Assessing the Magnitude of Polycyclic Aromatic Hydrocarbon Loading From Road Surface Runoff and Its Effect on Algal Productivity

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16. Abstract

The hypotheses of the study were that PAHs washing off roads would retard the growth of aquatic life-supporting algae and promote the growth of harmful, toxin-producing algae in estuaries, such as the Chesapeake Bay. Runoff from various road surfaces was tested for PAH concentrations. The tests revealed PAHs but also heavy metals in various concentrations.

The research results show that road surface runoff does not seem to affect algae detrimentally after a few days under normal summer conditions. Either the chemicals in the runoff degrade over time or bacteria in the runoff breaks down the chemical pollutants. In both cases, once the PAHs degrade, they seem to stimulate algal growth. The one exception is *Chaetoceros*, a beneficial algae, which was negatively affected by both 1 percent and full strength concentrations on newly paved and low traffic asphalt. Since PAH levels were relatively low for those two sites, it is possible that the response was not due to PAHs from these road surfaces but to heavy metals instead.

Brackish water algae, such as *Chaetoceros*, may experience toxicity from the PAHs and heavy metals. *Chaetoceros* contains significant fat reserves in which PAHs and heavy metals could accumulate. As those algae are consumed by an aquatic organism, such as oysters, the PAHs and heavy metals may become concentrated in the organism.

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A team of students and researchers gather runoff samples on a newly paved stretch of Route 4 in Port Republic, Md.

EXECUTIVE SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) are a suite of complex organic toxins that can originate from impervious road surfaces such as asphalt and sealants, as well as from oil spills and tire wear from heavy traffic. During rain events, impervious surfaces leach contaminants and empty via storm sewers into streams, rivers, and lakes. PAHs act as carcinogens and mutagens in these aquatic environments, affecting fish, insect larvae, and crustaceans.

Very little is known of the magnitude of PAHs loading from road surfaces and how various road surface types (including new green technologies) may differ in their retention or leaching of these toxins. Furthermore, little is known of how PAHs may affect the base of aquatic food webs, single-celled planktonic algae, and how PAH bioaccumulation in algae may transfer these toxins to higher trophic levels.

In order to assess the quality and quantity of PAHs released during simulated rain events, the study team collected and rinsed samples of leached compounds from various road surfaces actively used in Maryland. The selected surfaces all had different traffic intensities and seasonal precipitation patterns. We then examined the effect of PAHs on algal productivity with bioassays of representative beneficial (nontoxic) and harmful (toxic) species.

Results suggest that particulate matter on road surfaces, especially in high traffic areas, was a primary source of PAHs. Additionally, particulate matter from high traffic areas was also enriched in several types of heavy metals, namely copper, zinc, lead, chromium, and nickel. The research results show that road surface runoff does not seem to affect algae detrimentally after a few days of normal summer conditions. Either the chemicals in the runoff degrade over time or bacteria in the runoff breaks down the chemical pollutants. In both cases, once the PAHs degrade, they seem to stimulate algal growth. The one exception is *Chaetoceros*, a beneficial algae, which was negatively affected by both 1 percent and full strength concentrations on newly paved and low traffic asphalt. Since PAH levels were relatively low for those two sites (less than 10ng/L), it is possible that the response was not due to PAHs from these road surfaces but to heavy metals instead.

Brackish water algae, such as *Chaetoceros*, may experience toxicity from the PAHs and heavy metals. *Chaetoceros* contains significant fat reserves in which PAHs and heavy metals could accumulate. As those algae are consumed by an aquatic organism, such as oysters, the PAHs and heavy metals may become concentrated in the organism. A harmful freshwater species (*Microcystis aeruginosa*) was unaffected by most road surface leachate. Implications and suggestions for future study are provided.

INTRODUCTION

Impervious road surfaces have the potential to release chemical pollutants to nearby lakes, streams, and rivers where these chemicals may adversely affect aquatic organisms and, ultimately, humans.[9]Among some of the most common chemical pollutants in road surfaces are polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of complex organic molecules that can originate from petroleum-based products, including bituminous road surfaces and sealants. Road surfaces can also accumulate oils and contaminants from vehicle-related spills and tire wear. During precipitation, impervious road surfaces leach PAHs into runoff and stormwater discharge. While undeveloped land can act as a natural filter for these contaminants, impervious road surfaces quickly convey PAHs into nearby aquatic systems.

Effects on Aquatic Ecosystems

Many studies have examined how PAHs can affect aquatic organisms.[1, 2] Once in an aquatic environment, the majority of PAHs are quickly adsorbed by sediment particles or biotic material[3] (including living organisms and dead organic material) in which they can persist for several years depending on their molecular weight.[4]

PAHs are considered toxic to a variety of aquatic organisms, acting as carcinogens, DNA mutagens, and endocrine disruptors.[5, 6] For example, in finfish like flounder and trout, PAHs can interfere with estrogen production.[7, 8] Oysters and other bivalves are susceptible to PAHs because they have a poorly developed metabolic pathway that does not allow them to breakdown and deactivate PAHs.[9]

In aquaculture studies, the Pacific oyster (*Crassostrea gigas*) had marked decreased productivity when exposed to even low concentrations of PAHs.[10] Stream biota, such as insect larvae and crustaceans, are also adversely affected, resulting in reduced species diversity in affected areas.[11] Single-celled aquatic plants, like algae, can also be adversely affected by PAH concentrations.[12, 13] However, there are less studies about PAH effects on algae than other aquatic organisms.[12]

Algae are an ideal target group for toxicity bioassays because of their rapid growth, short generation times, and importance in most aquatic ecosystems. Their growth, termed primary production, provides the food and resources that dictate, both directly and indirectly, the production of many commercially important species, including oysters, striped bass, and blue crabs.[14, 15] Deleterious effects on algal productivity can be detected in less time than is needed to examine direct effects on larger, longer-lived organisms.

Since algae serve as the basis of the food web for most aquatic systems, inferences can be made about how PAH effects propagate through the entire system. For example, if PAH loading from a particular road surface decreases algal productivity, then the organisms that rely on algae as a food source would face either a decrease in food availability or a bioaccumulation of toxins.

