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Bulletin 72

Water Resources Research in Virginia—  
Annual Report for Fiscal Year 1972





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Water Resources Research in Virginia—  
Annual Report for Fiscal Year 1972

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Blacksburg, Virginia 24061



## PREFACE

The Water Resources Act of 1964, Public Law 88-379, and subsequent amendments authorized the establishment of State Water Resources Research Institutes or Centers in each of the 50 states and some territories. The purpose was to stimulate, sponsor, provide for, and supplement present programs for the conduct of research, investigations, experiments, and the training of scientists in the fields of water and of resources which affect water so as to assist in assuring the Nation at all times of a supply of water sufficient in quantity and quality to meet the requirements of its expanding population.

The Act authorizes appropriations every year (continuing indefinitely) to assist each participating state in establishing and carrying out the responsibilities of a competent, qualified Water Resources Research Institute or Center at one university in each state. It also provides for annual matching funds for the centers, and authorizes annual grants, contracts, matching or other arrangements with educational institutions including the Center, universities, foundations, private firms, individuals, and local, state, and federal government agencies to undertake research into any aspect of water problems related to the mission of the Department of the Interior which may be deemed desirable and are not otherwise being studied.

In August 1964, Governor Albertis S. Harrison, by letter to President T. Marshall Hahn, designated the Virginia Polytechnic Institute and State University as the center for the Virginia Water Resources Research in the Commonwealth of Virginia. The Center was established to plan and conduct competent research, investigations, and experiments of either a basic or practical nature, or both, in relation to water resources and to provide for the training of scientists through such research, investigations, and experiments. It also provides the mechanisms for cooperation in water resources research with other institutions of higher learning, private research groups, and action agencies throughout the state.

This is a summary of the eighth Annual Report submitted to the Office of Water Resources Research, U.S. Department of the Interior, in compliance with Section 506.1 of the Rules and Regulations Pursuant to the Water Resources Act of 1964 (Federal Register, December 3, 1964).

William R. Walker  
Director



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## **DIRECTOR'S STATEMENT**



## DIRECTOR'S STATEMENT

Problems of the state associated with water quality are the most numerous, the most easily identified, and the ones receiving the greatest amount of attention from the press, general public, and water regulatory agencies. Thus, concern is reflected in the Center's research activity in both the allotment and matching funds programs. Three projects involve improved techniques for monitoring the quality of water, two are concerned with improved waste treatment techniques, two deal with handling of waste water effluents, and two study the effects of land use regulations on water quality. Research designed to improve water management planning and evaluation of some existing management practices comprise the remainder of the Center's research program.

Much of the research conducted at the Center during this past year has found acceptance and is being utilized by practitioners in the field who are attempting to resolve water resource problems. Although all the projects are related to problem areas in Virginia, it is interesting to note that acceptance of the research results has been much greater outside of the Commonwealth than within. This would tend to lend credence to the old cliché that "a Prophet in his own land is without honor."

The results from the project on biological monitoring of industrial wastes (A-039) are now being used by Industrial Bio-Test Laboratory in the design of monitoring systems for five utility companies located in several states. Ecological consultants from Louisiana State University have found the information extremely helpful and indicated that fish, as sensors, may be incorporated into the monitoring system for the intake of the municipal water supply for the city of New Orleans.

The effect of detergent polluted waters on soil reaction and plant growth (A-41) has attracted the attention of Proctor & Gamble Company. Although phosphorus in detergents has received the greatest amount of publicity, the results of this research have indicated that the micronutrients (e.g., boron and arsenic) may have a significant effect on the future productivity of land. The spraying of waste water on agricultural lands is under intensive study by the U.S. Army Corps of Engineers and at several universities. The results of this research strongly suggest that the impact of detergents passing through waste treatment facilities must be considered in terms of their latent effects when ultimate disposal of the effluent is on the land. The economies of waste disposal in 1972 should not blind us to the possible destruction of the productivity of this natural resource at another point in time.

The project concerned with the penetration and mixing of heated water from nuclear power installations (B-041) has developed preliminary results now being used by Applied Physics Lab, of Johns Hopkins University, in their study of the Chesapeake Bay for the State of Maryland.

The use of Chironomidae and Ceratopogonidae for the monitoring of aquatic wastes (A-042) is showing great promise of developing a simple bioassay technique which may supplement, and in many cases replace, expensive chemical methods used in monitoring both industrial and domestic wastes. The state regulatory agency with responsibility for water quality is following the development of this project very closely and may attempt to incorporate some of the techniques showing promise into their monitoring program after the project is completed next year.

The city of Atlanta has under construction a new activated sludge plant with aerobic digesting facilities. Officials responsible for the design of this facility have maintained close liaison with principal investigators of project A-035, "Optimal Conditioning Procedures for Waste Activated Sludge Disposal." Some of the techniques developed in this project will become a reality in the sludge handling portion of this plant. Mr. Edward Rushbrook, Department of Water Resources, Montpelier, Vermont, has been very interested in the results of the project, and has indicated that the data developed therefrom may be used in formulating guidelines for the State of Vermont for waste water treatment facilities.

The preliminary results from a project entitled, "Coagulant Recovery and Reuse in Water Reclamation Systems," (A-040) show great promise for both water reclamation plants and conventional municipal water treatment plants. The consultant to the city of Denver, Colorado has indicated that alum recovery will be included in the design of the proposed reclamation plant. The results of this research were key elements in his recommending an alum recovery system for this new facility.

An interdisciplinary research program provides the opportunity to study the interaction of the social, political, economic, and technical aspects of water problems. Such a program offers the greatest promise for developing useful information for intelligent decision making among various alternatives. Until recently, the limited budget for the Center precluded the sponsoring of any interdisciplinary research. In June 1972, the National Science Foundation made a \$95,000 grant to the Center to study the physical, economic, and institutional considerations in deep well waste disposal. This 18-month study should not only provide a comprehensive evaluation of problems associated

with deep well waste disposal as viewed from several disciplines, but it should also give some insight into the mechanical and administrative problems of conducting interdisciplinary research in institutions of higher learning that are highly structured by disciplines. The experience of our Center as an effective vehicle for "bridging the discipline gap" may be as significant to other Centers as the research results themselves.



**ANNUAL ALLOTMENT PROGRAM**



# IMPROVING WATER QUALITY BY REMOVAL OF PESTICIDE POLLUTANTS WITH AQUATIC PLANTS

Project A-033-VA

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# IMPROVING WATER QUALITY BY REMOVAL OF PESTICIDE POLLUTANTS WITH AQUATIC PLANTS

## I. Uptake and Metabolism of Herbicides by Algae

### Introduction

Four algae were used in this study. Chlorella pyrenoidosa Chick, Scenedesmus quadricauda (Terp) Breb, Chlamydomonas reinhardtii Dangeard, and Euglena gracilis Chod. and Prov. var. urophora were obtained from the Indiana Culture Collection of Algae at Indiana University. These algae cultures were axenic and exhibited collection numbers 395, 77, 89, and 159, respectively.

The herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), 3-amino-s-triazole (amitrole), and 2-chloro-4-(ethylamino)-6-(isopropylamino-s-triazine) (atrazine) were selected for use in the various studies. These pesticides are important and of most interest because of (1) extent and volume of usage; (2) degree of hazard to man, fish, and wildlife; and/or (3) degree of persistence.

## II. Toxicity of Selected Herbicides Toward Algae

In order to study uptake of herbicides by algae, it was first necessary to determine the toxicity levels of the herbicide to the algae. Only by working at a relatively non-toxic herbicide concentration can one be assured that the algal cells were not being damaged during the uptake period.

Cell growth was used to determine herbicide toxicity. All four algae were used in the toxicity studies over a wide range of herbicide concentrations. The algae, taken from actively growing cultures, were streaked onto Bristol's agar containing the herbicide. The slants were then placed on racks in a growth chamber maintained at 23°C with continuous light of 1000 ft. candles intensity. All tubes were enriched with 1 g/l peptone as a shotgun vitamin source.

Since algae sometimes behave differently on agar and in water culture, it was decided to duplicate the toxicity study with water cultures. The technique differs only in that cells were inoculated into small, 20-ml glass vials containing Bristol's solution with the herbicide. Here again, peptone was added at the rate of 1 g/l. The necessary precautions were taken to insure axenic conditions. The vials were placed on a glass plate and illuminated from below with a bank of fluorescent lights. All vials were agitated daily for two weeks after which cell counts and O.D. measurements were made.

## Results

Growth inhibition on agar and in water culture was similar. It took over 100 ppmw 2,4-D to completely inhibit the growth of Chlorella and Chlamydomonas. Scenedesmus was more sensitive, showing no growth above 50 ppmw.

Amitrole was not found to be toxic up to 10 ppmw with any of the algae used. Atrazine, however, completely inhibited growth of all algae at 10 ppmw after two weeks.

Subsequent to the toxicity studies, other experiments were conducted on photosynthesis inhibition by atrazine. These experiments (to be discussed later) were conducted in pond water without peptone. It was observed that photosynthesis inhibition was much more severe at a given herbicide concentration than growth studies indicated. The logical explanation was that peptone was protecting the cells from herbicidal damage in the growth studies.

An experiment was designed to determine the magnitude of the protective action of peptone. Using an arbitrary concentration of 50 ppmw amitrole and 2,4-D, 0.2 ppmw for atrazine, it was determined that Scenedesmus growth inhibition was 25-35 percent greater in a medium without peptone as compared to one with peptone.

Work is presently underway to repeat the toxicity studies without peptone.

### **III. Uptake of Herbicides from Water by Algae**

Preliminary studies with 2,4-D indicated that uptake was pH dependent. Sorption of 2,4-D by the algae was also demonstrated to be essentially complete at 6 hours.

Six-hour uptake studies were conducted on all algae with all herbicides at pH values ranging from pH 4.7 to pH 8.3. Cells from actively growing continuous cultures were centrifuged and the cell pellet was resuspended in 50 ml pond water containing herbicide buffered at various pH values. The cell suspensions were shaken on a reciprocal platform shaker at 80 cycles/minute. 2,4-D-<sup>14</sup>C was removed from water at low pH; atrazine-<sup>14</sup>C uptake was favored at pH 7.1; and amitrole-<sup>14</sup>C was greatest at pH 7.5.

After having established the pH where optimum uptake occurred, all the algae were allowed to take up 2,4-D<sup>14</sup>C at 0.1 ppmw for 6 hours. Scenedesmus was most impressive in removing as much as 50 percent of the initial 2,4-D<sup>14</sup>C. Since some of the algae were relatively ineffective at the cell densities used, another experiment was conducted over 20 hours using different cell densities. Chlamydomonas was ineffective for removing 2,4-D<sup>14</sup>C from solution during 20 hours at cell densities up to 3.3 mg dry wt per ml. Euglena and Chlorella gave similar results. Scenedesmus removed as much as 67 percent of the initial 2,4-D<sup>14</sup>C label.

