

**THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO**



**DEPARTMENT OF RESEARCH  
AND DEVELOPMENT**

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**MONITORING FISH FROM LAKES WITHIN A SEWAGE  
SLUDGE-RECLAIMED STRIP-MINED AREA IN CENTRAL ILLINOIS**

**FINAL REPORT**

**FULTON COUNTY FISH STUDY**

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**1971-1976**

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## TABLE OF CONTENTS

	<u>Page</u>
LIST OF TABLES	ii
LIST OF FIGURES	iii
SUMMARY	iv
CONCLUSIONS	v
INTRODUCTION	1
ENVIRONMENTAL MONITORING PROGRAM	2
MATERIALS AND METHODS	5
Fish Collection and Analysis	5
Condition Factors and Length-Weight Relationships	5
Fish Tissue Preparation for Trace Metals Analysis	6
Analysis of Fish Tissue for Zn, Cu, Cd, Cr, Ni	7
Analysis of Fish Tissue for Hg	8
Methods of Statistical Analysis	9
Water Quality Parameters	9
RESULTS AND DISCUSSION	10
Analysis of Body Condition	10
Analysis of Fish Tissue Trace Metals Content	20
Analysis of Reservoir Water for Trace Metals	29
REFERENCES	33
ACKNOWLEDGEMENTS	35

LIST OF TABLES

<u>Table No.</u>		<u>Page</u>
1	Percent Occurrence in Catch of Each Species of Fish Collected from Reservoirs 3, 10 and 12 between 1971 and 1976	11
2	Amount of Sludge Applied as a Liquid Fertilizer on Fields within the Watershed of Reservoirs 3 and 12	12
3	Mean Condition Factor (K(TL)), Mean Total Length (TL) in Millimeters, and Number (N) of Individuals of Each Selected Species of Fish Collected from Reservoir 3 during July-November 1971-1976	13
4	Mean Condition Factor (K(TL)), Mean Total Length (TL) in Millimeters, and Number (N) of Individuals of Each Selected Species of Fish Collected from Reservoirs 10 and 12 during July-November, 1971-1976	14
5	Slope (n), Intercept (log c), and Calculated Weight at Selected Lengths for the Length-Weight Relationship of Largemouth Bass in Reservoirs 3, 10 and 12 Collected during July-November 1971/1972, 1975 and 1976	16
6	Slope (n), Standard Error (Sn) of the Slope, Intercept (log c), Number of Fish and Calculated Weight at Selected Lengths for the Length-Weight Relationship of Bluegill in Reservoir 3 Collected during July-November 1971-1976	17
7	Slope (n), Standard Error (Sn) of the Slope, Intercept (log c), Number of Fish and Calculated Weight at Selected Lengths for the Length-Weight Relationship of Bluegill in Reservoir 12 Collected during July-November 1971-1976	19
8	Concentrations of Trace Metals in Fillets of Fish Sampled from Reservoir 3 with Results of Statistical Analyses of the Mean Values	21

## LIST OF TABLES (CONT'D)

<u>Table No.</u>		<u>Page</u>
9	Concentrations of Trace Metals in Fillets of Fish Sampled from Reservoirs 10 and 12 with Results of Statistical Analyses of the Mean Values	23
10	Mean ( $\bar{X}$ ) and Range (R) of Fish Muscle Trace Metal Concentrations Found in Other Studies (ND = Not Detectable)	25
11	Mean Concentrations of Hg (Based on Dry Wt.) in Fillets of Fish from Reservoirs 3, 10 and 12 with Results of Statistical Analyses of the Mean Values (ND = No Significant Difference, NA = No Analysis for Hg)	28
12	Mean and Range of Levels of Mercury Found in Fish Muscle Tissue in Other Studies (All Values Converted to Dry Weight Basis by Use of the Dry/Wet Weight Ratio for Muscle Tissue of 0.203(11))	30
13	Annual Means of the Concentrations of Cu and Hg in the Water of Reservoirs 3, 10 and 12 from 1971-1976	31

## LIST OF FIGURES

<u>Table No.</u>		<u>Page</u>
1	Location of Sludge Application Fields in Watersheds of the Three Reservoirs of the Fish Monitoring Study	3

## SUMMARY

A fish monitoring program was conducted between 1971 and 1976 to determine effects upon fish in lakes within sewage sludge application watersheds. Fish body condition and tissue trace metals content were monitored before and after application. Body condition,  $K(TL)$ , of bluegill and green sunfish increased from 1.61 to 1.79 and from 1.69 to 1.97, respectively, in a lake (R3) receiving runoff from 216 digested sludge fertilized hectares (533 acres). No significant change in condition of green sunfish x bluegill hybrids, black crappie or yellow bullheads was detected between 1971/1972 and 1976. No difference in condition was detected for largemouth bass by use of length-weight relationships between 1971/1972 and 1976. Bluegill in a lake (R12) receiving runoff from 44 digested sludge fertilized hectares (110 acres) increased in condition between 1971 ( $K(TL) = 1.58$ ) and 1976 ( $K(TL) = 1.78$ ).

Concentration of the trace metals, Zn, Cu, Ni, Cd, and Cr in the fillets of fish from the lakes tended to decrease over the study period. Range of mean levels (based on dry weight) detected in the fish were: Zn (29 - 64  $\mu\text{g/g}$ ), Cu (0.43 - 5.09  $\mu\text{g/g}$ ), Ni (0.22 - 3.47  $\mu\text{g/g}$ ), Hg (0.52 - 2.86  $\mu\text{g/g}$ ), Cd (4.4 - 109.5  $\text{ng/g}$ ), and Cr (143 - 819  $\text{ng/g}$ ).

