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EVALUATION OF GRAPHITE FOR ENVIRONMENTAL TOXICITY USING THE STANDARD AQUATIC MICROCOSM

by Wayne G. Landis
Nancy A. Chester
Mark V. Haley
Dennis W. Johnson
Renee M. Tauber

RESEARCH DIRECTORATE

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The impact of a graphite dust on an aquatic ecosystem model, the Standard Aquatic Microcosm (SAM), was investigated. Graphite dust produced effects that resembled eutrophication in that a diversity decreased, ammonia increased, and a photosynthesis/respiration ratio of less than one was observed in the highest concentration. Compared to brass dust, graphite has much less potential to adversely impact aquatic ecosystems.

Keywords: toxicology, ecotoxicity, algae, nutrients, nitrate, (KT/KR)

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PREFACE

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EVALUATION OF GRAPHITE FOR ENVIRONMENTAL TOXICITY USING THE STANDARD AQUATIC MICROCOSM

1. INTRODUCTION

Over the last several years, a variety of single species assays have been developed using vertebrates, invertebrates, algae, and microorganisms that are useful in determining the effects of xenobiotics on individual species. Although these assays have proven to be very useful in protecting a variety of species, no information is available about the impacts of toxicants or many aspects of the dynamics and metabolism of an ecosystem. The goal of a multispecies assay is to mimic a natural ecosystem.

In a properly designed system, interspecific interactions both within and between trophic levels can be analyzed. Parameters of ecosystem metabolism (e.g., nutrient uptake, production, and respiration) can be important clues to the long-term impact of a material and should be included in the protocol. In addition, a multispecies assay should be able to provide data on the transformation and fate of the material in natural ecosystems.

Other criteria were also incorporated in the evaluation process and are similar to the criteria of Hammons.[1] Generality was an important criteria because a generic freshwater assay was desired. The behavior of the assay had to be well documented under the impacts of at least one well understood toxicant. Rejection standards or at least some first approximations of rejection standards had to exist so that a poor assay could be identified. Replicability within an experiment had to have been demonstrated. A standardized protocol that was sufficiently detailed for preparing of a standard operating procedure (SOP) had to exist. Finally, for long-term ecosystem level effects, a multispecies assay had to be highly repeatable regardless of the time of year, laboratory, or geographical location.

Using the above criteria as a guide, we selected the Standard Aquatic Microcosm (SAM) developed by Dr. Taub (University of Washington, Seattle, WA) as an ecosystem level assay. The SAM-protocol has undergone an extensive and lengthy period of research and development.[2,3,4,5,6] During the last 3 years, we participated in the Food and Drug Administration (FDA) supported round-robin evaluation of the method using copper sulfate as the toxicant.

In the round robin testing, the SAM was a reliable and repeatable assay.[7] A minor change in the protocol was made in the sterilization procedure to reduce the breakage of the test vessels during the course of the experiment. Several minor changes were made in counting the organisms that had no effect

on the repeatability (determined by the round-robin) of the assays. The amphipod stocks were also difficult to synchronize with the beginning of the SAM.

The impact of the graphite was more typical of eutrophication than acute toxicity. Nutrient metabolism was altered as well as the photosynthesis to respiration ratio. Compared to the other material assayed in the SAM, brass graphite is much less of an environmental risk in regard to aquatic toxicity and long-term effects.

2. METHODS AND MATERIALS

2.1 Toxicant.

Graphite Micro-260, a very fine dust, was supplied by the Asbury Graphite Mills, Incorporated (Asbury, NJ). Trace amounts of iron were detected using proton induced X-ray emission (PIXE) analysis.

2.2 Training.

During a 3 day workshop, three members of the Environmental Testing Group (ETG) received training on the SAM protocol in the laboratory of Dr. Taub (University of Washington). Subsequently, new employees were trained in-house. Dr. Taub's laboratory staff, especially Dr. Andrew Kindig, were unrelenting in supporting this research program.

2.3 SAM Protocol.

The 64-day SAM protocol has been described previously,[8] and the timing of events is presented in Figure 1. The microcosms were prepared by introducting 10 algal, 4 invertebrate, and 1 bacterial species into 3.0 L of sterile defined medium. Test containers were 4.0-L glass jars. An autoclaved sediment consisting of 200 g of silica sand and 0.5 g of ground chiten was autoclaved originally in the jar, but experience demonstrated that the resultant culture jars were very fragile, making the loss of replicate very likely. Two different processes were tried, and both were effective in reducing the fragility of the vessels. First, the autoclaved sediment consisting of silica sand and ground chiten was added after the separate medium and vessel sterilizations. Separate sterilizations improved the durability of the glass jars and did not add to the contamination of the microcosms. Another modification of the process was to follow the SOP but to immerse the culture vessel in a water bath to a point above the sand and chiten level during sterilization.

