Methods for Assessing the Effects of Mixtures of Chemicals
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Functional Processes of Ecosystems: Their Use in Assessing the Effects of Mixtures of Chemicals

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ABSTRACT

Techniques are being developed to assess the effects of chemicals on ecosystem function. Both energetic and nutrient cycling parameters are sensitive to chemical perturbations but few generalizations can be made in regard to their applications to the detection of stress effects in the field or the testing of chemical mixtures to predict ecological responses. Specific experimental and data reduction approaches helpful in elucidating potential interaction effects in field studies of complex effluents are suggested. Ecological testing of mixtures with both laboratory and field methodologies can provide essential information on the specific and integrated impacts of chemical components on essential ecosystem processes under specific conditions, but the predictive value of the results of these tests is largely unknown.

1 INTRODUCTION

The use of techniques for assessing ecological damage or predicting the effects of individual chemicals or mixed effluents on ecosystem properties or processes has been the topic of much recent discussion but has found little general acceptance in terms of standard practices for the monitoring of biological effects or safety evaluation of environmental chemicals. This situation is, in part, a response to an incomplete understanding of the complex interactions governing the functioning of ecosystems as well as a reticence by both scientists and regulators to attach tangible value to adverse changes in ecosystem-level parameters such as primary productivity and nutrient cycling.

However, as all organisms interact within the energy and material processing constraints which structure populations and communities as well as determine the functional efficiency of the ecosystem as a whole, the effect of chemical perturbations expressed through the response of ecosystem processes will encompass effects on varied individuals. Because an ecosystem is an integrated unit with fundamental characteristics which transcend the simple summation of component processes, analysis at the ecosystem level should not only reflect the response of component populations but should provide a comprehensive assessment of whole-system 'status'.

The use of ecosystem criterion variables to assess damage from chemical pollution in natural systems has been reviewed in detail elsewhere (Matthews *et al.*, 1982; Sheehan, 1984), as have their use in model ecosystems for the evaluation of ecotoxicity (Giddings, 1981; Suter, 1981). Although further development and critical evaluation of methods to assess pollutant effects on functional variables is needed, this paper will only briefly summarize this subject while addressing the more specific problem of biological monitoring and testing the effects of mixtures of chemicals on ecosystem function.

2 CHOICE OF FUNCTIONAL CRITERION VARIABLES

An ecosystem is basically an energy processing unit. The assemblage of organisms and their environment interact collectively through the exchange and incorporation of energy and materials. As functional processes within an ecosystem, energy and material movements are intimately related. Photosynthesis is regulated by essential element availability. An array of elements is necessary for the maintenance, growth, and reproduction of all species. Food and nutrients are often exchanged in the same community between producer and consumer although relative rates of usage are not equivalent. Therefore, a fraction of the energy processed by an ecosystem is expended in the recycling of nutrients and the retention of biomass.

The choice of appropriate ecological variables to describe system function will depend on the type of ecosystem, the relative importance of photosynthetic and allochthonous energy sources, the critical links in consumer regulation of energy and material flow, particularly with regard to essential elements, and the inherent variability in individual response variables. In general, criterion variables cannot be assigned *a priori* as they would be in a single species toxicity test (e.g. lethality); rather, they should be selected on a case-specific basis to provide a clear test of adverse impact on sensitive ecosystem processes. Variables measuring ecosystem metabolism (productivity, production/respiration ratio) have been shown to be more sensitive and replicable in response to chemical stress in aquatic microcosm tests (CV ~ 10–20%) than have measures of nutrient cycling and essential element retention (CV ~ 10–100%) (e.g. Brockway *et al.*, 1979; Giddings and Eddleman, 1979). The importance of detrital pathways in terrestrial systems suggests the importance of examining the response of heterotrophic microbial activity where tangible chemical impacts can take the form of suppression of the mineralization of natural organic litter or inhibition of the biodegradation (detoxification) of synthetic organic substances, or disruptions in specific microbial processes contributing to the cycling of essential nutrients (e.g. nitrogen fixation, sulphate reduction). Table 1 summarizes the utility of several functional variables as applicable to assessing chemical toxicity in general classes of laboratory microcosms. The ratings are an attempt to provide initial guidance; the choice of suitable criterion variables for specific test designs will depend on the test system, hypothesis, and toxic substance, as well as criteria previously mentioned.

