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## **VALIDATED APPROACHES TO RESTORING THE HEALTH OF ECOSYSTEMS AFFECTED BY SOIL POLLUTION**

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### **ABSTRACT**

The diagnosis of soil pollution in relation to the most commonly used remediation methods is an innovative way of tackling this problem. To this end, the theoretical framework of Ecosystems Health has been instrumental, since this area is presently emerging as a new language for general discourse on pollution. For example, the use of the term stressor for a pollutant implies the study of behavior responses in living organisms (at different levels of organization, from the cell to ecosystem) both in terms of impacts (toxicity) and tolerance towards the pollutant (their adaptation).

Two very common scenarios in the central Iberian Peninsula have allowed us to approach the understanding of this area of study: (i) old abandoned mines and, (ii) old solid waste (urban and industrial) landfills.

The soils of these systems are mainly polluted with heavy metals (Zn, Cu, Pb, Cd), they overlie different lithological substrates (granites, limestones and especially arkoses), and are highly representative of soils of the Mediterranean region (mainly regosols, luvisols and cambisols). The landfills of the mines or waste tips have slopes exceeding 40%, such that any leachates produced transfer pollutants to adjacent ecosystems at lower altitudes (stream bed pastures, marshes and surface water courses). Moreover, since the topsoil layer is usually sandy with low proportions of clay and organic matter, deep leachates also transport pollutants to ground water systems.

We focused on the autotrophic component of the affected ecosystems for several reasons: toxicity tests could be used to examine the physiological and behavioral responses of organisms (mortality, injury, metabolic changes) as well as population (population density, risk of extinction) or community (structure, diversity, biomass, nutrient flow changes) variables. The build-up of a heavy metal in the above-ground part of a plant (phytoaccumulation) consumed by herbivores is also detrimental for health, due to transfer to the trophic network. Root systems may play a role in phytostabilizing heavy metals and in preventing them from passing to deeper soil layers. Finally, given the erosion problems of fine materials in landfills and waste tip slopes, vegetation helps avoid the movement of topsoil layer pollutants to other ecosystems.

The methodological approaches validated by results obtained over the last twenty years can be summarized as: studies of polluted sites based on phytoecological sampling, analysis of soil chemical and physical properties, georeferencing of the heavy metals they contain for further sampling in areas showing the highest levels, collecting and chemically analyzing plants at these sites, and the use of soils with their seed banks to perform experiments in microcosms in controlled conditions. The idea is to use a combination of field and laboratory methods that simulate real scenarios in which soil pollution occurs.

## **INTRODUCTION: THE COMPLEXITY OF DEGRADED ECOSYSTEMS WITHIN THE FRAMEWORK OF SYSTEMS SCIENCE**

With the title “From Biodiversity to Biocomplexity”, the monograph of the June 2001 issue of *BioScience* was devoted to the multidisciplinary step we need to take if we are to understand our environment. The main questions addressed as challenges for biologists at this start of a century are perfectly apt to introduce the subject of the work we describe in this chapter. There is no question that the field of biocomplexity will be in the forefront of research endeavors in the years to come, given it is a property of all ecosystems; or in other words, it is the thread that links many complex systems that are structured or influenced by living organisms, their components or biological processes.

Interactions between live beings and all the facets of their external environment are an evident fact and research into these interactions, which involve multiple levels of biological organization and/or several spatial or temporal scales, is of great importance for everything related to environmental impacts. To undertake such research, long term data collection and monitoring are required to examine interactions at different spatial scales of the landscape and assess interplay between air, soil, water and the ecosystems they sustain. These issues will guide decision-making and help understand the functions of ecosystems and the changes they suffer in an ever-evolving world. Notwithstanding, the study of complex systems, among which we can include terrestrial ecosystems at polluted sites, is in large measure at a very early stage. Analyzing the individual components of a system offers no information about the properties of its combined components. The emergence of new properties when components change is a common phenomenon, but deciphering what makes new features emerge is a topic that has only just started to attract the attention of scientists.

With these brief considerations only, we can begin to understand the current impact that the topic of restoring degraded ecosystems is having for biologists because of its complex nature. Biocomplexity is nevertheless difficult to describe and experimentally assess owing to its non-linear nature. Indeed, understanding biological complexity requires the development of new paradigms that cross temporal, spatial and conceptual barriers. Certainly, in the journal mentioned it is recognized that the development of the ecosystemic focus and its use to try to understand and resolve environmental problems, has been one of the major advances of Biology in the past 50 years. For many years now this has been our approach (Hernández, 1989 and 1991) and in the subsequent sections of this chapter we describe some of the concepts we have established and tested to address the subject of restoring degraded ecosystems.

Remediating and recovering degraded ecosystems is really a topic of systemic reality. This dictates a need to juggle the basic principles of systems analysis (complexity, interaction, uncertainty, multicausality) in an epistemic focus on ecological restoration. The applicability of this focus stems from the fact that ecosystems are dynamic systems that evolve and coevolve with human activity, though always evoking their stability, a term frequently used in relation to the response of an ecosystem to perturbation. This in reality is the picture we are faced with: both EU community policies and global warming that affect the dynamics of abandoned cereal fields or grassland systems, along with the environmental impacts of new emerging systems in structured landscapes such as landfills. The remediation of landscapes by reforestation with tree species is met with changes in soil structure, a feature not usually considered in practice that complicates the system to be restored. In the case of solid waste landfills capped with soils taken from surrounding areas, we have to face the complexity that arises from the secondary ecological succession, arising from the seed bank of the covering soil, intermixing with the primary succession of these new ecosystems. Not only does the impact of waste disposal need restoring but the “waste system” itself also needs to be remediated.

## **ECOSYSTEMS' HEALTH**

The health of an ecosystem is one of the pillars that supports sustainable development, and is emerging as a new language for general discourse on pollution. Thus, ecosystems' health has been defined as an incipient science or systems approach to prevent, diagnose, and predict factors useful for managing the system and establish links between the health of an ecosystem and human health (Calow, 1995; Di Giulio and Monosson, 1996; Rapport et al 1995 and 2003). Thirty years ago research into geochemistry and health commenced as one of the UNESCO's lines of investigation in the Man and Biosphere (MAB) program. The hypothesis that heavy metals generated by geochemical actions in some tropical ecosystems, besides being related to the productivity of the system, could have effects on human health prompted us to assess the bioavailability of these metals in the main crops used for human consumption or forage in this region.

This was the basis for an experimental study conducted at several abandoned mine sites of the central Iberian Peninsula (Hernández and Pastor, 2007) and for our recently initiated study on tropical ecosystems (Hernández et al., 2007). The above flow diagram (Figure 1) indicates the general protocol inferred from our long-term results that has best served us to evaluate the relationship between the geochemistry and the health of an ecosystem.