Numbers of organisms, dissolved oxygen (DO), and pH were determined twice weekly. Nutrients (nitrate, nitrite, ammonia, and phosphate) were sampled and measured twice weekly for the

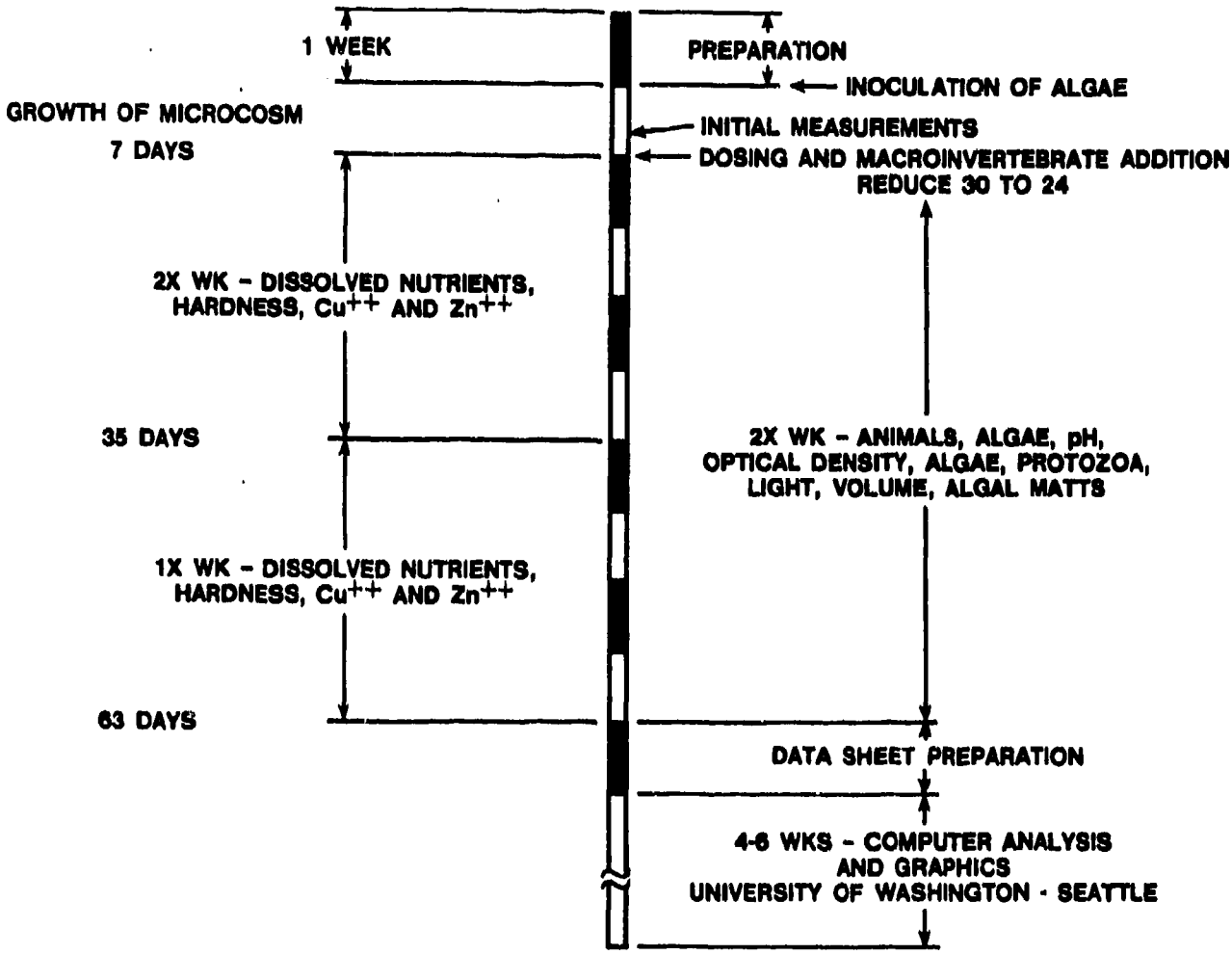


Figure 1. Timeline for the Standard Aquatic Microcosm (SAM).

first 4 weeks and only once weekly thereafter. Room temperature was 20 ± 2 °C. Illumination was 79.2 $\mu EM^{-2}sec^{-1}$ PhAR with a range of 78.6 to 80.4 and a 16/8 day/night cycle.

A stock suspension of the graphite dust was prepared for distribution to sample vessels. The material was weighed on a Cahn-28 electrobalance and dispensed into disposable polycarbonate tubes to which diluent was added to make a 1-mg/mL suspension. The sealed tube was placed in an ultrasonic water bath and manipulated until all the particles were as uniformly suspended as possible. The exposure concentrations were set so that a no effect and an effect level could be expected as estimated by Daphnia magna 48-hr EC50 and algal 96-hr growth assay results. Graphite was added to make concentrations of 0.01-, 1.00-, and 10.0-mg/L.

2.4 Data Analysis.

All data were recorded onto standard computer entry forms and checked for accuracy. The forms were then sent to the University of Washington for analysis. Parameters calculated included the concentrations of each of the species. DO, DO gain and loss, nutrient concentrations, net photosynthesis/respiration ratio (P/R), pH, algal species diversity, daphnid fecundity, algal biovolume, and biovolume of available algae. The statistical significance of each of these parameters compared to the controls was also computed for each sampling day.