Transient effects on primary productivity which cannot be detected in later changes in producer yield or short-term effects on decomposition of organic material which cannot be linked to essential element availability are of questionable value in ecotoxicological assessment. The identification of inhibition of microbial function as being either reversible, but with an extended period of delay before recovery, or persistent, causing a significant deficiency in activity, is critical to the recognition of adverse impacts in both field studies and controlled toxicity tests (see further discussion of response criteria in Domsch *et al.*, 1983). Stockner and Anita (1976) warn that exposures of 20–40 days are necessary in plankton-dominated aquatic systems to demonstrate a long-term habituation rather than a short-term shock response to toxic pollutants. The productivity response lag time in terrestrial ecosystems is expected to be long in

Table 1 Utility of functional variables in detecting significant adverse effects of chemicals in laboratory ecosystem models

Ecosystem criterion variable	Lotic models	Lentic models	Terrestrial plant–soil models
Primary production	G	G	M
Community production	G	G	P
Community respiration	G	G	G
P/R	G	G	M
Secondary production	M	M	P
Decomposition	M	M	G
Nutrient leaching	P	P	G
Nutrient availability	P	M*	G
Nutrient uptake	M+	M	M
Nitrogen fixation	P	G	G
Ammonification	P	M*	M
Nitrification	P	M*	G
Functional group diversity	G	?	?

G = good, sensitive and relatively frequently reported.
P = poor, insensitive or functionally unimportant or measurement problems.
M = medium, evidence inconclusive but utility has been suggested from field data.
? = unknown.
* = in water–sediment models.
+ = nutrient spiralling in stream models.

comparison to that of aquatic plankton-based communities because of the longer growth and reproductive cycles of high plants as well as the mediating effects of soil chemistry, biomass retention of nutrients, and herbivore–pathogen activity.

Demonstrating relationships between pollution effects on heterotrophic and autotrophic processes can be extremely difficult. Although the inhibition of natural decomposition processes has great potential impact on the ecosystem, the influence of short-term litter accumulation on nutrient cycles and ultimately the productivity of affected ecosystems is unclear. For example, studies of the accumulation of high levels of trace metals in forest soils have led to the hypothesis that heavy metals depress decomposition (e.g. Jackson and Watson, 1977). As nutrients are frequently a limiting factor in forest soils, decomposition rate controls the rate of primary production (Witkamp and Ausmus, 1976). However, at a distance from sources of extreme contamination, where metal concentrations are still elevated but there are no obvious signs of actual vegetative damage, there is little documentation of declines in primary productivity due to litter accumulation and subsequent nutrient retention. Such effects have, however, been predicted (Tyler, 1972). This example emphasizes the difficulty ecotoxicologists encounter in demonstrating comprehensive changes in ecosystem metabolism.

3 DETECTING AND QUANTIFYING THE ECOLOGICAL EFFECTS OF MIXTURES

In nearly all cases, the stress of pollution on natural ecosystems cannot be attributed exclusively to a single chemical but is the combined effect of many chemicals. Although some mixtures of chemicals exhibit simple additivity, many elicit less-than-additive effects or interact to provoke a more-than-additive response. Unfortunately, due to the complexity of monitoring the ecological response of natural systems to pollution, little has been done to elucidate pollutant interactions in the field, even though such interactions are cited as potential sources of error in field studies (Phillips, 1980).

The environmental behaviour of individual pollutants in a mixture may be quite different because of differences in mobility, partitioning transformation and persistence. These differences influence the pattern of exposure within an ecosystem. Studies, such as those of Huckabee and Blaylock (1973) using radiotracers in microcosms and in stream ecosystems, have demonstrated subtle differences in the transport and transformation of elements of the same general category (in this case toxic heavy metals). The problems of exposure estimation of mixtures are further complicated by the variety of potential sources of some chemicals and irregularities in their release to the environment. Conceptually the use of exposure commitment techniques analogous to those applied to individual test species (see Butler, 1978) can provide a framework for analysing the complex long-term patterns of multiple chemical exposure which may lead to detectable ecosystem response.