The ecosystems found at these sites are essentially those of grasslands and forage or cereal crops. In each of these settings, we find soils containing more than one heavy metal in their top layer as point-source pollution that affects both the plant populations and their consumers with the risk of transfer of these pollutants to ground and surface water aside from the food chain.

## **METHODS VALIDATED FOR DIFFERENT MEDITERRANEAN SCENARIOS**

According to the issues discussed in the preceding sections, we selected different sites on substrates and soils representative of the central Iberian Peninsula. The former include granite, limestone and gypsum and mainly arkosic substrates (Madrid facies),

and the soils are those also found across Spain (regosols, luvisols and cambisols). On the soils of arkosic regions in environments that are almost semiarid we find agrosystems of cereals, vines, fallow land, pastures and shrubs with a compacted topsoil layer poor in organic matter and of slightly acid pH. These three properties makes them good candidates for use as reference systems to address the revegetation of sites with soils impoverished by these or similar soil degradation processes that are to change use. A common example is the change from past industrial use to quality housing developments with green leisure zones.

The arkosic substrate also generates soils that are highly silted (scarce clay and much sand) besides containing slight amounts of carbonates. These two features reflected in agroecosystems and pastures of the area, make the latter ideal references for old soil-capped landfills with steep

slopes along which the finer elements of the covers are lost and transported downstream. Fallow systems are perhaps the most analogous to the most recently sealed urban solid residue (USR) landfills if not subsequently intervened with. Moreover, their understanding can also be a reference for the adequate restoration of agrosystems with naked soils.

Many factors of great ecological interest are often not included in environmental impact analysis (EIA) studies, despite the fact they need no vast amount of experimentation, nor do they require long sampling periods nor generate huge costs. Thus, for example, the analysis of real floristic composition and not only the potential composition (inferred from the literature), the diversity, the relative abundance of the main taxa, dominance, succession dynamics or the conservation state of the vegetation, although addressed in some studies and mentioned in the corresponding reports and statements, very few EIA are accompanied by fieldwork. Even fewer of these studies include soil analyses, despite integrated soil and plant variables providing relevant information on nutrient flow and on several functional properties of the ecosystem itself, which will need to be considered if restoration is in mind (Hernández et al., 1998a).

We have also observed that in many areas of central Spain, there are zones corresponding to old mining areas in which rubble landfills exist and soils are polluted to a greater or lesser extent by heavy metals. The plant communities they sustain are grassland and shrub-pasture formations and some wooded meadows used by cattle, sheep and wild animals. Given that most heavy metals and trace elements are components of biogeochemical cycles whose main compartments are the soil and vegetation, their importance in the food chain means that these elements warrant detailed investigation.

Heavy metals and trace elements can be absorbed by plants, be lost by deeper leaching to reach ground water, or can be lost through erosion (surface runoff), affecting surface water channels. The importance of the different transfer routes of these elements to other compartments of the trophic network varies considerably depending on the element in question, the plant species present or the use given to the pasture (whether used by livestock in situ or for fodder or elaborating feeds).

Several studies have shown that animals exhibit toxic element concentrations when grazing on polluted soils (Morcombe et al. 1994; Petersson et al., 1997; FAO, 2000; López Alonso, 2002; Wilkinson et al., 2003). Thus, it is necessary to control metals in terrestrial ecosystems. Although the work reported in this chapter has been undertaken

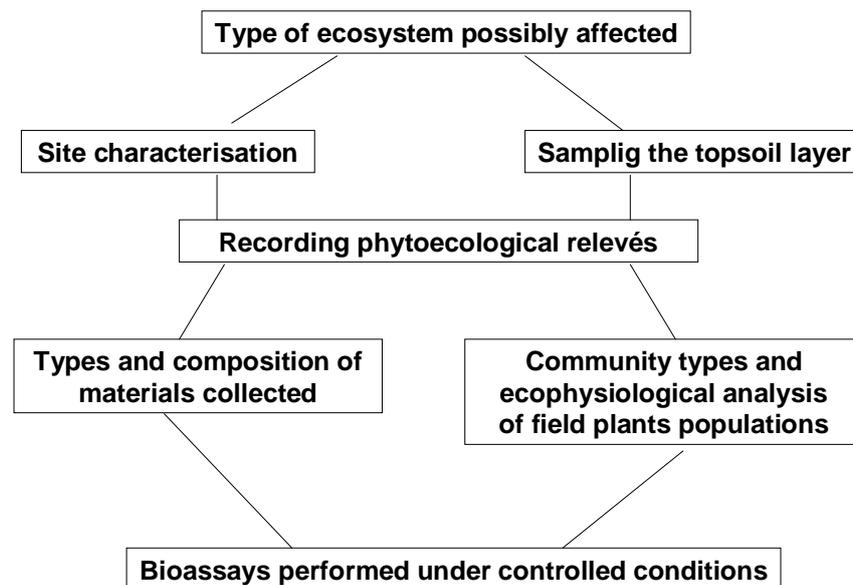
over the last twenty years, we will try to present a main line of progress regarding the solution of the problems arising during our efforts to ecologically restore the environments described above. Given the impossibility of detailing many results, the reader is referred to the corresponding bibliographic references cited. Summary tables of the results obtained are nevertheless provided as a general guide.

Before any effort is made to ecologically restore an area, a detailed description of the zone has to be made along with an estimate of its negative impact on the environment, the landscape and the structure and function of the ecosystems in the affected area. These preliminary evaluations should of course be conducted by multidisciplinary research teams.

Our present understanding of these issues is, nevertheless scarce, especially in terms of which wild species should be used to restore degraded Mediterranean ecosystems. Neither does the

literature provide many ecotoxicological analyses of grasslands. The experience described here can be, therefore, viewed as the ground-work for the remediation of soils contaminated with heavy metals.

