO Project (Cenci and Sena, 2006), to appraise the eventual differences in the biodiversity of soil that have resulted from different management practices. These practices are:

- Organic or ‘biological’ farming.
- Conventional ‘manure’ farming using animal manure and mineral fertilizers
- Sewage sludge ‘amended’ applications to soil.

The multidisciplinary BIO-BIO study has four main aspects:

- Temporal and seasonal (four samplings in one year);
- Chemical analysis of the different soil layers, 0-5 cm, 0-15 cm and 15-30 cm, to establish the presence of organisms;
- Physical measurements;
- Biodiversity and Bio-indication assessment, across an important pool of organisms encompassing the three management practices.

BIO-BIO Project

Cenci and Sena (2006) describe in detail the sub-projects that make up the BIO-BIO programme, but the following sections summarise the main findings of the project.

Area description, soil sampling, physical and chemical analysis

The soils of three study areas are on a nearly level land in the Po valley. The soil map units are documented in the ERSAF georeferenced soil database but brief descriptions are given below.

Soils of the 'Cascina Nuova' (manured) field
Representative soil type is, 'S Varese O' (soil mapping symbol: SVO), comprising deep, well drained soils developed in fluvial deposits; Taxonomic Class: coarse loamy, mixed, superactive, mesic Typic Dystrustepts (Soil Taxonomy, 1999).

Soils of the 'Cascina Orsine' (biological) field
Representative soil type, is 'Parosacco' (soil mapping symbol: PSA). comprising deep, poorly drained soils developed in fluvial deposits; Taxonomic Class: sandy, mixed, mesic Typic Humaquepts (Soil Taxonomy, 1999).

Soils of the 'Cascina Novella' (sludge) field
Representative soil type, is 'Valcova' (soil mapping symbol: VAC) comprising deep, moderately well drained soils formed in fluvial and glacio-fluvial deposits: Taxonomic Class: Fine silty, mixed, superactive, mesic Aquultic Haplustafs (Soil Taxonomy, 1999).

Table 1. Heavy metal concentrations and carbon contents at different soil depths

Site	Layer depth	Al	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
	cm	**%**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
Ca OR (Biologic)	0-5	4.68	6.7	0.22	33	12.1	0.04	18.7	18.3	61
	0-15	4.55	6.4	0.27	32	12.2	0.04	19.4	18.5	61
	15-30	4.69	9.7	0.33	34	13.1	0.05	20.4	17.4	61
Ca NU (Manure)	0-5	4.62	9.2	0.30	32	12.8	0.05	21.8	15.1	53
	0-15	4.07	7.5	0.24	31	11.2	0.04	18.2	16.9	57
	0-30	4.56	9.8	0.31	31	11.8	0.05	22.3	15.4	52
Ca NO (Sludge)	0-5	7.32	20.6	0.84	58	28.5	0.08	34.5	29.0	88
	0-15	6.94	21.0	0.79	61	30.2	0.09	32.0	22.7	84
	0-30	7.13	22.4	0.79	59	30.8	0.08	34.4	24.6	95
AM3 Corte Olona	Mean value	5.81	15.1	0.42	66	28.,0	0.08	42	22.3	84
Uncertainty		*3*	*10*	*15*	*8*	*8*	*24*	*8*	*9*	*7*

As an example, concentration of inorganic elements - dioxins, furans and PCBs - are shown in Table 1 and illustrated in Figure 2.

The results for the three study areas are in good agreement with concentrations of these elements found in the Pavia Province as a whole. The concentrations of the heavy metals in the areas treated with sewage sludge, do not show any significant increase by comparison with areas where no sewage sludge was applied. Furthermore, the concentrations for PCBs, dioxins and furans to were found to be well below the advisory limits, but the use of sewage sludge modestly raised concentrations of dioxins and furans in soil (Cenci et al., 2006).

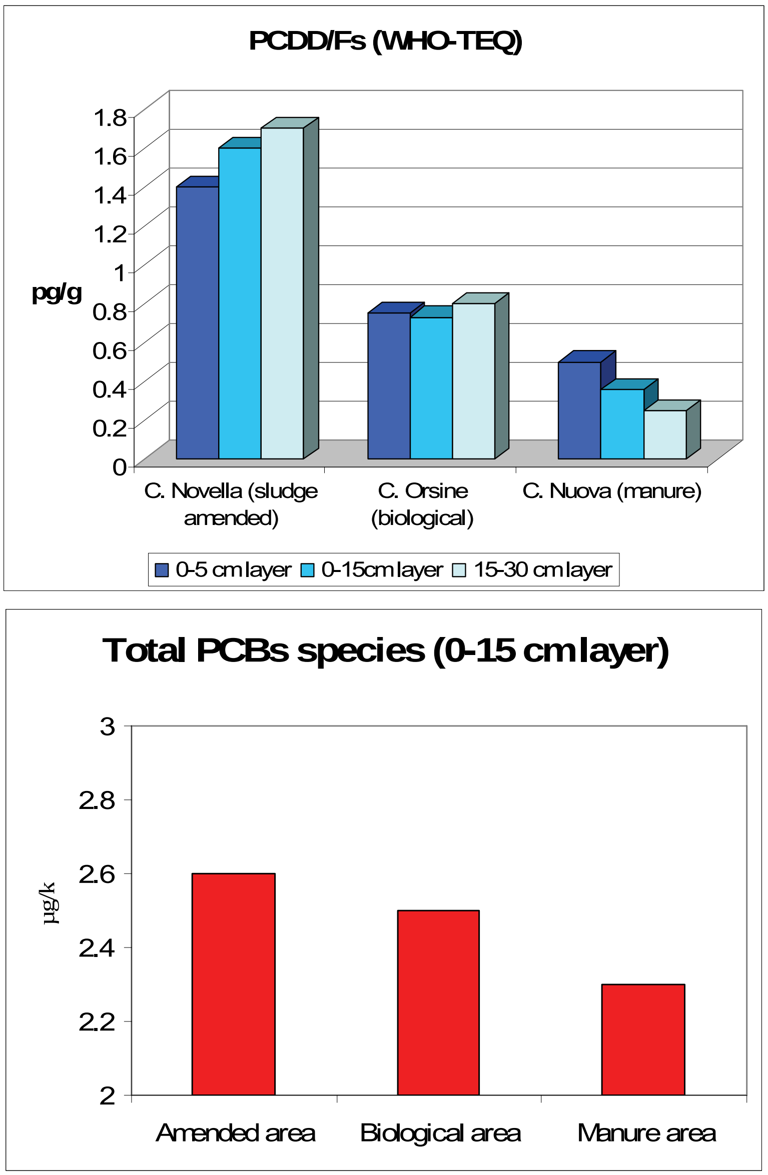


Figure 2. Concentration values of PCDD/Fs and the 17 species of PCBs (µg/kg) in the three study areas

Microbial indicators for assessing biological fertility status of soils

Use of bacteria, by analysing the products of their metabolism and identifying the main functional groups to which they belong, underlines the dual aspect of biodiversity and bioindication. The results obtained are influenced by a number factors that play important roles: humidity, organic matter content and the temperature of the soil (Pompili et al., 2006). The analysis of CO_2 alone, as a product of bacterial activity, is not sufficient to assess soil quality. High organic matter contents can mask heavy metal and organic compound contamination of the soil. For a complete interpretation, it is necessary to identify the functional groups of the bacteria (biodiversity), but this is not an easy task.

The concept of 'soil quality' is generally understood as the capacity of soil to function as a living system, able to fulfil all its biological functions, to sustain primary productivity, to promote the quality of air and water environments and to maintain the health of plants, animals and human beings (Doran and Parkin, 1994). Soil functions are now recognised as much broader in concept (Blum, 1993, 2005; De Groot et al. 2002), including Habitat Refugium (e.g. gene pool, seed bank), Cultural information (e.g. archaeological, education), Production Food (e.g. fodder; bre; raw materials; renewable energy), Engineering Technical (e.g. supporting industrial and socio-economic structures) and Regulation (e.g. buffering and filtering).

However, this report focuses on the biological functions of soil and in this respect, the concept of 'soil fertility', as the capacity of soil to sustain biological production, is a central issue, and all human and natural factors affecting production could rightly be considered as fertility factors (Sequi, 1989). Fertility factors belong to one of three distinct categories - physical, chemical and biological and the complex interaction of these three aspects makes up agronomic or integral fertility of soil, upon which productivity ultimately depends.

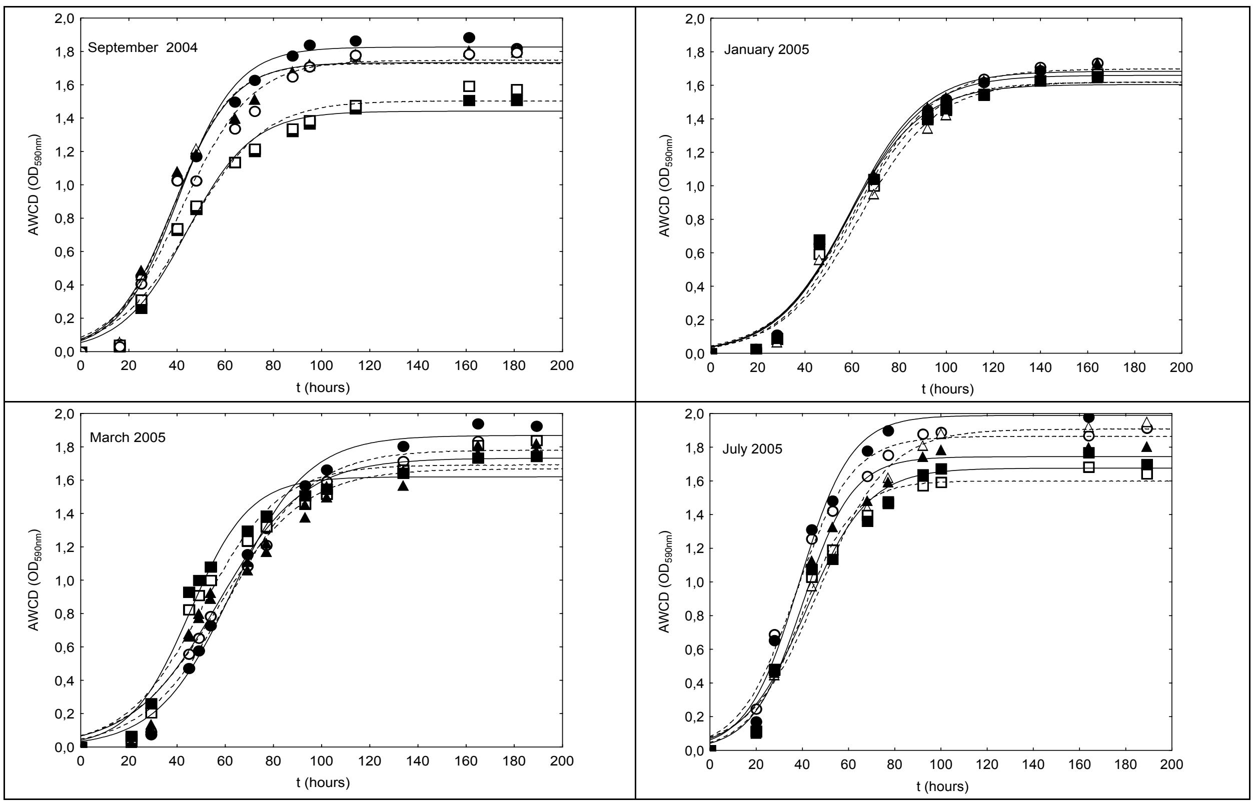
Physical fertility is controlled by soil structure and texture, chemical fertility results from the sum of available plant nutrients, whereas biological fertility relies on soil metabolic activity being defined as the overall reaction, both biotic and abiotic, to ensure a fertile medium for plant growth. Since biotic reactions are essentially microbial, it's possible to confuse soil metabolic activity as soil microbial activity. Whilst microbial activity indicates the wide range of activities carried out by micro-organisms in soil, biological or metabolic activity also reflects the activities of other organisms in the soil, for example plant roots (Nannipieri et al., 1990).

