

Illinois State Water Survey Division
WATER QUALITY SECTION
AT
PEORIA, ILLINOIS



SWS Contract Report 403

SITE-SPECIFIC PHYTOTOXICITY OF HEAVY METALS

by Wuncheng Wang

Prepared for the
U.S. Environmental Protection Agency

September 1986

Site-Specific Phytotoxicity of Heavy Metals

by
Wuncheng Wang
Water Quality Section
Illinois State Water Survey
Peoria, IL

Grant No. R810834-01-0

Project Officer
Clyde Bishop
U.S. Environmental Protection Agency
Washington D.C.

September 1986

Abstract

The objective of this study was to determine the toxicity of heavy metals in various river and lake waters. The water samples encompass a wide variety of water quality, from very soft to very hard. There were 18 sample stations, 10 in Illinois and 8 in the neighboring states of Indiana, Iowa, Missouri, and Wisconsin. Three metal ions were tested: barium, chromium (hexavalent), and nickel. The test organism was common duckweed, Lemna minor.

The results show that among the three metals, Ba phytotoxicity was the least and was influenced the most by the water quality of the test samples. Cr phytotoxicity was moderate and was influenced the least by the water quality of the test samples. Ni phytotoxicity was the greatest and was moderately influenced by the water quality of the test samples.

On the basis of these results, it is suggested that water quality criteria be modified, with consideration given to the site-specific water quality and the species of heavy metals.

CONTENTS

List	of	Figures.....v
List of Tables		vi
Glossary		vii
Acknowledgments		vii
1. Introduction		1
2. Conclusions		2
3. Literature	Review.....	3
Introduction		3
Biotic	Factors.....	4
A. Tolerance		4
B. Size and life stages		6
C. Species		7
D. Nutrition		9
Abiotic Factors		9
A. Organics		9
i. Synthetic organics		9
ii. Humic substances		12
B	pH.....	13
C. Temperature		14
D. Alkalinity and hardness		15
E. Inorganic ligands		16
F. Interactions		17
G. Sediments		23
H. In situ		24
Summary and Conclusions		26
4. Methods		27
A. Water samples		27
B. Duckweed culture		27
C. Duckweed test specimens		30
D. Test procedure		31
E. Test compounds		32
F. Growth medium		32
G. Statistical analyses		32
5. Results		34
A. Water quality		34
B. Nutrients		34
C. Controls		37
D. Repeatability		37
E. Ba toxicity		41
i. In duckweed growth medium		41
ii. In water samples (nutrient-enriched)		41
F. Cr toxicity		45
i. In duckweed growth medium		45
ii. In Illinois River water (enriched)		45
iii. In all water samples (enriched)		51

Concluded on next page

G.	Ni toxicity	56
	i. In duckweed growth medium	56
	ii. In Hayes Creek and Horseshoe Lake samples (enriched)	56
	iii. In Illinois River water (enriched).	56
	iv. In all water samples (enriched).	62
6.	Discussion	66
	A. Importance of aquatic vegetation	66
	B. Site-specific water quality criteria	67
	C. Reference toxicant	67
	References	69

Figures

1.	Sample stations	28
2.	Comparison of alkalinity of stations in 1956-1966 (Harmeson and Larson, 1969) and this study, in mg/L as CaCO ₃	36
3.	Negative controls	38
4.	Duplicate tests of enriched Skunk River water sample, 3/3/86, Ba toxicity	39
5.	Duplicate tests of enriched Skunk River water sample, 3/3/86, Cr toxicity	40
6.	Ba toxicity in duckweed growth medium, 7 tests	42
7.	Generalized exposure-effect curve, showing diameter of circles as degree of certainty about data points. From Whyte and Burton, 1980, p. 64. Reproduced by permission of SCOPE, * 15, Paris, France	43
8.	Ba toxicity in various water samples (enriched).	46
9.	Ba toxicity (IC50's) and SO ₂ concentration relationship.	48
10.	Cr toxicity in duckweed growth medium, 10 tests	49
11.	Duckweed growth in water controls (▲) and in Illinois River sample controls (●) and Cr toxicity in duckweed growth medium (▲) and in Illinois River samples (○)	50
12.	Cr toxicity in enriched Illinois River water samples, 13 tests	52
13.	Duckweed growth in growth medium and 10 surface waters (enriched) containing Cr ion	54
14.	Generalized Cr concentration-effect relationship in 18 surface water samples (enriched).	55
15.	Ni toxicity in duckweed growth medium, 7 tests	57
16.	Ni toxicity in enriched Hayes Creek and Horseshoe Lake samples, 4 tests	59
17.	Duckweed growth in water controls (▲) and in Illinois River sample controls (●) and Ni toxicity in duckweed growth medium (A) and in Illinois River samples (○)	61
18.	Ni toxicity in enriched Illinois River water samples, 12 tests	63
19.	Ni concentration-effect relationship in 16 surface water samples (enriched).	65

Tables

1.	Interactions of heavy metals in contact with aquatic organisms	19
2.	Mineral quality characteristics of selected stations in Illinois, median values in mg/L from 1950-1966 (from Harmeson and Larson, 1969).	29
3.	Nutrient stock solutions	33
4.	Water quality of test samples	35
5.	Duckweed growth (number of fronds) in water samples, 11/7/84, enriched with different levels of plant nutrients	36
6.	Ba toxicity in enriched water samples, in IC50's	44
7.	Classification of 8a toxicity in various enriched water samples	47
8.	Cr toxicity in enriched water samples, in IC50's	53
9.	Ni toxicity in enriched Hayes Creek and Horseshoe Lake samples, in percent growth inhibition	58
10.	Ni toxicity in enriched Illinois River water samples, in percent growth inhibition	60
11.	Ni toxicity in enriched water samples, in IC50's	64
12.	Classification of Ni toxicity in various water samples	64

Glossary

- Axenic culture - A culture treated to remove life forms other than the test organism.
- Colony - Several fronds (e.g., of duckweed) attached as a unit, usually consisting of mother and daughter fronds.
- Duckweed growth - Net increase of duckweed frond number during an incubation period.
- Duckweed growth medium - Double strength of algal growth medium (Standard Methods, 1985).
- Frond - A leaf-like structure; the fusing of leaf and stem of duckweed plants.
- IC50 - A toxicant concentration which causes 50% inhibition effect.
- Sample control - Ambient water samples enriched with duckweed growth medium.
- Water control - Deionized water samples enriched with duckweed growth medium.

Acknowledgments

This research project was supported by the U.S. Environmental Protection Agency, Grant 810834-01-0, Project Officer Mr. Clyde Bishop.

Judson M. Williams provided technical assistance throughout this study as well as graphic work and sample collection. Donald H. Schnepfer assisted in computer programming. Gail Taylor edited the report. Linda Johnson, Don Blakley, Dana Shackelford, Davis Beuscher, and David Hullinger all participated in water sample collection. David Hullinger and Dana Shackelford performed all chemical analyses. Linda Johnson typed the manuscript. Dr. Phillippe Ross reviewed and commented on the literature review section. Their assistance is sincerely appreciated.

1. Introduction

The development of water quality criteria is at a crossroad. On one hand, criteria are developed under laboratory controlled conditions with the goal of obtaining uniform and universal results. In other words, the results are necessarily repeatable since the essence of scientific method is repeatability. Many inter-laboratory, round-table studies have been conducted to test repeatability (Lemke, 1981).

On the other hand, the fundamental rule of environmental science is that there are no "standard" environmental conditions. It is inappropriate to derive a set of water quality criteria under laboratory conditions and then to apply them to various waters. For example, the National Academy of Sciences - National Academy of Engineering (National Research Council, 1972) reviewed the toxicity of lead, zinc, and copper to rainbow trout and found that the lethal concentration varied by two orders of magnitude, depending on the hardness of the water.

Consequently the trend in establishing water quality criteria is to take site-specific water properties into consideration. In guidelines prepared by the U.S. Environmental Protection Agency for deriving site-specific criteria, four methods are suggested, allowing each state to adopt a method appropriate for its local situation. Basically the derivation of site-specific criteria is based on national water quality criteria which are then modified by taking into account site-specific water quality characteristics such as resident species, heavy metal speciation, etc. (U.S. Environmental Protection Agency, 1982).

The objective of this study was to determine the toxicity of heavy metals in various river and lake waters. These waters encompass a wide variety of water quality, from very soft to very hard. Three metal ions were tested: barium, chromium (hexavalent), and nickel. The test organism was common duckweed, Lemna minor.

2. Conclusions

The duckweed toxicity test as conducted in this study is economical and sensitive. It offers an alternative/complementary test to general toxicity tests that use the common test species of daphnids and fathead minnows. Furthermore, it is a much more suitable test for assessing the phytotoxicity of herbicidal compounds.

The duckweed culture can be maintained with very little effort. In a long-term experiment, the stock culture remained vigorous and test specimens were available year-round.

Among three metal ions tested, Ba had the least phytotoxicity and was influenced the most by the water quality of the test samples. Cr had moderate phytotoxicity and was influenced the least by the water quality of the test samples. Ni had the greatest phytotoxicity and was moderately influenced by the water quality of the test samples.

These results suggest that the Cr water quality criterion may be adopted universally for all surface waters. The Ba criterion (5.0 mg/L) as stated by the State of Illinois (1986) for the protection of aquatic organisms can be substantially moderated according to water quality. The Ni criterion (1.0 mg/L) is extremely inadequate. At this concentration, a 30 percent growth inhibition of aquatic vegetation (based on common duckweed as the test organism) can be expected in almost all surface waters (Fig. 19) and 70 percent inhibition can be expected in extremely soft waters (Fig. 16).

The common practice for conducting biological toxicity tests is to include controls, commonly called negative controls. In this study, two types of negative controls were used throughout: water control and sample control. The negative controls measured the test organisms under favorable conditions. It is highly recommended, however, that a positive control also be used to measure the response of the test organisms to a universal, reference toxicant. Many organic and inorganic compounds have been suggested as reference toxicants. The results of this study suggest that Cr is a strong candidate to be the reference toxicant for surface waters.

3. Literature Review

This literature review encompasses aquatic environmental toxicities of heavy metals. The emphasis is on the effects of environmental factors on heavy metal toxicity to aquatic organisms. The effects of environmental factors on heavy metal uptake are also discussed. An attempt was made to cover as much literature as possible, but undoubtedly some reports have been missed inadvertently.

The literature review is generally divided into discussions of biotic and abiotic factors; each is then further divided. The literature shows divergent results. Nevertheless, the results can be summarized into four general laws of environmental toxicology that relate to quantity, organism, environmental conditions, and tolerance.

