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Ecological Responses of Freshwater Algae to Persistent Exposure to Household Detergent Residues

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Abstract: Domestic water supplies contain residues of household detergents which, upon discharge to freshwater systems, may contain ingredients such as surfactants, phosphates, fragrances, and preservatives that can change the physiology of primary producers. There is little evidence on long chronic low-level exposures due to the fact that most toxicity tests are conducted over a 24–96-hour period and only for one species. A 28-day experimental design is provided in which Chlorella vulgaris, Scenedesmus obliquus and a natural algal assemblage are exposed to linear alkylbenzene sulfonate (LAS) at the concentrations of 0, 10, 30, 100, 300 and 1,000 µg/L, in three replicate vessels each. Growth, chlorophyll a and b, photosystem II efficiency (Fv/Fm) and community diversity indices will be measured on Days 0, 3, 7, 14, 21 and 28. Quantitative endpoints are used to compile expected ranges of response. The reported concentrations of surfactant in rivers (0.2–37 µg/L) are very low when compared to 96 h EC50 values of 1.5–34 mg/L for Chlorella pyrenoidosa and S. obliquus, and 5.21 and 8.48 mg/L for inhibition of PSII. The chlorophyll a concentration decreased at 24 h and began to recover at 72 h, and only the chlorophyll b concentration decreased above 750mg/L, with PSII inhibition maximum at 24 h prior to partial recovery. These patterns suggest a species-specific, time-specific response and potential stimulation at low doses of nutrients. The framework provides concentration ranges, endpoints, and statistical procedures for testing cumulative effects of persistent exposure.

Keywords: Freshwater Algae; Detergent Residues; Linear Alkylbenzene Sulfonate; Chlorophyll; Photosynthesis; Community Diversity.

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I. Introduction

Chemicals enter freshwater from domestic, industrial, and agricultural processes, and domestic detergents are common sources of synthetic surfactants, phosphates, fragrances, and preservatives. These substances enter rivers and lakes via laundry, bathing and kitchen effluents, and remain in the water for extended periods after treatment due to incomplete removal (Ivanković & Hrenović, 2010). Surfactants have been reported in the water at concentrations of 0.2-37 µg/L; LAS have been detected in untreated sewage at concentrations of 2-487 mg/L at stations affected by sewage, and degradation products up to 487 µg/L at a concentration around a treatment plant outlet (Corada-Fernández et al., 2011).

Primary producers, freshwater algae, fix carbon, produce oxygen, and contribute to the growth of zooplankton, insects, mollusks, and fish. Algae are sensitive to pollution; changes in their growth, pigment content, photosynthetic activity, and community structure are widely used as indicators of water quality (Stevenson, 2014). Detergent residues can thus affect ecosystem productivity as well as the structure of sensitive algal groups.

The vast majority of toxicity information comes from short-term (24–96 h) acute tests at high concentrations with a single species, and the toxicity of each chemical has been used to rank the hazard of surfactants (Lechuga et al., 2016). There are still unresolved questions about the physiological effects of sub-lethal exposure over multiple weeks, and changes in community composition.

The aim is to define the ecological and physiological responses of freshwater algae to persistent detergent exposure. The objectives are to

(i) Evaluate growth and biomass,

(ii) Quantify chlorophyll a and b,

(iii) Assess photosynthetic performance,

(iv) Examine species abundance and diversity,

(v) Compare short-term and long-term responses, and

(vi) Identify concentrations associated with measurable adverse effects.

This paper continues from the previous one. Section II examines ingredients of detergents and toxicity impacts. The proposed 28-day experimental design, organisms, and statistical procedures are described in Section III. The quantitative endpoints are combined in Section IV for comparison. Section V covers ecological effects, cellular effects, and limitations. Last but not least, Section VI presents key findings and recommendations for further research.

II. Literature Review

2.1 Detergent Ingredients in Freshwater

The main cleaning agents are anionic (LAS and alkyl ether sulfates) and non-ionic (alcohol ethoxylate) surfactants that act on membranes and cellular physiology. Phosphates are used to condition water and provide nutrients. Optical brightening agents and preservatives are toxic agents whose toxicity may vary depending on the degradation and chemical composition of the product, and the actions of complete formulations cannot be predicted on the basis of the main surfactant alone (Brausch & Rand, 2011). Continued phosphorus control is the most powerful tool to prevent eutrophication in lakes, and excess phosphate fuels blooms, turbidity, oxygen depletion, and habitat degradation (Schindler et al., 2016). Enrichment and direct toxicity effects may, therefore, initially have opposing effects on algal biomass.