The microbial fraction represents a really important component of soil fertility because if it fails soil would become merely the mechanical support for plants. Micro-organisms, more than any other organisms, are highly adaptable to varying conditions and respond rapidly to changes (Hargreaves et al., 2003). For this reason they can be considered as reliable indicators of soil health and this is why they are usually used for soil status monitoring (Yakovchenko et al., 1996). In particular, measurements of microbial activity are actually included as indicators in many national and international monitoring programmes on soil quality. Usually an important criterion for an indicator is that it should respond promptly and accurately to perturbations (Holloway and Stork, 1991) because no individual measurement is enough as a single index of soil quality.

Micro-organisms respond rapidly to changing environmental conditions so that they are sensitive indicators of soil health and commonly used for soil status monitoring (Figure 3). The aim of this study was the characterization of three differently managed agricultural soils by using microbial indicators to assess soil biological fertility status. An extensive characterization of soil organic matter was carried out for all soils. Biochemical parameters included metabolic quotient, mineralization quotient and microbial quotient. Community level physiological profile analysis (CLPP) was used to investigate functional diversity of soil bacteria. Total amounts of fungi and bacteria were determined by direct microscopy. Indicators related to labile and humic organic matter fractions suggest significantly lower soil fertility and lower sustainability in the Sludge amended treatment. Differences between the Biological and Manure treatments were small.

A larger amount of nitrogen available for mineralization by soil microbes (mineralizable N) indicates higher biological soil fertility. A larger amount of hot water extractable carbon indicates a higher availability of food for micro-organisms. More intensive land-use involving soil tillage, fertilization and grazing, stimulates microbial decomposition and tends to result in a net decrease in the labile carbon pool and ultimately in a decrease in total soil organic matter, aggregate stability and biodiversity.

The lower levels of mineralizable nitrogen and hot water extractable carbon are in agreement with the significantly lower amount of total extractable carbon and the lower humic and fulvic acid fraction of organic carbon found in the Sludge treated plots compared to the Biological and Manure treatments.



Legend

● Biodynamic

▲ Manure

■ Sludge

— 0-15cm

---- 15-30cm

Figure 3. Community level physiological profile.

The differences between the site under Biological site and that under the Manure regime are less consistent. Potentially mineralizable nitrogen tends to be higher in Biologic, but this is statistically not significant. However, the potentially mineralizable carbon (C0) was significantly higher under the Biological regime.

Evaluating soil bio-hazards on Dictyostelium development

The Dictyostelium discoideum test features a rapid bioindicator with a large spectrum for soil analysis. The results obtained (Balbo and Bozzaro, 2006) reveal whether a soil is under particular stress or is generally contaminated. Such a test is employed to establish whether or not to continue with more specific investigations, although the test itself is not used to appraise the degree of soil contamination. At present, there are not many data in the literature for better assessment of soil quality.

The soil amoeba Dictyostelium discoideum is a low eukaryote, which has been widely studied for the investigation of several cellular processes such as cell motility, cell adhesion, development, chemotaxis and lately also to study the molecular mechanisms underlying drug resistance (Kessin, 2001; Alexander et al., 2006). Dictyostelium cells live and proliferate as solitary amoebae, feeding on bacteria by phagocytosis and dividing by binary fission. In nature, they live in the forest wood, decaying leaves and humid soil. Under laboratory condition, cells can be cultured on agar plates or in shaken liquid medium or in combination with bacteria.

Depletion of food triggers a developmental programme, whereby cells cluster together by chemotaxis giving rise to aggregates of approximately 105 cells. Each aggregate undergoes differentiation in at least two cell type and engages in a sequence of morphogenetic changes typical of a multicellular organism. The aggregates develop into fruiting bodies consisting of a "sorus", containing spores, hold by a slender stalk. The whole developmental programme is accomplished in approximately 24 hours. Moreover the two phases of Dictyostelium life cycle -growth and development- are temporally separate and mutually exclusive (Figure 4) (Kessin, 2001; Bracco et al., 2000).

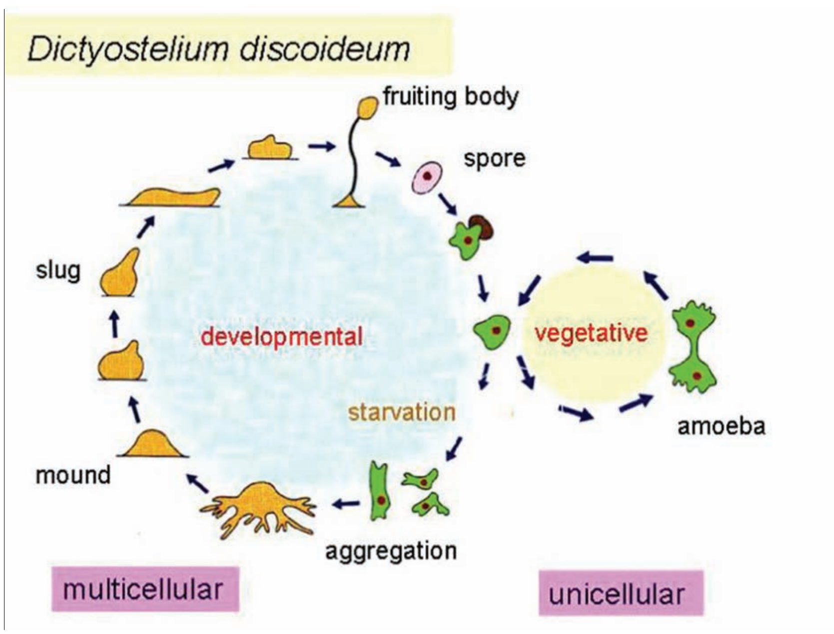


Figure 4. Dictyostelium discoideum life cycle

By contrast to bacteria and plants, and similar to animals, the Dictyostelium cell, is not protected by a cell wall, thus conferring to the amoebae high sensitivity to environmental stressors. These cellular and developmental properties and the rather unique capacity of the cells to develop both under submerged condition and on solid surface. The vegetative phase of Dictyostelium life cycle in which unicellular amoebae divide by binary fission. On the left the developmental stage, wherein cells depleted of food aggregate, by chemotaxis to form multicellular organisms of 105 cells. Each aggregate, containing two different cells types, organizes into a structure named fruiting body consisting of spores resting atop a cellular stalk. The spores will germinate in the presence of nutrients producing mitotically dividing cells.

Dictyostelium is a potentially attractive biosensor to detect the presence of bio-hazardous compounds in the extracellular environment, both on soil or water.

By exploiting all these characteristics, we have developed an easy, cheap and quick bioassay which allows to evaluate the influence of toxic substances on the rate of fruiting bodies formation. The assay detects biological effects of heavy metal on soil with varying sensitivity of the cells for heavy metals under submerged condition is higher of a factor 10 to 100. By comparing the Dictyostelium with other commonly used bioassay in a series of contaminated soils, the Dictyostelium sensitivity is comparable to that of other biosensor organisms such as Collembola and Earthworm (Hund-Rinke et al., 2002).

General results obtained with the Dictyostelium assay applied to soil samples from the three different farm management systems of Pavia province are presented in (Figure 5), show a difference between the two seasonal sampling. In C. Nuova ('manure' field), the results display an increase in fruiting bodies inhibition from 33% (November 2004) to 55 % (July 2005), ranging from slightly toxic to toxic. The C. Orsine ('biological' field) soil results are non toxic. The C. Novella ('sludge' amended) soil appears more toxic, inhibition varying between 60% and 49%; this robust reduction of fruiting bodies formation, should not be unequivocally attributed to the presence of bio-hazardous compounds in the soil. Most likely, the impaired development also results from the physical features of this soil being very calcareous and clayey in texture.

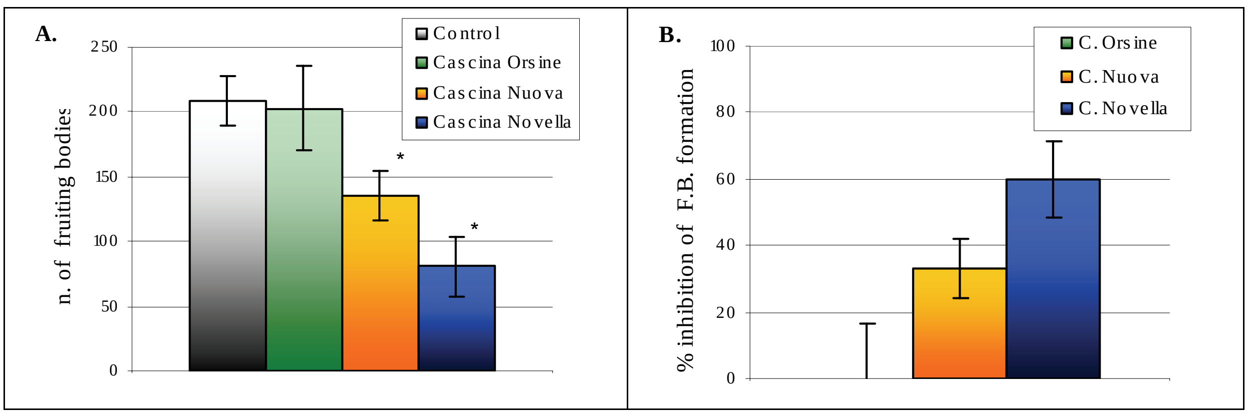


Figure 5 Analysis of the first of Pavia soil samplings with Dictyostelium test

A. Soil samples from the three farmlands collected on November 2004 were examined with the bioassay. Histograms display the mean values of the fruiting bodies formed in three independent assays whereas the error bars indicate the standard deviations.
The asterisk * indicates significant differences between raw data from control soil and each Cascina's sample (P<0.05).

B. Data are expressed as % inhibition of all the three farmlands compared to control soil.

F.B. Fruiting Bodies.

