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# Effect of Retorted-Oil Shale Leachate on a Blue-Green Alga (*Anabaena flos-aquae*)

Diane M. McKnight, Wilfred E. Pereira, Colleen E. Rostad, and Eric A. Stiles

*U.S. Geological Survey, Box 25046, MS 407, Federal Center, Denver, CO 80225*

In the event of the development of the large oil shale reserves of Colorado, Utah, and Wyoming, one of the main environmental concerns will be disposal of retorted-oil shale which will be generated in greater volume than the original volume of the mined oil shale. Investigators have found that leachates of retorted-oil shale are alkaline and have large concentrations of dissolved solids, molybdenum, boron, and fluoride (STOLLENWERK & RUNNELS 1981). STOLLENWERK & RUNNELS (1981) concluded that drainage from waste shale piles could have deleterious effects on the water quality of streams in northwestern Colorado.

In response to these possible water-quality effects, several pre-development studies have been conducted, describing the benthic macro-invertebrates and periphyton in Piceance Creek and the White River, which drain an area containing oil-shale reserves in northwestern Colorado (EVERHART & MAY 1973; ASHLAND OIL, INC. 1976; PENNAK 1974; U.S. Geological Survey, unpublished data). There were significant differences among these studies in species identifications and abundances (ERMAN 1981).

The approach in this study is to simulate the possible effects of leaching retorted-shale piles in laboratory bioassay experiments. We evaluated the effects of retorted-shale leachates on *Anabaena flos-aquae*, a common blue-green alga. CLEAVE et al. (1980) studied the effect of leachate from retorted shale produced by Paraho* and Union retorting processes on the growth of a green alga, *Scenedesmus*, a species indigenous to Lake Powell. They reported that addition of high concentrations (20%) of these leachates to culture medium decreased the growth of *Scenedesmus*, and that this effect could be attributed to the large concentration of dissolved solids. They suggested that an increase in concentration of dissolved solids could shift the phytoplankton species distribution towards dominance by blue-green algae over green algae and diatoms in Lake Powell. Blue-green algae also can be important soil microflora. STARKS & SHUBERT (1982) studied the succession of soil algae in an area regraded with saline and neutral overburden after surface mining. They found that several species of blue-green algae colonized the overburden, indicating an ability to tolerate the high concentrations of Ca, Na, and K. Their results indicate that blue-green algae also may become important in alkaline soils associated with the disposal of spent oil shale.

*Any use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey

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## METHODS

### Retorted-shale leachates

Retorted-oil shales from two different retorting processes were studied. Tosco II retorted shale is produced as a fine powder, whereas Paraho retorted shale varies in size and was ground to a fine powder in the laboratory. Leachates of Tosco II and Paraho retorted-oil shales were obtained in the following manner. Five hundred mL of distilled water were placed in a reservoir above a glass column (2.5-cm diameter) containing 100 g of shale retained by a 2.5-cm glass wool plug below and a 1.3-cm plug above the shale. The shale was leached for 9 days. The flow rate was greater through the Tosco II shale than through the Paraho shale and decreased with time in both columns; resulting in 350 mL of Tosco II leachate compared to 275 mL of Paraho leachate. The concentration of cations and trace metals in the leachates was measured by inductive-coupled plasma spectrometry (Table 1). Organic compounds in Tosco II and Paraho retorted-shale leachate were analyzed previously by PEREIRA & ROSTAD (unpublished data, 1982) by gas chromatography/mass spectrometry (Table 2). Synthetic-leachate solutions were prepared to reproduce the concentrations of major cations and anions.

TABLE 1. Composition of retorted-shale leachates and synthetic spent-shale leachates used in bioassay experiments [ISL = inorganic synthetic leachate; -- = not added; ND = not detected; NM = not analyzed]

| | Tosco II | | Paraho | | Piceance Creek[a] |
|---|---|---|---|---|---|
| | Leachate | ISL | Leachate | ISL | |
| pH | 8.0 | 8.2 | 12.0 | 11.8 | 8.2 |
| Cations (mg/L)[b] | | | | | |
| Na | 620 | 620 | 643 | 643 | 110 |
| Ca | 43.7 | 45 | 775 | 775 | 67 |
| Mg | 45.1 | 45 | 0.11 | -- | 42 |
| K | NM | -- | NM | -- | 3.4 |
| Sr | 0.63 | -- | 13.7 | -- | -- |
| Trace metals (µg/L) | | | | | |
| Mo | 1,920 | 19,600 | 2,840 | 19,600 | 12 |
| Fe | 95 | -- | ND | -- | 16 |
| Pb | 44 | -- | 16 | -- | < 1 |
| Cd | 10 | -- | 6 | -- | < 1 |
| Cu | 9 | -- | ND | -- | 2 |
| Zn | 8 | -- | ND | -- | < 3 |

