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Project Summary

Microcosms as Test Systems for the Ecological Effects of Toxic Substances: An Appraisal with Cadmium

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A two-phase set of experiments was conducted to address some of the problems inherent in ecological screening of toxic substances in aquatic microcosms, and to test two hypotheses concerning the response of ecosystems to perturbations. Phase I was a 4 X 4 factorial experiment (four levels of cadmium versus four levels of nutrient enrichment) with static microcosms designed to test the "subsidy-stress" hypothesis, and focused on the interactive effects of cadmium and nutrients. Phase II was a 2 X 4 factorial experiment (continuous and pulsed cadmium inputs versus phosphorus limited and non-limited inputs) with flow-through microcosms designed to test the "biomass increment" hypothesis, and focused on temporal aspects of system behavior (especially out/input for several elements) in response to nutrient limitation and chronic versus acute cadmium perturbations.

Phase I results supported the subsidy-stress hypothesis with respect to cadmium inputs: Increasing cadmium concentrations (0, 1, 10, 100 ppb) caused a decrease in the P/R ratio, a decrease in grazing herbivores, increase in nighttime respiration and fungi, all indicators of system stress. Since net daytime production and nighttime respiration increased with nutrient enrichment, there was no nutrient stress effect even at the highest level. There was a significant interaction effect of cadmium and nutrients with high nutrient levels reducing, somewhat, the stress effect of cadmium. Phase II results generally supported the biomass increment hypothesis and suggested a retention pattern for continuous, low concentration cadmium inputs similar to that of essential elements. Cadmium may have accumulated to a toxic threshold in some of the microcosms. Pulsed, high concentration cadmium inputs had significant effects on system behavior, depending on timing of inputs.

Conclusions relevant to toxicity screening in microcosms are: 1) Of the variables measured, community metabolism, community composition by trophic groups, and output/input ratios for NO_3-N, Mn and Fe, provided the best indicators of system response to cadmium. 2) Nutrient enrichment and phosphorous limitation significantly influenced cadmium effects on most of the variables studied. 3) Pulsed cadmium inputs early in succession significantly affected system response to cadmium pulses later in succession.

For screening a suspected toxic substance, we recommend a hierarchy of microcosm experiments including: 1) static microcosms (with and without sediments), 2) flowthrough microcosms (with and without sediments), and 3)

microcosm subsamples from specific natural ecosystems. Each step results in increased information about effects of a toxicant and each step more closely approximates natural ecosystems.

A bibliography of microcosm literature is presented at the end of the Project Report.

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Introduction

In accordance with the Toxic Substances Control Act of 1976, the U.S. Environmental Protection Agency is developing testing standards for evaluating potential hazards of chemicals before they are manufactured and released into the environment. To this end, interest is currently being shown in the use of microcosms for toxicant screening and predictive model validation.

The use of microcosms for these purposes is somewhat controversial because of the uncertainty involved in extrapolating results to natural conditions. When considered as generalized models of ecological processes, however, small scale microcosms might provide a means for evaluating gross effects of toxic substances on ecosystems because such microcosms do mimic certain functional properties of ecosystems. For example, a number of studies have demonstrated similarities between temporal processes in natural systems and in microcosms, including species succession, biomass accumulation, net production and community respiration, and radioisotope uptake and distribution. In addition, similar responses of natural and microcosm systems have been reported for various perturbations, including radiation, temperature, heavy metals, arsenic, organic toxicants, and nutrient enrichment. Thus, although quantitative extrapolation from microcosms to the real world results is not currently feasible, the qualitative response of microcosms to inputs of toxic materials under controlled laboratory conditions may provide a basis for a "first approximation" of the ecological effects of toxic substances.

The development of standardized testing procedures requires answers to several important questions, including:

1. Which ecosystem properties are most sensitive or best reflect ecosystem response to toxicant perturbations?
2. What influence will other environmental variables (e.g., pH, nutrient enrichment, light intensity, etc.) have on ecological effects of a toxic substance?
3. Will ecosystem response be a function of the timing or frequency of toxicant inputs with respect to stages of ecosystem development?
4. What degree of realism (biotic and abiotic complexity) should be incorporated into microcosms for use in toxicity screening?

