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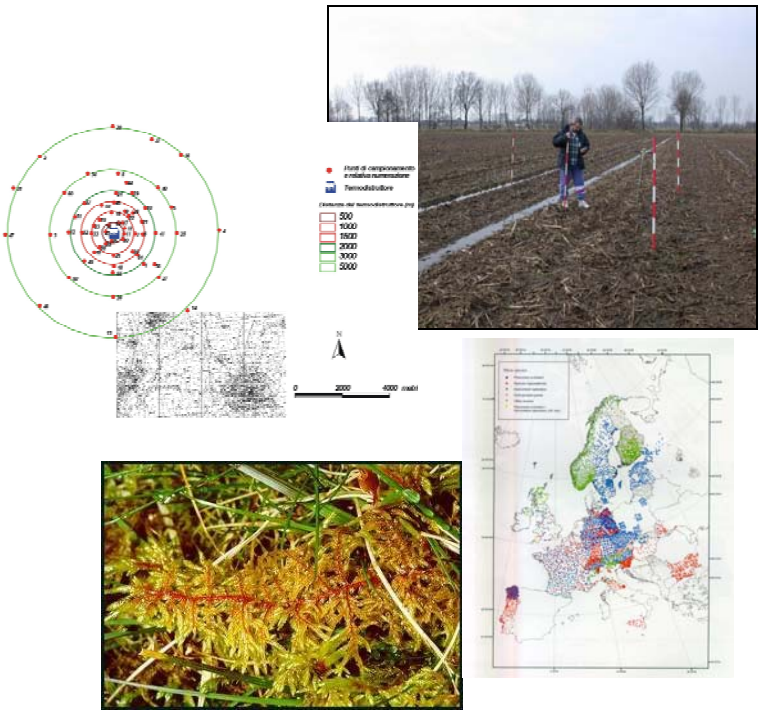
GUIDELINES FOR THE USE OF NATIVE MOSSES, TRANSPLANTED MOSSES AND SOILS IN ASSESSING ORGANIC AND INORGANIC CONTAMINANT FALLOUT

Roberto Michele Cenci



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Preface

The European Commission's Joint Research Centre in Ispra (Italy) aims to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

The Guidelines presented in this document, by adhering to the ideals of the Joint Research Centre and in a specific way the Institute for Environment and Sustainability, are a tangible example of equality and democracy that reflect the politics of the EU. The Guidelines have a strategic importance in both a scientific and social context as they allow researchers to converse in a single, common, scientific language. This transparency will reduce and minimize the possibility of errors and misunderstandings. They Guidelines operate by concurrently supporting and binding the diversity of approaches used by different researchers. The Guidelines represent a departure point for environmental studies aiming to evaluate the quality of air and soil. They are a reference to map out a common line that, on the one hand, will allow the comparison of results at a European level while, on the other, will enhance collaboration between individual researchers.

Through cohesion between different nations speaking a single scientific language, the Guidelines will strengthen the political concept of a single European Community.

From an operational point of view the Guidelines represent a novel innovation because they link the state of the soil with a bioindicator, in this case a moss. In this manner, the Guidelines allow us to obtain information about the quantity and origin of persisting organic and inorganic contaminants that are deposited on the surface of the soil. By using these Guidelines, it will be possible to observe and manage social, recreational, industrial and agricultural activities in a more effective manner. I am certain than these Guidelines will find widespread use at national and European levels.

Dr. Guido Schmuck

Director f.f. of the Institute for Environment and Sustainability, European Commission

Preface

The collaboration between the Joint Research Centre of European Commission (JRC/EC) and APAT on the issue of biological indicators, which started ten years ago with the Workshop on "*Biological monitoring of air quality in Italy*" by the Agency (called then ANPA), is now endorsed with the publication of these "*Guidelines for the use of native mosses, transplanted mosses, and soils in assessing organic and inorganic contaminants fall-out*".

After the publication of the Workshop Proceedings (ANPA 1999, *Serie Atti* 2/1999), we observed an increasing development of monitoring activities that supported a methodological refinement and enlarged the implementation framework, producing however non-homogeneous data throughout the Italian territory.

This volume reports in a simple and effective way the basic procedures that this kind of biological monitoring require, to regulate evaluation activities on atmospheric pollutants fall-out, so as to use common, widespread and shared methods, and to express the collected data according to comparable assessments, even among different Countries.

It represents a contribution to collate homogeneous ecological data, to improve environmental information, and to publish reports to support the politics of pollution prevention, reduction, and remediation.

Dr. Andrea Todisco

Director, Nature Protection Department, Agency for Environmental Protection and for Technical Services (APAT)

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GUIDELINES FOR THE USE OF NATIVE MOSSES, TRANSPLANTED MOSSES AND SOILS IN ASSESSING ORGANIC AND INORGANIC CONTAMINANT FALLOUT

Abstract

These guidelines on the use of mosses are of strategic importance in that they make it possible to harmonize the indications obtained from differing environments in terms of extent and, thereby, to compare them with results obtained using other methods. Thanks to their morphological features, mosses can be used as bioindicators to obtain fallout information for a significant number of organic and inorganic contaminants of human origin and determine the quantity of such contaminants deposited in soil. Analysis of both moss and soil is essential in identifying, and discriminating between, anthropic and natural sources of contaminant fallout.

Key words: mosses, soils, dioxins, heavy metals, radioisotopes.

