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14. The increase in diversity from the Lower Riphean to the Upper Riphean also supports the concept of the morphological evolution of stromatolites in the Precambrian.
15. I thank H. E. Andrews, E. S. Barghoorn, C. Jones, B. Kummel, and J. Sepkoski (Harvard University) and M. R. Walter (Yale University) for comments and aid in preparing this report. C. Jones drafted the figure.

17 June 1971

Eutrophication of Lake Water Microcosms: Phosphate versus Nonphosphate Detergents

Abstract. *The eutrophication potentials of a phosphate-containing detergent and two phosphate-free detergents, as determined in oligotrophic algal microcosms after activated sludge treatment, were not significantly different. All activated sludge effluents, including those from detergent-free waste water, lowered the algal diversity of the microcosms to about the same extent below that of the lake water controls.*

In their concern for the environment, federal and local legislators have introduced a deluge of bills aimed at controlling various pollutants. In particular, the detergent industry has been pressured to eliminate phosphates from detergent formulations. Some observers have expressed concern that excessive emotion and precipitous action may lead to ineffective and toxic detergent formulations (*1*), and yet the rush to pass new legislation continues.

Eutrophication is a surface water problem that has reached widespread proportions (*2*). Although there is some argument about which nutrients are the causative agents in eutrophication (*3*), many ecologists, limnologists, and environmental engineers agree that control and elimination of sources of phosphorus can control the nuisance blue-green algae blooms which often accompany eutrophic conditions in a lake. In general, only 50 percent or less of the total phosphate in lakes enters through domestic waste water (*4*); this source is, however, the one now receiving most attention.

Present-day domestic waste water contains about 10 mg of total phosphorus per liter, and one-half to two-thirds of this amount is from detergent phosphates (*5*). Thus, even with the elimination of detergent phosphates, about 3 to 4 mg of phosphorus per liter would remain in effluents from domestic waste water treatment plants, since conventional treatment generally does not remove much phosphate. Therefore, the real question is: Would a 50 to 60 percent reduction in phosphorus concentrations in domestic waste water significantly reduce the eutrophic conditions in our lakes?

I here report some direct experimental evidence indicating that the elimination of phosphates from detergents would make no significant improvement in eutrophic conditions in the lake receiving the resulting waste water effluent. The microcosm algal assay procedure developed by Mitchell and Buzzell (*6*) was used. The ecological significance of the various waste waters tested was assessed in terms of the resulting algal diversity calculated as Shannon's diversity index (*7*)

$$H_1 = -\Sigma P_i \log P_i$$

where $P_i = n_i/N$, n_i is the population of the ith species, and N is the population of the total community. The index ranges from zero for unialgal populations to unity for very diverse algal communities.

In general, the diversity of a lake's algal community diminishes with eutrophication (*8*). Thus, oligotrophic lakes would probably have diversity indices of from 0.7 to 1.0, and, as the lakes become eutrophic, the diversity index would drop to 0.3 or less.

Three detergent formulations were chosen for this study from store shelves to give a wide range of composition (Table 1). Product 1 was a phosphate-containing anionic surfactant formulation. The other two detergents were phosphate-free formulations, with nonionic and anionic surfactants. Both products 2 and 3 have been extensively advertised as "ecologically safe" for the environment.

A synthetic waste water was prepared whose composition was based on the glucose-peptone waste water designed by Wiener (*9*) to model domestic waste water in activated sludge treatment, but with the inclusion of a bicarbonate buffer in place of the phosphate buffer. A stock solution of the synthetic waste water was made up to 1 liter deionized water as follows: 16.0 g of glucose, 16.5 g of peptone, 2.5 g of urea, and 10.0 g of sodium bicarbonate for buffering. The syn-

Table 1. Composition and properties of detergents.