Introduction

Heavy metal toxicity to aquatic organisms can be affected by many environmental factors including alkalinity, hardness, pH, dissolved oxygen, temperature, turbidity, carbon dioxide, magnesium salts, phosphates, and chelating agents (National Research Council, 1972). The toxicity can be either potentiated or attenuated by these factors. An important effect of these environmental factors is the change of metal speciation, which plays a key role in metal toxicity (Florence, 1983; Baudo, 1981).

There are many review articles dealing with specific metals, including their aqueous chemistry, toxicology, and environmental behavior (Taylor, 1982; Taylor, 1983; Spear and Pierce, 1979; Taylor and Demayo, 1980; Demayo et al., 1980; Reeder et al., 1979). It is well recognized, however, that a metal pollutant rarely exists alone. In the case of water containing more than one toxicant, water quality standards for specific substances might need to be modified. In general the concentration-addition method has been shown to be applicable in most circumstances. Variations in uptake of specific substances in the presence of other toxicants do not appear to be related to the combined toxic potential of the mixture (EIFAC, 1980).

Other review articles containing relevant information can be found in the Annual Review series of the Water Pollution Control Federation. An interesting area which should receive greater attention is the microbial system. Metal toxicity to microorganisms can be moderated or enhanced by environmental factors including the presence of other competing or synergistic substances, formation of complexes with inorganic anions, pH, redox potential, temperature, suspended particulate organic matter, and dissolved organic matter (Stotzky, 1980; Babich and Stotzky, 1985; Babich et al., 1985). Microorganisms are known to

develop mechanisms for resisting metal toxicity, including energy-driven efflux pumps to keep toxic metal out, oxidation, biosynthesis of intramolecular polymers serving as metal traps, binding of metal ions to the cell surface, precipitation of metal ions, and biomethylation and transport of metal ions through the cell membrane (Wood and Wang, 1983). Higher plants generally keep toxic metal ions away from active metabolic sites by chelation at the cell wall, and the chelation process is highly metal-specific (Antonovics et al., 1971).

The factors which affect metal toxicity to aquatic organisms can generally be divided into two major categories: biotic and abiotic factors. Each group can be further divided into many subcategories. This literature review will be presented accordingly.

Biotic Factors

A. Tolerance

In environmental toxicology, tolerance, adaptation, and acclimation are used interchangeably. Tolerance is an important mechanism by which an organism reacts to an adverse environment. Duncan and Klaverkamp (1983) reported that white suckers' (Catostomus commersoni) tolerance of and resistance to Cd increased as a consequence of previous exposure. Mechanisms which might have been responsible for the decreased Cd toxicity included decreased uptake, increased excretion, redistribution of metals to less sensitive target sites, and/or induced synthesis of metallothionein for proteinaceous metal chelation. Benson and Birge (1985) showed that metal-contaminated fly ash pond minnows were significantly more tolerant of Cd and Cu than were hatchery minnows. At the exposure concentration of 6 mg/L Cd in moderately hard water, the median period of survival (LT50) for fly ash pond minnows was 50.5 h, compared with 6.8 h for hatchery minnows. The LT50 values for ash pond and hatchery fish exposed to 0.5 mg/L Cu in moderately hard water were 17.0 and 4.5 h, respectively. After being transferred to reconstituted water in the laboratory for 7 d, ash pond minnows significantly decreased their tolerance to Cd and Cu. Conversely, tolerance was increased in hatchery minnows following acclimation to the sublethal Cd concentration. Another study showed that fathead minnows exhibited similar induced tolerance to the Ag ion. The increased tolerance and resistance to Ag by acclimated organisms were lost if minnows were placed in a control water, indicating that the induced tolerance was not a sustained response (Birge et al., 1984).

Harrison (1983) examined two mechanisms of metal detoxification in the common mussel: the binding of Cu to metallothionein-like proteins and the incorporation of Cu into lysosome-like vesicles. Changes were noted in the kinds and quantities of Cu-binding proteins and in the latency of lysosomal enzymes occurring in the digestive gland cells. Chronic exposure to Cu resulted in Cu concentration in low molecular weight metallothionein-like proteins, while Cu concentration in high

molecular weight proteins was directly related to the mussel mortality.

The induced tolerance of fish to a toxic metal may also result from a factor other than the metal. Calamari *et al.* (1980) reported that fish acclimated in 320 mg/L CaCO₃ hardness but tested at 20 mg/L hardness reacted to metals in a different way from those maintained and tested at 20 mg/L. In this test procedure, chemical speciation of Cd was identical, yet the 48-h LC50 for the hardness-acclimated fish was approximately 7 times that for the control fish (0.677 and 0.091 mg/L Cd, respectively).

Plant species can also tolerate and adapt to environmental stress. Higher plants develop mechanisms for metal detoxification including exclusion, cell wall binding, organic acid complexation, enzymic adaptations, and permeability regulation (Thurman, 1981). Stockner and Antia (1976) cited literature examples for algal adaption and subsequent growth after exposure to pollutants. Experimental results showed that it might take as long as 20 to 40 d of exposure to pollutants for algae to acquire successful adaptations. Phytoplankton that initially tolerated only a low level of pollutant concentration could be trained to accept levels severalfold higher by repeated exposure. Wang (1986A) used algal communities from Peoria and Farmington sources (both in Illinois) to illustrate their acclimation to Zn toxicity. Peoria and Farmington algal communities exhibited different types of responses to Zn. In the dose-response relationship, one exhibited a concave-up response and the other, concave-down. When Peoria algae were acclimated to the environment of the Farmington sample, the response to Zn transformed into the same type as for the Farmington algae, and vice versa. After additional experiments, it was concluded that the Peoria algal community was acclimated to an elevated Zn concentration, while the Farmington community was used to a low Zn environment.

The tolerance to metal toxicity has also been studied *in situ*. Jones and McLean (1975) examined the aquatic flora of the rivers Ystwyth and Clarach, Wales, both of which are polluted by old lead mines. The results showed that Harmidium spp. were the most tolerant filamentous green algae and that the bryophyte Scapania undulate was tolerant of pollution. When Scapania was transplanted from its natural polluted site to a less polluted area there was no marked change in its metal content, but when the less tolerant Fontinalis squamosa was transplanted to polluted sites, its contents of Pb, Cu, Zn, and Mn increased within 6 weeks, and plants started to die and decay after 18 weeks. McNaughton *et al.* (1974) similarly studied clones of the broad-leaved cattail and soil samples from areas near a Zn smelter and a control location. They reported that the species was able to colonize in soils containing heavy metals, yet there was no evidence to indicate the evolution of species tolerant to heavy metals. Ernst (1975) indicated that plant species developed a metal tolerance specific only to those metals abundant in their habitat. The mechanism of resistance was probably not by the exclusion process because of the high metal content in resistant plants.

Stokes et al. (1973) reported that the lake waters in the Sudbury smelting area, Ontario, contained abnormally high levels of metals, especially Cu and Ni. Two algal species were isolated, Scenedesmus and Chlorella. In comparison with laboratory strains, the isolated species were found to be tolerant of the heavy metals Ni (Scenedesmus) and Cu (Scenedesmus and Chlorella). Stokes (1975A) further reported that Cu and Ni tolerance in the same organisms could be separated and that the expression of each was independent. Gregory and Bradshaw (1965) concluded that the tolerance mechanism of Agrostis tenuis was almost always metal - specific; tolerance to one metal did not automatically confer tolerance to another.

The tolerance of Chironomua tentans larvae to varying levels of heavy metals (Cd, Cr, and Zn) was studied (Lindstrom, 1980). Larvae from sediment low in heavy metals showed a lower rate of survival when exposed to a sediment high in heavy metals than larvae collected from sediments high in heavy metals exposed to sediment low in heavy metals.

In summary, an organism subjected to environmental metal toxicity will either survive or die. The surviving organism is the one which possesses mechanisms to tolerate the metal toxicity. The acquired tolerance is likely temporary and metal-specific.

B. Size and life stages

The size and more importantly the life stage of an organism have an important effect on its sensitivity to metal toxicity. It is known that the early life stage of an organism is more sensitive to toxicity than the juvenile and the adult. Nebeker et al. (1985) determined the no-observed-effect concentration of Ni to rainbow trout and reported that the early life stages were most sensitive when newly fertilized eggs were initially exposed, followed in sensitivity by eyed eggs, larval fish, and juvenile fish. Reish et al. (1976) tested two polychaetes and arrived at the same conclusion. Eaton (1974) observed that adult bluegill spawned at 239 ug/L Cd solution, but that most larvae were severely crippled 6 d after hatching at that concentration.

Using aquatic insects as test organisms. Smock (1983B) found that the metal concentrations of Co, Cr, Fe, Sb, and Sc in organisms decreased with increasing body size, indicating that surface adsorption was an important method of accumulation for these metals. While little or no correlation between concentration and body size was observed for K, Mn, and Na, Sykora et al. (1972) tested the effect of lime-neutralized ferric hydroxide on young brook trout. The results showed a trend towards smaller fish and lower weight with increasing concentration of suspended ferric hydroxide. Rainbow trout of 10-g body weight were 2.5 times more resistant to Cu lethality than the 0.7-g size (Howarth and Sprague, 1978).

In microbial toxicity tests, the size of the inoculum can also influence the toxicity results for heavy metals such as Cd (Truhaut et al. 1980).

C. Species

The toxicity of heavy metals is greatly influenced by the species of test organisms. Thorp and Lake (1974) determined Cd toxicity in soft water to selected freshwater invertebrates. LC50 values varied from 0.04 mg/L for amphipods, 0.06 mg/L for shrimp, and 0.84 mg/L for nymphs to well over 2000 mg/L for Trichoteran larvae. Studying a wide range of aquatic insects. Smock (1983A) reported that metal concentrations were highest in burrowing mayflies and some Chironomidae that ingest sediments along with detritus. Filter-feeders had the next highest concentrations, while carnivores and surface-feeders had the lowest. Rodgers et al. (1978) observed that duckweed was the only abundantly occurring macrophyte inhabiting a stream-swamp drainage system that received high quantities of coal ash and thermal discharges from a fossil fuel power plant, indicating that duckweed was hardy and/or adaptive in comparison with other species. Duckweed from unpolluted areas, however, was found to be sensitive to heavy metal toxicity (Wang, 1986B).

Other plant species also exhibit widely different responses to toxicity. For example, Ditvlum underwent osmotic disturbances in 10 and 10 mol/L Cu, with swelling of cell contents, while Phaeodactylum grew in continuous culture, surviving single doses of up to 10 mol/L Cu without diminution in growth with considerable uptake of Cu (Bentley-Mowat and Reid, 1977). The uptake of the heavy metals Zn, Cu, Pb, Cd, Mn, and Fe by aquatic plants clearly was species dependent. In general, submerged taxa had greater metal content than floating ones (Aulio and Salin, 1982; Van der Werff and Pruyt, 1982).