However, not all algae are created equal. Some algae can produce toxins or form massive population explosions (often called blooms or red tides) that have adverse effects on finfish, shellfish, and humans.[16] Therefore, it is important to distinguish these toxic algae from the beneficial, nontoxic algae described above. Also, it is possible that toxic algae may respond differently to PAH loading. For example, some toxic algae have a special enzymatic pathway (monooxygenase) that breaks down chemical contaminants and utilizes the detoxified compounds for energy.[9] It is highly possible that toxic algae may be unaffected by or even benefit from the presence of PAHs. Toxic algae's ability to metabolize these compounds could give the organisms a competitive advantage over algae that cannot.

Objectives

The objectives of this study were to characterize the types and concentrations of PAHs that originate from different impervious road surfaces and to assess the effect of the PAHs on the growth of different types of single-celled algae.

METHODOLOGY

Site Location and Sampling

We sampled road surfaces at four locations in Maryland in the summer of 2009. As seen in Figure 1, the sites can be classified as high traffic and old concrete, high traffic and old asphalt, newly paved asphalt, and low traffic and old asphalt. Samples for the high traffic intensity locations were taken on June 28 in Baltimore City. The samples at the other locations were taken on July 8 in southern Maryland. The high traffic and old concrete site is a few hundred yards north of the intersection of Liberty Road and Northern Parkway. The high traffic and old asphalt site is east of the intersection of Gwynns Falls Parkway and Reisterstown Road. The newly paved asphalt site is located south of the intersection of Broomes Island Road and Route 4. (When sampled, the asphalt was less than 24 hours old.) The low traffic and old asphalt site is located at a pull-off on the southbound side of Route 4.

Figure 1. Sampling Sites

Simulated rainwater was created by adjusting distilled water to a pH of 5.5, the pH of actual rainwater in this region. A sampling rig was developed to efficiently deploy and collect the simulated rainwater in a consistent surface area for each site.

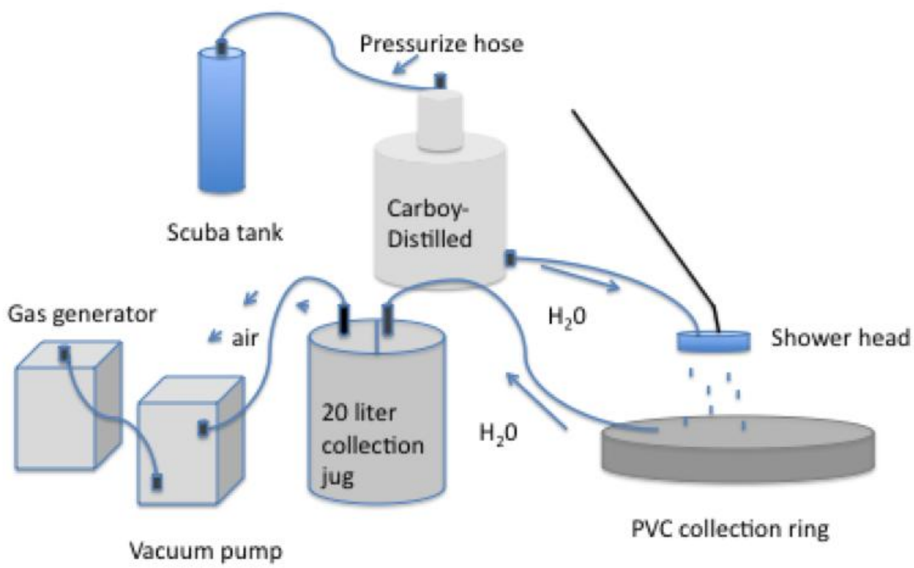


Figure 2. Diagram of Rig for Sampling Road Surfaces

The rig consisted of a PVC ring (53.34 cm diameter, 10.16 cm tall) outfitted with foam weather stripping around the circumference of the ventral side. The weather stripping was used to mold to imperfections in the road surface and create a waterproof barrier to hold the simulated rainwater inside the ring. Half-inch Tygon® tubing connected a 20-liter, Nalgene® carboy container filled with the simulated rainwater to a customized plastic showerhead that was 20.32 cm in diameter. A one-way nipple valve on top of the carboy cap allowed pressurized air from a scuba tank (3200 psi) to be pumped into the carboy. The pressurized air forced a steady stream of water through the showerhead and onto the road surface, simulating a rainfall event.

For a sampling, approximately 7 liters of simulated rainwater was applied to the ring, filling it with about 3.25 cm (1.3 inches) of water. The simulated rainwater was collected into a 20-liter, stainless steel jug with a vacuum system (3/4 HP) run by a gas-powered generator. This process was repeated at each site three times, for a total of nine ring deployments per site. The collected water was then placed on ice until it was returned to the lab and divided for organic analysis and biological assays. Metal samples were collected by hand with 10 ml Falcon tubes at the beginning, middle, and end of rain simulation. The 30 ml collected from each of the three rings were composited to make a 90 ml sample.

Organic Analysis

The organic analysis used between 16 and 19 liters of water from each location's triplicate samples. Within 24 hours of collection, these samples were passed through glass fiber filters and pre-cleaned XAD®-2 resin column. The filters, which were 293 mm in diameter with a 1 µm nominal pore size, were pre-baked at 550°C for 4 hours. The resin columns were manufactured by SUPELCO Analytical in Bellefonte, Pa. The glass fiber filters retained particle-bound material, while the XAD®-2 retained the dissolved phase PAHs. Both the filters and the XAD®-2 were Soxhlet extracted for 24 hours with approximately 500 ml of a 50:50 acetone and hexane mixture.

Prior to Soxhlet extraction, all of the samples were spiked with 100 ng of the following deuterated PAH surrogates: d_8-Naphthalene, d_{10}-Fluorene, d_{10}-Fluoranthene, and d_{12}-Perylene. Lab blanks consisting of clean filters and XAD®-2 blanks were also extracted for each sampling date. After the extraction was complete, the samples were liquid-liquid extracted with a 25-ml salt water rinse, double 25-ml DI water rinse, single 20-ml hexane back-extraction for filters, and triple 25-ml hexane back-extraction for dissolved samples. Sodium sulfate was then added to remove any remaining water. Extracts were then rotary evaporated to approximately 1 ml and transferred to autosample vials.

