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SOME EFFECTS OF LEAD-ZINC MINING

WASTEWATER ON A STREAM ENVIRONMENT

BY 758

ANTHONY RALPH DE ALONSO HANDLER, 1943

A

THESIS

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[signature]

ABSTRACT

The continuous discharge of lead-zinc mine and mill wastewater into some streams in southeastern Missouri may alter the ecology of those streams. A field and laboratory study was conducted to evaluate the potential pollution problem. Bee Fork Creek, the first stream in that area to receive tailings from mining operations, was selected for this study and periodically surveyed. Laboratory tests included static bioassays of lead, zinc, and copper, and the mode of action of the metals was investigated through post-mortem examination of the experimental fish. The test animals used in the bioassays were bluegill sunfish (Lepomis macrochirus, Rafinesque).

The heavy metals were found to be more toxic in water of low pH and alkalinity. Bluegills which died in the test solutions assayed showed destruction of the gill epithelium and penetration of the metals within gill cells. Analyses on the stream water revealed that noticeable changes in water quality have occurred, but although there was a rise in the concentrations of the heavy metals, these concentrations are still below the limits of acute toxicity. Significant ecological changes were observed downstream from the tailings discharge by the development of a profuse bacterial and algal growth. Recommendations for future study were made with respect to the various observed pollution effects.

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I. INTRODUCTION

History shows that in many regions of the United States land was misused in several manners, resulting in arid and unproductive areas later abandoned by the users, who searched for new land. When this was no longer possible, people became land conservationists, realizing that survival depended upon the land. The history of land depredation could be repeated in the future with the pollution of the Nation's natural water resources. Water has long been recognized as the lifeblood of socio-economic development, and cumulative demands are being placed on water resources by the increase in population and the upsurge in man's standard of living.

In this country, demands for usable water have been projected to increase to 51 percent of the total streamflow by 1980 and to 81 percent of streamflow by the year 2000 (1). These figures speak for themselves. Unless serious and objective action is taken toward water conservation, the United States may see its economic destiny limited by the lack of water. A general awareness of the pollution problem and of the importance of research as the principal pathway for resolving the many conflicting issues of pollution control has developed in the last twenty years. Government, private enterprise, and the general public have devoted considerable attention and resources in planning for national water needs (2). Conservation programs and water quality standards have been developed for the major waterways all over the country.

Among these norms and programs, the proposed water quality criteria for interstate waters between Missouri and Arkansas (3) are of outstanding importance for the development of the area constituting the Black

River Basin. The upper portion of the Black River runs in the South East region of the State of Missouri where farms and forests are the predominant features of a magnificent landscape. Meager land use and sparse population have kept the streams of this basin relatively unpolluted. Recently, however, rich mineral deposits have been discovered in this area, starting a wave of industrial progress whose impact is beginning to be felt. Lead, zinc and copper mines are already operating in the area and promoting the industrial growth of several underdeveloped Ozark counties.

Together with the natural enthusiasm for the forthcoming industrialization, a general concern for the future of the local streams has developed. The experience of other areas has demonstrated that pollution by lead and other heavy metals may have serious consequences upon the ecology of the streams receiving mining wastes. In addition, milling and flotation operations would contribute other chemicals, some of which may have unknown toxic properties. The mining companies in this area have decided to treat their effluents by holding the tailings in retention ponds before discharging them into the streams. This, however, does not prevent soluble metallic salts from reaching the streams, thus presenting a potential hazard for the maintenance of a healthy aquatic biota.

The economic importance of the mining development, allied with the need for preservation of the recreation areas and water resources has brought together the interest of companies and authorities. A research project (4) was initiated in 1968 at the University of Missouri-Rolla to investigate the initial shock and the following long term cumulative

effects of the mining wastes on the water quality and biota of the affected streams. This thesis presents some results found during the first year of investigation.

A. Purpose

The disposal of mining and milling wastes in streams of the presently developing "New Lead Belt" or Viburnum Trend of S. E. Missouri may deeply alter the aspects of such otherwise clear and unpolluted waters. The purpose of this study was:

1. To observe possible ecological changes in the affected streams due to mine discharges, with special reference to local sport fish.
2. To measure, periodically, parameters of water quality which could be changed due to mining wastes, with special reference to heavy metals.
3. To evaluate the effects of heavy metals on fish in water having characteristics similar to those of the streams being studied.
4. To investigate the mode of action of the heavy metals in their acute toxicity to fish.
5. To establish, finally, a first assessment of the effects of the discharge of mine effluents upon the water quality and biota of the streams in question.

B. Scope

In order to achieve the proposed objectives, field and laboratory work was performed. Bee Fork Creek, one stream of the region into which mine effluents have been discharged since 1967, was selected as the area

of study. Sampling stations were conveniently established along the stream. Field work comprised:

1. Collection of samples of water and biota.
2. Observations on the general stream conditions and characteristics of the bottom.
3. Measurements of selected parameters such as dissolved oxygen and temperature.

The studies and analyses performed in the laboratory involved:

1. Evaluation of physical and chemical characteristics of the water samples to determine turbidity, alkalinity, pH, hardness, fluorides, total dissolved solids, and heavy metals.
2. Examination and classification of biological populations.
3. Acute toxicity studies for the determination of the effects of the heavy metals on fish survival.
4. Autopsy and microscopical examination of the gills and other organs of the experimental fish.

The heavy metals studied were copper, lead, and zinc, which are the main constituents of the explored ore. For the toxicity studies, the chlorides of these metals were employed.

The experimental animals used in bioassays were bluegill sunfish, Lepomis macrochirus, Rafinesque.

II. THE AREA OF STUDY

The area selected for this study is located entirely within the boundary of the Clark National Forest, Reynolds County, Missouri. According to the 1967 Missouri census (5), Reynolds County has an area of 819 square miles with a population of 5,161 people, less than seven people per square mile, about one-tenth of the State average at that time. Except for the area around the mines and a few farms, the country is densely covered with oaks and shortleaf pines. More than 2,000 species of plants, and about 700 mammals, birds, reptiles, amphibians, and fishes inhabit the region (6). Figure 1 shows the general location of the study.

A. Bee Fork Creek

Bee Fork Creek is a tributary of the Black River. From its source, one mile southeast of Bunker, Missouri, the stream runs eastward on a bed of sand and gravel for approximately sixteen miles until it flows into the West Fork-Black River some three river miles above Centerville, Missouri. Its drainage basin covers an area of about 35 square miles. Flow measurements in two points of the stream yielded the results shown in Table I.

The clear waters of Bee Fork have served for many years as a natural spawning place for game fish of the region. Circular gravel nests of sunfishes can often be seen during the spring and summer seasons. Until two or three years ago, a fish hatchery was operated by the shores of the stream which attest to the good quality of its waters.

Although flowing completely within the Clark National Forest, Bee Fork has most of its banks adjacent to private lands. Recently, however,

some of this land has been acquired by the Federal Government for the purpose of developing a recreational camp, which will be the first one by Bee Fork (7).

TABLE I

Flow Characteristics of Bee Fork Creek (8)

Location of Measurement	Date	Discharge cfs
NE¼ sec. 25, T.32N., R.2W, at low-water bridge on County Highway TT, 5 miles east of Bunker, Missouri	9-30-65 1-26-66 8-10-66	10.00 4.55 1.54
NW¼ sec. 30, T.32N., R.1W., 100 ft. upstream from low-water bridge on County Highway, 5½ miles east of Bunker, Missouri	9-30-65 1-26-66 8-10-66	11.50 5.62 2.66

Bee Fork was the first stream of the New Lead Belt to receive waste waters from mining operations. At the beginning of this investigation and until very recently, Bee Fork was the only stream in the area to receive mining waste waters, hence its selection for these studies.

B. Fletcher Plant

Owned and operated by the St. Joseph Lead Co., Fletcher mine and mill, after four years of construction work and a capital investiment of 12.5 million dollars (9), started production in February, 1967 (10). Lead-zinc ore is mined on two levels, at 980 feet and at 1,116 feet (11). One of two vertical shafts is for men, materials and ventiliation, and the other is for automatically receiving and hoisting ore, previously crushed underground. On the surface, an automated flotation mill is controlled by a computer which continuously receives analytical information from an X-ray monitoring unit.

Fletcher Plant employs over 160 men, working with some of the most modern equipment in the world. The plant is able to process 5,000 tons of ore per day or 60,000 tons of lead per year (10), almost half of the total production of Missouri in 1966 (12).

Underground water is pumped from the mine at a rate of 5,000 to 10,000 gpm. Part of this water is used in the milling flotation operations, where several reagents are added. The flotation reagents used comprise a series of organic and inorganic compounds as listed in Table II.

TABLE II

Flotation Reagents Used in the Fletcher Plant (13)

Reagent	Conc. %	Feed lbs/ton of water
Sodium isopropyl xanthate	20	0.025 - 0.05
Diethyl dithiocarbamate	100	0.01 - 0.02
Mixed alcohols, 6 to 9 carbons	100	0.02 - 0.03
Zinc sulfate, $ZnSO_4$	15	0.04 - 0.05
Sodium cyanide, NaCN	10	0.0025 - 0.005
Copper sulfate, $CuSO_4 \cdot 5H_2O$	15	0.02 - 0.03
Sodium dichromate, $Na_2Cr_2O_7 \cdot 2H_2O$	10	0.0025

Flotation effluents are taken to a primary sedimentation pond where coarse suspended particles are settled out. The supernatant is then channeled, together with the rest of the underground water, into three ponds in series. Figure 3 illustrates a flow-diagram of the tailings of the Fletcher Plant. The total area of the three ponds is approximately 50 acres and detention time is estimated to be 850 hours (13). The tailings flow into Bee Fork via the West Fork Hollow.

C. Sampling Stations

Five sampling stations were established in the study area; three on Bee Fork Creek, one on the West Fork Hollow, and one on the West Fork Black River. The stations were assigned numbers according to an overall research project which includes other streams of the area (4). Forest Road 2313, which runs alongside Bee Fork most of its length, provided the means of access to the remote sampling points. The map of Figure 2 indicates the relative positions of the stations in the area.

1. Station 6

This station is located on Bee Fork Creek at the low-water bridge on County Highway TT, approximately two miles upstream from the mine discharges. The location of this station, using the map furnished by the Clark National Forest (14), is: Range 2 West, Township 32 North, Southeast Quarter of Quadrangle 24. Due to the unpolluted character of the stream at this point, this site was selected to be a control station. The stream is partially impounded by the bridge and has depths reaching eighteen inches. The water flows under the bridge through four large corrugated steel pipes.

2. Station FT

This station is located in the West Fork Hollow at the intersection with Forest Road 2313, about 200 yards from the point where the tailings reach Bee Fork. The location on the map is: Range 1 West, Township 32 North, Northwest Quarter of Quadrangle 30. The water passing this point is the effluent of the sedimentation ponds at Fletcher Plant, hence the selection of this point for sampling.

3. Station 7

This station is located on Bee Fork Creek at the intersection with Forest Road 2313, about one-half mile downstream from the mine discharge. Its location on the map is: Range 1 West, Township 32 North, Northern Half of Quadrangle 30. At this point, the stream water and the tailings are already well mixed, which was the reason for the selection of this station. The stream is partially impounded by the low-water causeway and usually flows over it with a depth of about one inch.

4. Station 8

This station is located on Bee Fork Creek at the intersection with Forest Road 2313, approximately two and one-half miles downstream from the mine discharge. Location on the map is: Range 1 West, Township 32 North, Southern Half of Quadrangle 21. This station was selected to allow observations on the persistance of the pollution and stream recovery. Bee Fork is much wider at this point, as well as shallower.

5. Station 9

This station is located on the West Fork Black River, some thirty yards upstream from the bridge on State Highway 21, about one mile North of Centerville, Missouri. Its location on the map is: Range 1 East, Township 32 North, Southern Half of Quadrangle 20. It was selected to allow a comparison between the water quality of Bee Fork and the Black River.

6. Points of Collection of Samples

With the exception of Station 9, all samples were collected at approximately the center of the stream flow. At Station 9, due to the considerable depth of the stream, samples were taken from the right bank (facing downstream) of the river.