2.2 Effects on Growth, Pigments, Photosynthesis and Cells

The swimming-velocity NOEC and EC50 values for a commercial laundry detergent were 10.8 mg/L and 34 mg/L, respectively, after 72 h exposure to E. gracilis, and the chlorophyll a and total carotenoids were significantly reduced at ≥50 mg/L, while photosystem II quantum yield inhibition reached a maximum after 24 h, following which there was a recovery trend (up to 800 mg/L) (Azizullah et al., 2011). Phenolic detergent degradation products are similarly toxic: nonylphenol is more inhibitory to growth, photosynthesis, and activity of photosystem II (PSII) than octylphenol, with 96 h EC50 values of 2.89 and 5.21 mg/L, respectively, for *Chlorella pyrenoidosa*, and 1.54 and 8.48 mg/L, respectively, for Scenedesmus obliquus (Oliveira et al., 2017). Maximum photochemical efficiency and growth suppression were observed in 6 freshwater microalgae exposed to NPE (0.5–3.0 mg/L), with species-specific responses resulting from antioxidant activity, and EC50 values of 96 h ranged between 1.005–1.534 mg/L (He et al., 2020). Reported mechanisms involve disruption of membranes, damage to photosystems, and oxidative stress.

2.3 Concentration and Exposure Duration

The toxicity is dose- and time-dependent. In a two-week LAS experiment with submerged macrophytes, little growth stimulation was observed below 5 mg/L, while concentrations higher than that produced growth inhibition and decreased chlorophyll (Wu et al., 2010). This evidence is not related to microalgae and only addresses concentration-dependent (biphasic) responses. With prolonged exposure, acclimation may occur (e.g., recovery in the yield of PSII) or cumulative damage may be sustained by the continuing stress (e.g., damage to PSII).

2.4 Limitations of Existing Work

The existing investigations are limited by short exposure duration (24–96 h), single-species design, use of purified chemicals rather than complete formulations, poor separation of nutrient enrichment from toxicity, and failure to account for the role of environmental modifiers, like interactions with light, temperatures, microbial activity, and interaction with sediments. Experiments with environmentally relevant concentrations and different species of algae are therefore needed to be conducted for longer periods of time.

III. Methods

3.1 Experimental Design

This study will be conducted over a 28-day period, and samples will be taken on days 0, 3, 7, 14, 21, and 28 in order to conduct an evaluation of the ecological and physiological effects of household detergent residue. In this experiment, both pure linear alkylbenzene sulfonate (LAS) isolates and a natural community in a freshwater ecosystem will be used in order to measure the effects of surfactants and also to conduct comparative studies. In addition to this, another set of experiments will be conducted in which a commercial detergent will be used to enable formulation studies. In order to develop this protocol, the basic guidelines of the standard 72-hour OECD 201 growth inhibition test—which requires at least five different concentrations and three replicates—have been extended to a longer test period. Most importantly, although based on the OECD method, this procedure acts as a non-OECD 28-day test protocol specifically tailored to capture cumulative physiological effects, potential acclimation, and community-level shifts under persistent low-level chemical exposure over an extended timeframe.

