EFFECT OF METAL PLATING WASTES ON THE ECOLOGY OF A WARM-WATER STREAM

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ABSTRACT

EFFECT OF METAL PLATING WASTES ON THE ECOLOGY OF A WARM-WATER STREAM

by Ronald R. Garton

The purpose of this study was to determine the effects of metal plating wastes upon the ecology of the Red Cedar River in southern Michigan. Water samples were analyzed for Cu, Ni, Zn, Cr^6, Cr Total and CN^- from stations selected upstream and for 10.3 miles downstream from the plating plant and numbers and types of invertebrates were determined at the same spots. Plating wastes could be detected for 10.3 miles below the effluent and strong negative correlations were found between wastes and all families of invertebrates except tubificids. Traditional methods of assessing pollution by indicator types, numbers of families, etc. were used. In addition, an index derived from the ratio of insects to tubificids is proposed as a measure of pollution and an optimum index value is suggested for the Red Cedar River. By means of a regression equation of insect-tubificid index on waste concentrations, maximum limits are suggested for plating wastes in the river.

EFFECT OF METAL PLATING WASTES ON THE ECOLOGY OF A WARM-WATER STREAM

By

Ronald R. Garton

A THESIS

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for the degree of

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I also wish to thank Dr. Gordon Guyer, Dr. Gerald Prescott, Dr. Peter Tack, and Dr. Niles Kevern for the time they gave as members of my guidance committee. And I wish to thank Dr. Duane Ullrey and his staff in Animal Husbandry who set such a fine example of interdepartmental cooperation in making laboratory facilities and equipment available for chemical analyses. Thanks also to fellow graduate students for helpful advice and to Alvin Jensen especially for assistance with computer programming.

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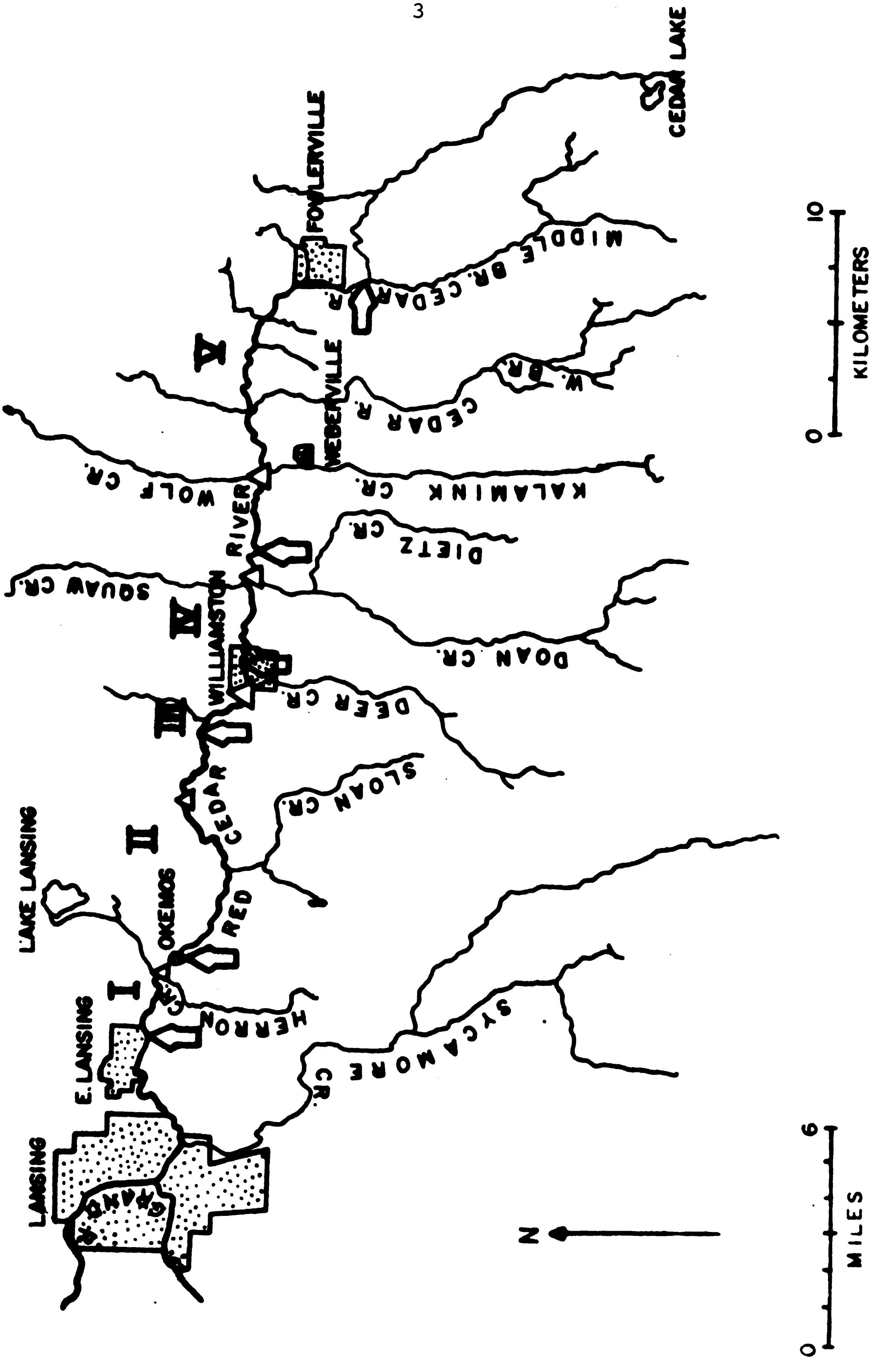
INTRODUCTION

This study was conducted in order to determine the effect of metal plating wastes on the invertebrate populations in the Red Cedar River, a warm-water stream in the southern lower penninsula of Michigan.

The Red Cedar River originates in Cedar Lake in Livingston County and then flows for approximately 45 miles in a northwesterly direction through Livingston and Ingham counties and finally flows into the Grand River in the city of Lansing. The river receives the water of 12 major tributaries and drains an area of about 472 square miles (Figure 1). The upstream portion of the river drains marsh and agricultural land used primarily for dairy farming and some raising of small grains. The land along the lower reaches of the river is more intensively farmed and the river is bordered by several small communities. Even in areas of intensive farming the fields seldom extend right up to the river banks so the river itself is lined with trees throughout the majority of its length.

The Red Cedar River has been the subject of several studies in past years by graduate students in the Fisheries and Wildlife Department of Michigan State University and by the Michigan State Water Resources Commission. For the

Figure 1. Map of Red Cedar River showing location of original study zones.


LANSING
GRAND R.
E. LANSING
LAKE LANSING
OKEMOS
I
II
III
IV
V
HERRON CR.
SYCAMORE CR.
RED CEDAR RIVER
SLOAN CR.
DEER CR.
WILLIAMSTON
DOAN CR.
SQUAW CR.
DIETZ CR.
WOLF CR.
KALAMINK CR.
WEBBERVILLE
W. BR. CEDAR R.
MIDDLE BR. CEDAR R.
FOWLERVILLE
CEDAR LAKE
N
0
6
MILES
0
10
KILOMETERS

studies done by Fisheries and Wildlife graduate students, the river was divided into five zones extending from Farm Lane bridge on the campus of Michigan State University to Van Buren Road bridge 30 miles upstream from campus and about one mile above the town of Fowlerville (Figure 1). This 30 mile section of the river drains an area of 355 square miles and the stream varies in width from about 20 to 80 feet. Average gradient is 2.4 feet per mile and discharge at the campus varies seasonally from an all-time low of 3cfs to a record high of 5510 cfs (King, 1964).

Past studies have been largely oriented toward characterizing and comparing different zones of the river in terms of chemistry and biology. In doing this, previous students have noted that productivity is much lower in Zone V, from Dietz Road bridge (10.32 miles below plating plant) to Van Buren Road bridge (0.94 miles above plating plant), than one would expect for a stream such as the Red Cedar River.

King (1964) found live molluscs to be absent for nine miles downstream from Fowlerville and found that aquatic insects were decreased in numbers of individuals and numbers of families in this area and that numbers of sludge worms (Tubificidae) were increased. This he attributed to metal plating wastes entering the river at the town of Fowlerville.

Linton and Ball (1965) found that the river supported extremely low numbers of fish and that fish populations did not recover until the lower stretches of the zone above Dietz Road bridge. This was in spite of the fact that the river in Zone V included the widest variety of major habitat types of any of the study zones and the river was receiving organic enrichment in this area, which might be expected to increase the amount of fish food present.

Accumulated evidence from these past studies clearly indicates that some factor is causing a detrimental effect upon the aquatic life in Zone V. Further, the most likely cause of this effect is a metal plating plant operated by Utilex Manufacturing Company which discharges plating effluent into the river at Fowlerville. This plant has had a long history of violations against established Michigan State water quality criteria for plating effluents and has been the cause of several fish kills in the past, the most recent of which is known to have occurred in the spring of 1961. This kill in Zone V, which could be traced for six miles downstream, was attributed to an accidental release of toxic materials from the plating plant (Parker, 1961).

Although effects of the plating wastes were noted by previous workers, the studies always encompassed such large areas of the river that no really intensive sampling was ever carried out in Zone V, so there was never any really adequate characterization of the type and extent of effects of the plating effluent. The plating effluent had an obvious detrimental effect upon the stream, but the exact nature of the effect was not known nor was it known how far the effects extended downstream from the effluent.

The present study was planned so as to concentrate chemical and biological sampling in that area of the river between Dietz Road bridge (10.3 miles below the plating plant) and Van Buren Road bridge (0.94 miles above the plating plant), formerly designated as Zone V, in order to determine the nature and extent of plating plant pollution and its effects.

DESCRIPTION OF STUDY AREA

That part of the Red Cedar River included in this study flows through woods and farm land for a distance of 11.26 river miles from Van Buren Road bridge 0.94 miles above Fowlerville to Dietz Road bridge, 10.32 miles below Fowlerville.

Elevation decreases from 900 feet MSL at Van Buren Road to 865 feet MSL at Dietz Road for a total drop of 35 feet in 11.26 miles. Mean gradient for this area of the river is 3.11 feet per mile as compared to the mean of 2.4 feet per mile for the entire river.

Vannote (1961) used hydrological data from February, 1958 to February, 1959 to compute relative amounts of discharge of the river and tributaries at various points in the watershed. This information makes it possible to approximate rate of flow in the upper reaches of the river from discharge records obtained by the United States Geological Survey at the gaging station at Farm Lane bridge on campus at East Lansing. According to Vannote, the flow at Van Buren Road bridge constitutes 27.43 percent of the flow at the campus and flow at Dietz Road bridge constitutes 50.04 percent of flow at the campus. These are approximations since rainfall over the entire watershed is not uniform from year to year.

High, low, and mean flow at the campus during each of the five sample periods is available from USGS records. Using these data at Farm Lane bridge and the conversion factors available from Vannote's hydrological study of the watershed, approximate high, low, and mean rates of discharge were computed for the upper and lower ends of the study area during each sampling series (Table 1).

The Red Cedar River has a widely varying rate of flow and may increase or decrease greatly in just a few days. This becomes especially important when pollutants are being released into the river at varying rates.

Although surrounded by farm land, the river in the study area is bordered by trees throughout most of its length and seldom has open fields close to the banks. There are very few people living immediately adjacent to the river in this area except where it passes through the town of Fowlerville.

In this area of the river there are three primary sources of domestic and industrial pollution. The first, and most important source, is the metal plating plant operated by Utilex Manufacturing Company in Fowlerville. The effluent of this plant enters the river at Fowlerville, 0.94 miles below Van Buren Road bridge and 10.32 river miles above Dietz Road bridge. The next pollutant is domestic

Table 1. River discharge in cubic feet per second during sampling program.

Sample Series	Date	Farm Lane Bridge at East Lansing			Van Buren Road 0.94 miles above plating plant			Dietz Road Bridge 10.32 miles below plating plant		
		High	Low	Mean	High	Low	Mean	High	Low	Mean
Pilot	30 Jun - 16 Aug '65	56.0	8.0	16.6	15.4	2.2	4.6	28.0	4.0	8.3
1	21 Jun - 6 Jul '66	71.0	33.0	46.9	19.5	9.1	12.9	35.5	16.5	23.5
2	1 Aug - 8 Aug '66	26.0	22.0	25.0	7.1	6.0	8.9	13.0	11.0	12.5
3	12 Sep - 27 Sep '66	31.0	24.0	27.7	8.5	6.6	7.6	15.5	12.0	13.8
4	31 Oct - 8 Nov '66	77.0	40.0	52.8	21.1	10.9	14.5	38.5	20.0	26.4

sewage from the Fowlerville sewage lagoons which discharge into the river 0.65 miles below the plating plant effluent. The last important source of domestic pollution in the study area is Kalamink Creek which picks up domestic sewage from the town of Webberville and discharges it into the river about seven miles below the plating plant. The effects of the domestic pollutants have been reported previously by Vannote (1961) and King (1964) but no major previous study has been concentrated on the effects of the plating wastes.

DESCRIPTION OF PLATING PLANT AND ITS OPERATION

The primary source of industrial pollution on the river is a metal plating plant operated by Utilex Manufacturing Company on the Middle Branch of the Red Cedar River at Fowlerville, Michigan. This plant, which employed 187 people in November, 1967, operates on a 16-hour day, six-day week basis with an annual one-week shut-down around July fourth. The two primary operations of the plant are zinc die casting and decorative plating of plumbing and automotive fixtures.