1. Introduction to the use of native mosses, transplanted mosses and soils in assessing organic and inorganic contaminant fallout

1.1 Introduction

In 1999 Italy's Environmental Protection Agency (ANPA) published a paper on the methods to be used in the case of mosses and soils [1]. After nearly a decade, a review is advisable. The attached Guidelines take account of numerous experiments in the environmental field and include a short description of bryophyte biology.

A great many pollutants are present in our environment. In addition to traditional gaseous air contaminants such as CO, CO2, SO2, O3 and NOx, a number of other substances, produced mainly by human activity, may be present in solid, liquid and gaseous form. Notable among these substances, which are constantly rising in number, quantity and importance, are persistent inorganic contaminants, radionuclides, dioxins, phuranes, polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs). Distributed across the various environmental compartments, these substances pose grave problems to plants, animals and man in so far as their accumulation may have consequences that are both unforeseeable and difficult to evaluate[2]. A study on heavy metals in the various environmental compartments showed the decisive role played by human activity in the global cycle of these elements. The main sources of anthropic pollution are the chemical, engineering and metalworking industries, power stations, domestic heating installations and the internal combustion engine [3].
In addition to the pollution levels recorded in densely populated and industrial areas, consideration must also be given to the dispersion of contaminants in the air and their transportation beyond national borders, possibly to remote areas devoid of direct anthropic pressure (e.g. Antarctica).
In view of the quantities of pollutants and the area concerned, evaluating air pollution and contaminant fallout requires new monitoring instruments to work alongside and, in some cases, replace, traditional methods.

1.2 Soils

Soil analysis is to be regarded as crucial in assessing organic and inorganic pollution in a given area. Furthermore, if soil analysis is supplemented by moss analysis, enrichment factors can also be assessed, allowing a better and more thorough interpretation of the findings.
Soil is constantly evolving, subjected as it is to major influences such as weather, morphology, vegetation, animals and human activity. "Soil" means the surface layer which, over the years, has developed into distinct layers or horizons [4].
Soil consists of minerals and organic matter. The minerals are generated by weather-related changes in rocks, changes which produce fragments of different sizes. Organic matter, on the other hand, consists of animal and plant organisms and the products derived from their decomposition.
Moving from the soil surface down towards the parent rock, both the percentage of organic matter and the effects of the air decrease, whereas the effect of rock increases.

If we look at a general example of a soil profile, we see that it consists of four horizons. The uppermost horizons (A and B) constitute soil in the stricter sense of the term: they are richer in organic matter and are host to most biologic processes; horizon C is located at a greater depth and consists of altered parent rock; horizon D is the bedrock. Horizons A and B comprise sub-horizons brought about by pedogenic factors or agents that play a role in soil development.
The main pedogenic factors are:

- Climate.
- The type of parent rock.
- The pedogenetic time span.
- Biological activity.
- morphology.

A fundamental limitation of soil analysis is that the results can be affected by the geological composition of the rocks, giving rise to a risk of misinterpretation. For example, high concentrations of a substance could be attributed to anthropic contamination, when the actual cause might be the geochemical nature of the land concerned. Soil analysis alone does not, therefore, constitute a sufficiently reliable means of determining contamination levels.

1.3 Biomonitoring

Full detailed and thorough information on the state and effects of air pollution can be obtained only through chemical and physical air analyses supplemented by bioassay tests.
Researchers have in recent years looked at various methods of assessing the deposition of heavy metals and organic contaminants in order to obtain adequate and sufficient information on both a large and a small scale and at reasonable cost.

Figures 1 and 2 show environmental biomonitoring in Europe, with mosses tested for the presence of 10 inorganic contaminants [5] [6].

Figure 1. [5] Moss-sampling areas in Europe in 1990-92.

The concept of "biomonitoring", i.e. environmental monitoring by means of living organisms, is based on the principle that a toxic substance can in some cases be detected in living organisms, thus revealing whether those substances are present in the environment and, by the same token, giving a rough initial indication of the quantities concerned. Generally speaking, all living organisms react to the various environmental pressures, be they natural or anthropic. As air pollution alters the environment, these changes are reflected in living organisms [7].
Consequently, organisms may be used in monitoring air pollution as both bioindicators and bioaccumulators. As bioindicators, their sensitivity to air contaminants makes it possible to produce an estimate of air quality in the area concerned (indirect method). In the case of the species that are most sensitive to pollutants, the main symptoms taken into account are as follows:

- Biochemical, physiological and morphological changes.
- Genetic damage.

A good bioindicator should therefore have the following characteristics:

- Known sensitivity to specific pollutants.
- Broad distribution in the area concerned.
- Sedentariness.
- Long life cycle.
- Genetic uniformity in the area in question.