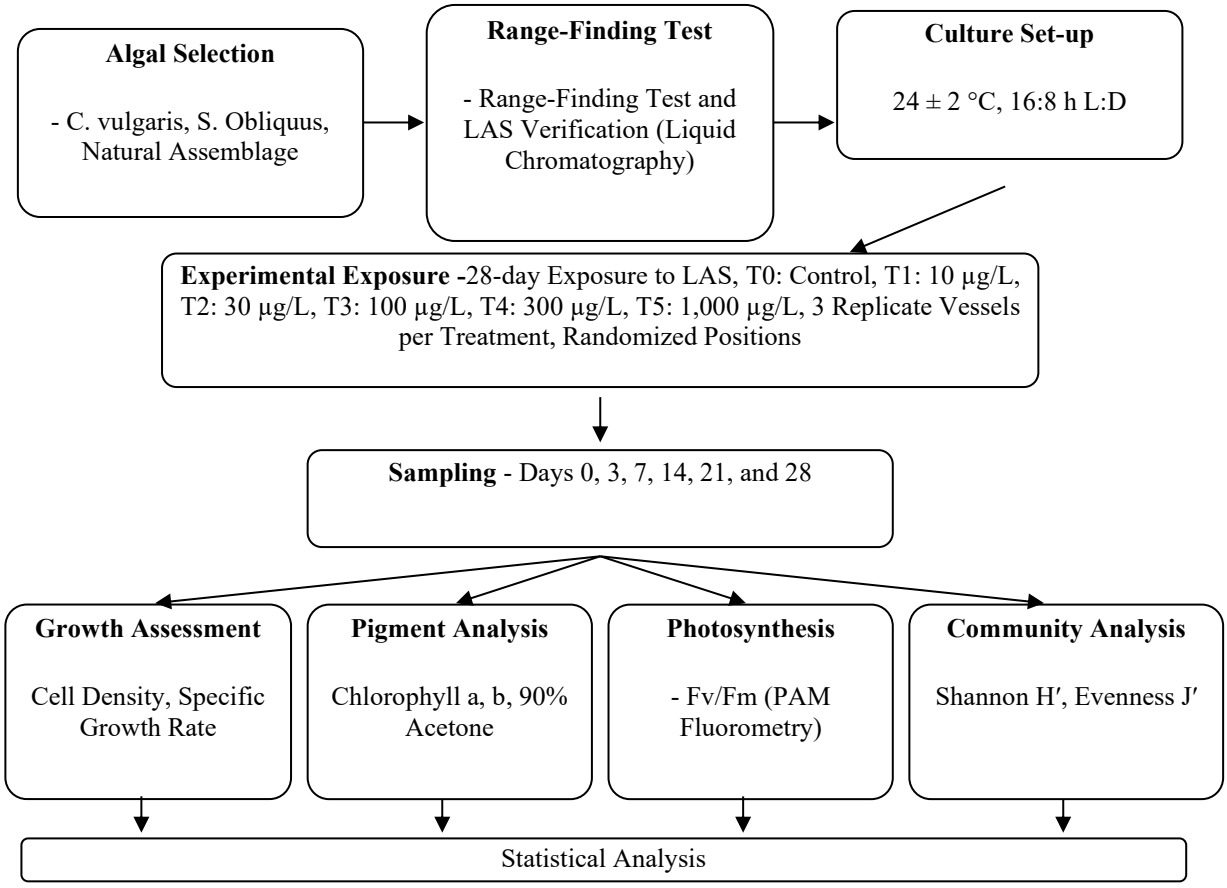


Figure 1: Methodological Flow of the 28-Day Exposure Experiment

Figure 1 summarizes the sequence from organism selection and range-finding to a six-treatment exposure, scheduled sampling, and four parallel measurement streams for growth, pigments, photosynthesis, and community structure. All streams go into one statistical analysis, with the vessel being the independent replicate. The experiment is preceded and repeated during exposure by analytical verification of the concentration of LAS.

3.2 Organisms and Exposure

Chlorella vulgaris and *Scenedesmus obliquus* are selected for fast growth and sensitivity to pollutants; *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* are optional additions. Cultures are grown at 24 ± 2 °C, 16 h light:8 h dark, 50–100 µmol photons m^{-2} s^{-1}, an initial density of 104 cells/mL, and standard freshwater algal medium. Treatment ranges from environmentally relevant to elevated LAS concentrations, and exposure has been confirmed by validated liquid chromatography. To avoid illumination gradients, the vessels are randomly positioned, and 36 experimental units are required for 2 species.

Table 1: Proposed LAS Exposure Treatments

Group	LAS (µg/L)	Category	Replicates
T0	0	Control	3
T1	10	Low	3
T2	30	Environmentally Relevant	3
T3	100	Elevated	3
T4	300	High	3
T5	1,000	Contaminated-Water Scenario	3

Table 1 lists the six treatments, each with three independent vessels per species. The lower range of concentrations is in the range of reported river and effluent concentrations, while T3-T5 test concentration–response patterns are under contaminated conditions. Concentrations are suggested design concentrations and should be tested and analyzed prior to the 28-day run to validate the chosen concentration.

3.3 Measurements and Statistical analysis

The measurements' mathematical formulations are shown in equations (1) to (8):

A hemocytometer is used to count cells:

$$N = \frac{\text{cells counted} \times \text{dilution factor}}{\text{volume counted}} \quad (1)$$

The specific growth rate (μ) is calculated as:

$$\mu = \frac{\ln N_t - \ln N_0}{t} \quad (2)$$

Growth inhibition is determined by:

$$I(\%) = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \quad (3)$$

Positive values of $I(\%)$ represent growth inhibition, while negative values represent stimulation.