## CONCLUSIONS

On the basis of the data presented, the following conclusions were made:

1. Reservoir 3. No species tested exhibited a significantly lower mean value of body condition for the 1976 collection than for the 1971 or 1972 preapplication collections. Of the five species tested, two species (bluegill and green sunfish) exhibited a significantly higher mean value of body condition during 1976 than during the preapplication years.

Reservoir 10. No change in body condition for green sunfish or for redear x green sunfish hybrids was exhibited between those collected during 1976 and those collected during 1971 and 1972, respectively.

There was no difference between 1972 and 1976 R10 redear sunfish, though the 1971 redear were in especially good body condition compared with redear collected in other years.

Reservoir 12. The bluegills were in better body condition during 1976 than during 1971 or 1972.

2. R3 and R10 Bass. The largemouth bass showed no change in weight with length between 1971/1972 and 1976.

R12 Bass. The largemouth bass were heavier at comparable lengths during 1976 than during the 1971/1972 preapplication period.

R3 and R12 Bluegill. Analysis of the length-weight relationships of bluegill supported the decisions based on their condition factors that, on the whole, bluegill were in better condition in postapplication than in preapplication years.

3. There was no detectable detrimental effect upon the body condition of the Fulton County fish species which can be linked with sludge distribution for the July-November collection period of 1971/1972 through 1976.
4. There was a tendency toward reduced levels of trace metals in fish muscle during the monitoring period, 1971/1972-1976, even though sludge was applied to fields within the watersheds of the reservoirs under study in generally increasing quantities during the same period. This was due perhaps to erosion control as a result of the berming and sloping of fields toward retention basins, which may have caused removal of sediments containing metals.

Routine analyses of surface water at the Fulton County site detected no pattern of increase in concentration of Zn, Cd, Cu, Cr, Ni, or Hg between 1971 and 1976.

5. There was no indication from analysis of the average concentrations of trace metals in the muscle tissue of the Fulton County fish species that sludge application caused any danger to the public health by increasing trace metals in fish fillets. None of the average values fell into ranges reported as harmful.



## INTRODUCTION

The Metropolitan Sanitary District of Greater Chicago (District) has been using digested sludge as a fertilizer and soil conditioner on a 6,284 hectare (15,528 acre) section of coal spoils in Fulton County, Illinois, since 1971. Sludge solids have been distributed to the land by means of large traveling spray vehicles (1972 through 1975) or incorporated into the soil by use of plows or discs (1976 to the present).

It was the intention of the District to grade the land to control runoff, increase the soil humus content by large additions of organic matter contained in the digested sludge, and restore the land to full agricultural productivity (1).

Fields developed from spoils were bermed and sloped toward retention basins so that all runoff could be collected and the quality checked before eventual discharge (2). The control of field runoff was intended to reduce the amount of substances, such as trace metals, which would be lost to the watersheds of neighboring lakes and streams, whether or not sludge application was carried out on these fields. Monitoring wells were strategically placed to observe groundwater quality (2).

Because of the many strip-mined reservoirs and occasional stream impoundments which had been stocked by farmers and local members of a hunting and fishing club, fish are the most numerous of the vertebrates in the study area, as well as the organism of greatest popular interest.

## ENVIRONMENTAL MONITORING PROGRAM

An environmental monitoring program was initiated in 1971 to determine the impact of sludge distribution upon selected biota in lakes and streams within the distribution watershed. The biological parameters monitored included bacteria, viruses, phytoplankton, benthic invertebrates, and fish. In the study reported here, the following were considered in terms of the effects of digested sludge application upon lacustrine fish populations within the District site:

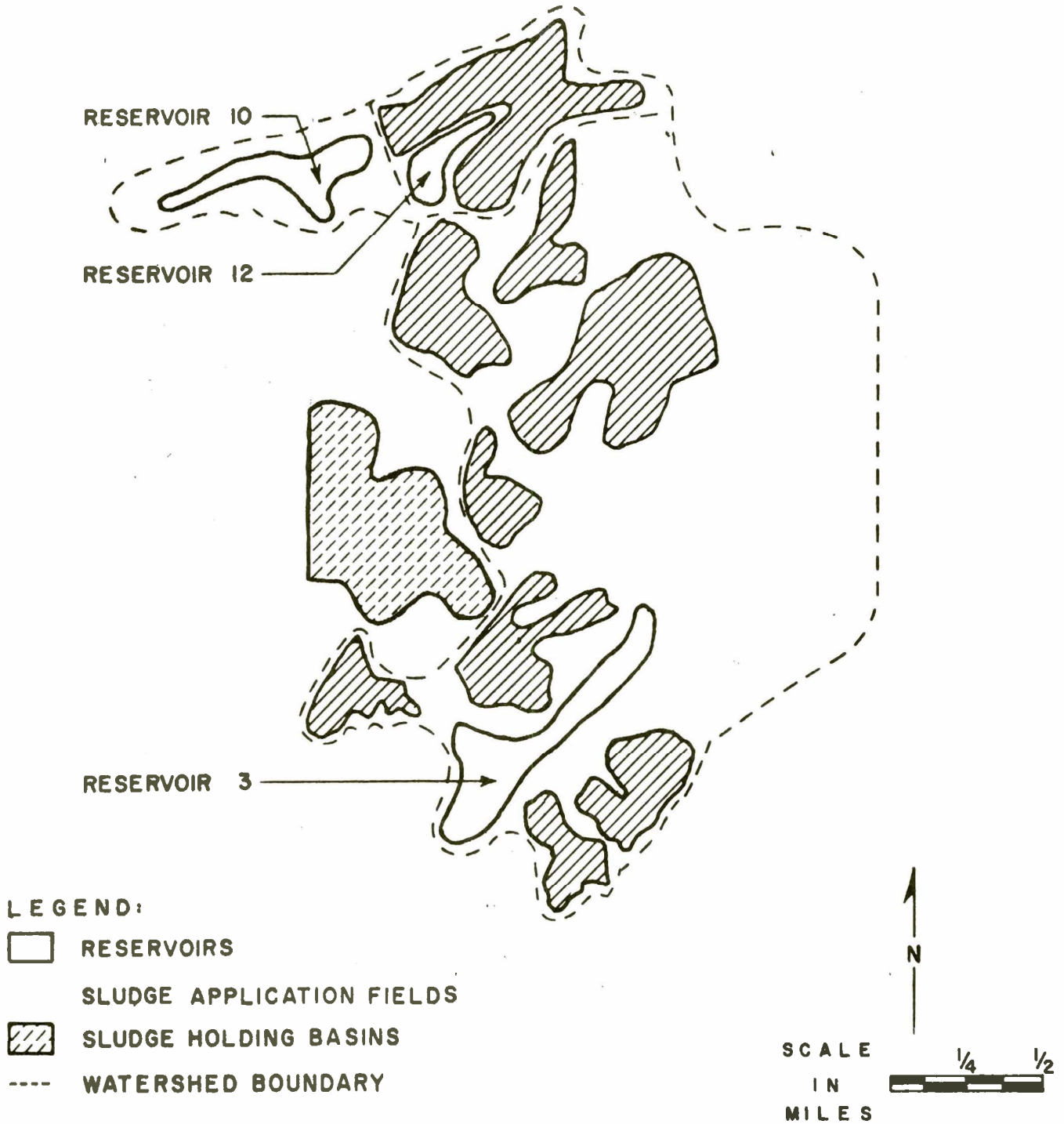
1. fish body condition--by use of the condition factor and length-weight relationship;
2. fish tissue trace metals content--including analysis of concentrations in fish fillets.