Wong and Beaver (1980) proposed an interesting two-species method for toxicity tests. One species, Chlorella fusca. was commonly found in lakes with high metal concentrations, while Ankistrodesmus was very sensitive to metals. By determining the maximum yield ratio between Ankistrodesmus and Chlorella. it was possible to compare toxic strength of harmful metals.

Using a multi-species community approach, Ruthven and Cairns (1973) found protozoa to be more resistant than Daphnia to phenol, K CrO., and Cu. Some protozoan species, however, were more sensitive than Daohnia to Zn, nitric acid, and HCl. The results suggested that there was no uniformity of toxic response to various toxic substances by different organisms. The multi-species approach has been advocated and discussed (Cairns, 1985). Multi-species toxicity tests obviously offer more than single species toxicity tests can. Following are three examples.

First, Hutchinson and Czyrska (1975) reported that when both Lemna valdiviana and Salvinia natans were present, the addition of 0.01-0.05 mg/L Cd resulted in greater toxicity to Lemna than when the same amount of Cd was added to Lemna alone. In contrast,

Salvinia grew a good deal better with Lemna in the presence of 0.01-0.05 mg/L Cd than it did when it grew by itself. The possible reason for this discrepancy is the species competition. Hutchinson and Czyrska observed that "under the stress of competition Lemna grows less well and cadmium levels are markedly increased in the tissues, while Salvinia grows better and the cadmium concentrations are correspondingly reduced." The inter-species interaction clearly cannot be obtained by using a single species test. Another example is food chains. Vinot and Larpent (1984) studied water pollution caused by uranium ore treatment works. They found that in certain streams, the fauna and flora disappeared completely. The study showed that ore tailings did not directly affect fish or crustaceans but only algae, the growth of which was either partly or completely inhibited, depending on the dilution provided by the watercourse. The toxic substances were found to be Cu and Zn, which caused the disappearance of the microalgae and which in turn, through the food chains, resulted in the disappearance of the higher trophic species leading to fish. The third example involves two freshwater oligochaete sp. (Chapman et al., 1982A). Comparison of data between mixed species tests and individual species tests indicated that organisms in the mixed species tests were significantly more tolerant of the toxicants Cd, Hg, and sodium pentachlorophenol.

Sugiura et al. (1982) used aquatic microcosms containing algae, protozoa, rotifers, oligochaetes, and bacteria. The effect of Cu stress was found to be greater in the early stage of heterotrophic succession than at later stages. At the later stage, it was assumed that the microcosm was more established and thus more resilient to the stress.

LeBlanc (1984) divided toxic substances into nonpesticide organics, pesticides, and metals and compared their acute toxicities to aquatic organisms. He found that all species analyzed - fresh and saltwater fish, invertebrates, and algae responded similarly to the toxicity of nonpesticide organics, while different groups of organisms responded differently to the pesticides. Different species of fish of the same family responded almost identically to the toxicity of pesticides, whereas fishes of different families reacted similarly, but to a lesser degree. No relation existed between the acute sensitivities of fish and invertebrates. A significant correlation was determined in acute sensitivities to metals between bluegill and fathead minnow, and between bluegill and Daohnia magna. The mode of toxicity of metals seems to be the same among species, although the degree of toxicity may vary.

It can be concluded that the test species is an important parameter for conducting toxicity tests. In environmental toxicology, it is advisable to consider a battery of organisms of different trophic levels, including algae, higher plants, invertebrates, and fish to encompass the entire food chain.

D. Nutrition

Nutrition of a test organism has a strong influence on its state of health and, consequently, a direct impact on its sensitivity to environmental toxicity. Seto et al. (1979) examined the effects of Cd on the duckweed Lemna gibba. They found that the ratio of chlorotic fronds to total fronds was greatly influenced by the difference in concentration of mineral nutrients. When cultured in a high-nutrient concentration, the chlorotic fronds did not appear in spite of high Cd concentration in the frond. On the other hand, when cultured in solutions with low concentrations of nutrients, the chlorotic fronds appeared in spite of low Cd content in the frond.

Johnels et al. (1967) studied Hg accumulation by pike (Cesoc lucius) and other fishes from Swedish lakes. They found higher accumulation rates in oligotrophic lakes than in more eutrophic ones with the same degree of Hg pollution. The Hg accumulation was apparently a function of many environmental factors including the role of organic matter in sorption and/or chelation, and activities of microorganisms responsible for Hg methylation.

Hart and Cairns (1984) defined aquatic ecosystem assimilation capacity as the ability of an ecosystem to assimilate a substance or stress without degrading or damaging its ecological integrity, namely the maintenance of structural and functional characteristics. They compared two types of lakes, one at the University of Michigan (eutrophic) and the other at Smith Mountain, Virginia (oligotrophic). It was found that eutrophic communities had a greater structural assimilative capacity than did oligotrophic communities. The eutrophic communities had a greater proportion of species that were tolerant of low concentrations of Cu. This is interesting in light of the fact that eutrophication decreases species diversity (Hooper, 1969).

The rates of metal uptake by organisms may vary among metals. Rudd and Turner (1983A) found that stimulation of primary production by addition of nutrients tended to increase Hg uptake and reduce the concentration of Se in the food chain, probably as a result of dilution of Se by greater growth of various aquatic organisms.

In summary, it can be concluded that the state of test organisms has an important effect on their susceptibility to metal toxicity. Generally, organisms which are more mature, healthier, and pre-conditioned to the stress can be expected to fare better than the young, less nourished, and un-conditioned.

Abiotic Factors

A. Organics

i. Synthetic organics. Many studies have tested the effects of organic compounds on metal toxicity and uptake. The majority of these test compounds are chelating substances. Muramoto (1980) studied the effects of chelating agents EDTA (ethylenediamine-tetraacetic acid), NTA (nitrilotriacetate), and

other agents on the toxicity of Cd, Cu, Zn, and Pb to carp. He reported that fish exposed to metals plus chelating agents (1:3 molar ratio) contained lower metal concentrations in gills and other parts than did fish exposed to metals alone. The chelators apparently inhibited metal accumulation. Nasu et al. (1983) indicated that only 30 uM EDTA was sufficient to prevent the absorption of Cu by Lemna paucicostata at a concentration of 5-10 uM, while 400 uM of EDTA was required to prevent the absorption of Cd at 5-10 uM. The growth of Lemna was inhibited in proportion to the amount of Cu and Cd absorbed.

Morris and Russel (1973) studied Cu toxicity to the brown alga Ectocarpus and found that the plant growth rate dropped rapidly with increasing Cu concentration, and stopped at a concentration of 0.45 mg/L. The presence of EDTA increased this critical concentration and the alga continued to grow up to a level of over 0.85 mg/L. Stokes and Hutchinson (1976) reported that Cu bound with EDTA was not toxic and that the strong chelator prevented Cu uptake by algal cells, while acetate had little effect on Cu toxicity. Nishikawa and Tabata (1969) used EDTA to eliminate the toxicity of wastewater from Cu mines, using Daohnia and dace as test organisms.

The infaunal amphipod crustaceans Rhepoxvnius abronius and Eohausterius sencillus are characteristic of nearshore sandy bottoms on the California coast. They are sensitive to moderate levels of heavy metals. In laboratory experiments, EDTA increased survival of the amphipods in sediments containing lethal levels of Cd (8.5 ug/g) (Oakden et al., 1984).

Andrew (1976) concluded that acute Cu toxicity to fathead minnows (Pimephales promelas) and Daphnia magna was much less when NTA was added. Similarly, Cu precipitates were not biologically active or toxic. Sprague (1968) found that NTA selectively chelated Cu first, then Zn afterwards, and proposed using NTA as an agent to detoxify heavy metals. Testing with Daphnia magna. Biesinger et al. (1974) reported that Cu and Zn toxicities were considerably less when NTA was added. Hongve et al. (1980) found that NTA had a superior detoxification capacity for Cu, Cd, Zn, and Pb because of a strong metal-NTA complex which did not affect the photosynthetic rate of natural phytoplankton. Hg-NTA complex, on the contrary, was more toxic than the ionic form of Hg. In a metal mixture of Cu-Cd-Zn-Pb-Hg, they observed that NTA dramatically enhanced the toxicity of the mixture to algal growth, possibly due to a synergistic effect. Sunda et al. (1978) found that after 96 h exposure to a given concentration of Cd, grass shrimp mortality decreased with increasing concentrations of NTA and increasing salinity. They attributed the protective effect of high salinity and NTA to the complexation of Cd. Chynoweth et al. (1976) tested varieties of organic compounds, glycine, cysteine, EDTA, NTA, citric acid, albumin, humic acid, and secondary sewage effluent. The results indicated that unbound Cu was more toxic to guppies than bound Cu.

The effects of other synthetic compounds on metal toxicity have also been studied. Calamari and Marchetti (1973) reported

that the toxicity of Cu and Hg and anionic surfactants to rainbow trout was "more than additive," while for the mixture of non-ionic detergent and metal, the toxic effect was "less than additive." Muramoto and Oki (1984) investigated the effect of a surfactant SDS (sodium dodecyl sulfate) on Cd and Ni uptake by water hyacinths. Exposure to Cd and Ni alone caused a decrease of biomass. Simultaneous administration of SDS did not alter this pattern. The addition of SDS decreased Cd uptake by plants, while it did not significantly affect Ni uptake. Borgmann and Charlton (1984) examined the toxicity of Cu to Daphnia magna in an artificial medium, medium plus algae, and water samples from Hamilton Harbor and Lake Ontario. Cu toxicity was greatest in the inorganic medium and lowest in the inorganic medium plus algae. After Tris addition, however, Cu toxicity was greatest in lake water and lowest in inorganic medium. The uptake of both Cu and Pb by the alga, Nostoc, decreased when citrate was added (Schecher and Driscoll, 1985). Cu-amino acid complexes were found to be less toxic than free Cu ions (Borgmann and Ralph, 1983).

The organic compounds, however, do not always reduce metal uptake and subsequently toxicity to aquatic organisms. Piccardi and Clauser (1983) reported that iris possessed a high affinity for absorption of heavy metals. Tests with Cu solutions indicated that a high amount of absorbed metal (82±8%) was in the roots. High concentrations of surfactants (both anionic and nonionic) had little effect on metal uptake. Triton X-100 did not affect the specific growth rate of Chlorella cultures when it was added to a culture medium (Wong, 1985). The compound, however, was found to be capable of enhancing Cd toxicity, particularly at the low concentration range. For Cu, the compound enhanced the metal toxicity only when Cu reached a high concentration. Canterford and Canterford (1980) investigated the effects of chelation on the toxicity of Cu, Zn, Cd, Pb, Hg, Ag, and Ti to a marine diatom, Ditylum, and indicated that the presence of EDTA in the culture medium did not lower the toxicity of all heavy metals. Among these metals, the free metal concentrations of Hg, Ag, and Ti were independent of EDTA concentration, suggesting little effect of EDTA on their toxicity. Ahsanullah and Florence (1984) tested organocopper toxicity to the adult marine amphipod, Allorchestes. They reported that water soluble ligands NTA, 8-hydroxyquinoline-5-sulfonic acid, and tannic acid ameliorated Cu toxicity by decreasing the concentration of the free Cu ion, while lipid soluble ligands such as oxins and potassium ethylxanthogenate increased Cu toxicity, presumably as a result of increased permeability through the cell membrane.