3.1 General Approaches

General approaches to the assessment of the effect of chemical mixtures in the field have included the correlation of ecological response variables with (1) concentrations of those pollutants in greatest abundance in various environmental pools, (2) concentrations of contaminants in the biota, (3) a specific fraction of the effluent (as with petroleum spills), (4) the arithmetic sum of concentrations of component chemicals (e.g. copper + zinc) (Tyler, 1976), (5) effluent discharge or application rates, (6) the composition or amount of precipitation (for air pollutants such as acid rain), or (7) indirectly, distance from the effluent source. These approaches have produced an abundance of data on the integrated effects of complex chemical mixtures but little direct information on the influence of chemical interactions in provoking ecological response.

The Whitecount Environmental Study is one example of the type of comprehensive long-term monitoring programme which has contributed valuable information to the understanding of the stress of complex mixtures on ecosystem processes (Legge *et al.*, 1978, 1981; Legge and Bogner, 1982). The output of effluent from sour gas processing facilities, the uptake of contaminants by foliage, and effects on tree growth were monitored from 1972 to 1980. Data from this intensive programme demonstrated that gas plant stack emissions could not account for total sulphur loading in the forest after 1979. Sulphur dust from a granulation facility was implicated in the further recent acidification and modification of nutrient cycling in the area. A consistent trend of decreased forest productivity was documented in a 1- to 3-km area around the gas plant over the period of the study.

3.2 Combined Experimental and Monitoring Approach

It is perhaps more informative to examine some experimental and analytical techniques which, combined with a field assessment programme, can be applied to clarify our understanding of the influences, on system function, of specific components in toxic mixtures. One experimental procedure which has assisted in these efforts is the combining of laboratory or *in situ* toxicity tests with survey data. Bartlett *et al.* (1974) used laboratory toxicity tests with algal cultures to explain the depression in primary productivity noted in portions of the Coeur d'Alene River extensively contaminated with heavy metals. Combinations of copper, zinc, and cadmium, in levels similar to those at Coeur d'Alene sites, were found to be approximately equivalent in toxicity to equal concentrations of zinc alone. The authors interpreted this to mean that zinc was the primary algicide.

Whitton and Shehata (1982) used laboratory tests with tolerant strains of blue-green algae to clarify observations of their dominance in natural communities near mines. They reported that the development of tolerance to any particular metal takes place easily but does not necessarily confer a general resistance to all metals. However, under laboratory conditions, strains of *Anacystis nidulans* grew at comparatively high levels of cobalt, nickel, copper and cadmium with only a slight decrease in final yield.

Eide *et al.* (1979) reviewed some of the limitations of laboratory phytoplankton toxicity tests in predicting the effects of heavy metals. They suggested the use of *in situ* dialysis enclosures holding indigenous phytoplankton in impacted areas to incorporate ambient environmental conditions, pollutant interactions, and community co-adaptations. Results of dialysis bag studies of a healthy natural community exposed to pollution *in situ* can then be compared with the long-term response of local microbial communities.

A second experimental approach which provides additional clarification of the influence of individual components on the toxicity of the mixture and information on site-specific phenomena is the combining of *in situ* manipulations with monitoring data. For example, Duchelle *et al.* (1982) examined the height growth of trees in open plots as well as in ambient chambers and chambers with a charcoal-filtered air supply. Plant growth was low in open plots, slightly greater in ambient air chambers, and greatest in filtered air chambers selectively removing atmospheric contaminants. Growth trends were inversely related to monitored ozone concentrations within the three test treatments. It would appear that the approach of modifying the pollutant mixtures may also be effective, in certain cases, in contaminated aquatic ecosystems. For example, certain heavy metals could be partially removed with chelators or the pH and chemistry changed with addition of a base. Such studies could be pursued with the use of microcosm enclosures or through whole-system modifications. This approach has seen limited application, as in attempts to experimentally substantiate observed effects of long-term lake acidification. Schindler *et al.* (1980) and Schindler and Turner (1982) have reported on controlled acid additions to a neutral environment, and Hultberg and Anderson (1982) have examined the impact of liming on the ecological functions of an acidic lake.