The soil fails to absorb water, creating condition for the development of the cells that cannot be compared with the other soils. These results highlight, in our opinion, the necessity to create a bank of standard soils with different physicochemical properties to be used as control soils in bioassays. In the absence of such controls the apparent toxicity of a given soil cannot be evaluated conclusively. Soil fertilized with manure and mineral compound results weakly toxic and toxicity varies in the course of the year. The soil fertilized with sludge results toxic, independently of the season.

Development and application of whole cell biosensors

This method, based on recombinant cell lines of the ciliated protozoan Tetrahymena thermophila for ecotoxicity screening, uses biosensors, is innovative and allows appraisal of toxicity of the soil, caused by inorganic elements and/or organic compounds (La Terza et al., 2006). At present the methodology is not able to discriminate between different types and contents of contaminants present in the soil. However, recent studies have shown the capability to recognize the different types of contaminants, but further studies will be necessary before the method is proven.

The analysis of complex environmental samples is primarily based on chemical analytical methodologies which, although accurate and sensitive, fail to provide data on bioavailability, potential synergistic/antagonist effects of the various toxicants on living organisms, as well as on the potential effect of unknown or chemically undetected substances.

Several recent reports showed that bio-reporter assays based on genetically modified cells (whole cell biosensors, WCB), represent a rapid, inexpensive and efficient alternative method for environmental monitoring (Baeummer, 2003; Baronian, 2004; Gu et al,. 2004; Hansen and Sorensen, 2001; Kohler, et al., 2000. These biosensors, that use whole cells as biosensing elements, instead of specific molecular entities (enzyme, antibodies, DNA), are able to provide an integrate view of the global cellular processes in response to noxious substances. The general approach for producing a biosensor using intact cells consists in fusing a stress-inducible specific promoter-DNA sequence from a well characterized gene regulation system to a reporter gene. The final genetic construct is inserted into the selected host cell. When the noxious substance is present, the expression of the reporter gene is induced, producing a signal that can be measured.

WCB biosensors have been obtained by transfecting cells of the protozoan ciliate Tetrahymena thermophila with a plasmid containing the coding sequence of the reporter gene Green Fluorescent Protein - GFP - (Chalfie and Kain, 1998) under the control of the stress inducible hsp70 promoter to generate a fluorescent bioreporter strain able to reveal a general condition of stress.

For the construction of the WCB, ciliates and, in particular Tetrahymena species, represent an ideal bio-material, since they offer a numbers of suitable characteristics to be used as biosensing elements of environmental sensors:

a) they occupy the first trophic levels and consequently are early warning indicators of cellular suffering;
b) they are available for most of the newly developed molecular genetic techniques;
c) they can be easily cultured and maintained in small volumes;
d) cell lines can be frozen and maintained in liquid nitrogen.

Moreover, the analysis of the recently sequenced macronuclear genome of Tetrahymena thermophila has revealed that this ciliate shares a degree of sequence conservation with human genes higher than that showed by other single-celled eukaryotic model organisms (Fillingham et al., 2002) yeast included.

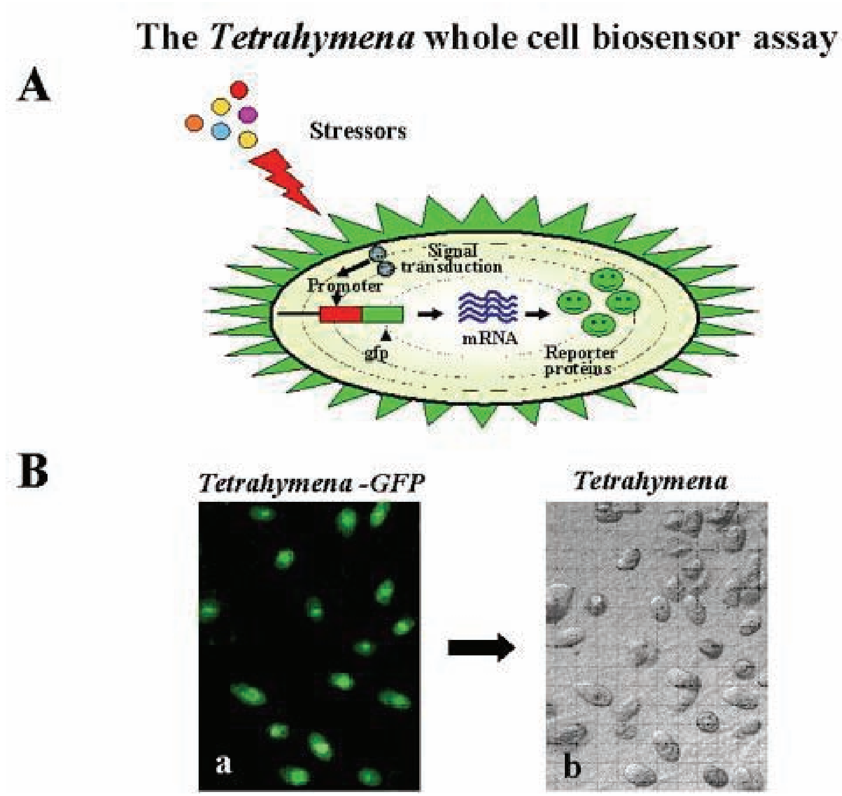


Figure 6. The Tetrahymena whole cell biosensor assay.

A) Representation by cartoon of the molecular events that elicit the hsp70-gfp gene induction following environmental stress exposure.
B) Recombinant Tetrahymena cells observed at the microscope after the exposure to stress: in a), cells showing gfp induction following stress and in b), the corresponding image in bright field.

These considerations make Tetrahymena an appealing bio-system for toxicity assessment, since it can provide information of direct relevance to human health and thus represent a valid alternative to the use of vertebrates in biomedical research. Tetrahymena is already widely used as a bioindicator: a database named TETRATOX has been established as a collection of toxic potency data for more than 2,400 industrial organic compounds (Schultz, 1997). For TETRATOX, the assay is a short-term, static protocol in which the 50% impairment growth concentration (IGC50) is the recorded endpoint. In many other cases lethality assays or inhibition of chemotaxis assays are also used (Chen and Leick, 2002).

In the Tetrahymena biosensor assay here established, the fluorescence emission represents the toxicity endpoint. By this assay, simple fluorescence microscopy techniques allow the real time and in vivo detection of fluorescence, without cell fixation requirement. This makes collection of experimental data easy and rapid, if compared with the classical physiological endpoint measurements such as loss of mobility, lower proliferation rate, etc.

This study developed and showed the validity of the Tetrahymena bioreporter assay for the assessment of the toxicity of soil elutriates coming from three agricultural farms under different agricultural management systems. The genetically modified cells are capable to produce a fluorescent signal at concentrations significantly lower than those detected by means of the more conventional lethality tests performed with the same organism. Thus, this assay is particularly suited to unveil sub-lethal concentrations of toxicants even in complex environmental samples such as soil elutriates and, consequently to furnish early warning data. Moreover, further advantages are offered by the fact that this assay allows the real time and in-vivo detection of the fluorescence.

The Tetrahymena whole cell biosensors were used to assess the potential toxicity of soil elutriates coming from three farms managed using different agricultural systems (conventional, organic and threaded with sewage sludge). In parallel to the Tetrahymena bioreporter assay, also classic lethality assays were performed. All data obtained suggest that in the presence of low levels of toxicity, the bioreporter assay allows a better evaluation of the toxicity displayed by the different elutriate samples with respect to the lethality assay.

To conclude, independently from the sampling periods, the descending order of toxicity revealed by both assays in the three farms was the following:

Cascina Nuova (manure) > Cascina Novella (sludge) > Cascina Orsini (biologic)

Also, the toxicity of the samples appears very low and totally absent in the summer elutriates of Cascina Orsini.

Table 2. Comparison of the LC20 and EC20 values obtained for the November 2004 and July 2005 elutriates.
The LC20/EC20 ratio highlights the higher sensitivity of the fluorescence (EC20) over the lethality (LC20) tests

November 2004 Elutriates	LC_{20} (%v/v)	EC_{20} (%v/v)	LC_{20}/EC_{20}	July 2005 Elutriates	LC_{20} (%v/v)	EC_{20} (%v/v)	LC_{20}/EC_{20}
Cascina Nuova	76	3	25.30	**Cascina Nuova**	100	6	16.67
Cascina Novella	83	4	20.75	**Cascina Novella**	>100	23	nc
Cascina Orsina	92	5.5	16.73	**Cascina Orsina**	>100	>100	nc

The results showed that the bioreporter assay allows a better evaluation of the toxicity displayed by the elutriate samples with respect to the lethality, assay in the presence of low levels of toxicity.

Nematode communities in three differently managed agricultural fields

This study demonstrates the use of nematodes to assess soil quality, although the results are marginally influenced by soil characteristics - organic matter, humidity and temperature (Biagini and Zullini, 2006). A reduction in biodiversity is closely correlated with contaminants present in soil For a better understanding of the loss in soil biodiversity, and to identify the causes, comprehensive data banks will be needed.

Soil biocoenosis includes a vast diversity of organisms depending on each other for carbon and energy. Among these organisms, microbes, mainly bacteria and fungi, are directly involved in organic matter decomposition and nutrient cycling. Other organisms, nevertheless, are also important in soil ecosystems, because they significantly affect microbial activity through trophic relationships (Wardle, 1999) and control populations of the lower trophic levels. The organisms grazing on bacteria and fungi contribute to increase the availability of nutrients for plants, otherwise immobilized in the microbial biomass (H. Ferris et al., 1998). This function, carried out mainly by protozoa and nematodes, is crucial for plant production and, thus, for the development of sustainable agriculture and forestry (Stork and Eggleton, 1992).

Many environmental factors and human disturbances affect soil ecosystems (Griffiths, 2003); particularly in agricultural systems these disturbances are tillage, amendments and pesticides (Freckman and Ettema, 1993). Intensive agricultural practices can have local negative consequences, such as a lower soil fertility and a reduced biodiversity. These changes mean also a profound alteration of biological regulation and nutrient availability (Matson, 1997). To preserve the soil health, practices to achieve a sustainable agriculture were developed. Substitution of synthetic compounds with organic matter in agricultural management led to better soil properties (United States Department of Agriculture, 1980).