[a]Average values of monthly data collected by U.S. Geological Survey (1980) from Piceance Creek below Rio Blanco, Colorado.

[b]No analysis was performed for K. STOLLENWERK & RUNNELS (1981) report K to have a lower concentration than Na, Ca, and Mg in Tosco II leachate, and a lower concentration than Na in Paraho leachate.

For the Tosco II synthetic leachate, Na, Ca, and Mg were added as sulfate salts, and the pH adjusted to pH 8.2 with 1N $H_2SO_4$ and 0.1N NaOH; $Na_2SO_4$ and $CaCl_2$ were added to the Paraho synthetic leachate, and the pH adjusted to 11.8 with 1N NaOH. For both synthetic leachates, the concentration of Mo was greater than the concentration in the actual leachates but similar to concentrations in leachates studied by STOLLENWERK & RUNNELS (1981). Molybdenum was added as ammonium molybdate because ammonium is readily assimilated by blue-green alga as a nitrogen source. Benzoic acids were added to cultures from a 30.5-mg/L solution of benzoic acid and 25-mg/L solution of a 3,5-dimethyl benzoic acid in distilled water. Benzoic acid and 3,5-dimethyl benzoic acid were found to be the most concentrated organic compounds in both spent-shale leachates (PEREIRA & ROSTAD, unpublished data, 1982). Naphthoic acids were added from a 20.5-mg/L solution of 2-naphthalenecarboxylic acid and a 10-mg/L solution of 1-naphthalenecarboxylic acid. These naphthoic acids were found in trace concentrations in Tosco II leachate (PEREIRA & ROSTAD, unpublished data, 1982).

TABLE 2. Organic acids in retorted-shale leachates
[ND = not detected]

| Organic acids (μg/L) | Tosco II | Parahoe |
|---|---|---|
| Benzoic acid | ND | 450 |
| 3,5-dimethylbenzoic acid | 540 | 7.0 |
| 1-naphthalene-carboxylic acid | 10 | ND |
| 2-naphthalene-carboxylic acid | 20.5 | ND |

## Culture of *Anabaena flos-aquae*

*A. flos-aquae* UTEX 1944 cultures were grown at 22°C in a water bath under continuous light in WC medium (GUILLARD 1975) amended with retorted-shale leachate or synthetic-leachate solutions, to a total volume of 250 mL in 500-mL polycarbonate flasks. In Experiment I, the concentrations of added retorted-shale leachate were 40% (100 mL leachate added to 150 mL WC medium), 8% (20 mL leachate added to 230 mL WC medium), and 0.4% (1 mL leachate added to 250 mL WC medium). The synthetic leachate solutions (Experiments II, III, and IV) were added to reproduce those concentrations of retorted-shale leachate. Control cultures were grown in WC medium with no addition of leachate or synthetic-leachate solution. Duplicate flasks were prepared for controls and for each concentration of shale leachate or synthetic-leachate solution. After addition of shale leachate, the flasks were inoculated from an *A. flos-aquae* culture exhibiting exponential growth (5 mL inocula in Experiments I and II and 2.5 mL inocula in Experiments III and IV resulting in similar initial chlorophyll *a* concentrations for all four experiments). The chlorophyll *a* concentrations in the cultures were determined by filtering aliquots through GFC glass-fiber filters, grinding and extracting the filters in 90% acetone, and measuring fluorescence with a Turner 110 fluorometer (STRICKLAND & PARSONS 1972). Cultures also were examined microscopically to observe any morphological effects. The cultures were sampled daily at the beginning of the experiments, and every other day after the cultures had reached stationary growth phase. Growth rate during exponential growth was computed by linear regression to the equation $\mu t = \ln [N(t)/N_0]$, where N is the chlorophyll *a* concentration as a function of time (GUILLARD 1975). The concentration of C-phycocyanin was determined by filtering 10 mL of culture on a GFC glass-fiber filter, and grinding and extracting the filter in cold phosphate buffer. Spectra were recorded with a Hewlett-Packard 8450-A UV-visible spectrophotometer; fluorescence was measured with an excitation at 610 nm; and emission scan from 600 to 800 nm with an Aminco-Bowman spectrofluorometer. The emission peak corresponding to C-phycocyanin occurred at 645 nm (CHAPMAN 1973).