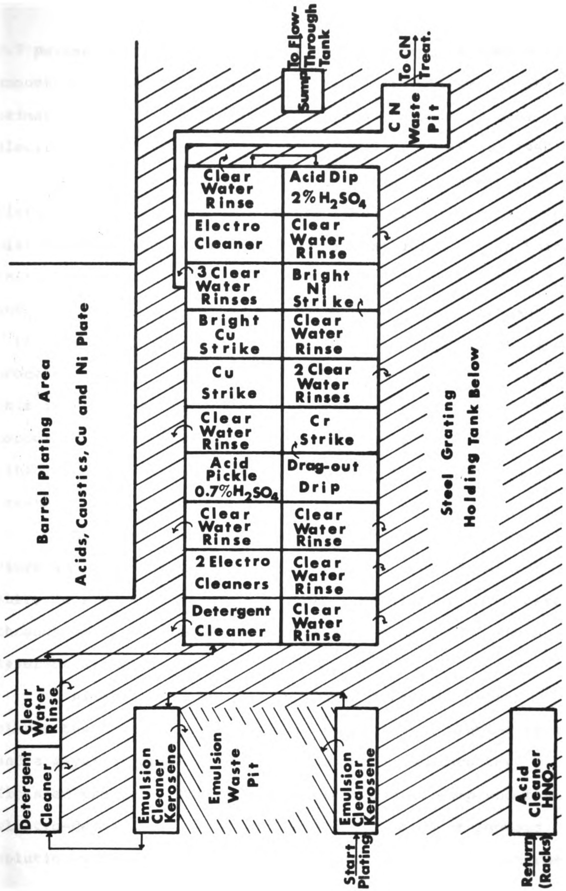
Pure zinc is purchased in ingot form and alloyed by the company with 4 percent aluminum before die casting to desired shapes. After casting, the parts undergo refining operations such as trimming, machining, buffing, and tumbling. Any washing or cooling operations here would be a source of zinc contamination of wastewater. Die cooling water is recycled with periodic addition of a phosphate base compound. Floor drains in the die casting area of the plant are presumed to discharge any spills or floor washings directly into the river (Caltrider, 1968).

After the fixtures have been cast and refined they are transported to the plating area and placed on racks to be carried through the plating operation. The plating sequence, diagramed in Figure 2, is as follows. The fixtures are first cleaned in two sets of kerosene emulsion cleaners to remove any dirt or residues from buffing or machining operations. Wastes from this operation are expected to flow into the emulsion waste pit and later be moved to an outside waste pit. However, Caltrider (1968) noted that drippings from the emulsion cleaner were falling into the metallic waste holding tank because there was no drip pan present. After the emulsion cleaner the fixtures go through two sets of detergent cleaners with a clear-water rinse in between to remove the kerosene emulsion.

After the second detergent cleaner come two electro cleaners. In these cleaners the fixtures are made the anode or cathode and an electrical current is set up through an alkaline solution. The cleaning is brought about by the chemical action of the alkali in conjunction with the mechanical action of vigorous hydrogen gas evolution if the work is the cathode (Richards, 1946). Cathode and anode may be inter-changed by an electrical switch during the process but with zinc castings it is generally preferred that the zinc be the anode as any film produced during cleaning is more easily removed in the subsequent acid dip (Metal Finishing Guidebook Directory, 1960).

From the electro cleaner the fixtures pass through a clear-water rinse and then into an acid pickle solution of

Figure 2. First floor plan of metal plating plant operated by Utilex Manufacturing Company, Fowlerville, Michigan. Adapted from Caltrider, 1968.


Barrel Plating Area
Acids, Caustics, Cu and Ni Plate
Steel Grating
Holding Tank Below
To Flow-Through Tank
Sump
To CN Treat.
CN Waste Pit
Clear Water Rinse
Acid Dip 2% H_2SO_4
Electro Cleaner
Clear Water Rinse
3 Clear Water Rinses
Bright Ni Strike
Bright Cu Strike
Clear Water Rinse
Cu Strike
2 Clear Water Rinses
Clear Water Rinse
Cr Strike
Acid Pickle 0.7% H_2SO_4
Drag-out Drip
Clear Water Rinse
Clear Water Rinse
2 Electro Cleaners
Clear Water Rinse
Detergent Cleaner
Clear Water Rinse
Clear Water Rinse
Detergent Cleaner
Emulsion Cleaner Kerosene
Emulsion Waste Pit
Emulsion Cleaner Kerosene
Acid Cleaner HNO_3
Start Plating
Return (Racks)

0.7 percent sulfuric acid. This acid may produce some small amount of etching to make the plating stick better but the primary purpose is for neutralization of the alkali from the electrocleaners (Metal Finishing Guidebook Directory, 1960).

After the acid pickle and clear-water rinse the fixtures go into the copper strike which is the first of the plating solutions. This copper strike solution puts a thin initial coat of copper on the castings to avoid blistering and then they go to the bright copper strike solution where a thicker coat of copper is applied by the electroplating process. Copper is applied in a copper cyanide solution and this is the source of the cyanide waste. After the bright copper strike the fixtures go through three clear-water rinses and this rinse water is sent to a special cyanide treatment tank before release to the river.

Next comes another electro cleaner and clear-water rinse and then another acid dip, this time into 2 percent sulfuric acid. After another rinse, the fixtures are passed through an electroplating solution of a nickel salt and a layer of nickel is deposited.

After nickel plating and three more clear-water rinses the fixtures are placed in the chromium plating vats and a layer of bright chrome is applied to produce the finished product. The most suitable chromium compound for electro-deposition is chromic acid, CrO_3, used in aqueous solution with small additions of other substances (Richards, 1946). In the best plating form the chromium is in the hexavalent state which is extremely toxic to living organisms.

After the chromium plating the fixtures go through a final series of rinses, shown in Figure 2, on the ground floor and then are taken to the second floor for two more clear-water rinses and a demineralized water rinse. The finished product is then removed from the racks and the racks are returned to the ground floor to be cleaned with nitric acid before being loaded up again for another plating sequence.

A similar operation is carried on in the barrel plating section but barrel plating is done in smaller batches for special pieces only.

Figure 2 illustrates the way the tanks for the entire plating operation are set up on an iron grating over a large holding tank so any spills or overflows in the operation go into the holding tank below. Small arrows show where rinse waters overflow into the holding tank as a normal part of the operation. Tanks holding stronger solutions may be expected to lose solutions from splashing, drippings, or accidents. In addition, all acidic and alkaline tanks are dumped into the holding tank approximately once a week (Caltrider, 1968). Treatment of wastes seems to be carried out in an incomplete manner and without sufficient controls to assure adequate results.

Cyanide is removed in the three rinses after plating in the copper cyanide solution and this rinse water is sent to one of a pair of concrete tanks built for cyanide treatment. The two tanks are filled on an alternate basis and the full tank is treated by addition of chlorine and sodium hydroxide while the other tank is filling. After treatment, the tank contents are tested for presence of cyanide and then released to the settling lagoon. Some cyanide still seems to get out but this is an improvement over the old method where cyanide was treated by a continuous stream of hypochlorite in one large flow-through tank.

Hexavalent chromium is treated by addition of sodium bisulfite to the first rinse tank following the chrome strike. Since hexavalent chromium is a yellow-orange color and trivalent is green, one can make a crude analysis by eye and this is the way it is done in the plant. Plant personnel add the sodium bisulfite to reduce the chromium at a rate such as to keep the rinse water "sufficiently" green by visual inspection as it overflows to the holding tank below (Caltrider, 1968).

Contents of the holding tank are pumped to an outside flow-through tank with two baffles so sludge can settle and floating oil can be skimmed off the top manually. This sludge and oil is dumped into a waste pit beside the tank and the liquid waste goes on into the lagoon with the treated cyanide wastes.

Metals such as copper, chromium, nickel, and zinc are commonly precipitated by pH manipulation in other plating plants but this is not the case here. The copper solution may receive this adjustment during cyanide treatment but is evidently not retained in the cyanide tank long enough for precipitation, if the proper pH is reached. No pH adjustment is made in an effort to precipitate the other metals.

The final phase of treatment occurs in the settling lagoon where metals and any solids have some chance to settle out. The settling pond is normally about 50 x 50 feet in area by 4 feet deep (Robinson, 1964) but, on one survey I witnessed, the lagoon was filled up to within six inches of the surface. At this time the flow-through tank was also nearly full. Settling in the lagoon obviously removes some of the metallic waste but the effluent is still a turbid blue-green in color. A 2 x 6" board skimmer is set up at the outfall to hold back surface oils but this is not always effective. Numerous stains show where the lagoon has overflowed so oils and metal wastes are carried into the river. With all treatment facilities working normally the effluent of the plant flows overland for about 75 feet from the lagoon to the Red Cedar River where its milky, blue-green presence may be traced visually for several hundred yards in the stream.

METHODS

Sampling Program

The object of the study was to determine effects of the metal plating wastes on the aquatic ecosystem, and specifically to detect any changes in numbers or types of invertebrates. In order to determine the effect of the plating wastes it was necessary to analyze the water for plating wastes both above and below the plating plant and to take enough bottom samples to adequately characterize the invertebrate communities in the stream, both above and below the plating effluent.

Such a sampling program was planned to give a detailed picture in those areas most affected by plating wastes and in the area of recovery. Sampling could be less intense in areas where recovery was more complete. Since there was no basis to judge how far the zone of pollution reached and where the recovery began a pilot study was conducted during the summer of 1965 to provide a general idea of the invertebrate distributions in the river before the sampling program was made final.

The pilot program was undertaken with the idea of sampling only invertebrates so no chemical analyses were included. Eighteen sample sites were selected with no attempt at randomization. Instead, sample sites were picked so as to be somewhat concentrated in the area of the plating waste effluent and, when possible, were picked so as to be easily accessible. Three of the sites were upstream from the plating plant, the farthest being just below Van Buren Road bridge 0.94 miles above the plating plant effluent. The other 15 sites were scattered downstream from the plant, with the last site 100 yards below Dietz Road bridge, 10.32 miles below the plating plant (Table 2).

At each sample site four sample spots were laid out on a transect across the river at a right angle to direction of flow. One sample spot was located four feet from waters edge on each side of the stream and the other two spots were located one third of the distance from each side of the stream. Each of the four spots on the transect was the site of a bottom sample.

During the period from 30 June to 16 August, 1965, bottom samples at each of the four spots on each transect were taken with a Petersen dredge designed to sample 0.68 square feet of bottom. At the time of sampling, the dredge contents were screened in a 30 mesh screen box to reduce the amount of fine sediment and debris and the invertebrates and larger debris were then placed in sample jars and preserved in formalin for return to the laboratory.

Table 2. Location of sample transects in miles below plating plant outfall.

Pilot series		Regular random series						
Station	Location	Stream area	Limits	Regular station	Series 1	Series 2	Series 3	Series 4
1	-0.92	1	-0.94 to -0.44	1	-0.89	-0.85	-0.90	-0.92
2	-0.44	2	-0.43 to 0.00	2	-0.27	-0.19	-0.43	-0.33
3	-0.04	3	0.00 to 0.26	P1	-0.03	-0.03	-0.03	-0.03
4	0.12	4	0.27 to 0.65	3	0.14	0.12	0.10	0.16
5	0.29	5	0.66 to 1.55	P2	0.14	0.14	0.14	0.14
6	0.53	6	1.56 to 2.71	4	0.45	0.35	0.47	0.36
7	1.26	7	2.72 to 3.83	5	0.87	1.39	0.84	0.75
8	1.74	8	3.84 to 4.89	P3	0.65	0.65	0.65	0.65
9	2.70	9	4.90 to 5.92	6	2.64	1.83	2.27	2.20
10	2.73	10	5.93 to 7.55	7	2.85	3.52	3.74	3.71
11	3.82	11	7.56 to 9.58	P4	3.83	3.83	3.83	3.83
12	5.94	12	9.59 to 10.31	8	4.56	4.07	4.15	4.12
13	7.53			9	5.88	5.42	5.61	5.24
14	7.57			10	6.81	6.79	7.43	6.93
15	9.55			11	8.24	9.03	9.16	9.01
16	9.60			12	10.10	10.19	9.94	10.15
17	10.13			P5	10.30	10.30	10.30	10.30
18	10.32							

In the laboratory the preserved samples were washed and contents were sorted by hand. Invertebrates were separated by family, counted and preserved in a solution of 70% alcohol in 2 ounce sample bottles. After counting, the invertebrates were identified to family and weighed for wet weight on a four-place Mettler balance. Using a method which King and Ball (1964) found to give a very close approximation to live, wet weight, I removed all invertebrates from the alcohol and soaked them in water for 30 minutes to counteract dehydration from the alcohol. They were then placed on small 30 mesh screens and spun for 30 seconds at 1,800 rpm on a small clinical centrifuge to remove excess water before being weighed on the Mettler.

Results of the pilot sampling series, presented in the results section, were then used to determine the character of the invertebrate populations and were thus used as a guide in laying out the final sample sites.

Based upon results of the pilot study, the river was divided into 12 sections for the final sampling program, two sections upstream from the plating plant and 10 sections downstream. The sections, designated with the help of county maps and 1/5000 scale aerial photos, were not of equal size but were chosen so that there were smaller sections, and thus more samples, in the areas of gross pollution and initial recovery (Figure 3 and Table 1).