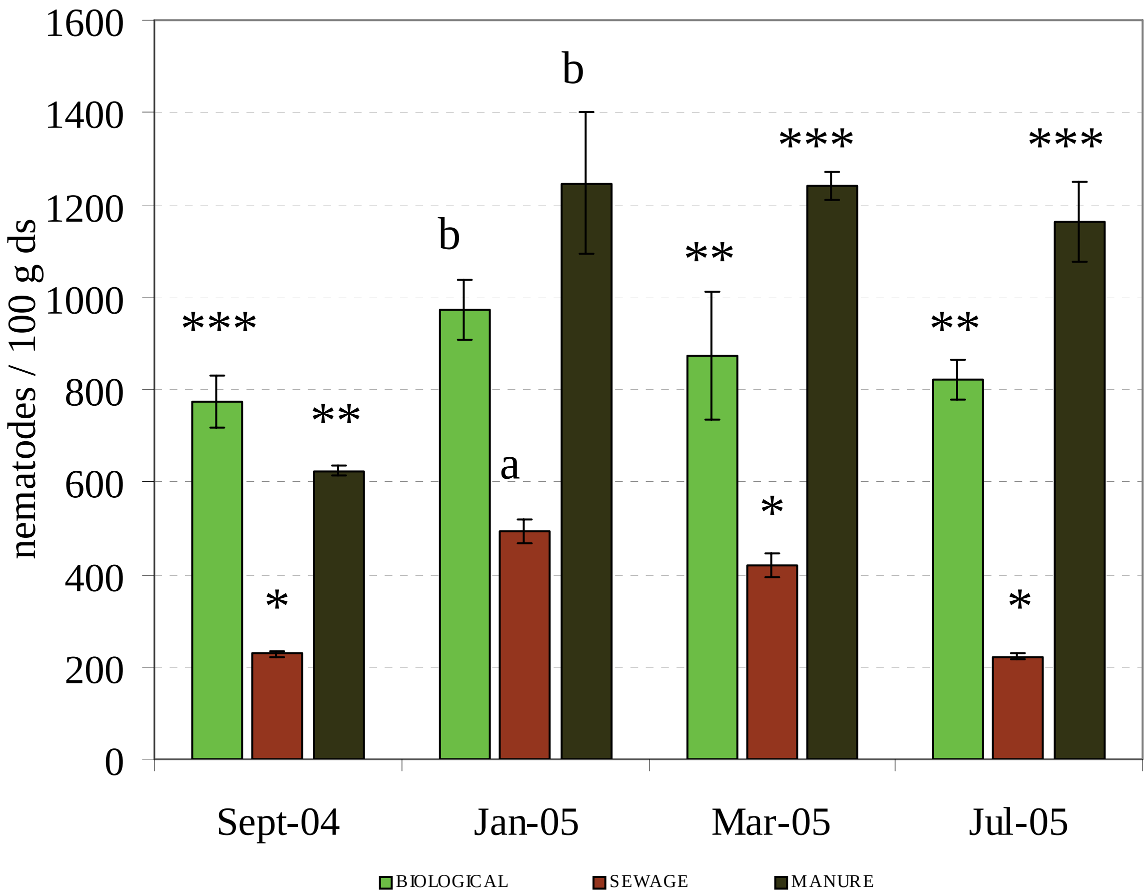
Many researches used soil organism as bioindicators of soil quality (Wodarz, 1992). Among soil organisms nematodes have propitious characteristics to monitor the environmental conditions (Bongers, 1999) even better than indices based on microbes, Collembola and mites (Neher, 2001). Nematodes were used to value the soil health in three agricultural fields differently managed. This valuation would be principally done on the basis of the presence/absence of the inputs and the amendment type.

Were sampled three sites, located in Pavia Province (northern Italy), under different agricultural practices, named “biological”, “sewage” and “manure”. The aim of this study was to value the soil health using nematode communities as bioindicators. The community of the biological-managed site community had the highest taxonomic and trophic diversity and maturity. The food web resulted fairly good structured and this site was in better conditions in comparison with the others. The sewage-managed site had the highest enrichment condition, but the lowest nematode density (Figure 7). Finally, the community of the manure-managed site was the worse and the least diversified and structured, being dominated by the plant feeders, especially by genus Paratylenchus, whose high density can be related to ecological degradation (Table 3).

All three plots had N-enriched resources and bacterial-dominated decomposition channels, especially the sewage-managed plot. In the sewage-managed plot the community was dominated by bacterial feeders, mainly Ba1 guild. This seems to indicate soil fertility. Therefore, this positive aspect must be pondered on the basis of the scarce nematodes density. The community has also a fairly good structure.

In the manure-managed plot, chemical fertilization and organic matter addition seemed to have an opposite effect on community dynamics. The excess of Paratylenchus among plant feeders seems to indicate degradation (Neher, 2001). Also the PPI value can be related to lower fertility in comparison with the other two plots. Moreover, the community was little mature and structured.

Finally, in the biological-managed plot the community had the highest taxonomic and trophic diversity and maturity and had a fairly good structure.



mean values, relative standard errors and statistical significance at * P<0.05, ** P<0.01, *** P<0.001 (according to Duncan's test (P<0.05) or Kruskall-Wallis test (P<0,05) respectively

Figure 7. Nematode densities (nematodes/100 g dry soil) in the three plots, for all sampling dates.

In conclusion, according to nematode community status, the plots can be arranged in this order:

Biologic (Cas. Orsine) > Sewage (Cas. Novella) > Manure (Cas. Nuova)

Table 3. Mean relative abundances of the main genera in the three plots.

taxa	Biologic Casina Orsine	Sewage Cascina Novella	Manure Cascina Nuova
Acrobeloides	6.3	1.9	4.1
Aphelenchoides	7.0	7.1	2.8
Cephalobus	5.1	7.9	1.7
Ditylenchus	3.7	11.1	1.7
Filenchus	7.2	8.5	1.1
Geomonhystera	0.6	0.0	9.0
Helicotylenchus	8.5	2.5	0.1
Mesorhabditidae	3.6	5.2	0.7
Panagrolaimus	7.8	0.9	8.6
Paratylenchus	15.7	0.7	45.8
Prismatolaimus	15.5	3.9	0.1
Rhabditidae	2.0	32.2	6.6

Evaluation of the environmental impact of agricultural management practices using soil micro-arthropods

QBS arthropods and collembola index uses the concepts of biodiversity to appraise soil quality. The QBS arthropods index is not able to identify soil contamination because it uses a hierarchical scale, the ‘Order of the Arthropoda’, that is not sensitive to external pressures or to contaminants present in soil. To obtain a good evaluation of soil quality, it is necessary to use the species level (QBS collembola) but this is a specialist field and therefore applying the method is very costly.

Soil fauna is an important component of soil systems because of its involvement in many aspects of organic matter decomposition, partial regulation of microbial activities, nutrient cycles and granular structure. Pollutants and other degradation factors can cause both quantitative and qualitative changes in fauna, which affect soil functioning (Bruce et al., 1997; Chauvat and Ponge, 2002; Gillet and Ponge, 2003). Use of soil bioindicators and test organisms may be helpful to detect environmental changes. Van Straalen (1998), in a review related to soil arthropod communities, specified that such bioindicators may play a role in soil monitoring measures.

Top-left: extracted specimens for the QBS-ar and Top-right for QBS-c calculation;
Bottom-left: Diplura; Bottom-right: Folsomia candida

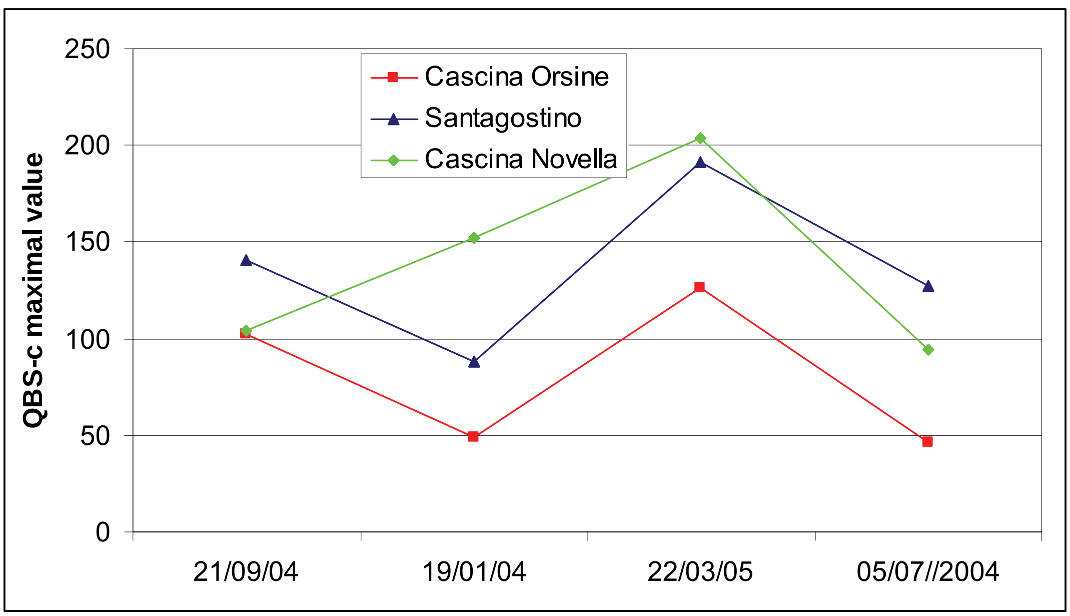
Figure 8. Berlese-Tullgren funnel, used for the soil microarthropods extraction

The types of invertebrate soil fauna used in monitoring pollutant effects include nematodes, enchytraeids and other oligochaetes, gastropods, springtails, isopods, arachnids (Cortet et al, 2000; Parisi et al., 2005; van Straalen, 2004). Some species in a single taxon may be specified as indicators of soil quality or as test organisms and used in toxicology tests.

In the collembolan taxon, Folsomia candida is the most frequently used species in both sub-lethal and lethal testing (Crommentuijn et al., 1993; Crommentuijn et al., 1995; Hopkin, 1997; van Gestel and Mol, 2003). Other collembolan species (Chauvat and Ponge, 2002) have been used in laboratory tests but have not reached the same level of routine use as has F. candida**.** Because of the species-specific differences in responses to contaminants, the tests conducted on F. candida provide partial indications as to the effects provoked by these substances on the collembolans; this information has also been useful to calibrate experiments on other species. Some collembolan species like Folsomia quadrioculata, F. fimetariodes, Isotoma minor and others species have been used to evaluate the effects of chemicals on collembola in field (e.g. Hopkin, 1997).

It is known that changes in the concentration of some metals in the soil or food can modify the species diversity and the density of the Collembola. When not altering the density, they can still influence the biology and reduce the survival potential, the rate of growth and the reproduction of species more sensitive to these elements. In many cases this effect is dose-dependent. In addition, variations in trace element concentrations in the soil can provoke effects on the fecundity of individuals. Tranvik et al. (1993) reported that reproduction in terms of eggs production in O. armatus was reduced by the presence of Cu and Zn.

The aims of this study (Gardi et al. 2006) were to evaluate the effects of three different agronomic management (biologic, manure and sludge) on the soil micro-arthropod communities, by using the QBS approach (Biological Soil Quality) (Figure 8). The effects of soils on the survival and reproduction of euedaphic Folsomia candida have also been evaluated.



Santagostino is Cascina Nuova (manure)

Figure 9. QBS-c maximal values for different sites and different sampling dates

The QBS indexes (QBS-ar and QBS-c) and the Folsomia candida soil test (Table 4) have been applied in order to evaluate the biological soil quality of three experimental sites, characterized by different agricultural regimen. The results however have probably been affected by a significant difference in soil characteristics among the sites and by the limited number of investigated sites; consequently, from the experimental data, it is difficult to rank the experimental sites in terms of biological soil quality.

The bioindicators have shown to be sensitive enough to detect the important seasonal variation in soil conditions and the effects of the main agronomic practices.