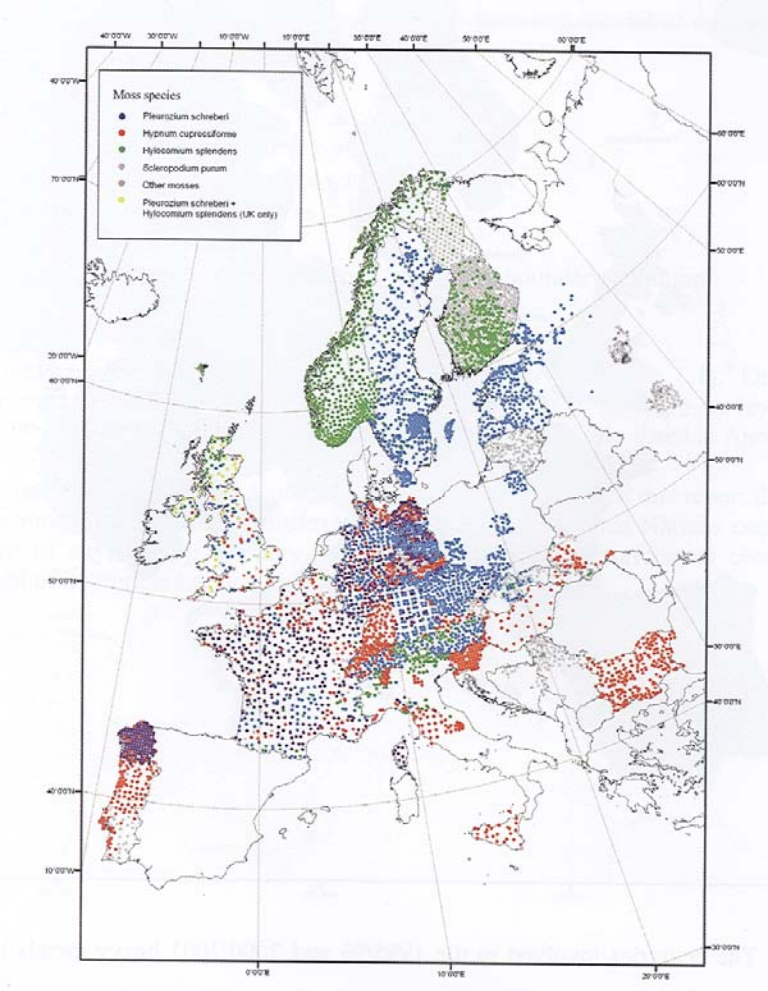

Figure 2. [6] Moss-sampling areas in Europe in 2000-01.

Bioaccumulators are based on the principle opposite to that of bioindicators, the focus in this case being on the species that are most resistant to atmospheric pollution and are capable of accumulating for a protracted period large quantities of contaminants such as heavy metals, organic compounds and radioisotopes (direct method). Thus, an organism can be used as a bioaccumulator if it features certain characteristics, in particular, a high tolerance to the pollutants concerned, this being essential to highlighting peaks of pollution.

More specifically, it must be capable of accumulating the substances in question, possibly with a linear correlation between contaminant concentration in the environment and in the organism.
It must also be widely distributed in the area concerned and have a reduced or zero capacity to absorb substances from the substrate.
The two strategies, which may be regarded as complementary, can generate data on pollution while at the same time ensuring effective integrated biomonitoring.
Bryophytes, which include mosses, are among the organisms most frequently used as bioaccumulators.

1.4 Bryophytes

Bryophytes are mostly terrestrial eukaryotic and autotrophic organisms that are taxonomically divided into Anthocerotae (hornworts), Musci (mosses) and Hepaticae (liverworts).
Bryophytes form an embryo (in such a primitive form that some researchers do not in fact regard it as an embryo) and alternate antithetic and heteromorphic generations, with a predominance of gametophytes (haploid generation) over sporophytes (diploid generation). The embryo is never fully autonomous as it is fed through the gametophyte.
Bryophytes are the simplest terrestrial photosynthesizing plants.
Despite the risk of transpiration entailing excessive loss of moisture through the plant surface, the fact that they occur outside water has two advantages:

- They can quickly obtain CO2 from the environment.
- They can absorb light direct from the atmosphere.

Their dissemination in what is a terrestrial environment has resulted in a number of changes in their reproductive process. Bryophytes are similar to algae in terms of sexual reproduction, since they produce male gametes that, thanks to the presence of flagella, are motile. Fecundation, however, requires water or dew to enable the male gametes to reach the female ones. Being water-dependent, the motion of gametes is on land limited to a short distance. As to reproduction by sporogony, bryophytes resemble terrestrial plants in that they produce meiospores that are carried by the wind, are without a flagellum and have a firm outer wall that prevents moisture loss.

1.5 Bryophyte morphology

With a view to providing a better understanding of moss reproduction, this section comprises a brief explanation of gametophytes and sporophytes.

1.5.1 Gametophytes

When germinating, the meiospore produces a thin filament, known as aprotonema, on which leafy shoots proliferate, giving rise to a gametophyte. When sexual maturity is reached, this serves to differentiate gametes.

In mosses, the gametophyte generally consists of an elongated part (stem), bearing a number of lateral flat appendices (leaves) and a basal part comprising hyaline cells (rhizoids) deprived of chloroplasts, which secure it to the substratum.
The stem may be several decimetres long and may be simple or have branches. Often, when found in large numbers, gametophytes form a pad that may cover extensive tracts of land.

1.5.2 Sporophytes

The sporophyte is a completely different organism. Firstly, it lives attached to the gametophyte, from which it derives its nourishment.
It generally comprises a foot embedded in the top part of the stem, extends into the seta, a thread-like portion with no lateral appendices, and culminates into a capsule containing the meiospores. At maturity, the capsule opens and frees the spores, which are scattered by the wind.