Figure 1. General Location of the Study in Reynolds County, Missouri

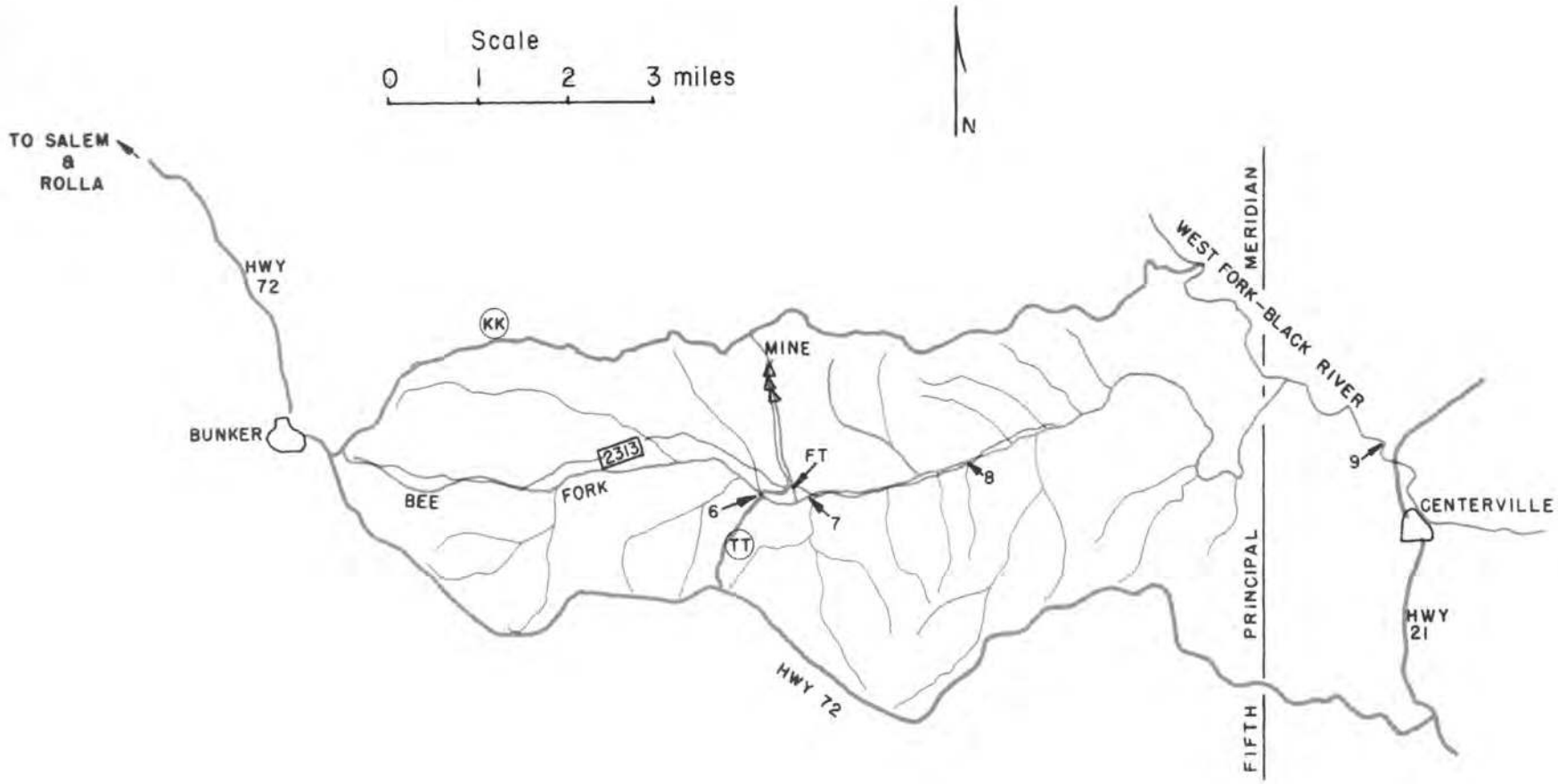

Figure 2. Location of the Sampling Stations

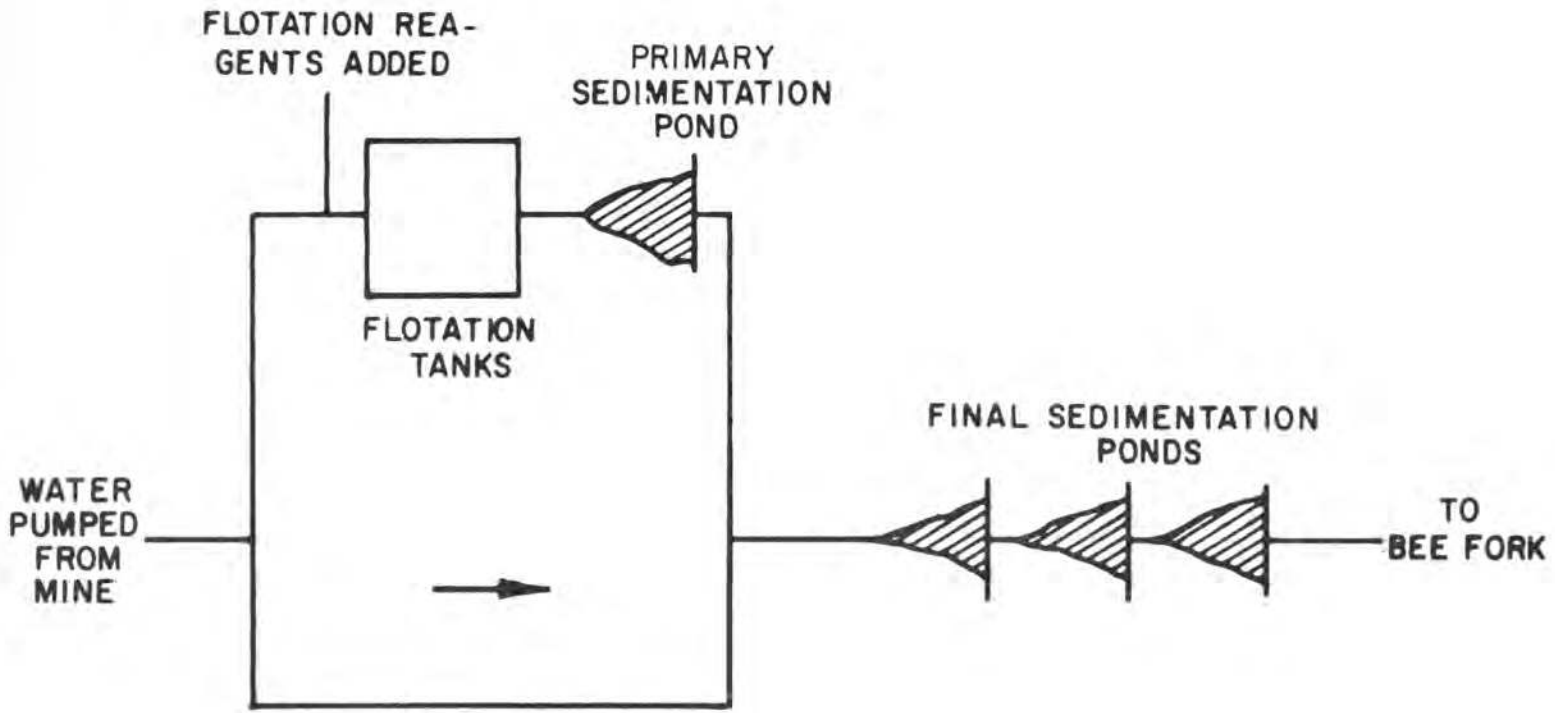

Figure 3. Flow Diagram of Mine Water at Fletcher Plant

Figure 4. Station 6, on Bee Fork Creek

Figure 5. Station FT, in West Fork Hollow

Figure 6. Station 7, on Bee Fork Creek

Figure 7. Station 8, on Bee Fork Creek

Figure 8. Station 9, on West Fork-Black River

III. REVIEW OF THE LITERATURE

A. Bioassay Studies Using Fish

The effects of a specific compound upon the several forms of life can be verified by experimentally assaying the compound on the animal or plant and observing the reactions of the organism under controlled conditions. In the case of aquatic life, fish has been the most employed experimental animal, due to its important position in the ecological cycle of a stream or lake, as well as in all aspects of its relationship with man. Although it would be rather difficult to establish which the first experiments of this type were, it is accepted that some of the first bioassays using fish were conducted more than a century ago, in Scotland, by Penny and Adams, as referred by Jones (15). In those experiments two species of fish were used, the minnow and the goldfish, the former for its prompt response to even slight changes in the environment, the latter for its high resistance to poisons, making possible a more detailed observation of the intoxication process. Fifty years later, in the United States, an important paper was published by Powers (16) to become a current reference for those interested in pollution effects. Powers' work was one of the first attempts to analyze the relationship between survival time and toxicant concentration under a statistical and mathematical approach.

Some years later, in Wales, fish were again employed for the evaluation of pollution effects, this time in a different fashion. Studying the destruction of river fisheries by pollution due to lead mining, Carpenter (17,18) placed a cage containing some minnows into a lead polluted stream. Her investigations on the mode of action of the

metal were performed by autopsy of the dead fish and histological examinations. Laboratory bioassay studies were also performed by Carpenter (19,20). In 1927, an important paper was published by Belding (21) in which the question of the influence of environmental and physiological conditions on the bioassay of a pollutant was raised. It was pointed out that the result of such studies might be significantly affected by factors such as species, sex, age, size, etc. of the fish used as well as temperature and oxygen content of the water, volume of solution per fish, etc. The need began to be felt for specific research aiming to a more thorough control of bioassays.

In 1937, Ellis (22) published the results of an extensive survey on water quality of rivers and streams of the United States, as well as the results of the bioassays of several substances commonly found in industrial and domestic wastes. His bioassays were conducted using both soft and hard water and environmental conditions were given careful attention. Another important feature of Ellis' work was the discussion of the mode of action of several pollutants. A distinction was then made between pollutants, according to the locus of injury and mode of action of the various active substances that the effluents contained. Later, in 1945, another major step was taken with the publication of a comprehensive book on bioassays by Hart et al. (23) which brought about the interest of several authorities. A mixed committee was formed to study the matter of standardization, and a final report was published in 1951 (24). Many modifications have been suggested since then, but the committee report is still the basis for the procedures outlined in Standard Methods (25).

There are three essential constituents of a bioassay: the subject, the stimulus, and the response (26). The latter can be observed and reported in many ways, but two major approaches are predominant. Since the degree of response is a function of time and concentration of the poison, its measurement can be accomplished either under constant concentration or constant exposure time. The American approach is to keep time constant, and the results of the bioassay are expressed in terms of the median tolerance limit, the concentration which will kill half of the test fish within a determined period of exposure. This period generally is 24, 48, or 96 hours. There is no basic reason for these specific numbers except the purpose itself of standardization.

The increase of survival time of experimental fish with a reduction on the concentration of the toxicant assayed suggests, and it has been experimentally observed (27,28,29), the existance of a point below which survival time will be independent of the toxicant concentration. The elimination of toxins by fish, as well as any organism, is an important mechanism of defense. Actually, it is only when the rate of detoxication is surpassed by that of toxication that a lethal effect may result (29). The search for this maximum safe concentration has been the object of several recent researches (30). Abram (27) summarized the approaches used in the studies for the determination of threshold concentrations in the following manner:

1. Long-term experiments in which survival time is related empirically to concentration.
2. Shorter-term tests with a narrow range of concentration, which kill only a proportion in each of the number of batches.

3. Comparison of survival time in a range of constant concentrations with survival time over the same range, but alternating with recovery periods in clean water.
4. Extrapolation of the results of short-term tests by assuming some type of mathematical function of known properties to describe the response distribution.

Long-term experiments are troublesome and difficult to carry out. Death by other factors, like disease, for instance, can occur (28) invalidating the results. Many fishes are difficult to keep under laboratory conditions, requiring special facilities, not always available to the investigator. This leaves, as a practical alternative, the extrapolation of short-term tests based on the assumption of a mathematical model (31). The choice of such a model is a function of the mode of action of the toxicant as pointed out by Haider (32). Lloyd (33) has also indicated that the mode of action is an important factor in the definition itself of the existance of a threshold concentration.

B. The Action of Heavy Metals on Fish

Studies of the toxicity of heavy metals to fish have been undertaken by several investigators. Carpenter (19) performed laboratory experiments with lead acetate and minnows. It was noticed that the toxicity to fish of dilute solutions of lead could be increased by renewing the solutions periodically. The survival time of fish killed successively in the same solution increased progressively, showing that the solution was becoming less toxic. Minnows exposed to 1 mg/l of lead survived after thirteen days when the solution was not renewed, but died in 48 hours when the solution was renewed seven times during

the experiment. Even at the low concentration of 0.4 mg/1, lead proved toxic to minnows within 24 hours when the solution was renewed nine times. This shows that lead will slowly precipitate out of solution, and the precipitate seems to be harmless to fish, as Carpenter suggested.

Based upon Carpenter's observations, Jones (34,35) studied the relative toxicity of salts of lead, zinc, and copper to the threespine stickleback (Gasterosteus aculeatus). The water used in his tests was described as very soft, with a calcium content of 1 mg/1. The pH ranged from 6.4 to 6.6 units in all tests, and solutions were renewed daily. Jones' results are summarized in Table III, compiled from a review by Doudoroff and Katz (36).