Table 4. Folsomia candida numbers of adults and young individuals

	Adults	Young individuals
Control soil	11 ± 1	385 ± 86
Cascina Orsine (biologic)	6 ± 3	97 ± 62
Cascina Nuova S. Agostino (manure)	11 ± 2	93 ± 43
Cascina Novella (sludge)	11 ± 1	97 ± 14

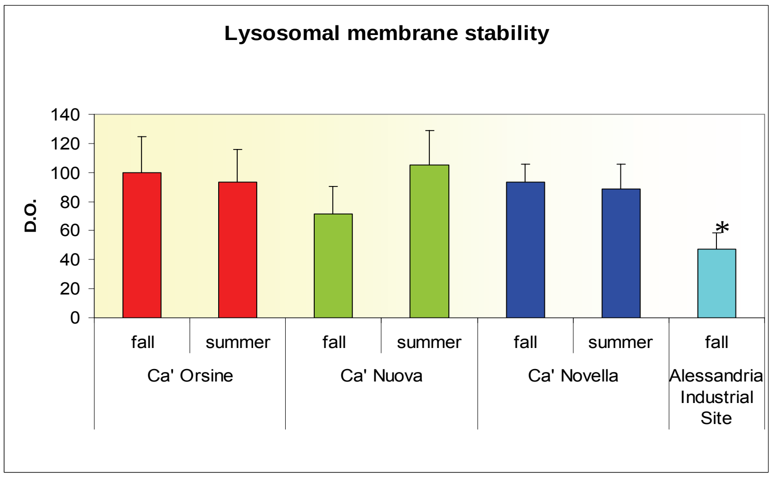
However, due to large differences at the three sites, not only in terms of agricultural regimen, but also in terms of agronomic history and soil characteristics, the experimental data obtained are not able to express a clear gradient of biological soil quality among the three investigated sites.

In the present research the main objective was to assess the effects of different agricultural systems (biologic, manure and sludge) on soil biological quality, but the errors induced by difference in soil characteristics, type of crop, agronomic history has been probably to high.

Evaluation of soil toxicity using a battery of stress biomarkers on the earthworm Eisenia Andrei

Use of earthworms in the laboratory to appraise soil quality is a bioindication and biodiversity method applied at the sub-level of the organism .The method is rapid but it does not give an indication of the type of contaminant present in the soil. Moreover, the earthworm data do not show a response in soils with moderate organic and/or inorganic contamination.

Among soil organisms, earthworms such as Lumbricus and Eisenia spp. (Anellida, Oligochaeta), are considered to be of particular interest to evaluate adverse effects of contaminants.



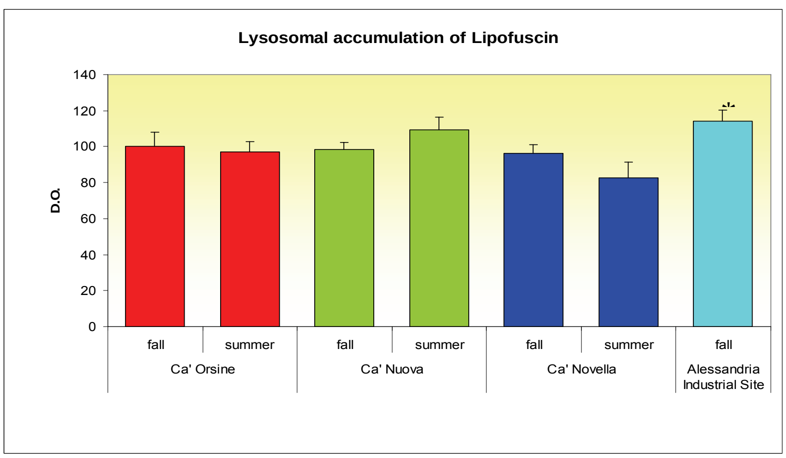


Figure 10. Lysosomal membrane stability in coelomocytes of Eisenia andrei and lipofuscin lysosomal accumulation in Eisenia chloragogenous tissue

Earthworms possess a number of qualities required in animals used for bio monitoring of terrestrial ecosystems. They are numerous, easy to sample, widely distributed and relatively immobile; they are in full contact with the substrate in which they live and consume large volumes of this substrate. On these organisms on developed a battery of stress biomarkers (i.e. parameters able to evidentiate the biological effects of the total charge of pollutants present in the environment) to detect the pollutant stress syndrome induced on worms by exposure to contaminants.

For the three soils, earthworms (Eisenia andrei) were exposed in climate chamber for 10 days to three natural soils sampled in two different seasons (fall and summer).E. andrei adults stress syndrome was investigated using a set of biomarkers of stress, such as lysosomal membrane stability, lysosomal accumulation of lipofuscin and neutral lipids, and Ca2+-ATPase activity, and a biomarker of exposure (AChE activity) suitable to reveal any toxic effects due to pesticides such as carbamate and organophosphorus compounds (See Gastaldi et Al., 2006).

Lysosomal membrane stability is recognized as an extremely sensitive indicator of cellular effects of pollutants in different species such as molluscs and fishes (Lowe et al., 1992; Moore et al., 1996). Lysosomal accumulation of lipofuscin was utilized because lipofuscin represents a lipid peroxidation end-product and its increase is related to the oxidative stress induced by pollutants. The lysosomal accumulation of neutral lipids is a useful indicator of alteration of lipid metabolism (Figure 10).

Ca^{2+}-ATPase activity plays a fundamental role in regulation of Ca^{2+} homeostasis and different toxic chemicals, that are able to produce oxidative stress in the cells as well as heavy metal ions, can affect the function of Ca2+-ATPase by acting on SH-residues (Figure 11).

Earthworms (Eisenia andrei) were exposed in climate chambers for 10 days to three different agricultural soils sampled at two different seasons (fall and summer).

The three soils were subject to different treatments: soil from Cascina Nuova (manure) was traditionally managed, soil from Cascine Orsine was subject to biological treatment, whereas soil from Cascina Novella was treated with sewage sludge.

The approach based on the integrated study of a battery of biomarkers has been validated in several laboratory experiments and field trials and it represents a valid screening tool in soil ecological risk assessment and an early warning index of soil pollution.
Results demonstrate that the earthworms exposed to the soil sampled in fall and summer seasons show no effects in terms of mortality and only a minimal level of oxidative stress as sub lethal physiological impairment.

The results demonstrated that mortality of earthworms was not affected in individuals exposed to the three soils sampled in two seasons and only a minimal level of oxidative stress as sublethal physiological impairment (with statistical significant change but lower than 20% and therefore of minimal biological injury in the animals exposed to Cascina Novella (sludge) and Cascina Orsine (biologic) soils).

Therefore the three sets of soil samples utilized in the analysis may be considered of good quality, being unable to induce sub-lethal toxic effects on E. andrei, utilized in the experiments.

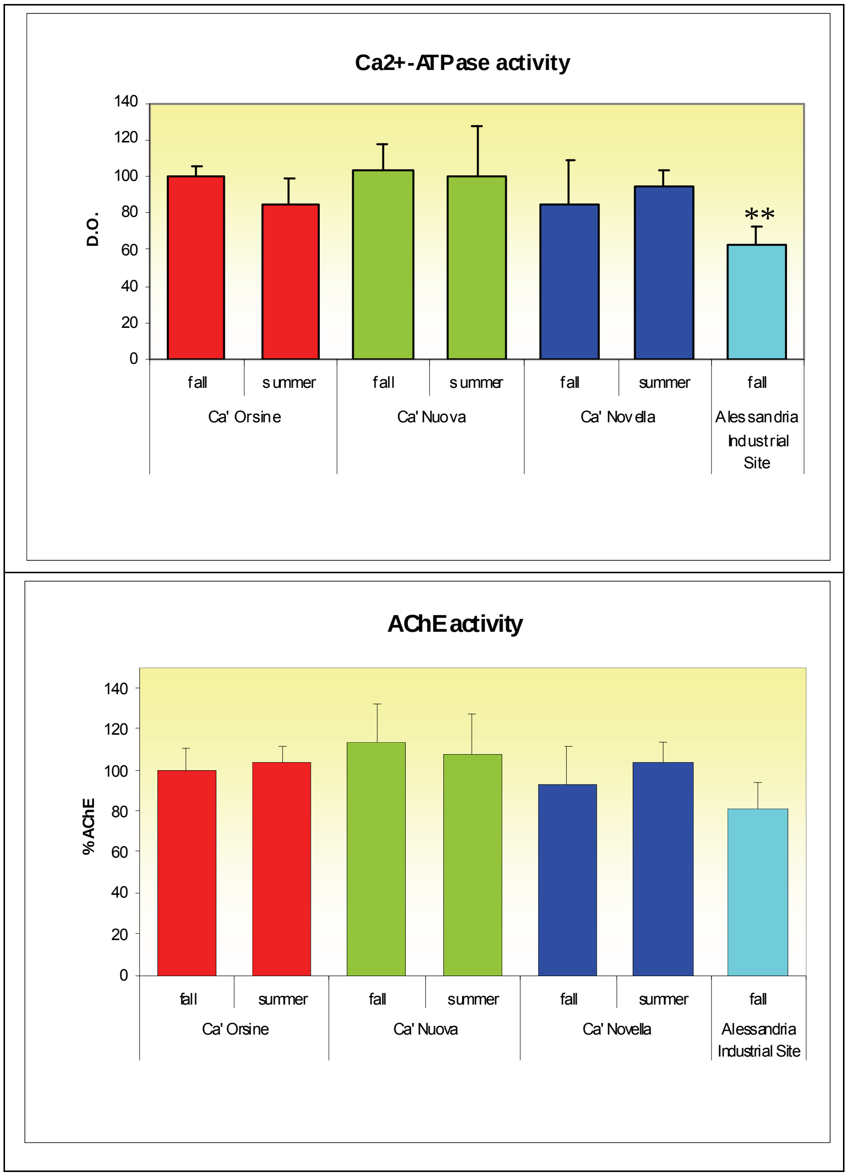


Figure 11. Ca2+-ATPase activity evaluated Eisenia andrei intestinal epithelium and AChE activity performed on earthworm homogenate

Earthworms used as indicators of agricultural management

Earthworms can be used as bioindicators, by measuring their biomass, or as indicators of biodiversity from the different earthworm species present, thus allowing good evaluation of soil quality. Agricultural practices, different cultivations, soil characteristics, humidity level and presence of specific contaminants are all parameters that significantly influence the final evaluation. Therefore it is possible to obtain similar responses for contaminated soils or soils with natural characteristics, such as high sand content or low water capacity and expertise in earthworm taxonomy is needed to apply such a method (Pérès et al., 2006).

Soil is a major interface between the lithosphere and the atmosphere. It could be regarded as an interactive system in which the physical, chemical and biological characteristics (soil structure, organic matter, soil solution, fauna and flora) are strongly related (Coleman and Odum, 1992). In order to understand soil functioning, it is necessary to assess the place and the role of each one of its components (physical, chemical, biological) as well as the interactions between these components (Table 5).

