Chapter 22
Markers, Indicators of Soil Pollution

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Abstract As an interface between the earth's crust, atmosphere, and hydrosphere, soil is a non-renewable resource that has multiple functions: biomass production, storage, filtration, and transformation of organic and mineral matter; source of biodiversity, habitats, species, and genes; environment for humans and their activities; and source of raw materials. Impact of pollution on soil quality has increased due to population growth and extensive exploitation of natural resources. Soil pollution destroys the physical, chemical, and biological balance, which ensures soil fertility. Soil pollution can inhibit enzyme activity, reducing the diversity of fauna and flora. The degree of retention of pollutants is influenced by the presence of other pollutants and their concentration, quantity of oxygen, humidity, temperature, pH, nutrients, bioaugmentation, products of co-metabolism, and so on.

Keywords Soil • Pollution • Ecosystems • Indicators • Plants • Biological activity • Biomonitoring

22.1 Soil Quality

Soil is a dynamic system, essential for human activities and ecosystems. The term "soil quality" themselves refers mainly to the agricultural productivity (fertility). Processes that can impact soil quality are: average emissions (industry and traffic); farming techniques (use of fertilizers and pesticides organic/mineral); and ground waste deposition. In general, the rate of pollutants production and dispersion exceeds the natural processes of biodegradation. Soil is a "living organism," and therefore training and development, and its evolution take place under the action of physical, chemical and biological factors. The fertility of soil ensues from these factors; it differs from rock that was formed under the influence of pedogenetic factors.

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Soil is a reservoir that accumulates pollutants from the air and water and is a natural interface between different systems (hydrosphere, biosphere, geosphere, lithosphere, and so on), and therefore constitutes an environment that can be integrated as a complex sorbent having specific properties. Soil is a component of the rural and urban environment, acting as a matrix with complex functions that constitute a two-way relationship with potential contaminants. Soil contains 93 % minerals and 7 % bio-organic substances or 85 % humus (structure), 10 % roots, and 5 % edaphon (soil organisms).

The 5 % of the total living creatures in soil are represented by 40 % fungi, 12 % earthworms, 5 % macro fauna, and 3 % micro fauna. The total living creatures in soil represent 0.35 % of the total weight of soil, but their importance for soil fertility is considerable and there are legitimate concerns about the biological balance (Postma and Lynch 2012).

Soil creatures belong to the flora and fauna and can be grouped into micro flora (algae, fungi, actinomycetes and bacteria), macro flora (plants with underground organs, roots, stems), micro fauna (protozoa: rhizopode, flagellates, ciliates), and macro fauna (flat worms and cylindrical, nematodes, enchitreide, lumbricide, insects, vertebrates). In this community, the conventional relationships are living together, prospering (metabiosis), mutual support (symbiosis), interdependence (parasitic), etc. Processes occur as a result of the activity of soil organisms' influence on fertility and hence on plant production (Sheng et al. 2012).

The complexity of biological and biochemical processes, assets, and basic soil in agrotechnical terms comprises: the formation of humus (humification), mineralization of organic matter, and release of elements used for plant nutrition (ammonification, nitrification and denitrification); enzymatic activity soil relationships between plant roots and soil microorganisms (nitrogen fixation); associations between soil microorganisms (commensalism, proto-cooperation, symbiosis, competition, amensalism, parasitism or predation); and the interactions between plant roots (favorable or antagonistic-*biochemical* inhibition /allelopathy/ exhibited) (Mavi et al. 2012).

The highlights of sustainable agricultural development schemes are: the utility planting of agroforestry belts to prevent erosion, protective effects, agrobiocenotic stability and balance, biodiversity and prevention of pollution by pesticides, etc. In open field farming system with a warming climate, the presence of pollutants and pests presents risk/disaster, stressing the importance of nurturing and the qualitative quantitative and qualitative potential of shelterbelts of the agroforestry farming system.

Under the global warming and the aridity conditions, the protective forest farm was recommended to control naturally the population of the oat leave beetle (*Oulema melanopus L.*) and limit the populations of other pests, such as aphides (*Sitobion avena Fabr*. and others) and thrips (*Haplothrips tritici* Kurd.) to levels below the economic damage threshold.

In terms of the biodiversity, the qualitative importance of the protective agroforestry farming system for biological pest limitation was highlighted as a model of sustainable and clean technology, as compared with the open field agriculture, conditions, and pest attacks in agroecoclimatic biocenoses, representing actual risk and disaster, which require treatment with insecticides.

Soil quality can be estimated by observing/measuring various properties/ processes (Butnariu and Goian 2005). Indicators can be used to determine soil quality indices.

Indicators should be easy to measure, cover the most extreme scenarios (soil types), including temporal variation, and be sensitive to environmental changes and soil management (Faria and Young 2010). Their selection should cover at least two major groups of flowering plants:

– The monocots species: *Avena sativa, Hordeum vulgare, Lolium perenne, Oryza sativa, Secale cereale, Sorghum bicolor, Triticum aestivum* and *Zea mays*.
– The dicotyledonous species: *Brassica campestris* Var. *Chinensis, Brassica napus* ssp. *napus, Brassica Rapa* ssp. *Rapa, Lactuca sativa, Lepidium sativum, Lycopersicon esculentum, Phaseolus aureus, Raphanus sativus, Sinapis alba* and *Trifolium ornithopodioides*.

Current climate changes, farm systems and technology trigger changes in agro-ecological culture. These changes include effects on soil and on crop development caused by technological mistakes, pollution, drought and heat, storms, torrents, landslides, floods and changes in the structure and abundance entomofauna pests able to destroy agricultural ecosystems. Geochemical environment is a result of living matter interaction with the local geochemical environment.

This interaction is reflected in the local route of atoms (biogenic migration).