1.6 Bryophyte ecology

Bryophytes are photosynthesizing organisms that live in a sub-arial environment or, in just a few cases, in fresh water (e.g. Fontinalis).
They are broadly distributed in a large variety of habitats, partly on land, on stones and rocks, and on tree trunks, branches and leaves.
Ecologically, mosses are very important. Thanks to their limited requirements, they are successful in colonizing habitats in which most other organisms fail to survive.
The presence of mosses on land is a highly relevant factor in the absorption of rainwater, reducing or eliminating the risk of water runoff and gradually allowing the water contained in them to reach the underlying soil.
As mosses have neither woody conduction tissue nor lignified supporting tissue, they absorb rainwater through their entire surface.
Dehydrated bryophytes do not die; they simply become quiescent and, if rehumidified, regain their vital functions.

1.7 Use of bryophytes as environmental indicators

In the late 1960s, a number of Swedish scientists used mosses to assess heavy metal pollution in Scandinavia [9]. Since then, the use of these organisms for environmental monitoring has been on the increase, extending to various types of organic and inorganic contaminants. Similarly, the size of the areas covered now ranges from less than a square kilometer to entire continents. Listing the studies that have been carried out thus far would take up too much space, without guaranteeing that no author had not been left out.
Most mosses derive their necessary nourishment from the atmosphere, since they have not developed a real root system or any water-conducting tissue. Contaminants are therefore absorbed through the surface of their leaves. This means that in mosses there can be a close correlation between the concentration of these substances in the plant and atmospheric deposition, since absorption from the substratum can be ruled out.

Generally speaking, bryophytes are good indicators of fallout of atmospheric contaminants on soil surfaces, and this for the following reasons:

- They tend to have neither a protective cuticle nor thick cell walls, as a result of which their tissues are readily permeable to water and minerals, including metal ions.
- The tissue making up the cell walls features numerous active sites (negatively charged groups), which act as efficient cationic exchanges [10].
- As minerals are obtained primarily from depositions of soluble salts and particles in the air, the substratum has little or no relevance in this respect. There are exceptions, however. It would appear that some mosses absorb metals and other contaminants from the soil, mainly through the capillary rise of water, making them inadequate for biomonitoring.
- New rather scanty biomass forms above the old, preventing any contact or interaction with the soil or its substratum.
- some moss species such as *Hylocomium splendens* (Hedw.) Schimp. of the Sphagnum type display distinct annual growth increments, making it easy to establish their age and the exposure time of the material used;.
- With the exception of some species, there seems to be no contaminant translocation between adjacent segments or between old and developing biomass.
- Many species are widely distributed (cosmopolite or circumpolar species) in specific habitats.
- Owing to their longevity and depending on the species and sampling method used, bryophytes may be used to assess depositions occurring over several years.

The main limitations of bryophytes as bioindicators are the following:

- In specific environmental situations (e.g. acid deposition or protracted rainfall), incomplete contaminant adsorption or a partial loss due to water runoff may occur.
- Sampling-point selection may be crucial, perhaps even to a greater extent than for other methods.

Lastly, it should be pointed out that transplanted bryophytes have been used in numerous studies aimed at assessing atmospheric contaminant deposition..
The case of a number of researchers who introduced tree trunks bearing *Hypnum cupressiforme* Hedw. moss in an industrial area in Wales is also of interest. The transplant died after only a few weeks, but the mosses went on accumulating metals [11].
The main problem with transplanted mosses, therefore, seems to be their use in habitats where they cannot survive because of adverse weather conditions or inadequate moss management from transplant to the final planting. Indeed, a large number of commonly used species are sensitive to drying and often stop growing or die in an inhospitable habitat.

2. Guidelines for the use of native mosses, transplanted mosses and soils in assessing the fallout of organic and inorganic contaminants

2.1 Using mosses as bioaccumulators

This section sets out guidelines for the use of mosses as bioaccumulators.
Mosses can be used to determine concentrations of the following contaminants:

- Heavy metals and metalloids.
- Macro elements.
- Radio-isotopes.
- Dioxins and furans.
- Polychlorobiphenyls (PCBs), and Polycyclic aromatic hydrocarbons (PAHs).

2.2 Native and transplanted mosses

2.2.1 General

The main limitation of native and transplanted mosses used as bioaccumulators of inorganic and organic contaminants resides is that they constitute a biological system and are therefore more prone to qualitative and quantitative changes. This drawback is, however, offset by the following major advantages:

- They feature low management costs compared with "traditional" techniques.
- They provide rapid results and information, thus making it possible to adopt prompter and more effective measures.
- They can be used in both large and small areas.
- They can be used in moss-free areas.
- They are easy to use in analytical and sample-dissolving procedures.
- They make it possible to reconstruct the past history of trace element deposition.
- They allow the deposition rate (mass/area x time) to be calculated on the basis of the concentration values.

2.3 Monitoring network

In planning environmental monitoring strategies attention should be paid to the size of the area concerned, with a view to making the right choices in terms of the monitoring network, the number of moss-sample collection or planting points. Moreover, information should be obtained on the morphology and use of the area and the anthropic pressures on it. Data on contaminant dispersal are regarded as essential, in so far as that they have a bearing on the density and the number of moss samples taken or transplanted.
Depending on the project requirements a regular grid with uniformly distributed samples may be used which will provide objective and independent information in accordance with predefined criteria.
Systematic sampling based on predefined grids should be preferred, in that the results can be compared to and combined with other studies. I.G.M. maps on a scale of

1:100 000 or 1:25 000 and representing squares measuring 1 x 1 km may accordingly be used. The sampling stations could be located at the grid intersections.