All algae tested tended to remove the amitrole-<sup>14</sup>C label from solution. Here again, Scenedesmus seemed to be most active. At a cell density of 3.2 mg dry wt/ml, Scenedesmus removed almost 70 percent of the amitrole-<sup>14</sup>C label from water containing 0.03 ppmw initial concentration.

Atrazine uptake was studied over 24 hours. Again, Scenedesmus appeared to be most effective in removing the label.

#### **IV. Photosynthesis Inhibition by Atrazine**

Atrazine is known to exert its primary effect by blocking the Hill reaction of photosynthesis. An experiment was designed to test photosynthesis inhibition by atrazine at different herbicide concentrations. All the algal species were tested. In general, actively growing cells were added to 50 ml Bristol's solution buffered at pH 8.3. The flasks (125 ml) were shaken on a reciprocal shaker in a growth chamber. The same light and temperature regime was used here as mentioned earlier. After the cells had acclimated for 6 hours, atrazine was added to flasks at 0.0, 0.01, 0.1, and 1.0 ppmw. After 6 hours more, 1.0  $\mu$ ci bicarbonate was added to all flasks. After 2 hours incubation with the bicarbonate, the cultures were filtered onto millipore filters with slightly reduced pressure. The filters were oven dried, wrapped around the inside of scintillation vials and counted after the addition of scintillation fluid.

##### Results

Photosynthesis was inhibited 100 percent, as compared to controls, in all algae at 1.0 ppmw atrazine. At 0.01 ppmw atrazine concentration, which would still be high as a residue in surface waters, photosynthesis was inhibited 30 to 40 percent in the various algae tested.

## V. Metabolism of Herbicides by Algae

As evidenced from the uptake studies, 2,4-D was only taken up by Scenedesmus and then only at low pH. Any metabolism of the 2,4-D molecule, therefore, would most likely occur at low pH values. To say the least, it is difficult to buffer a dense cell suspension over a period of several days. At best, one must resort to short contact periods of 18-24 hours. Using radio-labeled herbicides of high specific activity, and relatively high cell densities buffered at the most favorable pH values, one can effect considerable uptake during a metabolism study.

A metabolism study was conducted with Scenedesmus on all three herbicides. Scenedesmus was chosen because it was most active in removing the herbicides from water. Flasks (125 ml) were used as the reaction vessels. Each labeled herbicide was contained in 100 ml pond water buffered at specific pH values. Actively growing cells from continuous cultures were centrifuged down and washed up with the fluid containing the various herbicides. The cell suspensions were placed in a growth chamber and bubbled with air for 18 hours. At this time, microscopic examination of the fluid revealed the first appearance of a few bacteria. In order to attribute the metabolism to the algae, instead of bacteria, the reaction was terminated at this time. The suspension was centrifuged and the cells washed and extracted for metabolites. Since algae are known to form extracellular metabolites, the fluid was also extracted for metabolites.

Thin layer chromatograms (TLC) were prepared, developed, and then placed on X-ray film for exposure.

## VI. Aquatic Vascular Plants

### Methods and Materials

Research during the second year involving aquatic vascular plants included amitrole and diphenamid. The plants used were parrotfeather, water hyacinth, waterthread pondweed, and elodea. The research with each pesticide is reported separately. All these studies were conducted in a greenhouse with temperature range of 21°C at night to 27°C during the day. Nutrient solution (Hoaglands) was used at 1/10 strength for pondweed, elodea, and parrotfeather and 1/2 strength for water hyacinth. Light intensity ranged up to 6000 ft-c during midday.

Removal of pesticide from water was determined by evaluation of chemicals

remaining in solution at various intervals after initiation at  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  M concentrations. Controls without plants were utilized to correct for losses of pesticide through means other than plant uptake. The detection procedure utilized  $^{14}\text{C}$ -pesticides and liquid scintillation spectrometry.

Some pesticides were obtained from greenhouse cultures and propagated through rooted cuttings (parrotfeather and water hyacinth). Water hyacinth plants were partially rooted before cutting from the original plant. Parrotfeather was allowed to root from shoot cuttings, first under shade and after one week placed in full sunlight. The roots of these plants were placed in pesticide solution but shoots were above.

Waterthread pondweed and elodea were obtained from local water supplies as rooted plants. These species were submerged in pesticide solutions for uptake studies. Waterthread pondweed has a few floating leaves. Little or no plant transpiration was evident with this species.

Degradation of pesticides in aquatic vascular plants involved diphenamid and amitrole. The plants used were parrotfeather, water hyacinth, waterthread pondweed, and elodea. Plants were extracted and extracts partially cleaned of plant material by partitioning and/or chromatographic techniques before TLC separation of residues of pesticides and metabolites.

#### Diphenamid Removal and Degradation

Sixty parrotfeather plants (59 g shoot weight) were treated with 4 ppmw (500 ml) of diphenamid- $^{14}\text{C}$  (21.4  $\mu\text{c}$ ) through the roots. The shoots were extracted with benzene, evaporated to dryness, taken up in acetonitrile and partitioned with hexane. The acetonitrile was evaporated to dryness and residue taken up in benzene. The benzene (a few ml) was placed on an alumina column, developed with benzene, and three 500-ml fractions collected. In addition, three more 500-ml fractions were collected from the column but contained no pesticide and very low levels of metabolites. The 500-ml fractions were evaporated to 10 ml and 5 to 50  $\lambda$  spotted on TLC plates. The plates were developed with benzene: n-heptane saturated with water and methanol. Radioactive spots on TLC plates were detected with X-ray film. Diphenamid and certain selected metabolites observed in plants, such as tomato and ornamentals were placed on TLC plates for comparison and identification of degradation products in aquatic species.

Diphenamid was removed from water with all species. The rate of removal was slow but steady in most cases. The plants were allowed to remove herbicide for eight days and harvested immediately. Low levels of diphenamid

were not toxic to parrotfeather and growth continued. All species contained some diphenamid as the parent compound. Parrotfeather degraded the diphenamid to a mono-methyl derivative.

Water hyacinth plants were allowed to remove diphenamid as above except 2 ppmw and 4000 ml were used. Water hyacinth also degraded diphenamid to the N-methyl derivative and one additional unknown metabolite that moved slower on the TLC plates than the N-methyl compound. This product was not found in tomato or ornamental plants in previous studies. Parrotfeather contained about 46 percent diphenamid and 54 percent N-methyl derivative. Water hyacinth degraded significant quantities but was not as effective as parrotfeather. About 71 percent of the diphenamid was intact molecule, 18 percent was N-methyl derivative, and 11 percent unknown.

Waterthread pondweed did not remove as much diphenamid from water (about 4 percent in 20 days) and metabolism of the chemical was not apparent in plant extracts.

#### Amitrole Removal and Degradation

Elodea and water hyacinth removed amitrole from solution. In our first studies contamination with algae modified the results. In later studies, Cutrine\* was used in the water to control the algae and removal of amitrole was much greater than during the first study. This may have been an effect of Cutrine also. Water hyacinth being an emerged plant lost considerable water by transpiration as compared to evaporation only with elodea, a submersed plant. The increased water loss did not increase amitrole removal as was expected from previous studies using other pesticides.

Degradation occurred in water hyacinth as shown by a tracing of the autoradiograph of TLC plate. The metabolites and amitrole were extracted with ethanol, partially purified using 250  $\mu$  thick TLC plates and streaking the condensed extract at the origin, developing with benzene and acetone which left all the radioactivity of the  $^{14}\text{C}$ -amitrole and metabolites at the origin, extracting the silica gel G from origin with ethanol, and using thin (50  $\mu$ ) TLC plates for separation of products. Ammonium hydroxide-water-2-propanol solvent system was utilized and developed for 15 cm.

At least five unknown compounds were observed in the partially purified extracts. One moved faster than amitrole and four trailing at short distances (about 1 cm between each). Some amitrole was still present (approximately 1/4 of the extracted  $^{14}\text{C}$ ).

\*Cutrine is a registered trade name of the 3M Company

**OPTIMAL CONDITIONING PROCEDURES FOR  
WASTE ACTIVATED SLUDGE**

Project A-035-VA

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## OPTIMAL CONDITIONING PROCEDURES FOR WASTE ACTIVATED SLUDGE

The laboratory studies during the second year of the project were designed to define the mechanism responsible for the changes in activated sludge dewaterability that occur during aerobic digestion, and to evaluate the effects of the changes on subsequent synthetic polymer conditioning.

Laboratory-scale dewatering procedures were used throughout the investigation, however, the waste activated sludge samples used for experimental purposes were obtained directly from three different full-scale wastewater treatment plants prior to each test. The sludges, taken from the return sludge lines of two separate 6000 gpd extended aeration units and a 10 MGD conventional activated sludge plant, differed considerably in initial filterability, with sludge from the conventional plant being the most difficult to dewater. After determination of the initial dewatering properties, each sludge was aerobically digested for 9 to 12 days and changes in dewatering properties and conditioning response were measured.

Buchner funnel techniques were used to measure filterability. Specific resistance and the compressibility factor were assumed to be both indispensable and sufficient for the measurement of sludge dewatering properties and they were used for this purpose throughout the study. Initially, changes in suspended solids concentration, natural exocellular polymer concentration, pH, and alkalinity were monitored and related to filterability. As the study progressed, it became obvious that a physical parameter was needed and a technique for measuring the median particle size of the activated sludge floc was developed. During later runs, sludge samples were removed from the digesters and subjected to various handling and conditioning procedures, such as chlorination, anaerobiosis and polymer addition, and effects were measured.

The results showed that both the specific resistance and the compressibility factor of activated sludge are affected by aerobic digestion. The specific resistance decreased with aeration time, reaching a minimum after one to five days and then increased with further aeration to the extent that after 10 to 12 days of aeration the values were higher than the original ones. The compressibility factor increased with aeration time, reaching a maximum value after one to five days, and then decreased with further aeration. Since the lower the specific resistance and the higher the compressibility factor the better the dewatering properties of the sludge, the dewatering characteristics

of aerobically digested activated sludge were found to be optimum after one to five days of aeration and to deteriorate with further aeration.

Previous researchers have indicated that there is a strong direct correlation between exocellular polymer and activated sludge dewaterability. In this study, the natural exocellular polymer to suspended solids ratio consistently reached a minimum value after a day or two of aeration, and then dewaterability and natural polymer concentration were inverse. Possibly, the natural exocellular polymers in the sludges studied were at concentrations higher than the optimum for flocculation and when the concentration reached its lowest value after one or two days of aeration, optimum filtration characteristics resulted. However, there was strong evidence that the pH changes that occurred during aerobic digestion affected the flocculating properties of the exocellular polymer since the changes in specific resistance and compressibility factor during aeration could be correlated to change in pH.

It was found that changes in the median particle size with aeration time closely paralleled changes in compressibility factor. Furthermore, the specific resistance was found to be an inverse linear function of the median particle size. On the basis of these correlations, it was concluded that sludge dewaterability is strongly affected by any change in median particle size, and further studies were designed to investigate this conclusion more thoroughly.