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This delineation is not clear, because the accumulation of natural chemical elements is greater or less in living organisms (Butnariu 2012b). Cycles of chemical elements (geochemical cycles) have two phases: accumulation of pollutants into the structure of living systems and their release from dead organic matter. In the course of these cycles, there is a permanent exchange of pollutants between the soil, water, vegetation, and the lower troposphere. Macro elements recycling may reach high values compared to trace elements. Toxic cycles are based on the pollutants' course. There are antagonistic microorganisms, those that detoxify and others that enhance toxicity (Cytryn et al. 2012).

Metal pollution occurs in areas where large deposits are present on soil surface, but also where industrial pollution occurs continuously (Garrido et al. 2012).

The biogeochemical functions of living matter are mainly accumulation functions whose occurrence may or may not be dependent on the environment.

Living organisms accumulate pollutants when these are at large concentrations, and consequently the chemical composition of the plant reflects the mineral composition of the soil. Flora's tissues in the vicinity of metal areas has a higher metals concentration as compared to same species growing in environments with low metals concentration. These phenomena are based on the plant's ability to absorb the atoms of elements with low/null physiological importance when their concentration is high (Butnariu and Goian 2005). The plants accumulate pollutants selectively even and where they have low concentrations in biota. So, moreover to its fundamental physiological significance, a greater understanding of the stress response and the factors that modulate it may prove useful in understanding the significance for accumulates elements and in developing new approaches for biomonitoring system. (Schreck et al. 2011). The urochordates or tunicates [Ascidians (Sea Squirts)] accumulate vanadium found at low concentrations and use it in the synthesis of respiratory pigments. Diatomee *Phaeodactylum tricornutum* accumulate iron from the environment. There are circumstances where selective accumulation has no biological significance and the organisms are termed indicators of pollutant concentration.

Relationships among pollutants are based on multiple synergistic/ antagonistic interactions. The relations between abiotic and biotic factors are represented by specific composition, population density, and multiple interactions in ecosystem structure (Antiochia et al. 2007). Relationships between pollutants and abiotic factors are presented in interactions, changes in transport, transformation activities, etc., and are represented by alterations in specific composition, population density, accumulation, or reduction of pollutants.

22.2 Identifying Groups of Lichens, Mosses and Plants, Bacteria, Arthropods and Some Marker Enzymes, Considered Bio Product and Soil Quality Indicators

22.2.1 Lichens

Lichens represent an important species of bio-accumulators due to their ability to uptake pollutants from soil. Being highly sensitive to pollutants, lichens are used as bioindicators for different categories of pollutants: SO_2, NO_X, HF, Cl_2, O_3, peroxyacetic acid (PAA), metals, radioactive elements (Gupta and Igamberdiev 2010), fertilizers, pesticides and herbicides. For example, *Cladonia rangiferina* species can be used as an accumulative bioindicator of U, Fe, Pb and Ti. Other lichens, *Parmelia physodes* (L.), *Parmelia caperata* and *Evernia prunastri* (L.), are the best bioindicators species for specific monitoring of lead pollution.

Mercury is accumulated by lichens, such as *Alectoria capillaris, Alectoria tremontii, Hypogymnia physodes, Cladonia sp.* and *Collema sp*. The best known lichen bioindicator is *Hypogymnia physodes*.

22.2.2 Bryophyta (Mosses)

Mosses are also known to be high sensitive to pollutants. It is known that the number of bryophytes species has decreased significantly in urban areas and polluted industrial facilities. Some moss species have disappeared and the individuals of others have reduced in number (and biomass) in their distribution area (Dragović and Mihailović 2009).

Pleurozium schreberi, *Hylocomium splendens* and *Hypneum cupressiforme* were studied for pollution biomonitoring of eight metals (As, Cd, Cr, Cu, Fe, Pb, Ni, V and Zn) in Northern Europe and observed that react positively to increasing the concentration of metals. Mosses were found to accumulate radioactive elements more intensely than higher plants (Korobova et al. 2007). *Pleurozium schreberi* was used to monitor the precipitation of La, Zr, etc. as a result of nuclear tests, and species such as *Ceratodon* species, *Tortula ruralis* and *Bryum argenteum* were used to biomonitor concentrations of the radionuclide ^{137}Cs following the Chernobyl accident (Suchara et al. 2011). Testing bryophytes, an order of toxicity was established for some metals: Hg > Pb > Cu > Cd > Cr > Ni > Zn (similar to flowering plants).

Some moss species have the ability to accumulate metals up to extremely high concentrations. *Hylocomium splendens* originating from a copper mine, accumulated Pb, Cd, Cu, Zn at a concentration of 17,320 ppm (e.g., in comparison with the community of higher plants *Picea* 349.5 ppm, *Clintonia* 548.5 ppm, in the same environment) (Balabanova et al. 2010).

22.2.3 Higher Plants Bioindicator

Flowering plants and herbaceous species are known as accumulators. Species such as *Lolium perenne* L. and *Lolium multiflora* L. (plants common in parks, roadsides, and highways) are suitable as indicators of pollutants exposure. In addition to metals, these plants are indicators of sulphur and fluor. Additional grass species used as pollution bioindicators are: *Melandrium album* for Pb, *Thlaspi arvense* for Ni, Zn and *Solidago canadensis* for Pb. Species such as *Artemisia vulgaris, Calamagrostis epigeios, Chelidomium majus, Plantago major* and *Poa annua* can also be used as bioindicators of metals. *Equisetum arvense* plants were suggested as bioindicators of mercury, based on the research of the destroyed area of St. Helens volcano (USA) (Falk and Briss 2011) as well.