Some interesting results were reported by Nasu and Kugimoto (1981) showing that the reproduction rate of the duckweed Lemna paucicostata increased two- to threefold if 1 percent sucrose was added to the growth medium. The toxic effect of Cd and Cu on the reproduction rate was more noticeable when sucrose was added, while that of Cr (VI) was not affected by the sucrose addition.

Ferric iron is generally not considered highly toxic. Its effect on algal activity, interestingly, could be expressed on an equi-molar basis with EDTA (Wang, 1983). George and Coombs (1977)

found that Fe (III) complexes of citrate, EDTA, and 1,10-phenanthroline increased the rate of Fe uptake, whereas ferrichrome b and Fe (III) complexes of aceto- and benzo-hydroxamic acids caused a decrease.

ii. Humic substances. Natural water contains various amounts of dissolved and suspended organic matter. Brown et al. (1974) found that Cu toxicity to rainbow trout was quantitatively reduced when humic substances and suspended organic matter were present.

Zitko et al. (1973) used incipient lethal levels (ILL, a concentration level beyond which 50 percent of the population cannot survive indefinitely) to express the effect of humic substances on Cu toxicity to juvenile Atlantic salmon. The effect was very pronounced. The ILL'S of Cu, Cu + 5 mg/L humic acid, and Cu + 10 mg/L humic acid were 25, 90, and 165 ug/L, respectively. In the case of fulvic acid, the ILL's were 110 and 240 ug/L with the treatment of 5 and 10 mg/L fulvic acid, respectively. The results of other studies also indicated that Cu toxicity was reduced by natural organic substances (Brown et al. 1974; Sunda and Guillard, 1976; Sunda and Lewis, 1978).

The effect of humic substances on metal toxicity, however, is not always negative. Winner (1984) reported that the addition of humic acid to test water decreased the acute and chronic toxicity of Cu, but increased the acute and chronic toxicity of Cd, while humic acid had no effect on bioaccumulation of either metal. Laegreid et al. (1983) also observed that Cd in some lake water was more toxic to Selenastrum capricornutum than that in a growth medium. They suggested the possibility of dissolved organic-induced toxicity enhancement.

The complexing capacity of a water sample can be measured by selective ion electrodes. Buckley (1983) reported that the Cu complexing capacity of a sewage treatment plant effluent averaged 0.3 mg/L. Sixty-seven percent of the complexation was due to compounds of less than 10,000 molecular weight units. He also found that organic compounds in the effluent removable by activated carbon composed 88 percent of the total organic carbon and were responsible for 87 percent of the complexation. Similarly, Varini et al. (1981) found that Cu toxicity to Daphnia magna was mainly due to the fraction of low molecular weight compounds. Wilson (1972) observed that adding spent sulfite liquor to Cu solution increased the survival rate of Atlantic salmon. Gelatin and silica gel as gelling agents for microbial media were found to reduce the apparent toxicity of inorganic Sn (IV), whereas serine and hydroxyflavone enhanced it (Hallas et al., 1982).

In summary, the evidence generally indicates that organic substances can reduce the toxicity of metals such as Cu. It remains to be seen whether other metals can be affected similarly.

B. pH

The effect of pH on heavy metal toxicity is very complex: the effect is primarily dependent on the metal species. Nasu *et al.* (1983) demonstrated that both Cd and Cu inhibited the growth of Lemna paucicostata more at pH 5.1 than at pH 4.1. They also observed that Cd absorption was greater when initial pH was higher, but Cu absorption was not significantly affected by an initial pH between 3.6 and 5.1. Hargraves and Whitton (1976) isolated a common stream alga, Hormidium, from an acid mine drainage and found that its optimum range for growth was pH 3.5-4.0. The Zn toxicity to this alga increased at higher pH values. The binding capacity of the heavy metals Cu, Cd, and Zn on the blue-green alga, Chroococcus parvulus, was found to increase as pH increased from 4 to 7 (Les and Walker, 1984). Accumulation of Cd and Zn by the aquatic liverwort Scaevola also increased as pH increased (Whitton *et al.*, 1982). The increasing toxicity of Cu at high pH was attributed to the interactions of the metal ion with sulfhydryl-containing proteins or enzymes (Andrew, 1976).

Alternatively, there are many studies suggesting that metal toxicity can be reduced at higher pH values. Verma *et al.* (1985) observed that Hg (II) toxicity to fish decreased as pH increased. Robinson and Deano (1985) showed that Al and acidity had synergistic toxic effects. Michnowicz and Weaks (1984) reported that the alga Selenastrum capricornutum Printz grew significantly better in a test solution containing 0.2 mg/L As when it had previously been cultured at pH 8 than when cultured at pH 4. Babich and Stotzky (1982A) investigated the effect of pH on Ni toxicity using a broad spectrum of microorganisms, including bacteria, actinomycetes, yeasts, and fungi. They found that Ni toxicity was potentiated as pH was decreased to acidic levels. The results, therefore, implied that acid precipitation could cause direct injuries as well as indirect effects such as toxicity enhancement of heavy metals. An interesting, although seemingly contradictory, observation was that Ni accumulation by algae was highest at pH 8 (Wang and Wood, 1984). Using Chlorella for Hg toxicity tests, Rai and Khatoniar (1980) found that acidic pH enhanced Hg toxicity, while alkaline pH reduced it. Pb toxicity to fungi was potentiated under acidic conditions at pH 5 and 6 (Babich and Stotzky, 1979), while a significant increase of Pb uptake at lower pH values was also reported (Merlani and Pozzi, 1977; Lewis and McIntosh, 1984).

Using zebrafish egg hatching and larvae survival as a test procedure, Dave (1985) determined the pH effect on Al, Cd, and Fe toxicity. He reported that Al and Cd were more toxic at high pH levels while Fe was more toxic at low pH levels. Stendahl and Sprague (1982) studied the toxicity of vanadium pentoxide at pH 5.5, 6.6, 7.7, and 8.8. The V toxicity to 25-g trout was greatest at pH 7.7, at which level the predominating ion H_2VO_4 was apparently the most toxic form. HVO_4 prevailed at high pH and was calculated to be 60 percent as toxic as H_2VO_4 while the metal present as decavanadates at pH 6.6 and 5.5 was 50 percent as toxic as H_2VO_4 . O'Keefe *et al.* (1984) reported that Cd uptake by the water hyacinth increased as the pH increased from 2 to 5, while in Hoagland's solution, higher pH values decreased the uptake rate.

In summary, the evidence suggests that pH effect on metal toxicity is dependent on the metal species and is without a set pattern.

C. Temperature

An extensive, literature review on temperature effect upon chemical toxicity has been reported (Cairns et al., 1975A). The temperature effect on metal toxicity can generally be seen by two opposing results. On one hand, it was reported that temperature between 15 and 28 C had no significant effect on lethal concentrations of Cd, Cr, Cu, Hg, Ni, and Zn (Rehboldt et al., 1972). Similarly, temperature was found to have no effect on the rate of Hg uptake or elimination by freshwater clams (Smith et al., 1975). On the other hand, the majority of reports have indicated that temperature had an important effect on metal toxicity.

Bryant et al. (1984) studied temperature effects on Cr (VI) toxicity to invertebrates. In general, they found that Cr toxicity increased with increasing temperature. MacLeod and Pessah (1973) reported that the LC50 values for HgCl₂ to rainbow trout at 5, 10, and 20 C were 0.4, 0.28, and 0.22 mg/l, respectively, and the velocity of mortality (the reciprocal of time to death in h) was linearly related to temperature, $V = 0.002 + 0.0023t$, where t = temperature. Increased temperature also appeared to increase Zn toxicity to marine fish (Negilski, 1976), Zn toxicity to Lemna paucicostata (Nasu and Kugimoto, 1981), and Cd toxicity to larval fish (Middaugh et al., 1975). Other reports indicated that increased temperature resulted in increased uptake of heavy metals (Jackim et al., 1977; Frazier, 1976; MacLeod and Pessah, 1973). The increase in temperature also affected the turnover of metals in organisms. The biological half-lives of the heavy metals Co 60 and Zn 65 in shrimps were reduced by a factor of about 2 for an increase of 10 C (Van Weers, 1975). There is additional evidence indicating that temperature has an important influence on metal toxicity.

Braginskii and Shcherban (1978) found no acute toxicity of Cd, Cu, Mn, Ni, and Zn to six species of freshwater invertebrates in the temperature range 10-15 C, but the toxicities increased sharply at 25-30 C. It is apparent that temperature-mediated metabolic rate has an important role in metal toxicity. This viewpoint is further substantiated by Roch and Maly's experiments (1979). They observed that cold-acclimated (6 C) rainbow trout, exposed to lethal concentrations of Cd, survived longer than warm-acclimated (12 and 18 C) trout. The cold-acclimated trout survived higher Cd concentrations. Similarly, the cold-acclimated Atlantic salmon survived longer than warm-acclimated salmon (Hodson and Sprague, 1975). The survival time of fish in toxic concentrations of ammonia (albeit ammonia is not a metal species) also decreased markedly as temperature increased (Cairns et al., 1975B). Nevertheless, it should be pointed out that organisms

might recover better from metal toxicity at higher temperature than at lower temperature (Remacle et al., 1982).

It can generally be concluded that seasonal temperature variation can affect metal toxicity to aquatic organisms. The ecological implication is that water quality criteria should take this into account and that the criteria should be more stringent in summer than in winter.

D. Alkalinity and hardness

Alkalinity and hardness of a water sample normally go hand-in-hand and thus they are discussed here together.

There are many reports indicating that alkalinity and hardness can generally lead to detoxification of heavy metals. Brkovic-Popovic and Popovic (1977) indicated that the toxicity of the heavy metals Cd, Cr, Cu, Hg, Ni, and Zn to Tubifex was dependent on alkalinity and hardness, except that Hg was much less affected than the other metals. Slonim and Slonim (1973) reported that the lethal concentration of Be to guppies (96h LC50's) was a linear function of water hardness, between 10-400 mg/L (as CaCO₃). Howarth and Sprague (1978) reported that Cu toxicity to rainbow trout involved a complex response over a wide range of hardness and pH. The trend, however, can be generalized by stating that high hardness decreased Cu toxicity at any pH.