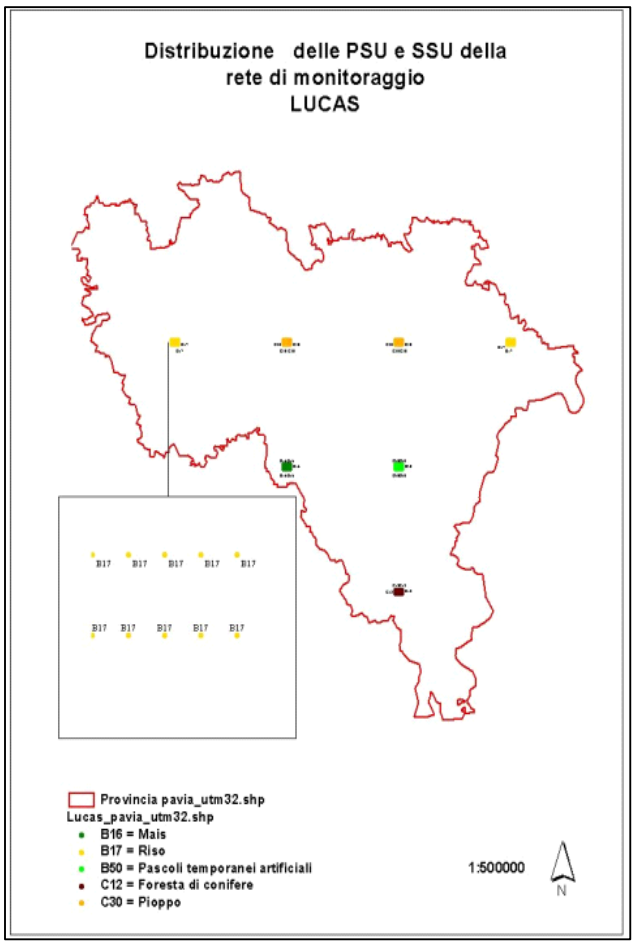

Figure 3. [12] L.U.C.A.S. grid (18 x18 km) for the province of Pavia.

The maps of the Inventario Nazionale Forestale (Italian Forestry Inventory), on the other hand, are based on grid squares measuring 3 x 3 km.

Two internationally accepted grids, I.C.P. Forest and L.U.C.A.S (based on squares measuring16 x 16 km and 18 x 18 km respectively - see Figure 3) may also be used.

In all the above-mentioned grids, submultiples may be used as required. Figure 4 shows an example of how the L.U.C.A.S grid nets may be used: the distance between sampling areas was 9 km.

It is advisable to use predefined grids for surveys of areas featuring highly specific sources such as smelting furnaces, incinerators, power stations, large industrial plants, etc. In such cases, moss samples may be taken from or placed in concentric rings around the facility, the number of monitoring stations decreasing as the diameter of the rings increases. Circles with a radius of 0.2, 0.5, 1, 3, 5, 8 and 10 km respectively and with 4 to 6 radii can certainly be used to cover and adequately describe the area to be monitored. Mosses should, where they do not occur naturally, be placed at the intersection point between the circle and its radius (Figure 5).

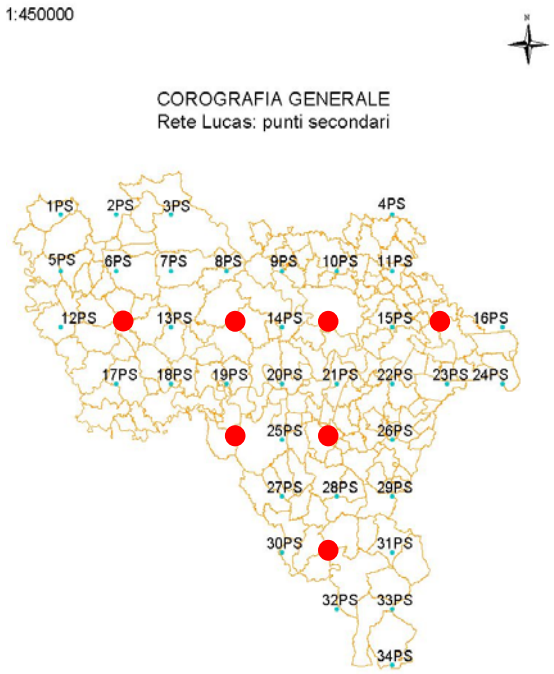

Figure 4. [12] Example of L.U.C.A.S. grid submultiples (9 x 9 km) for the province of Pavia. The red dots indicate the L.U.C.A.S. points.

Composite grids can be used for all surveys. A composite grid is obtained by superimposing a fixed grid on another grid based on a mathematical model, taking into consideration areas with potentially the highest fallout of the contaminants concerned. The points highlighted by the mathematical model, which will identify the areas with the highest probability of contaminant fallout, will be added to the fixed net grid sampling points.
In special cases, transects may be used along the intervals between moss sampling or planting points will depend on the type of survey and information sought. In urban centers devoid of native mosses, the option chosen will be to plant mosses along the roads or produce an ad hoc grid based on the requirements and objectives of the survey.