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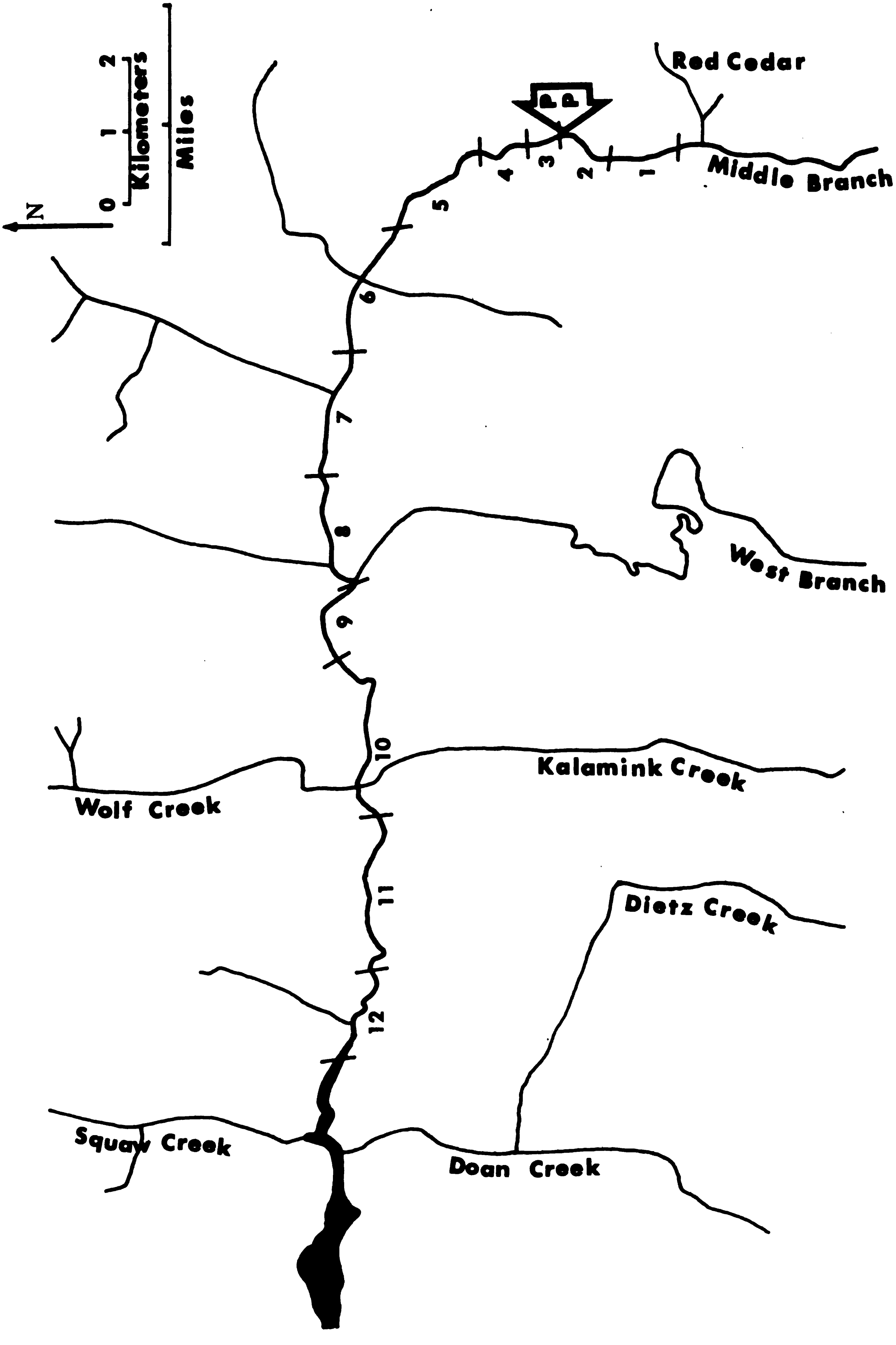
Figure 3. Map of final study sections.


Kilometers
0
1
2
Miles
N
Red Cedar
P.P.
Middle Branch
1
2
3
4
5
6
7
8
9
10
11
12
West Branch
Kalamink Creek
Wolf Creek
Dietz Creek
Squaw Creek
Doan Creek

For each of four sample series conducted throughout the summer of 1966, one sample transect was chosen at random in each section of the river. This was done by measuring river distance in each section in eights of an inch with a map measurer on 1/5000 aerial photos. A table of random numbers was then used to choose a number between zero and the total number of eights of an inch in each section. This number was then the number of eights of an inch measured on the photo from the upstream boundary of a sample section to the sample transect for that series. Thus, for each of the four sample series there was one randomly-chosen sample transect in each section of the stream (Table 1). Four dredge samples were taken on each transect as described for the pilot study.

In addition to the 12 randomly-selected sample transects, there were also five other sample transects, termed permanent sites. These were selected arbitrarily and were sampled at the same place during each of the four sample series.

During the four regular sampling series invertebrate samples were taken at four spots on each transect and samples were handled in the same manner as the samples taken for the pilot study. But, during the four regular sampling series, water samples were taken in addition to the bottom samples and these water samples were returned to the laboratory for chemical analysis for presence of plating wastes.

Duplicate water samples were taken in acid-washed polyethylene bottles at each sample transect and additional samples were taken directly from the plating plant effluent at various times during each sample series. Analysis for hexavalent chromium and cyanide was made within 24 hours. Samples were then acidified with 1 ml nitric acid per 500 ml of sample to prevent precipitation of metals and samples were stored for analysis for copper, nickel, zinc, and total chromium at a later date.

Chemical Analyses

Hexavalent chromium - Hexavalent chromium concentration was determined in the water samples within 24 hours, using the diphenylcarbazide colorimetric analysis given in APHA (1965). Color development was measured by a Beckman DU spectrophotometer.

Cyanide - Cyanide concentration was measured within 24 hours by the pyridine-pyrazolone colorimetric method described in APHA (1965). Color development was measured by a Beckman DU spectrophotometer.

Although colorimetric analyses were used for hexavalent chromium and cyanide, analyses for copper, nickel, zinc, and total chromium were done on a Jarrel Ash atomic absorption spectrophotometer.

Copper - Copper was measured directly from the water sample with no prior treatment. Standards were made up in concentrations such as to bracket the levels of copper found in the unknowns and then standards and unknowns were run at the same time and concentrations of unknowns were plotted from a standard curve. Copper was determined by atomic absorption using a wavelength of 3247 millimicrons with a fuel setting of 10 psi and an air setting of 15 psi.

Nickel - Nickel was also measured directly from untreated water samples. Standards were made up from $NiSO_4$ to bracket the concentrations of nickel in the river. Standards and unknowns were run at the same time and a standard curve was set up to determine concentration of unknowns. Samples were run on atomic absorption at a wavelength of 2318.5 millimicrons with air and fuel pressures both set at 15 psi.

Zinc - Zinc was also measured from untreated water samples and unknowns were determined by use of a standard curve. Standards made from zinc wire were run at the same time as the unknowns using atomic absorption at a wavelength of 2136.5 millimicrons and a fuel and air setting of 15 psi.

Total Chromium - Total chromium was also measured using the Jarrel Ash atomic absorption spectrophotometer but samples were first digested with nitric and perchloric acids by the following method. Twenty milliliters of nitric acid was added to 50 ml of sample and the combination was boiled down to about 10 ml in a 250 ml Phillips beaker with a glass bead. Then, five ml of perchloric acid was added and this combination was boiled down until the solution was clear and dense white fumes were given off. This solution was then cooled and diluted to 15 ml with deionized water.

Preliminary experiments indicated that some interference in the river water, presumably phosphates, caused lower values for total chromium standards in river water than in deionized water. But, addition of calcium carbonate got rid of the interferences so all samples were diluted after digestion with an equal part of 1000 ppm calcium carbonate to produce a final sample with 500 ppm $CaCO_3$. This final sample was run on the spectrophotometer using the flame emission method at a wavelength of 4255 millimicrons and air and fuel pressures of 15 psi. Standards made up from $K_2Cr_2O_7$ were run at the same time after being put through the same digestion and dilution procedures as the river water samples. Values for the river samples were then obtained from a standard curve.

RESULTS AND DISCUSSION

Water Analyses

Maximum concentration limits were set for copper, nickel, zinc, hexavalent chromium and cyanide in the Utilex Company effluent by an Order of Determination issued by the Michigan Water Resources Commission on 28 January 1953. Limits set were: 1.0 ppm copper, 1.0 ppm nickel, 0.5 ppm zinc, 2.0 ppm hexavalent chromium and 0.6 ppm cyanide. No limits were set for trivalent or total chromium. Since 1953 the effluent has been monitored 43 separate times by the engineers of the Water Resources Commission and waste limits have been exceeded a total of 126 times during these sample series. These limits were not only exceeded in the effluent but were often exceeded in the river itself, even after dilution. Table 3, listing maximum and minimun values for chemicals found in the river by Water Resources Commission engineers, does not show all violations but does give an idea of concentration ranges in the river.

Since the company has such a long history of violations during Water Resources Commission surveys, it is no surprise that allowable levels were exceeded during the present sampling program. Results for each of the six elements

Table 3. Maximum and minimum values for water chemistry determinations made by Michigan Water Resources Commission during period from 1953 to 1967.

Station (Miles below outfall)	pH		D.O.		Cu		Ni		Zn		Cr^6		Total Cr		CN	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Above Utilex	8.3	7.7	10.7	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Grand River Road (0.26)	8.3	7.3	10.5	2.5	1.1	0.0	0.9	0.0	0.2	0.0	0.4	0.0	0.2	0.03	0.94	0.0
Gregory Road (1.55)	8.1	7.5	9.9	1.8	0.35	0.0	0.9	0.0	0.1	0.0	0.34	0.0	0.14	tr	0.23	0.0
Nicholson Road (2.71)	8.0	7.7	10.1	4.1	0.2	0.0	0.5	0.0	0.1	0.1	0.24	0.0	0.02	0.0	0.22	0.0
Stow Road (3.83)	7.9	7.6	10.4	3.0	0.4	0.0	0.3	0.0	0.1	0.1	0.02	0.0	0.03	0.0	0.35	0.0
Gramer Road (5.92)	8.0	7.7	10.8	4.5	0.35	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.35	0.35

Zinc based on a very limited number of determinations.

All values except pH are in parts per million.

measured are here given separately, but it must be remembered that concentrations of single elements may be very misleading because of the possibility of additive and synergistic effects of the same elements in combination.

Copper - Results of analyses for copper concentrations in the water are listed in Table 4. Copper could be detected in the river water for at least 10.3 miles downstream in all four sample series. If means of copper concentrations downstream are compared with means of all samples upstream from the plating plant, we find that concentrations downstream through section 11 are significantly higher by a t-test at the 0.05 level. This would be for approximately 8.5 miles.

Just because an element such as copper can be detected in the water in above-normal limits does not necessarily mean it will have a harmful effect. Accumulated bioassay data do indicate harmful effects attributable to copper in aquatic systems but toxicity depends to a great extent upon associated factors such as water temperature, hardness, and dissolved oxygen. These associated factors are often not defined in bioassay reports so much of the data from the past is not comparable and cannot be used to set realistic limits of pollution.

Some workers have found concentrations from 0.1 to 1.0 mg/l copper to be non-toxic to most fish while others have found concentrations from 0.015 to 3.0 mg/l toxic to

Table 4. Average parts per million copper in each sample series.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all series
1	0.015	0.010	0.000	0.085	0.028
2	0.005	0.005	0.000	0.070	0.020
P-1	0.005	0.005	0.005	0.060	0.018
Utilex	0.870	1.250	1.345	1.530	---
Utilex	2.220	1.950	1.750	1.460	---
Utilex	---	1.600	---	---	---
3	0.180	0.250	0.285	0.140	0.214*
P-2	0.158	0.290	0.250	0.140	0.210*
4	0.110	0.320	0.245	0.140	0.204*
P-3	0.085	0.185	0.130	0.175	0.144*
5	0.090	0.275	0.090	0.140	0.149*
6	0.075	0.380	0.060	0.240	0.189*
7	0.075	0.165	0.067	0.185	0.123*
P-4	0.080	0.125	0.070	0.170	0.111*
8	0.087	0.105	0.075	0.190	0.114*
9	0.040	0.037	0.030	0.110	0.054*
10	0.037	0.035	0.022	0.115	0.052*
11	0.040	0.030	0.020	0.130	0.055*
12	0.045	0.012	0.010	0.100	0.042
P-5	0.022	0.015	0.010	0.100	0.037

*Denotes those means found to be significantly greater than the mean of all samples upstream from the plating plant. Determined by t-test at 0.05 level.

many kinds of fish, crustacea, molluscs, insects, phytoplankton, and zooplankton, especially in soft water (McKee and Wolf, 1963). Doudoroff (1952) found that synergism was an important factor to be considered. He found that fish could survive for eight hours with 8.0 mg/l zinc alone or 0.2 mg/l of copper alone but most fish died in eight hours in a combination of only 1.0 mg/l zinc and 0.025 mg/l copper. The extent of the synergistic effect is also affected by hardness of the water and other factors. And, even if past bioassay data are considered comparable and are used, they are measures of toxicity and even less is known about the deleterious effects various elements or compounds may have upon growth and reproduction.

As an approximation to be used in the interim until better data can be obtained, Tarzwell (1967) has suggested that 0.16 mg/l copper be set as the limit to be allowed in a waste anytime-anyplace. This would be the limit for copper alone and the limit would be lowered, as will be shown later, in the presence of other toxic substances. Even if synergism is ignored and 0.16 mg/l is taken as the maximum allowable in the Red Cedar River, we can see from Table 4 that this limit is exceeded for miles downstream from the plating plant. And, Tarzwell's limit is very generous compared to the limit of 0.02 mg/l set by the State of California for fish water and aquatic life (McKee and Wolf, 1963).

The maximum allowable concentration of copper in the Utilex Company effluent was set at 1.0 mg/l by the Order of Determination issued by the Michigan Water Resources Commission on 28 January 1953. Since that time this limit has been exceeded in 28 of the 43 sample series run on the effluent by Water Resources Commission engineers and it was exceeded in eight of nine sample series run on the effluent as part of the present study.

Nickel - Nickel concentrations detected during the sampling program on the river are shown in Table 5. Nickel could be detected for at least 10.3 miles below the plating plant in all four series and mean concentration values were shown to be significantly higher (t-test at the 0.05 level) in every section downstream from the plating plant when compared to the mean of all samples upstream from the plant.

Although toxicity of nickel varies according to the compound in which it is contained, it does not seem to be nearly so toxic as copper or zinc in the aquatic environment (McKee and Wolf, 1963). But, here again, simple toxicity is not the only measure of adverse effect and little is known of the effect upon growth and reproduction of aquatic organisms. It is in this conservative vein that the Mersey and Severn River Boards in England have established the limit concentration for nickel alone, or in combination with other heavy metals, at 1.0 mg/l total in industrial wastes (Klein, 1957).