3.3 Data Reduction Techniques

Multivariate and discriminant analysis techniques are perhaps the most powerful analytical tools available for the monitoring of mixtures. For example, accumulation of litter in woodlands near a lead–zinc–cadmium smelter was best explained by concentrations of zinc and cadmium and the acidity of the litter. A partial regression showed that these three factors accounted for 76% of the variation in litter weight at various sites. Multiple regression techniques were also used to demonstrate that cadmium, in forest litter, was implicated in the inhibition of breakdown at the latter stages of the mineralization process, when particulates were in the 0.5- to 4-mm size range (Coughtrey *et al.*, 1979). Andrews (1984) used principal component analysis to study physicochemical data and cluster analysis for biological data from the River Thames. He then compared the results to resolve the difficulty of quantifying natural and pollutant-derived influences on fish community abundance and composition. Similar methodology has been applied to the analysis of environmental factors controlling patterns of species composition in plankton communities (Green and Vascotto, 1978), and in terrestrial forest communities (Scale, 1982). A multivariate approach was used by Sayler *et al.* (1982) to evaluate the functional response to and recovery from coal-coking waste effluent by sediment microbes. Multivariate analysis of variance and discriminant analysis indicated that the accurate differentiation between contaminated and uncontaminated sediment required a minimum of nine estimates of community response. Total viable population density, ATP, alkaline phosphatase, and naphthalene and phenanthrene mineralization rates were the most highly weighted variables, with lipid and glucose uptake, nitrogen fixation, and sediment protein also contributing to the explanation of site variation. The authors also suggested that the ability to biotransform polyaromatic hydrocarbons is a retained physiological trait that will influence the system's future ability to respond to and to damp similar ecosystem perturbations. Two important results of this type of investigation are the identification of specific variables describing differences between communities, and demonstration of the contributions of these variables in describing community response to environmental variations, both natural (seasonal) and chemically induced.

Multivariate approaches should also be a significant aid in analysing data from model ecosystem studies where a large number of environmental and ecological variables are being measured simultaneously (e.g. Oviatt *et al.*, 1977). Although multivariate and numerical classification techniques can be powerful analytical tools in deciphering ecosystem response to chemical mixtures, these techniques are not self-interpreting. Appropriate data reduction methods need to be applied to data which have been carefully collected in properly designed sampling approaches. Of equal importance is a thoughtful interpretation based on ecological knowledge to maximize the value of information obtainable from impact assessment monitoring programmes. (See Green (1979) for details on impact study design.)

4 EXPERIMENTAL APPROACHES TO TESTING MIXTURES OF CHEMICALS

The biological testing of chemical mixtures has been largely based on the determination of the response of individuals or populations to whole effluent. While a limited number of researchers have evaluated the influence of chemical interactions on both aquatic and terrestrial species, few have tested the response of ecosystem processes to either the combined toxic effects of mixtures or, more importantly, to the interaction of effluent components. Prerequisites for ecosystem response tests should include the choice of appropriate system-level variables and the use of indigenous co-adapted communities and experimental conditions that approximate natural environments. In addition, the application of a proper experimental design which will allow reasonable limits of detection and the demonstration of interactions, if desired, should be included.

4.1 Aquatic Studies

4.1.1 Algal Assays

There has been a good deal of data collected from tests to predict the effects of sewage and waste water effluents on aquatic ecosystems. The objective of these procedures, in most cases, has been the assessment of nutrient addition accompanying effluent discharge on the biostimulation of primary productivity (e.g. Middlebrooks *et al.*, 1976). Algal assay procedures using pure cultures and natural water samples have also been applied to the toxicity testing of individual chemicals (e.g. Payne, 1976). *In situ* algal assays with native phytoplankton communities have been used to evaluate the effects of waste water effluent on lake primary productivity (Smith, 1975). In general, algal assay procedures have largely ignored the question of chemical interactions, with the exception of trace metal–chelator bioassays (Payne, 1976).