To address these questions and to further evaluate the potential utility of microcosms as ecological screening tools, we have conducted a series of experiments in which aquatic laboratory microcosms were exposed to a toxic substance. Because most of these questions are important, not only for screening protocol development but for ecosystem analysis, in general, we have designed the experiments to test two hypotheses which have been developed to explain ecosystem behavior in response to stress (toxic substances being a specific form of stress). The experiments were conducted in two phases, each addressing a different hypothesis.

Phase I

It has been suggested that ecosystems respond to environmental perturbations in a "subsidy-stress" fashion. At low to moderate levels of intensity, system inputs often act to subsidize or increase overall system function (e.g., the effects of nutrient enrichment or increase in temperature or productivity). Conversely, high levels of the same input can decrease system function or result in development of an entirely different system (replacement). The overall pattern is a unimodal, bell-shaped curve of system response along a gradient of increasing perturbation intensity. It also is hypothesized that relative variance of system response increases monotonically along the perturbation gradient. System response to a toxic or lethal input is hypothesized to be a stress at a levels of input. Complicating thes general response patterns are the influences of environmental and developmental gradients, such that system response to a given level of perturbatio might vary with environmental condtions or successional stages. Thes interactive effects are especially important considerations for toxicity screenin because test results will be unavoidabl biased by standard testing conditions An alternative to single factor experiments (i.e., varying levels only of a toxicant) might be a multifactor o factorial experimental design that would allow for consideration of the interaction of several factors simultaneously.

Phase I was designed to test the subsidy-stress hypothesis and to evaluate the influence of an environmenta variable (nutrient enrichment) on aquatic microcosm response to a toxic substance (cadmium). The experiment was arranged in a 4 X 4 factorial design with increasing levels of nutrient enrichment superimposed on increasing cadmium levels. Of particular interest were the interactive effects of nutrients and cadmium on several system level variables.

Phase II

A number of ecosystem studies suggest that nutrient output/input ratios are sensitive system level measures of ecosystem behavior and stress response. These studies indicate that the loss of essential elements from ecosystems often increases significantly after disturbance. For example, loss of calcium has been shown to be a sensitive indicator of stress. The "biomass increment" hypothesis suggests that nutrient output is an inverse function of the rate of biomass production within an ecosystem. Briefly, the hypothesis for an essential nutrient is: Prior to biotic colonization of an area, nutrient outputs are equal to inputs (barring abiotic uptake or loss). As biota become established and ecosystem development proceeds,, nutrient output becomes less than input as a result of biotic uptake and storage in growing tissues. At the time of peak net ecosystem productivity, the ratio of nutrient output/input is at a minimum, thereafter gradually increasing to unity as net productivity approaches zero at ecosystem maturity (steady state). A pulsed perturbation to the ecosystem results in an increase in nutrient output/input followed by secondary succession and an abbreviated repeat of the initial patterns of productivity and nutrient uptake. For nonessential elements output/input remains near unity throughout the entire sequence of events; for limiting quantities of essential elements, deflection of the output/input curve is related to the degree of limitation. In addition, it has been proposed that ecosystems must have a finite capacity to accumulate toxic elements, unless they have a capacity through microbial transformations to gasify the elements (as can occur with mercury, for example). As that capacity is approached, increasingly greater proportions of toxicant input should appear in system outputs, such that the retention pattern should fall somewhere between those of nonessential, not accumulated, and limiting elements. If this is true, the potential for an ecosystem to become a source of (rather than a sink for) toxic elements increases as the system approaches maturity.