Achille milefolium species, *Artemisia vulgaris*, *Plantago lanceolata* and *Amaranthus retroflexus* can be used as bioindicators for V and Zn. The species *Hypericum perforatum, Urtica, Hedera helix* show high concentrations of Pb, Cu, Zn, Cd, Hg, indicating increased concentrations of these elements. Plants such as *Vaccinium vitis* and *Vaccinium myrtilus* may indicate high levels of Cd, Mn and Pb.

Plantago lanceolata, since it is common in the urban and the rural areas and its leaves are large enough for analysis purposes, is used as a bioindicator. This specie was classified as an indifferent pseudo-metallophytes, which is able to grow on contaminated soils without abundant presentation or special vitality. Plants are systems designed to give an early warning of soil changes. Plants' early and massive reaction to the presence of pollutants through visible lesions is a good indicator of pollution (Hale et al. 2001, 2010).

22.2.3.1 Deciduous Trees and Shrubs

Leaves of deciduous trees accumulate pollutants from surrounding areas (roads and nearby factories) from soil and directly from air. The more sensitive species are considered to be *Betula pendula, Fraxinus excelsior, Sorbus aucuparia, Tilia cordata,* and *Malus domestica* (Franzaring et al. 2010). Current pollution effects can be observed on their leaves in various forms such as: small scorched patches, following acid rain; pale discoloration warning of the presence of sulfur dioxide, chlorine, fluorine, ozone, lead, etc.; expanding discoloration that turns into necrosis in parallel with increased pollution; and finally underdevelopment and malformation of leaves that indicate pollutant rich toxicity thresholds.

In the case of conifers and broadleaved evergreen leaves such as *Viburnum ritidophylum* the effects of pollution are presented throughout the growing season, similarly to deciduous plants.

22.2.3.2 Accumulator Indicator-Regarded Resistant Species

The following species are regarded as resistant (through hyper accumulation): *Eleagnus angustifolia, Populus canadensis, Salix Alba,* and *Sambucus nigra* (Komárek et al. 2007).

Relatively resistant species that accumulate pollutants are *Carpinus betulus, Quercus robur, Fagus sylvatica*, *Quercus palustris, Acer saccharum* and *Platanus acerifolia*.

For example, *Platyphyllos betula* species are indicative of beryllium soil contamination (Manousaki and Kalogerakis 2009). Trees that accumulate pollutants continuously present the morphological or the biochemical alterations.

Bioindicative information from trees is persistent, as long as they live. Table 22.1 summarizes the principal indicator plants for micro and ultra-micro elements pollutants (Butnariu 2012a).

These changes reflect the progress of the pollutants' stomata penetration, expressed as magnesium separation from the chlorophyll molecule and its transformation into phaeophytin (strongly correlated to humid conditions).

The uptake rates of the ions was in the order B $<$ Fe $<$ Mn $<$ La $<$ Zn $<$ Ga $<$ Cu for the tops and B $<$ Mn $<$ Fe $<$ Zn $<$ La $<$ Cu $<$ Ga for the roots. In the roots, the uptake rates of La, Cu and Ga was exceptionally high. The toxicity of the ions tested

Table 22.1 Indicator plants for microelements and ultra-micro elements and the significant symptoms/diseases

Metal	Indicator plants for micro and ultra-micro elements	Symptoms/diseases
Ag	*Robinia pseudaccacia*	Cause injury to trees and shrubs, chlorosis.
B	*Rosa rugosa*, *Acer campestre*, *A. hippocastanus*, *Morus alba, Platanus hybrida*, *Salix alba*, *Sambuccus nigra*	Susceptible to disease and bark beetle attacks, developed chlorotic spotting.
Be	*Betula platyphylla*	Display white flecks much like freckles.
Bi	*Robinia pseudaccacia*	Genotoxicity induced by the colloidal bismuth.
Ce	*T. occidentalis, Aesculus hyppocastanus*	Translocation into newly grown leaves.
Co	*Sophora japonica*	Problems that looks similar to fungal leaf spots, particularly those of fungus Marssonina.
Cr	*Koelrenteria paniculata*	Cr(VI) is highly toxic and mobile whereas Cr(III) is less toxic. Alterations in germination process as well as in growth of roots, stems and leaves, which may affect total dry matter production.
Cs	*Rosa rugosa*	Ultrastructural malformations of cell components.
Eu	*Rosa rugosa, Thuja occidentalis*	Aberration of cell cycle, disruption of cytoskeleton, deregulation of gene expression related with programmed cell death.
Fr	*Sophora japonica*	Has no biological role and is most likely extremely toxic, but in large quantities can provoke developed chlorotic spotting.
Ga	*Rosa rugosa, Thuja occidentalis*	Leaves did not unfold completely, had a needle shape.
La	*Rosa rugosa;*	Has a low to moderate level toxicity, but in large quantities can provoke developed chlorotic spotting.
Mo	*Robinia pseudaccacia*;	Leaf blade formations (whiptail) are typical visual symptoms.
Ni	*Rosa rugoza, Sophora japonica, T. orientalis, A. hyppocastanus*	Single white bands perpendicular to leaf veins appeared on primary leaves.
Sb	*Thuja occidentalis, Aesculus hyppocastanus*	Did not develop diagnostic fully expanded true leaves, or at harvest, these leaves were dead.
Sn	*Thuja occidentalis*	Susceptible to bark beetle infestations and display needle mottling and loss.
Th	*Rosa rugosa*, *Thuja occidentalis, Abies alba*, *Picea excelsa*	Has no biological role; most likely extremely toxic; passive absorption implies diffusion uranyl ions, organically bound Th^{4+} by soils endodermis of roots, does to their imperfect selectivity, increased permeability cell membranes.