Three reservoirs were selected as monitoring sites (Figure 1). Reservoirs 10 and 12 (R10 and R12) are both mine-spoil end cuts, having become filled with groundwater and rainwater following cessation of normal strip-mining operations. R10 is located outside of the distribution watershed. R12 receives drainage from two field runoff basins; thus R12 will receive runoff from 44 hectares (110 acres) of cropland fertilized with digested sludge. Reservoir 3 (R3) is a stream impoundment (originally serving as a silt catch basin for the mine company which formerly owned the land) which receives drainage from 1,600 hectares (3,954 acres), including 216 hectares (533 acres) of sludge fertilized cropland. R3 receives more drain-

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

FIGURE 1

LOCATION OF SLUDGE APPLICATION FIELDS  
IN WATERSHEDS OF THE THREE RESERVOIRS  
OF THE FISH MONITORING STUDY



age than any other reservoir on the District site.

Associated water quality parameters (e.g., nitrate-nitrogen and total phosphorus) were also included in the monitoring program.

## MATERIALS AND METHODS

Fish Collection and Analysis. Fish were collected from Reservoirs 3, 10 and 12 by use of 1.2 x 0.6 x 3.0 meter (4 x 2 x 10 ft) frame nets with 15.2 m (50 ft) shore leads; 38.1 m (125 ft) experimental gill nets; a 7.6 m (25 ft) bag seine with a bag of 0.64 cm (0.25 inch) square mesh and wings of 1.27 cm (0.5 inch) square mesh; a 230 volt, alternating current, boat-mounted, boom-electrofisher; a direct current, backpack-electrofisher operating off a 12-volt battery; and hook and line.

All fish were identified to species, weighed to the nearest gram, and measured for total length to the nearest millimeter. Subsamples from each species collection were frozen in plastic bags for trace metals analysis.

### Condition Factors and Length-Weight Relationships.

Length and weight data for fish collected during July through November, 1971 through 1976, were grouped according to year collected, species, and reservoir from which they were collected. Body condition has been expressed in this study by a factor, K (total body length used in computation) where:

$$K = \frac{W 10^5}{L^3}$$

and where: W = weight in grams, L = length in millimeters, and  $10^5$  is a factor to bring the value of K near unity (3).

The relationship of weight to length is expressed by the equation:

$$W = cL^n$$

where: W = weight in grams, L = length in millimeters, and c and n are constants.

We have used this relationship in its most usable form (3), the logarithmic form:

$$\log_{10}W = \log_{10}c + \log_{10}L$$

Fish Tissue Preparation for Trace Metals Analysis. Since other studies (4,5,6) have indicated a correlation between fish size (e.g., length or weight) with the concentrations of mercury in the muscle tissue, though much less so, if at all, with other metals (4,7), an attempt was made to analyze fillets from a broad size range of fish for each species in this study.

Fillets of the epaxial and hypaxial muscles were obtained from both sides of the fish, from just behind the gill cover to the caudal peduncle. All bones were removed from the fillets which were then cut into thin sections and placed on glass plates. The fillets were dried overnight (18 hours) at 60°C. The dried tissue was then ground in a Wiley Mill (to pass a 1-mm sieve) and the ground tissue was then stored in plastic vials.

Analysis of Fish Tissue for Zn, Cu, Cd, Cr, Ni. One gram of ground tissue was added to acid-cleaned, glass digestion tubes. Concentrated nitric acid (15 ml) was then added and allowed to stand for two hours at room temperature. The temperature was then gradually raised to 95°C to avoid excessive foaming. After the solutions had cleared and heavy fuming stopped, funnel caps were added to retard acid evaporation, and the tubes retained at 95°C overnight.

The funnel caps were then removed and the solutions allowed to evaporate to a few mls, again at 95°C. The tubes were then allowed to cool slightly, and 5 ml of HNO<sub>3</sub> and 2 ml of HClO<sub>4</sub> were added.

The tubes were gradually brought up to 160°C over a two hour period. When heavy white fumes appeared, funnel caps were added, and the solutions heated until they turned white. The caps were then removed, and the acid was allowed to evaporate to dryness.