One hundred nanograms of each of the following internal standards were added to the vials immediately prior to GC/MS analysis: d_{10}-Acenaphthalene, d_{10}-Phenanthrene, d_{12}-Benz[a]anthracene, d_{12}-Benzo[a]pyrene, and d_{12}-Benzo[g,h,i]perlyene. The masses of PAHs in the extracts were determined by gas chromatograph mass spectrometry (Agilent 6890/5973N) operating in select-ion mode. A DB-5 silica fused capillary column was used with helium as the carrier gas. The column, manufactured by Agilent in Folsom, Calif., was 25 m in length, 0.2 mm in diameter, and 0.33 μm in film thickness. The oven temperature was programmed from 45 to 280°C at a rate of 10°C per minute and held at 310°C for 16.5 minutes. To generate the PAH concentrations, the PAH masses were adjusted based on the surrogate recoveries and divided by the water volume. Values were compared to the blanks, with a value considered less than detectable if it was less than the average blank value plus 3 times the blank standard deviation.

Metals Analysis

Trace metals analysis required less sample volume, but the samples could not come in contact with the metal containers used for the organic analysis. As a result, we manually collected approximately 100 ml of water in plastic Falcon tubes at the beginning, middle, and end of each of the triplicate runs at the four sites. These three hand samples were then composited for analysis. To find the dissolved metals concentration, a portion of the water samples were filtered, reduced in volume, evaporated, and then analyzed by ICP-MS. To obtain the total metals (dissolved plus particle bound), the remaining portions of the water samples were acid digested, reduced in volume, and analyzed by ICP-MS. Subtracting the dissolved metals concentration from the total metals results in the particle based metals concentration. As with the organic analysis, both field and lab blanks were collected.

Algal Bioassays

Due to issues with obtaining the originally proposed beneficial diatom species (*Skeletonema*), two alternative species were used. Ultimately, the three algae species used to test sensitivity to road surface runoff were *Ankistrodesmus braunii*, *Microcystis aeruginosa*, and *Chaetoceros muelleri*. *Ankistrodesmus* is a single-celled Chlorophyte (green algae), *Microcystis* is a toxin-producing Cyanophyte (blue-green algae) that occurs in large colonies, and *Chaetoceros* is a single-celled/small chain-forming Bacillariophyte (diatom). Cultures of the freshwater algae, *A. braunii* and *M. aeruginosa*, were obtained from the University of Texas's Culture Collection of Algae and grown using Bold 3N media. *Chaetoceros*, a brackish water species, was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton and grown using F/2 media. *Ankistrodesmus* and *Chaetoceros* are both considered nontoxic, beneficial algae. They are beneficial as nutritious food for higher trophic levels. *Microcystis* is often toxic and is a nutritionally poor prey item for filter-feeding animals. Additionally, *Microcystis* has an enzymatic pathway that could allow the breakdown and utilization of PAHs. All cultures were maintained in Pyrex flasks at 20°C. In order to simulate environmental conditions, the cultures were alternately exposed to 14 hours of light and 10 hours of darkness.

Each road surface runoff was used within 24 hours of sampling to minimize degradation prior to testing. For each algal species, test tubes were spiked with one of three concentrations of runoff — 0 percent (control; no leachate added), 1 percent (dilution), or 100 percent (full strength; 100 percent leachate added). In addition to the leachate concentrations, Instant Ocean was added to the *Chaetoceros* tubes to maintain 10 ppt salinity. Growth was monitored once a day for up to seven days using a Turner Designs fluorometer (Model 10-AU-074). Measures of fluorescence indicate the amount of chlorophyll *a* in a sample. Chlorophyll *a* is the primary photosynthetic pigment found in all plants. Fluorescence values acquired from the fluorometer were converted to percent change from the initial observation. Results are presented in that form. The error bars in the results shown in Figures 5-7 reflect the standard deviation of the three replicates per treatment.

Statistical Analysis

For the algal bioassays, statistical differences were assessed using a student's T-test p-value, with p-values less than 0.05 indicating statistically significant differences among the treatments.