Figure 1. Protocol



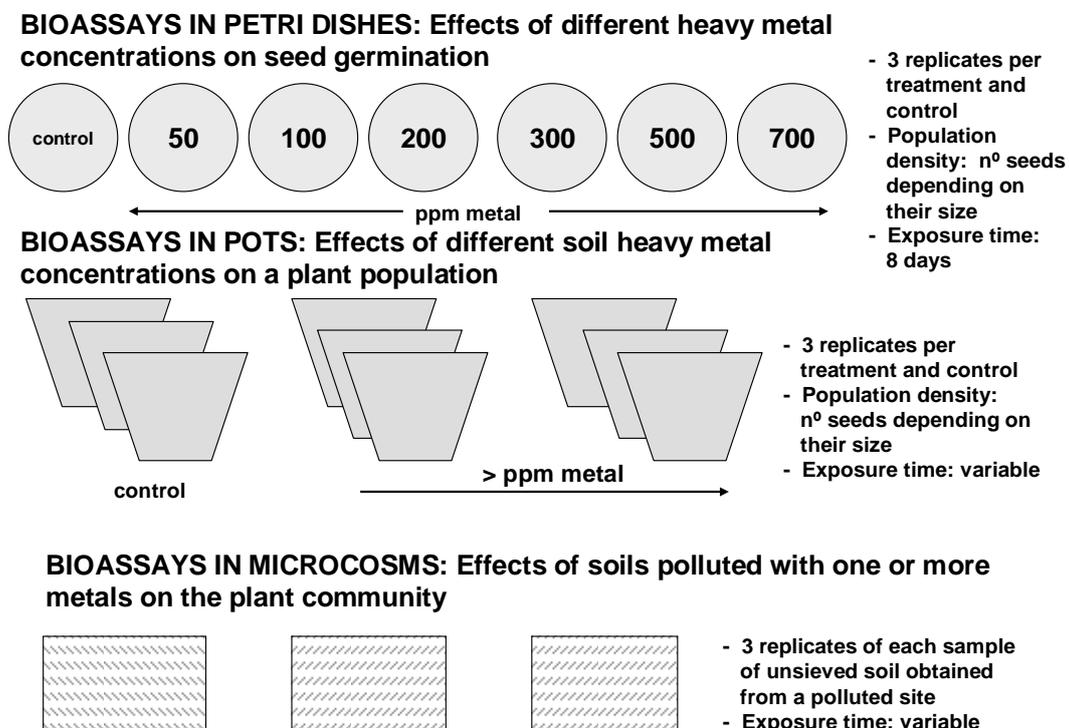
## **ASSESSING THE ECOTOXICITY OF SOIL POLLUTANTS AND DESIGN OF BIOASSAYS**

It is uncommon to find in the literature on the ecological restoration of terrestrial ecosystems valid protocols for use in studies on this subject. The following section describes certain concepts that may help decide upon the best procedure to use.

A *bioassay* is a method in which organisms (e.g., plants or animals), biological systems (e.g., tissues), or biological processes (e.g., enzyme activities) are used to measure the biological effects of a substance. In the context of managing sites with dangerous chemical residues, bioassays may be defined as the exposure of biological indicators to environmental samples collected in the field aimed at detecting the presence of toxicity and/or identify the toxic effects produced on the resident species. Generally a bioassay on a site containing hazardous residues involves laboratory tests (on the soil, soil leachates, water or sediment samples) using a standard set of test organisms under controlled lab conditions. Test organisms that have been widely used for ecotoxicology studies include *Selenastrum capricornutum* (a freshwater alga); *Daphnia magna* (a macroinvertebrate); *Pimephales promelas* (freshwater fish); *Rattus norvegicus* (rat) and *Lactuca sativa* (lettuce). Nonetheless, any nonstandard organism may be appropriate for a

bioassay when: a), the standard organisms used shows no response to known or likely pollutants; b) the response of a particular non-standard organism is more specific for the given pollutant; or c), if the response of a particular organism not included in the list of standards needs to be assessed. It is likely however that tests based on non-standard organisms will be more costly owing to the difficulty in obtaining, culturing or cultivating new organisms and consequently standardizing new bioassays (including quality assurance procedures). Moreover, a large number of preliminary tests using the non-standard organisms will be needed to confirm their validity.

Figure 2



A further feature to consider is the realistic planning of bioassays: (i) the design of bioassay studies involves establishing the field samples to be collected and their lab analysis; (ii) it is important that any project be completely planned in detail, from its

objectives to expected results, before embarking on any work. A poorly planned project will waste time and resources.

The steps of the protocol we followed for our bioassays designed for the purpose of restoring polluted soils were:

- Perception of a real-life possible ecotoxicity problem.
- Field and lab studies on the ecosystems possibly affected by the pollutants.
- Selection of species appropriate for evaluating toxicity based on the results of lab tests.
- Knowledge of the ecosystems possibly affected; knowledge of the chemical nature of the pollutants and their normal behavior or behavior in normal growth conditions of the populations and/or communities to be used in bioassays. Literature search on the case examined.
- Programming the different experimental designs necessary to fulfill the study's objectives. Designing an adequate statistical analysis optimizing the numerical treatment of the information with the number of situations or possible cases (to limit the number of samples analyzed). Establishing the exposure doses, time and the toxicity tests to use.
- Collecting the material needed for the experimental procedure.
- Modifying and validating the necessary techniques for the chemical and biological tests that will be needed, (Landis and Yu, 1999). To this end, the variables to be determined in the different bioassays should be analyzed, and according to the proposed objectives and the information obtained in the first step, tables should be drawn up to record data during biomonitorization. For this, we should consider the different levels of organization of living organisms (figure 3). Thus, the entire set of techniques should be designed to address the following issues: bioaccumulation/biotransformation/biodegradation; biochemical monitoring; physiological and behavioral monitoring; population variables; community variables (figure 4).
- Performing bioassays, analyzing the material used in the bioassays (population and community ecology, soil and plant chemical analysis, use of the most appropriate techniques to demonstrate the toxicity of contaminants).
- Numerical treatment of the information, analyzing and discussing the results.

It may be noted from the above description of our approach to scenarios of heavy metal soil pollution along with Figures 2 and 3, that an ecotoxicological analysis involves many complexity levels: spatial (geologic-edaphic), biological (populations), as well as many forms of ecotoxicological characterization. Hence, it is important to integrate different scales through the use of *microcosms*.

Figure 3. Inspired by Ramade, (1995)