TABLE III

Metal Concentrations for Various Survival Times of Fish (36)

Metal	Salt Tested	Concentration of Metal, mg/1, at which survival time averaged.			
		1 day	2 days	4 days	7 days
Cu	$Cu(NO_3)_2$	0.3	0.1	0.06	0.02
Pb	$Pb(NO_3)_2$	1.0	0.7	0.3	0.2
Zn	$ZnSO_4$	1.5	0.9	0.7	0.4

Pickering and Henderson (37) performed extensive tests to evaluate the acute toxicity of some heavy metals to different species of warm water fishes. Toxicity was reported in terms of the median tolerance limit, TL_m. Fishes tested were: fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), goldfish (Carassius auratus), and guppies (Lebistes reticulatus). The first three species were 1.5 and 2.5 inches long, and weighed one to two grams. The guppies were 0.75

to 1 inch long and weighed 0.1 to 0.2 grams. Static bioassays were conducted using two types of water: a soft water having a pH of 7.5, alkalinity of 18 mg/1 as $CaCO_3$, and total hardness of 20 mg/1 as $CaCO_3$, and a hard water having a pH of 8.2, alkalinity of 300 mg/1 and total hardness of 360 mg/1. Dissolved oxygen in all tests was reported at 7.8 mg/1 in the beginning of the experiments and always above 4 mg/1. No aeration was performed and water temperature was maintained at 25°C. Some of the results are summarized in Table IV. The values are expressed in terms of concentrations added, not the actually measured values.

C. Mode of Action

It has been already established that copper, lead, and zinc act upon the fish by impairing its respiratory function. Carpenter (19) observed that fish killed in toxic lead solutions presented symptoms of acute respiratory distress before death. A veil-like film covered the body of the fish. This film was also seen covering the gills and coming out of the edge of the operculum. The respiratory movements were extremely slow, feeble, and intermittent. Several factors seemed to lead to the conclusion that slow suffocation, with coagulation of the mucus around the gill, was the cause of death. Fish which survived at lower concentrations presented all the described symptoms during the first days, but finally recovered after the film sloughed off and was not renewed from the skin. However, if solutions were renewed periodically, the mucus coating continued to accumulate and death finally occurred. Analysis of the mucus revealed considerable quantities of lead in organic combination, probably in the form of albuminate. Further research by Carpenter (20) with salts of other heavy metals, including zinc and copper, showed a similar mode of toxic action for those metals.

TABLE IV

Median Tolerance Limits of Some Heavy Metals (37)

Salt	Test Fish	Dilution water	TL_m, in mg/l of metal		
			24-hr	48-hr	96-hr
$CuSO_4 \cdot 5H_2O$	Minnows	soft hard	0.040 2.71	0.035 1.86	0.025 1.76
	Bluegills	soft hard	0.86 10.7	0.74 10.2	0.66 10.2
	Goldfish	soft	0.094	0.043	0.036
	Guppies	soft	0.13	0.073	0.036
$PbCl_2$	Minnows	soft hard	8.18 482	5.99 482	5.58 482
	Bluegills	soft hard	25.9 482	24.5 468	23.8 442
	Goldfish	soft	45.4	31.5	31.5
	Guppies	soft	24.5	24.5	20.6
$ZnSO_4 \cdot 7H_2O$	Minnows	soft hard	0.88 34.5	0.78 33.4	0.78 33.4
	Bluegills	soft hard	5.75 40.9	5.11 40.9	4.85 40.9
	Goldfish	soft	9.07	6.44	6.44
	Guppies	soft	2.90	1.96	1.27

Ellis (22) agreed with Carpenter's conclusions, pointing out that the formation of insoluble compounds coated the gill filaments and filled the filament interspaces, thus preventing aeration of the blood. Death followed from a combination of anoxemia and carbon dioxide retention. The coagulation film anoxia theory was also supported by Westfall (38) and by Jones (34). The latter observed that the breathing rate of fish increased sharply in toxic solutions of lead, zinc and copper salts. It remained abnormally high until the fish were returned to clean water, or until shortly before death, when it fell rapidly. The rate of oxygen consumption, however, fell gradually until death, after an initial increase probably due to stimulation of activity.

The theory that the action of the metals is only external was contested by Behrens (39). Using a radioactive isotope as a tracer, he observed some penetration of lead into internal tissues of fish held in solutions of the chloride. Evidence in the same line was later presented by Haider (32), who studied chronic lead toxicosis in trouts. In acute toxicity, however, the action of the heavy metals was felt on the respiratory system only. Evidence on this line, although not in agreement with the coagulation film anoxia theory, was presented by Lloyd (40) who studied the acute toxicity of zinc to rainbow trout. Lloyd's conclusions were that the death of the fish should be attributed to damage to the gill tissues. More recent research tends to confirm this statement (41). Histological examinations of the gills of fish killed by lead, zinc, and copper salts showed that the epithelial cells separated from the filaments and from the lamellae.

Further research is needed to establish the exact nature of the toxic action of heavy metals on fish. Jones (15) mentioned the

possibility that this action could depend on the nature and quantity of the gill secretions, which may vary with each species. Some species may produce relatively little mucus, thereby leaving the gill tissues exposed to damage by the metals. Others may produce abundant secretion, which protects the tissues but leads to asphyxiation when precipitated. Whatever the case may be, however, it seems that the locus of injury is, undoubtedly, the gills. This is also indicated by the work of Mount (42), who developed an autopsy technique for detecting acute zinc poisoning in fish. The method is based on the fact that accumulation of the metal in the opercular bone is accomplished at approximately the same rate as in the gill tissues under exposure to sublethal concentrations. In acute toxicity, however, the gills accumulate zinc at a much faster rate. A similar procedure has also been developed for cadmium (43).

D. Importance of Environmental Factors

Several factors have long been recognized as having a definite influence on the toxic action of heavy metals and other poisons to fish (21).

Lloyd (33) stated that a decrease in temperature usually increases the survival time of fish in toxic solutions. His experiments with rainbow trout in solutions of zinc showed that the survival times increased more than twice when temperature was reduced from 22°C to 12°C. The experiments of Pickering and Henderson (27) seem to confirm that rule. The acute toxicity of zinc to the fathead minnow was increased by a factor of more than three times when temperature increased from 15°C to 25°C. Bluegills were also affected, but less markedly. Crandall and Goodnight (44) attributed this phenomenon to the fact that, since

fish are poikilothermous animals, their rate of metabolism is directly dependent upon environmental temperature. Thus, at a greater metabolic rate, the poisons would act more rapidly on the cells, and death would occur faster.

Dissolved oxygen concentration has also been seriously considered in bioassays. Pickering (45) exposed bluegills to toxic zinc solutions under varying dissolved oxygen concentrations. It was found that the environmental stress of low dissolved oxygen resulted in an increased mortality. The difference in the average TL_m values between low (1.8 mg/1) and high (5.6 mg/1) test concentrations of dissolved oxygen was a factor of 1.5. A similar relation was found by Cairns and Scheier (46). Lloyd (40) found that zinc was 1.5 times more toxic to trout at 3.8 mg/1 than at 8.9 mg/1 of dissolved oxygen. The trout used had not been previously acclimatized to the lower concentration.

Hydrogen ion concentration was found to have a decisive influence on the toxicity of sodium pentachlorophenate to fish (44). Survival times of fathead minnows more than doubled when the pH was increased from 5.9-6.0 to 7.5-7.6, and all fish survived at the pH of 8.9-9.0. These tests were conducted under 1 mg/1 of sodium pentachlorophenate at 18°C. Sprague (47) studied the toxicity of zinc and copper to young Atlantic salmon. He found that zinc sulfate was more toxic at lower pH values, due to precipitation of zinc at higher pH. An important study conducted by Mount (48), however, led to a conclusion directly opposed to the previous opinions. Mount tested the toxicity of zinc sulphate to minnows at three pH levels: 6, 7, and 8. Continuous-flow four day tests were performed. Although precipitation of the metal was visible at the highest pH, zinc was more toxic at pH 8 than at pH 7, and more

toxic at pH 7 than at pH 6. Sloughing of mucus was also greater at higher pH. Fish dying at the high pH was covered with the coagulated mucus, while fish killed at pH 6 appeared clean. Histological examination of the gills showed breakdown of epithelial cells in all cases with no differences due to pH variation. Mount concluded that the precipitated zinc seems to exert a toxic action when in suspension.

Jones (34) found that the addition of calcium salts to toxic solutions of lead and zinc reduced the toxicity of these metals. When 50 mg/l of calcium was added to test solutions, stickleback seemed unaffected by 1 mg/l of lead or 2 mg/l of zinc, concentrations that he had found lethal to the same fish in less than 24 hours in soft water. Copper, lead, and zinc have been shown to be more toxic in soft than in hard water. Mount (48) indicates that at any given pH, zinc toxicity decreased with increased hardness. According results were also reported by Pickering and Henderson (37). Although stating that some of the differences observed may have been caused by the decreasing solubility of the metals when the water becomes more alkaline, Lloyd (33) confirmed that the presence of calcium ions in the water reduced the toxicity of the heavy metals. Jones' suggestion that the presence of calcium prevented the precipitation of mucus (34) does not agree with the more recent works by Mount (48) and Lloyd (40). Lloyd (33) concluded that the reason why an increase in calcium ion concentration reduces the toxicity of heavy metals is still unknown.

E. Stream Pollution by Heavy Metals

Carpenter (17) studied the pollution by lead mining of several rivers in the Aberystwyth district of Cardiganshire, Wales. A definite

relation between the presence of lead in solution and poverty of fauna was established. After the cessation of mining and washing operations, a rapid increase in the flora and fauna of two streams was observed (18). This recovery process was described as having three distinct ecological phases, directly associated with presence of dissolved lead. Establishment of mollusca and fishes was only observed after several years, when not even intermittent presence of dissolved lead could be detected.

Jones (49) stated that the destructive effect of zinc pollution upon river fisheries may be as serious as that caused by lead mining. His studies of the river Ystwyth, in Wales, revealed that zinc pollution was the main factor for the absence of mollusca, crustacean, and fishes. The fauna of the mainstream was limited to aquatic insects and worms, which appeared to be very tolerant of zinc pollution. Similar remarks were made by Newton (50) in her study of the Rheidal river once polluted by lead and zinc mines.

Studies on the sublethal copper-zinc pollution of the Northwest Miramichi River, New Brunswick, Canada, were conducted by Sprague et al. (51). The authors observed that 10 to 22 percent of ascending salmon returned downstream during four years of stream pollution, compared with 1 to 3 percent before pollution. Copper and zinc levels in the stream were not enough to cause death of the fish, but avoidance by migrating adult salmon was evident. Young salmon populations decreased heavily in the stream every year after pollution, due to a low supply of eggs and poor survival rates.

The effect of zinc upon reproduction of fish have also been determined by Brungs (52). Long-term laboratory tests were conducted. It was found that reproduction was almost totally inhibited at concentrations

which had no effect on survival, growth, or maturation of the same fish. The number of eggs produced by female at 0.18 mg/l of zinc was only 17 percent of the eggs produced in the control unit containing 0.03 mg/l of zinc.

IV. EQUIPMENT, MATERIAL, AND PROCEDURES

In order to achieve the proposed objectives, field and laboratory work was performed. Field work comprised collection of samples of water and biota, observation of the general stream conditions, and measurements of some parameters such as temperature and dissolved oxygen.

The studies and analyses performed in the laboratory involved the determination of physical and chemical characteristics of the water samples, examination and classification of biological samples, bioassay studies, and autopsy and examination of the experimental fish.

A. Analytical Procedures

1. Turbidity

Turbidity measurements were made in the laboratory using a Hach Laboratory Turbidimeter Model 1860, which is a nephelometer. Calibration was accomplished by using a plastic standard furnished with the instrument. The values obtained with this instrument were not always in good agreement with those resulting from determinations by the Jackson Candle Turbidimeter. This procedure, however, was found to be simpler, faster, and more convenient with water samples having low turbidities.

2. Temperature

Air and water temperatures were determined, both in the field and in the laboratory, with the thermistor probe of a Precision Galvanic Cell Dissolved Oxygen Analyzer.

3. Dissolved Oxygen

Dissolved oxygen was measured using a Precision Galvanic Cell Dissolved Oxygen Analyzer. The instrument was calibrated against a sample of known dissolved oxygen measured by the Azide Modification of the Winkler Method, as recommended in Standard Methods (25, p. 406). The instrument was calibrated and used in the same day. Compensation for temperature variations was made by using a special slide-rule nomograph furnished with the instrument.

4. Hydrogen Ion Concentration

Measurements of pH were performed with a Beckman Zeromatic pH Meter with manual temperature control. Each time before use, this instrument was checked with a buffer solution having a pH of 6.86 at 25°C.

5. Alkalinity

Total alkalinity was determined by titration with 0.02 N standard sulfuric acid (25, p. 48). Methyl Orange indicator was used to show the titration endpoint.

6. Hardness

Both total and calcium hardness were determined by the EDTA Titrimetric Method (25, p. 147). A commercial titrant, Titraver*, and indicators, Univer* and Calver*, were used. The strength of the titrant was checked against a standard solution of 1 mg/ml calcium chloride, and corrections were made in the calculations.

7. Fluoride

Fluoride was determined using the SPADNS Method, as described in Standard Methods (25, p. 144). A Delta Water Analyzer Model 260 was the

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*Products of Hach Chemical Corporation

the photometer used. The reagents and procedures employed were in accordance with the manufacturer's specifications.