In the additional studies, the effects of a cationic synthetic polymer and of chlorine on the dewatering properties of aerobically digested sludge were evaluated. The artificial polymer reduced the specific resistance by increasing the median particle size, but the reduction was a function of how long the sludge had been aerated before the polymer was added. In some cases the longer the sludge had been aerated before the polymer addition, the higher the reduction in specific resistance, but in other cases the opposite was true. It is believed that this effect is also related to the change in pH during aeration. Chlorine addition produced an increase in the specific resistance of the sludges by reducing the median particle size. Both artificial polymer and chlorine were found to affect the compressibility but the trends were complex, although the overall effect for both was detrimental.

On the basis of the results obtained, it seems that measurements of the median particle size could very well be used to predict the changes in dewaterability of sludge, as measured by the specific resistance and compressibility factor, during aerobic digestion. When artificial methods of

sludge conditioning, like chlorination or polymer addition are used, the method seems appropriate to predict change in specific resistance, but not in compressibility factor.

Following definition of the mechanisms of change during aerobic digestion, the conditioning effect of synthetic polymers on activated sludge was more thoroughly investigated. Both cationic and anionic polymers were tested for conditioning effectiveness on the sludges as initially obtained. In all cases the addition of anionic polymer greatly worsened the filtration rates whereas the cationic polymers improved filtration. Following these experiments, a specific cationic polymer was selected for use throughout the sludge handling studies.

Ten to thirty mg/l polymer was added to each sludge as initially obtained and the effect on the specific resistance and compressibility factor of the sludges was measured. The specific resistance was improved by polymer addition in all tests, with improvement ranging from 40 to 98 percent depending on the origin of the sludge and the amount of polymer added. On the other hand, while polymer addition improved the compressibility factor of one of the sludges, it caused an 18 to 30 percent decrease in the compressibility factor of the other two sludges.

Following determination of the initial conditioning effects, each sludge was aerobically digested for 9 to 12 days and changes in the conditioning effectiveness of the same polymer doses were measured. As previously noted, without polymer conditioning the dewaterability of waste activated sludge is improved by the first 3 to 5 days of aerobic digestion but is worsened by further aeration. In general polymer effectiveness did not parallel these changes. The effect of polymer on the specific resistance of one sludge gradually improved over an 11-day aeration period whereas the effectiveness of the polymer on the sludge from the conventional plant (98 percent improvement initially) was totally neutralized by 3 to 5 days of aerobic digestion. The effect of aerobic digestion on the compressibility factor following polymer conditioning was both sludge dependent and polymer dose dependent.

After the aerobic digestion studies with low polymer addition were completed, the two worse sludges were selected for further study. The optimum polymer dose for both sludges prior to any laboratory handling was determined and then each sludge was aerobically digested for 40 days. The optimum dose was 130 mg/l for one sludge and 240 mg/l for the other. During the 40 days period, samples were removed at regular intervals and the filtration rate of the sludge was measured before polymer conditioning and

following addition of the optimum dose. A comparison of the two values shows that, for both sludges, addition of the initial optimum polymer dose always produced a large improvement in filtration except for days 5 through 8. During that period, for one day in each case, polymer addition actually decreased the filtration rate. After 10 days aeration, filtration improvement with polymer addition was always very dramatic but it was never better than the initial filtration rate.

A third polymer conditioning study was conducted wherein the change in optimum polymer dose with aeration was determined. The results show that aerobic digestion greatly decreased the polymer dose required for optimum filtration during the first 7 days. From an initial optimum dose of 125 mg/l, there was a linear change with time to a low of 25 mg/l at 7 days. There was no further change in optimum dose up to a digestion time of 20 days when the experiment was terminated. However, while the required dose decreased with aeration time, after 3 days the optimum filtration rate worsened with aeration time and after 7 days, the benefits of the decreased optimum dose were cancelled by the poor filtration rate.

The effect of both sludge chlorination and anaerobic storage on the polymer conditioning requirements of two of the raw sludges was studied. The addition of 100 mg/l HTH improved polymer conditioning of both studies while greater doses (250 and 750 mg/l) decreased polymer effectiveness. Anaerobic storage results were less consistent, but in general, 3 hours of anaerobiosis decreased polymer effectiveness whereas one day of anaerobiosis improved it.

From the results, it was concluded that the conditioning effect of polymers on waste activated sludge can be strongly affected by the sludge handling procedure used prior to mechanical dewatering. From a filtration rate standpoint, aerobic digestion is seldom beneficial when polymer is used for conditioning, and it will frequently cause a worsening of the polymer effectiveness. However, it may result in a considerable reduction in the amount of polymer required for optimum filtration.

## **Conclusions**

The experimental results of the investigation support the following conclusions:

1. Both the specific resistance and the compressibility factor of activated sludge are affected by aerobic digestion. Maximum filterability without

chemical conditioning can be obtained after 1 to 5 days of digestion whereas further aeration will produce dewatering properties worse than those initially present.

2. The specific resistance and the compressibility factor of activated sludge are very closely related to the median particle size of the sludge flocs as measured by sieve analysis. The relationship holds prior to and throughout aerobic digestion. Specific resistance is inversely related, compressibility factor is directly related.
3. Sieve analysis of activated sludge is a simple, reliable technique for predicting the dewatering properties of activated sludge. It is particularly useful for determining the conditioning effects of aerobic digestion.
4. The exocellular polymer concentration produced by microbial metabolism is not a major factor that determines sludge filterability during aerobic digestion.
5. Dewatering properties are adversely affected by chlorine addition and to some extent, by anaerobic conditions. The effects are reduced by aerobic digestion prior to the imposed chlorination or anaerobiosis. Long periods of anaerobiosis (24 hours or more) may actually improve filterability.
6. Activated sludge dewaterability can be improved by the application of synthetic cationic polymers. Anionic polymers are detrimental to dewatering as measured by filterability.
7. The conditioning effects of synthetic polymers cannot be improved by aerobic digestion. In fact, a gradual worsening with aerobic digestion generally occurs after the third day. However, the optimum polymer dose can be reduced by as much as 80 percent by 7 days of aerobic digestion. Digestion beyond 7 days produces no further improvement.



**NUMERICAL STUDIES OF UNSTEADY FLOW  
IN THE JAMES RIVER**

Project A-036-VA

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## NUMERICAL STUDIES OF UNSTEADY FLOW IN THE JAMES RIVER

The implicit method of solution of the continuity and momentum equations of flow in open channels was used to study problems of unsteady flow in natural rivers. A computer program was written to obtain particular solutions along the river with appropriate boundary conditions. The program was applied to three different types of problems in rivers. The first problem relates to flood flows in the James River. A storm occurring in December 1967 was analyzed and a comparison of measured and computer flows along the James showed good agreement. The second problem relates to the study of low flows from Holcomb's Rock, Virginia to Bent Creek, Virginia along the James River. In trying to model these flows, it was found that the program in single precision was unstable. However, when the same program was run in double precision, good agreement was obtained between measured and computed flows. The last problem was the analysis of waves in a pump-storage reservoir. Data were obtained on flows in Leesville Reservoir, on the Roanoke River, Virginia. The computer program was used to calculate reservoir levels and good agreement was obtained between measured and computed levels, indicating that this method could be used to study the propagation of waves of long wavelength in reservoirs.



**BIOLOGY AND CHEMISTRY  
OF SURFACE FRESHWATER MICROLAYERS**

Project A-037-VA

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# BIOLOGY AND CHEMISTRY OF SURFACE FRESHWATER MICROLAYERS

## I. Objectives

The objectives of this project as stated in the proposal were: (1) the construction and testing of three different surface microlayer sampling devices; (2) the collection of freshwater surface microlayers from a variety of aquatic ecosystems, under a variety of conditions; (3) the characterization of chemical and biological components of select freshwater surface microlayers in some detail. The first two objectives have been fully realized, and a considerable amount of data has been collected to aid in realizing of the third objective.

## II. Testing and Calibration of Three Surface Microlayer Samplers

Three surface microlayer samplers are being utilized. The surface sampling screen (SSS), the surface sampling tray (SST), and the surface sampling drum (SSD). The SSS has been in use since the very beginning of our work because it is the simplest, and most easily obtained of our samplers. The SST is a substitute for the "aquatic guillotine" proposed in the grant. It was designed and built with the aid of the Virginia Tech Industrial Research Center. Originally it was a one-meter square, stainless steel tray subtended by an aluminum frame and four cylindrical ballasts at the corners. Due to difficulties in handling the original model, we had a similar replica made. It works on the same principles, but is about one-third the size and appears to be more effective and much easier to manipulate. Also, it is sufficiently small to enable autoclave sterilization. We have had this similar SST since the first of October 1971, which was about the same time the SSD arrived. Due to late arrival of the SSD, much of the substantive work on this project started only in early Fall 1971.

Pertinent data for each of the three samplers are presented in Table 1. The work necessary for computing the mean depths of water collected by the samplers was carried out under ideal conditions in a long narrow wave study trough located in the Geology Department at Virginia Tech. This tank is 95 feet long, 4 feet wide, and 4 feet deep. It made it possible to set up artificial surface microlayers of known composition and sample them with the three devices. Tables 2 and 3 show the type of data we collected from these experiments. There is a definite correlation in the data presented in the first three tables. The SSD picks up the thinnest layer and also the greatest

Table 1

Calibration Data for Three Surface Microlayer Samplers

<u>Sampler</u>	<u>Width*</u> <u>(cm)</u>	<u>Length*</u> <u>(cm)</u>	<u>Area*</u> <u>(cm)</u>	<u>Volume of</u> <u>Water Per</u> <u>Area</u> <u>(ml)</u>	<u>Calculated</u> <u>Mean</u> <u>Sampling</u> <u>Depth</u> <u>(mm)</u>
Drum	60.0	120.0	7,200	37.6	.052
Tray	30.5	76.0	2,318	140.0	.603
Screen	40.0	75.0	3,000	98.8	.327

\*These values are for the collecting surface of the samplers

Table 2

Dry Weights of Cetyl Alcohol and AgCl ppt. Extracted from Water Which Was Collected from a Laboratory Trough by Three Surface Microlayer Samplers and One Subsurface Sampler

<u>Sampler</u>	<u>Cetyl Alcohol (gm/l)</u>	<u>AgCl ppt. (gm/l)</u>
Drum	3.5170	0.1333
Tray	0.1023	0.1350
Screen	0.0192	0.1494
Subsurface*	0	0.1097

\*10 cm from the surface

Table 3

Dry Weights of Octadecanol and AgCl ppt. Extracted from Water Which Was Collected from a Laboratory Trough by Three Surface Microlayer Samplers and One Subsurface Sampler

<u>Sampler</u>	<u>Octadecanol (gm/l)</u>	<u>AgCl ppt. (gm/l)</u>
Drum	5.490	0.134
Tray	0.122	0.147
Screen	0.291	0.121
Subsurface *	0	0.134

\* 10 cm from the surface

amount of long chain alcohols which made up our surface microlayer. The SSS and SST are comparable in their ability to collect a thin layer which is reflected in the amount of surface film collected. We conclude from these data that the SSD is the far superior device for collecting surface microlayers from freshwater ecosystems.