The tubes were allowed to cool, and then 1 ml of HNO<sub>3</sub> and 5 ml of triple-distilled water were added to dissolve the residue, warming the tubes, if necessary, until the solution was clear. The contents of the tubes were then poured into graduated polyethylene tubes with washings of triple-distilled water, and the final volume was brought to 20 ml.

Zinc and copper were determined using a Perkin-Elmer Model 403 atomic absorption spectrophotometer. Any sample with a reading for copper below 0.10 ppm was rerun on the

graphite furnace.

Cadmium, chromium, nickel, and copper (below 0.10 ppm) were determined using a Perkin-Elmer Model 306 atomic absorption spectrophotometer with peak read, equipped with a deuterium arc background corrector (for nickel and cadmium), a Perkin-Elmer HGA graphite furnace, a Perkin-Elmer AS-1 auto-sampling system, and a Perkin-Elmer Model 056 chart recorder.

Using the flame spectrophotometer, every fourth sample was followed by a blank, and every eighth sample was followed by a standard. Using the furnace, every fourth sample was followed by a 10 second automatic tube burnout, and then a standard was run. Results were taken directly from the peak read, with the chart recorder being used as a check. Any sample not reproducing a result within one unit was rerun using a fresh sample cup. Into each sampling cup, 0.30 ml of digested sample was pipetted using a micropipette. Every fifth cup contained 0.50 ml of standard. Of 40 samples in each run, 33 were of fish tissue, five were reagent blanks, and two were National Bureau of Standards bovine liver samples.

Analysis of Fish Tissue for Hg. A method by Hatch and Ott (8) was modified by the District laboratory for analysis of Hg. One quarter to one half-gram of ground tissue was digested with 20 ml of a 1:1 mixture of  $H_2SO_4$  and  $HNO_3$  at  $60^\circ C$ , and the mercury was reduced to metallic mercury with stannous chloride. Mercury was determined by flameless cold vapor



atomic absorption spectroscopy.

Methods of Statistical Analysis. Mean fish body condition factors,  $K(TL)$ , were analyzed by use of single factor analysis of variance with subsequent Student-Newman-Keuls (SNK) multiple comparisons (9).

Length-weight relationships were analyzed by use of analysis of covariance with subsequent SNK multiple comparisons of slopes and intercepts (9).

Mean fish tissue trace metal concentrations were analyzed by use of single factor analysis of variance with subsequent SNK multiple comparisons.

The 0.05 level of probability was chosen for statistical significance, unless otherwise noted.

Water Quality Parameters. All water quality parameters mentioned in the text were analyzed according to Standard Methods for Water and Wastewater Analysis (10).

## RESULTS AND DISCUSSION

Analysis of Body Condition. A list of fish species found in the three reservoirs under study, as well as their relative abundance, is given in Table 1. In general these reservoirs contained sunfish and bullheads. Fishing pressure was very low since the property was not open to the public.

The amount of sludge deposition on fields draining into R3 and R12 over the period 1972-1976 is listed in Table 2. Relatively little sludge was applied prior to 1974. A gradual shift from mostly spray application to mostly disc incorporation occurred during 1975.

Results of comparisons of mean condition factors for fish collected during July-November, 1971-1976 are listed in Table 3 and 4. No significant decrease in condition occurred for the species compared between "pre" (1971 and 1972) and "post" application periods. No significant change in condition was noted in Reservoir 3 green sunfish x bluegill hybrids, yellow bullheads or black crappie between these periods.

Comparisons of R3 bluegill K factor yielded the following results: for mean K(TL), 1972 and 1973 < 1971 < 1974 and 1975 < 1976. Comparisons of R3 green sunfish K factor yielded the following results: for mean K(TL), 1971 and 1972 < 1973 and 1974 < 1975 < 1976.

In general, no difference in mean condition factor was detected for Reservoir 10 redear, green sunfish or redear x

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 1

PERCENT OCCURRENCE IN CATCH OF EACH SPECIES OF FISH  
COLLECTED FROM RESERVOIRS 3, 10, AND 12  
BETWEEN 1971 and 1976

Species	Reservoir		
	R3	R10	R12
	-----PERCENT OCCURRENCE-----		
Bluegill	37	0	78
Green sunfish	9	23	5
Redear sunfish	1	32	3
Green sunfish x bluegill hybrid	15	0	<1
Redear x green sunfish hybrid	0	20	<1
Redear sunfish x bluegill hybrid	<1	0	1
Black crappie	11	0	0
Largemouth bass	11	22	11
Yellow bullhead	14	0	0
Black bullhead	1	0	2
Brown bullhead	0	2	0
Carp	<1	0	0

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 2

AMOUNT OF SLUDGE APPLIED AS A LIQUID FERTILIZER ON FIELDS  
 WITHIN THE WATERSHED OF RESERVOIRS 3 AND 12  
 FROM 1972 TO 1976

Reservoir	Year of Application					Total
	1972	1973	1974	1975	1976	
	----- Sludge Applied to Watershed ----- (metric tons dry solids)					
R3	171	391	8,559	11,060	15,746	35,927
R12	0	50	1,778	1,862	594	4,284

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 3

MEAN CONDITION FACTOR (K(TL)), MEAN TOTAL LENGTH (TL) IN MILLIMETERS, AND NUMBER (N) OF INDIVIDUALS OF EACH SELECTED SPECIES OF FISH COLLECTED FROM RESERVOIR 3 DURING JULY-NOVEMBER 1971-1976