(continued)

Table 22.1 (continued)

Metal	Indicator plants for micro and ultra-micro elements	Symptoms/diseases
U	*Rosa rugosa*, *Thuja occidentalis Abies alba*, *Picea excelsa*	Depleted on growth of tree, one of species showed evidence of hormesis.
V	*Rosa rugosa*, *T. occidentalis*, *R. pseudaccacia*, *A. hyppocastanus*	Trees lose leaves prematurely and look rather sickly.
W	*Robinia pseudaccacia*	Inhibitor of molybdoenzymes, it antagonizes molybdenum for Mo-cofactor, of these enzymes.
Zn	*Robinia pseudaccacia*	Causes severe Fe-deficiency chlorosis.

was in the order $Mn < Zn < B < Fe = Ga < La < Cu$ in the tops and $Mn < Ga < Zn < Fe = La < Cu < B$ in the roots (Wheeler and Power 1994).

These changes reflect the progress of the pollutants' stomata penetration, expressed as magnesium separation from the chlorophyll molecule and its transformation into phaeophytin (strongly correlated to humid conditions). The effects of pollution on bioindicator trees are apparent at the individual level in the form of morphological alterations (discoloration and necrosis), morphological changes (smaller leaves, malformed, shorter internodes, smaller flowers or miscarriage), premature leaf fall and reductions in crown transparency and branches desiccation. The correlation between pollution and accumulation in leaves show that tree leaves accurately reflect soil pollution and its quality.

Deciduous trees are a biologically effective "filter." Above a certain threshold of pollution, forests become vulnerable either directly though disrupted metabolic processes or indirectly through ecological imbalances.

22.2.3.3 Conifers

In comparison with deciduous trees, conifers are more sensitive indicators due to their leaves' (needles) life span (3–4 years) and their exposure to pollution also in winter periods.

Sulphur dioxide (SO_2) and hydrofluoric acid (HF) pollution may be indicated by direct needles analysis (as conifers are well known accumulators). For example, *Pinus, Picea abies, Pinus banksiana, Pinus nigra, Pinus silvestris, Pinus strobus* and *Larix decidua* are sensitive to SO_2 pollution (Acquaviva et al. 2012), while *Abies alba, Picea abies, Pinus ponderosa, Pinus silvestris* and *Pinus strobus* to HF (Samecka-Cymerman et al. 2006).

When dealing with metals, *Picea abies, Pinus silvestris, Pinus nrigra, Taxus baccata* and *Thuja occidentalis* are particularly suitable as indicators of Fe, Mn, Cu, Pb. Zn, Cd, Ag and Hg pollution. *Taxus baccata* plants were used to assess metal pollution and content (Pb, Cu, Cd, Ni, Cr, Hg) indicating a pollution reduction along the trees' development (Antiochia et al. 2007). Some conifers species, *e.g., Picea abies, Pinus banksiana and Pinus strobus,* were used to indicate pollution by photochemical oxidants, (Gupta and Sinha 2007).

The concentrations of uranium in the tissues of the conifers decreases in the following order: roots $>>$ stems $>$ twigs $>$ needles.

22.2.4 Microorganisms Role

Soil biological activity is largely concentrated in the topsoil (~30 cm). In the topsoil, biological components occupy a small fraction (<0.5 %) of the total volume and can reach up to 10 % of the total soil organic matter. These biological components are soil organisms, especially microorganisms. The soil contains a large and complex microbial diversity whose activity is essential for soil processes.

Direct detection of specific DNA sequences from soil bio-communities is an effective way to gather information on soil processes. However, soil activity does not depend on the number of genes present in the microbial community, but on their expression.

One approach is to extract genes directly from soil and detect the mRNA that is present (messenger RNA, the first transcription product). Soil surface heterogeneity is conveyed by colloidal matrices (mostly clay and organic matter, such as humus) able to adsorb nucleic acids, and enzyme inhibitors (mostly humic acids and ubiquitous ribonucleotids), which presents technical difficulties (Ahmad et al. 2012; Dong et al. 2012).

Heterotrophic organisms are those responsible for biosphere recycling, exploiting favorable thermodynamic chemical reactions to obtain energy and carbon from dead biomass. Their genesis lies in the oil fields as well as in microbial biodegradation of residual hydrocarbons in soil (Meynet et al. 2012). Microorganisms have ecological significance based on their ability to utilize gaseous or liquid hydrocarbons, solid aliphatic series, aromatic and asphalt, a process called bioremediation (Briones 2012).

Among the microorganisms involved in biodegradation of pollutants one can find bacteria, fungi, yeasts, and algae, bacteria and fungi being the most important groups.

22.2.5 Bacteria and Fungi ("The Benevolent Scavengers of Nature")

Among bacteria domain, aerobic bacteria such as: *Achromobacter sp., Acinetobacter sp., Actinomyces sp., Alcaligenes sp., Arthrobacter sp., Bacillus sp, Brevibacterium sp., Corynebacterium sp., Flavobacterium sp., Micrococcus sp., M. sp., Nocardia sp., Pseudomonas sp., Spirillum sp., Serratia sp., Rhodococcus sp.,* and *Vibrio sp.*, followed by anaerobic ones such as: *Geobacter metallireducens, Thauera aromatica,* *Desulfococcus multivorans, Clostridium sp.,* and *Desulfobacterium cetonicum*, are used as indicators. For example, the survival effect (toxicity) of genus *Rhizobium* under pollutant stress has been confirmed by many authors. When *Bradyrhizobium japonicum* was added (9×10^8 cells/mL) to two soils supplemented with different doses of sewage sludge, less than 1 % of the bacteria were present in both soils, after 42 days (Shentu et al. 2008). These drastic reductions were attributed to metals presence in sewage sludge.

As decomposers, fungi are often associated with woody vegetation (tolerating large amounts of tannins). Consequently, mushrooms are an organic part in the formation of soils and also playing a fundamental role in soil food chains. From the biological perspective, the parasitic species too are valuable, eliminating weak and sick individuals.