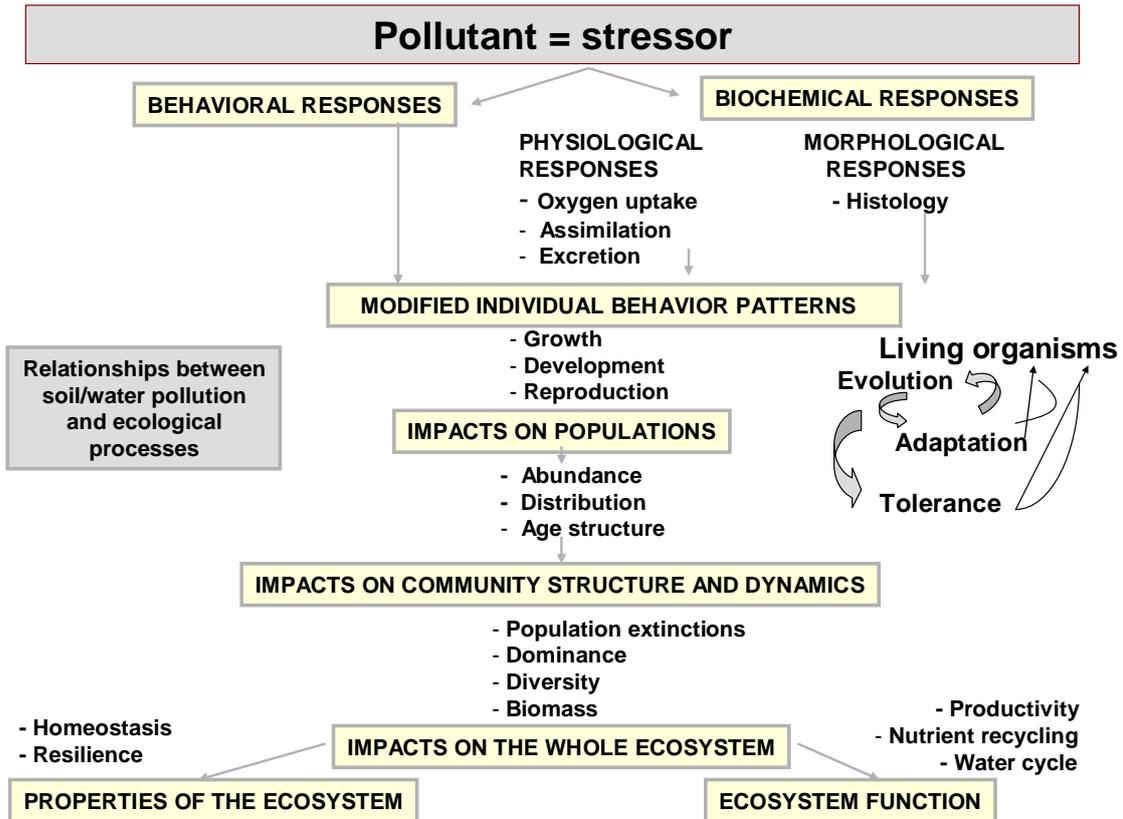
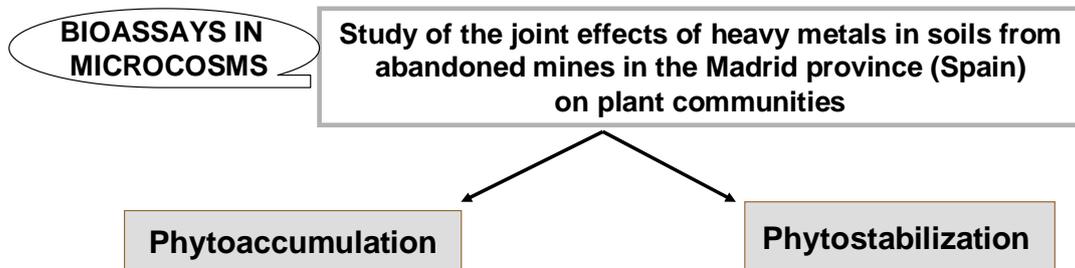


Figure 4



- Soil containing more than one heavy metal (mostly Cu, Pb, Zn and Cd) in its top layer (0-20 cm)
- Existence of a pollution gradient since these metals are unevenly distributed in the site's soils
- Three replicates per treatment and control Unsieved soil
- Time of exposure : 4 years

**Measures used to assess toxicity**

- Root biomass
  - Above-ground biomass
  - Plant biodiversity
  - Mean height of the plant community
- Productivity of the ecosystem

## CASE STUDIES

### ABANDONED MINE SITES WITH HEAVY METAL-POLLUTED SOILS

The soils of landfills and areas adjacent to old disused mines sustain different types of ecosystems and contain several heavy metals (Tables 1 and 2).

Owing to their efficiency, phytoremediation techniques (phytoextraction and phytostabilization) are considered the most appropriate for restoring these sites. A realistic application of such techniques is dependent on the ecotoxicologic diagnosis of the site in terms of its mineral paragenesis (see Tables 3, 4 and 5), and on knowledge of the response mechanisms of plant species, both at the population and community levels, to the combined action of several heavy metals.

Table 1. Total heavy metal contents (mg/kg) of polluted soils obtained from several sites of an abandoned silver mine (Guajaraz, Toledo) used to prepare microcosms for phytoremediation trials.

Soil sample	Zn	Cu	Pb	Cd	As
Control	250	16	410	0	0
1	5095	85	3850	37	326
2	2865	50	2430	11	284
3	835	20	1420	0	197
4	2940	46	2250	15	239
5	2290	22	1635	0	220
6	820	13	1205	0	193
7	1490	10	180	0	180
8	1005	20	1845	0	0
9	1585	24	1770	0	261
10	855	17	1220	0	0
11	515	9	720	0	294
12	4160	110	910	29	163
13	2410	31	1215	7	239

Table 2. Mean total (mg/Kg) and available (mg /100 g) heavy metal contents and organic matter percentages of soil samples taken from several zones of a disused copper mine (Garganta de los Montes, Madrid).

Soil origin	Zn	Cu	Pb	Cr	Cd	OM %
Landfill (rubble)	200 / 1.1	145 / 2.6	45 / 0	< 2	< 2	0.03
Landfill base	175 / 1.1	228 / 3.4	40 / 0	< 2	< 2	0.94
Floodplain	145 / 0.8	315 / 4.7	105 / 0	< 2	< 2	0.94
Ash woodlands	150 / 3.3	791 / 58.8	95 / 1.3	< 2	< 2	6.70
Temperate grasslands	100 / 1.7	741 / 1.7	47 / 1.1	< 2	4.5 / 1.1	2.69
Wet grasslands	800 / 4.2	1480 / 107	3750 / 1	< 2	340 / -	6.45

The chronological order of the steps to be taken for the ecological restoration of such sites we found to be most useful is indicated in Figure 5. This methodology may be viewed as an advance in ecotoxicological studies on native plants that play an important role in the trophic networks of ecosystems polluted with heavy metals. Knowledge of the main characteristics of the heavy metal contamination of a given site, along with the ecotoxicological problems they cause enables us to: a) to identify possible metal-accumulating, -tolerant or- excluding species that grow on landfills and areas adjacent to abandoned mines; b) to quantify the effect of different types of grass communities in terms of impairing the transport of the metals present in the polluted landfills and soils in leachates or surface runoff; c) design revegetation programs based on optimizing conditions for the phytoextraction, phytostabilization and physical stabilization of metals; d) evaluate potential “collateral environmental effects” associated with the different types of plant communities of the zones selected; e) establish potential benefits linked to the revegetation of the polluted sites, and f), to develop a protocol for lab experiments to obtain additional data on soil-plant relationships in these settings.

Figure 5

- Ecologically restoring abandoned mine sites whose soils are polluted with heavy metals**
- 1. Perform an ecotoxicologic diagnosis of the mine site**
  - 2. Identify any native plant species that could behave as phytoextractors and determine their ecological behavior within the study area**
  - 3. Evaluate through experiments performed in microcosms *the extent of metal* phytostabilization of the soils from the sites *with and without the use of chelating agents***
  - 4. Characterize the types of mixed-species vegetative covers that could be used to restore soils polluted with several heavy metals and determine the possible situations in which they may be used**

Table 3. Species numbers recorded in the plant communities of rubble landfills and ecosystems of the copper mine Garganta de los Montes, Madrid.