Phase II was designed to test these hypotheses and to evaluate the utility of output/input ratios of several elements as indicators of microcosm response to toxic element (cadmium) perturbations. Several other factors were incorporated into the experiment to determine: 1) the influence of pulsed versus continuous toxicant inputs on system response, 2) the effects of toxicant exposure early in succession, and 3) the influence of nutrient limitation on system response to toxicant exposure. The experiment was arranged in a 2 X 4 factorial design with phosphorus-limited (N:P = 100) and non-limited (N:P = 10) input regimes superimposed on four modes of cadmium input (zero input, continuous input, cadmium pulses early and late in succession, and a cadmium pulse late in succession).

Results and Discussion

Levels of nutrient enrichment used in Phase I (from N = 0.1, P = 0.01 to N = 10, P = 0.1 ppm) failed to produce a subsidy-stress response as far as productivity/respiration (P/R) relations were concerned; both P and R increased equivalently along the nutrient gradient. Whether higher levels of enrichment would disrupt P/R remains to be determined. In contrast, cadmium treatments (0 to 100 ppb) caused a significant decrease in P/R (deviation increasing with cadmium concentration) as predicted by the hypothesis. This effect was attributed to a decline in grazing herbivores (micro-crustacea were virtually eliminated at higher cadmium levels) and an increase in bacterial and fungal populations with consequent increase in community respiration. Accordingly, cadmium had an overall impact of switching energy flow from a grazing food chain to a detritus food chain.

Phase II results generally supported the "biomass increment" hypothesis. Outputs of B, Ca, Cu, Mg, Na and Zn, all essential but in excess of biotic demand, remained equal to inputs throughout the experiment. Two exceptions to the predicted trends were noted. First, maximum uptake of all essential elements did not coincide with maximum metabolic activity. Luxury consumption may have been responsible for the early occurrence of maximum phosphorus retention and might be expected to occur for other essential, limiting elements as well. Second, disturbances (i.e., cadmium pulses) that caused significant changes in metabolic activity were not reflected most strongly in the retention patterns of phosphorus, the element most limiting in system inputs. Outputs of NO_3-N, present in abundance relative to phosphorus, showed the strongest disturbance response, possibly as a result of selective cadmium effects on nitrogen metabolism. Results from Phase II also indicated cadmium retention patterns similar to those for essential elements and suggested that cadmium accumulation was a function of productivity. In the less productive systems, cadmium outputs approached input levels by the end of the experiment (286 days), whereas the more productive systems continued to accumulate cadmium. Because inorganic sediments were not present in the microcosms, cadmium must have been retained or stored in the biomass but it is not possible to tell from these data whether the mechanism was active biochemical uptake by living cells or sorption onto detrital materials.

Conclusions and Recommendations for Toxicity Testing

Results of these experiments suggest some tentative answers to the questions raised in the Introduction.

1. Which ecosystem properties are most sensitive or best reflect ecosystem response to toxicant perturbations?

Of the ecological variables measured in this study, community metabolism (net daytime production and especially nighttime respiration) and densities of various taxonomic groups provided the most consistent indicators of cadmium effects. The ratio of net production to community respiration (P/R) has been suggested as a useful measure of toxicant stress in microcosms but proved responsive to cadmium only in the static systems in our study; in the flowthrough systems, P_D and R_n both responded similarly, resulting in no net change in P/R. Reasons for this difference are not clear, but it does seem apparent that P_D and R_n, expressed individually, are important and easily measured variables in microcosm studies. Biomass and plant pigment concentrations were the least sensitive to cadmium of the variables measured. Biomass accumulation rates and pigment ratios might prove to be more useful. In the flowthrough systems, output/input ratios of NO_3-N, Mn and Fe showed significant responses to cadmium treatment. This illustrates the potential utility of output/input ratios (especially nitrogen) for toxicity screening and suggests further that some toxicants might selectively alter specific metabolic pathways. Estimates of rates of metabolism of certain essential elements (e.g., N, P, S) should be considered for use in microcosm screening tests.

2. What influence will other environmental variables (e.g., pH, nutrient enrichment, light intensity) have on ecological effects of a toxic substance?