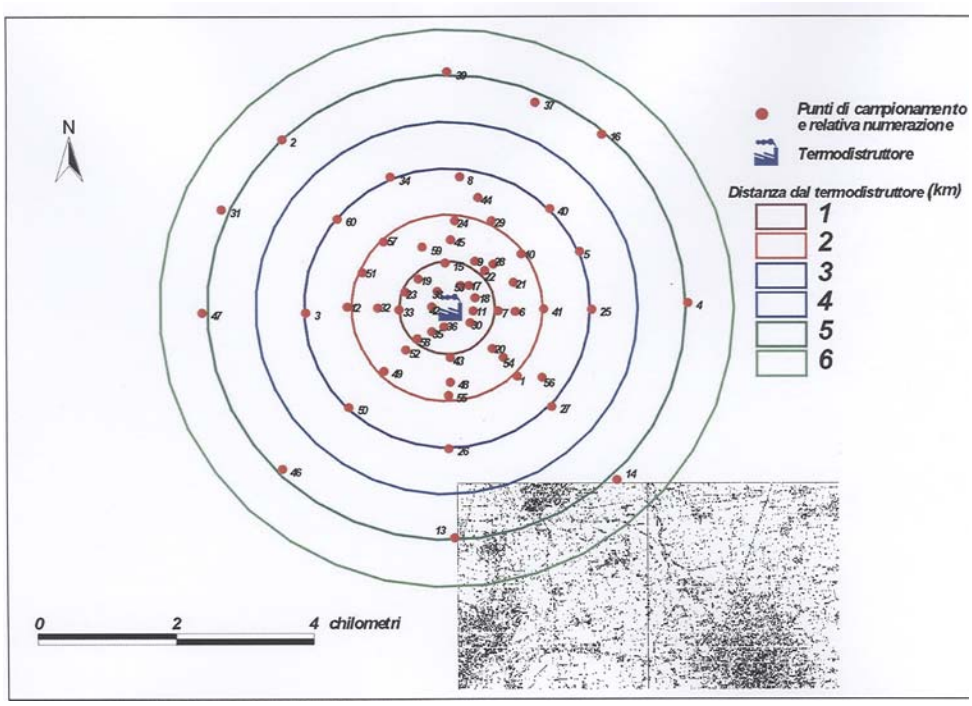

Figure 5. [13] Predefined grid overlay the various points of which are derived from a mathematical model. Used to assess fallout from a waste-to-energy plant.

2.4 Native mosses

Native mosses offer a record of heavy-metal depositions over a period of up to 5 or 6 years prior to collection, depending on the moss species used and the length of the thallus being collected. This method makes it possible to trace the history of areas ranging from thousands to just a few square kilometers or even fractions thereof.

The area identification sheet (Figure 6) is crucial and must contain the following:

- Sampling point code.
- Geographical coordinates.
- Name of location.
- A map 1:10 000 or smaller.
- A detailed photograph of the sampling point.
- A photograph giving an overall view of the area concerned.
- A description of how the area is used.
- The species of moss collected.
- A description of the type of substratum or soil sampled.

The procedure to be applied for the correct use of mosses as bioaccumulators is as follows:

- The distance between the moss-collection area and any houses/busy roads should be 200 meters or more. This applies to surveys aimed at assessing general depositions without direct local interferences. In other cases, however, this distance requirement may be waived.

The moss-collection area should cover 400 m2 (a square measuring 20 x 20 m). The amount of moss needed for analysis should be collected systematically from the whole area.

- In each sampling area collect the most abundant moss species. Give preference to the following species: *Hypnum cupressiforme* Hedw. (Figure 8), *Hylocomium splendens* (Hedw.) Schimp. (Figure 9) or *Scleropodium purum* (Hedw.) M. Fleisch. (Figure 10), using latex gloves when collecting them. The choice of these three moss species is based on numerous observations, in particular their widespread presence in Europe, the fact that they are widely used and their marked bioaccumulation.
- During collection, the moss should undergo preliminary cleaning, eliminating leaves, ground, twigs, conifer needles and other material.
- Place the collected moss in an envelope prepared with filter paper, on which all the necessary identification data should be written (sampling station code, collection place and date, etc.).
- In the laboratory, the moss should be placed on a bench with a PVC or ceramic surface and be cleaned, the latter being performed while wearing latex gloves.
- Cut the first centimeter of moss or different apical lengths (depending on how far back in time you want to go). Use scissors or tear them off by hand. Collect the quantity of fresh mass needed for analysis. Give preference to the greener thallus (Figure 11).

Punto di campionamento: **PS 27**
Coordinate geografiche:
Latitudine: **504643**
Longitudine: **4974937**
Altitudine: **245 m**
Località: **Spinosa**

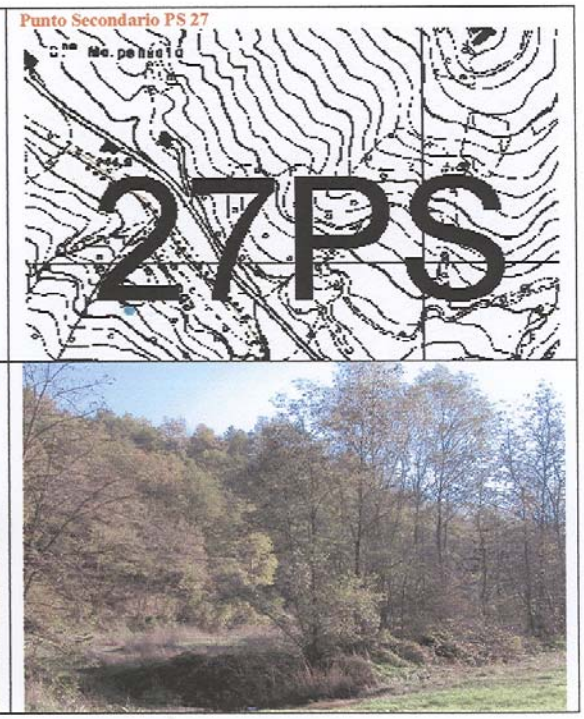

Figure: Mappa scala 1/10.000 (alto dx.), particolare del punto di campionamento (sx.), veduta generale dell'area (dx.)