Table 5. Average parts per million nickel in each sample series.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all Series
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00
P-1	0.00	0.00	0.00	0.00	0.00
Utilex	6.70	3.00	3.35	1.40	----
Utilex	3.80	8.80	2.10	3.50	----
Utilex	----	9.50	----	----	----
3	0.70	1.50	0.40	0.08	0.67*
P-2	0.40	1.50	0.37	0.08	0.59*
4	0.50	1.20	0.42	0.10	0.56*
P-3	0.30	1.00	0.35	0.10	0.44*
5	0.70	0.70	0.48	0.10	0.50*
6	0.20	0.85	0.28	0.19	0.38*
7	0.10	1.40	0.33	0.25	0.52*
P-4	0.30	1.10	0.31	0.20	0.48*
8	0.20	0.90	0.30	0.20	0.40*
9	0.10	0.70	0.29	0.18	0.32*
10	0.10	0.50	0.28	0.19	0.27*
11	0.10	1.00	0.25	0.20	0.39*
12	0.10	0.10	0.25	0.19	0.16*
P-5	0.10	0.10	0.24	0.10	0.14*

*Denotes those means found to be significantly greater than the mean of all samples upstream from the plating plant. Determined by t-test at 0.05 level.

A limit of 1.0 mg/l nickel was set for the Utilex Company effluent by the Michigan Water Resources Commission Order of Determination issued on 28 January 1953. This limit has been violated in 37 of 43 sample series conducted by the Water Resources Commission since 1953 and was exceeded in all nine sample series taken at the effluent in the present study. In addition, the limit set for the effluent was exceeded several times in the stream itself three or four miles downstream from the plating plant.

Zinc - Zinc concentrations determined during the sampling program are shown in Table 6. Zinc could be detected for at least 10.3 miles downstream from the plating plant in all four series. Mean concentrations downstream compared to mean concentration in all samples upstream from the effluent were found to be significantly greater (t-test at the 0.05 level) for every station downstream.

As in the case of copper and nickel, toxicity levels of zinc as determined by bioassays are not well defined and reports are often conflicting. Part of the conflict may be due to differences in temperature or hardness of the bioassay waters since descriptions of such parameters are not always included in older bioassay results. Jones (1938) found that for mature sticklebacks the lethal limit for zinc in water containing 1.0 mg/l calcium is only 0.3 mg/l but as much as 2.0 mg/l zinc is not toxic in water with 50 mg/l calcium. If this is true, toxicity of zinc would be reduced

Table 6. Average parts per million zinc in each sample series.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all series
1	0.010	0.015	0.010	0.000	0.008
2	0.030	0.020	0.000	0.000	0.012
P-1	0.010	0.016	0.000	0.000	0.006
Utilex	1.100	1.310	4.100	0.550	---
Utilex	0.980	4.300	0.370	0.985	---
Utilex	---	0.855	---	---	---
3	0.160	0.190	0.555	0.086	0.247*
P-2	0.145	0.195	0.510	0.086	0.234*
4	0.215	0.305	0.355	0.080	0.238*
P-3	0.190	0.145	0.195	0.120	0.162*
5	0.200	0.180	0.185	0.133	0.174*
6	0.060	0.190	0.085	0.110	0.111*
7	0.060	0.235	0.080	0.135	0.127*
P-4	0.135	0.200	0.055	0.130	0.130*
8	0.090	0.125	0.065	0.110	0.097*
9	0.045	0.095	0.040	0.065	0.061*
10	0.035	0.040	0.025	0.070	0.042*
11	0.020	0.040	0.015	0.050	0.031*
12	0.025	0.030	0.013	0.030	0.024*
P-5	0.040	0.020	0.020	0.040	0.030*

*Denotes those means found to be significantly greater than the mean of all samples upstream from the plating plant. Determined by t-test at 0.05 level.

in a river such as the Red Cedar where bicarbonate alkalinity ranges from over 100 to nearly 300 mg/l, depending upon river discharge rate at the time (Grzenda, 1960).

As previously mentioned in the copper section, Doudoroff (1952) found a synergistic effect in soft water between zinc and copper. The Water Research Board of England (Anon., 1960) tested for synergistic effects of copper and zinc and found a synergism present in soft water (15 - 20 mg/l as $CaCO_3$) but absent in hard water (320 mg/l as $CaCO_3$). Since the Red Cedar River is well above the soft-water range, we would expect little synergistic effect, if these findings are correct.

For fish, concentrations as high as 4.0 mg/l zinc have been reported not to have harmed trout exposed for 48 hours. Other workers have found 4.0 mg/l toxic at exposures of three days (McKee and Wolf, 1963). Toxicity among different kinds of fishes as found by various individual workers is so varied it is very difficult to put a definite value on zinc needed to cause death. It is even more difficult, if not impossible, to say what concentrations produce harmful effects on fish short of death.

Effect of zinc on invertebrates is even less well-known than the effect on fish but some work has been done to provide at least an estimate. Cairns and Scheier (1958) stated that 0.79 to 1.27 mg/l zinc was the 96 hour TLm for the snail, *Physa heterostropha*, in soft water at 20 degrees

C. and 0.62 to 0.78 mg/l at 30 degrees C. In hard water the results were 2.67 to 5.67 mg/l zinc at 20 degrees C. and 2.36 to 6.36 mg/l at 30 degrees C. Anderson (1950) states that in Lake Erie water at 25 degrees C. Daphnia magna were immobilized at very much less than 0.15 mg/l zinc chloride.

With so many varying, or even conflicting, reports on toxicity it is difficult to set realistic limits for zinc in wastes. The Mersey and Severn River Boards in England have adopted 1.0 mg/l zinc, alone or in combination with all heavy metals, as the limit concentration of zinc in wastes (Klein, 1957). Tarzwell (1967) suggests that 1.06 mg/l zinc be the maximum concentration allowed anytime-anyplace and bases this on 1/20 of average 48 hour TLm measurements. This figure is modified, as will be shown later, when other wastes are present in the same water.

The limit set on zinc in the Utilex effluent by the 1953 Water Resources Commission Order of Determination is 0.5 mg/l. This limit has been exceeded in 25 of the past 43 sample series of the Water Resources Commission and was exceeded in eight of nine sample series in the present study. In addition, the effluent limit was exceeded twice in the river itself below the plating plant.

Chromium - Ammounts of hexavalent chromium found during the sampling program in Utilex effluent and the river are shown in Table 7. At the time of the Water Resources Commission Order of Determination for Utilex in 1953 it was

Table 7. Average parts per million chromium^6 in each sample series.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all series
Utilex	0.85	0.03	0.30	0.045	
Utilex	0.78	0.40	0.30	0.125	
Utilex	---	0.27	0.03	---	
3		0.015			

Hexavalent chromium concentrations were below detection levels at all stations not listed in the table.

believed that hexavalent chormium was toxic to aquatic forms of life but that the trivalent form was much less harmful. With this belief as a basis, a limit of 2.0 mg/l hexavalent chromium was set for the effluent but no limits were set for chromium in the trivalent form. In order to meet the requirements, the plant operators have been adding sodium bisulfite to the chromium rinse water to reduce chromium from the hexavalent to the trivalent form before release to the river. Although done in a rather crude manner this has evidently been quite successful because hexavalent chromium limits have not been exceeded in Water Resources Commission surveys since June, 1964 and levels were well within limits during all nine sample series of the effluent in the present study. Only once could hexavalent chromium be detected in the river itself and this was just below the effluent and at a very low concentration.

If it was really true that hexavalent chromium was the only toxic form we would assume that chormium was of little consequence in the effluent or in the river. But, more recent studies have indicated that there is evidence to support the conclusion that hexavalent chromium is no more toxic toward fish than the trivalent form (McKee and Wolf, 1963) and some evidence even indicates that trivalent may be more toxic, although there is some uncertainty about this. Jones (1941) found the trivalent form to be more toxic to a flatworm, _Polycelis_ _nigra_, but Anderson (1950) found hexavalent to be most toxic to Daphnia magna in Lake Erie water so there may be some variation in toxicity between the two forms of chromium with various forms of aquatic life. In light of this evidence it appears that we must consider total chromium and not just one of the forms.

With chromium, as with the other metals discussed before, it is extremely difficult to set realistic limits for discharges based on present bioassay data so proposed limits vary somewhat. The Severn and Mersey River Boards in England have adopted standards to limit total concentration of all heavy metals, alone or in combination, including chromium, to 1.0 mg/l (Klein, 1957). McKee and Wolf (1963) have proposed the same limits for chromium (either form) to protect fish life in California but feel that a concentration of 0.05 mg/l must not be exceeded if other forms of aquatic life are to be protected. Fetterolf (1968) has informally acknowledged that this limit of 0.05 mg/l chromium appears justifiable at this time considering the chronic toxic effects of chromium. This does not allow for presence of other wastes at the same time, as will be discussed later.

Average concentrations of total chromium found during the present study are listed in Table 8. The limit of 1.0 mg/l was exceeded in four of nine sample series in the effluent and a limit of 0.05 mg/l would have been exceeded in every area of the river in each series. In every

Table 8. Average parts per million total chromium in each sample.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all series
1	0.07	0.08	0.05	0.10	0.075
2	0.07	0.08	0.09	0.10	0.085
P-1	0.10	0.08	0.17	0.10	0.112
Utilex	0.17	0.70	0.75	1.55	
Utilex	3.05	0.50	0.80	2.60	
Utilex	----	3.40	----	----	
3	0.35	0.29	0.28	0.21	0.282*
P-2	0.32	0.26	0.30	0.21	0.272*
4	0.28	0.28	0.25	0.15	0.240*
P-3	0.25	0.14	0.26	0.20	0.212*
5	0.24	0.20	0.31	0.15	0.225*
6	0.11	0.41	0.24	0.15	0.227*
7	0.11	0.15	0.20	0.17	0.157*
P-4	0.36	0.15	0.23	0.20	0.235*
8	0.14	0.13	0.22	0.18	0.167*
9	0.11	0.11	0.14	0.18	0.135*
10	0.10	0.09	0.29	0.17	0.162*
11	0.10	0.09	0.12	0.15	0.115*
12	0.14	0.09	0.15	0.16	0.135*
P-5	0.13	0.10	0.19	0.13	0.137*

*Denotes those means found to be significantly greater than the mean of all samples upstream from the plating plant. Determined by t-test at 0.05 level.

downstream section of the river the mean concentration of chromium was significantly higher (by t-test at the 0.05 level) than the mean concentration at all stations upstream from the effluent. In past surveys by the Water Resources Commission, chemists have regularly detected chromium concentrations over 3.0 mg/l and concentrations over 6.0 mg/l were not uncommon. This is not only far above what are proposed as safe limits for aquatic life but is also above the limit of 5.0 mg/l proposed by McKee and Wolf (1963) as the safe limit for chromium in water used for stock and wildlife watering.

Cyanide - A limit of 0.6 mg/l cyanide was set for Utilex effluent by the 1953 Water Resources Commission Order of Determination. Through 1965 this limit was exceeded 29 times in 43 series of effluent samples taken by the Water Resources Commission. One sample in 1965 had a cyanide concentration of 14.0 mg/l and many samples had cyanide in excess of 4.0 mg/l. Since installation of the new batch-treatment system for neutralization of cyanide wastes, the Water Resources Commission has reported one violation (1.0 mg/l cyanide) but all other tests were within limits and all effluent samples in the present study were within the limits set in 1953. See Table 9.

The fact that better control of cyanide treatment has enabled the company to meet the 1953 requirements is encouraging but much of the available bioassay data indicates

Table 9. Average parts per million cyanide in each sample series.

Sample	Series 1	Series 2	Series 3	Series 4	Mean of all series
Utilex	0.05	0.05	0.05	0.02	
Utilex	0.00	0.00	0.01	0.15	
Utilex	----	0.00	----	----	
3	0.02	0.00	0.03	0.03	0.020*
P-2	0.00	0.00	0.01	0.04	0.013*
4	0.03	0.00	0.03	0.04	0.025*
P-3	0.00	0.00	0.01	0.01	0.005*

*Denotes those means found to be significantly greater than the mean of all samples upstream from the plating plant. Determined by t-test at 0.05 level.

Cyanide concentrations were below detection levels at all stations not listed in the table.

that this limit may be too high to assure complete protection, especially during periods of extreme low river flow when the effluent may furnish a quarter or more of the total discharge of the river at Fowlerville. These periods of low flow were considered in the 1953 Order of Determination which called for a five to one dilution factor for wastes in the stream (Denniston, 1966).

Lethality of cyanide is largely associated with the proportion of CN^- which is in the form of HCN so toxicity increases with decrease in pH. Toxicity is also increased with elevation of temperature and reduction of dissolved oxygen. Nickel tends to complex with cyanide to reduce toxicity but zinc complexes are exceedingly toxic. This variability in toxicity makes it hard to set absolute limits for cyanides in wastes and especially hard to set limits in the Red Cedar River when temperature and dissolved oxygen vary from day to day and there are variable amounts of both nickel and zinc present with the cyanide. But, as an approximation, results of many bioassays indicate a lethal effect to fish in a few days at concentrations from 0.05 to 0.1 mg/l CN^- (McKee and Wolf, 1963). These values are for lethal effect and we still do not know the effects of lower concentrations on growth and reproduction of fish and other aquatic organisms.

After reviewing much of the research on cyanides, the Aquatic Life Advisory Committee of the Ohio River Valley

Water Sanitation Commission (1960) recommended that free cyanide in excess of 0.025 mg/l be considered unsafe in the waters of the Ohio River. Fetterolf (1968), while realizing the complexity of the problems in setting limits for cyanide, has suggested in an interdepartmental memo that 0.01 mg/l may be a realistic limit and bases this on 1/10 of the TLm found by various workers. This does not seem unreasonably conservative if we consider that highest concentrations in the Red Cedar River would usually come during periods of low river flow when the water temperature is highest and dissolved oxygen at its lowest.

Even with the new batch-treatment system in effect, some cyanide is released to the river (Table 9). This limit of 0.01 mg/l was exceeded in five of nine effluent samples in the present study and was matched or exceeded as far as 0.65 miles downstream from the effluent source.

Wastes in combination - It has already been stated that individual wastes were found in the effluent and in the river in concentrations greater than those allowed by the Michigan Water Resources Commission and much greater than those concentrations suggested as safe for aquatic life by various workers in the fields of toxicology and water pollution. But, this is only part of the story. Combination of the various toxic wastes produces a more lethal environment due to simple additive effect and a much more lethal environment if synergism, such as that between copper and zinc or zinc and cyanide, is considered.