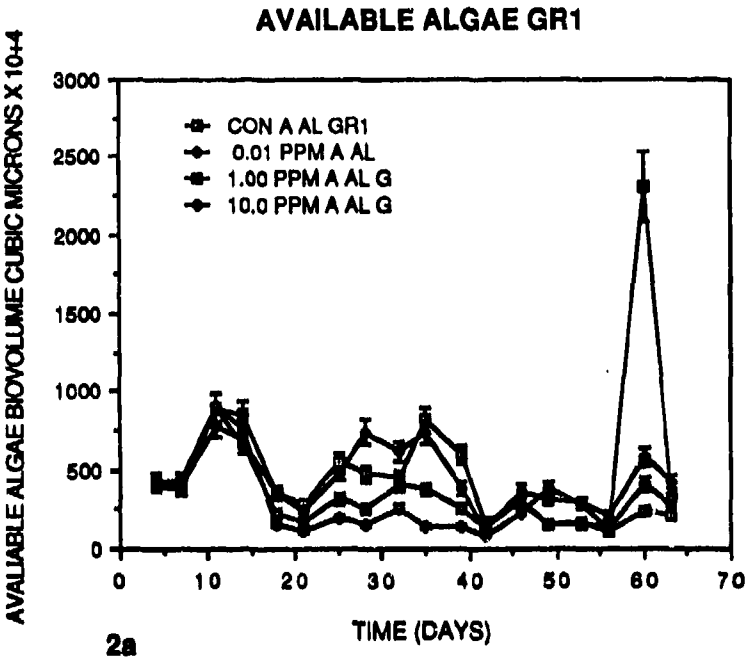
3. RESULTS

3.1 Interspecific Interactions.

In the graphite-SAM, the algal biomass (Figure 2a) remained remarkably consistent except for days 56 and 60. On these days, the medium dosage group showed an immense peak that appeared to some extent in each of the replicates at that concentration. The bloom was due to a sharp increase in Nitzschia kutsigiana and to a lesser extent Ankistrodesmus falcatus. Similarly, the daphnid populations tracked the control values rather well (Figure 2b). A clear dose response was not apparent.

3.2 Species Diversity.

Algal species diversity (Shannon-Weaver) demonstrated the variability of this measure of community structure (Figure 3). The highest concentration of graphite consistently exhibited the lowest species diversity after day 15 of the experiment. In general, the other dosage groups tracked well, especially the control and medium dosage groups.



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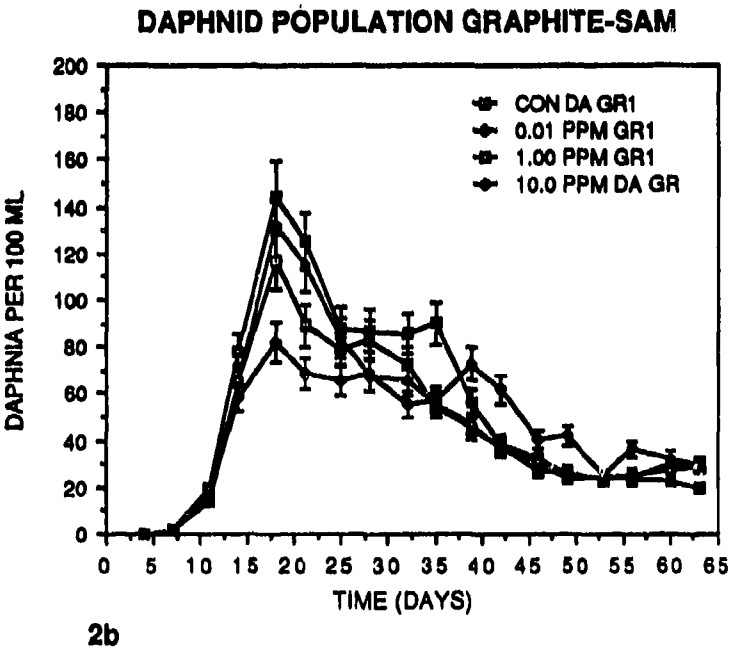

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Figure 2. Comparison of Algal and Daphnid Growth Patterns.