Note

Utilizzo del suolo: prato stabile

Descrizione dell'area:

circa 50 m a N-E: strada poco trafficata
circa 10 m a N-E: torrente Rile
circa 10 m a S-W: boschetto misto molto ampio di conifere, betulle,…

Muschio (*Brachythecium rutabulum* (Hedw.) Bruch & *al.*)

circa 10 m a S-W dal punto di campionamento del suolo
raccolto sui tronchi dei primi arbusti all'inizio del boschetto (forse o ontani o olmi)

Tavola 1. Informazioni relative al **Punto Secondario 27**

Figure 6. [14] Example of descriptive sheet for moss- and soil-sampling areas.

Figure 7. [12] 20 x 20 m moss- and soil-sampling area.

- Place the moss in a class-A glass lab crystallizer (i.e. a vessel 10 cm high with a 20-cm diameter), rinsed with double distilled water. Alternatively, a class-A glass lab beaker may be used, pouring enough double distilled water to cover the mosses and stirring with a glass rod for 10 to 15 seconds. Remove the mosses and place them in another crystallizer. This helps to eliminate coarse particles, usually soil. Cover the crystallizer containing the rinsed mosses with a watch glass.
- Place the container in a heater at 40°C for at least 48 hours. The 40°C temperature minimizes the loss of elements (e.g. Hg, Pb, etc.) and compounds through volatilization.
- After drying at 40°C for 48 hours, further dehydrate some grams of moss at 105°C to assess water loss.
- Grind the moss dried at 40°C in a grinding mill with an agate body and sphere. Alternatively, an agate mortar may be used. The granulometry of the moss powder must be less than 125 µm. This ensures good sample uniformity for quantities of 100 mg.
- Place the moss powder in a pre-washed polyethylene vessel with a double lid and 10 mm Teflon or glass sphere to homogenize the sample before each weighing operation.

The moss samples are now ready to undergo specific processes and analyses.

Figure 8. Moss of the *Hypnum cupressiforme* Hedw. species. (Photo by F. Sguazzin).

Figure 9. Moss of the *Hylocomium splendens* (Hedw.) Schimp. species. (Photo by F. Sguazzin).

Figure 10. Moss of the *Pseudoscleropodium purum* (Hedw.) M. Fleisch species.

2.5 Moss transplants

Transplanted moss may be regarded as a representation or record of the current situation, in that it provides indications on depositions which occurred between transplantation and collection. Exposure time should preferably not exceed 2 years. Needless to say, the moss is transplanted in areas where it does not occur naturally.
It is essential to find an area that is not subject to direct fallout and features a limited concentration of inorganic elements and organic compounds, in order better to assess deposition increments over time. The area should be densely covered in moss, allowing easy collection of the moss which is to be transplanted. Once such an area has been found, proceed as follows:

- Using a spade with a sharp edge, cut out an area of 35 x 45 cm of moss and underlying soil/substratum to a depth of 10-15 cm.
- Lift the moss and soil together and place them in a plastic crate (ventilated plastic crates of the type used for fruit are suitable for this purpose). Follow the same procedure for all the other crates needed for the survey (Figure 12).
- In the areas selected for placing the crated moss, dig a hole of the same size as the crate.

Figure 11. Moss stem cleaning and cutting operations.

- Place the crate in the hole so that the moss pad lies flush with the surrounding ground.
- The transplant area should be near some shade. If there is no shade, a small "fence" can be built with non-metallic material (bamboo canes or green plastic woven fabric) so as to obtain an area of shade. The shade should cover as much of the moss as possible (Figure 13). Care must be taken to avoid hampering lateral air circulation.
- Once the crate is in place, collect some moss samples to determine the initial concentration of the elements and/or compounds concerned. Wearing latex gloves, collect from the entire area the quantity (by weight) needed for analysis. Place the moss in an envelope, take it to the laboratory and process it as described above.
- Water the moss and underlying soil with double distilled water. The frequency and the quantity of water depend on the season. It should be borne in mind that the soil under the moss must remain moist at all times.
- Thereafter the moss may be collected every 2, 3, 4, 6, 12, 18 or 24 months, depending on the environment and study requirements concerned.
- In the interests of a more thorough interpretation of the findings, it is advisable to collect - and analyze - a single composite soil sample near each moss station.

Figure 12. Moss crates ready for transplant.

Figure 13. Creating a shaded area.

2.6 Variation coefficient

The intra-area variation coefficient (VC%), which covers any mistakes which may have occurred from sampling to analysis, should be estimated on the basis of one of the stations being studied. Alternatively, the results of Table 1 may also be used to obtain approximate data. This would show whether a change in the concentration of an element is attributable to depositions or to varying concentrations within a sampling area or station. The VC% should be assessed for both native and transplanted mosses.
Sampling should be carried out as follows:

- Identify an area where moss is abundant and forms a dense mat. Using a nylon thread, mark out a 1 square meter area subdivided into nine 33-cm sub squares.
- In each sub square, collect apical tissues of the same length (e.g. 2 cm). For collection and processing, see the instructions given in the previous section;
- From the solutions obtained following acid mineralization, determine the mean value and the percentage variation coefficient, analyzing each element five times.