Characteristics	Detergent		
	1	2	3
*Ingredients (% by weight)**			
Anionic surfactant	18		5
Nonionic surfactant	2	11	2
Sodium tripolyphosphate	50		
Sodium carbonate		65	21
Sodium silicate solids (from liquids)	6	8	
Sodium metasilicate pentahydrate			21
Sodium chloride			45
Sodium sulfate	14		4
Sodium carboxymethyl cellulose (65% pure)	<1	5	1
Water	10	10	
Brighteners, perfume, etc. (estimated)	<1	<1	<1
Properties			
Loose density (g/cm³)	0.33	1.04	0.85
Solution *p*H in the concentration used	9.7	10.8	11.3
Alkalinity (% Na_2O)†	9.6	42	19
Recommended amount (cups)	1¼	½	1
Grams per wash load	98	123	201
Solution concentration for the recommended amount of detergent (% by weight)	0.15	0.19	0.31

* Values are based on analyses of purchased samples carried out in the Monsanto detergent laboratory. † Titration to *p*H 4.

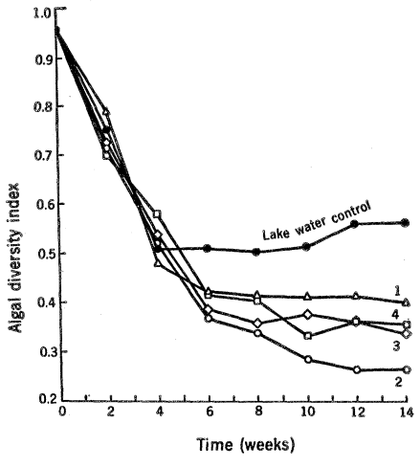

Fig. 1. Diversity indices of microcosms receiving 10 percent treated waste water: unit 1 from phosphate-containing detergent; unit 2 from nonphosphate nonionic detergent; unit 3 from nonphosphate anionic detergent; unit 4 from waste water with no detergent.

thetic waste water was prepared by diluting 10 ml of the stock solution to 1.0 liter with tap water. The final concentration of phosphorus was about 3 mg/liter (from the peptone), corresponding to domestic waste water without detergent phosphates. A synthetic waste water of this type may be more readily biodegradable than domestic waste water and hence may yield more inorganic nutrients in the treated effluent. However, the data in Table 2 do not support this expectation. This conclusion is based on the results of an experiment in which four activated sludge units (1.5 liters of total mixed liquor volume) were fed the synthetic waste water, with or without the detergents, on the 24-hour semicontinuous operation schedule recommended in the Soap and Detergent Association test procedure (*10*). The detergent concentrations (Table 2) were selected to approximate the concentration of conventional household detergents in the average domestic waste water and were based upon the amounts recommended for use by the manufacturers (Table 1).

Table 2 shows analyses of the daily effluents from the activated sludge treatment. Both unit 1, treating the phosphate-containing detergent, and unit 4, the control with no detergent, had an average soluble inorganic carbon (SIC) content of about 9 mg/liter and an average *p*H of 7.4. In contrast, the effluents of units 2 and 3, treating the detergents without phosphate, contained almost twice as much SIC as the treated waste water and had *p*H's about 0.5 higher. The titratable alkalinity of the four effluents showed this same trend. All the effluents had approximately the same soluble organic carbon (SOC) content, 5 to 6 mg/liter, an indication that none of the detergents hampered the biodegradation of organic carbon compounds by the activated sludge microorganisms.

Predictably there were no significant differences in the amounts of ammonia and nitrate nitrogen in the effluents, and there was no significant difference between the effluent phosphate concentrations of unit 2 and that of the control unit. As expected, the effluent from unit 1 contained about 2 mg more phosphate per liter than the control unit.

Effluent (600 ml settled but not centrifuged) from each of these activated sludge units was added in duplicate to laboratory microcosms prepared from mud (0.5 liter) and water (5.4 liters) taken from Table Rock Lake, an oligotrophic impoundment in southwestern Missouri. The initial characteristics of the lake water are given in Table 3. To maintain natural diversity 250 ml of the lake water was withdrawn and replaced with 25 ml of the respective effluent and 225 ml of fresh lake water, with its natural algal community, every 2 weeks. The control microcosms were made up with 0.5 liter of mud and 6 liters of lake water and were replenished with 250 ml of lake water.

The diversity indices of the microcosms are shown in Fig. 1. Each point represents the mean of the two duplicates. The difference between the two values was never more than 0.2 and was usually less than 0.1. The lake water controls developed rather stable algal communities with diversity indices between 0.5 and 0.6. This is below the 0.7 value for oligotrophic lakes but well above the 0.4 value for eutrophic lakes. The fact that the microcosm algal community was not as diverse as the lake community can be attributed to the differences between conditions in the laboratory and those in the natural environment. The diversity indices of microcosms receiving the activated sludge effluents were considerably lower than that of the lake water control (98 percent confidence level, *t*-test). The effluents from activated sludge units 2 and 3 caused even lower

Table 3. Characteristics of Table Rock Lake water.