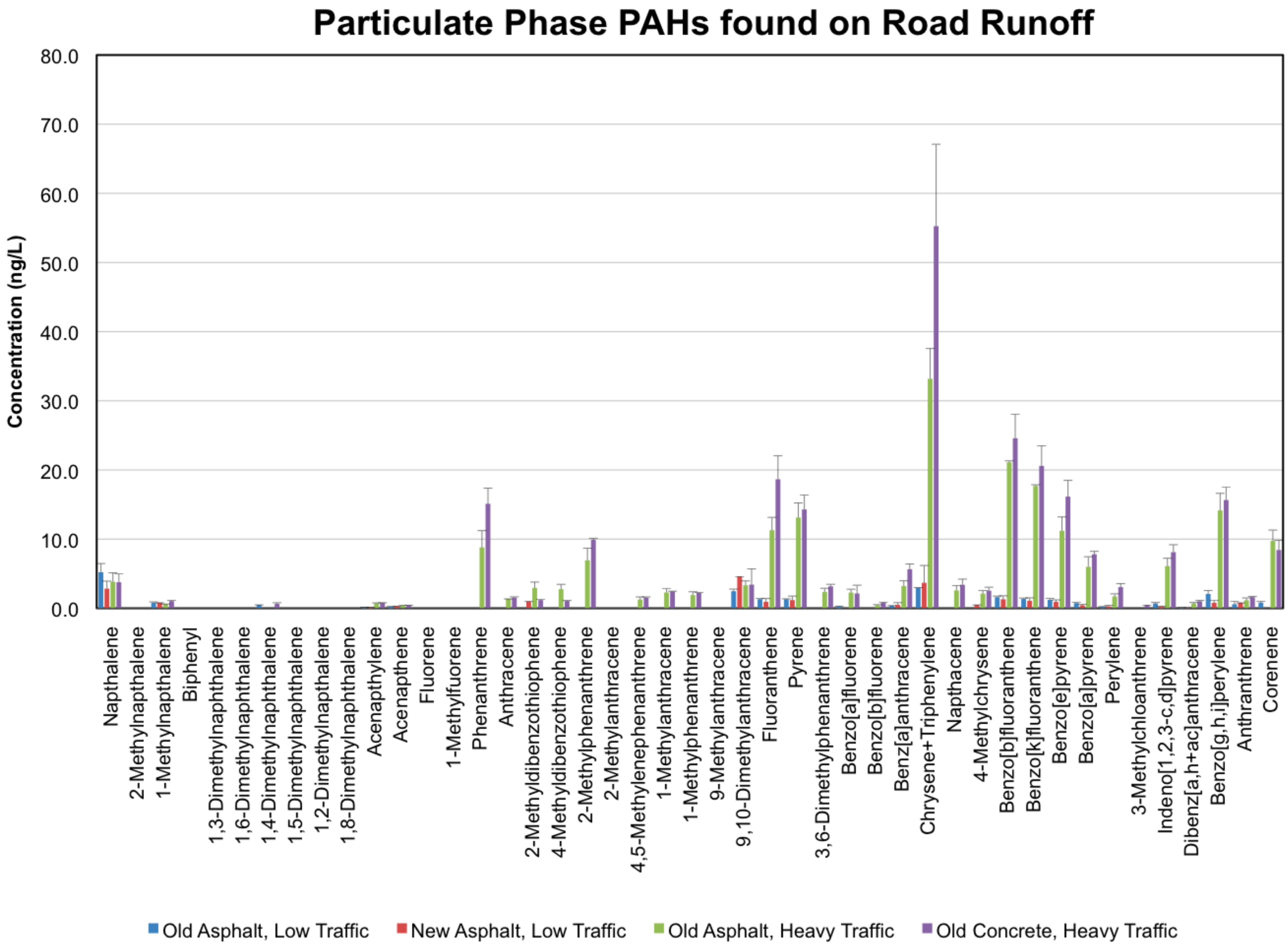
RESULTS

Overall, PAH leaching from the tested road surfaces varied. Though affected by road substrate (i.e., concrete or asphalt), the leaching appeared to be primarily dependent upon traffic intensity, the amount of fine particulate matter on the road, and the exposure concentration.

Road Surface Chemical Composition

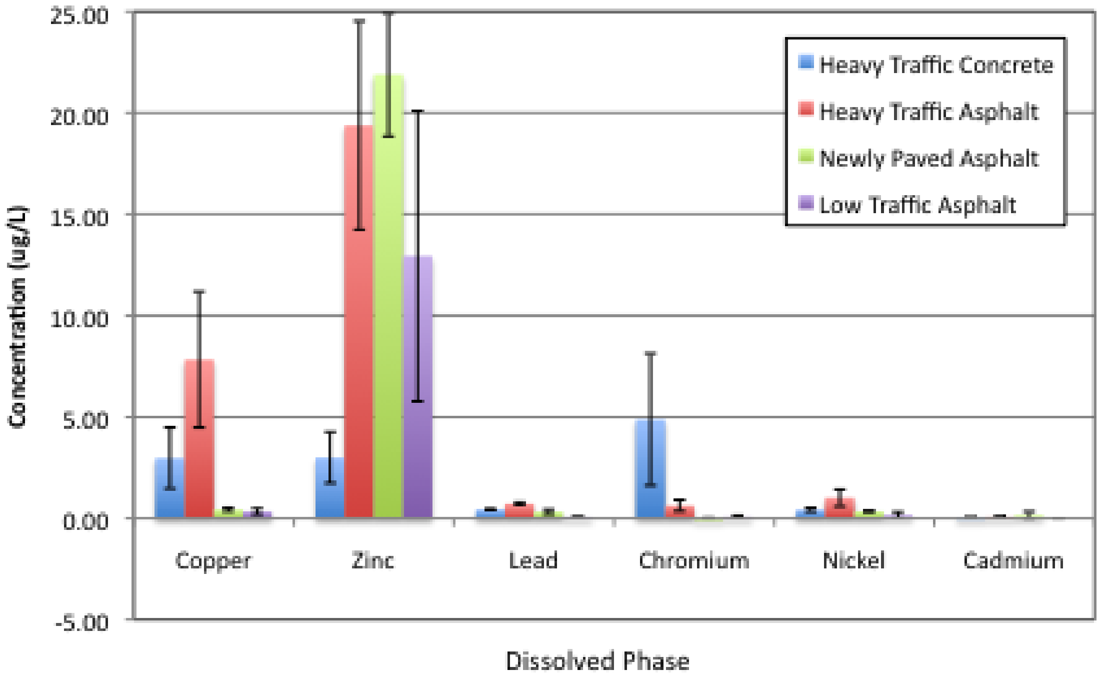
PAHs were seen in the particle phase on asphalt and concrete and in high and low traffic. As can be seen in Figure 3, many of the particle phase PAHs were found in relatively low concentrations (less than 10 ng/L). The PAHs that occurred in highest concentrations (greater than 10 ng/L) in at least two of the tested road surfaces included Chrysene + Triphenylene, Pyrene, Fluoranthene, Benzo [b] fluoranthene, Benzo [k] fluoranthene, Benzo [e] pyrene, and Benzo [g,h,i] perylene. The vast majority of the PAHs were seen in the particle phase, and the concentrations at the high traffic surfaces were significantly higher than at the low traffic site. All dissolved phase PAHs in the low traffic area were less than detectable. The metals analysis produced similar findings.

Heavy metals were another important component of the road surface leachate (Figure 4). Zinc was found in highest concentrations in three of the four different treatments, followed by copper, lead, chromium, nickel and cadmium. The concentrations of the particulate phase of these metals were an order of magnitude higher than the dissolved phase. The heavy traffic road surfaces were characterized by the highest concentrations of particulate phase metals, as well as most of the metals in the dissolved phase. The exceptions were the relatively high concentrations of dissolved zinc in the newly paved and low traffic asphalt treatments.