8. Total Dissolved Solids

Gravimetric analyses of total dissolved solids were run according to Standard Methods (25, p. 245). The samples were passed through a membrane filter having a pore size of 0.45 microns, and evaporated in porcelain dishes. After cooling overnight, the dishes were weighed in a Sartorius Analytical Balance Series. Duplicate samples were run for each test. Results were recorded only when they differed not more than five percent.

9. Heavy Metals

Analyses for heavy metals in the water samples were performed in the Geology Department of UMR. The instrument used was a Perkin Elmer Atomic Absorption Spectrophotometer Model 303 (53).

Analyses of copper and zinc concentrations in some samples from the laboratory test units were performed by simpler methods in the Sanitary Engineering Laboratory, since these samples were neither subject to interference from other ions nor required great accuracy in the results. The simpler procedure followed for copper determination was the Cuprethol Method (25, p. 131) and for zinc the Zincon Method (25, p. 320). The photometer used in both cases was a Delta Water Analyzer Model 260.

10. Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) determinations were performed by using the standard reflux method (25, p. 510). Twenty-milliliter sample size was used for each determination.

11. Biochemical Oxygen Demand (BOD)

The biochemical oxygen demand (BOD) of water samples were measured according to Standard Methods (25, p. 415). The samples were not diluted nor seeded, since the stream water already contained the necessary microorganisms. No preaeration of the samples was necessary, since the initial dissolved oxygen was always higher than the BOD by more than 1 mg/l. During each test, the bottles were incubated at 20°C, and kept in cardboard boxes to prevent exposure to light. Twenty-day tests were peformed, with one daily determination during the first five days and, thereafter, one determination for each additional five days.

B. Biological Procedures

1. Classification of Biological Samples

All samples of stream biota brought to the laboratory were examined and identified according to available keys and check-lists. Whenever necessary, microscopical observations were made, and photomicrographs were taken for future comparisons.

Fish were identified according to Pflieger (54). Identification of snails and crayfish was made according to Ward and Whipple (55), as recommended by the Missouri Department of Conservation (56). Algae were identified according to Palmer (57). All fish were identified as to genus and species, but other biota were classified as to genus only.

2. Bioassays for Evaluation of Toxicity

The purpose of the bioassay studies was to evaluate the acute toxicity of the heavy metals on fish. Although the determination of median tolerance limits (TL_m) was not the goal of investigation the procedures followed paralleled those outlined in Standard Methods under Routine Bioassay Method (25, p. 551).

For each experiment, five test samples were prepared and a control test was also performed in exactly the same manner as the other tests. Based on the reported TL_m values in the literature for similar experimental conditions and comparable water quality, an appropriate range of concentrations constituting a logarithmic series as suggested by Standard Methods (25, p. 554) was selected.

The test fish studied were acclimatized to the dilution water for at least ten days prior to any experiment. Two days before the test, an appropriate number of fish with uniform size were transferred from the acclimation aquarium to a smaller 3-gallon glass aquarium. Two days later, five fish were taken at random from the 3-gallon aquarium and placed in the control and test jars containing the solution to be assayed. The jars were aerated as described elsewhere.

Four-day tests were conducted on a regular basis with some tests extended up to seven days. The fish were not fed during the tests and the two days preceeding them. In the extended tests, the fish were fed daily during the last three days.

The reactions of the fish were constantly observed during the first four hours and periodically throughout the tests. Deaths were recorded as noticed and survivors counted after each 24 hours of exposure. Abnormal behavior, such as increased respiratory rate, loss of equilibrium, distress, was also noted and recorded. The fish were considered dead when no opercular or other movements could be detected during a five-minute observation even when the animal was touched with a glass rod (25, p. 555).

Water samples were taken from each test jar at the beginning of the bioassay and every 24 hours thereafter, and analyzed for pH, alkalinity,

hardness and the metal in question. Dissolved oxygen and temperature were measured not less than once every eight hours throughout the tests.

3. Autopsy Procedures

In order to establish the mode of action of the heavy metals in their acute toxicity to fish, all test animals which died during the experiments were dissected for examination of certain organs. Control fish were also periodically examined for comparison purposes.

Dissection and preliminary examinations were performed under a Bausch & Lomb SteroZoom binocular microscope Model BVB-73. Removed organs were further examined with the aid of a Bausch & Lomb DynaZoom binocular laboratory microscope, Model PB-25, equipped with a Polaroid camera.

The examined fish was laid on its right side and fixed to the wax of the dissection tray by three pins not crossing the body cavity. Using a scalpel, the left operculum was cut away, allowing observation of the gills and the interior of the mouth. Next, the body cavity was opened and a visual search was carried out for hemorrhagic and gross pathological conditions, such as enlargements and inflamations of the organs. Organs observed were the gas bladder, heart and liver.

After examination, the gills were carefully extracted and placed in 10 percent formaldehyde or 95 percent ethyl alcohol for further processing. The remainder of the dead fish was then discarded.

4. Preparation of Gills for Microscopical Examination

The Mallory-Parker stain for lead and copper (58) was the procedure followed in the preparation of the gills for microscopical examination

After the gills were extracted from the test fish, they were immersed in 95 percent ethyl alcohol or in 10 percent aqueous solution

of formaldehyde, depending upon whether the fish had been killed by lead or copper, respectively. Fixation was accomplished by immersing the tissues in these solutions overnight or longer.

Following fixation, the gills were embedded in paraffin and sectioned with a Jung AG rotary microtome*, regulated to cut 6- to 8-micron thick sections. The sections were placed on single-frosted microscope slides prior to staining.

Staining for copper followed the regular Mallory-Parker technique, as outlined below:

1. Xylene, absolute alcohol, 95 percent alcohol, distilled water.
2. Stain sections in hematoxylin for 2 to 3 hours at 54°C.
3. Wash in several changes of tap water 10 to 60 minutes.
4. 95 percent alcohol, 2 changes in absolute alcohol, 2 changes in xylene.
5. Mount in Caedex.

Some modifications (59) were introduced in staining the sections for lead, and the procedure followed was:

1. Xylene (5 min.), absolute alcohol (3 min.), 95 percent alcohol (3 min.), distilled water (3 min.).
2. Stain sections in hematoxylin for 1 hour at 54°C.
3. Dip in tap water long enough to remove excess stain from slide.
4. Dip in 95 percent alcohol, dip in absolute alcohol, place in xylene for one minute.
5. Mount in Caedex.

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*A product of R. Jung AG, Heidelberg, Germany.

A constant temperature of 54°C for hematoxylin staining was obtained with the use of a "Magni Whirl" constant temperature bath* Model MSB-1122A-1.

The stain resulted in lead appearing as a light to dark grayish blue, copper as intense blue, and the cell nuclei as deep blue.

Normal gill tissue was also processed for comparison.

C. Experimental Material

1. Collection of Samples

Water samples were collected and transported to the laboratory in 1-liter amber polyethylene bottles having a screw-type cover. All water samples intended for BOD or COD determinations were immediately placed in ice until processed.

Fish were collected with a minnow seine and transferred to an appropriate container so that they arrived alive at the laboratory. During the summer months, ice was placed in the containers to prevent excessive temperature elevation during transport.

Benthic samples were collected by hand or using a dip-net, and placed in plastic bags or in wide-mouth, rigid-plastic containers with tight covers.

2. Equipment for Bioassays

The equipment used in the bioassay studies consisted of a battery of wide-mouth glass jars of one-gallon capacity. Each jar was aerated by means of a glass tube of 4 mm inside diameter connected to the laboratory compressed-air supply by rubber tubing. Before being delivered

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*A product of Blue M Electric Company.

to each flask, the air passed through an activated-carbon filter. Screw-clips in the rubber tube allowed regulation of the air flow, so that an adequate dissolved oxygen level was maintained at all times without much agitation of the water in the test jars.

3. Metal Salts Tested

Reagent grade chlorides of copper, lead and zinc were used in the bioassays. A standard solution of each salt, containing 1 mg/ml of the metallic cation, was carefully prepared not more than one day before the beginning of each test. The diltuion water used for the preparation of all reagents was demineralized water obtained by passing tap water through a Bantam Demineralizer* Model BD-1.

4. Dilution Water for Bioassays

The dilution water used in the bioassays was the same water to which the fish had been acclimatized in the stock aquaria. The water was prepared in the laboratory by properly mixing water from a local well** with demineralized water. The characteristics of the dilution water are shown in Table V. The water in all aquaria was thoroughly aerated, as described in another section. Dissolved oxygen content was always above 6.5 mg/l, and temperature was $21 \pm 1^{o}C$.

5. The Experimental Fish

In order to determine the test-fish to be used in the bioassays, three species were first considered and brought to the laboratory for

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*A product of Barnstead Still and Sterilizer Co., Boston, Mass.
**Located at Dr. B. G. Wixson's residence, Line Barnitz Forest, Rolla, Missouri. The water is pumped from a depth of 75 meters, and was found to be relatively free from the heavy metals studied.

TABLE V

Characteristics of the Dilution Water

Characteristic	Concentration		
	Average	Maximum	Minimum
pH, units	7.9	8.1	7.8
Alkalinity, mg/l as $CaCO_3$	95	103	83
Hardness, mg/l as $CaCO_3$			
Total	100	106	90
Calcium	60	73	52

further evaluation. These were the northern studfish (Fundulus catenatus), the bleeding shiner (Notropis zonatus), and the bluegill sunfish (Lepomis macrochirus, Rafinesque), representative of the most numerous species in Bee Fork Creek. The fish were placed in a 15-gallon aquarium containing stream water at 21°C and fed commercial fish food.

The inadequacy of the northern studfish was promptly noticed, as it was difficult to feed and starved to death within a few weeks.

The bleeding shiner, endemic of the region, was found to be slightly adaptable to laboratory conditions. However, its fragility and susceptibility to diseases when crowded in the static waters of the aquarium makes this species impractical as an experimental fish. It died rapidly when taken out of the water for measurements, and required very gentle handling in transportation. It was also found extremely sensitive to temperature changes.

The best suited species for laboratory testing was found to be the bluegill sunfish. It feeds on most kinds of usual commercial fish-food after a short period of adaptation, generally less than two days, and is not as affected by crowding as the minnows. Common diseases, like tail and fin rot, can be easily controlled and the fish respond well and readily to treatment. Bluegills demonstrated a very high recovery capacity. They can be removed from the water for measurements or transport for periods up to one minute without visible damage, and are very resistant to handling.

Bluegills are distributed throughout the United States, and are probably one of the best known members of the sunfish family. They have long been recognized as important sport fish, and their meat is considered

"fine food" (60), which gives them economical importance. Sunfish are recommended test animals by Standard Methods (25, p. 549), and the bluegill has been widely used in bioassays (37,45,61).

Approximately 1,000 young bluegills, whose characteristics are given in Table VI, were obtained from the Missouri Department of Conservation*, and transported to the laboratories in two twenty-gallon polyethylene bins. In the laboratory, they were placed in fifteen-gallon glass aquaria equipped with an aeration apparatus consisting of two diffusion stones per aquarium.

Formalin baths were used to prevent external diseases, greatly favored by the static conditions of the aquaria. The treatment prescribed by Lagler (60) - 25 percent formalin 1: 4,000 for one hour - was found to harm the young bluegills to such an extent that death often occurred in abnormally high levels during the following 24 or 48 hours. The procedure was then changed, and fish were finally placed in a 1: 6,000 dilution of 25% formalin solution for one half hour, with no consequent mortality and apparently an effective control of external diseases. Internal infections were prophylactiely treated by daily addition of Terramycin** (4 mg/l) to the holding aquaria (28).

The fish were fed ground Purina trout chow daily in adequate amounts (24), and Permalife Tubifex worms were also periodically used as a food source.

The aquaria were cleaned by frequent removal of unconsumed food and other sediment by means of a syphon. Aquaria water was made up daily and completely renewed every two days. The aquaria were placed in an air conditioned laboratory with temperature maintained at 21°C.

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*Indian Trails Hatchery, Steelville, Missouri.
**A product of Chas. Pfizer & Co., Inc., New York, N. Y.

TABLE VI

Characteristics of the Experimental Fish

Species: Lepomis macrochirus, Rafinesque				
Physical Characteristics	Average	Maximum	Minimum	Number Observed
Length, cm	2.8	3.4	2.4	60
Weight, g	0.64	1.18	0.38	60
Coefficient of condition, K*	33.4	-	-	60
Age, weeks**	-	20	12	All

*Average of all calculated K values. K was taken as the weight in grams divided by the cube of the length, in cm (60).

**Estimated age of fish when tested, based upon information obtained from the Missouri Department of Conservation.

V. RESULTS

A. Ecological Observations

Three distinct ecological patterns were observed in Bee Fork Creek. Above the mine discharge the stream fauna is relatively rich, with several species of fish and other aquatic animals. The predominant fish species observed were the northern studfish (Fundulus catenatus), the bleeding shiner (Notropis zonatus), the bluegill sunfish (Lepomis macrochirus, Rafinesque), and the rainbow darter (Etheostoma caeruleum, Storer). Other fish occurring, but less frequently observed, were the green sunfish (Lepomis cyanellus, Rafinesque), and the smallmouth bass (Micropterus dolomieui, Lacepede). The fish were less than three inches long and the majority of the collected specimens measured less than two inches in length. Two benthic animals were usually found in the unpolluted part of Bee Fork: snails of the genus Goniobasis, and crayfish of the genus Orconectes. The snails were particularly numerous during the late summer and early fall months, but crayfish were present throughout the year.