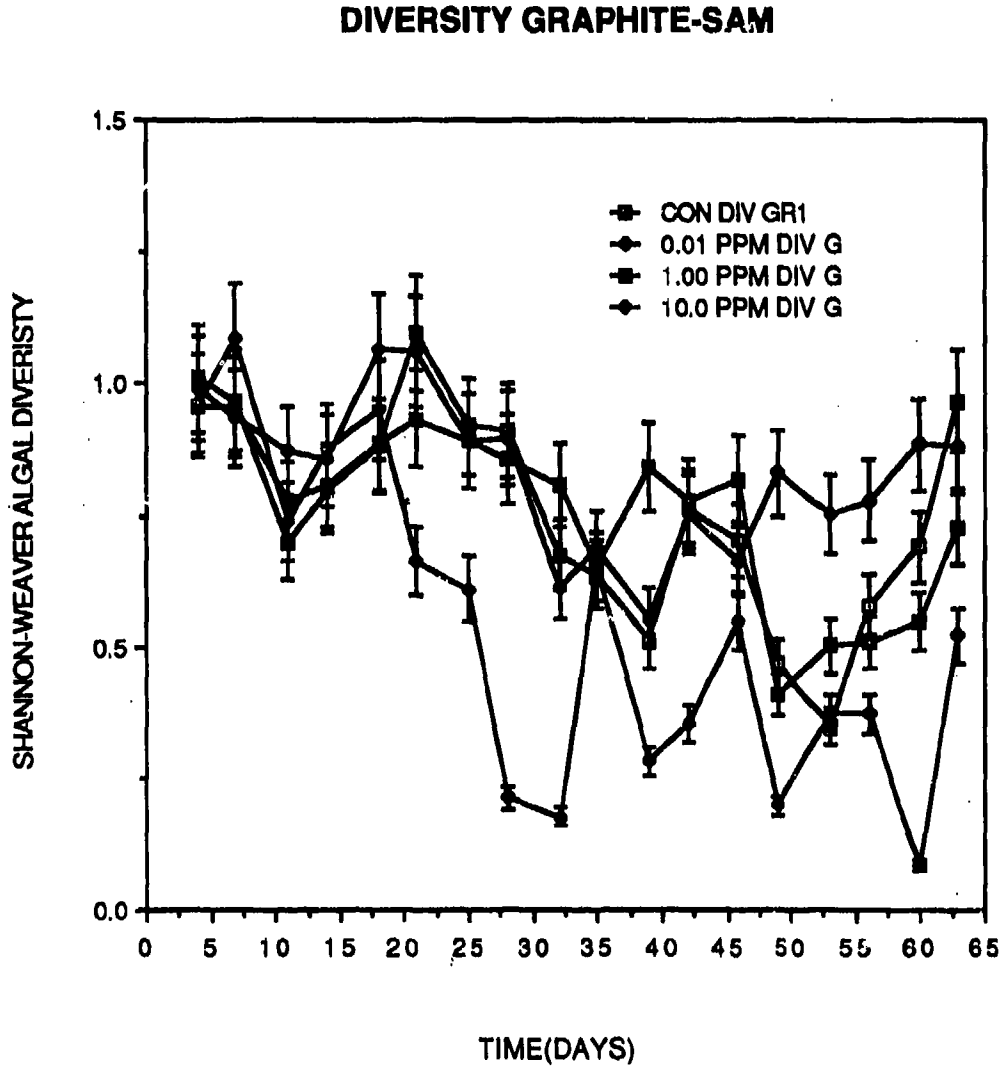


Figure 3. Species Diversity.

3.3 Nutrients.

Several of the nutrients, unlike the organisms, demonstrated a dose response relationship to the graphite. Phosphate deviated significantly from the control values (Figure 4). In the graphite-SAM phosphate concentration, the highest graphite concentration tracked the control and the two lower dosage groups until 3 weeks into the experiment. At that time, the phosphate steadily increased in the high dosage group. Nitrogen metabolism also demonstrated alterations in ecosystem metabolism (Figure 5). Nitrite at all dosages of graphite followed the same pattern (Figure 5a). At the highest concentration, the nitrite was slightly below the control values, while the two lower concentrations had nitrite levels slightly above the control. Nitrate (Figure 5b) is interesting because of the sharp fluctuations in the nitrate concentration with the two higher concentrations of graphite. The continuous increase in ammonia is most dramatic (Figure 5c). In the highest graphite concentration (10.0 ppm), the concentration of ammonia increased and continued to increase even at the end of the experiment. Generally, the two lower concentrations followed the pattern of the control. In the two highest concentrations, silicate remained at a level higher than the control after day 30 (Figure 6). Lastly, pH reflects many of these changes, especially in ammonia. After day 45, the pH of the microcosms at the highest concentration deviated markedly from all other groups, no doubt in part, reflecting the increase in basic ammonia ions (Figure 7).

3.4 Photosynthesis/Respiration Ratio.

The photosynthesis/respiration ratio fluctuated and often ranged to below one in the highest concentration of the graphite-SAM (Figure 8). In the 10-ppm graphite-SAM, the P/R ratio stayed below one for the first 46 days of the experiment. Large deviations above one were also exhibited by the other dosage groups although the large deviation on day 33 in the 0.01-mg/L group may have been spurious.

4. DISCUSSION

The SAM is capable of exhibiting a variety of effects due to the application of the toxicant. The graphite-SAM illustrated the effects of a material that has the potential of causing eutrophication. Species diversity in the highest concentration was lower than in the controls. Ammonia was elevated. The P/R ratio indicated that a great deal of respiration was occurring, less than the rate of photosynthesis. Many of the classical symptoms of eutrophication in full scale ecosystems were mimicked by this small scale system. Although it is difficult to imagine a pathway to incorporate inorganic carbon, the trace amounts of iron may have supplied a trace element that would induce the effects seen in the highest concentration.