Note: Dissolved PAH concentrations for all surfaces were below detectable limits and are not shown.

Figure 3. Types and Quantities of PAHs Detected in Particles Collected on Each Road Surface



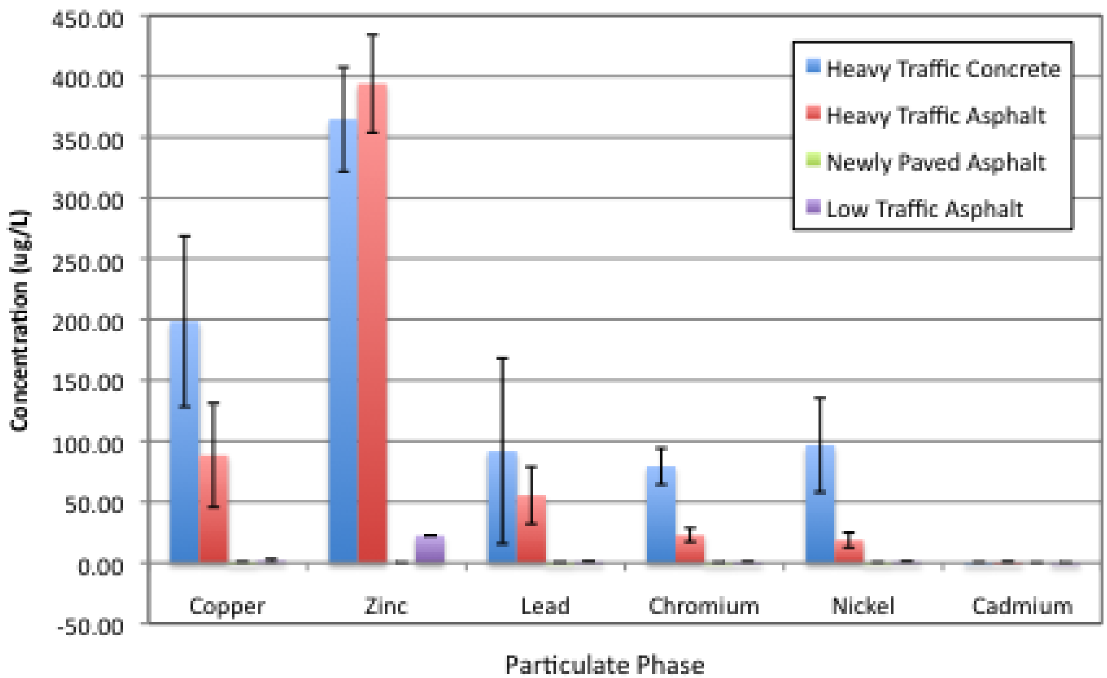


Figure 4. Heavy Metal Concentrations Found Within Each Road Surface Leachate

Algal Bioassays

As seen in Figures 5-8, the algal assay results suggest that each road surface affects each algal species differently. Furthermore, for each road surface sampled, there were differences in the responses to the exposure concentrations.

High Traffic Concrete and Asphalt

When tested on concrete and asphalt road surfaces with high traffic intensity, *Ankistrodesmus*, the beneficial algae, was adversely affected in the first few days of exposure to full strength concentrations (Figures 5 and 6). The strongest decrease was observed on asphalt, where growth became negative by Day 2 (Student's T-test p-value = 0.026). Concrete also exhibited significant decreases in growth, though not negative (p-value = 0.025). For both high traffic intensity roads, the 1 percent leachate concentration, which represents realistic concentrations, was highly variable and not significantly different from the control treatment. In both cases, growth in the first few days was faster in the 1 percent solution than in either the control or full strength leachate treatments. There were no significant differences in the growth of *Ankistrodesmus* among any of the treatments after seven days.

Except for the full strength treatment on asphalt, *Microcystis*, the toxic algae, did not exhibit statistically significant changes in growth at any concentrations on the high traffic intensity road surfaces (Figures 5 and 6). After being subjected to the full strength leachate for seven days, *Microcystis* showed a significant increase in growth. The *Chaetoceros* cultures crashed prior to the start of these algal bioassay experiments; therefore, there is no data available for this round of experiments.