Filamentous algae of the genus Spirogyra were often present, growing mainly close to the shores. Their growth, however, was always limited to small sporadic clusters. Microscopical examination of the water revealed the presence of many diatoms composed primarily of the genus Stauroneis.

A completely different environment was observed downstream from the mine discharge. At station 7, the stream bottom was entirely covered by a thick growth of algae and other organisms forming a sponge-like "mat". Microscopic examination of the growth showed large bacterial colonies combined with a prolific growth of the blue-green algae Oscillatoria.

The algal growth originated a biological net in which fine mineral particles and diatoms were entrapped. Diatom populations were visibly higher at this site. Parts of the "mat" occasionally floated and were carried downstream. Fish were seldom found in this area with only occasional single visitors spotted once or twice. Crayfish and snails were also absent. Figure 9 shows the difference between the stream bottom at stations 6 and 7.

At station 8, the stream bottom was cleaner, but crayfish and snails were not found to be present. Bleeding shiners were sometimes seen in small groups. In the early summer a water hole by the shores of Bee Fork, which was fed by a tributary small stream, was found to contain an abundance of crayfish. However the shells of dead crayfish were observed at station 8 in the water of Bee Fork Creek.

B. Analytical Results

The results of the analyses on water samples from the study area are presented in Tables VII, VIII, IX, X, and XI. The results are reported in terms of the average, maximum and minimum values found, as well as the number of measurements made. The temperature average was not reported, since most measurements were made during the warmer months of the year. The BOD studies were reported separately in Table XII and Figure 10. The rate of biochemical oxidation was found to be the highest at station 7, and the lowest at station 6. This rate was found to be fairly constant for any one station.

(a)

(b)

Figure 9. Different Bottom Environments in Bee Fork Creek: (a) Heavy Growth at Station 7; (b) Cleaner Bottom at Station 6.

TABLE VII

Characteristics of Water at Station 6

Characteristic	Average	Maximum	Minimum	No. of Analyses
Turbidity, units	1	3	0.2	15
Temperature, °C	-	26.5	4.0	15
Dissolved Oxygen mg/l	8.8	14.0	7.8	15
Dissolved Oxygen % sat.	101	104	96	15
pH, units	7.9	8.2	7.6	15
Alkalinity, mg/l as $CaCO_3$	130	160	60	15
Hardness, mg/l as $CaCO_3$ Total	120	150	60	15
Hardness, mg/l as $CaCO_3$ Calcium	85	100	32	15
Fluoride, mg/l F	0.08	0.16	0	8
Total Dissolved Solids, mg/l	140	160	100	8
Copper, µg/l	3.8	14.2	0.5	10
Lead, µg/l	4.6	9.0	0.5	8
Zinc, µg/l	14.0	4.5	0.5	12
Chemical Oxygen Demand, mg/l O_2	6.5	8.7	6.1	8

TABLE VIII

Characteristics of Water at Station FT

Characteristic	Average	Maximum	Minimum	No. of Analyses
Turbidity, units	8	70	2	15
Temperature, °C	-	25.5	4.0	15
Dissolved Oxygen mg/l	8.0	9.8	7.2	15
Dissolved Oxygen % sat.	92	100	65	15
pH, units	8.4	8.6	8.1	15
Alkalinity, mg/l as $CaCO_3$	185	260	160	15
Hardness, mg/l as $CaCO_3$ Total	180	195	160	15
Hardness, mg/l as $CaCO_3$ Calcium	96	110	84	15
Fluoride, mg/l F	1.35	1.58	1.05	8
Total Dissolved Solids, mg/l	380	390	360	8
Copper, µg/l	35.8	80	5.9	8
Lead, µg/l	13.0	26.0	5.0	8
Zinc, µg/l	29.8	105.0	2.5	11
Chemical Oxygen Demand, mg/l O_2	12.0	17.4	10.3	8

TABLE IX

Characteristics of Water at Station 7

Characteristic	Average	Maximum	Minimum	No. of Analyses
Turbidity, units	7	30	1	15
Temperature, °C	-	26.0	4.0	15
Dissolved Oxygen mg/l	8.8	14.2	7.6	15
Dissolved Oxygen % sat.	100	105	87	
pH, units	8.3	8.6	7.9	15
Alkalinity, mg/l as $CaCO_3$	145	165	100	15
Hardness, mg/l as $CaCO_3$ Total	155	190	110	15
Hardness, mg/l as $CaCO_3$ Calcium	90	130	58	15
Fluoride, mg/l F	1.00	1.54	0.34	8
Total Dissolved Solids, mg/l	360	375	350	8
Copper, µg/l	17.3	43.0	3.2	9
Lead, µg/l	19.7	50.0	5.4	9
Zinc, µg/l	19.7	57.0	4.0	14
Chemical Oxygen Demand, mg/l O_2	6.7	8.8	6.2	8

TABLE X

Characteristics of Water at Station 8

Characteristic	Average	Maximum	Minimum	No. of Analyses
Turbidity, units	2	15	0.5	15
Temperature, °C	-	26.5	4.0	15
Dissolved Oxygen mg/l	8.9	14.8	7.8	15
Dissolved Oxygen % sat.	102	107	96	
pH, units	8.4	8.6	7.9	15
Alkalinity, mg/l as $CaCO_3$	150	170	110	15
Hardness, mg/l as $CaCO_3$ Total	155	180	110	15
Hardness, mg/l as $CaCO_3$ Calcium	88	110	68	15
Fluoride, mg/l F	0.94	1.24	0.62	8
Total Dissolved Solids, mg/l	345	360	335	8
Copper, µg/l	7.2	17.0	1.8	7
Lead, µg/l	13.8	23.0	5.0	8
Zinc, µg/l	19.4	60.0	9.0	9
Chemical Oxygen Demand, mg/l O_2	6.5	8.8	6.1	8

TABLE XI

Characteristics of Water at Station 9

Characteristic	Average	Maximum	Minimum	No. of Analyses
Turbidity, units	0.4	0.5	0.2	8
Temperature, ^{o}C	-	26.0	4.0	10
Dissolved Oxygen mg/l	8.1	11.0	7.4	10
% sat.	98	106	77	
pH, units	8.2	8.2	8.1	10
Alkalinity, mg/l as $CaCO_3$	190	285	130	10
Hardness, mg/l as $CaCO_3$ Total	165	195	140	10
Calcium	98	140	84	10
Fluoride, mg/l F	0.20	0.23	0.15	6
Total Dissolved Solids, mg/l	225	240	215	4
Copper, µg/l	6.9	10.5	4.6	3
Lead, µg/l	28.0	-	-	1
Zinc, µg/l	41	110.0	4.0	3
Chemical Oxygen Demand, mg/l O_2	-	-	-	0

TABLE XII

Biochemical Oxygen Demand (BOD) of Bee Fork Creek Water at the Various Stations

Time of incubation, days	BOD, in mg/l*		
	Station 6	Station 7	Station 8
1	0.6	1.1	0.8
2	1.1	2.0	1.5
3	1.6	2.8	2.1
4	2.1	3.4	2.7
5	2.4	3.9	3.0
10	3.7	5.1	4.2
15	4.6	5.6	5.0
20	5.2	5.7	5.4

*Average of the results of four experiments.

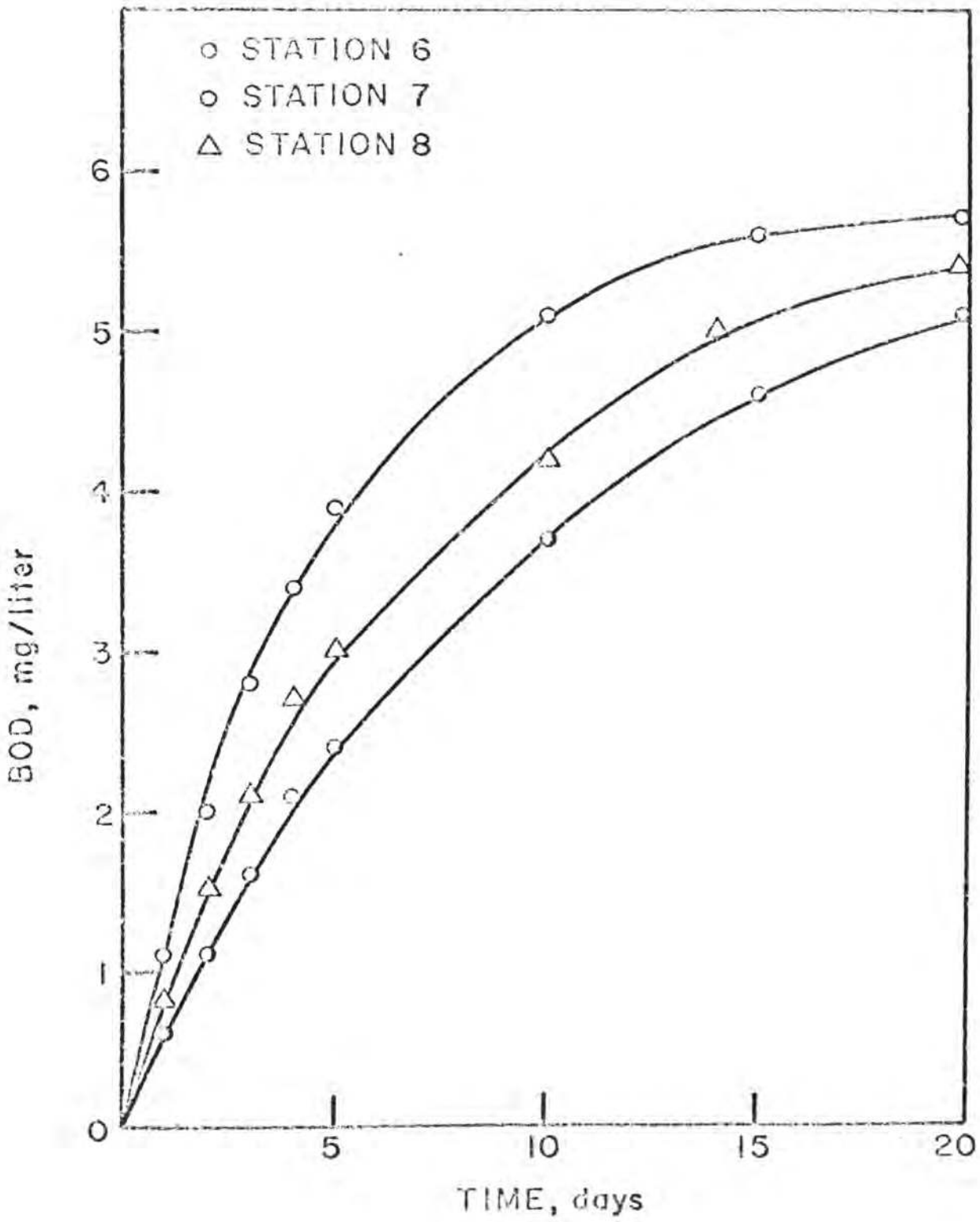

Figure 10. Comparison of Biochemical Oxidation Rates at Three Stations in Bee Fork Creek

C. Bioassay Results

1. Zinc

Zinc added to the water in concentrations up to 18 mg/l as Zn had no effect on survival of the tested bluegill sunfish. During the first 24 hours, the fish became sluggish and covered with coagulated mucus. After the mucus sloughed off, however, the fish recovered entirely and survived for the rest of the test period. Large amounts of a greyish-white precipitate were deposited at the bottom of the test jars but the precipitate demonstrated no acute toxicity to the fish. The lowest tested concentration which showed acute toxicity was 32 mg/l as Zn. At this concentration 80 percent of the fish died within 24 hours. The remaining fish, however, survived the 96-hour test. Measurements of the zinc concentration in the water showed a rapid decline of zinc in solution during the first 24 hours. The zinc concentration in the flask to which 32 mg/l had been added was only 1.3 mg/l at the end of 24 hours and 0.75 mg/l at the end of 96 hours. Analysis of the precipitate showed it to be mostly zinc carbonate (62).

When the water was acidified with hydrochloric acid, the pH dropped to 6.0 and the alkalinity to 22 mg/l as $CaCO_3$. Zinc remained in solution and the solution became much more toxic to fish, as illustrated by Table XIII. Fish which died in the low alkalinity solutions did not present the coagulated mucus on the body and gills, and no precipitate was visible.

TABLE XIII

Results of Bioassays of Zinc in Acidified Dilution Water

Zinc conc., mg/l Zn		Percent surviving after		
Nominal	Measured*	24 hours	48 hours	96 hours
18	17.0±0.8	40	0	0
10	9.4±0.5	100	100	60
5.6	5.3±0.2	100	100	80
3.2	3.4±0.1	100	100	100
1.8	1.8±0.1	100	100	100

*Average of measurements made after each 24 hours of test.