Plant families	Landfill	Landfill base	Floodplain	Ash woodland	Temperate grassland	Wet grassland
GRAMÍNEAE	8	8	6	6	2	2
LEGUMINOSAE	2	2	6	7	3	2
COMPOSITAE	6	4	4	6	2	0
OTHERS	14	16	9	9	4	4
Total	30	30	25	28	11	7
Plant cover %	60	39	80	95	85	95
MOST ABUNDANT SPECIES	<i>Melilotus alba</i> (15%)	<i>A. castellana</i> (6%) <i>M. alba</i> (4%)	<i>T.campestre</i> (23%) <i>A.castellana</i> (5%) <i>P.coronopus</i> (15%)	<i>A. castellana</i> (40%) <i>Adenocarpus</i> (8%)	<i>Corrigiola</i> (30%) <i>A. castellana</i> (9%)	<i>A. castellana</i> (60%) <i>H. lanatus</i> (2%) <i>T. pratense</i> (2%)

Table 4. Mean heavy metal contents (mg/Kg) of grassland species (grouped as families) containing at least three metals in their above-ground mass growing in soils of the copper mine Garganta de los Montes (Madrid).

Plant families	Cu	Zn	Cd	Cr	Ni	Pb
GRAMINEAE	566 ± 1568	223 ± 244	6 ± 0.9	7 ± 11	2 ± 4	3 ± 4
LEGUMINOSAE	28 ± 26	87 ± 22	3 ± 5	1 ± 1.4	1.3 ± 1.8	0 ± 0
OTHERS	37 ± 50	153 ± 175	6 ± 8	0.04 ± 0.09	0.9 ± 1.1	0.7 ± 1.5

Table 5. Plant species growing in soils containing one or more heavy metal in the central Iberian Peninsula

<i>Agrostis castellana</i>	<i>Convolvulus arvensis</i>	<i>Lolium rigidum</i>	<i>Spergularia purpurea</i>
<i>Anagallis arvensis</i>	<i>Crepis capillaris</i>	<i>Plantago afra</i>	<i>Stipa lagascae</i>
<i>Andryala integrifolia</i>	<i>Crepis vesicaria</i>	<i>Plantago coronopus</i>	<i>Thymus zygis</i>
<i>Avena barbata</i>	<i>Dactylis glomerata</i>	<i>Plantago lagopus</i>	<i>Trifolium striatum</i>
<i>Bromus hordaceus</i>	<i>Diplotaxis catholica</i>	<i>Plantago lanceolata</i>	<i>Trisetum paniceum</i>
<i>Bromus madritensis</i>	<i>Echium vulgare</i>	<i>Pulicaria paludosa</i>	<i>Vulpia ciliata</i>
<i>Bromus rubens</i>	<i>Hirschfeldia incana</i>	<i>Sanguisorba minor</i>	<i>Vulpia myuros</i>
<i>Bromus tectorum</i>	<i>Jasione montana</i>	<i>Scirpoides holoschoenus</i>	
<i>Carduus pycnocephalus</i>	<i>Leontodon taraxacoides</i>	<i>Sonchus asper</i>	

The following is brief description of the most appropriate protocol used for phytostabilizing heavy metals:

- a) Characterizing the plant communities growing at different abandoned mine sites.
- b) Analyzing heavy metals in the topsoil layer (0-10 cm) by stratified sampling of the different morphological units of the landscape and plant communities of the sites (landfills, slopes and pastures of affected and non-affected areas). The reference values provided by the EU and the US EPA should be taken into account.
- c) Chemical analysis of the root systems of the most abundant species that grow in the most polluted sites.
- d) Selecting sites polluted with three or more heavy metals and turfing up the topsoil layer (avoiding excessive handling) to set up microcosms. Experiments are run under controlled conditions for 3-4 years (approximate timeframe for a grass community to become established from the seed bank with the appearance of biannual and perennial species) and watered using deionized water. Yearly species relevés are recorded, species are cut back after flowering-fruitletting (to simulate consumption by herbivores or harvesting) and leachates are periodically collected to assess the export of pollutants.
- e) Examining the microcosms: determining root biomass and chemical analysis of the species (individual above-ground mass and combined root systems).
- f) Examining the effects of heavy metals in tissues of the root systems of individual species by electron microscopy. We use LTSEM, SEM-SE and controlled pressure SEM with an EDX detector to obtain information on the mineral composition of tissues and to locate the heavy metals in the tissue.

## **ECOSYSTEMS DEGRADED BY THE EROSION-POLLUTION DICHOTOMY**

The urban solid residue (USR) landfills of central Spain, which at the end of the 1980s were sealed, or capped, simply by applying a layer of soil taken from the surroundings, have also been the subject of our research efforts. From the perspective of revegetating these systems appearing in our landscapes, these sites are probably the most complex we have come across in terms of their ecological restoration and the environmental impacts they produce on the surroundings.

The characteristics of old USR tips makes their revegetation particularly difficult. These characteristics include: their height, orientation, slope, type and depth of the soils used to cap them along with their continued unauthorized use for waste disposal or other uses (e.g., for shooting practice); some waste dumps even have platforms with scarce slopes that are sometimes sown with cereals. Indeed, reality has surpassed the difficulties we initially predicted (Hernández, 1994). Accordingly, one of our main study tasks has been to identify the communities and autoecology of the species, mainly grasses, that grow on waste tip slopes, using as reference the study, also autoecological, of communities growing on the banks of rural roads in the same settings as these dumps. These are considered analogous, especially for the purpose of determining the mechanisms of the ecological succession involved in the spontaneous revegetation process (Estalrich et al., 1992 and 1997). They are also useful for identifying the best candidate species for mitigating erosion or the loss of fine soil components. In a second stage, we went on to analyze the behavior of many of these species in response to the salt and heavy metal pollution commonly suffered by these landfills (Adarve et al., 1998; Hernández et al., 1998; Pastor and Hernández, 2002 a, 2002 b and 2004; Pastor et al., 2003 and 2007).

From a scientific standpoint, this type of scenario allows us to gain insight into what we call the erosion-pollution dichotomy. This phenomenon is currently among the most frequent environmental impact problems and is practically undescribed in the literature. We consider the problem to be of special interest when realistically approaching restoration based on ecological principles.