Species		Year Collected					
		1971	1972	1973	1974	1975	1976
Bluegill	K(TL)	1.61	1.53	1.52	1.68	1.66	1.79
	TL	132	139	152	142	144	156
	N	71	55	57	209	227	97
Green sunfish	K(TL)	1.69	1.66	1.77*		1.87	1.97
	TL	138	141	157		153	134
	N	49	32	33		41	20
Green sunfish x bluegill hybrid	K(TL)	1.83	1.79	1.83	1.90	1.87	1.89
	TL	154	161	173	179	170	193
	N	66	60	34	96	32	10
Black crappie	K(TL)	1.32**		1.34	1.32	1.44	1.39
	TL	189		221	181	205	209
	N	17		12	102	71	15
Yellow bullhead	K(TL)	1.30	1.24	---	1.37	1.40	1.39
	TL	229	221	---	246	234	235
	N	55	30	1	45	90	46

\* 1973 and 1974 data combined

\*\* 1971 and 1972 data combined

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 4

MEAN CONDITION FACTOR (K(TL)), MEAN TOTAL LENGTH (TL) IN MILLIMETERS, AND NUMBER (N) OF INDIVIDUALS OF EACH SELECTED SPECIES OF FISH COLLECTED FROM RESERVOIRS 10 AND 12 DURING JULY-NOVEMBER, 1971-1976

Reservoir/Species		Year Collected					
		1971	1972	1973	1974	1975	1976
<u>Reservoir 10</u>							
Redear sunfish	K(TL)	1.95	1.87	1.88	1.79	1.85	1.81
	TL	190	181	194	175	161	157
	N	39	39	201	92	93	60
Redear x green sunfish hybrid	K(TL)	2.01	1.95	2.01	2.05	1.95	1.99
	TL	176	160	176	181	182	184
	N	52	33	96	52	58	37
Green sunfish	K(TL)	1.91	1.85	1.96	1.96	1.88	1.93
	TL	160	136	164	157	154	154
	N	56	21	32	58	134	80
<u>Reservoir 12</u>							
Bluegill	K(TL)	1.58	1.55	1.82	1.62	1.68	1.78
	TL	131	136	163	155	147	156
	N	235	356	90	233	305	98

green sunfish hybrids between "pre" and "post" application periods, though R10 redear exhibited a significantly higher mean condition factor in 1971 than in all other years (Table 4).

Comparisons of R12 bluegill K factor yielded the following results: for mean K(TL), 1972 < 1971 < 1974 < 1975 < 1973 and 1976.

Since the largemouth bass K factor was found to increase with increase in total length in certain years, and the range of lengths of bass compared was large, only bass length-weight relationships were compared between preapplication (1971/1972) and postapplication (1975, 1976) periods (Table 5).

Dissimilar changes in weight with increase in length occurred from 1971/1972 to 1975 for R3 bass (decrease) and R10 bass (increase). No change in weight with increase in length or in weight at comparable lengths was detected for R12 bass between these two periods (Table 5).

No difference in increase in weight at comparable lengths was detected for bass from Reservoirs 3 or 10 between 1971/1972 and 1976. However, a significant increase in weight at comparable lengths occurred from the "pre" to "post" application periods for R12 largemouth bass (Table 5).

In order to determine if increase in K factor with increase in length caused a bias in conclusions concerning condition for R3 and R12 bluegills, the length-weight relationships (Table 6) for each population in each reservoir were analyzed.

The slope,  $n$ , of the relationship  $\log_{10} \text{ weight} = \log_{10} c + n \log_{10} \text{ length}$ , displayed a great deal of variability among years.

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 5

SLOPE (n), INTERCEPT (log c), AND CALCULATED WEIGHT AT  
 SELECTED LENGTHS FOR THE LENGTH-WEIGHT RELATIONSHIP OF LARGEMOUTH  
 BASS IN RESERVOIRS 3, 10 AND 12 COLLECTED DURING JULY-NOVEMBER,  
 1971/1972, 1975, AND 1976

Reservoir Number	Year Collected	Slope (n)	Intercept (log c)	Total Length (mm)		
				175 --- Calculated Weight (g) ---	205	325
	1971/1972	3.317	-5.638	63	207	494
16	1975	3.037*	-5.000	65	192	425
	1976	3.146	-5.232	67	205	468
	1971/1972	3.038	-5.007	64	189	421
	1975	3.277*	-5.557	62	200	473
	1976	3.113	-5.165	66	199	451
	1971/1972	3.457	-5.983	59	203	502
	1975	3.565	-6.230	58	208	531
	1976	3.267	-5.504**	67	214	504

\*Significantly different ( $P < 0.05$ ) from 1971/1972

\*\*Significantly different ( $P < 0.01$ ) from 1971/1972



THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 6

SLOPE (n), STANDARD ERROR (Sn) OF THE SLOPE, INTERCEPT (log c)  
 NUMBER OF FISH AND CALCULATED WEIGHT AT SELECTED LENGTHS FOR THE  
 LENGTH-WEIGHT RELATIONSHIP OF BLUEGILL IN RESERVOIR 3 COLLECTED DURING  
 JULY-NOVEMBER 1971-1976

Reservoir and Parameters	Year Collected					
	1971	1972	1973	1974	1975	1976
<u>Reservoir 3</u>						
Slope (n)	2.858	3.217	3.061	2.614*	2.815*	3.251*
St. Error (Sn)	0.149	0.109	0.238	0.076	0.080	0.100
Intercept (log c)	-4.496	-5.281	-4.952	-3.947	-4.382	-5.298
Number of Fish	71	55	57	208	227	96
<u>Calc. Weight (g) at</u>						
100 mm	17	14	15	19	18	16
140 mm	43	42	41	46	46	48
180 mm	89	94	89	89	92	108

\*Slope (n) significantly different ( $P \leq 0.05$ ) than 3.0

The  $n$  calculated for the 1974 R3 bluegill (2.614) was significantly lower than those calculated for 1971, 1972, 1975 or 1976 collections. The slopes calculated for the 1974, 1975 and 1976 collections were significantly different than 3.0 indicating allometric growth for these fish. Values of  $n$  below 3.0 indicate greater growth in weight for the shorter individuals; values above 3.0 indicate greater growth in weight for the longer individuals.