Figure 11. Precipitated Mucus of Experimental Fish Which Died in Zinc Solutions of High pH.

2. Lead

When lead was added to the dilution water, a milky turbidity appeared within a few seconds, with the formation of a white precipitate. The turbidity was higher at high concentrations. Fish placed in experimental solutions, to which up to 56 mg/l of lead had been added, showed no signs of being affected by the metal. All fish survived the entire test period of 96 hours. The white precipitate settled to the bottom of the test jars within a few hours. Measurements of the lead content in the filtered* supernatant showed that almost all lead added had precipitated. The solution whose nominal concentration was 56 mg/l contained only 0.5 mg/l Pb. The lowest nominal concentration of lead causing death to fish was 100 mg/l, which killed all fish with 24 hours. The measured concentration of this solution was 19 mg/l. The fish presented the same coagulated mucus on the body and gills as in the experiments with zinc, but in lesser amounts.

When hydrochloric acid was added to the dilution water, lowering the pH to 6.0 and the alkalinity to 22 mg/l as $CaCO_3$, death occurred as shown in Table XIV. Precipitation of lead was still visible, but to a much lesser extent than before. A light turbidity was barely visible in the two highest concentrations. The dead fish showed no signs of precipitated mucus on the body or gills.

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*Filtered through No. 40 Whatman filter paper.

TABLE XIV

Results of Bioassays of Lead in Acidified Dilution Water

Lead conc., mg/l Pb		Percent surviving after		
Nominal	Measured*	24 hours	48 hours	96 hours
10	6.8±0.2	60	20	0
5.6	4.8±0.1	60	40	40
3.2	2.5±0.1	100	100	80
1.8	1.3±0.1	100	100	100
1.0	0.8±0.1	100	100	100

*Average of measurements made after 24 and 96 hours of test.

3. Copper

Copper was found to be the most toxic among the heavy metals studied. All fish exposed to 3.2 mg/l of copper (nominal concentration) were killed within 48 hours. This metal was found to remain in solution in alkaline waters longer than lead and zinc. Only very small amounts of precipitate were formed at the end of the experiments. As with the other metals, a film of coagulated mucus coated the dead fish. In the case of copper, the film had a blue color, and was less abundant. The results of the bioassays of copper are shown in Table XV.

D. Mode of Action

All fish dying in the toxic solutions assayed behaved similarly before death. A short initial period of high activity was followed by a phase of inactivity and signs of respiratory trouble. Opercular movements became irregular and increased in amplitude. The fish became sluggish and finally rested on the bottom of the test jars. Occasional swimming movements demonstrated a lack of orientation. The fish swam fast and in a disordered manner, sometimes striking the wall of the jar, ending by going to the bottom, almost motionless. At this point, even the weak currents created by the aeration apparatus were able to carry the fish, which presented no resistance. Death occured gradually, preceeded by an apparent loss of consciousness. In two instances, fish were opened for autopsy while the heart was still beating, although no response could be obtained to mechanical stimulation.

In all cases, the autopsy revealed no hemorrhages, enlargements, or inflammations in the liver or heart. The gas bladder contained some air, although it was not completely full.

TABLE XV

Results of Bioassays of Copper

Nominal conc., mg/l Cu	Measured conc., mg/l, and percent surviving after					
	24 hours		48 hours		96 hours	
	conc.	% surv.	conc.	% surv.	conc.	% surv.
3.2	2.6	20	-	0	1.0	0
1.8	1.1	40	-	40	0.2	40
1.0	0.7	100	-	100	0.2	100
0.56	0.5	100	-	100	0.2	100
0.32	0.4	100	-	100	0.2	100

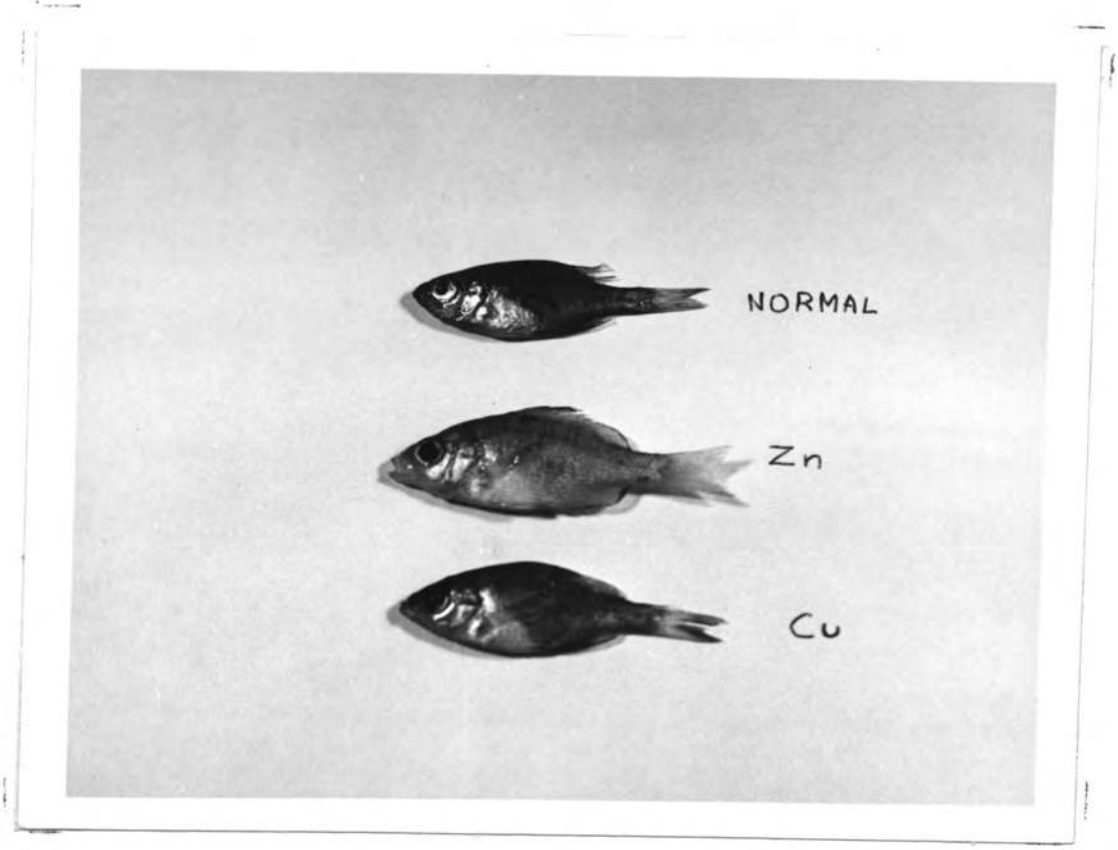

Figure 12. Comparison of Normal Fish with Fish Killed in Zinc and Copper Bioassays. Fish Killed by Zinc Were Coated with a White Film and Fish Killed by Copper Were Coated with a Blue-Gray Film.

Fish which died presenting mucus precipitation had the interior of the mouth, gills and opercular bones covered with the coagulated film. On the other hand, fish dying in acidified water were clean and not filmed.

Microscopical examination of the gills revealed that the tissues had been damaged to varying extent, as shown by the photomicrographs on the following pages. Since a method for staining zinc was not available, only copper and lead were observed and studied in the gill sections. Both copper and lead solutions penetrated the cells, as demonstrated by the four color pictures presented.

In all cases the gill tissue was visibly damaged, and copper and lead were detected within the cell wall. Considerable precipitation of the metals on the gills were only observed in fish which died in solutions of high pH and alkalinity.

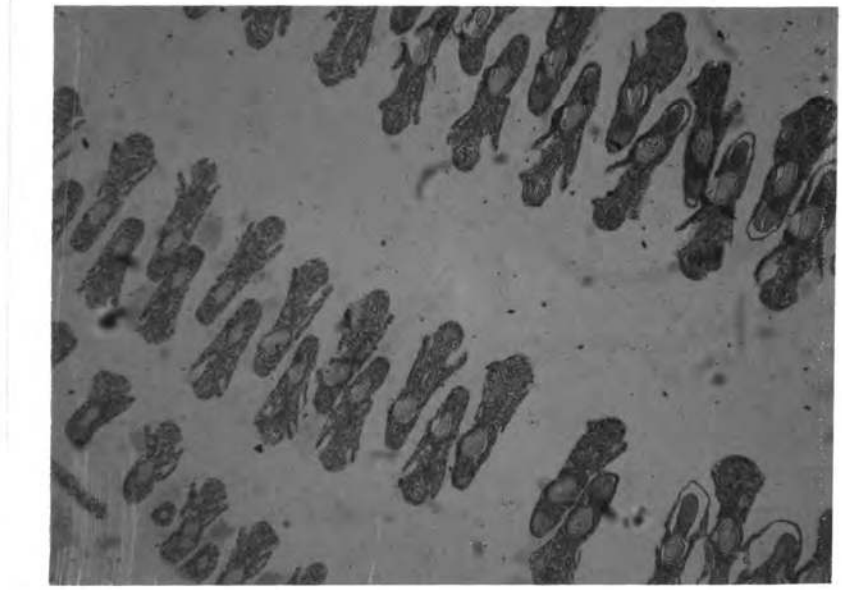

Figure 13. Photomicrograph of Normal Gill Filament in Transverse Section Showing Healthy Cell Tissue. x 100.

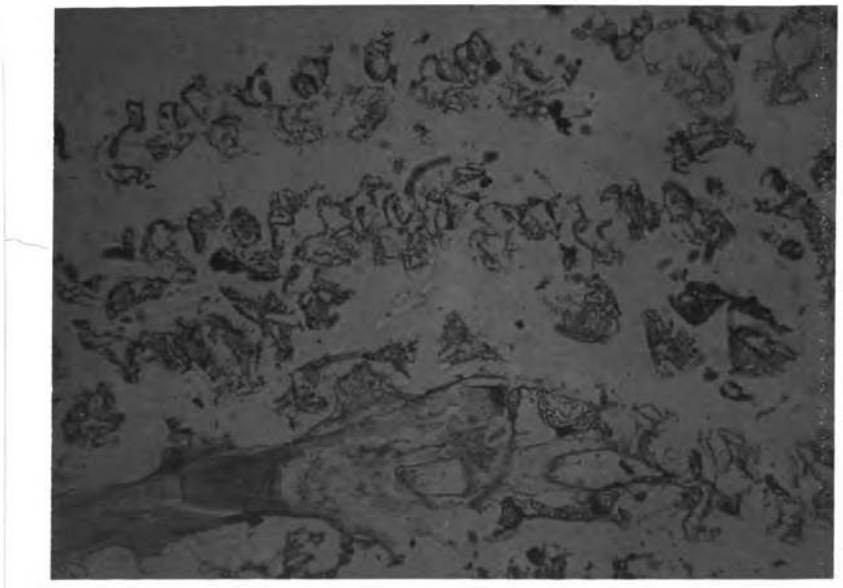

Figure 14. Photomicrograph of Gill Filament in Transverse Section Showing Cell Tissue Destroyed by Lead. x 100.

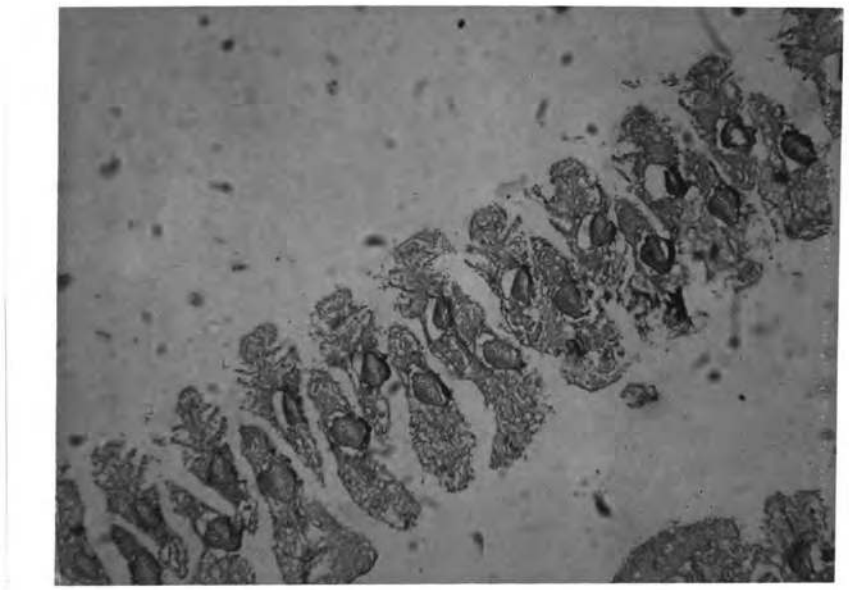

Figure 15. Photomicrograph of Gill Filament in Transverse Section Showing Cell Tissue Partially Destroyed by Copper. x 100.