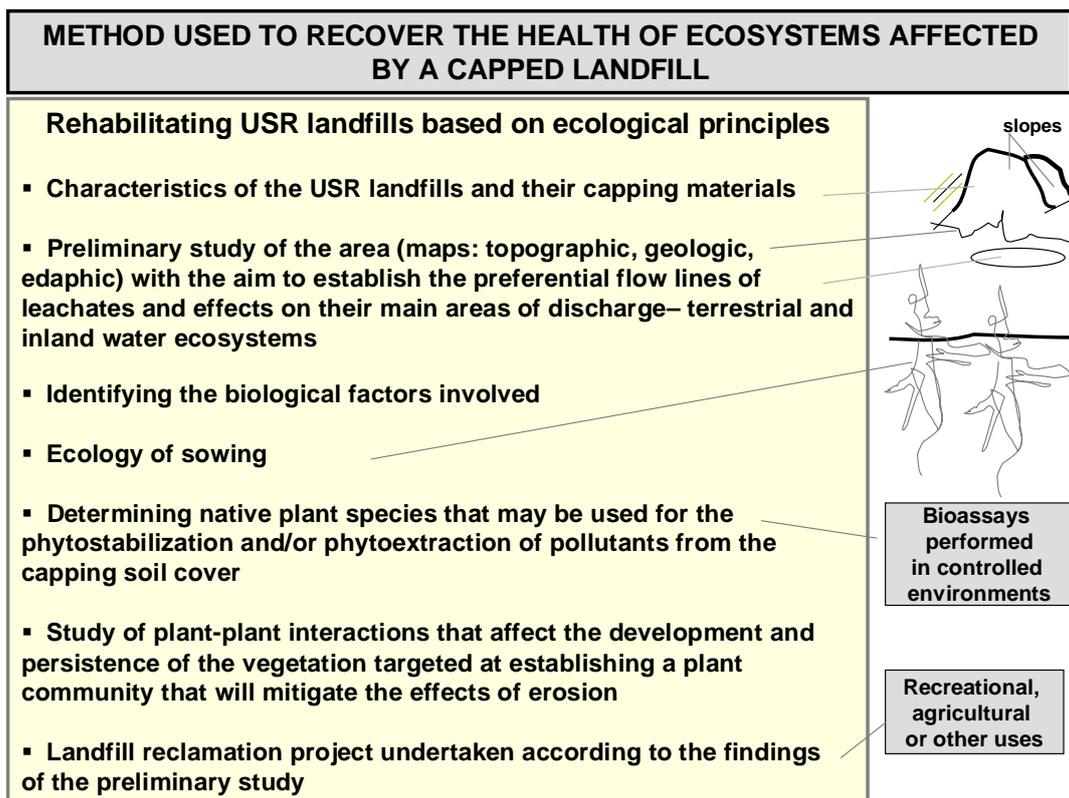
The scheme provided in Figure 6 indicates a need to start by determining the directions of ground water flows and their relationship with the surface water courses of the area (Adarve et al., 1994 a and b; 1996). This feature is linked to “distance contamination” or to the non-point source pollution observed in ecosystems outside the affected area (e.g., aquatic ecosystems in surface waters such as rivers or streams, and terrestrial ecosystems e.g., intoxication effects on animal populations that modify the structure of an ecosystem).

A further issue to consider, is the composition of leachates and directions of their main flows in relation to discharge zones, since this is linked to what we can denote “the spatio-temporal pollution of the ecosystems of the landfill environment”. The different level of the water table in discharge zones contributes to the “point-source pollution” of stable ecosystems in the environment (mainly marsh and streambed grasslands).

The nature and depth of the landfill’s soil cover is related to different aspects of the soil physics that help mitigate pollution, but that often determine that the slopes of the

capped landfill are not ideal for the development of the ecological succession in the landfill system. The loss or retention of compounds and chemical elements by the soil cover are related to what we refer to as “pollution in the landfill system”. Passage to the autotrophic component of that contamination is linked to pollution at a distance (of other ecosystems in the landfill’s surroundings) (Pastor et al., 1993; Urcelai et al., 1994 and 2000). Hence, it is also important that the study should examine ecotoxicological aspects analogous to the issues considered in the foregoing section (Pastor et al., 1994) within the conceptual framework of “risk analysis”.

**Figure 6**



Twenty years after many of these landfills were capped (Table 6), we intend to demonstrate the complexity that both their revegetation (spontaneous colonization of the vegetation from the seed bank of the soil cover) and phytoremediation using species that can adapt to their conditions supposes.

**a) Characterizing capped landfills: platforms, slopes and discharge areas**

The waste materials deposited in the landfills are of a mixed nature (urban solid, industrial and inert) and are not pretreated in any way. The soil used to cover the landfills is no deeper than 40 cm. Slopes are generally over 15 m in height and occasionally overlap due to subsequent reuse of the landfill by tipping waste on top of the sealing soil cover. Gradients are high, and frequently surpass 40%. These slope features besides affecting the plant colonization of these systems, also affect the extent of the leachate discharge area as surface runoff. Even when a landfill has a single slope, runoff occurs as a fan and thus differentially affects the biodiversity of the discharge zone. Moreover, a single slope often shows considerable variation in soil factors apart

from exhibiting obvious differences with respect to the cover soils derived from different substrates.

Table 6. Present characteristics (2006-07) of USR sealed landfills on arkosic, limestone and gypsum substrates in the *Comunidad de Madrid* (Spain)

USR landfills	1 <sup>st</sup> year of capping	Ecosystem Main discharge	After-uses	No. slopes
<b>Granites and gneiss</b>				
Colmenar Viejo	1986	Stream and pastures grazed by cattle	Itinerant shepherding; fencing and reforestation with pines	3
San Lorenzo			Open burning, itinerant shepherding; houses constructed in the discharge area	3
El Escorial		Stream and ash woodland	Unauthorized rubble disposal/cattle grazing	4
<b>Arkosic</b>				
Móstoles	1986	Stream and wetland	Cereal cultivation and itinerant shepherding; cereals and horse-riding paths	3
Villaviciosa	1987	Slope and wetland	Recreational use	1
Navalcarnero	1989	Sheep pasture	Cereal cultivation; shooting practice; reused for waste disposal; housing	-
El Álamo	1995	Wetland	Restored using covers comprised of native grasses	2
<b>Limestone, loams, clays</b>				
Alcalá de Henares	1986	River	Sown with acacias; other trees; grasses irrigated with river water	1
Torrejón de Ardoz 1	1982	Wetland	Pines; reused for waste disposal and continuously infilled by the wetland	3
Torrejón de Ardoz 2	1991	Wetland	Reused for rubble disposal; newly sealed in 1994; fenced off but unauthorized dumping continues	several
Mejorada del Campo	1986	Slope, streambed and river	Itinerant shepherding; shooting practice; restructured by the high-speed train, or AVE; reforested with pines; sown with alien grass species	3
Getafe	1986	Wetland	Reused for inert waste and rubble disposal	12 overlapping
Pinto 1	1986	Slope	Reused for waste disposal; use currently controlled	4
Pinto 2 a	-	Cereal crops	Reforested with pines	3
Pinto 2 b	-	Cereal crops	Reused for waste disposal	3
La Poveda	-	Stream	Reused to deposit rubble from the AVE works	2
Arganda	1987	Stream and streambed pastures	Uncontrolled - waste and inert materials continue to be deposited	2
<b>Gypsum</b>				
Aranjuez	1990	Stream	Open burning, steep slopes corrected by breaking-up after capping	1

Our phytocological and edaphic studies performed in the past years both on the capping soils (Table 7) and soils of the discharge zones have revealed certain features that may be of help when planning to ecologically restore this type of environmental impact setting. The following is a systemized list of the main questions addressed: differences in the values of some edaphic variables and biodiversity between the landfills and the reference ecosystems, as well as differences in edaphic variables among the different landfill slopes.

