EXPLOITING MICROBES AND PLANTS TO CLEAN UP PESTICIDE CONTAMINATED ENVIRONMENTS

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Background

Microbes and plants are among the most important biological agents that remove and degrade waste materials to enable their recycling in the environment. Whilst these agents are adapted to tackle most naturally occurring substances, many modern synthetic chemicals present a challenge to their remediating capabilities. The fact that some recalcitrant chemicals can persist in the environment for long periods is in itself indicative of the limitations of natural degradation processes. A particular example is that of the potent insecticides (organochlorines, organophosphates and carbamates) that were first developed during the 1940s to 1960s. The widespread use of synthetic pesticides in agriculture and public health that followed, led to fears over the persistence of these toxic chemicals in the environment, bioaccumulation in the food chain and risks to non-target species.

Although most newer pesticides are less persistent or bioaccumulative, many are more mobile in the environment. Through seepage and run-offs, and continuous cycles of volatilisation and condensation, pesticides used on land can end up in aquatic environments, and traces of pesticides have even been found in rain, fog and snow (Rice, 1996; Dubus *et al*., 2000) (Figure 1)

Environmental contamination by pesticides generally falls into two broad categories:

- A diffused low-level contamination from continued use of pesticides in agriculture and public health and remnants of persistent pesticides used in the past.
- Heavy pollution of soil and surface water/ground water in defined areas due to disposal or accidental releases of concentrated pesticide formulations.