Symbiotic associations through mycorrhiza, ensure the lives of many trees, shrubs and herbaceous species. Fungi are natural indicators of pollution. Mushrooms easily accumulate metals, pesticides and radioactive substances. Chemical analysis of fungi reveals the “black box” of pollution status of a certain habitat, e.g.: mushrooms are highly sensitive to acid rain (absent when acid rain is present).

Fungi involved in hydrocarbon degradation are part of the genera: *Alternaria, Apergillus, Cephalosporium, Cladosporium, Fusarium, Graphium, Geotrichum, Mucor, Penicillium, Rhizopus* and *Trichoderma*. Genus *Achromobacter* was used in the degradation of carbazole and phenanthrene (*Achromobacter xylosoxidans*).

Of the genus *Bacillus* species *Bacillus firmus* is remarkably able to degrade completely acenaftilena, anthracene, benzo[β]fluoranthene and reduce concentrations of naphthalene, dibenzo[a,h]anthracene and indeno[123–c,d]pyrene (Schneider et al. 1996). *Bacillus licheniformis* is able to degrade completely anthracenebut only to reduce other hydrocarbon concentrations. *Bacillus subtilis* completely degrade acenaphthene, anthracene and benzo[b]fluoranthene and reduce the concentration of naphthalene, indeno[123–cd]pyrene and toluene (Magyarosy et al. 2002). *Bacillus pumilus* is effective in achieving the biodegradation of hydrocarbons degradation rate of 86.94 %. *Brevibacterium* genus isolated from soil degraded 40 % of the hydrocarbons within 12 days. *Mycobacterium* species that are able to degrade hydrocarbons are *M. lacticola, M. luteum, M. phlei* and *M. rubrum*.

The last three species degrade gasoline, oil and paraffin (Liang et al. 2011).

Of the *Pseudomonas* genus is *Pseudomonas alcaligenes* noted that degrades naphthalene, benzo[b]fluoranthene and indeno [123–cd] pyrene and reduce the quantities of anthracene, benzo[a]anthracene and benzo[ghi]perylene and *Pseudomonas putida* which has the ability to degrade organic solvents such as toluene or naphthalene (Björklöf et al. 2009) at fungi the genera *Aspergillus*, *Penicillium, Paecilomyces* and *Fusarium* are able to biodegrade hydrocarbons. *Penicillium* genus, has the ability to degrade 90 and 75 % phenanthrene (Vacca et al. 2005), and *Cladosporium resinae* species is able to degrade aliphatic hydrocarbons.

Genus *Rhizopus* (filamentous fungi, soil, fruits, vegetables and stale bread) was isolated and studied in terms of the ability of biodegradation of hydrocarbons (Chikere et al. 2011).

Biodegradation takes place in several stages and is not the result of one specific body (many strains of microorganisms acting synergistically). Between some species as *Nocardia* and *Pseudomonas*, which can degrade cyclohexane association is established phenomenon of synergism. *Nocardia* by using ciclohexanone, produce intermediate compounds, which are taken by Pseudomonas; and *Pseudomonas* produce growth factors (biotin), required *Nocardia* bacteria growth (Christ et al. 2005).

Penicillium and *Rhodococcus* have been shown to be effective in the degradation of polycyclic aromatic hydrocarbons, while *Rhodococcus* with *Aspergillus terreus* had synergistic relationships (Kılıç 2011). Microorganisms and microbial communities are an integrated measure of soil quality, an aspect which cannot be obtained by physical or chemical measurements and / or analysis of large organisms. To prevent irreversible environmental consequences, bacterial parameters were found to be sensitive to pollution, may be included in evaluation studies and monitoring strategies for contaminated soils.

22.2.6 Invertebrates

The group of invertebrate animals found in soil is made up of worms and arthropods.

The species of *Arthropoda* living in soils comprises different organisms (arachnids, myriapods, insects, etc.) that feed on plant debris or fungus mycelium. Arthropods (*Aranea, Acari* and *Insecta*) in soil, especially mites, are ubiquitous in terrestrial species.

Mites contribute indirectly to the decomposition cycle of organic matter and nutrients, regulating other populations of invertebrates. Animals in soils are key regulators of nutrients in the soil report. Most species of *Mesostigmata* are sensitive to pollution. Information obtained about arthropod species can be used to characterize almost any aspect of an ecosystem accurately (Antunes et al. 2011).

22.2.7 Metabolic Substances

There are many metabolic substances (bioproducts) that can be used as indicators of soil quality; these include sterols, antibiotics, proteins, enzymes, etc.

22.2.7.1 Ergosterol

Ergosterol (ergosta-5,7,22-thrien-3β-ol) is a vital component and the main endogenous sterol cell membrane of fungi, actinomycetes, and some microalgae (micosteroli).

Microorganisms are able to synthesize compounds isoprene residues bonds with acetic acid CoA participation. The most common micosterols are ergosterol and other sterols zimosterolul in smaller amounts. Ergosterol concentration is an indicator of increased fungal activity of organic compounds and mineralization activity.

Metals and some fungicides reduce metabolic activity by between 18 and 53 %, but do not affect ergosterol content. Other fungicides reduce the ergosterol content of biomass (Robine et al. 2005). Research conducted in grasslands and arable soils have established a link between amount of ergosterol in the hyphae of fungi and soil stability.