## RESULTS AND DISCUSSION

### Experiment I.--Retorted-shale leachates

The compositions of the retorted-shale leachates used in the bioassay experiment are presented in Table 1. Both leachates are enriched in dissolved solids compared to typical freshwater in the oil-shale region. The Paraho leachate has a greater pH and greater Ca concentration than the Tosco II leachate. These differences probably result from partial decomposition of carbonate minerals into oxides at the higher temperatures of the Paraho retorting process and then hydrolysis of oxides during leaching (STOLLENWERK & RUNNELS 1981). Also, the concentrations of Mo ($\approx$2000-3000 (g/L) are much greater than in typical freshwater.

Even at the greatest concentration of Tosco II leachate, the growth rate of *A. flos-aquae*, as determined from chlorophyl *a* concentrations, was comparable to the growth rate in the control cultures; the final biomass was 60% of that in the control cultures (Table 3). These 40% Tosco II-leachate cultures were, however, very yellow in comparison to the control. The spectra of phosphate buffer extracts of the control and 40% Tosco II-leachate cultures are shown in Figure 1. The significance of these spectra is that there is a major peak at 616 nm in the control-culture spectrum and only a slight inflection in the Tosco II culture spectrum. This absorbance peak at 616 nm corresponds to C-phycocyanin, a biliprotein. Biliproteins usually represent 1 to 10% of the dry weight of blue-green algae; they are the principal pigments of the photosynthetic lamellar system, and therefore have a masking effect on the chlorophylls and carotenoids, giving blue-green algae their characteristic bluish color (CHAPMAN 1973). The absorbance at 616 nm in the spectrum for the control culture corresponds to a C-phycocyanin concentration of 0.82 mg/L. Analysis by fluorescence showed that the concentration of C-phycocyanin was 3.4 times greater in the control culture than in the 40% Tosco II culture. This decrease in biliprotein content caused by the Tosco II leachate represents a major shift in the photosynthetic system of *A. flos-aquae*. Microscopic examinations