Table 1. [15] Variation coefficients (%) for some elements obtained in a grid in the province of La Spezia.

Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Ti	V	Zn
20	12	32	12	18	22	7	23	11	21	17	20

2.7 Deposition rate

The deposition rate (DR) is obtained on the basis of concentration values in moss, and is expressed per unit of mass (mg) of the element concerned in the unit area (m2) over the unit of time (1 year). It is obtained by using moss concentration on the basis of the following formula:

$$DR = \frac{C_{EL}}{(F.E.)\times(T_a)\times(F_R)} \quad [5]$$

Where:

CEL Concentration of the element in the moss, expressed in mg/kg.
F.E. Efficiency factor for the element concerned.
Ta Period covered by the moss stems expressed in years.
FR Rühling's factor [log10 (element concentration in moss) = 0.59 + 1.0 log10 (atmospheric deposition)] =~ 4

To determine the deposition rate, specific information and data are necessary, in particular a correct assessment of the annual growth of the moss. The data obtained are

usually regarded as general fallout data and provide information on the type of pressure to which the area is being subjected.

2.8 Collection and processing of superficial soil samples

The collection and analysis of the soil/substratum help to assess the "soil effect" (i.e. the increase in concentration attributable to the deposition on moss leaves of terrigenous particles from the soil) and, at the same time, obtain information on concentrations of contaminants in the soil and, to some extent, determine their quality.
The enrichment factor (EF) [16] is derived from the moss element concentration/moss Al concentration ratio, divided by the soil element concentration/soil Al concentration ratio.
Aluminium is the preferred element for EF purposes, since concentrations in soil are of the order of one percent and thus can hardly be influenced by fallout of anthropic and/or natural origin.
If the EF exceeds 10, the concentrations found in mosses are to be attributed to human activity or are of natural origin, e.g. volcanic. With an EF of 10 or less, the origin of fallout is due mainly to soil or to the substratum.
For soil sampling a superficial layer should be collected, the thickness of which will vary depending of the type of survey to be carried out, the type of anthropic pressure and the use to which the soil is put.
The thickness of the superficial layer should be as follows:

- 0-2 cm for monitoring recent fallout (road edges, accidental spillages, built-up areas, etc.).
- 0-5 cm for industrial plants, woodland, etc.
- 0-30 cm for agricultural areas.

For soil collection, proceed as follows:

- Use an area of exactly the same size as that where the moss was collected (a square measuring 20 x 20 meters) (Figure 7).
- Remove the bedding and collect, from the entire area, at least 20 subsamples each weighing 100-150 g, which are to be mixed in the field to form a single composite sample.
- After collecting the (composite) soil and placing it in a crystallizer or glassware vessel, remove by hand any stones, twigs or coarse material and place the soil sample in a plastic bag writing down the same information as for mosses.
- Dehydrate in a laboratory heater at 40o C for 48 hours.
- Pour through a 2-mm sieve.
- Grind the fraction of 2 mm or less with an agate sphere mill and place the soil thus ground in a pre-washed polyethylene vessel with a double lid and a 10-mm Teflon or glass sphere to homogenize the sample before each weighing operation.

The soil samples are now ready to undergo specific processes and analyses for the study concerned.

2.9 Aqua regia extraction

For the mineralization of moss and soil samples, it is advisable to use the ISO 11466 method. This offers a high extraction performance for inorganic contaminants including platinum, palladium and rhodium. By using a microwave and aqua regia, a level of soil and moss mineralization comparable to the above-mentioned ISO method can be obtained.

2.10 Analytical methods

Analytical equipment is neither described nor reported. It should be suited to the objectives and yet sensitive enough to assess the concentrations of the elements and compounds to be monitored in the moss and soil.
Lastly, it is essential to use an appropriate number of certified standard materials such as those certified by N.B.S. (National Bureau of Standards), C.R.M. (Certified Reference Material), N.I.S.T. (National Institute of Standardization) and N.R.C.-C.N.R.C. (National Research Council Canada).

2.11 Conclusions

Writing guidelines invariably entails a risk of making mistakes, of not being sufficiently exhaustive and even of upsetting people.
However, this exercise also conveys a strong message to all those who are interested in environmental monitoring, a message in favor of working together in a large group, comparing results and benefiting from the experience of many colleagues, including some that one may not have actually met in person.
This can help provide mankind with a deeper understanding of what occurs in the environment, the air and the soil by establishing, by means of a common thread or language, i.e. a standard method, a link between a range of experiments.
For all their limitations, these guidelines can contribute usefully to fostering understanding and cooperation on air and soil environmental monitoring projects covering areas both large and small and involving numerous researchers operating in this field.

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Abstract
These guidelines on the use of mosses are of strategic importance in that they make it possible to harmonise the indications obtained from differing environments in terms of extent and, thereby, to compare them with results obtained using other methods. Thanks to their morphological features, mosses can be used as bioindicators to obtain fallout information for a significant number of organic and inorganic contaminants of human origin and determine the quantity of such contaminants deposited in soil. Analysis of both moss and soil is essential in identifying, and discriminating between, anthropic and natural sources of contaminant fallout.

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