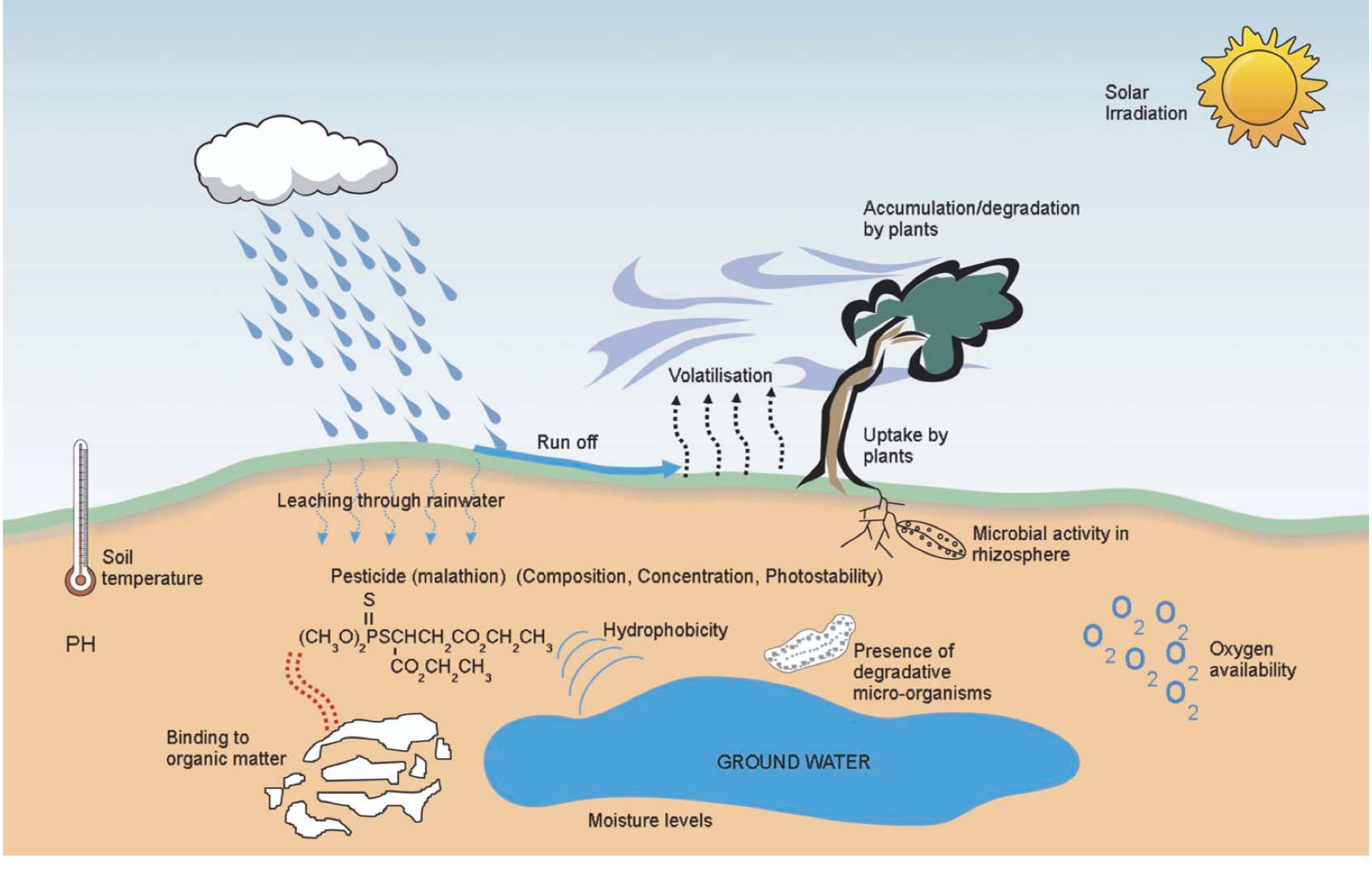

Figure 1. **Microbial communities and plants work in synergy to breakdown hazardous substances in the environment.**

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Table 1. Remediation strategies for contaminated soils.

Remediation Strategy	Advantages	Disadvantages
Chemical inactivation such as immobilisation or oxidation	Fast	Use of chemicals may be costly, and may give rise to added contamination
Incineration	Fast Reduction in volume	High costs of transportation, problems of combustibility of soil matrix and toxic emissions
In situ vitrification	Reduces leaching and soil volume	High costs to generate required temperatures (1600–2000 °C).
Stabilisation/solidification (binding to resins)	Reduces leaching	The cost of binding resins may be very high
Thermal desorption (high temperatures in the absence of oxygen to vaporise or destroy pesticides)	Less heat required than for incineration The matrix is not incinerated, reduced emissions.	Gaseous emission controls required
Vapour stripping (contaminated soil surrounds a tunnel to which vacuum is applied, removing volatile waste by a series of filters)	Generates little waste Fairly cost effective.	Only suitable for volatile contaminants
Bio- and phytoremediation (metabolising properties of micro-organisms or plants are harnessed for environmental clean-up)	Low cost, low maintenance, environment-friendly. Can be adapted for use *in situ*	Is slow when compared to incineration or chemical deactivation methods

Research into decontamination strategies has tended to focus on small areas contaminated with high concentrations of pesticides, especially those compounds that pose an immediate threat to the environment and human health. In contrast, a diffused low-level contamination of the environment has received little attention, except where there has been a legislation to limit the amount of residues in a given commodity, such as foodstuffs, drinking water *etc*.

The natural processes that break down toxic chemicals in the environment have become the focus of much attention to develop safe and environment-friendly deactivation technologies. The processes involved in pesticide biodegradation, such as oxidation, hydroxylation, aromatic ring cleavage, hydrolysis, dehalogenation, dealkylation, or conjugate formation, have been well studied in recent years. This has provided a basis for the targeted use of microbes and plants in enhanced remediation of contaminated sites (Mulbry and Kearney, 1991; Chaudhry *et al*., 2002). Both bioremediation (using microbes) and phytoremediation (using plants) offer the potential for low-cost, low-maintenance, environment-friendly and renewable resources for *in situ* remediation of contaminated environments that are far more cost-effective than any *ex-situ* decontamination technique (Table 1).

Microbes or plants?

The choice of using microbes, plants or both in a remediation effort depends on the extent of contamination, nature of the chemicals present, and the amount of time available for decontamination. The decontamination rates achieved by bioremediation technology are generally slower than those achieved by some physical and chemical methods. The fundamental constraint to the success of either of the technologies is the ability of microbes or plants to grow in an environment that might be heavily contaminated with organic and inorganic chemicals. The hydrophobic nature of most pesticides presents a major obstacle in their uptake by microbes or plants. Indeed, the uptake and translocation of highly hydrophobic pesticides through plant roots may only be limited in most species. Thus, whilst plants have been shown to possess useful enzymatic mechanisms to degrade most pesticides (Chaudhry *et al*., 2002), their main application in phytoremediation has been to ‘bio-extract’ inorganic pollutants, such as toxic heavy metals. There are other constraints to the use of plants alone in remediation; plant growth is dependent on a number of environmental factors, such as availability of nutrients and water, soil type and pH, *etc*. The maximum benefits of phytoremediation may, therefore, be achieved in long-term applications, or when used in conjunction with other immediate remedial actions. Despite such limitations, plants are known to absorb a wide range of air-borne chemicals through the foliage surface. Natural vegetation may, therefore, act as a continuous sink for many environmental pollutants including semivolatile pesticides (Simonich and Hites, 1995).