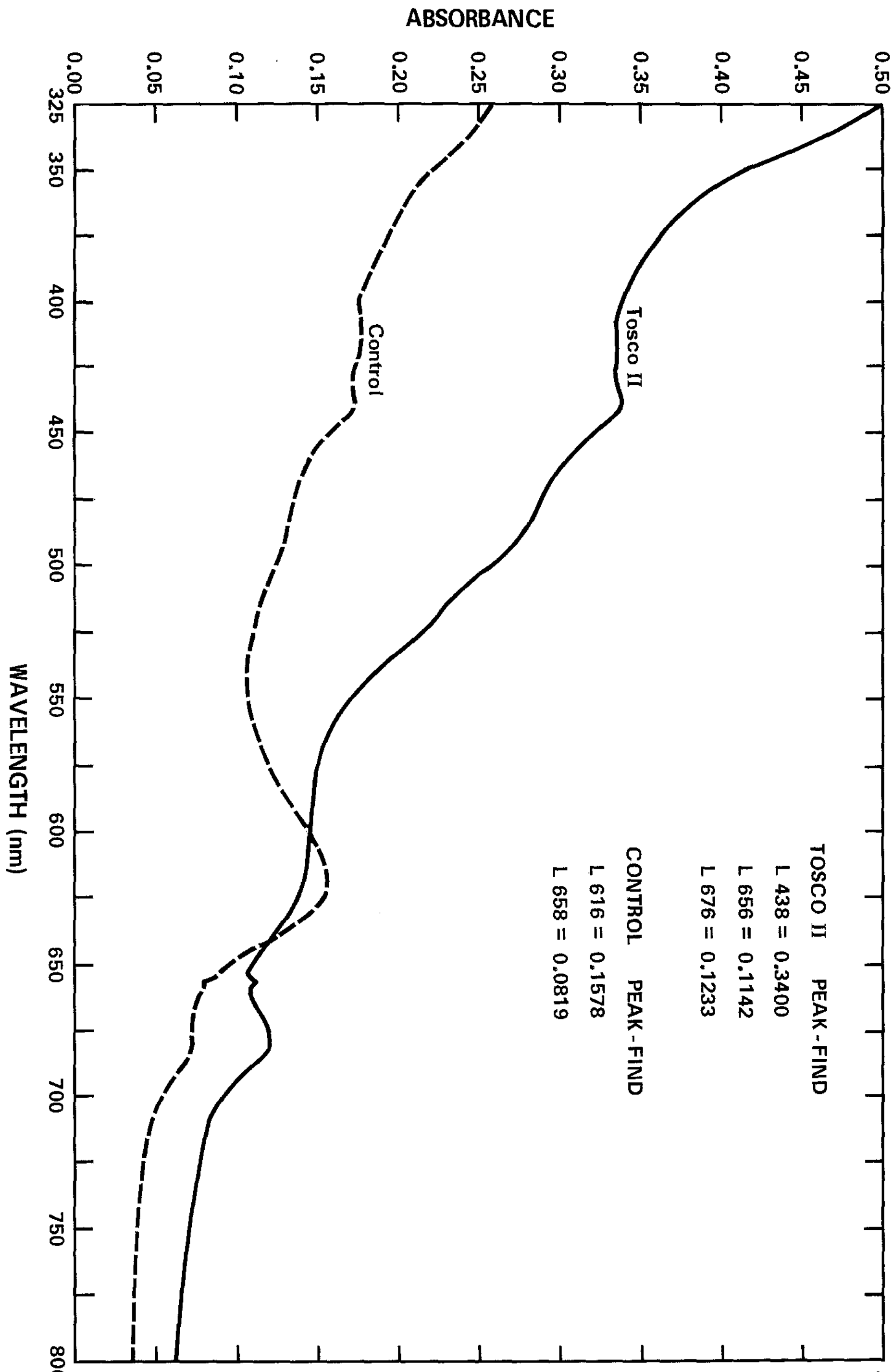


Fig. 1. Effect of Tosco II retorted-shale leachate on the spectra of photosynthetic pigments extracted in cold phosphate buffer.

(accomplished using a Nikon LabPhot microscope at 1,500 total magnification with phase contrast) of the *Anabaena* trichomes showed no visible morphological changes in the 40% Tosco II culture.

TABLE 3. Growth of *Anabaena flos-aquae* in culture media with added retorted-shale leachate
[Reported data are the mean-values for duplicate cultures]

| Culture | Growth rate* ($day^{-1}$) | Chlorophyl *a* concentration in stationary phase (mg/L) |
|---|---|---|
| Control | 0.45 | 1.37 |
| Tosco II retorted-shale leachate (percent) | | |
| 40 | 0.48 | 0.80 |
| 8 | 0.54 | 1.36 |
| 0.4 | 0.48 | 1.34 |
| Paraho retorted-shale leachate (percent) | | |
| 40 | No growth | No growth |
| 8 | growth after lag phase | 1.42 |
| 0.4 | 0.52 | 1.81 |

*Computed by a linear regression to the equation $\mu t = \ln[N(t)/N_0]$ for first 6 days, where $\mu$ = growth rate, t = time, N = chlorophyll *a* concentration, and $N_0$ = initial chlorophyll *a* concentration.

The intermediate concentration of Tosco II leachate had a slight stimulatory effect on the growth rate, and had no effect on the final biomass. This culture also had a slight yellow color probably caused by a decrease in the C-phycocyanin content. The least concentration of Tosco II leachate had no significant effect on the growth of *A. flos-aquae*.