Rai and Khatoniar (1980) observed that 1 mg/L Hg (HgCl₂) was highly toxic to the alga Chlorella. and that up to 20 mg/L Ca counteracted the Hg toxicity and stimulated the growth of Chlorella. Similarly, Cd toxicity to oak leaf could be minimized by Ca (Fuhrer, 1983). Weis (1980) showed that Zn retarded limb regeneration in the fiddler crab, Uca peregulator, and the effects of Zn (1-5 mg/L) were potentiated under conditions of decreased salinity (7-8 ‰ as opposed to 30 ‰), possibly owing to a lower Ca concentration.

Stephenson (1983) examined the effects of water hardness, temperature, and the size of freshwater shrimp on the susceptibility to Cu toxicity. Among these variables, he found that only water hardness had statistically significant effects on LC50 values. Cu was 4-6 times more toxic in soft water than in hard water. Ajmal and Khan (1984) reported that Cd toxicity to saprophytic and nitrifying bacteria decreased as hardness increased, with a minimum at 320 mg/L (as CaCO₃) hardness for nitrifying bacteria and 400 mg/L for saprophytic bacteria. Poston et al. (1984) reported that U(VI) acute toxicity (48h LC50) to Daphnia magna diminished by a factor of 7.5 as mean water hardness and alkalinity increased from 70 and 57 mg/L to 195 and 130 mg/L, respectively.

There are two schools of thought for interpreting the effects of metal detoxification by alkalinity and hardness. The first hypothesis involves a biological mechanism for the observed effects. As mentioned in the section on tolerance, Calamari et al. (1980) reported that Cd toxicity to salmon was vastly

different if the fish was acclimated in hard water, even though it was tested in soft water. Apparently a biological mechanism may be involved in the detoxification processes. Additional evidence of a biological mechanism is that the fecundity of Daohnia magna was correlated with CaSO₄ content, in the range 91-2100 mg/L (LeBlanc and Surprenant, 1984). It is likely that when a metal toxicity test involving invertebrates is performed, the varying amounts of Ca can influence the test results accordingly. On the other hand, there is evidence pointing to a chemical mechanism as the cause of metal detoxification to organisms.

Slonim and Slonim (1973) attributed the detoxification of Be by water hardness partly to the buffering capacity and Ca antagonisms in hard water solution. There may be a competitive uptake between toxic metals and Ca in hard water. For example, Kinkade and Erdman (1975) found that the initial uptake of Cd in a simulated freshwater ecosystem was faster in hard water than in soft water. However, the total concentration of Cd was greater in the organisms cultured in soft water than in those in hard water. Similarly, Chapman and Dunlop (1981) reported that Zn uptake by freshwater protozoa (Tetrahymina pyriformis) was reduced from 0.5 ug/L to 0.1 ug/L per 1 million cells by adding 500 mg/L Ca alone or 500 mg/L Ca + Mg. Mg alone was about half as effective in reducing Cd toxicity. The mechanism is possibly due to competition at the cell membrane in the presence of Ca and Mg. Poston et al. (1984) attributed the loss of U(VI) toxicity to the complexation of the U ion with the carbonate ion.

There is also indirect evidence suggesting the effects of alkalinity and hardness on metal uptake by plants. Franzin and McFarlane (1980) investigated the aquatic ecosystem in the vicinity of a base-metal smelter located at Flin Flon, Manitoba. They reported that metal contents in plant material were different from what were expected on the basis of metal concentrations in the water samples. Varying Ca contents in lake water were speculated to be the cause of the discrepancy.

In summary, alkalinity and hardness can be considered as a "buffer" in natural water for moderating heavy metal toxicity.

E. Inorganic Ligands

Inorganic ligands such as Cl⁻, OH⁻, PO₄³⁻, etc., have been shown to influence heavy metal toxicity to aquatic organisms. The results are complex. For example, Babich and Stotzky (1978) found Zn toxicity to fungi, bacteria, and coliphages was unaffected, lessened, and increased, respectively, by the addition of high concentrations of NaCl. They reported that the increased Zn toxicity in the presence of NaCl was not due to synergism between Zn and Cl and elevated osmotic pressure, but to the formation of a complex Zn-Cl species. Babich and Stotzky (1983) further reported that Ni toxicity to microbes (bacteria, actinomycetes, and yeasts) in marine systems was reduced by increasing salinity.

In general, Cr(VI) toxicity to invertebrates decreased with increasing salinity. For Coroohium volutator, however, there was

a decrease in toxicity with increasing salinity over the range 5-30 ‰, and increased toxicity caused by further salinity increases up to 40 ‰ (Bryant et al., 1984). Hallas et al. (1982) found that replacement of SO_4^{2-} with NO_3^- did not change Sn toxicity to estuarine microorganisms, while the replacement of Cl with NO_3^- decreased Sn toxicity. Piccardi and Clauser (1983) observed that Iris pseudacorus survived 3 mg/L Cu in the presence of NaCl, without showing signs of stress. This was likely due to decreasing Cu absorption by the plant in the presence of NaCl. Hg ions did not exhibit synergistic effects with reduced salinity and merely acted additively (Gray, 1976). Frank and Robertson (1979) reported that the median lethal concentration (96h LCS0) of Cr to the blue crab, Callinectes sapidus, was 89 and 98 mg/L at 15 and 35 ‰ salinity, respectively. In comparison, the LC50's of Cd were 4.7 and 11.6 mg/L at the respective salinities. Jones et al. (1976) found that both high and low salinities increased the toxicity of Cu to the polychaete Nereis diversicolor. They suggested that the cause was synergistic action, instead of increased bioaccumulation of Cu. Jackim et al. (1977) observed that a decrease in salinity depressed Cd accumulation in marine bivalves.

PO_4^{3-} is widely distributed in natural water and may have some effects on metal toxicity. Sanders (1979), using algal cultures low in phosphorus, found that As toxicity to algal growth could be suppressed by increasing the phosphorus concentrations. Similarly, Cd uptake by Chlorella was observed to decrease as PO_4^{3-} increased (Khummongkol et al., 1982), and Zn-P interactions were significant in influencing Selenast rum cell yield results (Kuwabara, 1985). It is likely that PO_4^{3-} competes at the sorption sites against As and also forms non-dissolved chemical species such as Cd-PO_4 or Zn-PO_4 , resulting in a decrease of the metal bioavailability.

Other ligands have also been examined. Andrew et al. (1977) studied effects of the ligands carbonate-bicarbonate, orthophosphate, and pyrophosphate on Cu toxicity to Daphnia at constant pH and total hardness. They found that mortality rates and reciprocal survival times were directly correlated with Cu and Cu hydroxy $[\text{Cu}(\text{OH})_n]$ ion activities. Cu toxicity was negatively correlated with activities of soluble CuCO_3 and other complexes. Riedel (1984) determined the acute toxicity of Cr(VI) to the diatom Thalassiosira in artificial media containing 3.2 and 0.32 ‰ salinity and varying SO_4^{2-} concentrations. The increase of salinity was found to decrease Cr(VI) toxicity. Furthermore, Cr(VI) inhibition was a function of the ratio of Cr(VI) to SO_4^{2-} . Inhibition occurred when the ratio exceeded about 500:1. It was speculated that SO_4^{2-} and CrO_4^{2-} compete at the same sites, resulting in reduced Cr(VI) toxicity.

F. Interactions

One strength of biological toxicity tests is that the tests reflect the combined integrated effects of all toxic constituents present in a test sample. Wastewaters discharging into natural waters frequently carry more than one toxic or potentially toxic

substance. The combined toxic effects of all constituents are often not equivalent to the summation of all individual effects - they are sometimes greater, other times less.

The interactions between toxicants, however, are very complex. There are many variables influencing the interactions, such as metal species, test organisms, and lethal versus sublethal concentration. The joint actions are commonly described as 1) synergism, where the combined toxic effect of a mixture is greater than the sum of the individual toxicities, 2) antagonism, where the combined toxic effect of a mixture is less than the sum of the individual toxicities, and 3) non-interactive, or additive, where the combined effect is identical to the sum of the individual toxicities. All three types of interaction were found between Fe and Zn in different sources of algal samples (Wang, 1985).

The interactions between heavy metals appear to be without a set pattern. For example, interactions between the most studied metal pair, Cd and Zn, varied from antagonistic to synergistic as observed by various researchers using different test organisms (Table 1). Thorp and Lake (1974) reported that Cd and Zn interaction was dependent on the quantity of toxic metals. Synergistic interaction between Cd and Zn was observed by Hutchinson and Czyrska (1975) and Marshall *et al.* (1981), while antagonistic interaction was reported by Spehar *et al.* (1978), Weis (1980), Attar and Maly (1982), Singh and Yadava (1984), and O'Keefe *et al.* (1984). Hutchinson and Czyrska (1975) found that Zn at 0.05 and 0.08 mg/L had a stimulatory effect on both Lemna and Salvinia. The stimulatory effect of Zn was suppressed by the presence of Cd at 0.01 to 0.03 mg/L. The mixture of Cd and Zn showed a noticeable synergistic effect. Spehar *et al.* (1978) indicated that survival of flagfish larvae exposed as embryos was significantly decreased in both individual Cd and Zn concentrations. They further noted that the mixture of these metals did not further suppress the survival, suggesting antagonism between these metals. Weis (1980) used limb regeneration in the fiddler crab as the indicator and found an antagonism between Cd and Zn.

Other binary metal mixtures have also been studied. Stauber and Florence (1985) reported that the marine diatom Nitzschia closterium was more tolerant to Cu toxicity when cultured with normal Fe levels (0.79 mg/L) than when cultured in an Fe-deficient medium (0.079 mg/L). They speculated that colloidal Fe(OH)₃ binding to the diatom cell membrane sorbed Cu and prevented it from penetrating into the cell. Stokes (1975B) observed that Cu and Ni had strong synergistic effects on algae isolated from Sudbury, Ontario. The results of uptake and toxicity of Ni in the presence of Cu supported the theory that Cu increased the permeability of the cell. Cu once inside the cell was firmly bound. On the other hand, Anderson and Weber (1976, 1977) experimented with mature male guppies and found that the binary solution containing Cu and Ni exhibited strict additive interaction. It is possible that these metals acted with a common mode of lethal action.