Numerous microbial strains have been shown to degrade or ‘bio-fix’ a wide range of environmental chemicals. The bacterial species found to be most useful in bioremediation belong to the genera *Flavobacterium*, *Arthrobacter*, *Azotobacter*, *Burkholderia* and *Pseudomonas*. In particular, some strains of *Pseudomonas* have been reported to degrade a large number of organic chemicals. For example, strains of

Pseudomonas sp. and *Klebsiella pneumoniae* have been shown to possess hydrolase enzymes that are capable of breaking down s-triazine herbicides, such as atrazine, which because of aqueous solubility and persistence could leach into groundwater. Similarly, a number of enzymes such as oxygenases, hydroxylases, hydrolases and isomerases present in *Pseudomonas* and *Alcaligenes* sp. have been shown to degrade the herbicide 2,4-D (Mulbry and Kearney, 1991). Both bacteria and fungi can degrade OP pesticides through hydrolytic cleavage, and pyrethroids (e.g. permethrin) through cleavage of the ester bonds. With the exception of dithio-carbamates, microbial degradation of all types of carbamate pesticides has also been demonstrated; for example, a rapid hydrolysis of carbaryl has been reported due to presence of the enzyme carbaryl esterase in *Pseudomonas* sp. (Mulbry and Kearney, 1991). A few strains of *Pseudomonas* have also been genetically altered to confer ability to degrade recalcitrant chemicals, such as chlorobenzenes that are commonly used in pesticide synthesis (Wackett *et al*., 1994). The white-rot fungi *Phanerochaete chrysosporium* have enzymes that not only enable them to degrade lignin and cellulose, but also breakdown many recalcitrant chemicals including halogenated-phenol ring-containing compounds, such as the wood preservative pentachlorophenol, which is a particularly persistent pollutant in industrial wastes from paper and leather tanning industry (Evans and Bucke, 1998).

Many recalcitrant chemicals are also known to be transformed by microbes to products that are more efficiently absorbed and translocated by plants. Thus, a combination of bio- and phyto-remediation in the immediate vicinity of the plant root mass (rhizosphere) could enhance the degradation process of pesticides. This interaction could be further improved by manipulating microflora in the rhizosphere, for example, through the introduction of known bioremediating species of microbes. The synergy should greatly enhance the overall rate of remediation, especially under conditions that promote the growth of both microbes and plants.

Problems and solutions

There are a number of difficulties that have hindered the full-scale commercial adoption of bio- and phyto-remediation. Tests carried out on the remediating efficacy of microbes or plants under controlled laboratory conditions do not usually simulate true field situations. There is a general lack of reliable techniques to prove efficacy of remediation in the field, and ordinary sampling techniques often fail to reveal the real levels of a pollutant in a heterogeneous field. An inherent problem with bioremediation in soil environments is the fact that target substances may not be readily available for uniform dispersal, due to low solubilities and high binding capacities. This could lead to regions of high concentration of pesticides that often prevent microbial activity, a problem that has proved a major stumbling block in utilisation of bioremediation to its maximum potential. The use of mechanical aids, for example ploughing, biological means such as the use of biosurfactants, or the use of microfauna and macroinvertebrates to disturb the soil matrix can enhance bioremediation *in situ* (Hamby, 1996).

The use of cell-free enzyme preparations to degrade organic pollutants is also gaining popularity, as it is not subject to many of the limitations that are associated with microbial growth under field conditions. One example is the use of an aqueous fire-fighting foam containing OP-hydrolase to degrade a number of OP compounds (LeJeune *et al*., 1998). Bioremediation could also benefit from advances in techniques such as microencapsulation for a slow or timed release of bioremediators to overcome problems encountered under field conditions, and to enhance the persistence of microbial or enzymatic preparations to achieve maximum benefits.