Table 5. Pedological and physico-chemical characteristics of the different sites

Agricultural management	Land use	Texture	CEC (meq/100 g)	Soil classification	pH	C % (0-30 cm)	C org % (0-30 cm)	Al mg/kg (0-30 cm)	Cd mg/kg (0-30 cm)	Cu mg/kg (0-30 cm)	Pb mg/kg (0-30 cm)	% Sand (0-30 cm)	% Loam (0-30 cm)	% Clay (0-30 cm)
1.Orsine *Organic farming (Bd)*	Maize (M)	Loam-sandy	13.2±1.2	Brunisol (with some feature of hydromorphy)	6.1									
	Barley/Pea (C)	Loam-sandy	11.4±1.9	Brunisol	6.2									
	Temporary Pasture (P)	Loamy	12.5±0.4	Brunisol	6.2	1.32	1.02	46400	0.27	12.4	18.06	68	27	5
2. Nuova *Traditional* (Td)	Permanent Pasture (P)	Sandy	15.8±0.5	Brunisol	6.6	1.05	0.9	44167	0.29	11.10	15.9	73	22	5
3. Novella *Fertilized (Be)*	Maize (M)	Loam-clay	18.7±0.9	Brunisol (with some feature of hydromorphy)	6.9	0.92	0.7	71300	0.80	29.8	25.4	34	56	10
	Rice 1 (R1)	Loam-sandy	20.6±3.3	Reductisol	7.1									
	Rice 2 (R2)	Loamy-clay	16.2±1.7	Brunisol	6.7									
	Wheat (C)	Loamy-clay	18.7±0.9	Brunisol	6.9									6.9

Moreover, soil characteristics are strongly influenced by environmental conditions (mesological and human pressures). In that way, the agricultural soils which permit the vegetable production are submitted to anthropic constraints (mechanical or chemical). If the practices carried out then allowed the increase of the outputs, they were also associated with the degradation of the soil quality, in relation to the process of run-off or compaction, the decrease of soil biodiversity, in relation to several pressures as soil contamination and decline in soil organic matter. Soil considered as a support of biodiversity, does not have any tool to assess the biological quality. In order to improve this, some projects have tried to create relevant biological indicator of soil quality.

In temperate regions, the earthworms in term of biomass constitute the principal component of the total faunal biomass (Lee, 1985). They have a large influence on soil physical, chemical and biological properties and thus are considered as "ecosystem engineers". In agro ecosystems, as in many other environments, their role in promoting soil fertility is important. Furthermore, because of their strong interaction with soil, earthworm populations are also profoundly affected by agricultural practices, such as soil tillage, crop residues, the use of fertilizers and pesticides, etc. (Edwards, 1983; Chan, 2001) and also by mesological conditions (Pérès, 2003). So, earthworms may be used as bio indicators of soil because they are easy to rear and classify and are very sensitive to both chemical and physical soil parameters.

The BIO-BIO project has investigated the earthworm responses of different agricultural managements identified in the project: organic, manure and sludge. Earthworm biomasses in cultivated soils are usually lower than 50 individuals/m^2 (Gerard and Hay, 1979). In cereal agro system, their abundance can be lower than 10 individuals/m^2 until disappearing, whereas in pasture they can reach 400 individuals/m^2 (Bachelier, 1978). Concerning the three agricultural managements studied in this work, the earthworm population parameters as abundance and biomass were very low, especially under pastures: 32.6 individuals/m^2 under temporary pasture in organic farming management despite the fact that there is no tillage since September 2003, and no use of pesticide; 46.5 individuals/m^2 under permanent pasture in traditional management despite the no tillage and the fertilisation.

This result suggests that the constraints of these sites strongly altered earthworm population. This observation is reinforced by the distribution of the ecological groups: in most cases, anecic and epigeic species missed. However, the three ecological groups which characterized earthworm populations have complementary function in soil. Thus, the unbalance noted in the different study sites underlines the strong actual or former anthropic constraints. Moreover, only few earthworm species were recorded and most of them were rare. These species are characteristic of the wet soils observed in Pavia region, resulting from the formation of the alluvial plain and its strong anthropization.

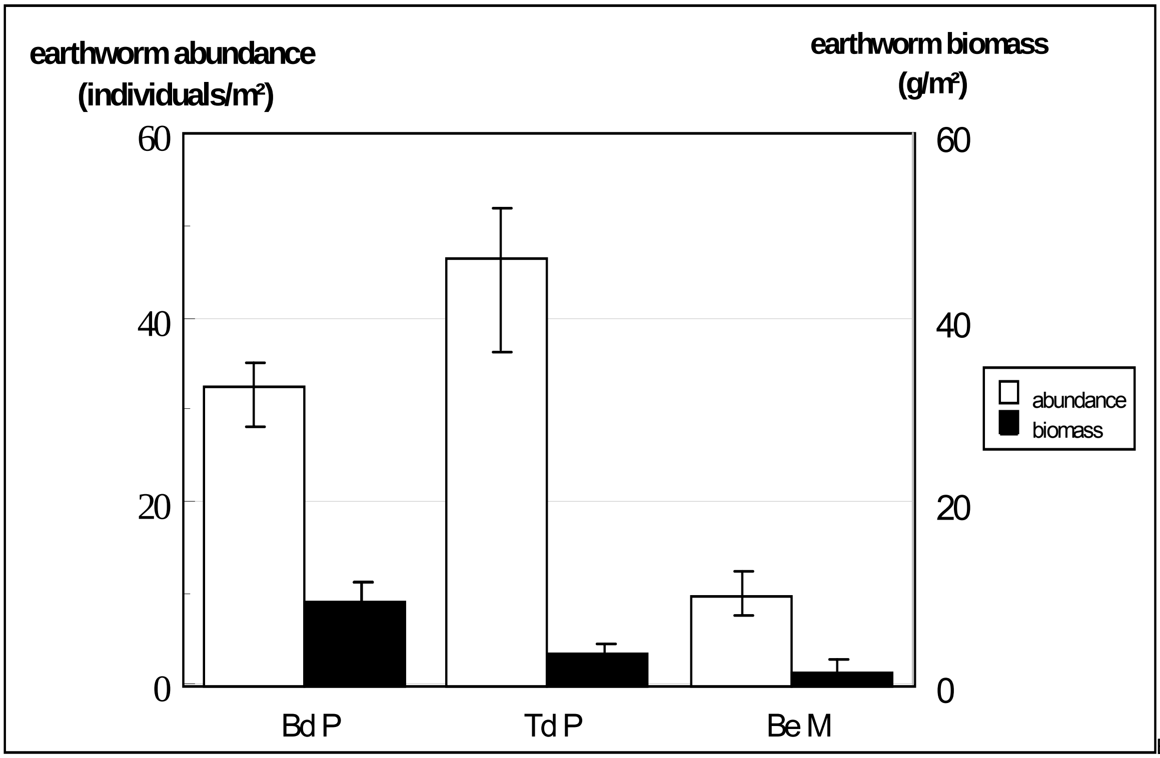
The difference of earthworm abundance and biomass between organic farming (Cascina Orsine, biologic) and traditional managements (Cascina Nuova, manure), even if it was not significant (32.6 individuals/m^2 and 46.5 individuals/m^2) (Figure 12) could be explained by the fertilization in traditional management and also by the no plough of soil. In both pastures, epigeic species miss (in traditional management, Acp was rare).

This finding contrasts with several studies which showed that epigeic species are very important in pasture (Pérès, 2003). In temporary pasture in organic farming management, this miss could be explained by the high predation in alluvial plain. In organic farming, this result could be explained by the fact that this pasture is a temporary one included in a rotation: in September 2003, the plough could have strongly decreased the earthworm population as several studies have showed (Curry et al., 2002).

The absence of anecic species in permanent pasture in traditional management could not be explained by the management which should be benefit for anecic species (enough food resource, no soil tillage); only the cow trampling could explain this absence.

On the other hand, Cascina Nuova (traditional) site presents a more sandy soil texture than Cascina Orsine site (organic), which could have a bad influence on earthworm population: sand has a direct bad effect because of the abrasive properties, and has an indirect bad effect by creating a filter soil (Pérès et al., 1998) (Table 6).

Concerning the structure of earthworm population in maize in fertilized management, the low values of abundance, biomass and species richness could be explained by several parameters.
As we have observed for the temporary pasture, the soil tillage is well known to markedly decrease earthworm population. So the plough realised each year depressed the fauna population (Figure 13; Table 7).



BdP (Pasture)(Cascina Orsini, biologic); TdP (Pasture)(Cascina Nuova, manure); BeM (Maize)(Cascina Novella, sludge)

Figure 12. Earthworm abundance and biomass in three agricultural managements (mean+S.E., n = 3)

Moreover, the use of some pesticides could have negative impact on the development of some earthworm species (abundance, biomass, reproduction) that influenced the specific structure of the population (Cluzeau et al., 1987; Texier, 1995; Ablain, 2003).

Furthermore, the chemical soil analysis of this site shows that soil contents high values of element as Cu, Zn, Pb. A part of those elements could come from the pesticides and also from the sewage sludge.

Earthworms are particularly sensitive to copper. Malecki et al. (1982), studied the effect of different heavy metals on Eisenia foetida; copper, given as nitrate, reduced reproductive rates at 100 mg/g. Found that if copper concentration is >80 mg/g, earthworms are almost completely eradicated from orchards. Paoletti et al. (1998), observed a negative correlation between copper and earthworms in vineyards of north-eastern Italy. Thus, most of the characteristics of the fertilized management site (land uses, pedological constraints) could explain the earthworm population structure recorded under maize.

The study of the specific structure of earthworm population showed some differences related to the agricultural management. Octodrilus transpadanus, which is the most abundant species recorded in this study, presents a large distribution in central Europe, and is recorded in all the different soil types (Bouché, 1977); its abundance is especially large in wet soils as marsh or banks. So, most of the characteristics of the fertilized management site (land uses, pedological constraints) could explain the earthworm population structure recorded in maize.