22.2.7.2 Glomalin

Glomalin is a glycoprotein produced by the hyphae and spores of arbuscular mycorrhiza fungi (*Glomus*) in soil and roots. Glomalin store carbon in the form of protein and carbohydrates (glucose). Glycoprotein penetrates organic substances, which are attached to particles of sand or clay. Glomalin contains about 30–40 % carbon to form associations with soil. This material aerates grained soil and fixes the carbon into it, and increases air permeability and water storage capacity in the soil. Due to glomalin, soil structure is favourably changed. Glomalin-related proteins in soil have been studied as a biochemical marker in the ground, mainly because of their stability. Glomalin is a complex substance, that is water-resistant to biodegradation, has adhesive properties (Vodnik et al. 2008), for the soil activated by photosynthetic plants (through root exudates, and in particular by root exudates of plants symbiosis producing mycorrhizal fungi). Mycorrhiza mushrooms biosynthesize phytohormones, which accelerates root development. Use of *Medicago sativa* plants inoculated with *Glomus mosseae* and *Glomus intraradices* showed a higher stability of unpolluted soil (1–2 mm) and the overall stability of soil was positively correlated with the soil and mycorrhizal root volume and low the non-mycorrhizal soil (Bedini et al. 2009). Polluted soil is poorly mycorrhizal and is low in glomalin.

22.2.7.3 Enzyme Activity

In general, the activities of soil enzymes change earlier than other parameters, thus representing early indicators of soil quality change. Methods for determining enzyme activities are more appropriate, offering indicative data in a shorter time than microbiological analyses of the soil biodegradative process.

Soil enzyme activities may be used to indicate the change in the plant-soil system, because enzymes are closely related to the nutrient cycle and soil biology and it is easy to quantify and integrate information about the status of microbial and physico-chemical properties of soil and changes in proportion to changes in the soil.

The enzyme locking mechanism is based on the reaction with amino pollutants, imino, and sulfhydryl protein, some metals competing with major elements, replacing them in metal enzymes. Other pollutants can harm cells and cause biochemical disorders, such as nitrogen and phosphorus mineralization, cellulose degradation, and nitrogen fixation. Studies of individual enzymes (with conflicting results) showed temporal and spatial variability (Garcia-Ruiz et al. 2009). Enzyme activity was associated with indicators of biogeochemical cycles, degradation of organic matter, and soil remediation processes, so that they can establish with other physical or chemical properties, soil quality. Enzymes are called indicators because they are closely related to organic matter, have physical, microbial activity and biomass of soil; they provide information on changes in quality and are evaluated quickly.

Enzymes have different origins (bacteria, fungi, plants, and macro invertebrates), different locations (intra or extracellular), different matrix association (alive or dead cells, clays or/and humic molecules), and various laboratory test conditions.

β-glucosidase (β-GLU) is an indicator of soil quality due to its importance in catalytic reactions for the degradation of cellulose, glucose being released as a source of energy to maintain microbial biomass and metabolic activity in soil. It plays a role in energy availability in the soil, which is directly related to the content of C and the ability to stabilize soil organic matter.

In general, microorganisms produce an enzyme complex where xylanases are associated with cellulases, β-GLU, etc. It protects the substrate mycelium, which lyses against other existing bacteria in the soil that could be used as a source of carbon and nitrogen, thus keeping it to air mycelium growth (Bonet et al. 2012). β-GLU activity is inhibited by the presence of metals and other pollutants.

Phosphatase (PHO). Phosphorus is essential for plant growth. Much is immobilized due to the intrinsic characteristics of the soil (pH) that affect nutrient availability, enzyme activity, and soil amendment equilibrium solid phase. PHOs are a group of enzymes that catalyze the hydrolysis of phosphoric acid esters and anhydrides, but participate in the metabolism of phosphorus compounds (nucleotides, sugar phosphates, and polyphosphates).

Because of their activity in acidic and alkaline soil conditions, phosphomonoesterases were the most studied.

PHO's role in the mineralization of soil organic phosphorus substrates. PHO activity in the soil can be influenced by soil properties (number of aerobic bacteria, ammonification level, the number of active bacteria in ammonification, nitrification degree, and the number of fungi).

PHO is a consequence of increased activity caused by the accumulation of quantities of organic matter and pollutants, representing a detoxification mechanism for microorganisms (Wang et al. 2012). Microorganisms in polluted environments are characterized by pronounced acid phosphatase synthesis, an activity that results in the formation of phosphates, used to precipitate cellular metals as metal phosphates.

Dehydrogenase (DEH) This enzyme catalyzes the release of the hydrogen ion (proton) from the molecular complex and is involved in redox processes. Its activity reflects good soil microbial activity (respiratory potential of soil microbiota). The sensitivity of DEH activity to pollution can be explained by the fact that the dehydrogenase is active inside living cells, intact, unlike other enzymes that act outside the cell. DEH activity was found to be sensitive to pollution with Cd, Pb and Zn (Wyszkowska and Wyszkowski 2010).

Intensity of the microbial metabolism in soil can be assessed by measuring dehydrogenases. Soil microflora activity is due to come, capable of multiplication and is the result of DEH's, which are components of the enzyme (indicator of biological redox systems).

Actual and potential activity due to the proliferation of living organisms and DEH interfere in H dislocation in the soil, being reductase. DEH activity is an indicator of overall soil biological activity. Determination of the activity is a global test of biological activity, DEH, reflecting the activity of microflora and anaerobic microorganisms, having the advantage that it can be applied to a number of samples.

Catalase (CAT) (the enzyme first investigated in soil) is produced by microorganisms and plants, and is characterized by high persistence. Its role is to break down toxic hydrogen peroxide, which is formed in the aerobic respiration of microorganisms being produced as a result of mitochondrial electron transport and due to various hydroxylation and oxygenation reactions. It correlates with the amount of humus, the pH, and the number of microorganisms in the soil. Accumulation, like the destruction of CATs in soil, is determined by mineral fertilization in strict interaction with the development of soil microflora. In soils deficient in nutrients and energy material, the degree of accumulation of CATs is increased as a result of mineral fertilization, the absolute level of this enzyme's accumulation in soils cannot be reached with high fertility.