The effects of combination were recognized in England when the Mersey and Severn River Boards adopted a limit of 1.0 mg/l copper or zinc or nickel, etc., with a maximum total concentration of 1.0 mg/l (Klein, 1957). This is much more restrictive than the system of setting limits for individual wastes with no combination limit but it still does not emphasize the fact that 0.5 mg/l cyanide and 0.5 mg/l zinc may be much more toxic than 1.0 mg/l of zinc alone.

Tarzwell (1967) in his Interim Report on Water Quality also recognized the presence of additive or synergistic effects and recommended use of the following formula to set limits for combinations:

$$\frac{Ca}{La} + \frac{Cb}{Lb} \cdot \cdot \cdot + \frac{Cn}{Ln} \leq 1$$

Ca, Cb ... Cn are the measured concentrations of toxic materials in the water.

La, Lb ... Ln are the respective permissible concentration limits derived for each individual material.

The formula can be used in two different ways. Concentrations and allowable limits may be given as 24-hour averages or as maximum amounts allowed "anytime-anyplace." In setting the permissible limits (usually 1/20 of 48-hour TLm) it is assumed that limits are chosen with an adequate safety factor to allow for synergism. This may not prove to be true in all cases but it appears that there has been no adequate data to propose a better system.

Since concentration of metals from the plating plant effluent is so variable in the Red Cedar River, and because a "slug" of effluent could cause great harm in a very short time, the "anytime-anyplace" limits are the most practical ones to use in this case.

Maximum amounts of wastes found in each section of the river are given in Table 10. If these amounts are used in the formula proposed by Tarzwell, with the maximum allowable limits suggested by most recent studies, the resultant sums exceed the allowable in every section of the river downstream from the plating plant effluent. In section three, just below the plant, the sum is 14.84 and in section 12, nearly 10 miles downstream, the sum is 4.7. Both are many times the allowable sum of 1.0.

The formula proposed by Tarzwell is based on past bioassay data and was proposed with the realization that this might not be perfect but is the best available at this time. Another method will be proposed later in this thesis and comparisons will be made at that time.

Table 10. Maximum amounts of each waste found in each section of the river.

Section	Miles to section center	Plating wastes in parts per million					
		Cu	Ni	Zn	Cr^6	Cr Total	CN
1	-0.69	0.08	--	0.01	--	0.10	--
2	-0.22	0.07	--	0.03	--	0.10	--
Effluent	0.00	2.22	9.50	4.10	0.85	3.05	0.15
3	0.13	0.29	1.50	0.56	0.02	0.35	0.04
4	0.45	0.32	1.20	0.36	--	0.28	0.04
5	1.10	0.28	1.00	0.20	--	0.31	0.01
6	2.13	0.38	0.85	0.19	--	0.41	--
7	3.27	0.19	1.40	0.24	--	0.36	--
8	4.36	0.19	0.90	0.13	--	0.22	--
9	5.41	0.11	0.70	0.10	--	0.18	--
10	6.74	0.11	0.50	0.07	--	0.29	--
11	8.57	0.13	1.00	0.05	--	0.15	--
12	9.95	0.10	0.24	0.04	--	0.19	--
Maximum single limits for wastes anytime-anyplace		0.16	1.00	1.06		0.05	0.01

Tarzwell's formula for combinations:

$$\overset{Cu}{\frac{?}{0.16}} + \overset{Ni}{\frac{?}{1.00}} + \overset{Zn}{\frac{?}{1.06}} + \overset{Cr}{\frac{?}{0.05}} + \overset{CN}{\frac{?}{0.01}} = \leq 1$$

With maximums found in section 3 Red Cedar

$$\frac{0.29}{0.16} + \frac{1.50}{1.00} + \frac{0.56}{1.06} + \frac{0.35}{0.05} + \frac{0.04}{0.01} = 14.84$$

With maximums found in section 12 of Red Cedar

$$\frac{0.10}{0.16} + \frac{0.24}{1.00} + \frac{0.04}{1.06} + \frac{0.19}{0.05} + \frac{0.00}{0.01} = 4.70$$

Invertebrate Sampling

Traditional methods of analysis - In the case of the Red Cedar River, as in most instances of pollution, the primary point to be considered is the effect on the biological community. A combination of chemical determinations and past bioassay data would enable us to make a rough prediction of effects in the river but it would be almost impossible to assess the effect of the infinite combinations of physical factors throughout a year or more. Because of the variability in concentration and types of pollutants, Hynes (1963) and previous workers have considered that the relatively immobile invertebrates are the best indicators of pollutional effects. They require much more time to repopulate an area than do fish and they give a good indication of the least favorable as well as average yearly conditions in a body of water.

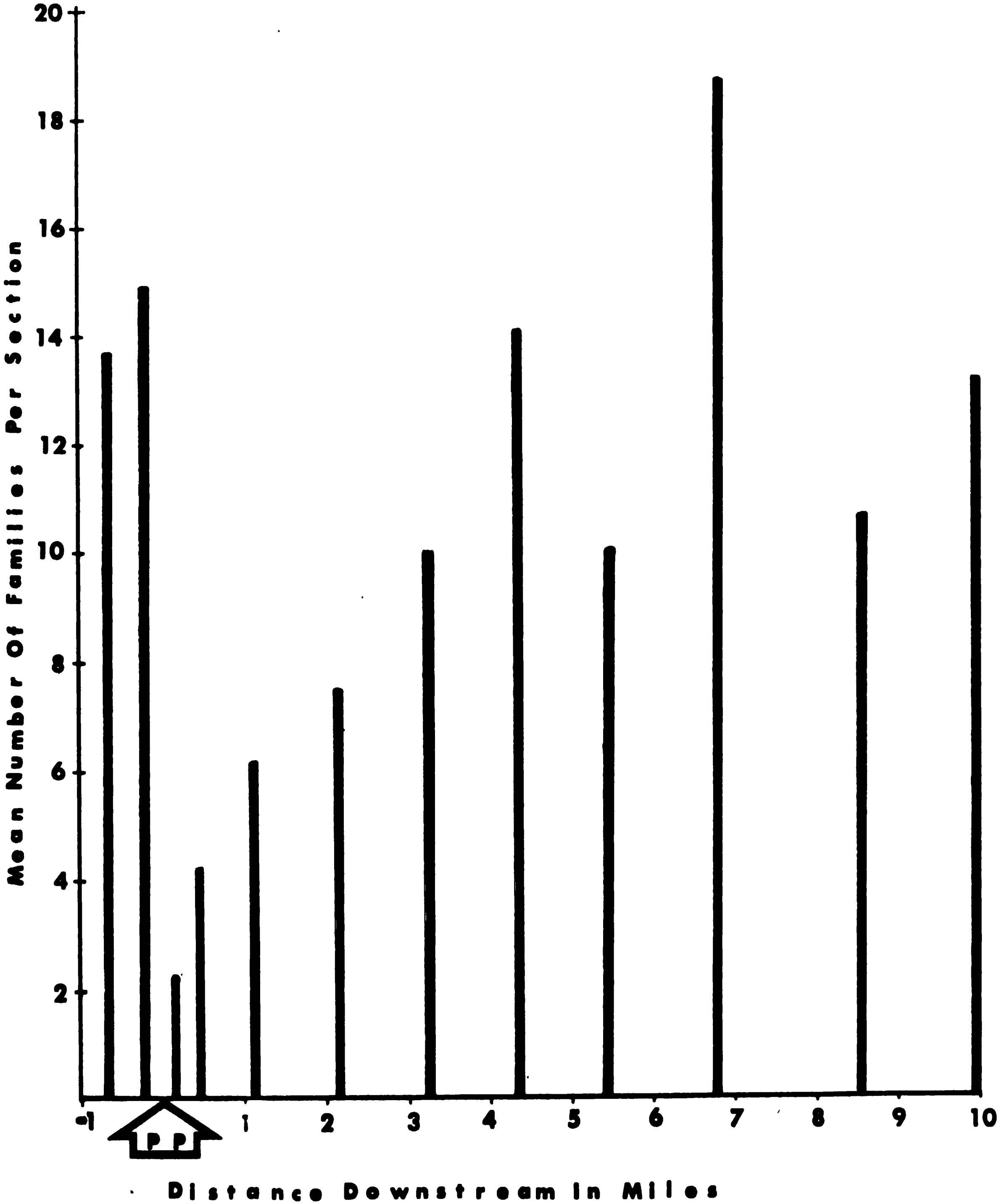
Although there is wide-spread agreement as to the validity of using invertebrates as indicators of pollution, there is some disagreement as to how the data should be handled in order to best detect pollution effects. In this study the invertebrates were identified to family and effects of pollution were assessed by some of the frequently used methods such as indicator types, numbers of families present and community types. In addition, a more easily used index of pollution is proposed which may be compared to more time-tested methods.

A basic theory in ecology is that a natural, unpolluted environment will contain more niches, and thus support more types of animals, than will an environment which has been made less favorable due to pollution or other artificial changes. Thus, in a clean, unpolluted stream, conditions would be favorable for many families of invertebrates. But, if the stream becomes polluted, the conditions are such that only part of the original inhabitants are able to survive. With fewer families present those remaining have less competition so are often found in much greater numbers than would be present in a clean environment.

This is exactly the situation found in the Red Cedar River (Figure 4). Upstream from the plating plant effluent the river averaged about 14 families per dredge. Just below the effluent the average number of families dropped to two and family numbers increased gradually downstream as plating wastes became less concentrated.

In addition to the change in numbers of families present in various areas of the river, there is also a definite pattern shown in the recovery of families downstream from the source of pollution. This is demonstrated in Tables 11-15 which show the numbers of individuals in each family upstream and downstream from the plating plant outfall. In the upstream sections many families are present and none have excessively high numbers of individuals. Just below the plant there is usually only one family present,

Figure 4. Mean number of invertebrate families found in each study section.


Mean Number Of Families Per Section
20
18
16
14
12
10
8
6
4
2
-1
P P
1
2
3
4
5
6
7
8
9
10
Distance Downstream in Miles

Tables 11 - 15. Numbers of invertebrates per square meter in each sample series.

T = Pollution tolerant

F = Facultative

I = Intolerant to pollution

Classification as to tolerance to pollution is based upon past experience of personnel of Michigan Water Resources Commission (Personal communication with Ron Willson, 1968).

PILOT PROGRAM
1965
30 June -
16 August

	Pollution tolerance		Stations and Distance Below Plating Plant																
Station Number			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Miles			-0.92	-0.44	-0.04	0.12	0.29	0.53	1.26	1.74	2.70	2.73	3.82	5.94	7.53	7.57	9.55	9.60	10.13
Kilometers			-1.48	-0.71	-0.06	0.19	0.44	0.85	2.03	2.78	4.34	4.39	6.15	9.56	12.12	12.18	15.37	15.45	16.30
			Numbers of Animals per Square Meter																
Taxonomic Group	Organic	Metals																	
Tubificidae	T		169	59	193	993	2758	19,211	54,931	10,496	5221	485	3601	634	32	71	63	24	43
Tendipedidae	F		816	193	737			67	4	4	95	650	319	2376	2017	516	477	634	690
Tabanidae	F		4	35	12			12				8	59	59	4		8	12	
Pyralidae	F								12	4		12	24	35	12			20	
Tipulidae	F								4						4			39	
Hydrophilidae	F								20	4	32	16	4	16					
Simuliidae	F								47			102	1872		410	12		516	
Hydropsychidae	F								20				331		351		43	1474	4
Dytiscidae	F		16							4	4	20	4	8					
Sialidae	F		4	8	24						71	35	28	51	99	12	75	67	20
Sphaeriidae	T	I	4	8	4						16	4	4	8	8				16
Veliidae	F		4								8					12			
Corixidae	F											4		4					
Naucoridae	F											4							
Rhagionidae	F											4							
Ceratopogonidae	F				20							4		12	4		4		
Elmidae	F		20	59	536							4		43	91	20	24	55	43
Coenagrionidae	F		28										43	43		8			
Hydracarina	F												4	4	4				
Gyrinidae	F													4	114		4	16	
Chrysomelidae	F													4					
Aeschnidae	F													4					
Amphipoda	F	I	95	1580										4	16	4			
Hydroptilidae	I	I												4	4		8		
Planorbidae	F	I			4									8	12				
Baetidae	I	I												4	8	12		12	12
Agrionidae	I	I													20				
Limnephilidae	I	I													20	8	12	24	4
Physidae	T	I	71	99		4									20				
Syrphidae	T														4				
Hirudinea	T		35	83											28	8		4	
Lumbriculidae	F														8			4	
Mesoveliidae	F																	4	4
Heptageniidae	I	I																8	
Perlidae	I	I																4	
Stratiomyiidae	F																		4
Viviparidae	F	I			12														
Limnaeidae	F	I		4	8	4													

SERIES 1

1966
21 June - 6 July

			Stations and Distance Below Plating Plant																
Station Number			1	2	P1	3	P2	4	P3	5	6	7	P4	8	9	10	11	12	P5
Miles			-0.90	-0.27	-0.03	0.14	0.14	0.45	0.65	0.87	2.64	2.85	3.83	4.56	5.88	6.81	8.24	10.10	10.30
Kilometers			-1.45	-0.43	-0.05	0.23	0.23	0.72	1.05	1.40	4.25	4.59	6.16	7.34	9.46	10.96	13.26	16.25	16.57
	Pollution tolerance		Numbers of Animals per Square Meter																
Taxonomic Group	Organic	Metals																	
Tubificidae	T		3050	126	362	7892	20,945	22,490	8428	8428	201	75	9558	260	528	1135	1107	114	3042
Sialidae	F			4		4						4		8	4	16	24	16	
Tendipedidae	F		118	303	39		142	177	12	12	12		75	24	75	75	32	338	4
Coenagrionidae	F						8		4	4		24		4		8			
Hydrophilidae	F		4				4				12			4	8		8	8	8
Tabanidae	F										4					12			
Simuliidae	F													630	99				
Pyralidae	F										4			4	16	4			
Dytiscidae	F											4				4			
Gyrinidae	F												8		8	8			
Stratiomyiidae	F			4	8								4				4		
Elmidae	F		39	32	71									4	8	24	51	347	39
Belostomatidae	F													4					
Hydropsychidae	F			8											20				
Psychomyiidae	I	I													4			8	4
Ceratopogonidae	F		43	20	32										4		8	35	
Limnephilidae	I	I													4				
Amphipoda	F	I	4	272	4												4		
Rhyacophilidae	I	I																8	
Corixidae	F		8	20														12	
Viviparidae	F	I		8															
Ephemeridae	I	I	4																