4.1.2 Polychlorinated Biphenyls (PCBs) and Oil as Mixtures

The experimental assessment of the toxic effects of isomeric mixtures of PCBs or of fractions of petroleum mixtures provides insights into testing functional responses in aquatic systems.

The effect of mixtures of PCB isomers on the primary productivity of naturally occurring phytoplankton communities has been assessed in the laboratory with isolates cultured in water taken from their natural habitat (Kricher and Bayer, 1977; Södergren and Gelin, 1983). The results of these studies indicate that primary productivity was transiently depressed after a single exposure. The solvent (either acetone or ethanol) used to bring the PCB mixture into solution was shown to influence the percentage depression in productivity. A similar interaction of the solvent acetone with benomyl and captan was observed in fungal assays (Burrell and Corke, 1980). Phytoplankton cultures from an oligotrophic lake were shown to be more vulnerable to PCBs than isolates from a eutrophic lake. Södergren and Gelin (1983) explain this result as an experimental artifact arising from a relationship between cell density and the severity of productivity suppression. Carbon-fixation rates were found to be similar in both types of community when exposed at a level based on a PCB/particulate organic carbon ratio.

In situ exposures of phytoplankton communities to PCBs, either in BOD bottles (Södergren and Gelin, 1983) or in dialysis membrane bags (O'Connors *et al.*, 1978; Powers *et al.*, 1977), have provided information on the types and severity of response under conditions closely approximating natural light, temperature, and nutrient fluxes. An important finding of these studies, substantiated by experiments examining pesticide effects on algal assemblages (Maly and Ruber, 1983), is that these compounds have longer-term effects on species composition in addition to temporarily depressing primary productivity. Composition changes have been suggested to have a significant impact on the transfer of energy to consumers due to reductions in plankton size and nutritional quality (Fisher, 1975; O'Connors *et al.*, 1978).

Short-term studies of heterotrophic glucose uptake in aquatic sediments amended by PCBs provided inconclusive results and led Sayler *et al.* (1979) to conclude that information on long-term multifaceted effects of PCBs is essential to demonstrate conclusively that this type of mixture significantly perturbs heterotrophic activity.

The effects of variously altered water-soluble fractions of crude oil have been examined in the laboratory with flask cultures of natural marine microbial communities (Henson and Hayasaka, 1982). Labelled glutamic acid uptake was found to be sensitive only to the emulsified water-soluble fraction, as a result of three- and eight-day exposures.

The influence of water-soluble fractions of crude oil and of oil dispersants on nitrate generation by sandy beach microfauna has been assayed with simple miniature sand columns (Harty and McLachlan, 1982). These provided a rapid relative measure of inhibition of nitrification, with a reasonable degree of repeatability, but allowed only a short-term assessment (approx. two days). McLachlan *et al.* (1981) used a similar apparatus to make *in situ* assessments of microbial activity over longer periods (four months).

The longer-term effects (up to 1.5 years) of oil fractions on microbial processes in sediments have been assayed by Griffiths *et al.* (1982a, b) using *in situ* techniques. Sediments amended with various organic compounds were exposed to fresh and weathered crude oil at a number of different concentrations and were then incubated *in situ*. The level of activity of enzymes that hydrolyse structural polysaccharides was reduced while activity in enzymes that hydrolyse storage polysaccharides was stimulated. Changes were also observed in nitrogen fixation and denitrification rates, redox potentials, carbon dioxide and methane production rates, and glucose uptake and mineralization rates. These studies take into account the influence of macrofauna, exerted primarily through burrowing activity.

As the potential interactions of oil fractions and dispersants are of great interest when attempting to predict their combined impact in aquatic systems, Wells *et al.* (1982) have suggested a bioassay-partitioning relationship for single-species toxicity tests. Their approach is to subject an unknown mixture of compounds to partitioning into non-toxic solvents such as mineral oil. It may be possible to identify the chemical nature and partitioning characteristics of the toxic species from the reduction in toxicity in given solvents. Such an approach could be modified to assess functional parameters and should be applicable to a number of complex effluents which consist of organic and inorganic components in both dissolved and particulate form.