Characteristics	Initial value
*p*H	7.4
Soluble inorganic carbon (mg of C per liter)	12
Soluble organic carbon (mg of C per liter)	< 2
PO_4 (μg of P per liter)	0
Total P (μg of P per liter)	< 10
NO_2 (μg of N per liter)	0
NO_3 (μg of N per liter)	50
NH_3 (μg of N per liter)	0
Total algae (cell/ml)	199
Algal genera	17
Diversity index	0.93

Table 2. Activated sludge treatment of detergents.

Characteristics	Activated sludge unit			
	1	2	3	4
	Influent			
Detergent formulation	1	2	3	Control
Detergent added (mg/liter)*	60	87.5	146	None
SIC (mg/liter)†	11	16	15	10
SOC (mg/liter)†	95	108	96	90
	Effluent			
SIC, average (mg/liter)‡	9.5	18.8	17.2	8.7
SIC, difference (mg/liter) §	0.7 ± 0.8	10.2 ± 0.9	8.5 ± 1.0	
SOC, average (mg/liter)	6.4	4.9	5.5	5.2
SOC, difference (mg/liter)	1.3 ± 0.8	− 0.2 ± 0.7	0.2 ± 0.9	
NH_3, average (mg of N per liter)	1.2	1.2		1.2
NO_3, average (mg of N per liter)	9.5	11.0		10.0
NO_3, difference (mg/liter)	0.8 ± 1.3	0.8 ± 1.0		
PO_4, average (mg of P per liter)	2.8	0.5		0.7
PO_4, difference (mg/liter)	2.1 ± 0.5	0.2 ± 0.2		
*p*H, average	7.4	7.8	7.9	7.3
*p*H, difference	0 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	
Alkalinity (meq/liter) ||				
To *p*H 7	0.17	0.40	0.28	0.20
Total	0.91	2.00	1.51	0.75

* Based on the amounts recommended for use by the manufacturer. † Soluble inorganic carbon or soluble organic carbon in centrifuged samples (Beckman carbon analyzer model 915). ‡ Averages based on 19 to 20 samples over a 12-week period. § Differences from the control were calculated individually for each sampling, averaged ± 2 standard deviations from the arithmetic mean. || Amount of acid needed to titrate a sample to *p*H 7 or to the breakpoint near *p*H 4.8 to 5.

diversities than the effluents from unit 1, but the differences are significant only at the 95 percent confidence level.

All the waste water microcosms developed eutrophic conditions, with growths of blue-green algae well in excess of 1000 cell/ml, predominantly *Phormidium* accompanied by two to three other algal genera. The concentrations of algae in the lake water controls remained below 500 cell/ml with the green algae *Staurastrum* and *Ankistrodesmus* often predominating, accompanied by 7 to 12 other species.

On the basis of these results, it is evident that domestic waste water will produce eutrophic conditions in receiving waters. However, the data are not in support of the often stated position that the simple elimination of the phosphates from detergents will significantly decrease the rate of eutrophication caused by the resulting waste waters. Furthermore, the data show that the "ecologically safe," high-alkalinity, high-carbonate detergents offer no improvement.

Substitution of untested detergent formulations of this sort may appear to be an easy way out politically, but there is no indication that this technique will reduce eutrophication. Eutrophication may actually increase as the result of additional alkalinity, which would be still another factor added to our overall pollution problem. A much more effective idea would be the construction of facilities for the removal of all nutrients from waste waters in those areas where algal control is a problem.