Table 1. Interactions of Heavy Metals in Contact with Aquatic Organisms

Toxicants	Test organisms	Interactions	References
Binary			
Cd-Zn	freshwater shrimp	Less than additive when less than 1 toxic unit Additive when >1 toxic unit	Thorp and Lake (1974)
Cd-Zn	aquatic plants	synergistic	Hutchinson and Czyrska (1975)
Cd-Zn	freshwater fish	antagonistic	Spehar <u>et al.</u> (1978)
Cd-Zn	fiddler crab	antagonistic	Weis (1980)
Cd-Zn	freshwater zooplankton	synergistic	Marshall <u>et al.</u> (1981)
Cd-Zn	invertebrate	antagonistic	Attar and Maly (1982)
Cd-Zn	freshwater algae	antagonistic	Singh and Yadava (1984)
Cd-Zn	aquatic plants	antagonistic	O'Keefe <u>et al.</u> (1984)
Cd-Ni	freshwater algae	synergistic	Prasad and Prasad (1982)
Cd-Pb	freshwater algae	synergistic	Prasad and Prasad (1982)
Cd-Fe	freshwater algae	antagonistic	Gipps and Coller (1982)
Cd-Cu	freshwater algae	antagonistic	Bartlett <u>et al.</u> (1974)
Cu-Fe	marine algae	antagonistic	Stauber and Florence (1985)
Cu-Mn	freshwater algae	synergistic	Christensen <u>et al.</u> (1979)
Cu-Ni	freshwater algae	synergistic	Stokes (1975B)
Cu-Ni	freshwater algae	synergistic	Hutchinson and Stokes (1975)

Continued on next page

Table 1. Continued

Cu-Ni	freshwater fish	non-interactive	Anderson and Weber (1976)
Cu-Pb	freshwater fish	antagonistic	Ozoh (1979)
Cu-Zn	freshwater fish	synergistic	Anderson and Weber (1977)
Cu-Zn	marine phytoplankton	synergistic antagonistic (depending on species)	Braek <u>et al.</u> (1976)
Fe-Zn	freshwater algae	non-interactive antagonistic synergistic (depending on algal sources)	Wang (1985)
20 Hg-Se	freshwater fish egg	synergistic	Huckabee and Griffith (1974)
Hg-Se	freshwater community	antagonistic	Rudd and Turner (1983B)
Ni-Hg	fungus	antagonistic	Babich and Stotzky (1982B)
Ni-Zn	fungus	antagonistic	" "
Ni-Pb	fungus	antagonistic	" "
Ni-Pb	freshwater algae	non-interactive	Prasad and Prasad (1982)
Cd.Cu.Zn	Chinook salmon freshwater fish	non-interactive or antagonistic	Finlayson and Verrue (1982)
Cd.Cu.Hg.Pb	aquatic plants	synergistic	Jana and Choudhuri (1984)
As,Cd,Cu,Hg,Pb,Zn	freshwater zooplankton	synergistic	Borgmann (1980)

Table 1. Concluded

<u>Mixture</u> Cd.Cu.Zn	freshwater fish	synergistic	Eaton (1973)
Cd.Cu.Pb.Zn	aquatic plants	synergistic	Nakada <u>et al.</u> (1979)
As.Cd.Cr.Cu Fe.Hg.Ni ,Pb Se ,Zn	freshwater algae	synergistic	Wong <u>et al.</u> (1978)

The metal interaction, furthermore, is complicated by the species of test organisms. Braek et al. (1976) studied the combined effects of Cu and Zn on three marine diatoms and one dinoflagellate. They found that the mode of interaction was unpredictable. For example, with A. carteri and T. pseudomana the metal interactions showed a clear case of synergism, while the metals exhibited antagonism in the case of the diatom P. tricornumtum. Wang (1985) found that Zn was much more toxic than Fe to the natural algal community. The binary solution of Zn and Fe acted differently according to the sources of algal population. Three types of interaction - synergistic, antagonistic, and non-interactive - were all observed.

There are other seemingly contradictory results in the literature. The mixture of Hg and Se showed a greater depression of hatchability of the eggs of carp, Cyprinus carpio, than of eggs exposed to these metals singly, suggesting synergistic toxic effects (Huckabee and Griffith, 1974). The bioaccumulation of Hg and Se, however, indicated that the presence of Se reduced the uptake of Hg. The reduction appeared to depend on the position of the organism in the food chain. For fish the reduction was proportional to the amount of Se accumulated (Rudd and Turner, 1983B).

Borgmann (1980) experimented with binary mixtures from the toxic metal ions As, Cd, Cu, Hg, Pb, and Zn using biomass production kinetics of freshwater copepods. The average observed growth time increased, suggesting significant synergism. Similarly, Jana and Choudhuri (1984) indicated potentiated toxicity in metal combinations among Cd, Cu, Hg, and Pb compared with individual metals toward mature leaves of Potamogeton. Vallisneria. and Hvdrilla. Finlayson and Verrue (1982), however, found that the binary solutions of Cd, Cu, and Zn were either additive or antagonistic in their lethality to Chinook salmon.

The complex responses of various test organisms to binary metal solutions, however, has not discouraged researchers from studying mixtures containing three or more metals. Eaton (1973) found that Cd, Cu, and Zn mixtures at sublethal levels acted synergistically toward the fathead minnow. Using a mixture containing Cd, Cu, Pb, and Zn, Nakada et al. (1979) showed synergistic interaction in the case of Elodea. Wong et al. (1978) indicated that 10 metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, and Zn) were not toxic to algae when presented individually at the levels established by the Great Lakes water quality criteria. The mixture of these metals at the same concentration, however, was strongly inhibitive.

Cherry et al. (1977) observed that the addition of Hg at 40 ng/L appeared to induce greater increase of heavy metal uptake by bacteria than did the addition of Cu at 2 mg/L in brackish and ash basin water. In fresh water, however, the reverse was found.

In summary, toxicity tests of complex effluents represent a great challenge to aquatic toxicologists, regulatory agencies, consulting engineers, and industrial concerns. The conflicting

results in the literature are apparently because of the use of different test organisms, test methods, toxic substances, and the like. More studies on toxicity interaction are required before a degree of predictability can be achieved.

G. Sediments

Natural waters contain various qualities and quantities of suspended sediments. These sediments have important effects on metal toxicity to and bioaccumulation by aquatic organisms. Pesch and Morgan (1978) exposed the adult male polychaete, Neanthes arenacedentate, to Cu with and without clean sand. They observed that Cu toxicity was lower with sand than without sand: the 28-d LC50's were 0.1 and 0.044 mg/L Cu, respectively. A possible explanation was the adsorption of Cu by sand, causing the loss of Cu concentration. Cd bound with bentonite was absorbed by the soft-shell clam, Mya arenaria, as fast as that in the chloride form, while Cd bound in humic acid, albumin, and estuarine sediment showed much slower uptake by the clams (Phelps, 1979). Pesch (1979) also experimented with adult male worms exposed to 0.1 ± 0.015 mg/L Cu in seawater in continuous-flow bioassay. The different sediment types significantly influenced the Cu-induced mortality. The time of 50 percent mortality was 7.8 days without sediment, 36.5 days with sand, 54.5 days with the sand-mud mixture, and 50.0 days with mud. Similarly, Graney et al. (1984) studied the accumulation of Cd in the Asian clam, Corbicula fluminea, using different substrates. The greatest tissue accumulation of Cd occurred in the no-substrate environment, while the lowest occurred in an environment containing a mixture of sand, clay, and organic matter. The environment with a mixture of sand, silt, and clay exhibited a median effect. Nay and Van Hassel (1983) investigated variations of Pb, Ni, Cd, and Zn concentrations among six species of fish from a highway-contaminated stream and reported that association with stream sediment appeared to influence whole-body metal accumulation, while the metal contents in muscle were not correlated.

There are many studies indicating that sediment substances effectively reduce metal toxicity. Brinkhurst et al. (1982) showed that the tolerance of oligochaetes to Cd and Hg was increased by the presence of sediment. Hongve et al. (1980) reported that the addition of 7.6 mg/L lake sediment at 0.64 nephelometric turbidity units consistently reduced toxic effects of Cd, Cu, Hg, Pb, and Zn to the natural phytoplankton photosynthesis. Hardy et al. (1981) observed that the clam Protothaca showed a linear uptake of Cd. When 3.6 g/L washed sediment was added, however, the uptake was reduced to 17 percent of that in the sediment-free sample. Phelps (1979) found that the uptake of the clam Mya arenaria exposed to 10 mg/L total Cd was reduced to 33 percent of the uptake of the control when 0.1 g/L of estuarine sediment was added. Sediments also effectively reduced Ni toxicity to bacteria, actinomycetes, and yeasts (Babich and Stotzky, 1983), Hg toxicity to and accumulation by fish and other aquatic organisms (Turner and Rudd, 1983), and pollutant effects on aquatic oligochaetes (Chapman et al., 1982B). Ahsanullah et al.

al. (1984) exposed the burrowing shrimp, Callinassa australiensis, to Cd-contaminated water and sediment. They found that the shrimp accumulated Cd from water at a rate commensurate with the Cd concentration in the water and the duration of exposure. Sediments effectively removed Cd from the aqueous phase and thus kept Cd from being bioavailable to the shrimp. In contrast, Hartman and Martin (1984) showed that suspended sediments increased the short-term toxicity of glyphosate to daphnids at all concentration levels.

Different types of lake sediments can also affect metal toxicity and availability. Schierup and Larsen (1981) reported that eutrophic, sewage-polluted lake sediments contained up to 80 times the heavy metal concentrations of oligotrophic, non-polluted lake sediments. The metal availability, however, was greater in the latter than the former.

Aquatic macrophytes exhibited further complicated patterns. Mayes et al. (1977) determined the uptake of Cd and Pb by Elodea canadensis. They found that plants grown in the same water but in sediments from different sources had significantly different concentrations of Cd and Pb. Additionally Elodea samples rooted in the sediment from the same sources but grown in different waters with different levels of metals also accumulated significantly different amounts of Pb and Cd. The results indicated that both water and sediment were important sources of metal intake for rooted aquatic vegetation. Welsh and Denny (1980) suggested two pathways for the transfer of metals from sediments to the shoots. Pb accumulation in the shoots was considered the result of adsorption from the water. Cu accumulation was suggested to be due mainly to absorption by the roots and translocation within the plant to the shoots.

H. In situ

As indicated previously, many studies showed that metal toxicity to aquatic organisms is greatly affected by environmental factors. The results of in situ studies, in contrast to controlled laboratory studies, consequently become even more complex. The seasonal cycle alone may cause variation in the results. Brunge et al. (1976) reported that the nominal total Cu 96-h LC50 values to fathead minnows ranged from 1.6 to 21 mg/L, apparently influenced by the source of dilution water: a natural stream downstream from a sewage treatment plant. Storch (1977) used a natural community of Lake Erie phytoplankton for determining the effects of Fe(III). The results showed that Fe(III) might either stimulate or inhibit algal photosynthesis depending on the concentration and the time of year. The stimulation, 10 to 18 percent, occurred in late summer when 0.05 mg/L was added. Erickson (1972) found that the Cu inhibition on the growth of Thalassiosira pseudonana in unenriched seawater samples varied with season and location of collection. Fe toxicity to fresh and marine water phytoplankton was variable over time (Wang, 1983; Wilson and Freeberg, 1980).