4.1.3 Mixtures Containing Metals

A combination of low pH and above-normal levels of metals and metalloids may exist in aquatic systems receiving acid precipitation. Such mixtures present different but equally complex problems for toxicity testing. For instance, ^{14}C-glucose mineralization in sediment microbial cultures has been used to assay the effects of mercury, lead, arsenic, selenium, and acidity, introduced individually and in combination (Baker *et al.*, 1983). Low pH markedly reduced microbial activity and enhanced the effects of individual metals and metalloids. Metals, in turn, affected culture pH. The effect of the combination of the four elements resembled that due to mercury alone, but additive interactions could not be ruled out. These experiments demonstrate that the interactions between sediment microorganisms and pH plus heavy metals and metalloids are indeed complex, as microorganisms themselves may modify the environmental conditions which, in turn, affect the bioavailability and toxicity of the mixture.

A more sophisticated approach to the determination of toxic interactions of mixtures in aquatic ecosystems has been demonstrated with factorial studies of heavy metal combinations. In a three-day laboratory bioassay using water and phytoplankton from the Red Sea, Ibragim *et al.* (1980) demonstrated both stimulation and suppression of ^{14}C assimilation, depending on the combination of metals and the length of exposure. For example, lead, cadmium + copper, and lead + cadmium + copper were stimulatory on day 1, while mercury and copper were inhibitory. On days 2 and 3, only lead or lead + cadmium were stimulatory, while mercury and mercury + lead were inhibitory.

Taking this approach a step further, Kaitala *et al.* (1984) have assessed the interactions of copper (20 μg/l), cadmium (40 μg/l), zinc (80 μg/l) and pentachlorophenol (PCP; 80 μg/l) on pelagic primary productivity (^{14}C assimilation) and heterotrophic activity (^{3}H-glucose uptake), in brackish water, during five-day incubation periods, *in situ*. Results were expressed in half-normal plots on which significant effects due to metal or time interactions appear as clear deviations from the straight line. A close linkage of both heterotrophic activity and primary productivity response to the test compound was observed. Heterotrophic activity responded more rapidly than productivity and the effect on the test system was greatest for copper addition. The effect of cadmium on productivity was quite different from that of copper or zinc, as productivity did not recover to the base levels. The interaction of zinc with cadmium reduced the inhibition caused by the cadmium. PCP did not have a detectable effect on productivity and exerted only minor influence on heterotrophic activity, but PCP interactions with copper had a pronounced inhibitory effect on the recovery of productivity following copper suppression.

In these types of studies, where experimental duration is greater than the generation period of the microbial community, the terms *inhibition* and *stimulation* refer to changes in the 'state' of the system rather than actual direct stimulation or inhibition of measured variables by the combinations of toxic substances. Changes in a test system are mediated through nutrient and substrate availability as well as trophic and competition interactions.

However, studies of short (e.g. five-day) duration must still be considered predictive only of short-term response. Longer-term interaction studies would necessitate the use of larger, more complex microcosm test chambers within a similar factorial design framework. Although a variety of aquatic microcosm designs have been demonstrated to be functionally responsive to chemical mixtures (e.g. Giddings, 1982; Porcella *et al.*, 1982; Rodgers *et al.*, 1979), incorporation of these model test systems into a complex factorial experiment may prove unwieldy. Such test systems may, however, be applicable to simple joint interaction studies. For the present, microcosm tests should be applied to the assessment of effects of mixtures on various metabolic and nutrient cycling parameters through time. Such studies could provide indications of habituation response and the functional resilience of the system.

4.2 Terrestrial Studies

4.2.1 Laboratory Methods

Laboratory and simple *in situ* studies of the response of soil microbes to air pollutant mixtures suggest some potential approaches to short-term testing of functional response in terrestrial ecosystems. As with sediment microbial communities, soil microbial response to pollutant mixtures is detectable with generalized metabolic measures such as soil respiration and dehydrogenase activity (e.g. Killham *et al.*, 1983), or with more specific metabolic parameters such as glucose mineralization (e.g. Strayer and Alexander, 1981) or hydrogen oxidation (e.g. Rogers and McFarlane, 1982). Effects on specific nutrient transformations and on nutrient retention are also widely reported.