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Asymmetry, Its Importance to the Action and Metabolism of Abscisic Acid

Abstract. *Unlabeled and ^{14}C-labeled enantiomorphs of abscisic acid (ABA) were obtained through acetylcellulose chromatography and tested as inducers of abscission, as inhibitors of seed germination, and as antagonists of gibberellic acid-induced synthesis and release of α-amylase. The activity of the* **R** *isomer was either equal to or less than that of the naturally occurring* **S** *form. Greatest differences were in the inhibition of root-related growth. In excised beam axes, although uptake of* **S**-[^{14}C]*ABA is faster, the internal concentration of* **R**-*ABA is higher because of faster conversion of* **S**-*ABA to inactive metabolic products. In axes a reversal in chirality is less important to the physiological action of ABA than to its metabolism.*

Abscisic acid (ABA) has been implicated in the regulation of a host of physiological responses in higher plants (*1*). Apparently the **S** isomer is the only naturally occurring form, but the other enantiomorph can be obtained by resolution of the synthetic racemate (*2*). This makes ABA the only optically active plant hormone for which both enantiomorphs are available. The only record of the relative effectiveness of these enantiomorphs consists of a statement by Milborrow to the effect that **R**- and **S**-ABA are equally active in inhibiting the coleoptile growth of dissected wheat embryos (*3*). We report a new procedure for the resolution of **RS**-ABA, the synthesis of **R**-[2-^{14}C]- and **S**-[2-^{14}C]ABA, and data on the relative effectiveness, uptake, and metabolism of these optically active substances.

RS-ABA can be separated from the accompanying *trans* analog obtained in the synthesis of Roberts *et al.* (*4*) by crystallization from ethyl acetate–toluene (1:3). Resolution is achieved in two steps; enantiomorphic enrichment on an optically active chromatographic column and selective solubilization of the optically active component with a hydrocarbon solvent containing a low percentage of a hydrogen-bonding compound (*5*). Chromatography of 80 mg of **RS**-ABA on a column of acetylcellulose (Woehlm) and elution with 3.3 percent ethanol in toluene in subdued light at 4°C leads to about 5 percent enantiomorphic enrichment. The **S** isomer is eluted first. Extraction of the combined, enriched fractions from several runs with 2.5 percent *n*-butanol in isooctane (by volume), thin-layer chromatography on silica gel GF_{254} with a benzene, ethyl acetate, acetic acid system (50:5:2), to remove accompanying *trans*-ABA, yields crystalline **S**- and **R**-ABA of optical purity comparable to that obtained by Cornforth *et al.* (*2*).

S-[2-^{14}C]ABA and **R**-[2-^{14}C]ABA were synthesized by the same procedure as that used by us for **RS**-[2-^{14}C]-ABA (*6*). **RS**-1-Hydroxy-4-keto-α-ionone (*4*) was partially resolved on acetylcellulose columns with 3 percent ethanol in toluene at 4°C, yielding enriched **S** isomer in the material eluted first. Extraction with 7 percent *n*-butanol in hexane (by volume) yielded oils that

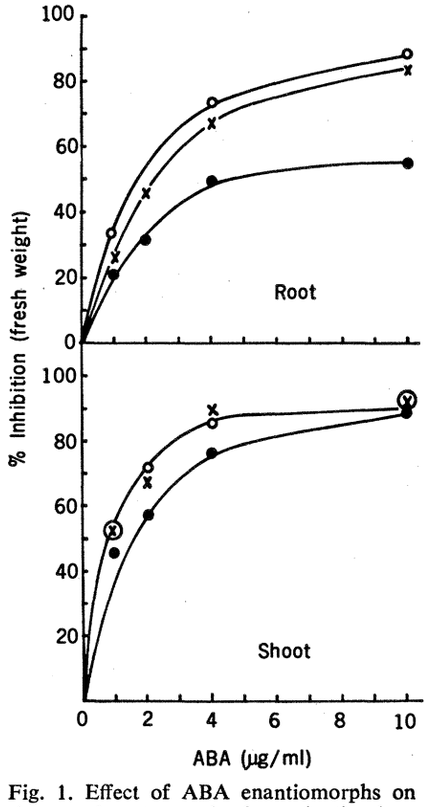

Fig. 1. Effect of ABA enantiomorphs on shoot and root growth of germinating barley seeds, *Hordeum vulgare* (var. Himalaya). The seeds were incubated in the dark at 26° to 27°C for 56 hours in 0.01*M* Hepes buffer *p*H 7.1 with 50 μg of chloramphenicol per milliliter. An average of 20 seeds was used per assay, and germination was close to 100 percent even in the presence of ABA. Shoots and roots were removed from the seeds, and their fresh weight was determined. ○, **S**-ABA; **X**, **RS**-ABA; ●, **R**-ABA.