SERIES 2
1966
1 August -
8 August

Stations and Distance Below Plating Plant

Station Number	Pollution tolerance		1	2	P1	3	P2	4	P3	5	6	7	P4	8	9	10	11	12	P5
Miles			-0.90	-0.19	-0.03	0.12	0.14	0.35	0.65	1.39	1.83	3.52	3.83	4.07	5.42	6.79	9.03	10.19	10.30
Kilometers			-1.45	-0.31	-0.05	0.19	0.23	0.56	1.05	2.24	2.94	5.66	6.16	6.55	8.72	10.93	14.53	16.40	16.57
			Number of Animals per Square Meter																
Taxonomic Group	Organic	Metals																	
Tubificidae	T		213	1541	957	7774	39,022	31,161	42,907	33,860	30,641	693	3893	10,827	1076	485	55	32	800
Pyralidae	F			8	4	4		47	20	8	47		20	12		4			
Tendipedidae	F		292	756	957			165	8	110	24	181	12	256	1048	296	55	319	311
Sialidae	F			12	12			4		28		79		8	67	75		35	99
Coenagrionidae	F		24	130	12			20	32		4		240	43	4	12		4	24
Ceratopogonidae	F			20				47	4				4	51		16			
Culicidae	F								4	8				8					4
Corixidae	F								4				8		4				
Haliplidae	F			1					4										
Libellulidae	F										4		4						
Dytiscidae	F										4								
Gerridae	F										4								
Tabanidae	F			87							20		28	51		32			
Hydrophilidae	F										4		43	63		8			
Leptoceridae	I	I		4								4	24	20	12	4	4	12	
Elmidae	F		79	303	528								4		43	130	16	248	485
Gomphidae	F												4	4					
Baetidae	I	I	8	8									8					24	16
Gyrinidae	F			4									4			12		20	
Hydroptilidae	I	I											12			260			
Agrionidae	I	I											4			4			
Hebridae	F													4					
Chrysomelidae	F													4		4			
Limnephilidae	I	I												12		8			8
Hydracarina	F														4				
Ephemeridae	I	I		20												4			12
Hydropsychidae	F			8												59	63	138	4
Rhyacophilidae	I	I	4													20			
Sphaeriidae	T	I		134	4											177			
Psychomyiidae	I	I														4		6	4
Lumbriculidae	F			51												4			
Muscidae	F															4			
Corydalidae	F			4															
Amphipoda	F	I	91	879	4														
Planariidae	F			39															
Viviparidae	F	I		8															
Heptageniidae	I	I		16															
Planorbidae	F	I		4															
Glossiphoniidae	T			20	4														
Belostomatidae	F			4															

SERIES 3
1966
12 September -
27 September

Stations and Distance Below Plating Plant

Number of Animals per Square Meter

Station Number			1	2	P1	3	P2	4	P3	5	6	7	P4	8	9	10	11	12	P5
Miles			-0.92	-0.43	-0.03	0.10	0.14	0.47	0.65	0.84	2.27	3.74	3.83	4.15	5.61	7.43	9.16	9.94	10.30
Kilometers			-1.48	-0.69	-0.05	0.16	0.23	0.76	1.05	1.35	3.65	6.02	6.16	6.68	9.03	11.95	14.74	15.99	16.57
Taxonomic Group	Pollution tolerance Organic	Pollution tolerance Metals																	
Tubificidae	T		307	623	2163	101,924	53,107	111,943	125,993	35,342	25,299	22,517	13,451	16,355	1036	8376	1288	47	1245
Coenagrionidae	F		524	59	28		4	102	102	35	571	753	396	894		12		16	256
Elmidae	F		331	4295	1619		8	8	16			20		12	20	839	1032	772	1328
Amphipoda	F	I	6138	1800	181		4									8			8
Pyralidae	F		16					16	4	4	51	189	35	91		4	4	106	16
Chrysomelidae	F							4					8	28					
Tendipedidae	F		745	493	1281			532	3711	201	1651	2463	1261	4641	209	469	3207	796	2104
Psychomyiidae	I	I						4		4									
Libellulidae	F			12				4		4	16	12							
Ceratopogonidae	F		8	512	461				8		67	16	35	43		71	24	8	63
Hydrophilidae	F								47		264	705	477	1048		110	28	4	4
Corixidae	F			79	16				28	4				12					79
Agrionidae	I	I							8					4				99	
Saldidae	F		776						4		4		32						
Belastomatidae	F								4										8
Notonectidae	F								8										
Culicidae	F		4							4			4	4				4	
Tabanidae	F		8	59							83	59	134	106	4	39	55	4	47
Erpobdellidae	T			4	4						4								
Sialidae	F			16	8							4	4	67	47	51	71	39	71
Gyrinidae	F											4		8					
Planariidae	F		4	12	4							12		4		20	8	59	4
Leptoceridae	I	I	16	12	4							8		24		63	28	51	32
Gomphidae	F											4							
Dytiscidae	F		4										4						
Hydropsychidae	F				4								4	91		229	423	7348	
Sphaeriidae	T	I	102	146	55								4	32	4	35	75		236
Psychomyiidae	I	I	4										4					20	8
Rhagionidae	F			4	4								4	16				4	
Simuliidae	F													39			63		
Limnaephilidae	I	I		4											12	12		12	16
Amnicolidae	F	I	437	4												43			
Glossiphoniidae	T		55		8											20			
Plarorbidae	F				4											8			
Tipulidae	F																28	35	4
Heliopsychidae	I	I																8	
Baetidae	I	I	99															355	20
Ephemeridae	I	I		4	4														
Mesoveliidae	F		1																
Ancylidae	F	I	16																
Heptageniidae	I	I	4																
Physidae	T	I	24	24															
Lumbriculidae	F			16	8														

SERIES 4
1966
31 October -
8 November

	Station Number		1	2	P1	3	P2	4	P3	5	6	7	P4	8	9	10	11	12	P5
	Miles		-0.85	-0.33	-0.03	0.14	0.14	0.36	0.65	0.75	2.20	3.71	3.83	4.12	5.24	6.93	9.01	10.15	10.30
	Kilometers		-1.37	-0.53	-0.05	0.23	0.23	0.58	1.05	1.21	3.54	5.97	6.16	6.63	8.43	11.15	14.50	16.33	16.57
	Pollution tolerance		Stations and Distance Below Plating Plant — Number of Animals per Square Meter																
Taxonomic Group	Organic	Metals																	
Tubificidae	T		181	110	1478	85,652	85,652	62,031	90,545	85,155	8380	12,088	8337	14,011	5189	14,696	2455	2041	1182
Coenagrionidae	F		268	24	32	12	12	8	51	51	323	209	355	292	4	8		4	8
Elmidae	F		118	1576	1320			4				8	4	12	165	256	768	406	556
Pyralidae	F		8		8			4		43	24	8	43	20		4			
Saldidae	F							4											
Dytiscidae	F								4	24		4	8		4				
Hydrophilidae	F			4					4		43	150	595	666	20	102	4		4
Tendipedidae	F		808	607	2273				359	240	599	595	855	1277	4673	883	1694	1643	1225
Notonectidae	F								12	4									
Culicidae	F									4									
Sialidae	F			4	8						20		12	32	12	87	4	32	28
Tabanidae	F			71	12						71	12	193	32	8	12	59	55	8
Ceratopogonidae	F		20	804	788						8	4	59	244	106	126	398	181	79
Aeschnidae	F		4								4	8	4						
Haliplidae	F											4							
Amphipoda	F	I	2132	414	142							4							
Corixidae	F		4	24								28	4	4	35		32	67	338
Libellulidae	F											28	4	16					
Glossiphoniidae	T		12	12	4							4	4						
Gomphidae												4					4		
Sphaeriidae	T	I	4	55	32								20		12	32	16		351
Planariidae	F												8	8		8			
Leptoceridae	I	I	24		4								8	39			4	8	
Belostomatidae	F				4								4						
Simuliidae	F													8		28			
Hydropsychidae	F		4											20		473	102	4	
Psychomyiidae	I	I	4		4									4	8			16	8
Haliplidae	F													4					
Stratiomyiidae	F															4			
Tipulidae	F		16	16												12	35		4
Lumbriculidae	F		12	59												8			
Agrionidae	I	I														12			
Hydroptilidae	I	I														4	4		
Rhagionidae	F				4												4		
Baetidae	I	I	35															8	4
Ephemeridae	I	I																4	24
Limnephilidae	I	I	8	8	8														4
Gyrinidae	F		4																
Amnicolidae	F	I	229		83														
Heptageniidae	I	I			4														
Physidae	T	I	16																
Viviparidae	F	I		4															

Tubificidae, and the number of individuals may exceed 100,000 per square meter. As the effluent is diluted out farther downstream families return in relation to their ability to withstand the plating wastes.

The Michigan Water Resources Commission from past experience has labeled some families as intolerant to plating wastes (Personal Communication with Ron Willson, 1968) and these families are indicated in the tables. In nearly all instances these are the families which are absent for the greatest distance below the plating plant. In some cases when intolerant families are represented by only one or two individuals just below the plant, their presence is probably due to drift and not to the fact that they normally live in that area.

The five tables represent five different sample series taken at different times of the year so the numbers of families present vary but the pattern is the same in every one whether samples were taken in spring with few families present or in late summer when many more families were found.

Correlation analysis - If the drastic changes between stations upstream and downstream from the plating plant are really due to plating wastes, there should be a statistical correlation between wastes and numbers of families and individuals in the river. Analysis for such correlations was run on the CDC 3600 computer using the 11 most prominent families above the plant and the six metals in the effluent. Data for the analysis was taken from the three upstream stations and only three stations downstream to avoid interference by effluent from the Fowlerville sewage lagoons. As shown in Table 16, there was a positive correlation between Tubificidae and all plating wastes except hexavalent chromium and there were negative correlations between wastes and all other invertebrates and between Tubificidae and other invertebrates. All animals except Tubificidae are inhibited by the plating wastes and it almost appears that the Tubificidae receive a beneficial effect. This is probably due to lack of predation and competition since they are the only survivors in the area and not due to any benefit from the wastes.

A similar correlation analysis was run using all families present in that section of the river. Results, which are on file and available at Institute of Water Research, Michigan State University, are essentially the same and so were not included here due to unwieldy size of the intercorrelation matrix. It would be possible to use the correlations to set up lists of relatively intolerant families including Baetidae, Tabanidae, Amphipoda, Glassiphoniidae and a short list of more tolerant families composed primarily of Tubificidae so we could describe "clean" or "polluted" water communities. But, although used extensively in the past, there seems little to be gained from this. As

Table 1[illegible]. Intercorrelation matrix for 11 families and 6 metals.

	Tubificidae 1	Tendipedidae 2	Ceratopogonidae 3	Sialidae 4	Baetidae 5	Tabanidae 6	Coenagrionidae 7	Elmidae 8	Amphipoda 9	Glossiphoniidae 10	Sphaeriidae 11	Cu 12	Ni 13	Zn 14	Cr^6 15	Cr Total 16	CN 17
Tubificidae 1	1.0000																
Tendipedidae 2	-0.2388	1.000															
Ceratopogonidae 3	-0.2135	0.3706	1.0000														
Sialidae 4	-0.1996	0.3800	0.0532	1.0000													
Baetidae 5	-0.1078	0.0713	-0.0593	-0.0140	1.0000												
Tabanidae 6	-0.1966	0.1613	0.3028	0.2533	0.0861	1.0000											
Coenagrionidae 7	-0.0707	0.2118	-0.0281	-0.0185	0.9424	0.1272	1.0000										
Elmidae 8	-0.2440	0.3052	0.6646	0.3439	-0.0543	0.4889	0.0100	1.0000									
Amphipoda 9	-0.1534	0.1133	0.0054	-0.0473	0.8767	0.1226	0.9182	0.0231	1.0000								
Glossiphoniidae 10	-0.1183	0.1888	0.0331	0.0904	0.9025	0.1549	0.8560	-0.0145	0.7675	1.0000							
Sphaeriidae 11	-0.2162	0.3117	0.2223	0.2699	-0.0311	0.2841	0.0046	0.3766	0.0045	-0.0039	1.0000						
Cu 12	0.5486	-0.3656	-0.2373	-0.2474	-0.1491	-0.2618	-0.1664	-0.3492	-0.2158	-0.1724	-0.3374	1.000					
Ni 13	0.1435	-0.3178	-0.2172	-0.1525	-0.1109	-0.2053	-0.1479	-0.2575	-0.1596	-0.1235	-0.2300	0.7926	1.0000				
Zn 14	0.5727	-0.3368	-0.2650	-0.2000	-0.1245	-0.2322	-0.1417	-0.3041	-0.1797	-0.1379	-0.2604	0.8308	0.5095	1.0000			
Cr^6 15	-0.0945	-0.1259	-0.0728	-0.0711	-0.0354	-0.0656	-0.0586	-0.0825	-0.0510	-0.0395	-0.0735	0.2609	0.5394	0.0858	1.0000		
Cr Total 16	0.4314	-0.3803	-0.2415	-0.2151	-0.1987	-0.2916	-0.2355	-0.3047	-0.2729	-0.2004	-0.3217	0.8430	0.7089	0.7477	0.2420	1.0000	
CN 17	0.6828	-0.2972	-0.2241	-0.1956	-0.1091	-0.2021	-0.1161	-0.2529	-0.1571	-0.1216	-0.2264	0.3580	-0.0180	0.3716	-0.1339	0.3981	1.0000

Hynes (1963) has pointed out, these ideal communities seldom occur as predicted due to the variability in nature so they can be misleading. And, in this case, simple family lists as shown in Tables 11-15 accomplish the same purpose.