Table 7. Soil variables recorded for the soil covers (mean values and standard deviation) of landfills overlying substrates representative of the central Iberian Peninsula five years after they were capped.

Soil variables	Granites and gneiss	Arkoses	Limestones	Gypsums
pH	7.0±0.2	7.1±0.4	7.6±0.1	7.6±0.3
OM (%)	1.5±0.5	0.6±0.3	1.5±0.03	0.36±0.3
Total N (%)	0.080±0.030	0.033±0.010	0.094±0.056	0.048±0.004
P (mg/100g)	21.1±19.2	13.2±9.0	6.3±2.5	9.0±1.3
Na (mg/100g)	1.8±0.7	6.9±3.7	1.3±0.2	1.9±1.5
K (mg/100g)	17.2±9.5	21.8±3.8	32.3±9.9	13.1±1.1
Ca (mg/100g)	350.0±172.9	335.0±144.5	715.0±65.0	1396.7±1108.2
Mg (mg/100g)	9.6±3.6	37.2±23.3	25.1±2.8	6.60±1.7
Zn (mg/Kg)	125.5±69.8	83.5±146.0	57.5±10.5	33.3±5.7
Cu (mg/Kg)	8.7±19.4	150.9±730.2	13.0±11.0	5.0±5.2
Pb (mg/Kg)	7.7±5.6	72.8±297.0	28.5±3.5	0.0±0.0
Cd (mg/Kg)	0.0±0.0	1.5±3.1	0.0±0.0	0.0±0.0
Cr (mg/Kg)	0.0±0.0	4.4±4.5	0.0±0.0	0.0±0.0
Ni (mg/Kg)	22.2±3.6	15.7±8.1	22.5±3.5	17.7±1.2
Co (mg/Kg)	0.0±0.0	1.5±2.3	0.0±0.0	0.0±0.0

From this analysis, we were able to conclude that the events occurring and/or after-uses given to the landfills after their initial capping, along with the particular characteristics of each landfill pose many difficulties for the phytorestitution of their cover soils. The reasons for this are explained in more detail in the following section.

### b) Cover soil-vegetation eco-chemical relationships

Floristic relevés were recorded in twenty capped landfills of the central peninsula found on different substrates (granite and gneiss, arkoses, gypsum, limestone and marl). We also examined specialization of the flora to the different levels of disturbance as well as the adaptive strategies used by the species colonizing these environments, manifested by different biological features. Notwithstanding, based on the observations made (Tables 8 and 9), we focused on certain characteristics of the perturbation effects on the species and communities that most often appear in this type of setting.

Evident differences related to the diversity of both the plant species (all herbaceous) and nematodes in the soil (a soil mesofaunal indicator of nutrient recycling) were noted between the soil cover of the landfills and the corresponding reference ecosystems (Urcelai et al. 2000). An initial response to ecochemical relationships was that associated with the salinity of the landfills. The data shown in Table 10 indicate high levels of anions, especially of chloride ions soils detected in soils devoid of vegetation, and those provided in Table 11 reflect salinity differences among zones. These observations prompted an analysis of the link between salinity and pasture species to understand the ecological behavior of these plants and apply this knowledge to our restoration strategies (Adarve, et al., 1998; Hernández et al., 1998 b; Pastor et al., 2002 a and b).

Table 8. Significantly different results (99.9%) obtained in 36 soil samples taken from landfills overlying arkosic materials (in the fifth year after their initial sealing) and 55 samples collected from reference ecosystems of the same region.

Biotic factors	Landfills	Reference ecosystems
Cover. Total vegetation (%)	34.9 ± 17.1	60.6 ± 25.0
Mean vegetation height (cm)	14.9 ± 9.1	22.7 ± 10.2
Plant diversity (no. sp / m <sup>2</sup> )	15.5 ± 7.3	29.5 ± 11.4
Density of nematodes (no./100 cm <sup>3</sup> )	45.6 ± 38.3	122 ± 50.7

Table 9. Plant biodiversity (species richness) variation in the discharge areas of three landfills of the *Comunidad de Madrid* five years after their initial sealing affected by surface runoff (N: reference ecosystem; A to H: Landfill sites).

Alcalá			Torrejón					Móstoles								
N	A	B	N	A	B	C	D	N	A	B	C	D	E	F	G	H
75	36	46	61	32	45	35	50	73	32	32	35	35	37	40	35	71

Table 10. Levels of anions (mg/Kg) recorded in the soil cover of three landfills found on an arkosic substrate six years after they were initially capped according to the revegetation arising from the seed banks of the cover soil used.

USR landfill	Sulfates	Chlorides	Nitrates	Fluorides
<b>Mejorada</b>				
Soil under Gramineae	10.5	14.8	10.0	1.2
Soil under Leguminosae	23.4	20.8	7.3	1.3
Naked soil	47.8	374.4	36.7	3.3
<b>Móstoles</b>				
Soil under Gramineae	11.0	5.6	0.9	1.4
Soil under Leguminosae	15.9	10.6	0.9	0.9
Naked soil	11.0	3.3	0.9	0.9
<b>Navalcarnero</b>				
Soil under Gramineae	11.1	8.3	3.8	0.9
Naked soil	123.4	145.6	43.3	0.9

Table 7 indicated that Zn is a metal found in greatest quantities in all the landfills. Along with the high chloride contents, this led us to design a set of bioassays conducted under controlled conditions applying different concentrations of Zn chloride to the soil and then sowing with several native species collected from the field (analogous to those growing on the landfills), as well as other commercially available ecotypes: pasture gramineas (*Lolium rigidum*, *Hordeum murinum*, *Bromus hordaceus* and *B. rubens*), commercial gramineas (oats, wheat, maize), pasture legumes (*Trifolium subterraneum*, *T. glomeratum*, *T. tomentosum* and *Lupinus angustifolium*) and forage plants (alfalfa, vetch and lupin), along with the crucifer *Hirschfeldia incana* (Pastor et al., 2003 and 2004).