### **III. Biological and Chemical Data from Three Aquatic Ecosystems Studied During the Last Year**

Water was collected from three sources for chemical and biological evaluation: (1) a farm pond located about 2 miles west of Blacksburg off State Route 624; (2) an oxidation pond which serves Oak Forest Trailer Park near Merimac, Virginia; and (3) Claytor Lake, Virginia. Samples were taken with each of the three devices under consideration in this study, as well as a dip sample (the most commonly used method of collecting water) and a subsurface sample to compare with the surface samples. Once obtained, the water was examined for numbers of viable bacteria (spread plate counts). Identification and counts of algae (direct microscopic), analysis for several inorganic compounds (Hach Kit), dry weight of particulate matter, and coliform bacteria (Most Probable Number (MPN) tests and plate counts on selective media).

Table 4 presents some of the most recent data on viable counts of bacteria and fungi. In the majority of cases this year, the numbers of bacteria and fungi were greater in the surface microlayer, than in subsurface water. Often this difference is 10 to 100 times more. Early data of this type prompted us to examine the coliform group of bacteria more closely. Starting in late March, we did MPN determinations on all the water which we collected. Table 5 shows the results of these tests. In over half the samplings, the number of coliforms was found to be greater in the surface slicks, than in the subsurface or dip samples. Table 6 gives some results of a more detailed study of the coliform group using differential and selective media. More work is being done to try to verify the preliminary grouping as shown in this table.

Tables 7-12 give a breakdown of what algae genera were present in each sample, and in what numbers. These data have not been evaluated completely, but some trends are evident, e.g., from the oxidation pond data can be seen the *Chlamydomonas* tends to occupy the surface slick in high numbers, decreasing with depth. Whereas the number of Merismopedia increases with depth.

Table 4

Viable Counts of Bacteria and Fungi Expressed in  
Number of Organisms Per ml of Water

<u>Sampler</u>	<u>Pond Water Agar</u>	<u>Potato Dextrose Agar</u>
<u>Farm Pond--March 30, 1972</u>		
Drum	$6.7 \times 10^3$	$5.0 \times 10$
Tray	$4.6 \times 10^3$	$1.0 \times 10$
Screen	$3.8 \times 10^3$	$3.8 \times 10$
Subsurface*	$4.9 \times 10^3$	$1.0 \times 10$
Dip	$4.9 \times 10^3$	$1.5 \times 10$
<u>Farm Pond--May 4, 1972</u>		
Drum	$6.3 \times 10^4$	$2.0 \times 10^2$
Tray	$7.8 \times 10^3$	$5.9 \times 10$
Screen	$6.9 \times 10^4$	$2.3 \times 10^2$
Subsurface*	$9.2 \times 10^3$	$1.5 \times 10$
Dip	$1.0 \times 10^4$	$1.2 \times 10^2$
<u>Claytor Lake--April 6, 1972</u>		
Drum	$3.2 \times 10^4$	$1.1 \times 10^2$
Tray	$6.0 \times 10^3$	$3.6 \times 10$
Screen	$5.6 \times 10^3$	$5.3 \times 10$
Subsurface*	$2.9 \times 10^3$	$0.3 \times 10$
Dip	$6.2 \times 10^3$	$1.5 \times 10$
<u>Claytor Lake--May 11, 1972</u>		
Drum	$1.0 \times 10^6$	$4.7 \times 10^7$
Tray	$8.3 \times 10^4$	$4.4 \times 10$
Screen	$1.1 \times 10^6$	$1.9 \times 10^2$
Subsurface*	$6.4 \times 10^4$	$1.8 \times 10$
Dip	$9.3 \times 10^3$	$4.0 \times 10$

Table 4  
(Continued)

<u>Sampler</u>	<u>Pond Water Agar</u>	<u>Potato Dextrose Agar</u>
	<u>Oxidation Pond--April 20, 1972</u>	
Drum	$9.5 \times 10^6$	$1.2 \times 10^3$
Tray	$1.0 \times 10^7$	$1.9 \times 10$
Screen	$1.0 \times 10^7$	$9.6 \times 10$
Subsurface*	$8.1 \times 10^6$	$4.3 \times 10$
Dip	$1.0 \times 10^7$	$4.3 \times 10$

\*10 cm from the surface

Table 5

The Most Probable Number (MPN) of Coliform Bacteria Per 100 ml of Water

<u>Sampler</u>	<u>MPN</u>	<u>MPN</u>
	<u>Farm Pond</u>	
	<u>3-30-72</u>	<u>5-04-72</u>
Drum	2	1,070
Tray	1	730
Screen	0	540
Subsurface*	4	540
Dip	4	540
	<u>Claytor Lake</u>	
	<u>4-06-72</u>	<u>5-11-72</u>
Drum	150	≥ 2,400
Tray	46	265
Screen	185	260
Subsurface*	76	225
Dip	40	59
	<u>Oxidation Pond</u>	
	<u>4-20-72</u>	<u>5-18-72</u>
Drum	≥ 240,000	160,000
Tray	≥ 240,000	200,000
Screen	200,000	147,000
Subsurface*	166,000	73,000
Dip	92,000	160,000

\*10 cm from the surface

Table 6

Viable Counts (Per ml) of Enteric Bacteria Isolated  
on Three Types of Differential Media

<u>Sampler</u>	<u>Desoxycholate Agar**</u>	<u>Eosine Methylene Blue Agar</u>	<u>Salmonella- Shigella</u>	<u>Hektoen Enteric Agar*</u> Coliform	<u>Proteus</u>
<u>Farm Pond--March 30, 1972</u>					
Drum	1.0 x 10	3.5 x 10	1.5 x 10 <sup>2</sup>	4.0 x 10	3.0 x 10 <sup>3</sup>
Tray	1.5 x 10	3.0 x 10	4.0 x 10	4.5 x 10	3.6 x 10 <sup>2</sup>
Screen	2.0 x 10	2.0 x 10	1.5 x 10	0.5 x 10	5.5 x 10 <sup>2</sup>
Subsurface†	nil	1.5 x 10	4.0 x 10	nil	1.3 x 10 <sup>2</sup>
Dip	1.5 x 10	1.0 x 10 <sup>2</sup>	2.5 x 10	5.5 x 10	3.0 x 10 <sup>3</sup>
<u>Farm Pond--May 4, 1972</u>					
Drum	4.4 x 10 <sup>2</sup>	5.0 x 10 <sup>2</sup>	3.7 x 10 <sup>2</sup>	9.5 x 10	4.8 x 10
Tray	6.3 x 10	2.5 x 10	6.3 x 10	3.5 x 10	0.5 x 10
Screen	1.1 x 10 <sup>2</sup>	1.1 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	1.0 x 10	3.5 x 10
Subsurface†	3.3 x 10	4.0 x 10	2.0 x 10	1.0 x 10	5.3 x 10
Dip	2.7 x 10	0.5 x 10	2.5 x 10	1.0 x 10	0.5 x 10
<u>Oxidation Pond--April 20, 1972</u>					
Drum	1.8 x 10 <sup>3</sup>	2.5 x 10 <sup>3</sup>	2.1 x 10 <sup>3</sup>	1.3 x 10 <sup>3</sup>	6.5 x 10 <sup>3</sup>
Tray	1.8 x 10 <sup>3</sup>	4.0 x 10 <sup>3</sup>	3.0 x 10 <sup>3</sup>	3.8 x 10 <sup>3</sup>	4.0 x 10 <sup>3</sup>
Screen	3.5 x 10 <sup>3</sup>	1.1 x 10 <sup>3</sup>	8.2 x 10 <sup>2</sup>	2.2 x 10 <sup>3</sup>	6.9 x 10 <sup>3</sup>
Subsurface†	1.2 x 10 <sup>3</sup>	2.0 x 10 <sup>3</sup>	4.1 x 10 <sup>3</sup>	2.3 x 10 <sup>3</sup>	3.4 x 10 <sup>3</sup>
Dip	3.0 x 10 <sup>3</sup>	1.2 x 10 <sup>3</sup>	2.2 x 10 <sup>3</sup>	7.0 x 10 <sup>2</sup>	3.4 x 10 <sup>3</sup>
<u>Claytor Lake--April 6, 1972</u>					
Drum	2.5 x 10	3.0 x 10	2.6 x 10 <sup>2</sup>	3.5 x 10	4.2 x 10 <sup>2</sup>
Tray	2.0 x 10	3.0 x 10 <sup>2</sup>	4.5 x 10	0.5 x 10	8.1 x 10 <sup>2</sup>
Screen	0.5 x 10	3.5 x 10	1.9 x 10 <sup>2</sup>	1.0 x 10	2.3 x 10 <sup>2</sup>
Subsurface†	2.0 x 10	7.5 x 10	1.0 x 10 <sup>2</sup>	2.0 x 10	1.2 x 10 <sup>2</sup>
Dip	1.0 x 10 <sup>2</sup>	1.4 x 10 <sup>2</sup>	5.5 x 10	1.5 x 10	2.4 x 10 <sup>2</sup>

Table 6  
(Continued)

Claytor Lake--May 11, 1972

Drum	$3.3 \times 10^2$	$6.9 \times 10^2$	$6.1 \times 10^2$	nil	$3.6 \times 10^2$
Tray	nil	$1.1 \times 10^3$	$2.0 \times 10$	$0.5 \times 10$	$6.5 \times 10$
Screen	$5.0 \times 10$	$4.8 \times 10$	$1.9 \times 10^2$	nil	$1.2 \times 10^2$
Subsurface†	$8.0 \times 10$	$1.9 \times 10$	$3.0 \times 10$	$1.0 \times 10$	$5.0 \times 10$
Dip	nil	$3.0 \times 10$	$2.0 \times 10$	nil	$2.0 \times 10^2$

\* From which three characteristic colony types were counted.

\*\* Only the pink colonies were counted.

† 10 cm from the surface.