The decreased growth of *A. flos-aquae* in culture medium amended with Paraho retorted-shale leachate is shown in Figure 2. There was no growth at a concentration of 40% Paraho leachate. Within a day after addition of the Paraho leachate, a white-floc precipitate was observed, which, on analysis, proved to be mainly $CaCO_3$. The $CaCO_3$ precipitation may have prevented growth by coating the *Anabaena* cells in the inoculum. There was a smaller quantity of precipitate in the 8% Paraho cultures; these cultures eventually grew after a lag phase (4 and 6 days). The 8% Paraho culture with the shorter lag phase reached the same final biomass as the control cultures. Again, the lag phase was probably caused by $CaCO_3$ precipitation.

The least addition of Paraho leachate (0.4%) caused a small but significant increase in both the growth rate and the final biomass of *A. flos-aquae*. The growth enhancement may have been caused by the increase in major cation concentration (from 13 to 16 mg/L of Ca) or of some micronutrient.

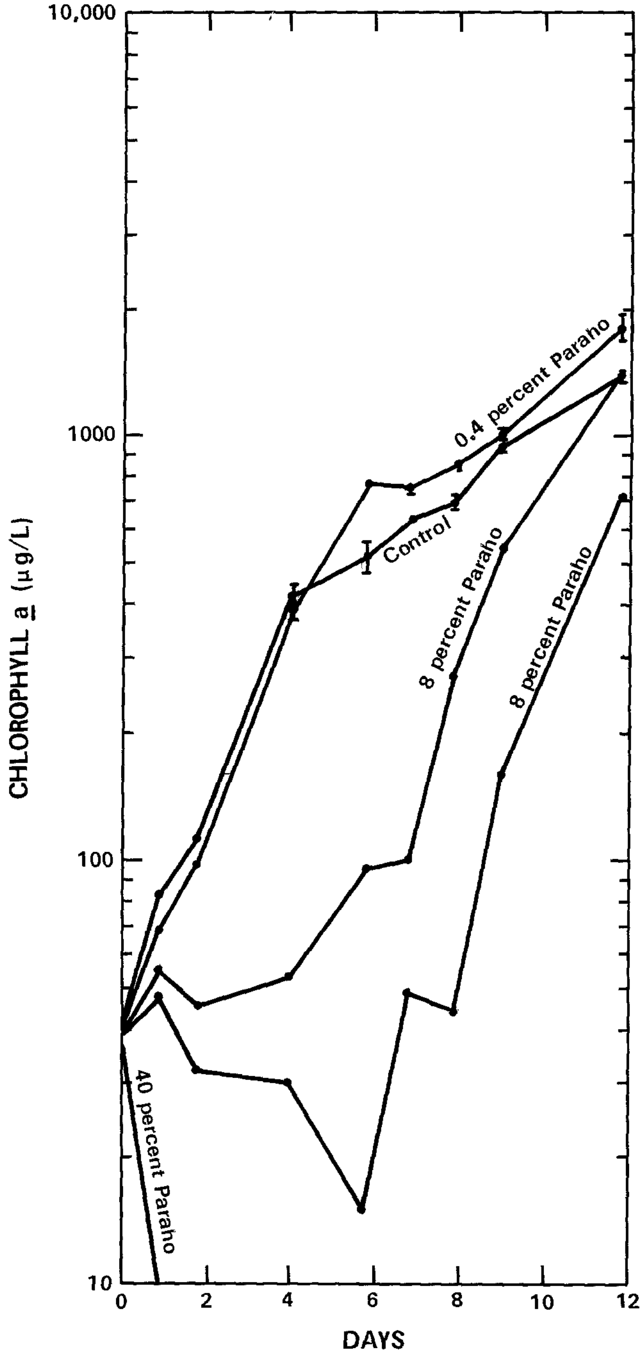


Fig. 2. Effect of Paraho retorted-shale leachate on the growth of *Anabaena flos-aquae*. The range and mean chlorophyll *a* concentrations for duplicate cultures are shown except for the cultures with 8% Paraho leachate.

Experiment II.--Synthetic spent-shale leachates

Salt solutions were prepared to reproduce the major cations in the Tosco II and Paraho retorted-shale leachates (Table 1). Molybdenum also was added at a concentration of 19,600 µg/L; which is greater than in either the Tosco II or Paraho leachates of this study, but comparable to concentration of Mo reported by STOLLENWERK & RUNNELS (1981) in retorted-shale leachates. Cultures also were grown with only the Mo addition.