Chlorophyll is extracted in 90% acetone and quantified spectrophotometrically using the formulas:

$$\text{Chl } a = 12.7A_{663} - 2.69A_{645} \quad (4)$$

$$\text{Chl } b = 22.9A_{645} - 4.68A_{663} \quad (5)$$

(measured in μg/mL using a 1-cm cuvette and normalised to dry biomass).

Photosystem II efficiency is determined from the pulse amplitude modulation fluorometry measurement after the same dark adaptation:

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (6)$$

Community diversity is calculated using the Shannon index (H') and evenness (J'):

$$H' = -\sum p_i \ln p_i \quad (7)$$

$$J' = \frac{H'}{\ln S} \quad (8)$$

Normality (Shapiro–Wilk) and variance homogeneity (Levene) are tested, and the effects of treatment and time are analyzed by a repeated-measures mixed-effects model and Tukey comparisons. The dose–response regression method is used to estimate EC10 and EC50, community differences are estimated by PERMANOVA, and associations between growth, pigments, and photosynthesis are estimated by Pearson correlation. Significance was determined at $p < 0.05$, and results are presented as mean ± SD and confidence intervals. Replicate = the experimental vessel, NOT the individual count.

IV. Results

This section summarizes numerical results which define regions of expected responses for the experiment proposed. The results of the 28-day experiment will be measured and inserted after completion.

4.1 Occurrence of Detergent Residues

Concentrations of reported dissolved surfactant in rivers range between 0.1 and 37 µg/L, while treated effluents range between 11 and 50 µg/L LAS and untreated sewage is up to 6.7 mg/L LAS, which means an increase of up to three orders of magnitude compared with non-affected rivers.

4.2 Growth and Biomass

Nonylphenol was more toxic than octylphenol to both microalgae, with species' sensitivity to each chemical being the opposite, as *S. obliquus* was more sensitive to nonylphenol and less sensitive to octylphenol than *C. pyrenoidosa*. Macrophytes are documented to have low-dose growth stimulation at < 5 mg/L LAS.

Table 2: Quantitative Endpoints used to Define Expected Responses

Organism	Exposure	Endpoint	Value
Euglena Gracilis	72 h, detergent	Swimming Velocity NOEC / EC50	10.8 / 34 mg/L
Euglena Gracilis	72 h, detergent	Chl a and Carotenoid Decline	≥50 mg/L
Euglena Gracilis	72 h, detergent	Chl b Decline	> 750 mg/L
Chlorella Pyrenoidosa	96 h	EC50 Nonylphenol / Octylphenol	2.89 / 5.21 mg/L
Scenedesmus Obliquus	96 h	EC50 Nonylphenol / Octylphenol	1.54 / 8.48 mg/L

Table 2 compiles endpoints from 72–96 h tests that anchor the expected concentration–response behavior. The sensitivity of the pigments varies, and so does that of the species: Chlorophyll a and carotenoids are about 15 times more sensitive than chlorophyll b; the sensitivity of the species varies between the different chemicals up to a factor of 5.5. 72 h values are values for a complete formulation, and 96 h values are values for single compounds and cannot be directly exchanged.

Figure 2 compares nonylphenol and octylphenol toxicity to two green microalgae. The EC50 values are lower in both species, indicating higher toxicity, with Scenedesmus obliquus being the more sensitive species (1.54 mg/L). S. obliquus is the least sensitive to octylphenol (8.48 mg/L). The species ranking for the chemicals is reversed, showing that sensitivity is not transferable across algal taxa.