Table 6. Mean abundance (individuals/m^2) and biomass (g/m^2) of earthworm species in three agricultural managements (mean, n = 3)

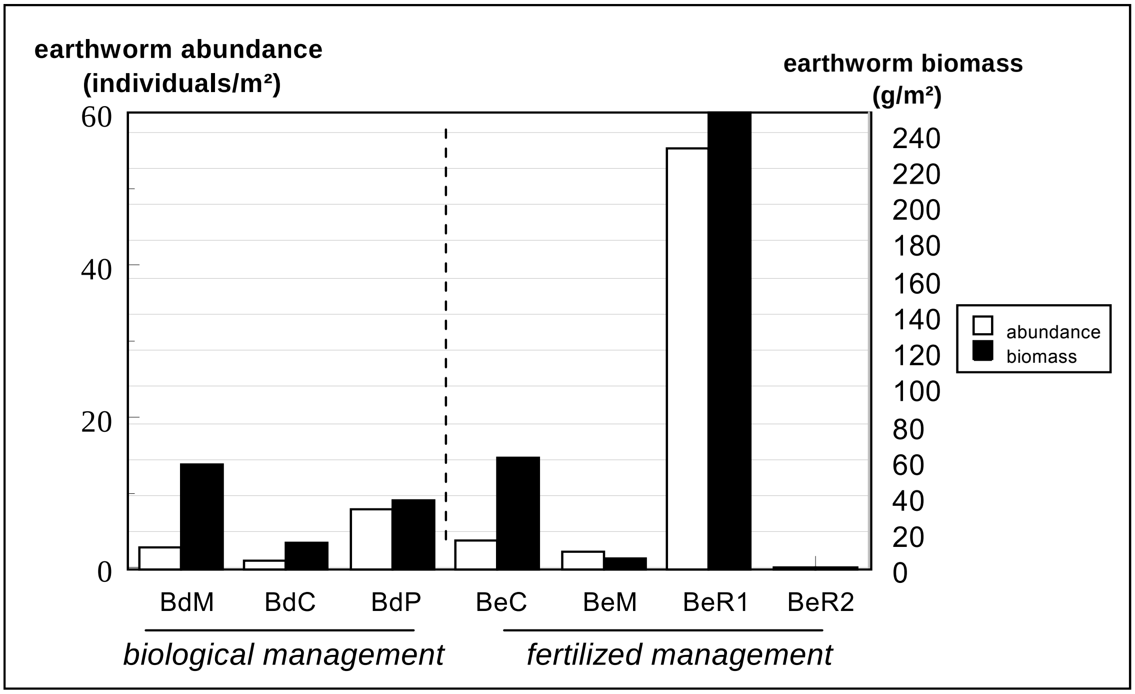
Management	**Abundance of earthworm species (individuals/m^2)**					
	O. transpadanus	***E. tetraedra***	***A. a. antipai***	**A. c. *paratypicus***	**Lumbricus sp.**	***M. dubius***
BdP	**32.7**	0	0	0	0	0
TdP	0	0	**24**	0.3	0.3	**22**
BeM	2.3	**7.3**	0	0	0	0
	Biomass of earthworm species (g/m^2)					
BdP	9.2	0	0	0	0	0
TdP	0	0	2.5	0.2	0.5	1.5
BeM	0.5	0.7	0	0	0	0

BdP (Pasture) (Cascina Orsini, biologic)
TdP (Pasture) (Cascina Nuova, manure)
BeM (Maize) (Cascina Novella, sludge)

The study of the specific structure of earthworm population showed some differences related to the agricultural management. Octodrilus transpadanus, which is the most abundant species recorded in this study, presents a large distribution in central Europe, and is recorded in all the different soil types (Bouché, 1977); its abundance is especially large in wet soils as marsh or banks.

Observed in both organic farming management and fertilized management, this species missed in traditional management. In this last site, the sandy texture generates very important variations of soil water content that could explain the absence of this endogeic species. Even if this pasture is permanent, it seems that the embankment and the pedological conditions involved a decrease of the earthworm population and allowed the installation of small less vulnerable species.

However, the agricultural management could partly explain the results observed, but the land uses and the mesological and pedological constraints explained additional differences. Thus, the earthworm species should be an indicator, but in our study more an indicator of mesological conditions than of agricultural management.



BdM (Maize)Pasture)(Cascina Orsini, biologic)
BdC (Pois/Orge)(Cascina Orsini, biologic)
BdP (Pasture)(Cascina Nuova, manure)
BeC (Froment) (Cascina Novella, sludge)
BeM (Maize) (Cascina Novella, sludge)
BeR1 (Rize1) (Cascina Novella, sludge)
BeR2 (Rize2) (Cascina Novella, sludge)

Figure 13. Earthworm abundance and biomass in the different land uses within two agricultural managements (mean+S.E., n = 3)

The earthworm population recorded within a same agricultural management, appeared to be strongly influenced by the land uses and pedological context. Within the organic farming management, the plough realised each year in the crop rotation, appeared to have been unfavourable for earthworm populations in terms of abundance (4.3 individuals/m^2), and biomass (3.5 g/m^2). This bad effect was not balanced by the organic input (cow manure) realised at the same time. This finding confirms the major negative impact of soil tillage on earthworm population.

Furthermore, several studies have described the toxicity of slurry, depending on the ammoniac content. In maize, the absence of plough and the presence of cultural residues on soil surface (food resource and protection from predation and climate constraints) explained the values of fauna. This confirms the need to protect soil surface in order to improve biological soil quality.

The global characteristics of the temporary pasture (grass cover, organic input, no tillage) explained the highest values of earthworm population (even if these values are not as large as those found in the literature), and confirmed that within a same agro-pedological context, the pasture system is the most favourable for earthworm population (Pérès et al., 2006).

Three species were recorded, but only Octodrilus transpadanus was not rare. The low value of endogeic species in temporary pasture was explained by the large quantity of juveniles, and thus a growth ratio (juvenils/adultes) which showed the restoration of the earthworm population. The large biomass and low abundance values for maize (BdM) and crop rotation (BdC) were linked to the presence of adults and also the presence of Aporrectodea caliginosa meridionalis (BdM and BdC) et Lumbricus rubellus rubellus (BdM).

Within the fertilized management, the earthworm populations were strongly affected by the pedological conditions: the hydomorphic and anoxic conditions observed in Rice2, explained the so low abundance of earthworms. This reductisol presented chemical and physical conditions that only epigeic species (Eiseniella tetraedra), because they always stay at the soil surface, could accept. The earthworm population was marginal in Rice2, compared to earthworm population in Rice1 (231 individuals/m^2), where the soil was a Brunisol. This finding suggests that pedological constraints could alter significantly greater earthworm population than land uses.

Table 7. Mean abundance (individuals/m^2) of earthworm ecological groups in three agricultural uses in organic farming management (mean, n = 3)

Land use	Ecological groups (individuals/m^2)		
	Epigeic	endogeic	Anecic
Maize (BdM)	0	10.3	1.7
Crop rotation (BdC)	0	4.0	0.3
Temporary pasture (BdP)	0	32.6	0.0

BdM (Maize)(Cascina Orsini, biologic);
BdC (Barley/Pea)(Cascina Orsini, biologic)
BdP (Pasture)(Cascina Orsini, biologic)

Moreover, spread of sewage sludge explained as well the low values observed in maize and cereal crop. The well known toxicity of Cu, Pb and Zn on earthworms combined to the recent soil tillage (BeC) and to the late harvest of maize in autumn (that compacted soil surface) could inform on the absence of anecic species.

However, the study of the toxicity is not easy, because accumulation and toxicity of element are very variable depending on the earthworm species (Kruse and Barett, 1985; Barrera and Andres, 2001) and the ecological groups (Ireland, 1979; Ash and Lee, 1980). Octodrilus transpadanus which was the most common species in this study, was not present in Rice2, confirming that the agro-pedological conditions were too restricted for this species. Eiseniella tetraedra, which was recorded under Rice2, was also observed under maize. This finding suggests that soil maize is frequently saturated. This is confirmed by the hydromorphic features observed in the soil sample. The earthworm species appeared to be good indicator of soil characteristics.

The use of earthworms and micro organisms as indicators of agricultural management is thus possible. However, the results observed in this study demonstrated that parameters as abundance, biomass, species structure of earthworm population are strongly influenced by the agricultural practices (soil tillage, organic input ...) and the pedological context (physical and chemical characteristics). Thus in order to assess the relevance of these biological population parameters as bioindicators of agricultural management, it would be necessary to compare different agricultural managements by maintaining other things unchanged (e.g. land uses and pedological context).

Impact of different agricultural practices on soil genotoxicity

Clover as a bioindicator is suitable for assessing the organic component and/or persistent inorganic contaminants present in soil. The toxicity of these contaminants modifies DNA but such an approach is not suitable for identifying soil contamination; chemical and physics analysis is needed to identify the quantity and type of contaminants. The method is highly sensitive to the modest variations in organic and/or inorganic contamination in soil that can induce variations in DNA.

In the recent past, soil quality has attracted special attention the world over. A good soil quality is in fact fundamental to protect and improve long-term agricultural productivity, water quality, and habitats of all organisms including people. Because of its high retention capacity, soil is very vulnerable to contaminant accumulation.

Agriculture practices can introduce an abundance of substances into soils reducing their quality. Among these substances genotoxic compounds are of great concern. Genotoxicity is in fact one of the most dangerous effects of contaminated soil, since many xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticides, are demonstrated to be DNA damage inducers (Klassen, 1995). Genotoxic compounds in soil can reduce crop productivity, can induce the build-up of resistance plant species and can negatively affect living organism health. For this reason it is important to evaluate the impact of various agricultural management systems on soil genotoxicity.

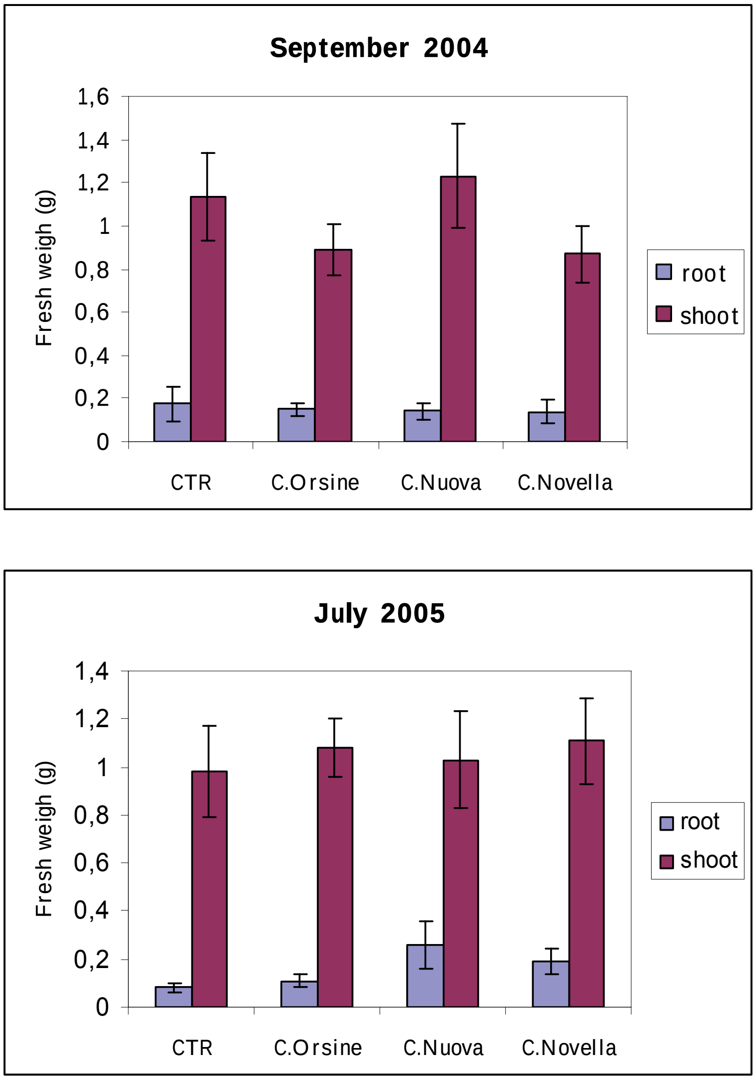
In this regard, physical and chemical methods for soil analysis do not provide sufficient information, since most soil genotoxics are unknown and the standard chemical analyses can assess the dangerousness of pollutants only in relation to the concentration of major contaminants and not also to the exposition time and to their bioavailability. Moreover soil pollutants can induce additive, antagonistic or synergistic effects and soil microflora can convert non-genotoxic compounds to genotoxic derivatives (Watanabe and Hirayama, 2001). In contrast, biological methods allow a direct assessment of genotoxic potential of soil stressors. Biological data can be used to estimate the environmental impact on ecosystem and individual organisms, including humans.