Nutrient enrichment and phosphorus limitation significantly influenced the cadmium response of most of the variables measured. In general, the poorly enriched microcosms were more sensitive to cadmium than their highly enriched counterparts. The importance of this finding for toxicity screening in microcosms is that standard testing conditions, such as levels of nutrients and other factors, are likely to influence test results. This unavoidable bias can be minimized or at least accounted for by conducting screening tests in matrix or factorial experimental designs that include potentially interacting factors. In particular, if tests are run in microcosms of site-specific derivation, then environmental factors important in a given geographic area (e.g., salinity, pH, temperature extremes) could be incorporated into the test, along with toxicant levels, for a more meaningful evaluation. Any number of factors could be included in such a scheme (including several toxicants), but experimental costs would increase with each factor. Judicious choice of potentially important factors would be required.

3. Will ecosystem response be a function of the timing or frequency of toxicant inputs with respect to stages of ecosystem development?

The mode of toxicant introduction into microcosms is an important consideration for toxicity testing. Because toxic substance inputs into natural ecosystems occur over wide ranges of frequency and magnitude, one-time additions of a toxicant to microcosms might not provide a meaningful evaluation of ecological effects. In the present study, cadmium was added to the static microcosms only at the beginning of the experiment, precluding any consideration of toxicant input dynamics. We addressed this problem in flowthrough microcosms by applying cadmium in pulses at several stages in succession. Results showed that cadmium pulses early in succession significantly affected system response to later pulses, possibly due to selection for tolerant organisms. We also compared flowthrough microcosm responses to continuous, chronic versus acute, pulsed cadmium exposure. Continuous 10-ppb Cd inputs may have caused a toxic threshold response, but results are inconclusive.

4. What degree of realism (biotic and abiotic complexity) should be incorporated into microcosms for use in toxicity screening?

Generally, the microcosms used in this study (small, 6-liter volume with naturally derived communities) were sensitive to moderately low concentrations of cadmium (100 ppb). The lowest concentrations, however, caused no response in the static system (1 and 10 ppb Cd) and a possible but inconclusive response in the flowthrough systems (10 ppb Cd). In contrast, others have found significant ecological responses to low levels of cadmium and copper in relatively large, ecologically complex, outdoor microcosms. This suggests a possible direct relationship between outdoor microcosms. This suggests a possible direct relationship between microcosm size (or complexity) and toxicant sensitivity, but the relationship is not clear. Conversely, it has been suggested that the most sensitive systems (i.e., least resistant to perturbation) are relatively low in "functional complexity." Until some empirical means is found to evaluate functional complexity, however, this problem will be difficult to resolve. It is also possible that physical or chemical properties (e.g., pH or water hardness) of the various microcosms are related to their sensitivities. In any event, our results suggest that small laboratory microcosms are potentially useful for estimating gross ecological effects of toxic substances, perhaps as an early phase in multiple-stage testing followed by later but more selective studies in more complex systems.

A Hierarchical Approach to Toxicity Screening

Based on results from this and other studies, we suggest that a potentially useful screening protocol for aquatic ecosystems might consist of a series of factorial experiments in aquatic microcosms of increasing complexity: 1) relatively simple static microcosms (with and without sediments), 2) flowthrough microcosms (with and without sediments), and 3) detailed but selective studies in microcosm subsamples from specific ecosystems. The factors to be included in each experiment will have to be determined on a case-by-case basis depending on available information concerning properties and expected distribution of each chemical. Measurements in each experiment should include, but not necessarily be limited to, community metabolism, community composition (relative abundance of major trophic groups), and dynamics of essential elements (metabolism of N, P, S, etc., and input-output relationships in flowthrough systems). The inclusion of sediments might require additional measurements (e.g., redox potential, microbial activity, sediment characteristics).

The advantage of such a hierarchical approach is that each step yields increasingly more information and serves as a guide for subsequent experiments. In addition, each step more closely approaches the real world.

The complete report, entitled "Microcosms as Test Systems for the Ecological

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The complete report, entitled "Microcosms as Test systems for the Ecological Effects of Toxic Substances: An Appraisal with Cadmium," (Order No. PB 81-209 595; Cost: $15.50, subject to change) will be available only from:

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