Both the 40% and the 8% synthetic Tosco II addition increased the growth rate of *A. flos-aquae* (Table 4). However, on comparison with the cultures with added ammonium molybdate, it appears that the increased growth is mostly due to the ammonium molybdate. Blue-green algae as well as other algae assimilate ammonium more readily than nitrate, (WARD & WETZEL 1980) the nitrogen source in the WC culture medium; and the ammonium probably is responsible for the growth-rate increase.

TABLE 4. Growth of *Anabaena flos-aquae* in culture media with added synthetic retorted-shale leachate solutions

[Reported data are mean values for duplicate cultures]

| Culture | Growth rate* ($day^{-1}$) | Chlorophyl *a* concentration in stationary phase (mg/L) |
|---|---|---|
| Control | 0.60 | 1.35 |
| Tosco II synthetic leachate (percent) | | |
| 40 | 0.68 | 1.66 |
| 8 | 0.67 | 0.88 |
| Paraho synthetic leachate (percent) | | |
| 40 | no growth | no growth |
| 8 | 0.74 | 1.38 |
| Ammonium molybdate (19,600 mg/L) | 0.65 | 0.97 |

*Computed by a linear regression to the equation $\mu t = \ln[N(t)/N_0]$ for first 6 days.

The cultures with the added synthetic Tosco II leachate did not appear yellow, as did the cultures with the actual Tosco II leachate added. This observation was verified by measuring the C-phycocyanin concentration in the control and 40% synthetic Tosco II cultures. The spectra of the phosphate-buffer extracts were similar, both having a peak at 616 nm corresponding to about 0.45 mg/L of C-phycocyanin and fluorometric analysis confirmed this result. Thus, we concluded that the decrease in the biliprotein content of *A. flos-aquae* caused by high concentrations of Tosco II leachate is not a result of the increased concentration of major cations and anions or of Mo.

The high concentration of the synthetic Paraho leachate had the same effect as the high concentration of the actual Paraho leachate. There was precipitation of $CaCO_3$ and no growth. Five days after the addition of the synthetic-Paraho leachate, the precipitate was removed by filtration and the culture medium reinoculated with *A. flos-aquae*. These cultures grew at a rate that was similar (0.60 $day^{-1}$) to that of control cultures. We conclude that the inhibitory effect of high concentrations of Paraho leachate is due to the precipitation of $CaCO_3$ and that, once the $CaCO_3$ has precipitated, the remaining dissolved inorganic constituents in the Paraho leachate are not inhibitory.

There was no observable precipitation in the 8% synthetic Paraho culture. In fact, these cultures grew significantly faster than the other cultures. Although ammonia may have contributed to some of the growth stimulation, changes in the major cations also may have been important.

## Experiment III.--Benzoic acids

PEREIRA & ROSTAD (unpublished data, 1982) found that the two most concentrated organic acids in the retorted-shale leachate were benzoic acid and 3,5-dimethylbenzoic acid. The concentrations of these organic acids in the cultures from addition of the leachates (Table 2) were less than those used to preserve foods, but were possible causes for some of the effects of the spent-shale leachates not attributable to the inorganic constituents tested. The benzoic acids were added in concentrations corresponding to the 40, 8, and 0.4% retorted-shale leachates; 0.5, 0.06, and 0.006 mg/L for benzoic acid; and 0.5, 0.05, and 0.006 mg/L for 3,5-dimethylbenzoic acid. However, no significant effect on growth was observed even at the greatest concentrations. The growth rates were between 0.53 and 0.51 $day^{-1}$ in cultures with added benzoic acids, compared with 0.52 $day^{-1}$ in the control cultures. Further, there was no visible change in color or morphology of the algae. The conclusion from this experiment is that the growth effects such as the decrease in C-phycocyanin that could not be attributed to the major salts in retorted-shale leachates are likewise not attributable to the two most concentrated organic acids.