Non-domestic landfills and other sites, with a long history of pollutant dumping, are especially problematical from a remediation standpoint. These sites may harbour a wide variety of contaminants, often with low bioavailability, which further declines with time (Hatzinger and Alexander, 1995). The presence of co-contaminants (*e.g.* phenols) can result in further complications as they can inhibit the activity of microbial communities present to degrade other pollutants (Allard and Neilson, 1997). It is, therefore, desirable to utilise those indigenous and non-indigenous microbes and plants that have diverse degradative properties; and most importantly which would less likely be suppressed by the presence of co-contaminants. Further difficulties may be encountered with long-standing contaminated sites where pesticides may have chemically bound to soil or penetrated deep into the soil subsurface, or even into groundwater. The survival of aerobic microbes under these conditions of low oxygen would be limited, and the use of anaerobic microbial communities could be advantageous. Similar considerations apply to the use of plants, since the species most suitable for use in phytoremediation at a particular site would be those able to grow under field conditions. In fact, it has often been demonstrated that the indigenous plants and synergistic communities of microbes present at a contaminated site are those best suited for remediation purposes. This is due to natural selection over time of those species/ strains that are capable of exploiting the contaminated environments (Romantschuk *et al*., 2000). Thus, research into bio- and phyto-remediation has also been aimed at generating the environmental conditions that give maximal growth of indigenous microbial communities or plants with the ability to remove and/or degrade contaminants *in situ* (Allard and Neilson, 1997).

A case study

Some bacterial species, such as *Arthrobacter* sp., *Pseudomonas dimunuta* and *Flavobacterium* sp., have been shown to breakdown organophosphate pesticides (Shelton and Somich, 1988; Mulbry and Kearney, 1991). We studied the ability of microflora in soils collected at different locations in the UK to breakdown diazinon, an organophosphate compound used in sheep-dip formulations for the control of sheep scab mite (Parker, 1997). The disposal of large volumes of sheep-dip leftovers presents a hazard to the environment, especially in the vicinity of watercourses. The

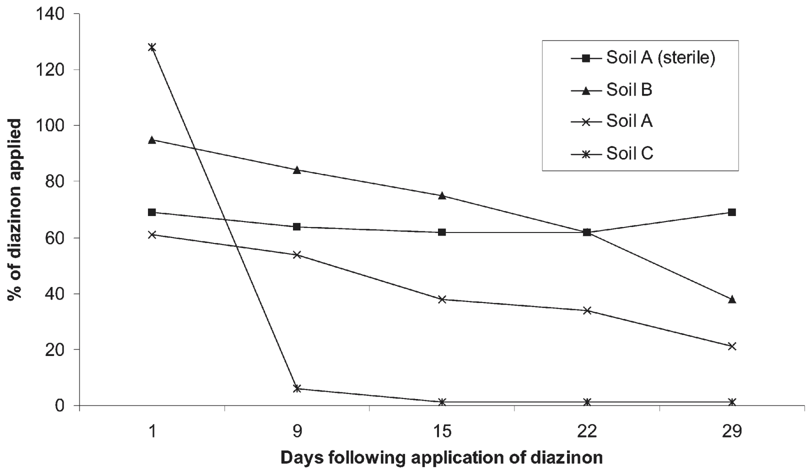

Figure 2. **The breakdown of diazinon by different soil samples.**

holding tanks before release into watercourses. Selected bioremediators may also be used in a 'bioreactor' set up to provide a safe and cost-effective means of deactivating large stocks of unwanted persistent pesticides that pose a continuous hazard to the environment in some developing countries (FAO, 1998).

Future outlook

During the last five decades, improvement in crop yields and product quality has mirrored the increase in pesticide usage. However, this has also contributed to an increasing public pressure to minimise the use of toxic chemicals in the environment. The regulations regarding the approval of pesticides have become much more stringent than in the past. This has shifted the emphasis towards finding alternative methods of pest control, especially those involving biotechnology and that aim to rely on little or no use of pesticides. However, the fast pace of biotechnology in recent years has also met with an equal public disapproval, and all the signs are that the use and disposal of pesticides will continue for some considerable time to come. In particular, developing countries will continue to encounter major problems associated with pesticide usage and disposal. This will inevitably lead to a continued need for long-term strategies to tackle such issues. There are already emerging commercial uses of bio- and phyto-remediation principles, and considering the vast range of applications, these sectors are likely to see a rapid growth in the future. The anticipated growth in demand for more cost-effective ways for environmental cleanup and hazardous waste management are likely to drive expansion of the remediation technologies.

One such area into which the use of biological remediation technologies is likely to expand involves tackling the growing worldwide problem of sewage wastes. In the past, up to 50% of the UK sewage sludge was dumped at sea. Since the banning of dumping at sea in 1998, there has been a significant increase in its disposal at landfill sites. Sewage sludge contains useful nutrients that can be utilised as a fertiliser for plants. However, its use on agricultural lands has been limited because of the presence of toxic heavy metals and certain organic pollutants including pesticides. A remediation approach can be useful in deactivation and removal of these contaminants from sludge materials, whilst using it as a fertiliser to grow short-rotation crops that can be used for non-food purposes, such as in energy generation.