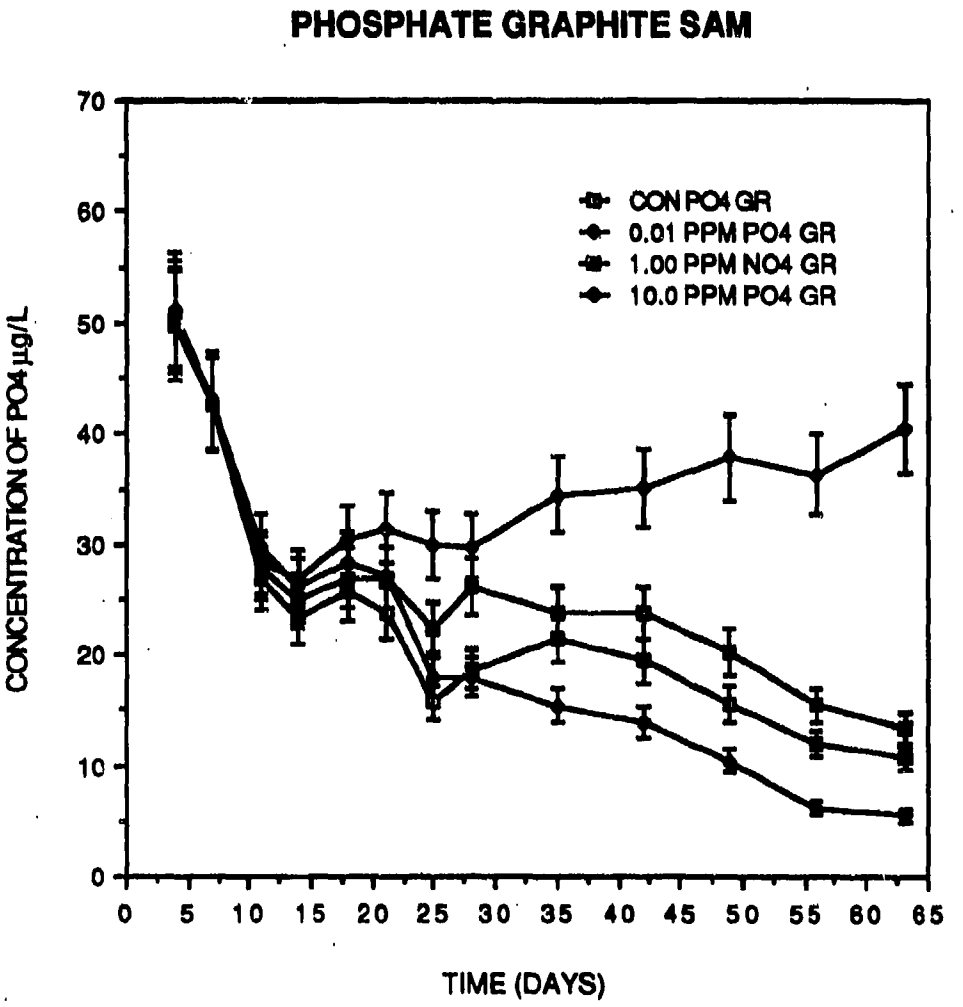
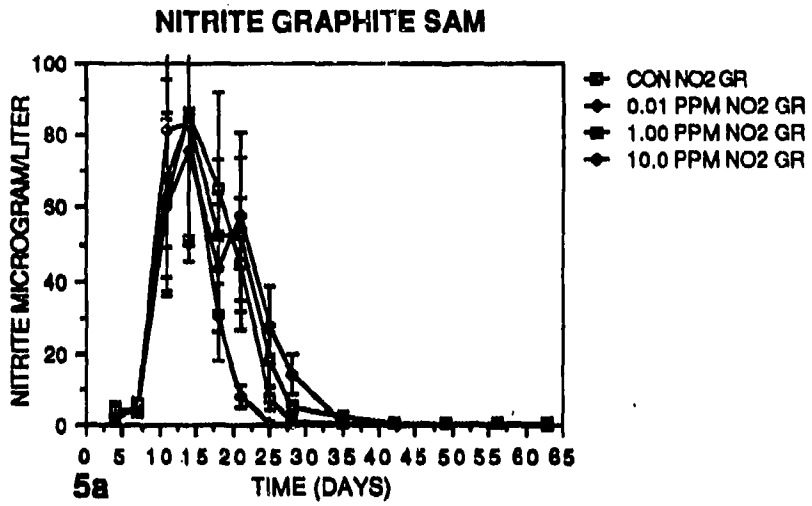


Figure 4. Phosphate Metabolism.



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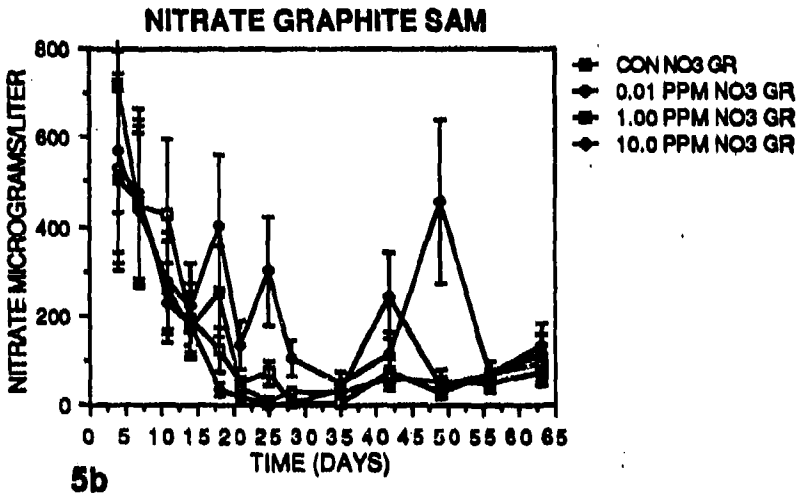