DEH and CAT activities decrease with depth, due to reduced oxygenation (Cookson et al. 2007). T enzyme activities (β-GLU, PHO, DEH, and CAT) of polluted soils have values that differ from than normal.

22.3 Answer to Plant Defenses to the Soil Contamination

Phenolic compounds represent a group of secondary metabolites with antioxidant properties and are involved in plant adaptation to stress conditions.

During normal processes of growth and development, plants are subjected to various types of biotic and abiotic stress, such as drought, high salinity, mineral nutrient deficiency, ultraviolet light, extreme temperatures, hypoxia, and toxicity of metals, herbicides, fungicides, gaseous pollutants, and pathogen attack. Phenols are considered important biomarkers for phytotoxic effects of heavy metals and other pollutants. Increased production of phenolic compounds is probably a part of the defense mechanism of the plant.

Phenolic compounds are of interest because of their role in allopathic ecological processes, their role in a plant's protection against herbivores, and their involvement in the response to stress, such as competition between and/or interspecific to or pollution. Phenols are produced in the cytoplasm and form droplets in vacuoles, which later evolve into one vacuole filled with phenols.

Under these conditions of degenerating cytoplasm, organelles disappear, eventually leading to the release of cytoplasmic and/or vacuolar contents and mesophyll cell death.

Secondary metabolites are accumulated as a result of stress conditions. Impregnation of cell wall phenolic esters, suberisation of cores, and lignification defense responses appear to stabilize the cell wall architecture against degradation. Plants can synthesize and accumulate phenolic compounds, as a physiological response to soil contamination.

These compounds occur as a result of changes in various pathways of plant metabolism and not by submission in the external environment, because the epidermis (both cells and their external walls) are intact. In some regions of the leaflets, areas of necrosis are observed, cells having disintegrated after accumulation of phenolic compounds. The epidermis does not show significant changes in the general shape of epidermal cells or stomata.

In the fine sections (seen in the electronic microscope) in leaf mesophyll cells collected from unpolluted areas (witness), numerous chloroplasts with defined borders, with small dens formations, were observed. Cells have large central vacuoles (Gorshkova et al. 2010).

Sections through leaves harvested from polluted areas show varying degrees of disorganization in the mesophyll. In leaf mesophyll cells containing chloroplasts that upload large starch granules some of which are transient, cytoplasm shows signs of degeneration (Carpita et al. 2001). In some sections, there are sometimes electrono-transparent formations in the cytoplasm and vacuole. In most cells, there are electrondense deposits along the cell walls (Diotallevi et al. 2010). In many mesophyll cells of leaves collected from polluted sites, the vacuoles were larger than the control (these vacuoles push the cytoplasm to the cell wall). Therefore, the shape of the chloroplasts appears flattened (lenticular as compared to mesophyll cells from controls, harvested from unpolluted areas). In some cells, the vacuoles are filled with electrondense material, while in others they are completely transparent (Korbas et al. 2008). The tonoplastis remains intact in both cases, which suggests a continuation of various cellular components within vacuoles. Electrondense deposits, probably degenerate residues of vesicles, can be seen in intercellular spaces.

In leaves, along cell walls, compact electrondense material storage is observed, with some even being inlaid in the wall. These deposits are present in intracellular spaces and the cell periphery (Migocka et al. 2011). In a more advanced stage of degeneration, they were observed in mesophyll cells near the upper epidermis. They contain chloroplasts that the plastidial envelope tilacoid membranes degraded and system changes within the grain. Inside chloroplasts, electronodense particles are visible (Lindeboom et al. 2008).

Cellular organelles gradually degenerate as a result of cell death. At this stage of cell necrosis, no the form of organization remains, core is no longer observed, as mitochondria and vacuoles merge with the cytoplasm, leading to complete degeneration. Electrondense deposits are unevenly spread cells still adhering to the walls (McCann et al. 2007).

The presence of phenol compounds in the cell is not an unusual phenomenon for many plant species, but their precipitation in the cytoplasm and vacuole is an indication of impaired cellular metabolic pathways as a result of stress induced by various polluting substances (DalCorso et al. 2010). The presence of these deposits may be due to the fact that the properties of phenols change depending on the amount of pollutants in the environment (Simmler et al. 2010; Butnariu and Coradini 2012).

Some phenolic compounds accumulate around the chloroplast envelope and cytoplasmic vesicles, and others around the tonoplast. Sometimes, these compounds were observed even within cell walls (Yu and Jez 2008). The presence of phenolic deposits near the plasma membrane and double membranes surrounding organelles, chloroplasts, and mitochondria is consistent with subcellular sites of their biosynthesis. The appearance and accumulation of phenolic products in vacuoles and in the area apoplast (cell wall) have a role in eliminating the free radicals produced by cell metabolism (Balakrishna et al. 2009; Butnariu 2012a).

Flavonoids located near membranes prevent the lipid oxidation that affects them when subjected to the action of free radicals. Researchers have identified the role of antioxidant phenolic compounds' accumulation in leaf mesophyll cells in areas exposed to pollution, and suggest that they help reduce oxidative stress and limit the damage to the photosynthesis mechanism (Singh et al. 2012).

The accumulation of metal ions in cells stimulates the production of phenolic compounds, and therefore, the presence of metals (lead) could be one of the factors that determine the accumulation and precipitation of polyphenolic compounds in vacuoles, cytoplasm, or intercellular spaces (Bu et al. 2012; Butnariu and Giuchici 2011).

The effects of polyphenols occurring at the cellular level as a result of oxidative stress response are still unclear. While small amounts of polyphenols may be protective, their production in large quantities as a result of the sustained action of pollutants is lethal to cells, leading initially to microlesions, and subsequently to necrosis even in different areas, depending on the intensity of the action of pollutants.