The degree of dissolved oxygen saturation varies according to

season. Experimental results have shown that the toxicity of Cd to aquatic insects increased as dissolved oxygen concentration increased (Clubb et al., 1975). This finding can be explained by the increase in the amount of Cd found in the insects as dissolved oxygen increased. They also reported that oxygen consumption increased as dissolved oxygen increased. Similarly, Karbe et al. (1975) reported that a correlation existed between heavy metal accumulation in mussels and oxygen saturation.

Taylor and Crowder (1983) analyzed soil-sediment samples and plant tissues. They found no correlation between metal concentration in tissues or leaves and Zn, Mg, or Ca in soil-sediment, but a high degree of correlation between Cu, Ni, Fe, and Mn concentrations in the root zone and the reproductive tissues and soil-sediment. Accumulation of Fe and Mn in all plant tissues was correlated with concentrations in the soil-sediment material.

The combination of Cu concentration and sediment particle size had an effect on benthic macroinvertebrates, although the sediment particle size itself appeared to have no effect on microinvertebrate distribution (Kraft and Sypniewski, 1981). Foster (1982) studied algal flora at several stations on streams drained through mining regions. Both the total algal abundance and the number of species were depressed at sites of high metal concentrations. It was found that the degree of metal pollution, rather than the polluting metal concentration per se, determined the species present.

Spehar and Carlson (1984) compared Cd toxicity in in-site and laboratory water. Their results of acute tests with several species showed that Cd was less toxic in site water than in laboratory water. Furthermore, acute tests conducted monthly in site water showed that Cd toxicity varied by more than a factor of 3 over the year. Wang (1986C) examined the effect of river water on the toxicity of Ba, Cd, and Cr(VI) to common duckweed, Lemna minor. The results indicated that Cd toxicity was slightly reduced, Ba toxicity was almost nil, and Cr(VI) toxicity changed very little. The removal of Ba toxicity was due to barium sulfate precipitation.

An interesting although little studied effect is that of light illumination on metal toxicity (Dongmann and Nuernberg, 1982). They found that the toxicity thresholds of Cd and Ni on the marine diatom Thalassiosira rotula decreased with increasing illumination. The results can be explained by the greater algal metabolic rates due to the availability of light energy.

Engler et al. (1982) conducted solid-phase plant bioassays testing the availability of heavy metals. They reported influencing factors such as redox potential, organic matter content, total sulfur content, and pH. They observed that the availability of Cd and Zn for plant uptake increased in upland conditions, while As increased under flooded conditions.

Summary and Conclusions

In summary, the aquatic environmental toxicology of heavy metals is very complex. Many discoveries have been reported only recently, and undoubtedly many more will be reported in the future. On the basis of this literature presentation, there appear to be four basic laws of environmental toxicology:

First Law (Quantity) -

Any Chemical Can Be Toxic;
The Question is Quantity.

Second Law (Organism) -

Toxicity Is Not a Constant;
It Depends on the Individual Organism.

Third Law (Environmental Conditions) -

Toxicity Is Not a Constant;
It Can Be Potentiated or Attenuated
under Various Environmental Conditions.

Fourth Law (Tolerance) -

Toxicity Is Not a Constant;
Organisms May Possess Repair Mechanisms and
Develop a Tolerance Toward It.

4. Methods

A. Water samples

During the 19-month study period (September 1984 to April 1986), many ambient water samples were taken. Fig. 1 depicts the locations of the 18 sample stations, of which 10 were in the state of Illinois and 8 were in the neighboring states of Indiana, Iowa, Missouri, and Wisconsin. The selection of the ten in-state stations was based on 1) geographical distribution and 2) water quality considerations. According to a 10-year, monthly monitoring program, these waters encompassed a wide spectrum of water quality, from low to high alkalinity, softness to hardness, and low to high amounts of total dissolved solids, Table 2 (Harmeson and Larson, 1969).

All the in-state stations were sampled during the first year of the study. Two in-state stations (at the Illinois River and Lake Michigan) and all eight out-of-state stations were sampled for the remaining months of the study. The out-of-state stations were at Lake Geneva, Wisconsin, and seven major rivers, all in areas bordering the state of Illinois. These waters are considered typical midwestern streams draining through intensive farming areas.

The water samples were collected by using the surface grab method. All stations were visited at least twice, and one station (Illinois River at Peoria) was visited 13 times. Water quality parameters were analyzed according to Standard Methods (1985).

B. Duckweed culture

The source of duckweed (Lemna minor) stock culture was a ground-water recharge pit located inside the property of the Illinois State Water Survey. This water body is in an enclosed area and no pollutants are known to enter it. The duckweed stock culture is the same as that used in previous studies (Wang, 1986B, 1986C). Furthermore, this stock culture has been offered as a reference species for duckweed toxicity tests as a part of the American Society for Testing and Materials standard development protocol (Wang, 1986D).

The culture was maintained in the laboratory in a polypropylene tub, 20-L capacity. Approximately 15 L was maintained by adding cold tap water weekly to compensate for evaporation loss. Plant nutrients were added in a quantity twice the concentration (2X) recommended by Standard Methods for algal growth medium (Standard Methods, 1985). The plants were illuminated with continuous cool-white fluorescent light at 3300 lux. Room temperature was maintained at 25-27 C.

Under these favorable conditions, duckweed grew vigorously and provided sufficient test specimens year-round. The only time a difficulty was encountered was when insect infestation reached

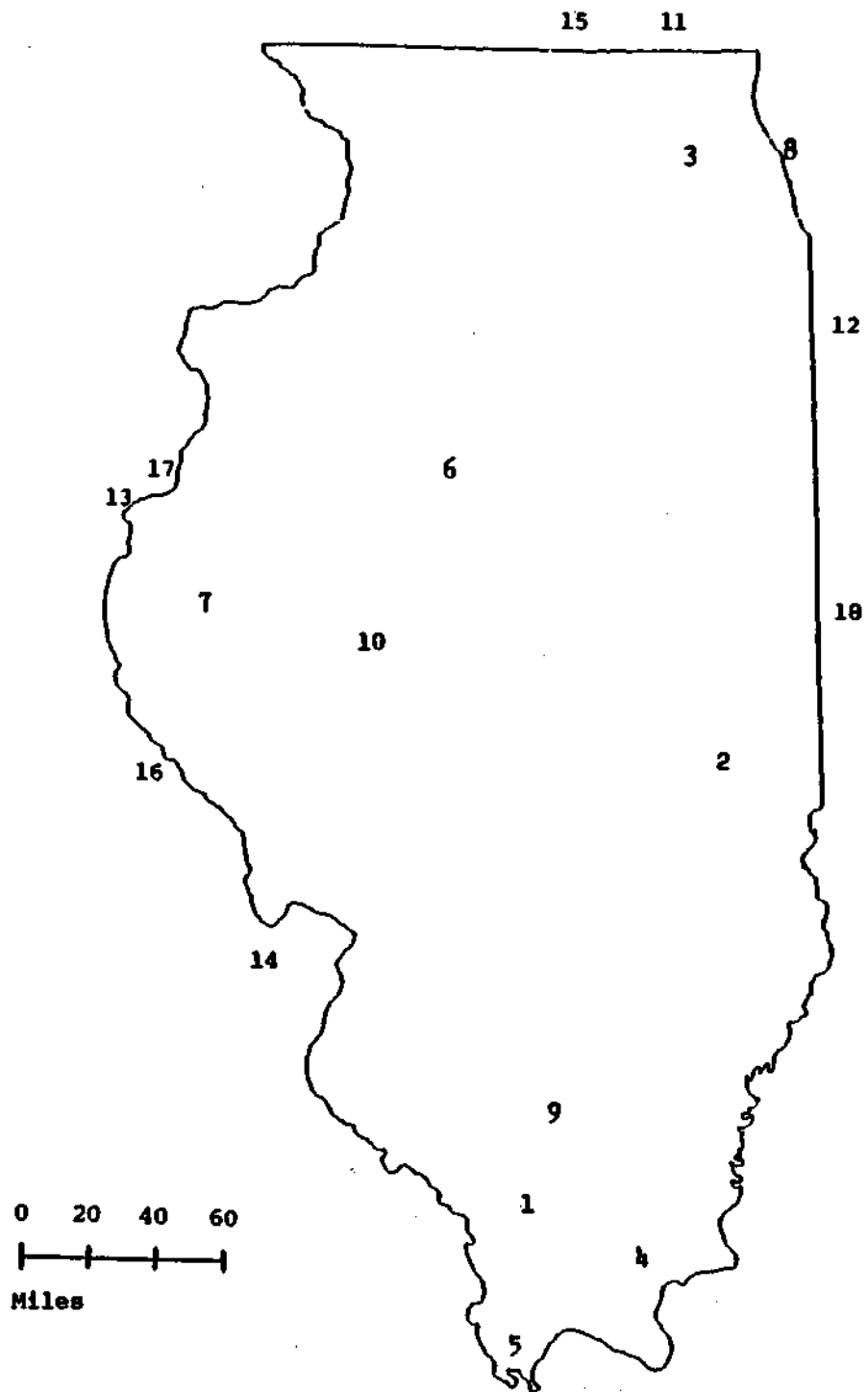


Fig. 1. Sample stations.

Table 2. Mineral Quality Characteristics of Selected Stations
in Illinois

Median values in mg/L from 1956-1966 (from Harmeson
and Larson, 1969)

Station no.	Station	Alk	Hardness	TDS
1	Beaucoup Creek @ Rte. 127	80	585	995
2	Embarras River @ Camargo	205	375	350
3	Fox River @ Algonquin	252	318	400
4	Hayes Creek @ Glendale	45	60	110
5	Horseshoe Lake @ Miller City	NA	NA	NA
6	Illinois River @ Peoria	160	260	400
7	LaMoine River @ Colmar	205	250	320
8	Lake Michigan @ Glencoe	118	130	165
9	Rend Lake @ Ina	60	135	260
10	Sangamon River @ Oakford	240	300	380

NA - Not available

TDS - Total dissolved solids

such proportions that the duckweed stock was damaged to a noticeable degree. Nevertheless, the duckweed grew fast and was never in short supply. The insect problem was solved completely with only one spray of a broad-spectrum commercially available insecticide. The duckweed was not affected by the spray.

Many snails were present in the stock culture. They consumed a negligible amount of duckweed plants considering the duckweed biomass and the rate of multiplication. No attempt was made to eliminate the snail population. There were algae present in the tub. Since duckweed formed a dense mat at the water surface, algae had no chance to reach bloom proportions.

C. Duckweed test specimens

Twenty-four hours before the toxicity experiments were initiated, duckweed specimens were selected for tests. A scoopful of duckweed from the stock was dispersed in a small tub containing cold tap water. The selection criteria for test specimens were that the colonies be healthy-looking and uniform, with two fronds of approximately equal size per colony. Plants which were over-sized, under-sized, irregularly shaped, discolored, insect bitten, and the like were not used. Plastic forks, instead of forceps, were used in order to prevent injuries to the duckweed plants. The selected specimens were kept away from light.