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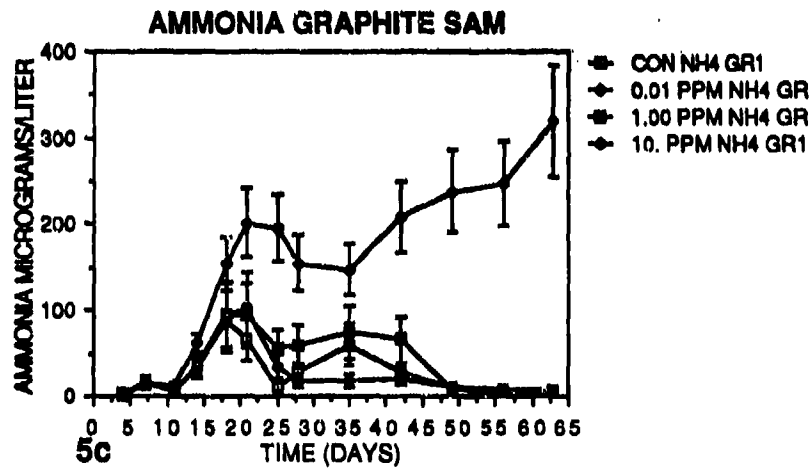

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Figure 5. Nitrogen. In the highest concentration, 10.0 ppm, the concentration of ammonia continued to increase even to the end of the experiment.

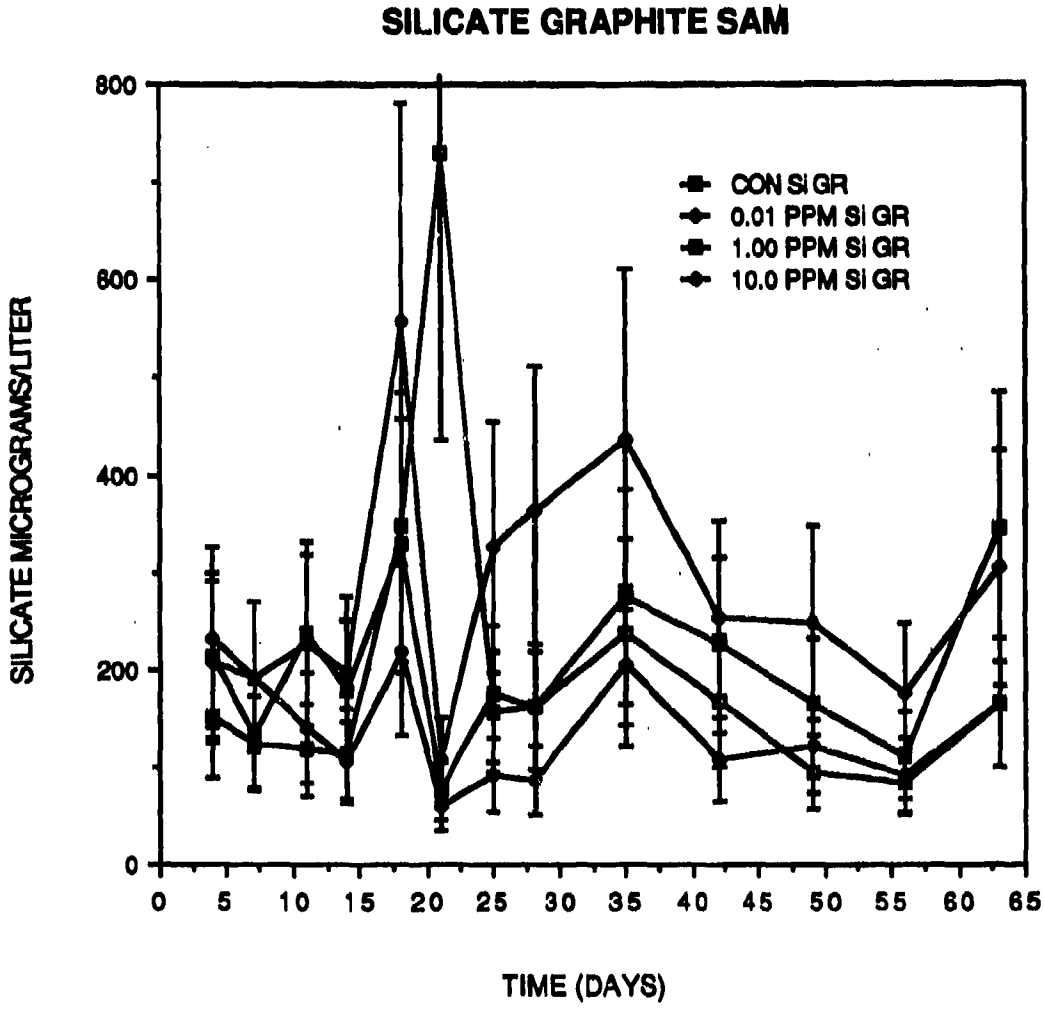

Figure 6. Silicate. In the two highest concentrations, silicate remained at a level higher than the control after day 30.