Principal components analysis - Although the correlations between invertebrates and wastes are quite strong (Table 16), the effect upon different families is so varied that we might suspect that environmental factors other than plating wastes are also involved. In fact, variability in nature is already so high that we would never expect the plating effluent to be anything but one of many factors affecting the stream. For this reason data on the same 11 families and six metals for three stations above and three below the plating plant were used for a principal components analyses.

The principal components analysis is a statistical method initially used in psychology to separate non-correlated factors having an effect on behavior (Seal, 1964). In this case the method was used to separate non-correlated factors each of which had an effect upon the total variability of invertebrates in the three upstream and three downstream sample stations. The columns labeled P_1 .... P_{17} (Table 17) are vectors of factors which ~~e~~affect each family or metal by the relative amounts shown in the columns. At the bottom of each column is given the relative amount of

Table 17. Factor loading matrix for principal components analysis.

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	P_{13}	P_{14}	P_{15}	P_{16}	P_{17}
Tubificidae	0.5843	0.1969	-0.3926	0.4911	0.0487	-0.0116	0.1193	0.2131	-0.1002	-0.2947	0.0546	-0.2110	0.0653	0.1128	0.0192	-0.0180	-0.0095
Tendipedidae	-0.5509	-0.1652	0.0947	0.2081	0.3379	-0.5736	0.0551	0.0897	-0.3700	0.0595	-0.1318	0.0502	0.0329	-0.0235	0.0095	-0.0078	-0.0254
Ceratopogonidae	-0.4154	-0.3331	0.2361	0.4433	-0.4930	-0.3571	-0.0261	0.0322	0.0931	0.0827	0.2180	-0.0604	-0.1487	0.0432	-0.0213	-0.0236	0.0140
Sialidae	-0.3549	-0.2479	0.1710	0.2257	0.7227	0.0875	-0.3296	0.1252	0.2384	-0.0248	0.0792	-0.0350	-0.1091	0.0328	-0.0157	-0.0030	0.0091
Baetidae	-0.4602	0.8589	0.0441	0.0449	-0.0015	0.0322	0.0088	-0.0090	0.0879	0.0241	0.0498	-0.0084	0.0477	-0.0208	-0.1399	-0.0563	-0.0950
Tabanidae	-0.4515	-0.1541	0.2161	0.4139	-0.1135	0.5712	-0.1752	-0.0249	-0.4186	0.0858	0.0020	0.0003	-0.0266	0.0226	-0.0062	-0.0081	-0.0043
Coenagrionidae	-0.5032	0.8297	0.0333	0.1134	0.0085	-0.0380	0.0497	0.0093	-0.0226	-0.0507	-0.1180	0.0013	-0.0036	0.0090	-0.0917	-0.0117	0.1214
Elmidae	-0.5092	-0.3967	0.2679	0.5127	-0.2243	0.0448	-0.0794	0.0531	0.2778	-0.1490	-0.2219	0.0590	0.1713	-0.0719	0.0173	0.0181	-0.0119
Amphipoda	-0.5141	0.7781	0.0402	0.0334	-0.0859	0.0202	0.0452	-0.0292	0.0609	-0.0868	-0.1677	0.0360	-0.2313	0.0763	0.1214	0.0265	-0.0376
Glossiphoniidae	-0.4905	0.7720	0.0829	0.1232	0.0681	-0.0249	-0.0521	0.0310	0.0193	0.1387	0.2413	-0.0301	0.1839	-0.0503	0.1222	0.0477	0.0131
Sphaeriidae	-0.4284	-0.3123	0.1279	0.2202	0.2529	0.1855	0.7160	-0.1646	0.0923	0.0688	0.0430	-010212	-0.0147	0.0101	-0.0028	0.0069	0.0014
Cu	0.8412	0.2525	0.2846	0.2746	0.0345	-0.0698	-0.0094	-0.1142	-0.0545	-0.0098	-0.0087	-0.0922	-0.0760	-0.0996	-0.0741	0.1418	-0.0151
Ni	0.6469	0.1884	0.6590	-0.0414	0.0436	-0.0062	0.0072	-0.0631	0.0133	0.0694	-0.1038	-0.2570	-0.0122	-0.0964	0.0604	-0.0953	0.0071
Zn	0.7560	0.2420	0.0898	0.3633	0.1002	-0.0326	0.0073	-0.2478	-0.0407	-0.1749	0.1260	0.3105	-0.0366	-0.0831	0.0300	-0.0595	0.0083
Cr^6	0.2420	0.0730	0.6828	-0.2639	-0.0509	0.0754	0.1770	0.5697	-0.0480	-0.0928	0.0684	0.1398	-0.0048	0.0240	-0.0116	0.0139	-0.0010
Cr Total	0.8241	0.1768	0.2532	0.2276	0.0380	-0.0584	-0.0431	-0.0960	0.0896	0.2482	-0.0939	0.0984	0.0961	0.2455	-0.0072	0.0039	-0.0020
CN	0.5073	0.1442	-0.5518	0.3683	-0.0135	0.0885	0.0736	0.3828	0.0898	0.2867	-0.0861	0.0577	-0.0761	-0.1201	0.0109	-0.0198	0.0036
Relative Amount of Variance	5.2526	3.3598	1.7527	1.5119	1.0384	0.8535	0.7223	0.6615	0.5088	0.3500	0.2756	0.2616	0.1815	0.1323	0.0695	0.0410	0.0269
Percent	30.9	19.8	10.0	8.1	6.0	5.0											
Cumulative Percent	30.9	50.70	60.70	69.60	75.60	80.60											

Table 17. Factor loading matrix for principal components analysis.

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	P_{13}	P_{14}	P_{15}	P_{16}	P_{17}
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Coenagrionidae	-0.5032	0.8297	0.0333	0.1134	0.0085	-0.0380	0.0497	0.0093	-0.0226	-0.0507	-0.1180	0.0013	-0.0036	0.0090	-0.0917	-0.0117	0.1214
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Sphaeriidae	-0.4284	-0.3123	0.1279	0.2202	0.2529	0.1855	0.7160	-0.1646	0.0923	0.0688	0.0430	-010212	-0.0147	0.0101	-0.0028	0.0069	0.0014
Cu	0.8412	0.2525	0.2846	0.2746	0.0345	-0.0698	-0.0094	-0.1142	-0.0545	-0.0098	-0.0087	-0.0922	-0.0760	-0.0996	-0.0741	0.1418	-0.0151
Ni	0.6469	0.1884	0.6590	-0.0414	0.0436	-0.0062	0.0072	-0.0631	0.0133	0.0694	-0.1038	-0.2570	-0.0122	-0.0964	0.0604	-0.0953	0.0071
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Cr^6	0.2420	0.0730	0.6828	-0.2639	-0.0509	0.0754	0.1770	0.5697	-0.0480	-0.0928	0.0684	0.1398	-0.0048	0.0240	-0.0116	0.0139	-0.0010
Cr Total	0.8241	0.1768	0.2532	0.2276	0.0380	-0.0584	-0.0431	-0.0960	0.0896	0.2482	-0.0939	0.0984	0.0961	0.2455	-0.0072	0.0039	-0.0020
CN	0.5073	0.1442	-0.5518	0.3683	-0.0135	0.0885	0.0736	0.3828	0.0898	0.2867	-0.0861	0.0577	-0.0761	-0.1201	0.0109	-0.0198	0.0030
Relative Amount of Variance	5.2526	3.3598	1.7527	1.5119	1.0384	0.8535	0.7223	0.6615	0.5088	0.3500	0.2756	0.2616	0.1815	0.1323	0.0695	0.0410	0.0266
Percent	30.9	19.8	10.0	8.1	6.0	5.0											
Cumulative Percent	30.9	50.70	60.70	69.60	75.60	80.60											

variance attributed to each factor and from this is figured the percent of variance attributed to each factor.

An important characteristic of principal component analysis is that it separates out the factors, shows the effect of each factor on each metal and each family, and indicates how much of total variance is attributed to each factor, but it does not identify the factors. Identification of the factors is based upon all available evidence and is dependent to a large degree upon the amount of evidence and the experience of the researcher. So, a weakness of the method is that it never conclusively proves the effect of any one identifiable factor in the environment. The main advantage is that, with non-correlated factors, it enables one to at least recognize that these factors are present and to hypothesize as to their nature.

In this case the primary aim is to see how much of the total variance can be attributed to the plating effluent. Factor P_1 which accounts for 30% of variance is almost certainly due to the effluent. Metals and tubificids all increase and all other forms decrease in relation to this factor. Other methods of analysis, such as the family lists, have already shown that this is the effect of the plating effluent on the stream. When we consider the great natural variability in a stream, 30 percent is a large amount to be caused by one factor, especially when we know that the effect of this factor is detrimental.

Other major factors such as P_2 and P_3 may be related to season, temperature, stream discharge or any number of environmental forces not even suspected. Factor P_3 seems especially likely to be season or discharge (or this may be one factor) since most invertebrates increase as summer progresses and metal concentrations increase with decrease in stream discharge. It is probably impossible to identify all forces in the environment but it is still important to be able to recognize that these forces are present and to see that, while the plating plant effluent is not the only factor contributing to overall variance in the stream, it is the major factor in this area.

Index of pollution - Family occurrence lists such as shown in Tables 11-15 and lists of indicator species have been used extensively in the past as measures of pollution and a combination of these indicators has been accepted by most biologists as quite reliable. However, these methods require a great deal of time and effort and much of the work must be done by persons with good training in taxonomy. Even when the time and effort are spent, the results are often meaningless to anyone but an aquatic biologist. Some biologists such as Hynes (1963) and probably most engineers claim that we need simpler methods of quantifying pollutional effects to make the results more useable by industry and understandable by the lay public.

The fact that Tubificidae can thrive in concentrations of plating wastes which eliminate all other forms of invertebrates suggests that some relationship between tubificids and other forms might be an indicator of pollution, at least from the plating plant. King and Ball (1964) recognized this relationship and proposed a ratio of aquatic insect weight to tubificid worm weight as an index of domestic and plating plant pollution in the Red Cedar River.

This ratio worked quite well for King but it had one major disadvantage. If worms were absent the index reached infinity and this made it impossible to calculate means, variance, etc. Substitution of King's index into a simple mathematical formula solves this problem while retaining the basic idea of the index.

The basic idea of the formula is that if a number t goes from 0 to infinity,

then $x = \frac{1}{1 + t}$ goes from 0 to 1.

Since the index $\frac{\text{Insects}}{\text{Tubificids}}$ goes from 0 to infinity,

it is substituted for t.

$$\text{So the index } x = \frac{1}{1 + \frac{\text{Insects}}{\text{Tubificids}}} = \frac{\text{Tubificids}}{\text{Tubificids} + \text{Insects}}$$

and goes from 0 to 1.

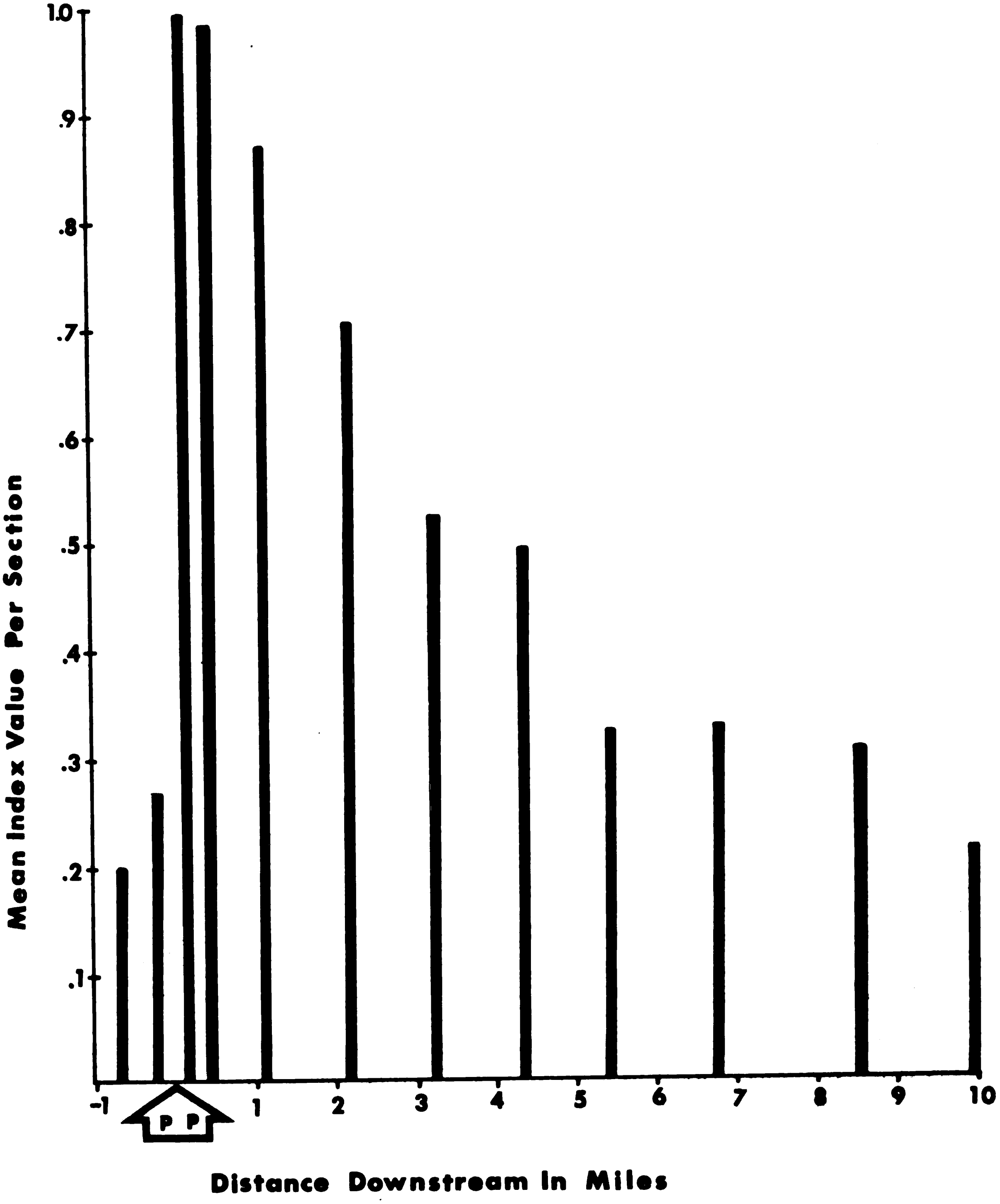
If insects are absent the index will be 1 and if tubificids are absent the index will be 0. An index of zero would seldom occur since we would almost always have at least a few tubificids in a well-balanced clean-water system.