Values of  $n$  calculated for 1971 and for the 1972 collections were numerically equal to those calculated for the 1975 and 1976 collections, respectively, and the inability to detect a difference for the former two from 3.0 is probably due to the smaller sample size and greater standard deviation of the regression coefficient ( $S_n$ ). The  $n$  calculated for 1973 (3.061) was significantly different than those calculated for 1972 (3.217) and for 1975 (2.815).

Values of  $n$  for 1971 and 1975 and for 1972 and 1976 were not found to be significantly different from each other. Tests of the intercepts ( $\log c$ ) of each of the respective combinations revealed that weight was significantly higher in the "post" application years (1975 and 1976) which agrees with the conclusions of the  $K$  factor comparisons.

The values of  $n$  (Table 7) for R12 bluegills were significantly greater than 3.0 for all years and no difference could be detected among them. Values of  $\log_{10} c$  were then compared and it was concluded that in terms of weight at comparable lengths:

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 7

SLOPE (n), STANDARD ERROR (Sn) OF THE SLOPE, INTERCEPT (log c),  
 NUMBER OF FISH AND CALCULATED WEIGHT AT SELECTED LENGTHS FOR THE  
 LENGTH-WEIGHT RELATIONSHIP OF BLUEGILL IN RESERVOIR 12 COLLECTED  
 DURING JULY-NOVEMBER 1971-1976

Reservoir and Parameters	Year Collected					
	1971	1972	1973	1974	1975	1976
<u>Reservoir 12</u>						
Slope (n)	3.171*	3.234*	3.235*	3.150*	3.189*	3.187*
St. Error (Sn)	0.036	0.026	0.049	0.048	0.035	0.042
Intercept (log c)	-5.164	-5.309	-5.260	-5.121	-5.184	-5.159
Number of Fish	235	356	90	233	305	98
<u>Calc. Weight (g) at</u>						
100 mm	15	14	16	15	16	16
140 mm	44	43	48	44	46	48
180 mm	97	97	108	96	102	107

\*Slope (n) significantly different ( $P \leq 0.05$ ) than 3.0

(1971 and 1972) and 1974 < 1975 and 1976. This is similar to the K factor comparison except for the inability to detect a difference between 1971 and 1972.

Results of analysis of mean condition factors and of length-weight relationships for the fish from Reservoirs 3, 10 and 12, reported previously (18, 19), showed no detrimental effects upon these fish which could be attributed to liquid fertilizer application. These results included the analysis of fish condition during March-April (pre-spawning period), 1972-1974, and May-June (spawning period), 1971-1974.

Analysis of Fish Tissue Trace Metals Content. Results of the analysis of fish fillets for the trace metals Zn, Cu, Cd, Cr and Ni for fish collected from Reservoirs 3, 10 and 12 are listed in Tables 8 and 9.

Most of these mean values were within the ranges of values found by other authors (4, 11, 12, 13) for fish muscle tissue (Table 10).

In general, the mean levels of trace metals in the fillets of fish from the three reservoirs exhibited a tendency to decrease over the study period, 1971-1976. Several species, however, contained significantly higher concentrations of a metal in 1976 than in the 1971/1972 preapplication period, as shown below:

Reservoir 3: green sunfish x bluegill hybrids, a higher zinc level;

Reservoir 12: bluegills, a higher zinc level;

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 8

CONCENTRATIONS OF TRACE METALS IN FILLETS OF FISH SAMPLED  
FROM RESERVOIR 3 WITH RESULTS OF STATISTICAL ANALYSES OF THE MEAN VALUES

Species	Year Collected	Number	Trace metal, Mean (dry weight)				
			Zn µg/g	Cu µg/g	Cd ng/g	Cr ng/g	Ni ng/g
Largemouth bass	1971/1972	36	40	1.79	13.3	306	362
	1973	14	37	5.87	22.0	523	373
	1974	30	56	0.91	7.7	282	785
	1976	30	44	0.75	12.9	287	456
Difference among means:			**	**	ND†	**	ND
Black crappie	1971/1972	15	42	2.54	12.6	353	683
	1974	15	37	0.89	11.8	152	508
	1976	15	31	0.43	11.5	261	540
Difference among means:			**	**	ND	ND	ND
Bluegill	1971/1972	32	63	5.09	109.5	424	3472
	1974	31	47	2.75	101.9	398	1353
	1976	30	53	0.92	6.1	584	872
Difference among means:			**	ND	**	ND	*

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 8 (CONTINUED)

CONCENTRATIONS OF TRACE METALS IN FILLETS OF FISH SAMPLED  
FROM RESERVOIR 3 WITH RESULTS OF STATISTICAL ANALYSES OF THE MEAN VALUES

Species	Collected	Number	Trace metal, Mean (dry weight)					
			Zn µg/g	Cu µg/g	Cd ng/g	Cr ng/g	Ni ng/g	
Green sunfish	1971/1972	36	41	4.00	17.4	447	1778	
		20	50	1.94	13.0	558	638	
		20	42	0.98	9.8	819	883	
Difference among means:			**	ND	ND	ND	ND	
Green sunfish x bluegill hybrids	1971/1972	25	54	1.84	32.9	425	1087	
		1974	29	48	1.21	13.1	238	222
		1976	10	64	1.37	8.6	415	968
Difference among means:			**	ND	**	**	**	
Yellow bullhead	1971/1972	25	43	3.25	86.2	188	345	
		1974	25	32	2.58	12.2	155	803
		1976	25	33	1.37	8.7	449	407
Difference among means:			**	**	**	ND	**	

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

†ND = No significant difference

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 9

CONCENTRATIONS OF TRACE METALS IN FILLETS OF FISH SAMPLED FROM RESERVOIRS 10 AND 12 WITH RESULTS OF STATISTICAL ANALYSES OF THE MEAN VALUES