Polyphenolic deposits are located near the cell wall, but inside it they appear in intercellular spaces and they adhere to the primary wall. Lead has an affinity to bind to pectin and cellulose, more easily than to hemicellulose (Rodríguez-Durán et al. 2011; Butnariu 2011). The middle lamella of the cell wall is composed of pectic substances in nature and primary cell wall contains mostly cellulose and hemicellulose (appearing as electronodense deposits in cell walls, binding reveals pollutants).

Lead acts as a pollutant, by binding to pectic substances in the middle lamella structure and the composition of the primary cell wall (Sayadi and Ellouz 1995).

Electronodense deposits in the intercellular spaces were also reported in maize (*Zea mays*). Their presence suggests the transport of lead through the cell wall apoplast, and precipitation and accumulation in these areas. The changes induced by pollutants are related to the sclerenchyma structure whose morphogenesis is visibly affected (Salgado et al. 2005). In addition to normal sclerenchyma fibers with uniform walls and moderately thickened and lignified areas, a thin-walled fiber, sometimes wavy, appears which reduces significantly the functionalities for supporting the stem (Tiveron et al. 2012). Necrotic cells within which large amounts of tannin are accumulated are observed adjacent to sclerenchyma beads.

Influence of pollutants leads to ultra-structural changes that are evident in all the analysed species. The most common changes are related to the accumulation of polyphenolic products in both leaf mesophyll cells and epidermal cells (Burk and Ye 2002). Polyphenols biosynthesis following exposure to stress can support the premise that these substances are major structural biomarkers that can be used to highlight the influence of pollutants on plants, both woody and herbaceous (Hale et al. 2001). As well as the presence of polyphenols (flavonoids), other ultrastructural changes were observed. Thus, chloroplasts are affected by the presence of pollutants, mechanisms that block starch leaching during the night (stroma accumulate, leading to the disruption of the membrane system), and other changes (Fini et al. 2011; Butnariu and Corneanu 2012). Impaired chloroplast photosynthesis efficiency decreases (as determined by blocking parallel with stomatal clogging ostioles solid deposits).

SOD analysis proves that the enzyme is present in media *Plantago media, P. lanceolata, Lotus corniculatus, Mentha sp., Potentilla anserina, Polygonum sp., Geranium sp., Linaria vulgaris* and *Cornus sanguinea*, and its level was significantly different depending on the amount of pollutant (Jozefczak et al. 2012). Pollutants induce synthesis of SOD in *Polygonum sp.*, where the level of the enzyme is over four times that in the control sample.

The species *C. sanguinea* and *Geranium sp.* that have the ability to defend themselves against the action of pollutants through increased synthesis of SOD, behave similarly, while *P. lanceolata, Mentha sp., P. anserine* and *L. vulgaris* have a different behaviour, since SOD synthesis is inhibited by pollutants. SOD can be considered as a marker of pollution, for some species, but in *Plantago, Mentha* or *Potentilla*, this indicator is not significant (Li and Hu 2005). For catalase, it is found that there is variability in terms of the constituent components. Considered as the next step in removing the reactive forms of oxygen, the catalase in different studied species is distinguished by intense catalase activity in *Polygonum sp.* and *L. vulgaris*, while the species *C. sanguinea, Plantago sp. and P. anserina* have reduced enzyme activity values.

Pollution significantly inhibits catalase activity in the species *Geranium sp. and L. vulgaris,* and stimulates it in the species *P. lanceolata, Mentha sp.* and *P. anserina*. The presence of cholinesterase indicates that in the species *Trifolium* *pratense* enzyme activity is more intense than in the species *L. corniculatus* (Keunen et al. 2011).

In conclusion, SOD may be a biomarker for species *Polygonum sp., Geranium sp.* and *C sanguinea* and catalase for *Mentha sp., P. anserina, L. vulgaris, P. lanceolata* and *C. sanguinea* (Roberts et al. 2004). For different degrees of pollution, enzyme activity (superoxide dismutase, catalase, and cholinesterase) is characteristic of each species.

22.4 Final Remarks

The management of contaminated soils using plants, microbes, and other biological systems to degrade/convert environmental pollutants under controlled conditions to a level at which they become harmless or that is lower than the limit set by regulatory authorities is an on-going challenge for researchers, industry, and regulators. Thus, the identification of indicators of soil pollution and bioremediation, applied for the rehabilitation of contaminated soil ecological reconstruction, provides an alternative that is feasible and economically and environmentally sustainable. In this context, we can use a number of biological agents that include both distributed heterogeneous microbial communities and plants with different origins. The sustainability of soil depends on the one hand on the characteristics of some physical, chemical, and biological properties that effect the soil's relative stability, and, on the other hand, how natural and anthropogenic factors act on it. Given the role of soil is actually an essential active mediator of the processes, which is the basis of life on earth, a complex biomonitoring system to identify and remove sources of pollution in order to maintain maximum ecological potential is necessary and important for soil quality.

Accurate biomonitoring is essential for anticipating risks to the environment and human health. In the future, further studies are needed to understand the genetic diversity of the microbial populations that are susceptible and tolerant to pollutants and the interactions between pollutants and soil microorganisms in natural conditions. There are several biological properties of the soil that can be used as indicators of soil quality, alone or in combination with other chemical or physical properties. However, they are far from universal and should be chosen according to the situation. From another point of view, there are many properties that are difficult to quantify and interpret, but require simpler and less costly observations, similarly, should be used and properties that are sensitive to changes in management. Appropriate strategies should be established for sampling and analysis with many variants results as essential factors to consider when using biological indicators as markers indicators of soil pollution.

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