We also examined the effects of soil Zn contamination on individual species (Pastor et al. 2003) and the plant communities that grow in the ecosystems of the natural surroundings of the landfill areas. Only a few community species were able to tolerate

high soil Zn levels and may thus be considered appropriate as phytoremediators of soils with similar concentrations of this metal, around 700 ppm (Tables 12 and 13).

Table 11. Soil conductivity values recorded ten years after capping in a single landfill (Mejorada) with several slopes and a landfill (Móstoles) whose only slope has very different zones in its low, middle and high parts

Soil cover of the Mejorada landfill				Soil cover of the Móstoles landfill			
		pH	Conductivity ( $\mu\text{S}/\text{cm}$ )			pH	Conductivity ( $\mu\text{S}/\text{cm}$ )
SLOPE 1	Soil 1	7.8	297	ZONE 1	Soil 1	7.1	706
	Soil 2	7.8	361		Soil 2	7.1	484
	Soil 3	7.9	460		Soil 3	7.4	452
SLOPE 2	Soil 1	7.6	395	ZONE 2	Soil 1	7.3	450
	Soil 2	7.7	553		Soil 2	7.4	494
	Soil 3	7.7	514		Soil 3	4.2	669
SLOPE 3	Soil 1	7.9	564	ZONE 3	Soil 1	3.4	1032
	Soil 2	7.6	551		Soil 2	3.2	1882
	Soil 3	7.6	810		Soil 3	7.4	394
SLOPE 4	Soil 1	7.9	282	ZONE 4	Soil 1	2.1	2810
	Soil 2	7.7	364		Soil 2	2.7	2690
	Soil 3	7.9	405		Soil 3	2.6	2620

Table 12. Plant diversity and cover determined in a bioassay using microcosms prepared with soil containing different concentrations of zinc chloride.

	Treatment (ppm Zn)	Mean and SD
Diversity (total no. vascular sp.)	0	20.0 $\pm$ 2.0 a
	300	11.7 $\pm$ 2.3 a
	500	6.0 $\pm$ 1.0 b
	700	4.7 $\pm$ 0.6 b
% Cover vascular sp.	0	70.0 $\pm$ 8.9 a
	300	53.7 $\pm$ 2.1 b
	500	30.0 $\pm$ 4.0 c
	700	14.7 $\pm$ 4.2 d
% Cover mosses	0	17.0 $\pm$ 2.6 a
	300	2.0 $\pm$ 2.0 b
	500	0.3 $\pm$ 0.6 b
	700	0.3 $\pm$ 0.6 b

Table 13. Zn contents (mg/Kg) of the above-ground mass of several species of the community grown in microcosms containing different soil Zn chloride concentrations.

Species	Control	300	500	700
<i>Vulpia myurus</i>	74,6	228	765	1157
<i>Polypogon maritimus</i>	75,0	278	-	957
<i>Juncus buffonius</i>	75,3	120,5	149	893
<i>Echium sp.</i>	259	811	-	-
<i>Scirpoides holoschoenus</i>	28	36,5	64	-
<i>Trisetum panicetum</i>	63	373	-	-
<i>Lolium rigidum</i>	-	255	-	-
<i>Gaudinia fragilis</i>	-	251	-	-
<i>Anagallis arvensis</i>	-	86	-	-

As we examined behavior at the species and community level in controlled conditions (microcosms), we also performed experiments in field conditions. These experiments revealed some responses of the plants to physical abiotic factors (slope gradient and orientation) and biotic factors (such as the species growth habit in relation to the horizontal soil cover, rooting types in shallow soils, and possible seed production). These features need to be considered for revegetating landfills besides ecochemical interactions.

The real situation shown by sealed waste landfills, whether solid urban residues, industrial residues or mixed (as most of those in the Comunidad de Madrid), means we are faced with a rather complex restoration process mainly due to the following characteristics:

- 1) Their many slopes with steep gradients and different orientations are environmental factors that any revegetation project will have to deal with.
- 2) The scarce layer of capping, or sealing, material (soils of the landfill surroundings) along with the steep slopes leads to the loss of fine materials that affect the implantation of root systems and cause fertility losses of the soils proper to the landfill system and silting of discharge areas (this often occurs in wetlands where the water table fails to crop out or in surface water channels in which sediments build up).
- 3) Besides these effects of physical degradation processes, those arising from the contamination that the landfills also exhibit determines that any restoration process will have to jointly deal with both types of effect, which we denote the erosion-pollution dichotomy of soils. In many cases, plant species that could be implanted to prevent erosion are unable to tolerate any of the pollutants found in these systems, or vice-versa.
- 4) In landfills capped with soils from their respective surroundings, the two processes of ecological succession intermix: on the one hand, there could be a primary succession since a new community starts in these “recently arising” systems, and on the other, a secondary succession could arise through germination of the seed bank present in the soil used to cover the landfill.

The two most important questions that should be highlighted are: a need to arrest erosion and its effects such as silting of water courses, eutrophication and pollution of surface waters; and in a subsequent stage, a need for adequate surveillance to avoid the constant reuse of a sealed landfill for waste disposal, which will hinder any restoration project.

## **CONCLUSION**

After several years of exploring the best way of going about restoration based on ecological principles, we would like to highlight the following conclusions. The first is the highly complex nature of the processes involved in restoring ecosystems degraded through human actions. In our introduction to this subject, we mentioned the basic aspect of the complexity of ecosystems. This complexity is exaggerated by any human land management measures, mainly inappropriate land use, waste disposal and use of fertilizers. Without a good understanding of the “normal” behavior of an ecosystem, we cannot even attempt to identify the components of the ecosystem affected by an environmental impact and will not be able to undertake a realistic restoration. This determines the importance of finding a reference ecosystem that resembles in as much as possible the degraded ecosystem, unless of course, we have information of the previous state and behavior of the system before its perturbation.

Despite this complexity, we feel the different methodologies used to restore the terrestrial ecosystems of the degraded scenarios most frequently arising in the Mediterranean semi-arid and arid environments of Spain could render autochthonous plants with a capacity to restore polluted soils and help detain the erosion of sites with pronounced slopes. Our approach has also provided combinations of plant species that may be used for sites whose soils are polluted with several heavy metals. Although we used low cost techniques requiring a considerable length of time to adapt them for use in polluted environments, they all offer information for the restoration of specific polluted sites of the area. Thus, it seems that no general ecological restoration concept can be applied.

The many levels of complexity involved in an ecotoxicological study: spatial (geologic-edaphic), biologic (populations), and the many possible ways of evaluating the ecotoxicological nature of an environment means we should integrate scales through the use of *microcosms* (see figure 9).

Experiments in microcosms provide a quantitative idea of the optimal field conditions for the phytostabilization (in highly polluted soils) and phytoextraction (in moderately polluted soils) of metal contaminants. Both field and laboratory work is needed to identify the plant species that determine community structure in these environments and to complement previous bioassays by establishing further levels of heavy metal tolerance and toxicity.

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