Species	Year Collected	Number	Trace metal, Mean (dry weight)				
			Zn μg/g	Cu μg/g	Cd ng/g	Cr ng/g	Ni ng/g
R12 Largemouth bass	1971/1972	13	48	2.73	26.5	336	439
	1974	17	57	1.25	27.9	587	2273
	1976	30	37	0.91	13.5	501	758
Difference among means:			**	**	ND†	ND	ND
R12 Bluegill	1971/1972	30	40	1.78	74.1	730	825
	1974	31	35	1.86	13.5	653	1010
	1976	30	46	1.29	20.9	519	488
Difference among means:			**	ND	*	ND	*
R10 Largemouth bass	1971/1972	46	42	2.46	32.7	313	375
	1974	36	52	3.06	17.3	251	246
	1976	30	37	1.19	11.7	331	406
Difference among means:			**	**	*	ND	*

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 9 (CONTINUED)

CONCENTRATIONS OF TRACE METALS IN FILLETS OF FISH SAMPLED  
FROM RESERVOIRS 10 AND 12 WITH RESULTS OF STATISTICAL ANALYSES  
OF THE MEAN VALUES

Species	Year Collected	Number	Trace metal, Mean (dry weight)				
			Zn µg/g	Cu µg/g	Cd ng/g	Cr ng/g	Ni ng/g
R10 Green sun- fish	1971/1972	20	30	1.72	13.0	329	747
	1974	19	31	2.37	14.1	196	1647
	1976	20	29	0.96	24.7	363	594
Difference among means:			ND	ND	ND	*	*
R10 Redear sunfish	1971/1972	30	38	1.36	34.0	273	486
	1974	25	40	0.50	11.1	143	930
	1976	28	42	0.65	4.4	547	377
Difference among means:			ND	**	ND	**	**
R10 Redear x Green sunfish Hybrids	1971/1972	15	35	1.21	11.5	251	428
	1974	7	41	3.53	5.4	152	437
	1976	18	32	0.84	8.8	259	326
Difference among means:			**	**	ND	ND	ND

\* Significant at the 0.05 level  
 \*\* Significant at the 0.01 level  
 † ND = No significant difference



THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 10

MEAN ( $\bar{X}$ ) AND RANGE (R) OF FISH MUSCLE TRACE METAL CONCENTRATIONS  
 FOUND IN OTHER STUDIES (ND = NOT DETECTABLE)

Location and Species		Trace metals, Dry weight basis					Reference	
		Zn μg/g	Cu μg/g	Cd ng/g	Cr ng/g	Ni ng/g		
<u>Indiana "Contaminated Area"</u>								
25	Largemouth bass	$\bar{X}$	43		75		(11)	
		R	30-61		10-311			
	Bluegill	$\bar{X}$	68		431		(11)	
		R	30-158		61-1308			
<u>Indiana "Uncontaminated Area"</u>								
	Largemouth bass	$\bar{X}$	37		12		(11)	
		R	18-66		10-48			
	Bluegill	$\bar{X}$	48		76		(11)	
		R	35-65		46-148			
<u>Illinois River Fish</u>								
	Various species	$\bar{X}$	13-23	0.34-1.20	25-150	300-800	400-900	(12)
		R	4-37	0.25-120	20-425	100-1350	200-1400	

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 10 (CONTINUED)

MEAN ( $\bar{X}$ ) AND RANGE (R) OF FISH MUSCLE TRACE METAL CONCENTRATIONS  
 FOUND IN OTHER STUDIES (ND = NOT DETECTABLE)

Location and Species	Trace metals, Dry weight basis					Reference
	Zn μg/g	Cu μg/g	Cd ng/g	Cr ng/g	Ni ng/g	
<u>Eutrophic Lake (Michigan)</u>						
Largemouth bass*	$\bar{X}$		180			(4)
	R		100-240			
Yellow bullhead*	$\bar{X}$		170			(4)
	R		105-245			
Hybrid sunfish*	$\bar{X}$		195			(4)
	R		130-255			
<u>Wisconsin (Various waters)</u>						
Largemouth bass*	$\bar{X}$	18-36	ND	ND-350		(13)
Crappie*	$\bar{X}$	23-71	ND	ND-500		(13)
Bluegill (and "sunfish")*	$\bar{X}$	24-88	ND	200-650		(13)

\*Indicates a conversion to a dry weight basis by use of the dry/wet wt. ratio for muscle tissue of 0.203 (11).

Reservoir 10: redear sunfish, a higher chromium level.

The mean values for zinc (64  $\mu\text{g/g}$  and 46  $\mu\text{g/g}$ , respectively, for the R3 hybrids and R12 bluegill) from the 1976 collection were much lower than the 500  $\mu\text{g/g}$  (based on dry weight) zinc concentration considered dangerous in fish for human consumption (11, 14). The maximum concentrations of zinc determined for these fish from R3 (121  $\mu\text{g/g}$ ) and R12 (71  $\mu\text{g/g}$ ) were also well below the 500  $\mu\text{g/g}$  limit. Therefore, these concentrations do not pose a danger to the public health.

Since no sludge application occurred within the watershed of Reservoir 10, the high level of chromium concentration of 547  $\mu\text{g/g}$  in R10 redear sunfish fillets (Table 9) was probably within the natural range of variation for this metal in the muscle tissue of this species.