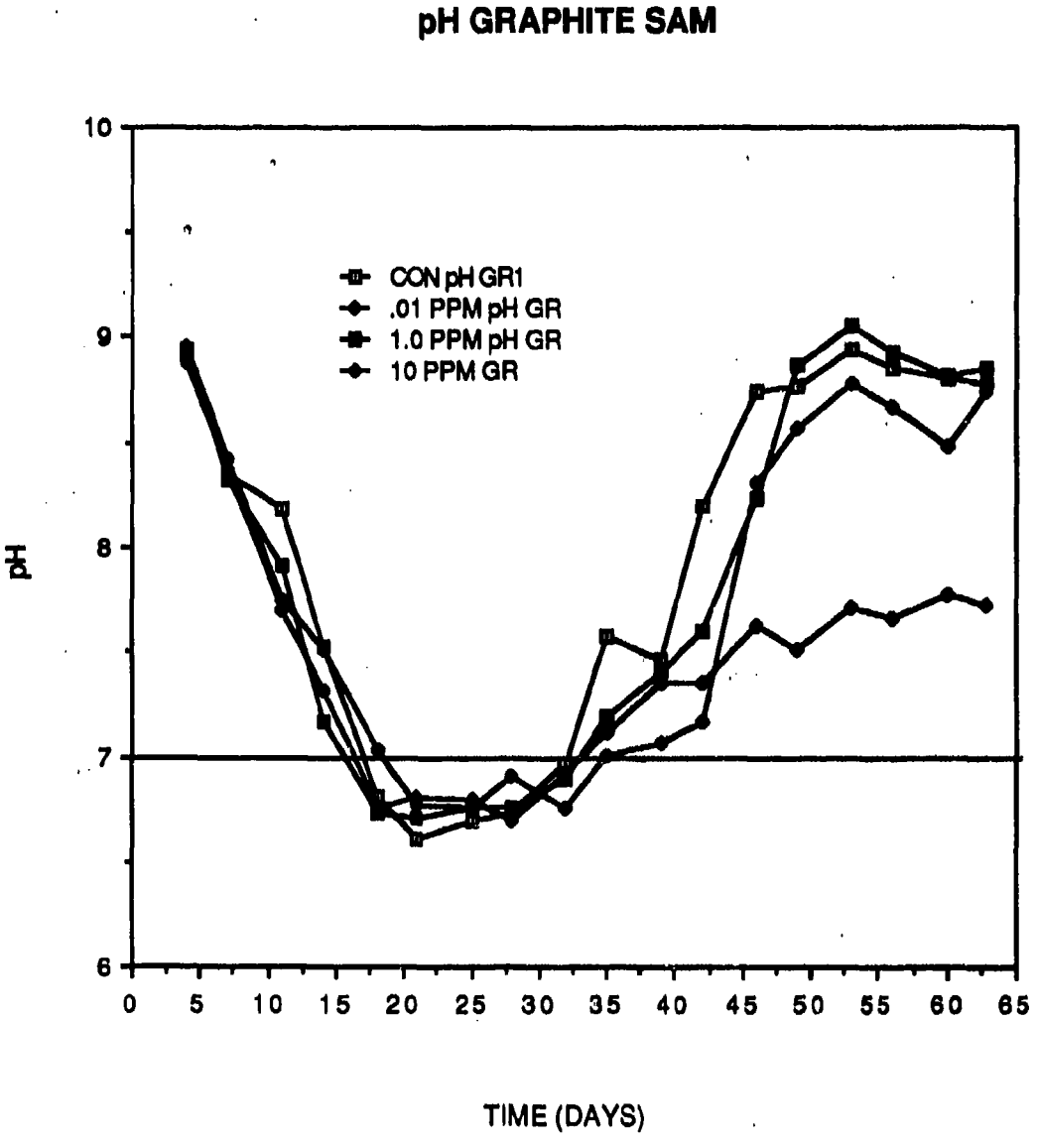

Figure 7. pH. After day 45 the pH of the microcosm at the highest concentration deviated markedly from all other groups, no doubt in part, reflecting the increase in basic ammonia ions.