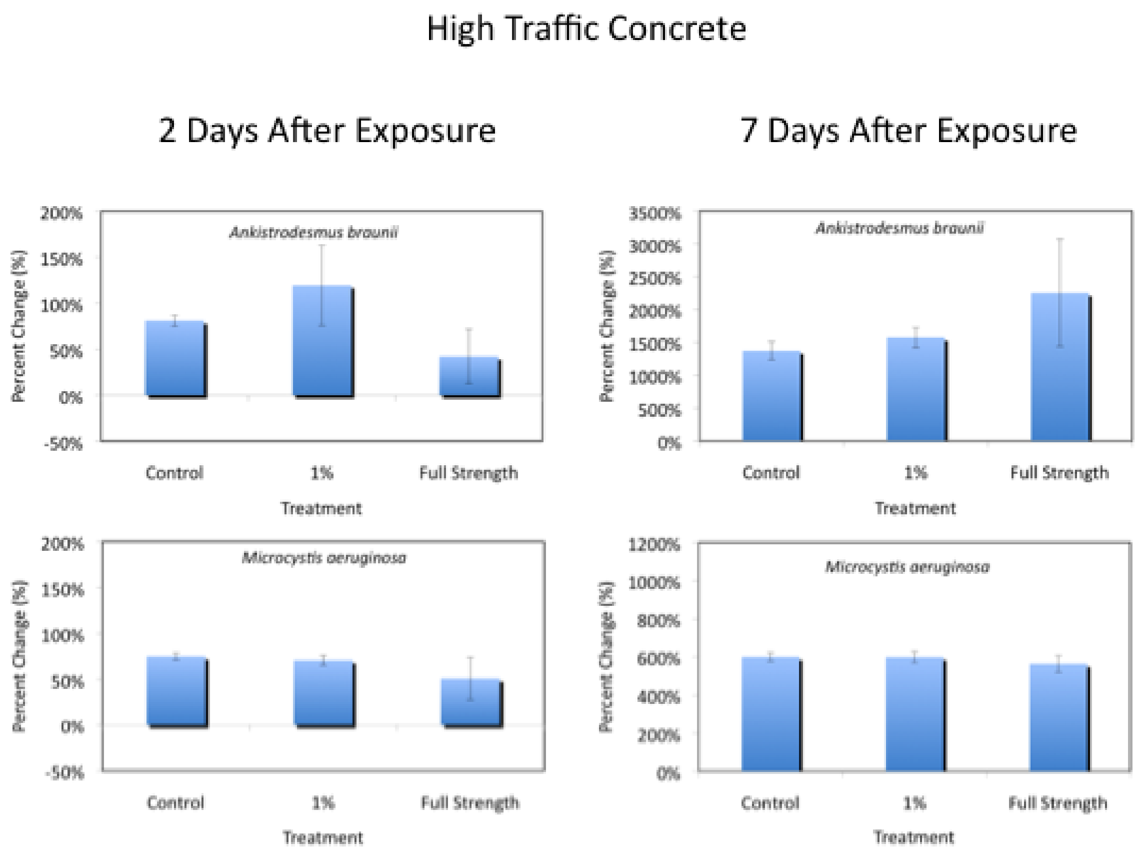


Figure 5. Algal Bioassay Results at the Beginning (Day 2) and End (Day 7) of the Exposure to the High Traffic Concrete Leachate

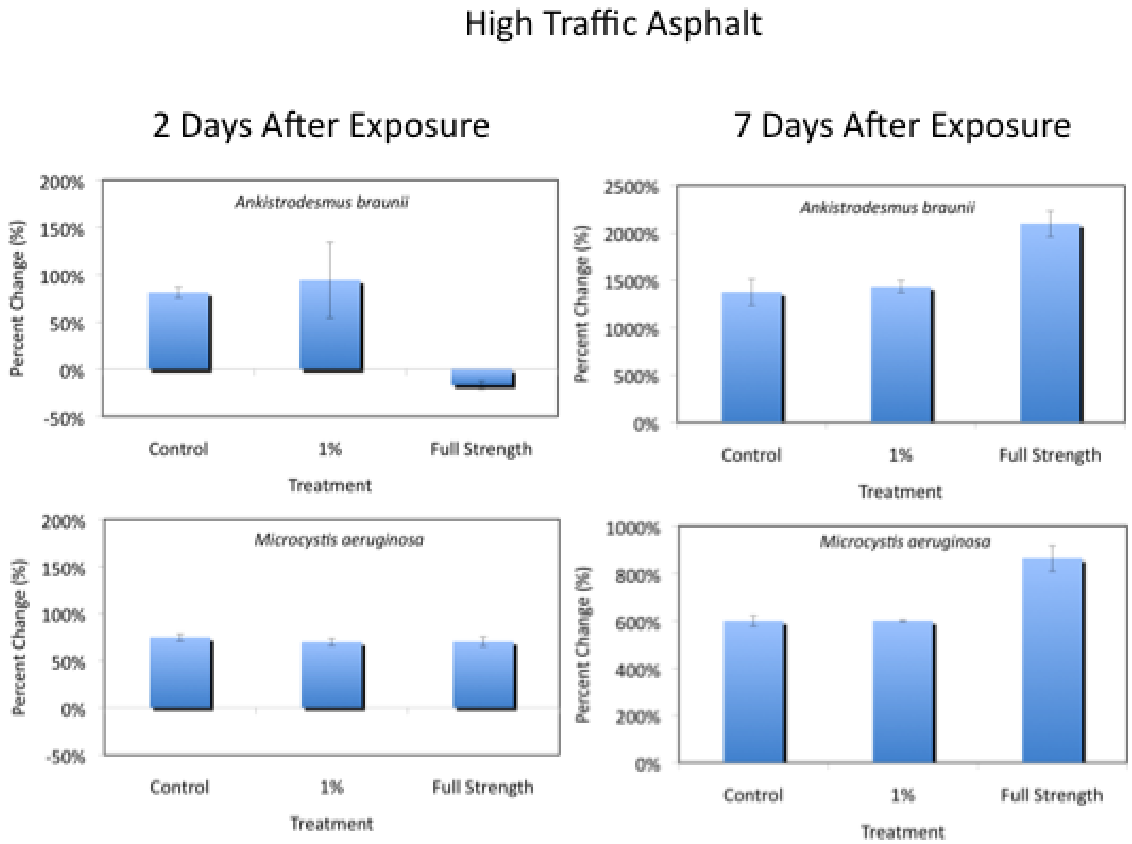


Figure 6. Algal Bioassay Results at the Beginning (Day 2) and End (Day 7) of the Exposure to the High Traffic Asphalt Leachate

Newly Paved and Low Traffic Asphalt

There were no significant differences in the leachate treatments relative to the control for the newly paved and low traffic asphalt sites for *Ankistrodesmus*. As seen in Figures 7 and 8, bioassay results from the full strength concentrations on the newly paved and low traffic asphalt sites did not lead to decreases in growth for *Ankistrodesmus*. Instead, growth in the first few days was higher but not statistically different than the control treatment (p-values > 0.05). Furthermore, after seven days of exposure, concentrations of *Ankistrodesmus* were nearly identical, regardless of treatment.

The growth rate of the diatom *Chaetoceros* was negatively impacted by the addition of the leachate from these two sites (Figures 7 and 8). Increasing the leachate's concentration accelerated statistically significant decreases in the growth of the *Chaetoceros* cultures. The largest effect was observed in the newly paved asphalt treatment. Inhibition of growth was still observable and statistically significant after seven days of exposure.

After two days, there were no significant differences in the growth of *Microcystis* in the leachate treatments and the control for either the newly paved or low traffic asphalt (Figures 7 and 8).

Growth of *Microcystis* in the full strength leachate showed a decrease relative to the control after seven days.