Higher plants can be considered sensitive and efficient bio-indicators of genotoxicity. They can be exposed for periods of few minutes to days or weeks. They are easy to handle, inexpensive and although the genotoxic effects observed in plants can not be extrapolated directly to human populations, the finding of plant bioassays may be taken into account for these purposes (Guimarães, 2000) (Figure 14).

The present report (Citterio and Sgorbati, 2006) examines agricultural activity in an environmental context and focuses on farming systems as the main vehicle for maintaining or improving soil and living organism health. In particular, the impact on soil genotoxicity of the three following different agricultural management systems were investigated: biodynamic ecological farming system (Cascina Orsine, biologic), traditional agriculture system (Cascina Nuova, manure), agriculture system using stabilized sewage sludge (Cascina Novella, sludge).

Soil genotoxicity was assessed by using the plant bio-indicator Trifolium repens L. cv Regal, since its documented sensitivity to organic and inorganic compounds (Dueck et al., 2003). DNA damage induced by agricultural soils in the test-plant was detected with Amplified Fragment Length Polymorphism (AFLP), which is a very sensitive molecular tool allowing the detection of DNA fragmentation and uniform or chromosomal mutations (Bagley et al., 2001; Citterio et al., 2002). Results obtained for the three different systems were analysed and compared.

Soil is a fundamental natural resource for agriculture. Successful farmers recognize that preservation of healthy, high-quality soils is essential to profitable and sustainable crop production.

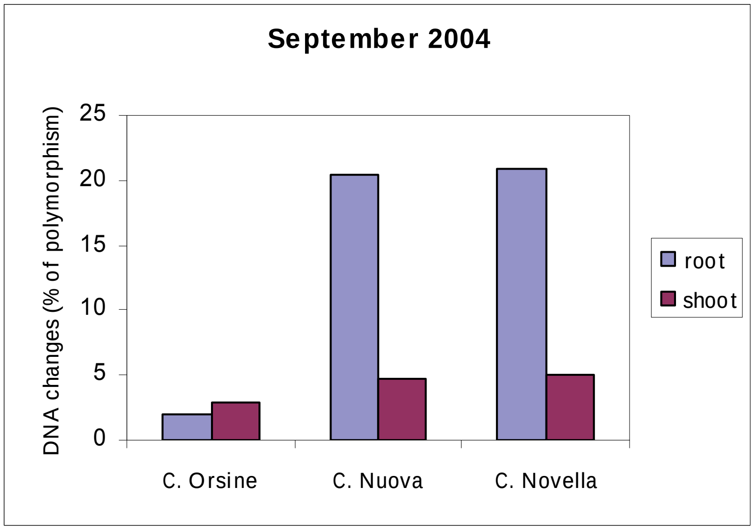


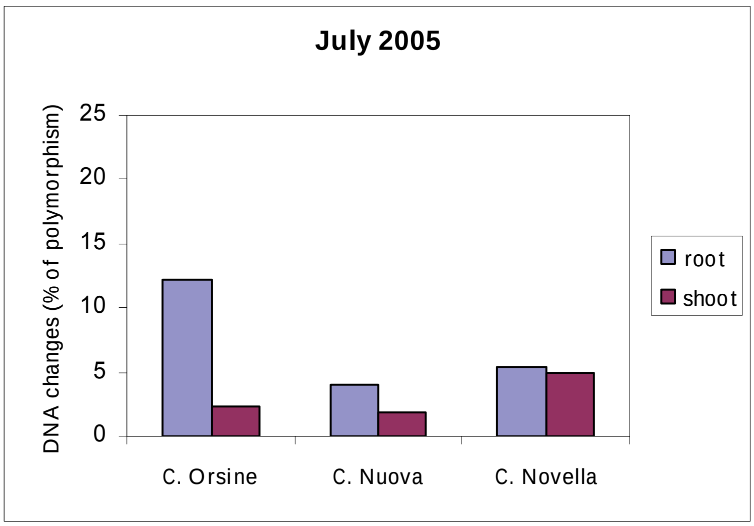
No significant difference among plants grown in the three agricultural soils and no difference between September and July bio-indication experiments can be observed (ANOVA, $P<0.05$).

Figure 14. Fresh weight (mean±standard deviation) of test plants grown in the differently treated farming soils.

One aspect of soil quality is related to the presence of genotoxic chemicals which can reduce crop yield and negatively affect human health. In farming soils, this type of xenobiotics can essentially originate from atmosphere deposition, from irrigation water and/or from agriculture practices.

The chemical analyses revealed that in the present case study atmosphere and fertilizers were two of the genotoxic chemical sources.





(∗) indicate statistically significant differences as compared to controls (P<0,05)

Figure 15. Analysis of polymorphism (P% = no. of polymorphic loci/no. of total loci) detected by AFLP in the DNA from the shoots and the roots of plants exposed to farming soils.

For example the considerable amount of polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) found in all the three soils are likely ascribed to atmospheric depositions, whereas the higher amount of heavy metals detected in Cascina Novella (sludge) soil is likely due to the recurring application of sewage sludge.

Nevertheless, as explained in the introduction chemical analytical tools are not sufficient to establish the genotoxic potential of a soil and only the combination with a bio-indication system can help to assess the impact of different farming practices on soil genotoxicity. Experimental results showed that after plant harvest (September 2004 experiment) the only soil which did not induced any significant alteration in test-plant DNA was that from Cascina Orsine (biologic), where a biodynamic agriculture (BD) has been practiced for 25 years. However the same soil just after preparation for the new sowing (July 2005 experiment) induced DNA changes only in the roots. This suggested that Cascina Orsine BD practices introduced in the soil genotoxic substances or compounds that soil microrganisms and plants converted to genotoxic derivatives.

A change in bioavailability of genotoxic substances should be also considered, although soil pH did not significantly change from September 2004 to July 2005. It is likely that these genotoxics were organic compounds because no DNA damage was detected in the test-plant shoots. Usually, for their chemical properties, organic substances such as PAHs are most retained in the root whereas heavy metals are transported to shoot inducing DNA damage also in this organ. The other two farming soils induced DNA damage in both September and July experiments (Figure 15). After plant harvest (September 2004) the “genotoxic activity” of the two soils was very high and both test-plant roots and shoots were affected. These genotoxicity levels were higher than those assessed just after soil preparation (July 2005). It means that, in spite of the introduction of fertilizers, at least part of the genotoxic compounds detected in September 2004 experiment were degraded or make less available or eliminated from the first 0-30 cm of soils before July 2005 sampling.

This result needs a discussion considering that no increase in soil pH was detected after soil preparation and that the AFLP data were reliable because many repetitions were carried out. We can make different hypotheses. We can suppose that the previous soil treatments (July 2004) introduced in the soils more genotoxic chemicals than that performed in July 2005 or that Cascina Nuova and Cascina Novella practices introduced in the soil non genotoxic substances which need time to be converted in genotoxic derivatives by soil microrganisms. A further hypothesis is that irrigation water used during summer 2004 plant cultivation contained genotoxics. Only additional experiments will help to clarify this issue and to assess the real impact of the three type of farming practices on soil genotoxicity. On the base of the present findings, Cascina Orsine (biologic) agricultural management system seems the best farming approach to maintain soil quality with regard to genotoxicity.

Conclusion

The concepts of biodiversity and bioindication are interconnected and require clarification for public consumption. Biodiversity is commonly understood to be the sum of the different organisms on Earth, estimated to total 13 million. Plant and animals species above ground are in decline, thousands species being lost every year because of pollution, reduction of habitats, a burgeoning human population, fragmentation of land and introduction of exotic species. However, the main overarching cause is economics, and it seems that the restoration of biodiversity above ground can be managed if the interests of the individual are subordinated to those of society as a whole.

To study and investigate soil biodiversity is a difficult task because of the complex interactions that exist in soil and the need for considerable expertise to undertake the necessary investigations. The factors that influence biodiversity are diverse: some are natural, for example soil acidity, water retention, temperature and organic matter content, others are anthropogenic, for example human population pressure. In a handful of soil, there are a hundred billion bacteria, many of these unknown to science and Mankind is not in a position to create them. It is difficult to evaluate biodiversity below ground, and it is not possible to use biodiversity to appraise biodiversity.

The previous sections outline some general ideas of what needs to be done in this field, whilst, at the same time, suggesting the basis for further studies. Bacteria, collembola and earthworms, which cover the three nutritional nets, are the most useful bio-indicators for appraising the evolution of biodiversity and assessing soil quality. To evaluate the biodiversity 'in soil' means to appraise the quality of the soil. Only integrated studies, that take into consideration the chemical, physical and biological nature of soil, will lead to a full understanding of soil biodiversity.

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Abstract
To study and investigate soil biodiversity is a difficult task because of the complex interactions that exist in soil and the need for considerable expertise to undertake the necessary investigations. The factors that influence biodiversity are diverse: some are natural, for example soil acidity, water retention, temperature and organic matter content, others are anthropogenic, for example human population pressure. This report summarises the results of the multidisciplinary BIO-BIO study of biodiversity and bioindication, conducted within the Pavia Project, which had as its principal objective the evaluation of the quality and health of soil in Pavia Province, Lombardy, in northern Italy. The area under investigation covered 3000 km^2 and the project took into account of the different uses of soil. International standard methods were adopted for the identification of sampling points, the collection, treatment and analysis of the samples for heavy metals, macro-elements, dioxins, furans, soil acidity, physical properties (water retention, pore size, geochemical profile, etc.) and biological data (bacteria and terrestrial mosses). The differences in soil biodiversity that have resulted from different management practices, namely: organic or ‘biological’ farming; conventional ‘manure’ farming using animal excreta and mineral fertilizers; and sewage sludge ‘amended’ applications to soil, have been studied on a seasonal basis (4 sampling per year) and analysis of soil samples taken at 0--5 cm; 0-15 cm and 15-30 cm depth. Some general ideas of what needs to be done in this field are outlined, whilst, at the same time, suggesting the basis for further studies. Bacteria, collembola and earthworms, which cover the three nutritional nets, are the most useful bio-indicators for appraising the evolution of biodiversity and assessing soil quality. To evaluate the biodiversity ‘in soil’ means to appraise the quality of the soil. Only integrated studies, that take into consideration the chemical, physical and biological nature of soil, will lead to a full understanding of soil biodiversity.

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