## Experiment IV.--Naphthoic acids

Naphthoic acids are known to be plant auxins that stimulate growth at low concentrations and inhibit growth at high concentrations (BUTA & STEFFENS 1974). Naphthoic acids were not found in Paraho leachate, but were found at low concentrations in Tosco II leachate and were possible causes of the decrease in C-phycocyanin. Naphthoic acids were added at concentrations of 0.056, 0.011, and 0.002 mg/L for 2-naphthalenecarboxylic acid and of 0.028, 0.006 and 0.001 mg/L for 1-naphthalenecarboxylic acid. Again, no significant effect on growth, color, or morphology was observed. The growth rates were between 0.41 and 0.44 $day^{-1}$ in cultures with added naphthoic acids, compared with 0.44 $day^{-1}$ in control cultures. We conclude that the decrease in C-phycocyanin may have been caused by other trace organic compounds or a combination of constituents in the Tosco II leachate.

## SUMMARY AND CONCULSIONS

The bioassay experiments reported here were designed to help in the evaluation of the potential environmental effects of development of the oil shale reserves in Colorado, Wyoming, and Utah. STOLLENWERK & RUNNELS (1981) had warned that the water quality of Piceance Creek and the White River may be adversely impacted by increased concentrations of dissolved solids, Mo, B, and F. CLEAVE et al. (1980) found that growth of a green alga from Lake Powell was inhibited by 20% concentrations of Paraho retorted-shale leachate; they speculated that blue-green algae may be favored by addition of leachates from waste-shale piles. We have found that retorted-shale leachates have major effects on the growth of a common blue-green alga at concentrations of 40%; the Paraho retorted-shale leachate inhibits growth, and the Tosco II retorted-shale leachate suppresses the cellular content of C-phycocyanin, a biliprotein important in the photosynthetic lamellar system. At concentrations of 8%, the leachates have minor effects; the Paraho leachate induces a lag phase and the Tosco II leachate increases the growth rate. At a concentration of 0.4%, the Paraho leachate increases growth and the Tosco II leachate has no effect. The inhibitory effect of the Paraho leachate at the greatest concentration was shown to result from precipitation of $CaCO_3$. After $CaCO_3$ precipitation, 40% Paraho leachate probably no longer affects growth. However, the other growth effects, including the decrease in C-phycocyanin, were not attributable to either changes in the concentration of major cations or Mo, or exposure to benzoic acids or naphthoic acids in the retorted-shale leachates. The decrease in C-phycocyanin may be caused by other trace organic compounds or a combination of several constituents.

These results have implications in terms of potential changes in blue-green algal populations from leaching of retorted-shale piles. First, where the leachate is significantly diluted (1% or less) by freshwater, no effects can be anticipated. The concentrations of chemical constituents leached from retorted-shale are likely to be very low in large water bodies such as Lake Powell or the Colorado River; therefore, it is unlikely that development of oil shale reserves will favor blue-green algae if they are not presently important phytoplankters in these surface waters.

In the immediate vicinity of the retorted-shale piles, leachate may alter blue-green algal populations both in streams and in soils. Variable environmental conditions will control leaching mechanisms in the retorted-shale piles and may result in concentrations of leached constituents much different from those in the laboratory leachates. Although environmental factors (light, temperature, nutrient availability, etc.) which constrain natural blue-green algal populations in the immediate vicinity of spent-shale piles are much different than those controlling the laboratory *Anabaena* cultures, some of the effects observed in laboratory experiments may occur. The decrease in biliprotein content caused by Tosco II leachate may decrease the ecological fitness of blue-green algal populations, and lead to their replacement by algae whose photosynthetic systems are not affected by the leachate. Although precipitation of $CaCO_3$ was dependent on the chemistry of the Paraho leachate and the culture medium used in these bioassay experiments, it is likely that $CaCO_3$ also will precipitate at some point in the mixing of surface waters with waters draining Paraho retorted-shale. Where $CaCO_3$ precipitation occurs, the water-quality problems probably will be similar to those from the precipitation of hydrous iron oxides in acid mine drainage. Also, algal species favored by $CaCO_3$ deposition may be abundant as in travertine-and marl-depositing environments (WETZEL 1960). Downstream from the reach of stream or area of soil where the $CaCO_3$ is precipitated the blue-green algal populations, and other microbiota, may not be altered if the chemistry of the water after $CaCO_3$ precipitation is not much different from the normal water chemistry.

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