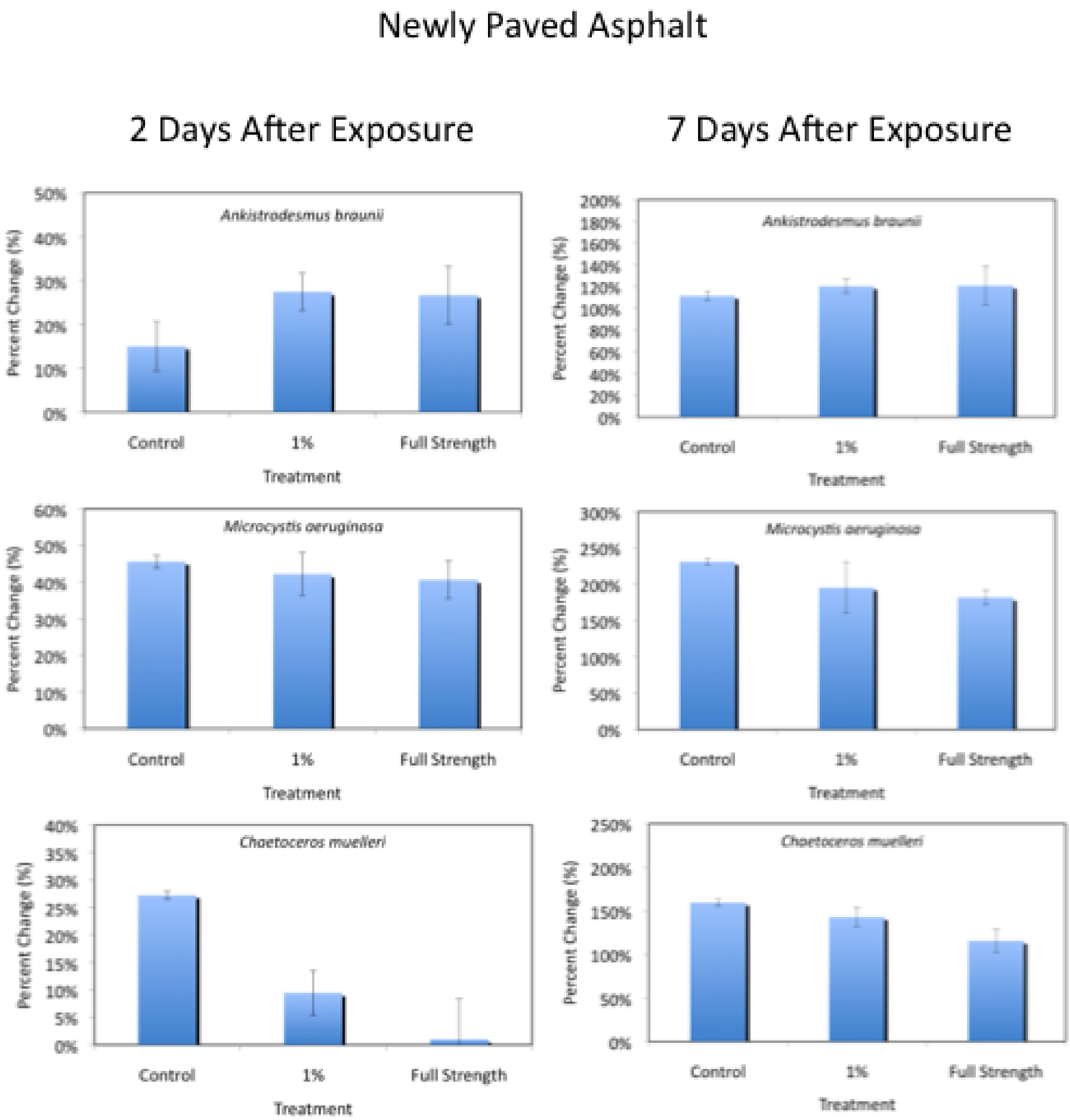


Figure 7. Algal Bioassay Results at the Beginning (Day 2) and End (Day 7) of the Exposure to the Newly Paved Asphalt Leachate