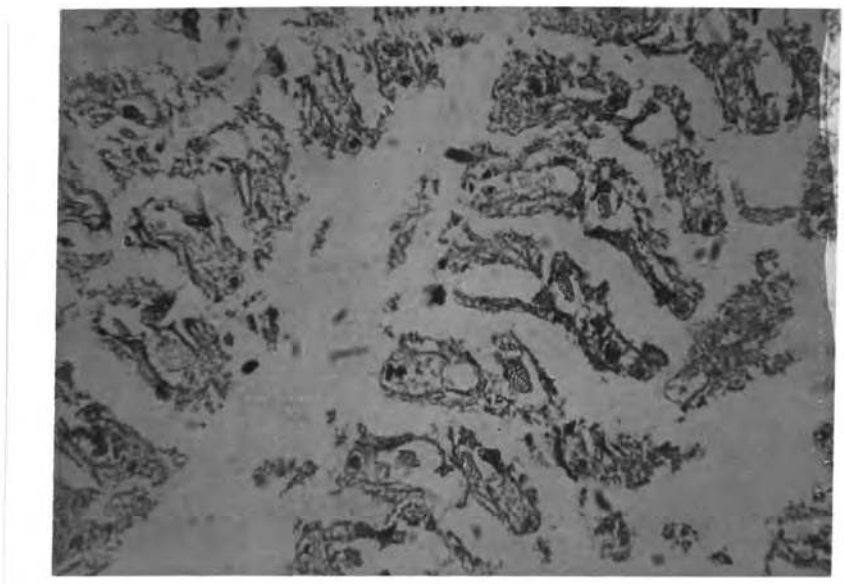

Figure 16. Photomicrograph of Gill Filament in Transverse Section Showing Cell Tissue Almost Completely Destroyed by Copper. x 100.

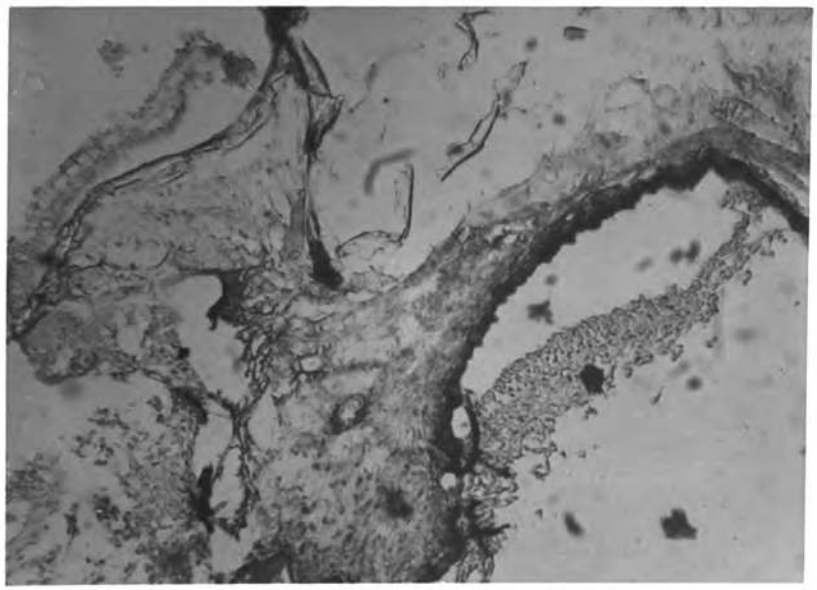

Figure 17. Photomicrograph of Gill Arch in Transverse Section, Showing Penetration of Lead (Dark Blue) within Tissue. x 100.

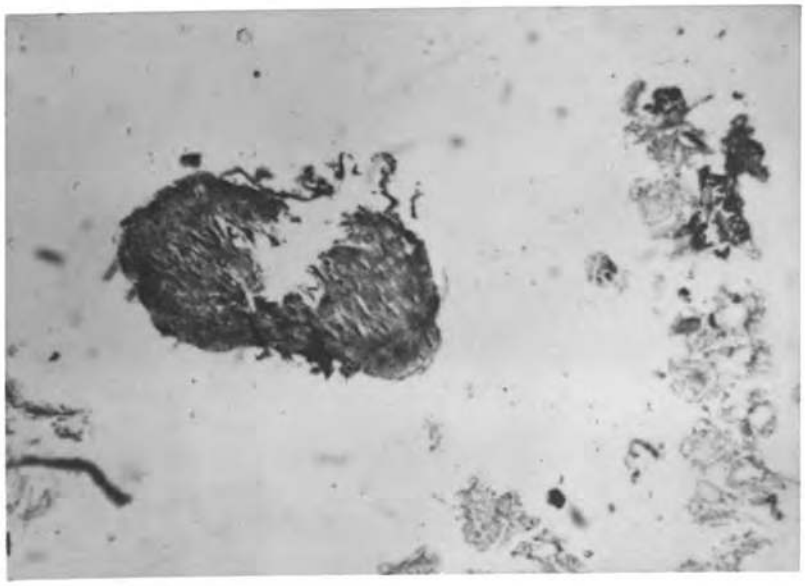

Figure 18. Photomicrograph of Gill Arch in Transverse Section, Showing Complete Penetration of Tissue by Lead (Dark Blue). x 40.

Figure 19. Photomicrograph of Gill Filament in Longitudinal Section, Showing Penetration of Copper (Very Dark Blue). x 100.

VI. DISCUSSION

Undoubtedly, discharges of mine tailings into Bee Fork Creek have deeply altered the physiognomy of that stream, as observed in the preceeding results. The ecological changes observed in the lotic environment may be derived from several factors. First, the mining waste water may contain substances which could serve as an energy source for symbiotic action within the mat growth developed at station 7. If such is the case, the seizure of the clean stream bottom by this mat would originate adverse conditions for the presence of other organisms. Snails attach themselves to clean rocks and gravel. Crayfish also require an accidented or burrowing stream bottom for protection. Secondly, the waste may contain substances which are toxic or detrimental to certain organisms. The most sensitive organisms could have been eliminated, reducing competition among the remaining forms. Predatory action could also have been reduced by this means. Hence, the creation of favorable conditions for the development of an undesirable ecological pattern. Finally, a combination of both actions is also possible. Any conclusions based on the observations made during this study, however, would be premature. Further study of the constituents of the wastewater, and the interactions of the organisms which constitute the stream pollution mats would need to be carried out for an accurate evaluation of the phenomenon. It is evident, however, that the unusual aquatic environment prevailing in the lower parts of Bee Fork Creek can be directly related to the industrial operations effluent discharge.

The results of the COD tests indicated a slight increase in organic matter, possibly caused by the tailings. This may be due to the organic

flotation reagents, but might also result from the decomposition of submerged vegetation in the sedimentation ponds at Fletcher Plant. The BOD studies, showing higher biochemical activity below the waste discharges, indicated that the organic matter present in the mine tailings is biologically degradable by the organisms living in the stream, at least to a certain extent.

The changes in water quality, although of considerable amplitude for certain parameters, lack a conclusive character in most cases. One such example is the increase in fluoride concentration, from 0.08 mg/l at station 6 to about 1 mg/l at the downstream stations. The origin of such an increase can be traced to the mine subsurface water but effects on the stream biota, if any, cannot be concluded yet.

It appears that copper, lead, and zinc do not present any problems of acute toxicity at the levels at which they have been released into Bee Fork up to now. Much higher concentrations had no acute effects on fish survival, as demonstrated by the laboratory bioassay studies. The present concentrations of these heavy metals are actually lower than the threshold limits of lethality (29) and other deleterious effects (52) reported in the literature. Copper was found to be the metal whose concentrations were closer to detrimental levels. If any action is taken in the future concerning the treatment of the mine tailings for the reduction of the heavy metal concentrations, copper should receive first attention.

The increase in the concentrations of the heavy metals was followed by a rise in total and calcium hardness. This may function as a balancing factor in the overall situation, since calcium has been reported to have an antagonistic effect on the toxicity of heavy metals.

An interesting point in the results was that lead concentration in the tailings was smaller than at stations 7 and 8. This fact suggests the possibility of another source of contamination, located between stations 6 and 7. It is possible that rain water, passing through the exposed dumps of mine refuse, oxidize galena to lead sulfate, which dissolves and can occasionally reach Bee Fork via an underground stream. A similar situation in the Rheidol River was observed by Newton (50).

The combined action of the metals was not studied and this observation could lead to interesting conclusions regarding the absence of fish below the mine wastewater discharge. It is possible that synergistic action between zinc and copper (15) may increase the sensitivity of the fish to the metals, thus causing them to avoid that area.

The results of the studies on the mode of action were quite interesting and revealing. They showed that the metals always penetrated within the cells, even when the cell membrane was not broken. It would seem reasonable to conclude that, under chronic exposure to subacute concentrations, the heavy metals could be carried into the blood stream of the fish, affecting other internal organs. The mode of action, then, would not be the same, and extrapolation of results of short-term tests could lead to erroneous conclusions. This is in agreement with the findings of Haider (32) who observed the presence of lead in the liver, kidneys and heart of fish exposed to chronic metal poisoning.

Another important fact is that destruction of the gill epithelium was accomplished to a lesser extent in fish developing a coagulated mucus than in those not presenting the film. This seems to confirm

Jones' (15) suggestion for a dual mode of action in acute toxicity. However, the deciding factors were the characteristics of the experimental water, rather than fish species.

VII. CONCLUSIONS

The following conclusions can be drawn from the results of this study.

1. Considerable changes in water quality and stream ecology have taken place at Bee Fork Creek, which can be directly or indirectly related to the mining operations. These changes were:
   a. An increase in turbidity, pH, alkalinity, hardness, fluoride, total dissolved solids, and heavy metals content of the water.
   b. A reduction in the population of fishes and benthic animals in a two-mile stretch of the stream immediately below the mine and mill tailings discharge.
   c. The development of an intense growth of algae and bacteria which completely covered the stream bottom immediately below the mine wastewater discharge.
2. The mine discharges have not caused noticeable changes in temperature or dissolved oxygen in Bee Fork Creek.
3. Concentrations of heavy metals in the stream have not reached levels which were found or reported as acutely toxic to fish.
4. Under static bioassay conditions, copper, lead and zinc were less toxic in water of high pH (7.9) and alkalinity (95 mg/l as $CaCO_3$) due to the formation of insoluble precipitates of the above metals.
5. In the process of acute poisoning by copper, lead and zinc, death occurs in Lepomis macrochirus as the result of impairment of the respiratory function.

6. All bluegill sunfish which died in the experimental solutions showed destruction of the gill epithelium.
7. The penetration of copper and lead was observed within gill cells even when the cell membrane was not broken.
8. The precipitated mucus on the body and gills of the experimental fish was observed with all methals, but occurred only within water of high pH and alkalinity.

VIII. RECOMMENDATIONS FOR FUTURE STUDY

This study is part of an overall research project for the area entitled "Stream Pollution in the New Lead Belt of S. E. Missouri", which is studied in part at the Sanitary Engineering laboratories of the University of Missouri-Rolla. Issues such as the detection and evaluation of toxic effects of flotation reagents, as well as the development of treatment processes for mine and mill wastewater, are now under investigation or have already been programmed for the near future.

The results of this study, however, suggest that more research is needed into the biological interactions among the organisms which constitute the growth observed at station 7. Such information could be applicable to understanding the biological approach to the treatment of the wastes in question.

The chronic effects of heavy metals in concentrations presently found in the stream have not been studied, and little or no information can be found in the literature. Long-term studies using fish and other animals (such as crayfish and snails) should be performed for a more complete evaluation of the stream pollution problem. Such studies should give special attention not only to the mode of action of these metals, but also to possible synergism or antagonism occurring among them when present together in the stream.

The effects of low concentrations of fluoride upon stream life also need to be established through bioassays. The lack of information on this subject points out the need for further research and study concerning possible detrimental long-term effects of fluoride on stream biota.

BIBLIOGRAPHY

1. "Summary Report of Senate Select Committee on National Water Resources," Journal Amer. Water Works Assn., 53, 371 (1961).

2. Kerr, R. S., "Planning for National Water Needs," Journal Amer. Water Works Assn., 53, 1081 (1961).

3. "Proposed Water Quality Criteria for Interstate Waters Between Missouri and Arkansas," Missouri Dept. of Conservation, presented at a Public Hearing at Doniphan, Mo., July 15, 1966.

4. Wixson, B. G., "Preliminary Stream Pollution Surveys in the "New Lead Belt" of S. E. Missouri," paper presented at the 1968 Annual Meeting of the Missouri Water Pollution Control Assoc., Jefferson City, Missouri (1968).

5. Long, L. H., editor, "The 1969 World Almanac," Newspaper Enterprise Association, New York, N. Y., p. 643 (1968).

6. "Recreation Guide to National Forests in the Eastern Region," U.S. Dept. of Agriculture - Forest Service, Washington, D. C., p. 9 (1968).

7. Tryon, C. P., Watershed Scientist, Clark National Forest, Rolla, Mo., Personal Communication (1969).

8. "Water Resources Data for Missouri, 1966. Part 1 - Surface Water Records," USDI, Geological Survey, Rolla, Mo., p. 178 (1967).