Table 7

Identification and Counts/ml of Algae Found in Water Collected by Three Surface Microlayer Samplers (and One Subsurface Sampler) from an Oxidation Pond on September 24, 1971

<u>Genera</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface*</u>
<u>Chlamydomonas</u>	64,546	341	49,205	2,614
<u>Trachelomonas</u>	3,977	500	114	227
<u>Merismopedia</u>	10,227	45,910	20,909	126,479
<u>Pediastrum</u>	455	nil	nil	nil
<u>Euglena</u>	1,591	1,250	nil	1,477
<u>Chlorella</u>	10,227	21,136	2,955	10,114
<u>Selenastrum</u>	nil	568	nil	795
<u>Ankistrodesmus</u>	227	455	nil	341
<u>Scenedesmus</u>	114	114	nil	568
<u>Eudorina</u>	nil	nil	341	nil
Total	91,364	70,274	73,524	142,615

\*10 cm from the surface

Table 8

Identification and Counts/ml of Algae Found in Water Collected by Three Surface Microlayer Samplers (and One Subsurface Sampler) from an Oxidation Pond on October 14, 1971

<u>Genera</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface*</u>
<u>Chlamydomonas</u>	1,250	20,568	5,909	7,614
<u>Trachelomonas</u>	1,591	2,046	5,114	2,046
<u>Merismopedia</u>	50,796	10,555	15,682	4,639
<u>Pediastrum</u>	227	1,023	568	796
<u>Euglena</u>	227	1,611	1,364	1,364
<u>Chlorella</u>	1,931	4,432	6,250	4,639
<u>Selenastrum</u>	1,023	796	2,273	1,611
<u>Ankistrodesmus</u>	nil	341	455	nil
<u>Scenedesmus</u>	nil	227	nil	nil
diatoms	1,023	nil	nil	nil
Total	58,068	41,599	37,615	22,709

\*10 cm from the surface

Table 9

Identification and Counts/ml of Algae Found in Water Collected by Three Surface Microlayer Samplers (and One Subsurface Sampler) from an Oxidation Pond on November 11, 1971

<u>Genera or Group</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface*</u>
<u>Chlamydomonas</u>	2,614	1,477	1,136	568
<u>Trachelomonas</u>	340	909	1,136	681
<u>Merismopedia</u>	7,841	16,932	16,591	20,796
<u>Pediastrum</u>	nil	nil	nil	nil
<u>Euglena</u>	nil	nil	nil	113
<u>Chlorella</u>	1,610	795	1,818	1,136
<u>Selenastrum</u>	1,610	1,364	1,477	909
<u>Ankistrodesmus</u>	nil	113	227	113
<u>Scenedesmus</u>	nil	nil	nil	nil
diatoms	nil	nil	nil	nil
<u>Eudorina</u>	nil	nil	nil	nil
Total	14,015	21,590	22,385	24,316

\*10 cm from the surface

Table 10

Identification and Counts/ml of Algae Found in Water Collected by Three Surface Microlayer Samplers (and One Subsurface Sampler) from Claytor Lake on April 6, 1972

<u>Genera or Group</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface*</u>
<u>Chlamydomonas</u>	369	57	45	23
<u>Trachelomonas</u>	45	nil	nil	nil
<u>Chlorella</u>	nil	nil	nil	142
<u>Ankistrodesmus</u>	17	nil	6	nil
diatoms	642	57	11	6
<u>Asterionella</u>	nil	23	nil	6
Total	1,073	137	62	177

\*10 cm from the surface

Table 11

Identification and Counts/ml of Algae Found in Water Collected  
by Three Surface Microlayer Samplers (and One Subsurface Sampler) from an  
Oxidation Pond on April 20, 1972

<u>Genera or Group</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface*</u>
<u>Chlamydomonas</u>	2,273	909	568	909
<u>Trachelomonas</u>	795	455	568	229
<u>Merismopedia</u>	682	113	113	229
<u>Phacus</u>	17,500	38,864	26,705	37,160
<u>Euglena</u>	113	113	nil	nil
<u>Chlorella</u>	1,136	nil	341	nil
<u>Selenastrum</u>	nil	nil	113	nil
<u>Ankistrodesmus</u>	229	113	nil	113
<u>Scenedesmus</u>	nil	nil	113	113
diatoms	341	nil	341	113
other	nil	3,864	7,273	3,296
Total	23,069	44,431	36,135	42,162

\*10 cm from the surface

Table 12

Identification and Counts/ml of Algae Found in Water Collected by Three Surface Microlayer Samplers (and One Subsurface Sampler) from a Farm Pond on May 4, 1972

<u>Genera or Group</u>	<u>Drum</u>	<u>Tray</u>	<u>Screen</u>	<u>Subsurface</u>
<u>Trachelomonas</u>	45	11	nil	nil
<u>Merismopedia</u>	nil	nil	23	nil
<u>Chlorella</u>	nil	6	nil	nil
<u>Ankistrodesmus</u>	nil	nil	nil	17
<u>Scenedesmus</u>	51	6	6	11
diatoms	250	108	186	34
Total	346	131	215	62

\*10 cm from the surface

#### **IV. Summary**

The three samplers have been constructed and calibrated. The SSD is superior to the SST and SSS in its ability to pick up the thinnest layer of surface water. Three different aquatic ecosystems have been examined to determine if there is a significant difference between surface microlayers and subsurface water. In all parameters studied, there is indeed a significant difference between surface microlayers and the subsurface water directly below them.



**THE APPLICATION OF BIOLOGICAL MONITORING  
SYSTEMS TO SIMULATED INDUSTRIAL WASTE  
DISCHARGE SITUATIONS**

Project A-039-VA

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## THE APPLICATION OF BIOLOGICAL MONITORING SYSTEMS TO SIMULATED INDUSTRIAL WASTE DISCHARGE SITUATIONS

A pollution monitoring system, which uses fish as sensors, was tested with zinc, copper, calcium, and temperature changes.

In each experiment, six fish were exposed to dechlorinated tapwater containing no toxicants or additives for five days in order to establish normal breathing and movement patterns for each fish. The movements were detected by a specially-constructed light beam apparatus, and the breathing signals were picked up by stainless steel electrodes in the water and recorded on a medical polygraph. On the sixth day, the test conditions started. Two of the fish were not exposed to the test conditions and were kept as controls for the 11-day duration of the experiments. The specific experiments are described below.

Starting on day 6 of experiment 4, the water temperature was made to follow a diurnal cycle (approximately a 6°C change) similar to that reported for the Potomac River on June 11, 1961 at a location free of thermal effluents. No stress detection occurred, at least as measured by the movement apparatus. The data on the breathing of the fish have not been analyzed yet. This preliminary result is encouraging because it indicates that the pollution monitoring system will not give a warning in response to a natural fluctuation in water temperature. In experiment 12, the water hardness was increased by increasing the calcium concentration from approximately 7.5 mg/l to 140 mg/l. In order to determine whether calcium was antagonistic to zinc in experiment 16, the calcium concentration was first increased to approximately 140 mg/l, and then 3 mg/l zinc was introduced. To test for a possible synergistic effect, 0.44 mg/l copper and 1.50 mg/l zinc were introduced together in experiment 8. This mixture is equivalent in toxicity to 2.94 mg/l zinc, if the toxicities of zinc and copper are additive, and 2.94 mg/l zinc is just below the detection threshold of the monitoring system (based on previously completed experiments). If the combined toxicity of copper and zinc is more than additive then we would expect to obtain a warning from the monitoring system. The data from the copper-zinc experiment have not been analyzed yet.

Eleven other experiments, conducted at one-month intervals, were designed to determine how often the fish in the monitoring system would have to be replaced if they were continuously exposed to a "biologically safe

concentration" (0.075 mg/l) of zinc. Approximately 100 fish were kept in a continuous flow of water containing 0.075 mg/l zinc, starting on August 4, 1971. Starting on the fifth week of exposure, and at one-month intervals thereafter, six fish were taken from the holding tanks and exposed in the monitoring system to the same zinc concentration to which they were accustomed (0.075 mg/l) for six days, then to approximately 3 mg/l zinc for four days. If the fish did not become resistant to the zinc or desensitized in some way, they would show warning responses to 3 mg/l zinc. An additional test was conducted with the fish that had been exposed to a low zinc concentration for ten months. After being exposed to 0.075 mg/l zinc for six days and to 3 mg/l zinc for four days, they were exposed to progressively higher zinc concentrations until a response was obtained. The data from these experiments have not been analyzed yet. During the first experiment (after the fish had been exposed to the "biologically safe concentration" for one month), there were technical problems with the apparatus, and the results may not be usable. Experiments run after exposure periods of three and six months were disrupted by chlorine which entered the water system in such large quantity that the dechlorinator was overloaded, and an experiment run after nine months of exposure was disrupted when the electric power to Derring Hall (where the experiments took place) was turned off. Some data can be salvaged from these disrupted experiments, and the question of whether the fish lose their capacity of responding to 3 mg/l zinc after exposure to 0.075 mg/l zinc can probably be answered.

**COAGULANT RECOVERY AND REUSE  
IN WATER RECLAMATION SYSTEMS**

Project A-040-VA

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## COAGULANT RECOVERY AND REUSE IN WATER RECLAMATION SYSTEMS

The initial work conducted during the first portion of the study period for this project enabled the development of analytical procedures for determination of the amounts of chemical coagulant that are contained in typical chemical sludges. In addition to use of atomic absorption spectrophotometry, special attention was given to the application of a standard wet chemistry method for determination of aluminum. This method has been applied to determine recoverable aluminum in various typical alum sludges.

During the first year of the project, the principal studies have been aimed at assessing the optimum procedure for recovery of aluminum from alum rich chemical sludges similar to those found in conventional water treatment plants and in many tertiary wastewater treatment facilities. Seven alum sludges, representing a variety of treatment conditions, were selected for detailed investigation. In each case the aluminum content of the sludge was determined, the recoverable aluminum at various pH levels as the sludge was acidified was measured, and the volume and characteristics of the remaining solids were evaluated.

One of the principal problems associated with alum recovery systems has been the separation, following acidification, of the remaining solids from the supernatant containing the recovered aluminum. In order to effectively reclaim alum, it is apparent that the residual solids must be separated rapidly under acidic conditions and that the settled sludge must be susceptible to rapid dewatering and drying. A portion of the reported work was designed to bring about improved settleability and dewatering of the remaining solids by polyelectrolytic conditioning of the acidified alum recovery system.

The work indicates that substantial improvement in the operating characteristics of an alum recovery system may be achieved by judicious selection and use of an appropriate polymer for the purpose of conditioning the acidified system for rapid and effective separation of the remaining solids.

The third purpose of this research is to determine the effectiveness of the recovered coagulant during reuse. Preliminary indications are that no difficulty will be experienced in substituting recovered alum for fresh alum.



**EFFECTS OF DETERGENT POLLUTED WATER ON SOIL  
REACTION AND PLANT GROWTH**

Project A-041-VA

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## EFFECTS OF DETERGENT POLLUTED WATER ON SOIL REACTION AND PLANT GROWTH

A greenhouse experiment was conducted to determine the effect of detergents contained in irrigation water on the growth of corn (Zea mays L.) on a Davidson clay loam and a Norfolk fine sandy loam. The soils were maintained at approximately field capacity with solutions containing various concentrations of either a heavy duty non-enzyme laundry detergent (Bz), or a heavy duty enzyme detergent (Tx). The detergent concentrations used were 0, 20, 80, 1600, 4800, 8000, 10,000, 12,000, and 14,000 ppm.

Yield increases were observed on the Davidson clay loam on treatments that received 800, 1600, 4800, and 8000 ppm Tx and 1600 ppm Bz. The only yield increase which was observed on the Norfolk fine sandy loam occurred on the 800 ppm Tx treatment. These yield increases were attributed to an increase in phosphorus availability from the phosphorus contained in the detergents.