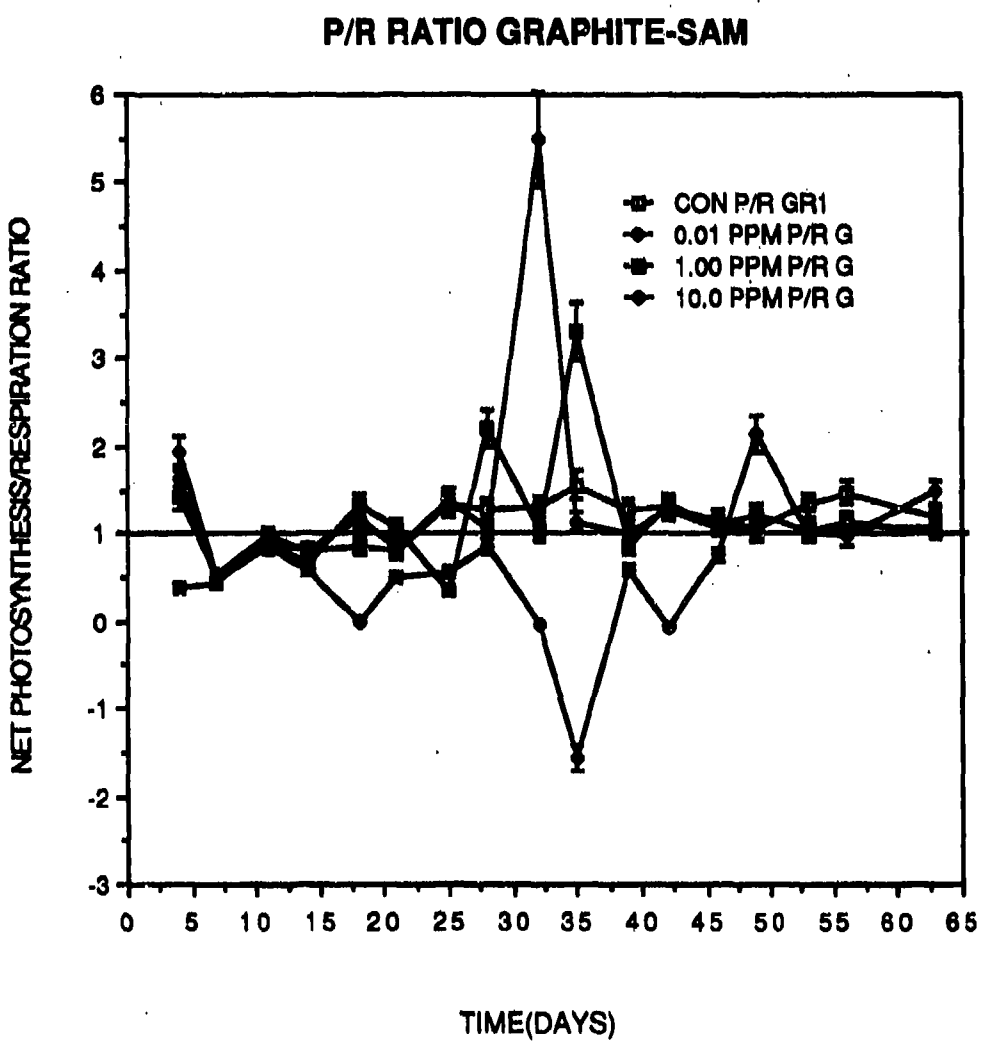

Figure 8. Photosynthesis/Respiration Ratio (P/R).

In evaluating toxic effects, individual criteria such as species diversity, biomass, P/R ratio, and nutrient cycling cannot be used individually to identify an impact. For example, as traditional theory has it, ecosystems that are undergoing stress are forced back to earlier successional stages and therefore species diversity drops. In the graphite-SAM, the diversity of the algae was consistently lower only in the highest concentration (10 mg/L). Two explanations come to mind. First, the SAM protocol is examining the system during its early development and maturation. In a developing ecosystem, species diversity is normally lower irrespective of any contaminant. In addition, excess nutrients allow the organisms to compensate for toxicant-induced inefficiencies. Kindig et al. has previously shown that mature microcosms without excess nutrients, are more susceptible to environmental stress.[9] The use of developing microcosms is justified, however, in that many aquatic systems undergo seasonal development with a succession of biota depending on temperature, light, and colonization. Secondly, the idea that a toxic or eutrophic effect automatically manifests itself in a decrease in diversity is an erroneous assumption. Landis, using derivations of the resource competition models of Tilman, has demonstrated in the two species case that a xenobiotic or nutrient can increase species diversity.[10,11] The brass-SAM illustrated the insensitivity of diversity as an indicator of stress or alteration in ecosystem metabolism.[12] However, the combination of the alteration in nutrient metabolism and diversity indicate an effect upon the ecosystem at the highest concentration.

The importance of understanding interactions among populations is crucial in estimating the long-term impact of introducing a xenobiotic. A toxicant can impact many levels of an ecosystem from the replication of the DNA to the interactions of the communities trophic levels. The most severe shortcoming of a single species assay is that, other than toxicity or fate directed by the species under examination, all other levels of community interaction are bypassed. These interactions not only include the classical predator-prey and competition, but the toxicant may be directly affected by the variety of extracellular materials produced by an ecosystem and progressive transformation of the material during its metabolism by various species. At our present level of understanding, we are unable to predict community interactions on the basis of acute assays only.

As useful as multispecies assays such as the SAM may be, they certainly cannot mimic every aspect of an aquatic ecosystem. Scale effects are crucial. The SAM, because of its homogeneity of habitat and size, would be devastated by large predators such as fish. Unless the laws of physics can be altered, the turbulent mixing, boundary layer, and stratification found in many aquatic ecosystems cannot be adequately

simulated on the scale of a typical microcosm. Attempts to do so can be misleading.

Although there are limitations to the ability of the SAM to mimic full-scale ecosystems, significant alterations in community metabolism were noted. Signs of acute or chronic toxicity were not apparent, but the P/R ratio of generally less than one and the increase in nitrogenous waste may have had detrimental effects if the experiment had been continued. However, the graphite material assayed in the SAM was much less toxic than the similar dust comprised of brass. Overall, graphite poses much less of a threat to the environment than brass.

5. CONCLUSIONS

- Graphite at the concentration of 10 mg/L did demonstrate ecosystem-level effects in species diversity and nutrient metabolism. The effects resembled eutrophication.

- Acute toxicity was not apparent at the concentrations assayed.

- Compared to brass, the graphite has much less of an impact on aquatic ecosystems.

- The SAM is capable of mimicking eutrophication at the community level.

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