9. Hayes, W. C., "Mining in Missouri," Missouri Business, 18, 7:6 (1968).

10. Arundale, J. C., "The Mineral Industry of Missouri in 1967 (Preliminary)," Mineral Industry Surveys, USDI, Bureau of Mines, Washington, D. C. (1967).

11. Arundale, J. C. and Martin, J. A., "The Mineral Industry in Missouri," preprint from the 1966 Bureau of Mines Minerals Yearbook, U.S. Government Printing Office, Washington, D. C., 15 pages (1967).

12. Arundale, J. C., "National and International Significance of Missouri Mineral Resources," Missouri Business, 18, 7:31 (1968).

13. St. Joseph Lead Co., Personal Communication (1968).

14. Clark National Forest Sportsman's Map (Salem, Potosi, Centerville Ranger Districts), Forest Supervisor, Rolla, Mo. (1963).

15. Jones, J. R. E., "Fish and River Pollution," Butterworth & Co. (Publishers) Ltd., London, 203 pages (1964).

16. Powers, E. B., "The Goldfish (Carassius carassius) as a Test Animal in the Study of Toxicity," Illinois Biological Monographs, 4, 2 (1917).

17. Carpenter, K. E., "A Study of the Fauna of Rivers Polluted by Lead Mining in the Aberystwyth District of Cardiganshire," Annals of Applied Biol., 11, 1 (1924).

18. Carpenter, K. E., "The Lead Mine as an Active Agent in River Pollution," Annals of Applied Biology, 13, 395 (1926).

19. Carpenter, K. E., "On the Biological Factors Involved in the Destruction of River Fisheries by Pollution Due to Lead-Mining," Annals of Applied Biology, 12, 1 (1925).

20. Carpenter, K. E., "The Lethal Action of Soluble Metallic Salts on Fishes," British Journal of Experimental Biology, 4, 378 (1927).

21. Belding, D. L., "Toxicity Experiments with Fish in Reference to Trade Waste Pollution," Transactions American Fisheries Society, 57, 100 (1927).

22. Ellis, M. M., "Detection and Measurement of Stream Pollution," Bulletin U.S. Bureau of Fisheries, 48, 365 (1937).

23. Hart, W. B., Doudoroff, P. and Greenbank, J., "The Evaluation of the Toxicity of Industrial Wastes, Chemicals and Other Substances to Fresh-Water Fishes," The Atlantic Refining Co., Philadelphia, Pa., 317 pages (1945).

24. Doudoroff, P., Anderson, B. G., Burdick, G. E., Galtsoff, P. S., Hart, W. B., Patrick, R., Surber, E. W. and Van Horn, W. W., "Bio-Assay Methods for the Evaluation of Actue Toxicity of Industrial Wastes to Fish," Sewage and Ind. Wastes, 23, 1380 (1951).

25. "Standard Method for the Examination of Water and Wastewater," 12th edition, American Public Health Association, New York, N. Y., 769 pages (1965).

26. Finney, D. J., "Statistical Methods in Biological Assay," 2nd edition, Hafner Publishing Co., New York, N. Y., 668 pages (1964).

27. Abram, F. S. H., "The Definition and Measurement of Fish Toxicity Thresholds," Advances in Water Pollution Research, Proceedings 3rd Intl. Conf. Water Poll. Res., Volume I, Water Pollution Control Federation, Washington, D. C., p. 75 (1967).

28. Smith, J. W., "The Identification and Evaluation of Toxic Effects of Organic Micropollutants," Doctoral Dissertation, University of Missouri-Rolla, Rolla, Mo. (1968).

29. Chen, C. W. and Selleck, R. E., "A Kinetic Model of Fish Toxicity Threshold," paper presented at the 41st Annual Conference of Water Pollution Control Federation, Chicago, Ill. (1968).

30. Mount, D. I. and Stephan, C. E., "A Method for Establishing Acceptable Toxicant Limits for Fish - Malathion and the Butoxyethanol Ester of 2,4-D," Transactions American Fisheries Society, 96, 185 (1967).

31. Selleck, R. E. and Chen, C. W., Formal Discussion - Paper I-4, "The Definition and Measurement of Fish Toxicity Threshold," by F. S. H. Abram, Advances in Water Pollution Research, Proceedings 3rd Intl. Conf. Water Poll. Res., Volume I, Water Pollution Control Federation, Washington, D. C., p. 91 (1967).

32. Haider, G., Formal Discussion - Paper I-4, "The Definition and Measurement of Fish Toxicity Thresholds," by F. S. H. Abram, Advances in Water Pollution Research, Proc. 3rd Intl. Conf. Water Poll. Res., Volume I, Water Pollution Control Federation, Washington, D. C., p. 88 (1967).

33. Lloyd, R., "Factors that Affect the Tolerance of Fish to Heavy Metals Poisoning," Biological Problems in Water Pollution, 3rd Seminar, 1962, U.S. Public Health Service, Cincinnati, Ohio, p. 181 (1965).

34. Jones, J. R. E., "The Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback (Gasterosteus aculeatus, L.) and the Effect of Calcium on the Toxicity of Lead and Zinc Salts," Journal of Experimental Biol., 15, 394 (1938).

35. Jones, J. R. E., "A Further Study of the Reactions of Fish to Toxic Solutions," Journal of Experimental Biol., 25, 22 (1948).

36. Doudoroff, P. and Katz, M., "Critical Review of Literature on the Toxicity of Industrial Wastes and their Components to Fish - Part II: the Metals, as Salts," Sewage and Ind. Wastes, 25, 802 (1953).

37. Pickering, Q. H. and Henderson, C., "The Acute Toxicity of Some Heavy Metals to Different Species of Warm Water Fishes," Proc. Purdue Univ., Ind. Waste Conf., p. 578 (1964).

38. Westfall, B. A., "Coagulation Film Anoxia in Fishes," Ecology, 26, 283 (1945).

39. Behrens, B., "Zur Pharmakologie des Bleis. IV. Mitteilung. Uber den Mechanismus der Bleivergiftung von Fischen," Arch. Exp. Pathol. und Pharmakol., 137, 311 (1928).

40. Lloyd, R., "The Toxicity of Zinc to Rainbow Trout," Annals of Applied Biol., 48, 84 (1960).

41. "Water Pollution Research, 1960," Her Majesty Stationary Office, London (1961).

42. Mount, D. I., "An Autopsy Technique for Zinc-Caused Fish Mortality," Trans. American Fisheries Society, 93, 174 (1964).

43. Mount, D. I. and Stephan, C. E., "A Method for Detecting Cadmium Poisoning in Fish," the Journal of Wildlife Management, 31, 168 (1967).

44. Crandall, C. A. and Goodnight, C. J., "The Effect of Various Factors on the Toxicity of Sodium Pentachlorophenate to Fish," Limnology and Oceanography, 4, 53 (1959).

45. Pickering, Q. H., "Some Effects of Dissolved Oxygen Concentrations Upon the Toxicity of Zinc to the Bluegill, Lepomis macrochirus, Rafinesque," Water Research, 2, 187 (1968).

46. Cairns, J., Jr. and Scheier, A., "The Effect of Periodic Low Oxygen Upon the Toxicity of Various Chemicals to Aquatic Organisms," Proc. 12th Ind. Waste Conf., Purdue University, Lafayette, Ind., p. 165 (1958).

47. Sprague, J. B., "Lethal Concentrations of Copper and Zinc for Young Atlantic Salmon," Journal Fish. Res. Board Canada, 21, 17 (1964).

48. Mount, D. I., "The Effect of Total Hardness and pH on the Acute Toxicity of Zinc to Fish," Air and Water Poll. Intl. Journal, 10, 49 (1966).

49. Jones, J. R. E., "A Study of the Zinc-Polluted River Ystwyth in North Cardiganshire, Wales," Annals of Applied Biol., 27, 367 (1940).

50. Newton, L., "Pollution of the Rivers of West Wales by Lead and Zinc Mine Effluent," Annals of Applied Biology, 31, 1 (1944).

51. Sprague, J. B., Elson, P. F. and Saunders, R. L., "Sublethal Copper-Zinc Pollution in a Salmon River - A Field and Laboratory Study," Advances in Water Pollution Research, Proc. 2nd Intl. Conf. Water Poll. Res., Volume I, Water Pollution Control Federation, Washington, D. C., p. 61 (1965).

52. Brungs, W. A., "Chronic Toxicity of Zinc to the Fathead Minnow (Pimephales promelas, Rafinesque)," U.S. Dept. of the Interior, Federal Water Pollution Control Administration, Newton Fish Toxicology Laboratory, Cincinnati, Ohio, prepublication copy (1968).

53. Tibbs, N., "Determination of Trace Amounts of Copper, Lead, and Zinc in Streams of S. E. Missouri," Master Thesis, University of Missouri-Rolla, Rolla, Mo., in preparation.

54. Pflieger, W. L., "A Check List of Fishes of Missouri, with Keys for Identification," Missouri Department of Conservation, Jefferson City, Mo., 63 pages (1966).

55. Ward, H. R. and Whipple, G. C., "Fresh-Water Biology," John Wiley & Sons, New York, N. Y., 1111 pages (1918).

56. Dieffenbach, W. H., Fishery Biologist, Missouri Dept. of Conservation, Personal Communication (1968).

57. Palmer, C. M., "Algae in Water Supplies," U.S. Public Health Service Publication No. 657, U.S. Government Printing Office, Washington, D. C., 88 pages (1962).

58. Glick, D., "Techniques of Histo- and Cytochemistry," Interscience Publishers, Inc., New York, N. Y., p. 23 (1949).

59. Quinn, M. L., Research Assistant, UMR, Personal Communication (1968).

60. Lagler, K. F., "Freshwater Fishery Biology," 2nd edition, Wm. C. Brown Company, Publishers, Dubuque, Iowa, 421 pages (1956).

61. Gilderhus, P. A., "Some Effects of Sublethal Concentrations of Sodium Arsenite on Bluegills and the Aquatic Environment," Transactions American Fisheries Society, 95, 289 (1966).

62. Bolter, E., Associate Professor, UMR, Personal Communication (1968).

VITA

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APPENDIX

APPENDIX

Missouri Department of Conservation

Proposed Water Quality Criteria for Interstate Waters
Between Missouri and Arkansas
Presented at a Public Hearing at Doniphan, Missouri
July 15, 1966

Black River Basin a Tributary to the White River Basin

The method of sample collection, sample preservation, the analyses and measurements to determine water quality, and the accuracy of the results shall be in accordance with the Twelfth edition of "Standard Methods for the Examination of Water and Wastewater" or methods mutually agreed upon by the State Water Pollution Control Agencies. Stream sampling shall occur in all cases after adequate mixing of waste discharges with the stream has been accomplished unless otherwise specified.

I. Black River

A. Water Uses

The Black River in Missouri and Arkansas is used for recreation, and agricultural purposes, including irrigation.

B. General Criteria

Waste discharged to the Black River shall not create conditions in the stream which will adversely affect public health or the use of the waters for the following purposes: source of public or industrial water supply, propagation of fish or wildlife, and for agricultural, mining, industrial, recreational or other legitimate uses.

C. Specific Criteria

1. Flow

The criteria stipulated shall be met at all times regardless of flow.

2. Stream Criteria

a. pH

The pH shall be between 7.0 and 8.5 following adequate mixing in the stream. A pH above 8.5 in the stream must not be due to waste discharges.

b. Coliform Group

The coliform group shall not exceed 1000 per 100 ml. as a monthly arithmetical average (either MPN or MF count) during any month of the recreational season (May through September); nor exceed this number in more than 20% of the samples examined during any such month; nor exceed 2,400 per 100 ml. (MPN or MF count) on any day during the recreational season except during periods of storm water runoff.

c. Dissolved Oxygen

A minimum of 5 mg/l of dissolved oxygen shall be maintained in the stream.

d. Temperature

Waste discharges shall not elevate the temperature of the stream more than 5 degrees Fahrenheit above the natural cross section stream temperature, and at no time will the temperature of the stream be elevated above $90^{o}F$.

e. Wastes Potentially Toxic or Detrimental in Low Concentrations

Substances which may be toxic to humans, fish and wildlife, or detrimental to agricultural, mining, industrial, recreational or other legitimate uses shall be limited to non-toxic or non-detrimental concentrations in the stream.

f. Taste and Odor Substances

Taste and odor producing substances shall be limited to concentrations in the stream that will not interfere with the production of potable water by modern water treatment processes or impart unpalatable flavor to the flesh of fish, or result in noticeable offensive odors in the vicinity of the water, or otherwise interfere with the reasonable use of the water.

g. Turbidity

Waste discharges shall cause no measurable increase in the turbidity of the stream.

h. Color

There shall be no color that will cause substantial visible contrast with the natural appearance of the water.

i. Excessive Nutrients

There shall be no wastes discharged which cause nuisance growths of algae, bacteria or fungi in this stream.

j. Oil

There shall be no visible oil in the stream.

k. Solids Deposits

There shall be no man-made deposits of solids either organic or inorganic in nature on the stream bed.

l. Specific Conductance

Waste discharges shall not elevate the specific conductance above 500 micromhos in the stream.

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