Abnormal growth characteristics, such as plant bending, the development of leaf constrictions, interveinal chlorosis, mottling, and tip die back were observed in corn on many treatments. Yield decreases occurred on the Davidson clay loam on treatments that received 8000, 10,000, 12,000, and 14,000 ppm Bz and 14,000 ppm Tx. Yield decreases also occurred on the Norfolk fine sandy loam on treatments that received 1600, 4800, 8000, 10,000, 12,000, and 14,000 ppm Bz and 4800, 8000, 10,000, 12,000, and 14,000 ppm Tx.

Corn yields were statistically compared with soil pH, electrical conductivity of soil saturation extracts, the exchangeable sodium percentage of the soils, and the boron concentration in plant tissues to determine the factor responsible for yield decreases. Based on these comparisons, it was concluded that the soluble salts contained in the detergents, mainly sodium, were responsible for the decreases in yield on all treatments except where Tx was applied to the Norfolk fine sandy loam. In the latter case, the general salinity effects as measured by electrical conductivities could not be separated from specific sodium effects as measured by exchangeable sodium percentage determinations. It was also concluded that soil pH or boron contained in the detergents was not responsible for yield decreases in this study. The maximum amount of arsenic applied in any treatment through detergent applications was 98  $\mu\text{g}$ . This level was applied to the Davidson clay loam in the 14,000 ppm Bz solution. That amount of arsenic would be sufficient to

increase the arsenic concentration of the soils by a negligible factor of 0.043 ppm.

Plants withstood higher concentrations of detergents on the Davidson clay loam than on the Norfolk fine sandy loam. The severity of plant injury was less on the Davidson clay loam probably because of its larger cation exchange capacity and larger surface area.

From the results of this study, it seems that household laundry detergent formulations or their breakdown products pose little, if any, hazard as a soil pollutant at concentrations normally found in domestic sewage or sewage sludge. However, investigations should be conducted to determine if any of these substances would accumulate in toxic quantities in the more arid regions where leaching is reduced or absent or if continued applications of these materials could result in boron toxicity or increase in the pH of certain soils to such an extent that micronutrient deficiencies would develop.

**THE DIFFERENTIAL USE OF CHIRONOMIDAE AND  
CERATOPOGONIDAE (DIPTERA) IN THE MONITORING  
OF VARIOUS WASTES**

Project A-042-VA

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## THE DIFFERENTIAL USE OF CHIRONOMIDAE AND CERATOPOGONIDA (DIPTERA) IN THE MONITORING OF VARIOUS AQUATIC WASTES

Collections of the littoral were made at three sources of suspected pollution. (a) Federal Mogul Corporation, Blacksburg, Virginia on Cedar Run Creek (metal pollution); (b) Green Hill Meat Packers Company on Roanoke River on Route 460 between Christiansburg and Salem, Virginia (organic pollution); and (c) Glen Lyn Power Plant on New River at Glen Lyn, Virginia (thermal pollution).

The technique was designed specifically to collect Diptera breeding in the littoral edge of streams. Weekly samples were taken above and below each pollution outfall. At each sampling station, two subsamples were taken which consisted of 500 ml mud from the one-inch surface along the stream edges. One subsample was for larval identification, and the other subsample was used for rearing and adult identification.

In the laboratory, the larval subsamples were separated using a sugar flotation method and a series of Tyler sieves. The adult subsamples were placed in rearing cages and adult Diptera were collected as they emerged several days later. All material collected was sorted and identified. In many cases, the genus and species of immature forms were not known well enough to identify closer than family or genus. (This emphasizes the need for basic studies on systematics of these groups of Diptera).

The invertebrate fauna obtained from the sorted subsamples are listed in Table 1. For convenience the taxa are grouped according to abundance. The Oligochaetes, Crustacea, and Hydracarina will not be treated in detail.

Of the 31 taxa collected, only 12 are numerous enough or encountered regularly enough to be considered important. Oligochaetes so dominated the fauna that they tended to overshadow the other groups. However, the Oligochaetes were present in such a wide variety of sizes and reproductive fragments that any discussion at this level of organization was meaningless. Also, the collecting technique was not designed to trap this group systematically.

In comparing the various sites and faunal composition above and below the respective outfalls, it becomes clear that a noticeable decrease occurs in total numbers as well as in diversity in the samplings below the outfall. This is

Table 1

Taxa Found in the Sorted Subsamples, Adjusted  
for Uniform Sample Size

	Federal Mogul Corporation		Green Hill		Glenn Lyn		Total
	Above	Below	Above	Below	Above	Below	
Abundant and rarely absent from any sample							
Oligochaeta	880	600	390	185	500	31	2386
Ceratopogonidae	72	23	72	21	101	4	293
Chironomidae	20	31	40	19	40	2	154
Tipulidae	37	30	19	9	0	1	96
Stratiomyidae	38	13	6	3	0	0	60
Dolichopodidae	31	12	9	1	4	0	57
Common: taken regularly though rarely or never in large numbers							
Ephydriidae	2	1	7	0	0	0	10
Tabanidae	2	7	1	0	0	0	10
Collembola	0	3	2	1	3	0	9
Hydracarina	3	0	0	2	4	0	9
Hydrophiliidae	1	3	2	1	1	0	8
Elmidae	1	0	1	1	1	2	6



particularly true at Cedar Run where the station below the Federal Mogul Corporation had 41 percent fewer organisms and 23 percent fewer taxa than the station above the outfall. On the Roanoke River, the station below the Green Hill Company outfall had 75 percent fewer organisms but had the same number of taxa with different distribution above the outfall. On New River, the stations above and below the outfall showed a less varied fauna overall, but the reductions in total numbers present and taxa present were no less obvious. Ninety-two percent fewer organisms and 17 percent fewer taxa were found. In New River, it was discovered during the summer that not only was there a thermal outfall, but also a considerable outfall of fly ash which would drastically affect bottom fauna, particularly littoral fauna.

On a percentage basis, Chironomids and Tipulids as a whole are least affected by the various outfalls. In every case, however, the effect produced by the fly ash and the thermal effluent at Glen Lyn was most drastic.

Table 2 represents the taxa obtained from adult rearing subsamples. With the exception of two entomophagous aquatic Hymenoptera, all the adults to emerge were Diptera. Losses occurred in every taxon below the outfalls, the notable exceptions being at the Roanoke River where Tipulids, Chironomids, and Dolichopodids increased by a factor of at least 2 at the sites below the outfall. This is hardly surprising since certain species of these groups tend to increase in the presence of organic pollution. The most striking result is that the Ceratopogonids represented the only taxon not drastically affected by the outfall in Glen Lyn into the New River. Even the Oligochaeta population was much reduced.

Table 3 is a list of families and/or genera so far encountered in this study. Further identifications are being made at present.

It is evident that certain groups of the Diptera are affected by both heavy metal and thermal or fly ash pollution. Determination of the species most notably affected is being made. It is hoped that more thorough collection in 1972 will lead to determination of species of Diptera that are most affected by certain types of toxic pollution.

Table 2

Reared Adults

<u>Taxon</u>	<u>Federal Mogul Corporation</u>		<u>Green Hill</u>		<u>Glen Lyn</u>		<u>Total</u>
	<u>Above</u>	<u>Below</u>	<u>Above</u>	<u>Below</u>	<u>Above</u>	<u>Below</u>	
Tipulidae	50	16	54	132	26	0	294
Chironomidae	7	10	12	25	9	0	63
Ceratopogonidae	121	30	220	190	43	34	638
Psychodidae	0	3	5	0	0	0	8
Dolichopodidae	5	0	2	8	7	1	23
Tabanidae	0	0	1	1	0	0	2
Stratiomyidae	0	0	0	0	1	0	1
Hymenoptera	1	0	0	0	0	0	1
Total	184	59	294	355	86	35	1030

Table 3

List of Families and/or Genera So Far Encountered

Collembola	Ephemeroptera
Isotomidae	Ephemeridae
	Hexagenia sp.
Hymenoptera	Baetidae
Braconidae	Bactis sp.
(Chalcoidea): Mymaridae	Isonychia sp.
Tipulidae	Psychodidae
Eroptera (=Polymeda) sp.	Pericoma sp.
Limnophila spp.	Psychoda spp.
Pilaria sp.	Maruina sp.
Limonia sp.	
Tipula spp.	
Trimicra sp.	
Eriocera spp.	
Chironomidae	Ceratopogonidae
Hydrobaenus sp.	Palpomyia spp.
Cryptochironomus spp.	Atrichopogon spp.
Chironomus spp.	Culicoides spp.
Polypedilum spp.	Bezzia spp.
Orthocladus sp.	Stilobezzia spp.
Diamesa sp.	Parabezzia sp.
Pentaneura sp.	Forcipomyia sp.
	S. coquilletti
Stratiomyidae	Anthomyidae
Stratiomys sp.	Limnophora sp.
Nemotelus sp.	
	Tabanidae
	Tabanus sp.

**AQUATIC FUNGI OF THE LOTIC ENVIRONMENT  
AND THEIR ROLE IN STREAM PURIFICATION**

Project A-043-VA

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## **AQUATIC FUNGI OF THE LOTIC ENVIRONMENT AND THEIR ROLE IN STREAM PURIFICATION**

Fungi and bacteria are heterotrophic organisms which derive their energy from the decomposition of organic matter. An important aspect of the ability of these organisms to decompose organic materials concerns what effect this may have on the purification of streams and rivers. With these considerations in mind, a study of the aquatic fungi of rivers was undertaken. This work dealt specifically with the aquatic phycomycetes, a group of fungi which are commonly encountered in rivers but have received virtually no study.

The objectives of this project are:

1. to determine the aquatic fungal flora of rivers;
2. to determine the changes that occur in the flora when effluents are introduced; and
3. using studies with selected fungi in the laboratory, to determine their possible role as purifiers of polluting effluents.

Samples were collected from the New River within the boundaries of the Radford Army Ammunition Plant, Radford, Virginia. Collection stations were located above, at, and below the point of effluent discharge from the ammunition plant. At each station collections were made within the following zones of the river:

1. the main channel,
2. the shoreline--including the edge of the river and the associated wet soil (This area is variable depending on the amount of water in the river.), and
3. flood plain-uplands--areas near the river which are only occasionally covered with water.

### **General Conclusions**

1. A more diverse fungal flora is found at the shoreline than in the other two general areas.

2. The flora of the upland areas was distinct from that of the main channel indicating that the fungi found in the main channel were not derived from the surrounding soils as a result of runoff during rains.
3. The introduction of an effluent causes about 50 percent reduction in the number of species found.

A representative filamentous aquatic phycomycete (Achlya sp.) from the river was obtained in pure culture. Laboratory studies are being conducted to determine the effects of some common classes of pollutants upon the growth of the fungus. Areas under investigation are heavy metals, respiratory poisons (e.g., cyanide), detergents, pH stress, and osmotic stress. Preliminary results indicate that 7 to 10 ppm of zinc, 7 ppm of cyanide, and a 3 percent solution of manitol (osmotic stress) cause a significant reduction in growth.