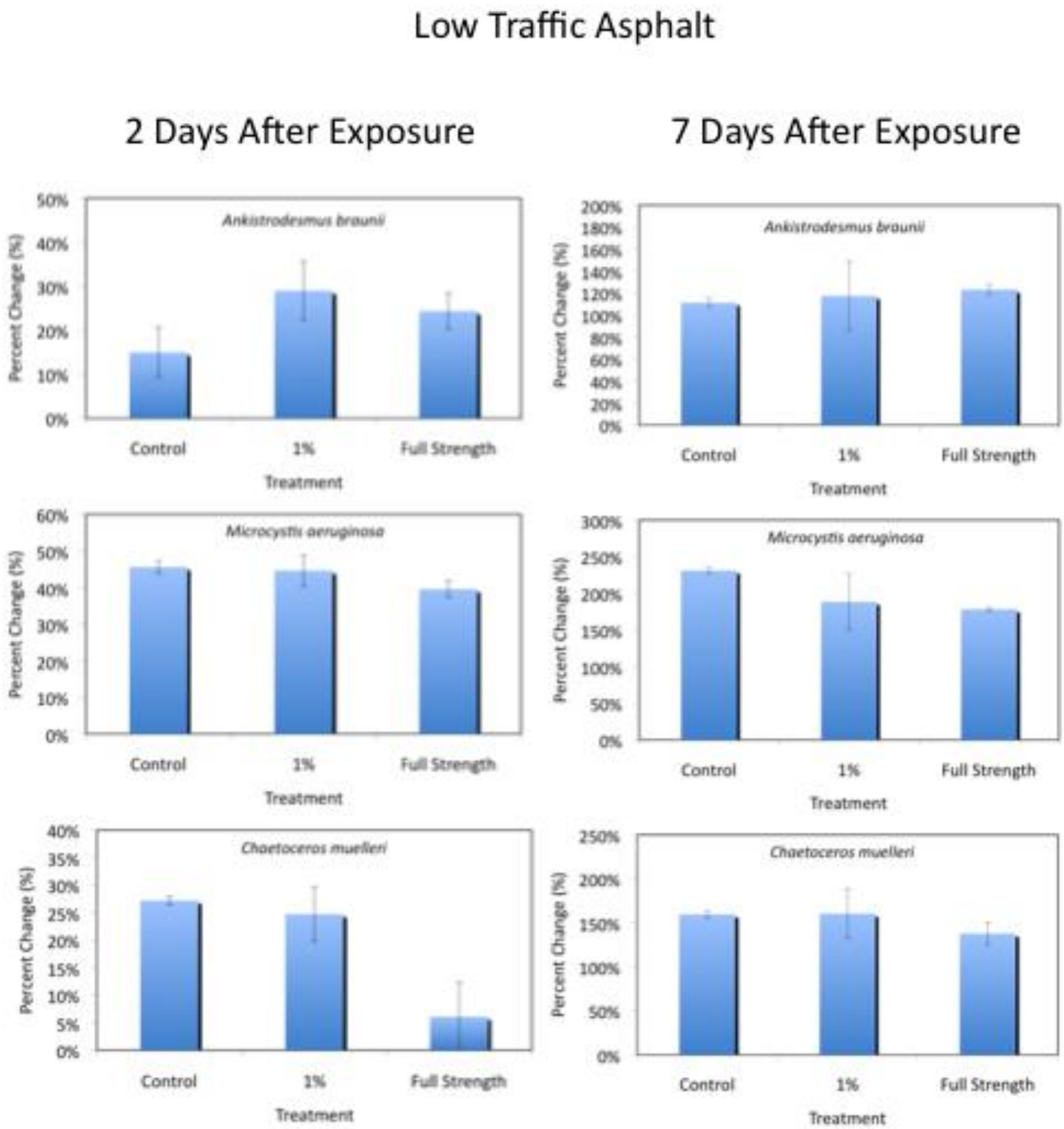


Figure 8. Algal Bioassay Results at the Beginning (Day 2) and End (Day 7) of the Exposure to the Low Traffic Asphalt Leachate

CONCLUSIONS

Effects of PAH and Heavy Metal Loading on Algae

Road surfaces contain distinct PAH and heavy metal signatures, lending to observable differences in the algal response to such contaminants. With most road surfaces, realistic concentrations did not significantly decrease algae growth. Many of the effects only became significant at full strength concentrations, which suggests that road surface runoff may not adversely affect algae in surrounding aquatic systems after a few days of normal summer conditions.

The one exception to the above statement is *Chaetoceros*, a beneficial algae, which was adversely affected by both 1 percent and full strength concentrations on newly paved and low traffic asphalt. (Note: *Chaetoceros* was unavailable for the high traffic treatments.) Since PAH levels were relatively low for those two sites (less than 10ng/L), it is possible that this response was not due to PAHs from these road surfaces. Instead, heavy metals (e.g., zinc from Figure 4) may have adversely affected *Chaetoceros*. In another study, PAHs showed little or no adverse effects on another diatom, *Thalassiosira* sp., at a variety of different concentrations, supporting the idea that the diatom in this current study was impacted by the metals in the road surface leachate.[17]

There are several possible reasons for this diatom's sensitivity. First, the media (f/2) and salinity (10 ppt) needed to grow this brackish water diatom were different from the other tested species. It is possible that the dissolved salts in the media may have interacted with the road surface leachate, increasing the availability or toxicity of the heavy metals in the runoff. If this is the case, a follow-up experiment involving other brackish water organisms would be advisable.

Second, *Chaetoceros*, like other diatoms, contains significant fat reserves in which heavy metals could accumulate.[18] Third, *Chaetoceros* may be more susceptible to pollutants than the other species tested. *Chaetoceros* and other similar centric diatoms have been found to be sensitive to other pollutants (e.g., arsenic).[19]

Duration of Leachate Toxicity

Despite negative or reduced growth in the first few days after exposure to high traffic treatments, cultures of *Ankistrodesmus*, another beneficial algae, had the most growth with the full strength exposure levels. This suggests that either the chemicals in the runoff may degrade over time or the bacteria in the leachate breaks down the chemical pollutants. In both cases, once the PAHs degrade, they may provide a nontoxic form of organic nutrients that stimulate algal growth. Based on examination of the time courses for each experiment, we can conclude that this breakdown occurs within the first three days of the experiment.

This time period has an important implication. For example, if storm water loading into an aquatic system is followed by several days of cloudy, less than optimal conditions for algal growth, then it is possible that bacteria may have already degraded any PAH contaminants by the time conditions become favorable for algal growth. This, in conjunction with the non-significant results of the 1 percent concentrations, further suggests that PAH loading from road surface leachate may not detrimentally affect algal populations and may instead stimulate algal growth.

Beneficial Algae vs. Harmful Algae

Leachate concentrations had different effects on beneficial and harmful algae. As hypothesized, *Microcystis*, a toxin-producing algae, did not appear to be significantly affected by any road surface or leachate concentration. Though genetic testing was not completed in this study, it is likely that *Microcystis* has the enzymatic pathway required to break down and utilize PAHs. It should also be noted that cell size has been shown to be a significant factor in the sensitivity of different phytoplankton species to PAHs.[17] Since *Microcystis aeruginosa* forms colonies of hundreds of small cells, this morphological configuration may also be a factor in enabling its growth in the presence of PAHs.

Final Thoughts and Future Studies

This study provided a necessary analysis of the quality and quantity of PAHs and heavy metal leaching from existing road surfaces. Overall, freshwater algae may not be affected at realistic concentrations, but brackish water algae may experience toxicity. This suggests that PAHs and heavy metals may have more significant impacts on estuarine environments and organisms.

It is difficult to compare and rank the tested road surfaces by their impact on algal growth. This is due to the fact that the sampling schedule and sites prohibited testing all four road surfaces simultaneously. In further studies, it is recommended that road surfaces be chosen within the same region to enable these types of comparisons.

The results from a study completed this year indicate a significant difference in the lethal sensitivity to PAHs between cultured phytoplankton and natural populations of phytoplankton.[17] These results show that natural populations of phytoplankton are less resistant to PAHs than those phytoplankton grown in culture. In order to more accurately assess the impacts of PAHs on phytoplankton, future studies should use natural phytoplankton to best reflect the effects of road surface leachate in the actual aquatic environment.

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