Rapid advances in biotechnology are also likely to drive further innovation and growth in bio- and phyto-remediation sectors. The engineering of pesticide-degrading enzymes or enhancing their levels in selected microbes and plants has already been demonstrated at laboratory scale (Timmis *et al.*, 1994). Another technology under development involves engineering microbes and plants with genes that express antibody fragments with binding sites against specific chemicals. At present the efficacy of 'plantibodies' against atrazine, and paraquat has been demon-

aim of the study was to isolate diazinon-degrading microbes from soils that had been previously exposed to OP pesticides. The bioremediating ability of the collected soil samples (A, B and C) was compared against a soil sample that had been sterilised by autoclaving. Soil A was composed of heavy clay with flints that had been exposed to various pesticides (including OPs) in the past, although not for several years. Sample B was from an organic soil rich in manure that had been exposed to OPs in the past. Soil C had organic matter on top of clay, and was exposed to OPs previously and in the recent past. Each sample was mixed with sterilised topsoil in 1:19 ratio, and added with a commercial sheep-dip formulation 'Topclip' containing 60% w/w diazinon to achieve a concentration of 100 mg of the active ingredient kg^{-1} of soil. Sub-samples were drawn after a period of 24 hours following pesticide application and subsequently at approximate weekly intervals for four weeks. Diazinon was extracted from the soils in ethyl acetate, and analysed by gas chromatography using a range of diazinon standards (Figure 2).

The results showed an increased breakdown of diazinon by soils A and C when compared to the sterilised soil sample. In particular, the soil sample from a Yorkshire farm (soil-C) showed good degradation of diazinon (only 2% remaining after 29 days), whilst soil-B did not differ significantly from the sterilised sample. The degradation of diazinon by soil C, therefore, appeared to be the result of microbial activity. The results were also in agreement with the well demonstrated phenomenon (often termed as 'soil memory') that once exposed to a compound, the soil microflora retain their ability to degrade the same compound upon re-exposure. It has been shown that repeat applications of some chemicals may enhance the metabolising properties of soils, due to enhanced growth of the selected microbial communities.

This successful study also illustrates the huge potential for isolating other bioremediating microbes from natural environments against other pesticide compounds. Using such bioremediators, industrial effluents, such as those from pesticide manufacturing facilities, could be deactivated in strated (Longstaff *et al*., 1998), but there is a vast scope for developing transgenic plants that absorb, bind and inactivate a specific chemical, or even a class of chemically related pollutants. Such advancements in knowledge not only need to be adopted by researchers but also by the marketing, user and regulatory sectors involved. Moreover, a greater collaboration between research institutions and industry, as well as other sectors especially geology and engineering is required to rectify the problems associated with demonstration of the true potential and commercial recognition of these techniques.

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Helen Atterby, Neil Smith, Qasim Chaudhry and *David Stead* at the Central Science Laboratory of the Department of Environment, Food and Rural Affairs in York are involved in research into different aspects of bioremediation. Of particular interest are biodegradation of environmental chemicals, bioremediation of organic wastes to control malodours, and manipulation of microbial activity in rhizosphere to enhance the process of phytoremediation.

A BOOK FROM THE BRIGHTON CONFERENCE

Pesticide Behaviour in Soils and Water

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This 450-page book is a full record of the proceedings from the two and a half day symposium that ran concurrently with the BCPC Conference – Weeds 2001, held in Brighton, UK, 12–15 November 2001.

Introducing the symposium, Professor Allan Walker said, "It is important to understand the basic processes that occur in soil and the interactions that take place between them, so that we can devise appropriate practical management strategies to reduce the potential for environmental contamination."

Following the introductory session, which provided an overview of the quantitative assessment of pesticide interactions within the soil, consideration was given to the role of long-term sorption kinetics and soil pH along with sorption to hard surfaces and behaviour in sediment-water systems.

Looking at things on a larger scale, the next session focussed on the complexities of lysimeter and field-data and discussed the assessment of field-scale variability of herbicide transport. Degradation is the main route for loss of pesticides from the environment, and the following session described how studying the behaviour and ecology of pesticide degrading organisms can improve our understanding of the biodegradation processes.

Methods that can be used to measure and describe variability and uncertainty in mathematical modelling were the focus of the session on 'Quantitative Aspects', whilst a number of papers were presented on the different approaches that can be taken to protect soil and water from contamination by pesticides.

The final papers addressed some of the current issues relevant to the regulation of environmental exposure and risk assessment and the implementation of risk assessment strategies for pesticides in soil, water and air.