**THE REMOVAL OF SOLUBLE MERCURY FROM WASTE  
WATER BY COMPLEXING TECHNIQUES**

Project A-044-VA

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## THE REMOVAL OF SOLUBLE MERCURY FROM WASTE WATER BY COMPLEXING TECHNIQUES

Organic Polyelectrolyte flocculants were investigated for their usefulness in removing soluble mercury from aqueous solution. It was hoped that the polymers used may have characteristics which would enable them to complex with mercury. When used as the only additive to a mercury solution, both cationic and anionic flocculants proved to be completely ineffective.

Ion exchange materials have been developed from rubber buffing scraps coming from the tire recapping industry. Large quantities of this rubber are available at nearly zero cost. A cation exchange resin has been produced from this rubber by a simple sulfonation reaction using 98 percent sulfuric acid. Diffusion characteristics and loading capabilities are superior to commercially available resins with respect to mercury removal.

An anion exchange material has also been produced from the scrap rubber by a quaternary-ammonium substitution. The rubber is first reacted with chloro-methyl ether in the presence of a Friedel-Crafts catalyst. The final reaction involves reacting the chloro-methylated rubber with tri-methyl-amine. The result is a substituted quaternary-ammonium group. This resin is expected to perform as well as the cation exchange material with respect to removal of anionic mercury-chlorine complexes.

Both types of ion exchange material have good physical strength and durability. This was achieved by chlorination of the rubber before the substitution reactions. Apparently, the chlorination prevents excessive crosslinking during substitution, which if not prevented, would cause brittleness.

Loading capability was determined by evaluation of Freundlich isotherms at 80°F. Diffusion characteristics were extracted from kinetic rate data assuming the reaction to be diffusion controlled. Comparisons were made with Dowex 50W-X8 (cation exchange) and Dowex 1-X8 (anion exchange). These resins were suggested by Dow specifically for the removal of soluble mercury from water. In each case, the rubber based ion exchange materials appeared superior to the Dow resins with regard to loading capability and diffusion characteristics. The modified rubber was also successfully regenerated and used again with identical results.

Future work will involve further evaluation of the anion exchange material, studies of chemical stability, and pressure drop studies. Scrap rubber is presently available at zero cost and the substitution steps are not expensive, especially for the sulfonation reaction. Presently, cationic and anionic exchange resins cost between one and two dollars per pound.

The removal of ionic mercury from water solutions with several types of hair has been investigated. Human, hog, and cattle hair, as well as, a modified form of cattle hair obtained from a tannery, were screened for mercury removal ability. The tannery hair contains sulfhydryl groups instead of the naturally occurring disulfide crosslinkages. The tannery hair had a slightly lower kinetic rate of mercury removal than the other types of hair, but the amount of mercury reacted per gram of hair at equilibrium, for tannery hair was much greater than those of the other hair tested.

On the basis of the screening results, tannery hair was chosen for comparison with Dowex 50W-X8 cation exchange resin and Dowex 1-X8 anion exchange resin. The tannery hair exceeded both of the resins tested in the kinetic rate of mercury removal, as well as in the amount of mercury reacted per gram of material at equilibrium. The mercury solutions used in the tests contained both the cationic and anionic forms of mercury. The pressure drop through a fixed bed of tannery hair was found to be similar to those published for the two ion exchange resins. Tannery hair is available for twelve to fourteen cents per pound, whereas the Dowex ion exchange resins tested cost more than one dollar per pound.

## **MATCHING FUND PROGRAM**



**ANALYSIS OF WATER RESOURCE  
ADMINISTRATIVE AGENCIES**

Project B-025-VA

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## ANALYSIS OF WATER RESOURCE ADMINISTRATIVE AGENCIES

The research phase of the project, now essentially complete, has consisted of a comprehensive analysis of the legislative mandates and operating procedures of Virginia administrative agencies having responsibilities related to water resources utilization and management.

This research has indicated that the Commonwealth is in a rapidly evolving period with respect to the administrative and institutional framework within which water resources are managed. Many deficiencies in the previously existing structure have been or are being eliminated; however, certain fundamental inadequacies still exist which limit the effectiveness of the state's program.

One unresolved problem concerns state water policy. Various legislative declarations which serve as elements of a water policy exist, but no comprehensive statement has been enacted. Policy-making authority has also been delegated to certain agencies, but results have primarily been limited to physical planning. The consequence of this absence of a definitive policy is a situation under which, for example, it can be ascertained that both water power development and scenic river preservation are within the policy of the state, with little guidance provided for determining which use shall take precedence over the other in a specific case. The establishment of a comprehensive policy indicating priorities would assist rational decision making in this and other cases of conflicting water use.

The findings of the study also indicate the need for modification of individual agency responsibilities in certain instances. Although much emphasis has recently been placed on coordination of agency activities, further improvements are needed. For example, no clearly defined relationship exists between the agency with comprehensive water resources planning authority and the various agencies whose operations are intimately related to implementation. Some aspects of water resources management are delegated to agencies outside the mainstream of the State's program and are not within appreciable influence of the agencies whose major responsibilities are in this area. Coordination needs improvement in several cases where two or more agencies possess varying degrees of joint responsibility for specific water resource activities.

Recommended solutions to these problems will take the form of legislative changes relative to establishment of water policy; interagency transfers of responsibility; and specific coordination mechanisms to replace existing voluntary, informal procedures.

**FUNCTION OF THE MARSHES IN REDUCING  
EUTROPHICATION (AGING) OF ESTUARIES OF THE  
MIDDLE ATLANTIC REGION**

Project B-027-VA

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## FUNCTION OF THE MARSHES IN REDUCING EUTROPHICATION (AGING) OF ESTUARIES OF THE MIDDLE ATLANTIC REGION

Seasonal phosphorus and nitrogen movement through two Virginia salt marshes within the York River tidal basin is being determined. The marshes sampled are an oligohaline marsh dominated by grass species Spartina cynosuroides and a mesohaline marsh dominated by S. alterniflora.

Sampling stations were established on a principal drainage creek in a physically isolated section of each marsh. Concentrations of orthophosphate, total dissolved phosphorus, total phosphorus, ammonia, dissolved Kjeldahl nitrogen, total Kjeldahl nitrogen, nitrite, and nitrate are measured hourly over a complete tidal cycle beginning at low slack water. In addition, simultaneous measurements of current velocity and tide height in the creeks are taken and used in conjunction with the phosphorus and nitrogen concentrations to compute net transport of phosphorus, nitrogen, and water. Salinity, dissolved oxygen, air and water temperature, along with carbon-14 primary productivity, are also determined hourly.

Results obtained thus far indicate that the marshes contribute orthophosphate as well as dissolved and particulate organic phosphorus to the York River. The orthophosphate contribution is in part a result of upland weathering and subsequent runoff into the marsh while the organic phosphorus is the product of decomposition of dead marsh flora that had previously assimilated inorganic phosphorus supplied both by runoff and by York River water. Thus, the salt marshes serve to convert inorganic to organic phosphorus compounds. Since organic phosphorus is not readily assimilated by aquatic autotrophs, the marshes in effect lower the amount of phosphorus available to York River phytoplankton and macrophytes. If phosphorus is proved limiting to the river's primary productivity and if the organic phosphorus is transported to the ocean before it is digested, the marshes prevent further eutrophication of the York River estuary. Data on nitrogen flux indicate that the marshes serve as a sink for nitrate and nitrite and a source for dissolved and particulate organic nitrogen compounds and ammonia. The complexity of the nitrogen cycle makes it difficult at this time to attribute this change to any one process. Nitrate is probably assimilated by autotrophy and released as reduced nitrogen upon degradation of dead plant material.

Other processes affecting nitrogen flux are nitrogen fixation, and denitrification. Denitrification may be particularly important as marsh

sediment below one centimeter is anaerobic as evidenced by hydrogen sulfide production. Nitrogen has recently been cited as the nutrient limiting primary productivity in coastal waters. Thus, salt marshes, by affecting nitrogen, may affect productivity in the coastal environment.

Seasonal differences in the flux of nutrients in the marsh system have been revealed with greater losses of nitrate occurring in the summer months. While more particulate nitrogen and phosphorus loss occurs in the late fall and early spring than during the active growing season, higher levels of orthophosphate occur in the marsh in the summer than any other time. This probably accounts for the higher algal productivity observed in the marsh during the summer months.

Seasonal patterns are also evident in the flux of detritus as have been shown for the nutrients. The magnitude of flux is greater for both particulate and dissolved organic carbon during the summer and fall, with the summer period showing the greater flux of material. The quantity of carbon present in particulate form exceeds that of the dissolved and the patterns of flux as a function of tide are different.

In addition to the measures of carbon for the flux of detritus, collection of samples for ATP analysis was initiated in January. To date, only the samples from the January, Ware Creek study have been analyzed for ATP. These ATP results are, however, quite encouraging for they indicate a flux of living material out of the marsh while carbon figures for the same period are inconclusive.

**EVALUATION OF FLOOD INSURANCE IN A  
DISASTER AREA**

Project B-030-VA

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## EVALUATION OF FLOOD INSURANCE IN A DISASTER AREA

Base data on the study town of Buena Vista, Virginia have been collected and key punched. General damage tables have been prepared for each half foot of flood water elevation for houses having market values from \$2000 to \$50,000. Damage tables have also been generated for household contents. A computer program has now been completed to analyze costs associated with various levels of insurance coverage for floods of different frequency. These results are now being analyzed and compared with conventional relief costs, reviewed in terms of loss in income tax revenue due to casualty deductions, and examined to determine whether flood insurance should be compulsory in terms of structures subject to 10-year, 20-year, 50-year floods, etc.

In addition, evaluation of proposed amendments to the federal Flood Insurance Act will be included in the research. The damages generated as the result of Hurricane Agnes have given rise to new concerns about the program and why it has not received more acceptability.



**RECOVERY OF STREAMS AFFECTED  
BY ACID MINE DRAINAGE**

Project B-034-VA

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## RECOVERY OF STREAMS AFFECTED BY ACID MINE DRAINAGE

The project involved two main areas of exploration. The first was an experimental shock treatment of a healthy stream to note the effects of a short term stress, and the recovery processes involved in restoration of the aquatic community to its previous complexity after the stress. The second approach was the study of rivers and streams which are already affected by acid mine discharges. These streams and rivers were selected with acid discharges in the upstream regions, and downstream portions with improved water quality and improved biological diversity.

The work on a short term shock of low pH was carried out on a small, very productive stream near Blacksburg, Virginia. Mill Creek is a spring fed trout stream with total phosphate averaging 0.07 ppm, and  $\text{NO}_3 - \text{N}$  averaging 2.2 ppm. Total alkalinity values varied between 220 and 250 ppm while total hardness varied between 240 and 280 ppm. The pH remained consistently high (above 8.0) as did dissolved oxygen (6 to 10 ppm). Temperatures varied seasonally  $1^\circ\text{C}$  to  $20^\circ\text{C}$  with some diurnal fluctuation during the warm summer months,  $15^\circ\text{C}$  at 3:00 a.m. to  $20^\circ\text{C}$  at 3:00 p.m.; estimated mean temperature for the stream is between  $10$  and  $12^\circ\text{C}$ . A total of 101 taxa were identified from samples taken from Mill Creek. To provide two areas of similar habitat, one for experimental purposes, and the other maintained as a reference area, a 150-foot long straight riffle section was divided in half along 100 feet of its length. This produced two areas of nearly comparable habitat. The experimental shock acidification was carried out using concentrated sulfuric acid. Several small sandbag dams were constructed upstream of the experimental area, and flow was alternately reduced in reference and experimental sections by 50 to 80 percent. During reduced flow, concentrated sulfuric acid was added to the experimental section. The pH was reduced to 4.0 or below and maintained at that level for 30 minutes. Acid was neutralized with concentrated sodium hydroxide at the downstream end of the divider.