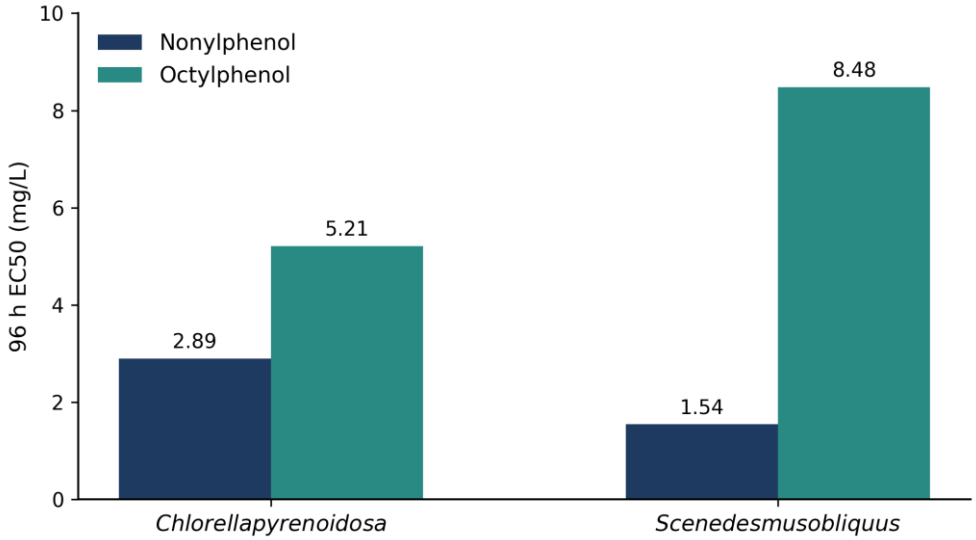


Figure 2: Ninety-Six-Hour EC50 Values Of Nonylphenol and Octylphenol

4.3 Chlorophyll and photosynthetic performance

Photosynthetic impairment was transient, with chlorophyll a and carotenoids significantly inhibited at ≥50 mg L-1 detergent while chlorophyll b was only inhibited above 750 mg L-1. Photosystem II quantum yield was maximally inhibited after 24 h and then demonstrated a recovery trend.

4.4 Community composition and short- versus long-term responses

The data available comparing 24 h exposure with 28 d exposure at the same concentration, or reporting diversity indices exposed to surfactant. Acute tests show maximum physiological inhibition within 24 hours and partial acclimation, but stimulation at low doses of nutrients and loss of sensitive species under chronic stress have not been tested. The contrasts are the key comparison of the experiment that is being proposed.

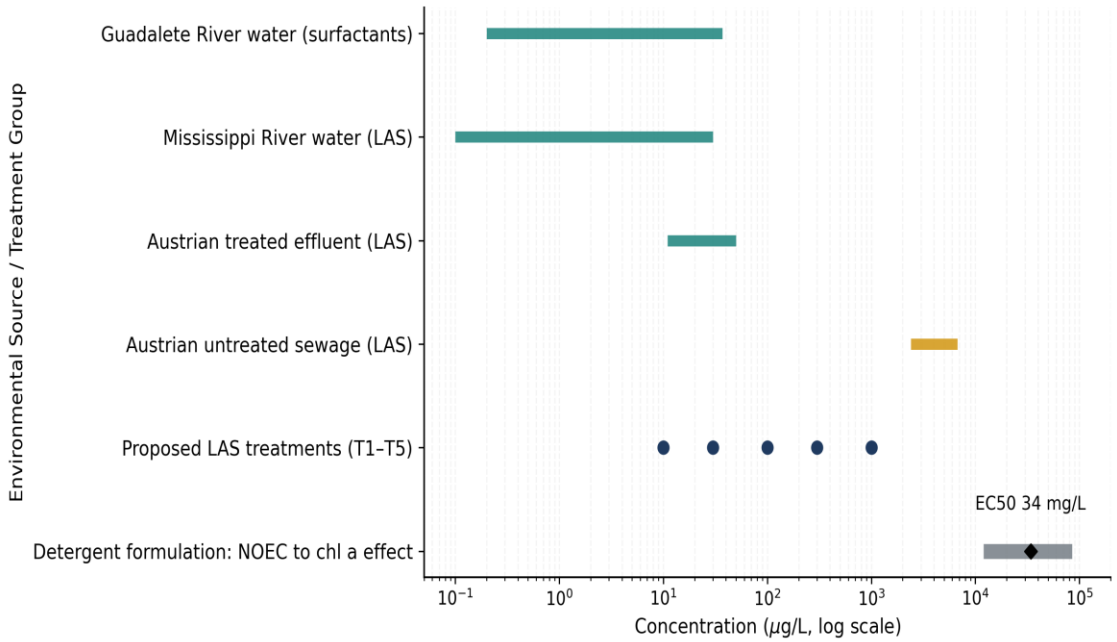


Figure 3: Reported Environmental Concentrations vs Proposed Treatments and Acute Effect Levels

Figure 3 places reported surfactant concentrations in rivers, effluents, and sewage beside the proposed LAS treatments and 72 h effect levels of a commercial detergent on a logarithmic axis. The upper levels of the river and effluent (T1-T3) correspond to time periods that are one to two orders of magnitude shorter than the contaminated conditions (T4-T5), and the acute effect levels are one to two orders of magnitude greater, emphasizing the need for environmentally realistic, longer exposures.