Results of the analysis of fish fillets for mercury from Reservoirs 3, 10 and 12 are listed in Table 11. Reservoir 12 bluegill and Reservoir 10 largemouth bass contained higher concentrations of mercury in 1976 than in 1971/1972. In the case of the R12 bluegill the values of mercury determined during 1974 and 1976 were higher than in the preapplication years (though these latter years were not different from each other). The mean values do not exceed the Food and Drug Administration (FDA) guideline for mercury of 2.5  $\mu\text{g/g}$  (converted from 0.5  $\mu\text{g/g}$  based on wet weight using the ratio for muscle tissue of 0.203 (11)). The maximum level found in the R12 bluegill fillets was 0.45  $\mu\text{g/g}$  which is 44 percent greater than the FDA guideline.

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 11

MEAN CONCENTRATIONS OF Hg (BASED ON DRY WT.) IN FILLETS OF FISH FROM RESERVOIRS 3, 10 AND 12 WITH RESULTS OF STATISTICAL ANALYSES OF THE MEAN VALUES (ND = NO SIGNIFICANT DIFFERENCE, NA = NO ANALYSIS FOR Hg)

Reservoir and Species	Year of Collection						Difference among Means
	1971/72		1974		1976		
	Num-ber	Hg $\mu\text{g/g}$	Num-ber	Hg $\mu\text{g/g}$	Num-ber	Hg $\mu\text{g/g}$	
<u>Reservoir 3</u>							
Largemouth bass†	32	2.86	1	1.00	30	1.78	**
Bluegill	30	1.40	30	1.35	30	1.58	ND
Green sunfish	36	0.52	15	0.71	20	0.71	ND
Yellow bullhead	19	1.58		NA	19	1.20	ND
<u>Reservoir 10</u>							
Largemouth bass	38	1.09		NA	30	1.43	**
Green sunfish	17	0.84		NA	17	0.63	*
Redear sunfish	19	0.92		NA	19	0.69	ND
<u>Reservoir 12</u>							
Largemouth bass	20	1.71		NA	30	0.85	**
Bluegill	29	0.88	19	1.38	30	1.62	**

† Fourteen bass collected from R3 during 1973 were also analysed and included in the statistical analysis (Mean Hg conc. = 0.83  $\mu\text{g/g}$ ).

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

Largemouth bass from R10, which received no sludge within its watershed, also showed a significant increase in mercury between 1976 and 1971/1972. Such an increase within a fish species thus may be within normal natural variation. Also, since the concentration of 1.62  $\mu\text{g/g}$  Hg in R12 bluegill was not significantly different from the 1.43  $\mu\text{g/g}$  concentration in R10 bass, this level was within the range of means of fillet concentrations in fish from lakes unaffected by sludge application in the study area. The maximum level of Hg for R10 bass collected during 1976 was 2.73  $\mu\text{g/g}$  which also exceeds the FDA guideline.

The levels of Hg determined for fillets of Fulton County fish lie within the range determined by other investigators (4, 6, 16, 17) (Table 12). Therefore, the change in fillet concentration determined so far does not seem to be biologically significant.

Analysis of Reservoir Water for Trace Metals. Analysis of water from Reservoirs 3, 10 and 12 did not yield mean levels of the metals Zn, Cd, Cr, or Ni which were higher than the minimum detection limit (0.1, 0.01, 0.02 and 0.1 mg/l, respectively) for the routine analysis of these metals.

Results of analysis for Cu and Hg are given in Table 13. In neither case were concentrations of these metals appreciably higher in postapplication years.

There is no indication that the levels of trace metals in the waters of the reservoirs have caused any increase in the fish. The tendency for lower concentrations in the fillets in the post-

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 12

MEAN AND RANGE OF LEVELS OF MERCURY FOUND IN FISH MUSCLE TISSUE IN OTHER STUDIES (ALL VALUES CONVERTED TO DRY WEIGHT BASIS BY USE OF THE DRY/WET WEIGHT RATIO FOR MUSCLE TISSUE OF 0.203 (11))

Location and Species	Hg ( $\mu\text{g/g}$ ), dry weight		Reference
	Mean	Range	
<u>Eutrophic Lake (Michigan)</u>			
Largemouth bass	2.34	0.95-4.50	(4)
Yellow bullhead	0.43	0.27-0.68	(4)
Hybrid sunfish	1.28	0.68-1.91	(4)
<u>Mississippi</u>			
Largemouth bass	-	0.25-3.70	(16)
<u>South Dakota</u>			
Black crappie	-	1.20-3.30	(17)
Black bullhead	-	0.40-1.80	(17)
<u>West Coast</u>			
Pacific hake	0.43-1.92*	-	(6)

\* Range of means.

THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO

TABLE 13

ANNUAL MEANS OF THE CONCENTRATIONS OF  
Cu AND Hg IN THE WATER OF RESERVOIRS 3, 10 AND 12 FROM 1971-1976

Parameter	Reservoir	YEAR SAMPLED					
		1971	1972	1973	1974	1975	1976
Cu (mg/liter)	3	0.08	0.15	0.03	0.03	0.01	0.07
	10	0.05	0.14	0.04	0.04	0.04	0.03
	12	0.04	0.17	0.05	0.26	0.02	0.02
Hg (µg/liter)	3	ND*	0.3	0.3	0.1	ND	ND
	10	ND	0.2	0.2	0.1	ND	ND
	12	0.02	0.2	0.2	0.2	ND	ND

\* ND = Below level of detection, 0.1 µg/liter for Hg.

application years is in accord with the generally low values of Cu and Hg in later years.

It should be noted that the routine analyses for the metals Zn, Cd, Cr, Cu and Ni in the reservoir water were carried out by use of an atomic absorption technique (10) for which the minimum detection levels for these metals were not as low as for the same metals in the fish tissue analyses. For the reservoir water analyses, the minimum detection levels were tenfold higher for Cu and Cd, twentyfold higher for Cr, and one hundredfold higher for Ni and Zn, than for the fish tissue analyses. Therefore, any changes in levels of Zn, Cd, Cr, Cu and Ni concentrations of the reservoir water which were below the minimum detection levels would not have been noticed.



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