Previous studies on Mill Creek indicated a very rapid recovery with community structure and density results showing return to normal within 28 days of the experimental shock. Sampling was carried out to repeat this experiment with additional sampling to assay the effect of drift borne organisms on the recovery of the damaged section. Drift samples were collected for four weeks prior to the experimental acidification, and on a regular basis after the acidification. Preliminary analysis of these results show

downstream by the addition of dilution water from several tributaries, along with neutralization of acid drainage by unpolluted alkaline waters from Back Creek and Laurel Run. By station 10 (the farthest downstream) water quality was stabilized by restored pH, decreased sulfate and iron concentrations, and low acidity to alkalinity ratios. Water quality showed variation which may be attributed to changes in stream discharge. Since the acid drainage maintains a rather constant flow, differential dilution and neutralization occurred with changes in stream discharge. Water quality conditions during the May sampling period showed overall improvement throughout the whole stream. July water quality conditions showed increased effect of the acid drainage due probably to decreased stream discharge and dilution.

Biologically, the stream showed severe effects of the acid mine drainage. Upstream reference stations maintained a high diversity ( $\bar{d}$ ) and density,  $\bar{d}$  3.2 and above, density 80 to 400 organisms/ft<sup>2</sup>. The reference stations showed little fluctuation due to variations in stream discharge. Collections downstream showed variation in  $\bar{d}$  and density with changing discharge. Station 3, the acid source, showed moderate diversity during the May high discharge period,  $\bar{d} = 2.99$ , but low diversity during the July low discharge period  $\bar{d} = 0.81$ . The two stations immediately downstream from the acid drainage (stations 4 and 5) showed the most severe effect of the acid drainage on Indian Creek. The  $\bar{d}$  values remained relatively high at these stations, but the density dropped from 33 and 11 organisms/ft<sup>2</sup> during May to 5 and 14 organisms/ft<sup>2</sup> in July. The acid drainage was not well mixed by station 4 and this might account for increased densities due to a suitable habitat unaffected by the acid drainage which was maintained on the left bank. Station 5 is the most severely affected station in Indian Creek because the acid drainage is well mixed at this station, and there are no tributaries to dilute and chemically neutralize the acid waters. Station 6 was a healthy tributary with  $\bar{d}$  values above 3.7 and densities above 110 organisms/ft<sup>2</sup>. Stations 7 and 8, downstream from this tributary, showed improved biological communities with  $\bar{d}$  of 2.9 or above and moderately high densities of 40 to 80 organisms/ft<sup>2</sup>. Station 9 was a healthy tributary with  $\bar{d}$  values of 3.7 or above, and densities of 110 organisms/ft<sup>2</sup> or greater. Station 10 showed moderately high  $\bar{d}$  values 3.3 for May and 2.7 for July, and variable density 40 to 120 organisms/ft<sup>2</sup>.

Analysis of the bottom fauna community for the relative frequency of tolerant vs. non-tolerant organisms helps illustrate the recovery process, and the effect of varying acid drainage concentrations on the bottom fauna. During the May high discharge period the macrobenthic community was dominated by non-tolerant organisms. These organisms probably drifted

downstream and became at least temporarily established in habitats made suitable by improved water quality. During the July low discharge period the bottom fauna was dominated by tolerant organisms. The changes in water quality made habitats unsuitable for the non-tolerant organisms which either died or emerged, and the tolerant forms dominated. The balance between tolerant and non-tolerant organisms was a good indication of recovery. The upstream reference stations, and the unpolluted tributaries maintained communities balanced with tolerant and non-tolerant organisms. Those stations which were under constant acid drainage stress were dominated by tolerant organisms. Station 10, the station furthest downstream, maintained a macrobenthic community balanced with tolerant and non-tolerant organisms. This indicates recovery in this section of the stream.

Table 1

Water Chemistry - May 1971

Station Number	1	2	2a	3	4LB	4RB	5	6	7	8	9	10
Free Acidity ppm as CaCO <sub>3</sub>	0	0	NR	0	0	0	0	0	0	0	0	0
Total Acidity (Cold) ppm as CaCO <sub>3</sub> (Hot)	2	2	NR	20	4	4	2	3	2	2	2	2
	0	2	NR	20	4	0	2	0	2	2	0	0
Total Hardness gpg as CaCO <sub>3</sub>	22.1	2.21	NR	4.89	2.79	2.21	2.44	1.86	2.68	2.33	1.28	1.86
Calcium gpg as CaCO <sub>3</sub>	1.74	1.28	NR	2.79	2.09	1.63	1.74	1.39	1.63	1.63	.93	1.51
Fe ppm	0	0	NR	.3	.1	0	0	0	.02	.04	0	0
SO <sub>4</sub> ppm	14.5	14.0	NR	82	41.6	26.5	28.2	11.5	28.8	20.0	9.5	24.0
pH	7.0	7.0	NR	4.0	5.8	6.5	6.5	7.0	6.5	6.8	7.0	6.8
CO <sub>2</sub> ppm	1.76	1.76	NR	14.8	3.52	1.76	1.76	1.76	1.76	1.76	1.76	1.70
Phenolphthalein Alkalinity ppm as CaCO <sub>3</sub>	0	0	NR	0	0	0	0	0	0	0	0	0
Methyl Purple Alkalinity ppm as CaCO <sub>3</sub>	18	10	NR	0	12	14	12	18	14	18	14	12

Table 2

Water Chemistry - July 1971

	<u>1</u>	<u>2</u>	<u>2a</u>	<u>3</u>	<u>4LB</u>	<u>4RB</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
Free Acidity ppm as CaCO <sub>3</sub>	0	0	0	0	0	0	0	0	0	0	0	0
Total Acidity (Cold) ppm as CaCO <sub>3</sub> (Hot)	2	2	2	52	6	2	2	2	2	2	2	2
	0	0	0	56	6	0	2	0	0	2	0	0
Total Hardness gpg as CaCO <sub>3</sub>	3.49	3.03	3.49	10.16	4.78	4.43	4.43	2.68	3.38	3.84	1.51	3.49
Calcium gpg as CaCO <sub>3</sub>	2.91	2.33	3.03	6.17	3.38	3.14	3.61	2.09	3.03	3.14	1.39	2.68
Fe ppm	0	0	62	3	1.5	1.25	.25	.12	.5	.25	.12	.12
SO <sub>4</sub> ppm	12.2	15.5	27.1	195.8	65.8	53.9	59.5	8.9	49.7	48.2	7.1	42.0
pH	7.5	7.0	7.0	7.4	6.0	6.5	7.0	7.0	6.9	7.0	7.0	7.0
CO <sub>2</sub> ppm	1.76	1.76	1.76	51.4	3.52	1.76	1.76	1.76	1.76	1.76	1.76	1.76
Phenolphthalein Alkalinity ppm as CaCO <sub>3</sub>	0	0	0	0	0	0	0	0	0	0	0	0
Methyl Purple Alkalinity ppm as CaCO <sub>3</sub>	30	28	34	0	8	16	12	32	14	14	20	12



**THE EFFECTS OF TERTIARY TREATED WASTE FOR  
NUTRIENT REMOVAL OF EUTROPHICATION OF MODEL  
STREAMS AND RESERVOIRS**

Project B-039-VA

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## THE EFFECTS OF TERTIARY TREATED WASTE FOR NUTRIENT REMOVAL ON EUTROPHICATION OF MODEL STREAMS AND RESERVOIRS

Special experimental apparatus was needed to carry out the objectives of the project. A special, enclosed, photoperiod room, with dimensions of 9-1/2 ft wide by 13-1/2 ft long, was constructed to enclose 6 model experimental streams, each 4 feet in length. Next, a head box was constructed to feed tap water into each stream and to serve as a "seed source" of plants and animals to colonize the streams. Some difficulty was experienced in the Winter, in growing organisms in the streams, but since mid-March there has been a profusion of growth of algae and diatoms, all over the bottom and sides of the streams.

The first experiment introduced sewage effluent which had been treated for phosphorus removal by a tertiary process utilizing iron precipitation, performed in the sanitary engineering laboratories. At present this experiment is still in progress and a second experiment of this part is planned from June to August. The iron precipitation process simulated an actual tertiary treatment plant as it would operate under real circumstances. Total phosphate phosphorus in the resulting treated water has ranged from 0.105 to 0.260 mg/l  $\text{PO}_4 - \text{p}$ . This treated water is monitored weekly for alkalinity, pH, hardness,  $\text{PO}_4 - \text{P}$ ,  $\text{NO}_3 - \text{N}$  and chloride ion content.

Earlier, last winter, a problem arose when the tap water, used to feed the streams, was found to contain excessive amounts of phosphate, up to 1.62 mg/l as orthophosphate. Consequently, a great deal of time and effort was devoted to locating a suitable method and to find a system to remove phosphorus from the tap water. A company and removal process (ion-exchange using special anionic resins) was located and the equipment has been ordered and is to be placed in operation in early June, after the first experiment is concluded.

The first experiment contrasts control stream (mean orthophosphate phosphorus of approximately 0.70 mg/l) with the experimental streams, which receive, in addition to the phosphorus in the tap water, the tertiary treated effluent water in different strengths. Even though the experiment is still operating, it appears that there is little significant difference in primary productivity between the control and experimental streams. Primary productivity has been measured weekly using several methods, such as placing slides in the stream and removing several periodically for analysis, of biomass

accumulation and chlorophyll content. Also, we are attempting to use the  $^{14}\text{C}$  method to measure primary productivity. In addition, air and water temperatures of the streams are monitored daily, and alkalinity, hardness, pH, phosphorus, nitrate - nitrogen and chlorides are monitored weekly.

**PENETRATION AND MIXING OF HEATED JETS  
INJECTED INTO WATERWAYS WITH APPLICATION  
TO THE THERMAL POLLUTION PROBLEM**

Project B-041-VA

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## PENETRATION AND MIXING OF HEATED JETS INJECTED INTO WATERWAYS WITH APPLICATION TO THE THERMAL POLLUTION PROBLEM

During the first year, the development of the approximate analysis and the small scale experiments have been completed. On the basis of the small scale results, a refined, larger scale apparatus has been designed and constructed. This test channel has a 12 in. x 24 in. cross-section, and special care has been taken with the uniformity of the approach flow. Tests began during the latter part of May.

Work on the exact numerical treatment of the full, three-dimensional problem is proceeding on schedule. Check-out computations on simple, limiting cases has been completed.

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