V. Discussion

Synthesized evidence supports the main hypothesis to the extent that, in principle, the detergent-associated chemicals significantly inhibit the growth of algae as well as the concentration of pigments and the efficiency of PSII, with the level of inhibition being highly dependent on chemical structure, species targeted, and exposure time. A species reversal in response to nonylphenol and octylphenol shows that the green microalgae have different defenses. It is important to recognize that community-level changes can readily appear as a result of species' differential sensitivity, even when overall production is not that much affected, so that changes in community structure may be more sensitive bioindicators than overall production alone in chronic pollution situations.

5.1 Possible Mechanisms

In cell membranes, amphiphilic surfactants penetrate directly, changing the permeabilization of cells, the transport of nutrients, and impacting cell viability, which is in line with observed morphological and metabolic changes in cell exposure to LAS. In addition, reactive oxygen species cause oxidative stress, leading to the damage of important pigments, proteins in cells, and DNA. The reduction in the efficiency of PSII directly reduces the rate of photosynthesis and, in turn, the rate of carbon fixation and cell division. At the same time, detergent phosphorus provides nutrients which initially boost biomass production, creating higher oxygen demand when the organic matter is decomposed. Therefore, experimental designs should take care to differentiate direct effects of surfactants from nutrient enrichment, by making comparisons between pure compounds and complete formulations.

5.2 Ecological Consequences

At the ecosystem scale, decreased photosynthesis reduces primary production in the aquatic food chains. The change in species composition affects the nutritional quality of the zooplankton that graze on the phytoplankton; certain species are replaced by other species that are more tolerant. Excessive nutrient-induced algal blooms also decrease water clarity and decrease dissolved oxygen levels. Conventional tests in the lab cannot account for chronic effects, although typical environmental levels in river water are well below the acute toxicity limit. The question to be addressed is whether a continuous low-level exposure over long periods of time causes measurable cumulative physiological damage or a change in the community structure, but without immediate, short-term algal mortality.

5.3 Limitations

The conclusions drawn have several inherent limitations. It is not possible to fully account for natural environmental fluctuations in light, temperature, organic matter, and sediment interactions in controlled laboratory systems. Commercial detergents are mixtures of chemicals that can have additive, synergistic, or antagonistic effects on toxicity. Also, LAS may change, adsorb, or break down with time, so that actual exposure levels can only be confirmed by analysis.

VI. Conclusion

A continued and chronic exposure of freshwater algae to chemical stress is caused by household detergent residues. Surfactants, especially linear alkylbenzene sulfonate (LAS), are found in rivers and in treated effluents, and local inputs to the river around wastewater outfalls increase the concentration of the surfactant many-fold. Evidence reports the effects of these detergent-associated chemicals on algal growth, pigment content, and photosynthetic efficiency, which varied with species, chemical structure,

concentration, and exposure time. Nonylphenol proved to be more toxic than octylphenol to both of the algae tested, and the sensitivity of the pigments varied, with chlorophyll a being most sensitive and, in most cases, more sensitive than chlorophyll b and carotenoids. The inhibition of Photosystem II was maximal within 24 h and only partially recovered, suggesting acclimations during prolonged exposure. In addition, there is a second pathway that is introduced by the detergent phosphorus – nutrient enrichment can lead to increased biomass in the first place, and reduced oxygen availability in the second. High concentrations of surfactant, nonylphenol-type compounds, and untreated wastewater inputs were thus the most significant associations. The design proposed corresponds to the main gaps of short single-species tests with 28 days using environmentally relevant to elevated LAS concentrations, replicated vessels, verified exposure, and community-level endpoints. There is still no direct evidence of persistent low-level exposure, and the impact of community change is still tentative until experimental data are available. Recommendations include better wastewater treatment, careful detergent dosing and use of biodegradable detergents, further studies of realistic detergent mixtures, interactions between detergents and P, sensitivity of diatoms and cyanobacteria, and long-term exposure (continuous exposure > 28 days). Future research should focus on testing realistic detergent mixtures, surfactant-phosphate interactions, and the sensitivity of diatoms and cyanobacteria.

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