A simple laboratory test procedure might follow the approach of the one-day hydrogen bioassay proposed by Rogers and McFarlane (1982). This procedure has been demonstrated to be responsive to a wide variety of gaseous, liquid, and solid pollutants and, of particular interest, complex mixtures such as industrial waste water from various sources, smelter baghouse dust, and chemical process sludge. The simplicity of the apparatus and the test suggests that it may be particularly applicable to factorial experiments examining interactions. Such tests, however, should be considered only as a screen for short-term change.

4.2.2 Microcosm and Lysimeter Studies

Soil microcosm and lysimeter studies will be essential to the demonstration of the longer-term effects of mixtures on microbial processes under conditions better mimicking nature. For example, Jackson *et al.* (1978) employed a microcosm test to demonstrate that smelter baghouse dust in high concentrations stimulated leaching of essential elements. Microcosms could contribute substantially to our understanding of pollutant interactions if chemical transformation and partitioning kinetics are monitored simultaneously with functional responses. For example, Kaufman (1977) used a laboratory soil microcosm to demonstrate the effect of one pesticide on the degradation of others. However, none of the various microcosm systems for testing the effects of chemicals on soil processes, reviewed by Suter (1981), appear to be appropriate for testing the interactions of the numerous combinations of chemical components likely to be found in many complex effluents. As with aquatic ecosystems, tests of multiple interactions between chemicals and soil processes must, for practical reasons, be confined to simple, replicable experimental techniques.

Lysimeter studies have proven effective in assessing the impact of air pollutants on nutrient dynamics in relatively complex plant–soil environments. Norwegian researchers used lysimeter experiments under greenhouse conditions to demonstrate the adverse effect of artificial acid precipitation on essential element and nutrient retention (e.g. Abrahamsen and Staunes, 1980; Bjor and Teigen, 1980). One advantage of this type of experimental technique is the development of an input/output budget of essential plant nutrients. However, the response to mixtures including organic chemicals, in terms of the nutrient budget of a test system, appears to be much more complex and difficult to interpret. Metabolism of organic chemicals can immobilize nutrients, masking leakage from stressed biota. Measurement of nutrient availability for growing plants may be needed to clarify effluent impact under such conditions.

4.2.3 Field Studies

Controlled chamber and field studies have provided, in general, the most accurate estimates of the effects of mixtures on productivity in terrestrial systems. The yield of agricultural crops is often the focus of such studies, and a number of techniques are available to simulate various types of airborne pollution with different soil systems and agroecosystems (Suter, 1982). In the experimental testing of simple gaseous mixtures, the effects of ozone and sulphur dioxide on potato yield have been evaluated under the semicontrolled conditions of fumigation chambers (Foster *et al.*, 1983). The impact of the same two contaminants on soybean yield was investigated, under slightly less controlled conditions, using a linear gradient field exposure system without enclosures (Reich *et al.*, 1982). Both experimental procedures implicated ozone as primarily responsible for reduced plant yields. The chamber study had the experimental advantage of replication and good control of exposure, while the field gradient approach incorporated the influence of near-ambient fluctuations in environmental conditions.

Field studies of mixtures of chemicals in environments other than agroecosystems would appear to be necessary in order to determine site-specific effects from potentially serious air pollution mixtures thought to have potentially serious impacts on productivity and nutrient cycling, and as a means of validating microcosm extrapolations.

5 CONCLUSIONS

Methodologies for the monitoring of biological effects and safety evaluation of the effects of mixtures on ecosystem function are in a comparatively early stage of development. Field survey data can be made more informative by the use of laboratory and *in situ* experiments to allow for potential interaction phenomena. The relationships among chemical and ecological variables may be clarified with discriminant analysis procedures. Interaction tests on a large number of chemicals require a factorial design and simple, replicable test systems. Microcosm and field tests can contribute to our understanding of the effects of mixtures through simultaneous measurement of chemical transformations and partitioning and the response of ecosystem processes. Validation of microcosm extrapolations and site-specific responses must come from controlled field experiments.

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