The revised formula was used to calculate an index value for all sections of the river based on all samples in each section. The result (Figure 5) shows much the same pattern as a simple list of families but the curve is more even. Mean index values at the lower end of the study area had nearly returned to the levels found upstream from the plating plant, after having almost reached maximum values of 1.0 just below the plating effluent. This is much the same pattern found in the chemical analyses for metal concentrations. Concentrations 10 miles downstream were almost down to the levels found above the plating plant.

Multiple regression analysis - If the index is really a measure of the "health" of the river, and if this "health" is influenced primarily by the plating plant effluent, there should be a measurable relationship between index and metals so we could predict the effect of one upon the other. To determine this relationship, metals and index values for all stations in all four regular sample series below the plating plant effluent were used to compute a multiple regression of index on the plating plant metals.

The resultant regression equation is: Index = $0.15 + 0.47\ \mathrm{Cu} + 0.05\ \mathrm{Ni} + 0.42\ \mathrm{Zn} + 9.10\ \mathrm{Cr}^6 + 1.46\ \mathrm{Cr}^T + 10.03\ \mathrm{CN}^-$. The equation is significant at less than 0.0005. A comparison of regression and error sums of squares

Figure 5. Relation of mean insect-tubificid index to distance downstream from the plating plant. Calculated using both regular and pilot series data.


Mean Index Value Per Section
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
-1
P P
1
2
3
4
5
6
7
8
9
10
Distance Downstream In Miles

indicates that 57% of the index variability in the river below the plating plant can be explained by regression of the index on the plating plant wastes (Li, 1964). Regression coefficients and standard errors of coefficients are as follows:

Variable	Regression Coefficient	Standard Error
Constant	0.1571	0.0842
Cu	0.4763	0.5239
Ni	0.5266	0.1029
Zn	0.4290	0.4231
Cr^6	9.1750	16.5505
Cr^T	1.4679	0.4919
CN	10.0363	3.1528

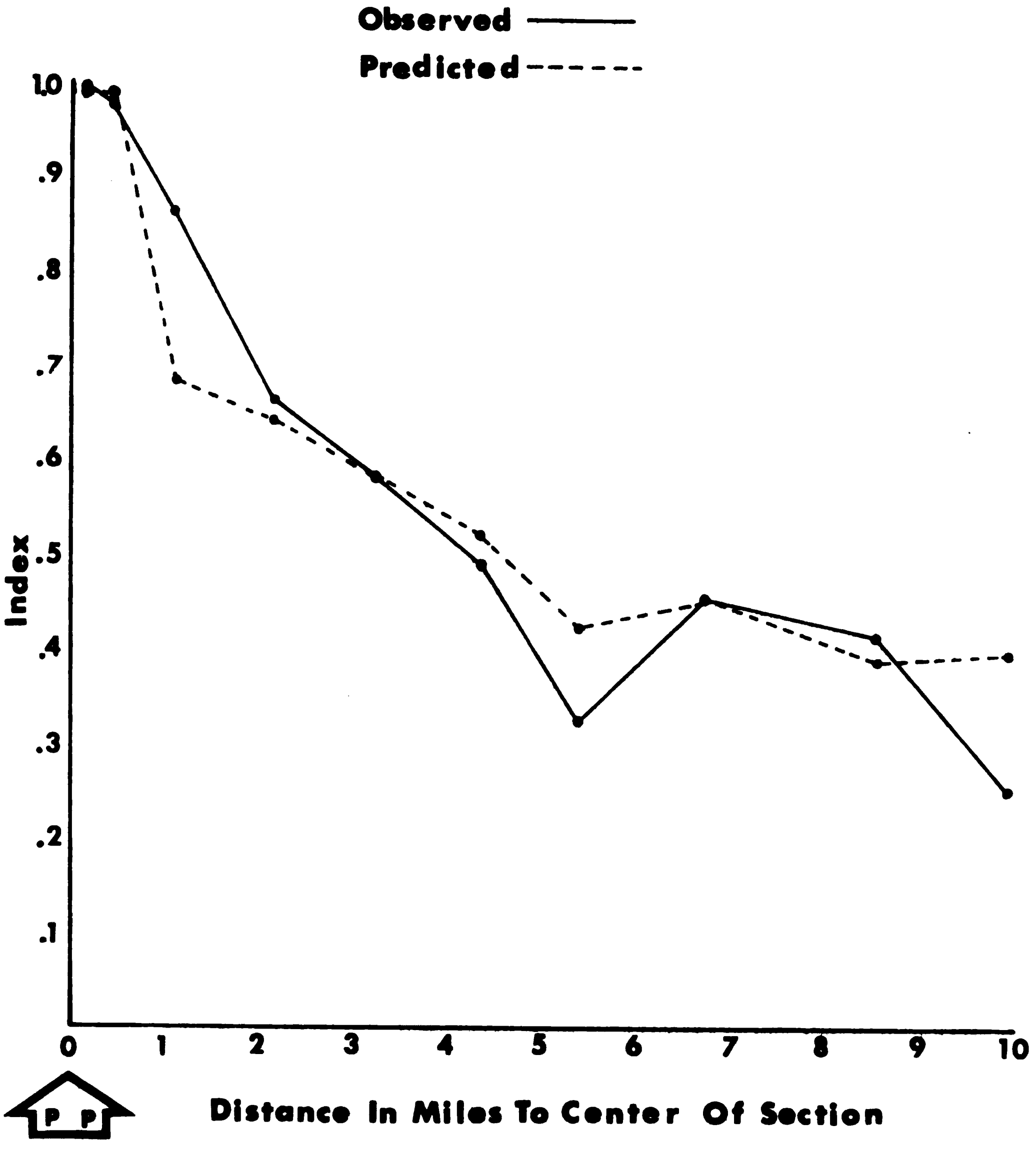
From the regression coefficients it would appear that total chromium and cyanide have the most effect upon the index. Hexavalent chromium also shows a very great effect but the standard error is very high, due to the fact that Cr^6 was absent from all but one of the river samples. It is not surprising that cyanide has a pronounced effect since the toxicity of cyanide is well-known. But, it is interesting to note that chromium is so important since it was thought to be relatively harmless in the trivalent state and was not even limited by the 1953 Order of Determination of the Water Resources Commission. Complete data and analysis are available on file at the Institute of Water Research, Michigan State University.

If the regression of index on metals is really worthwhile we should be able to predict the index from metal concentrations. This was done and a plot of observed and predicted indices is shown in Figure 6. The multiple correlation coefficient is 0.7593 which is quite good for a highly variable biological system. At the same time, we should be able to determine a desirable index level and use the regression equation to set waste limits to ensure the maintainance of such an index level.

As a measure, the index may not be meaningful by itself, but it can be tied in with other things to help determine a desirable index. Fish production for recreation is a primary concern in this area and fishing has long been deteriorating on the Red Cedar River. Linton and Ball (1965) have indicated that fish populations in the river are drastically reduced for about eight or nine miles below the plating plant. If this is the case it appears that we cannot have an acceptable recreation fishery in the river in an area where the insect-tubificid index is higher than about 0.4.

If 0.4 is taken as the maximum allowable index we see that plating wastes are at the maximum allowable limits about nine miles below the plant. This point in the stream corresponds to section 11 and mean metal concentrations in mg/l found there are as follows: Cu 0.05, Ni 0.39, Zn 0.03, Cr^6 -0.0, Cr^T 0.12, CN^- -0.0. Use of these mean concentrations in the regression formula results in a predicted index of 0.39.

Figure 6. Relation of observed to predicted insect-tubificid index values. Indices are based upon data from the four regular sample series in which both invertebrates and water samples were collected.


Observed
Predicted
Index
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
4
5
6
7
8
9
10
P P
Distance In Miles To Center Of Section

If these same values are used in the formula proposed by Tarzwell (1967) for maximum allowable wastes the following results are obtained:

$$\begin{array}{ccccccccc} \text{Cu} & & \text{Ni} & & \text{Zn} & & \text{Cr}^{T} & & \\ \frac{0.056}{0.16} & + & \frac{0.39}{1.00} & + & \frac{0.03}{1.06} & + & \frac{0.12}{0.05} & = & 3.07 \end{array}$$

From this, we would assume that wastes are still too high because the results of Tarzwell's formula are not supposed to exceed 1.0. It appears that his formula may be a bit more conservative than necessary but he may not be far off when we consider long-term effects on the population. Linton (1967) claims rock bass populations are declining in the river so, if this is due to plating waste, an index value closer to 0.3 may really be needed. If this is true we might have to consider concentrations in zone 12 as too high so maximum allowable amounts would be closer to the amounts proposed by Tarzwell's formula and a great deal lower than were proposed by the Water Resources Commission. To maintain an index below the 0.3 range with the metals present in the same proportions found in section 11, maximums would have to be about half the average amounts found in section 11. These would be 0.026 Cu, 0.20 Ni, 0.015 Zn, 0.06 Cr^{T} expressed as mg/l. The resultant index would be 0.27. If these are used in Tarzwell's formula the result is 1.52 so still supposedly too high.

The fact that Tarzwell's formula is high is due primarily to the very low limit (0.05 mg/l) which is used for total chromium as suggested by Fetterolf (1968). This limit may be lower than necessary but we cannot be sure at this time.

Based upon invertebrate populations in the Red Cedar River and fish populations in the corresponding areas, I believe maintainance of an insect-tubificid index not much greater than 0.3 would ensure use of the river for warm-water sports fisheries. To maintain this index and general state of "health" of the river the following are proposed as maximum concentrations of plating wastes in the river: 0.056 mg/l Cu, 0.39 mg/l Ni, 0.03 mg/l Zn, 0.00 mg/l Cr^6, 0.12 mg/l Cr^T and 0.00 mg/l CN^-. This should assure a maximum index value of 0.39 below the effluent and an index of 0.3 or better could be expected within less than a half-mile distance downstream. If these limits are maintained in the upstream sections near the plant outfall, we could expect to be able to maintain sports fish populations there (excluding any other pollution or habitat problems) and could expect the river further downstream to be even better.

SUMMARY

Metal plating wastes discharged by Utilex Manufacturing Company into the Red Cedar River at Fowlerville, Michigan are causing a decrease in productivity of fish and bottom organisms in the river for many miles below the effluent. In the present study, water was analyzed for plating wastes above and for 10.3 miles below the plating waste outfall and standing crop estimates were made of invertebrates in the same areas in order to determine the effect of the plating wastes on numbers and types of invertebrates in the stream.

Nickel, zinc and total chromium were found in significantly (by t-test at 0.05 level) higher concentrations for 10.3 miles downstream as compared to concentrations upstream from the plating plant. Copper was increased significantly for over eight miles. Cyanide and hexavalent chromium could not be traced nearly so far but they were present in the river for as much as one-half mile downstream from the effluent. All six waste elements were found in the river in quantities which have been found to be detrimental to aquatic organisms.

Families of invertebrates in the river were reduced from an average of about 14 families upstream from the

plating plant to two right below the effluent. Families of invertebrates begin to appear again downstream from the effluent according to their tolerance for plating wastes and an average of 12-14 families is attained again in the river about seven miles below the waste effluent.

Use of correlation analysis has shown that all families of invertebrates except Tubificidae are adversely affected by the plating wastes. Tubificids seem relatively insensitive and increase in numbers in higher concentrations of wastes, presumably because of lack of competition and predation. Use of principal components analysis has shown that over 30 percent of variability among invertebrates at three sample stations upstream and three stations downstream from the plating plant can be attributed to the plating wastes.

A revised version of an insect-tubificid ratio (King and Ball, 1964) is proposed as an easier method of assessing pollution in a river such as the Red Cedar. The weight ratio $\frac{\text{Tubificids}}{\text{Tubificids + Insects}}$ averaged about 0.25 upstream from the plant. Just below the effluent the ratio exceeded 0.99 and it slowly declined downstream to a value of about 0.3 ten miles downstream from the effluent.

Multiple regression analysis was used to determine the relationship between insect-tubificid index and plating waste concentrations in the river. The following regression equation resulted: Index = 0.15 + 0.47 Cu + 0.05 Ni + 0.42 Zn + 9.10 Cr^6 + 1.46 Cr Total + 10.03 CN^-. Regression coefficients indicate that total chromium and cyanide have most effect upon the index in the Red Cedar River. Cr^6 seems very important also but is subject to a very large standard error.

Since Linton (1967) found fish production greatly reduced in areas which had an index value much greater than 0.3, it is suggested that this index be used as an indication of general "health" of the river and that an index value of 0.3 to 0.4 be considered the maximum allowable in the river. With 0.3 to 0.4 as the maximum allowable index value the regression equation was used to arrive at a recommendation of maximum amounts of wastes to be allowed in the Red Cedar River. Maximum wastes recommended as allowable are: 0.056 mg/l Cu, 0.39 mg/l Ni, 0.03 mg/l Zn, 0.00 mg/l Cr^6, 0.12 mg/l Cr Total, 0.00 mg/l CN^-. If these limits are not exceeded, an index below 0.3 to 0.4 can be expected to be maintained below the plating plant and downstream stretches of the river should improve in numbers and types of invertebrates and presumably in fish production.

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