The advisability of using two-frond colonies instead of three- and four-frond colonies has been questioned because some duckweed species (particularly Lemna gibba) have very few two-frond colonies. L. minor was preferred over other species for two reasons. First, L. minor is a native North American species that is widely distributed, while L. gibba is much less common. The selection of L. minor as a test species for environmental protection thus makes more sense than the selection of L. gibba. Second, the selection of two-frond colonies permits use of a narrow age group, whereas three- and four-frond colonies consist of two or more generations.

Another interesting point has been raised concerning using an axenic culture (a culture treated to remove other life forms) for duckweed toxicity tests. Axenic cultures of duckweed have been reported in the literature. However, this was not attempted in this study. The reason is that the methods used to achieve an axenic culture (membrane filtration, chemical treatment, and autoclaving) invariably alter the water quality of ambient water samples. For example, autoclaving would result in calcium carbonate precipitation, alkalinity and hardness reduction, and pH elevation. Membrane filtration would remove particulate matter, which is a part of the ambient water quality, possibly affecting heavy metal toxicity. These treatments would defeat the purpose of this study.

It may be asked whether there is any interference to the experiments from other life forms, especially algae. The duckweed

plants were usually attached with algae; also the lake and stream waters contained varying algal populations. Duckweed plants, being floating organisms, are not affected to a large extent by algae. This is especially true for a 96-h exposure period. During this short time, algae were relatively few. Any tests of longer duration might result in algal blooms, making interpretation of duckweed toxicity test results difficult.

D. Test procedure

The toxicity test experiments were performed by using 236-mL fruit jars. For toxicity studies, a series of metal solutions were prepared using a 45 percent dilution scale. From a 1-L solution (either deionized water or ambient water sample) containing the highest metal concentration and duckweed growth medium (double strength of algal growth medium. Standard Methods. 1985), a 450-mL portion was withdrawn which served as the highest metal concentration solution. The remaining solution was replenished with plant nutrients to the original concentration, diluted with the same water, and mixed vigorously. A second 450-mL portion was withdrawn, which served as the second-highest solution (55 percent metal concentration of the first solution). The procedure was repeated until there were seven test solutions, in concentration series of 100, 55, 30, . . . 2.8 proportion. Each 450-mL portion was divided into three equal portions, which were placed in three fruit jars. These were considered triplicates.

Two kinds of controls were prepared. For the water controls, duckweed growth was tested in a solution containing plant nutrients in deionized water (i.e., duckweed growth medium). It is also of interest to determine duckweed growth in the ambient water samples containing plant nutrients. This is called sample control. Water control and sample control tests were conducted throughout this study along with the toxicity tests.

Immediately before the selected duckweed specimens were tested, they were examined again for any irregularity or multiplication. Only the plants which met the original criteria were used. The duckweed plants were added at the rate of 30 fronds, or 15 colonies, to each jar. A constant illumination was provided by cool-white fluorescent bulbs at an intensity of 6456 lux. Each jar was covered with a watch glass to prevent excessive evaporation loss and to keep foreign objects, debris, insects, and the like from falling into the jars. Temperature was maintained at 25-29 C. On three occasions temperature exceeded this range and the experiments were repeated. The incubation time was 96 h.

At the end of incubation, the number of fronds in each jar was counted with the aid of a lighted magnifying glass. Each recognizable, protruding bud was counted. The net increase in frond number was indicative of duckweed growth and the response to toxicity.

E. Test compounds

Three reagent grade chemical compounds were tested, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, K_2CrO_4 , and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. Three stock solutions were prepared, containing 40,000 mg/L Ba, 10,000 mg/L Cr, and 2,000 mg/L Ni. The maximum metal concentrations in all tests using ambient water samples were 400, 100, and 20 mg/L for Ba, Cr, and Ni, respectively. In other words, a 10-mL metal stock solution was added to 1 L water to make the highest metal concentration. Toxicity tests using duckweed growth medium were also conducted. In this case, the maximum metal concentration was decreased to 200 mg/L Ba, 100 mg/L Cr, and 5 mg/L Ni in order to obtain the proper dose-response range.

The chemical assay of the stock solutions showed that the determined concentrations of Ba, Cr, and Ni were 95, 100, and 95 percent of calculated concentrations, respectively.

F. Growth medium

At least six duckweed growth media are discussed in the literature. It would be a major task to compare them. For this study, it was decided to adopt the algal growth medium (Standard Methods, 1985) for the duckweed culture and test solutions. The reasons are that this medium is widely accepted, even though there are reservations about it primarily because of the micronutrients tin and selenium, and that algae and duckweed are both plant species. The advantages are obvious in terms of comparison, development, and standardization of these tests.

The duckweed growth medium used in this study, as mentioned earlier, was double the strength (2X) of the algal growth medium. The reason is given later (see "Results" section, "Nutrients" subsection). The algal medium outlined in Standard Methods is composed of 6 stock solutions, each of which contains one chemical compound, and 1 solution containing a mixture of micronutrients. Because some compounds are compatible and together are unlikely to produce chemical changes, it was decided to combine a few of them in order to reduce the number of nutrient stock solutions. The solutions are shown in Table 3. It can be seen that in this manner, only 3 solutions (A, B, and C) are used regularly, instead of the 7 separate stock solutions outlined in Standard Methods.

G. Statistical analyses

IC50 values (the concentration which causes 50 percent inhibition in comparison with the control sample) and 95 percent confidence limits were calculated by the binomial method (Stephen, 1977), using a personal computer. This method produced more realistic values than either the moving average or probit method. Linear regression analysis was also used. The t-test was performed to determine any significant difference between mean values of test groups.

Table 3. Nutrient Stock Solutions

Solution A		
NaNO ₃		25.5 g/L
NaHCO ₃		15
K ₂ HPO ₄		1.04

Solution B		
MgCl ₂		5.7
CaCl ₂ ·2H ₂ O		4.41
	2 2	

Solution C		
MgSO ₄	7H ₂ O	14.7
H ₃ BO ₃		0.186
MnSO ₄	H ₂ O	0.264

1 mL of Solution 0, as follows:

ZnCl ₂		3.27 mg/L
CoCl ₂		0.78
CuCl ₂		0.009
NaMoO ₄	2H ₂ O	7.26
FeCl ₂		96

Two mL each of solutions A, B, and C are used to make 1 L solution of duckweed growth medium

The methodology described in this section was largely used as the basis of the "Proposed New Standard Guide for Conducting Static Toxicity Tests with Duckweed," for the American Society for Testing and Materials, currently in draft #6 (Wang, 1986D). Interested readers can contact the author for a copy.

5. Results

A. Water quality

Water quality characteristics of the test samples are given in Table 4. The station numbers in this table correspond to the locations in Fig. 1. A comparison of Tables 2 and 4 indicates that the water quality of the in-state stations during this study was similar to that during 1956-1966 (also see Fig. 2).

The pH values of samples from all 18 stations were in the range of 7-8 (Table 4), except for a sample from Hayes Creek, pH 6.32. This station is located inside Shawnee National Park. Other water quality characteristics of this station are extremely low alkalinity (10-15 mg/L) and hardness (37-38 mg/L). The station with the hardest water is Beaucoup Creek. This station is possibly under the influence of oil extraction activities. It has a very high level of total dissolved solids, 995 mg/L (Table 2).

The samples from two glacial lakes, Lake Michigan and Lake Geneva, were moderately alkaline and moderately hard. Surprisingly, Rend Lake, an artificial impoundment, was less alkaline and less hard than Lake Michigan.

B. Nutrients

The methods used in this study were similar to those in previous studies (Wang, 1986B, 1986C). Early in this study, it was decided to test the effect of nutrient content on duckweed growth. Two samples were used, from the Fox River and Lake Michigan. Of these two samples, the Fox River contained considerably more plant nutrients than Lake Michigan. For example, the total phosphorus and nitrate-nitrogen were 0.12 and 1.58 mg/L for the Fox River sample, and 0.01 and 0.13 mg/L for the Lake Michigan sample. The experiment was conducted by adding different levels of plant nutrients -- 1X, 2X, and 3X -- according to the level recommended in Standard Methods (1985). Control samples, with no nutrients added, were also used.

The results in Table 5 clearly show that in the case of the Lake Michigan sample, duckweed growth was significantly greater ($P < 0.05$) with regular (1X) strength plant nutrients than without nutrients (control sample). In the case of the Fox River sample, duckweed growth was significantly higher than in the control sample only at double strength (2X) plant nutrients. At triple strength (3X), there was no significantly greater growth of duckweed. Consequently it was decided to use double strength of algal growth medium in all tests. This was called the duckweed growth medium.

Table 4. Water Quality of Test Samples

Station No.	Station	No. of samples	pH	Range		Turb+
				Alk*	Hard*	
1	Beaucoup Creek @ Rte. 127	2	7.64-7.77	89-116	274-400	50-114
2	Embarras River @ Camargo	2	8.00-8.10	136-214	245-320	6-70
3	Fox River @ Algonquin	2	8.13-8.29	214-227	290-318	17-26
4	Hayes Creek @ Glendale	2	6.32-7.25	10-15	37-38	3-11
5	Horseshoe Lake @ Miller City	2	6.92-7.63	46-73	54-78	11-21
6	Illinois River @ Peoria	13	7.85-8.16	158-236	232-364	33-105
7	LaMoine River @ Col mar	2	7.80-8.06	168-177	252-257	14-16
8	Lake Michigan @ Glencoe	5	7.95-8.12	108-115	140-146	0-137
9	Rend Lake @ Ina	3	7.32-7.78	40-53	84-87	29-54
10	Sangamon River @ Oakford	2	7.95-8.08	186-210	185-298	54
11	Lake Geneva @ Williams Bay. Wl	3	8.08-8.26	186-194	237-242	3-4
12	Kankakee River @ Schneider, IN	3	7.85-8.15	182-207	308-311	13-16
13	Mississippi River @ Fort Madison, IA	3	7.57-8.09	50-160	82-256	70-109
14	Missouri River @ St. Charles, MO	3	7.95-8.12	164-188	225-265	38-258
15	Rock River @ Janesville, Wl	3	8.05-8.32	114-236	160-310	8-28
16	Salt River @ Rte. 79, MO	3	7.30-7.96	50-95	80-129	16-246
17	Skunk River @ Rte. 61, IA	3	7.48-8.27	90-227	126-314	57-414
18	Wabash River @ Perryville, IN	3	7.82-8.34	156-224	244-325	26-156
		Total	59 samples			

* in mg/L as CaCO₃

+ in NTU

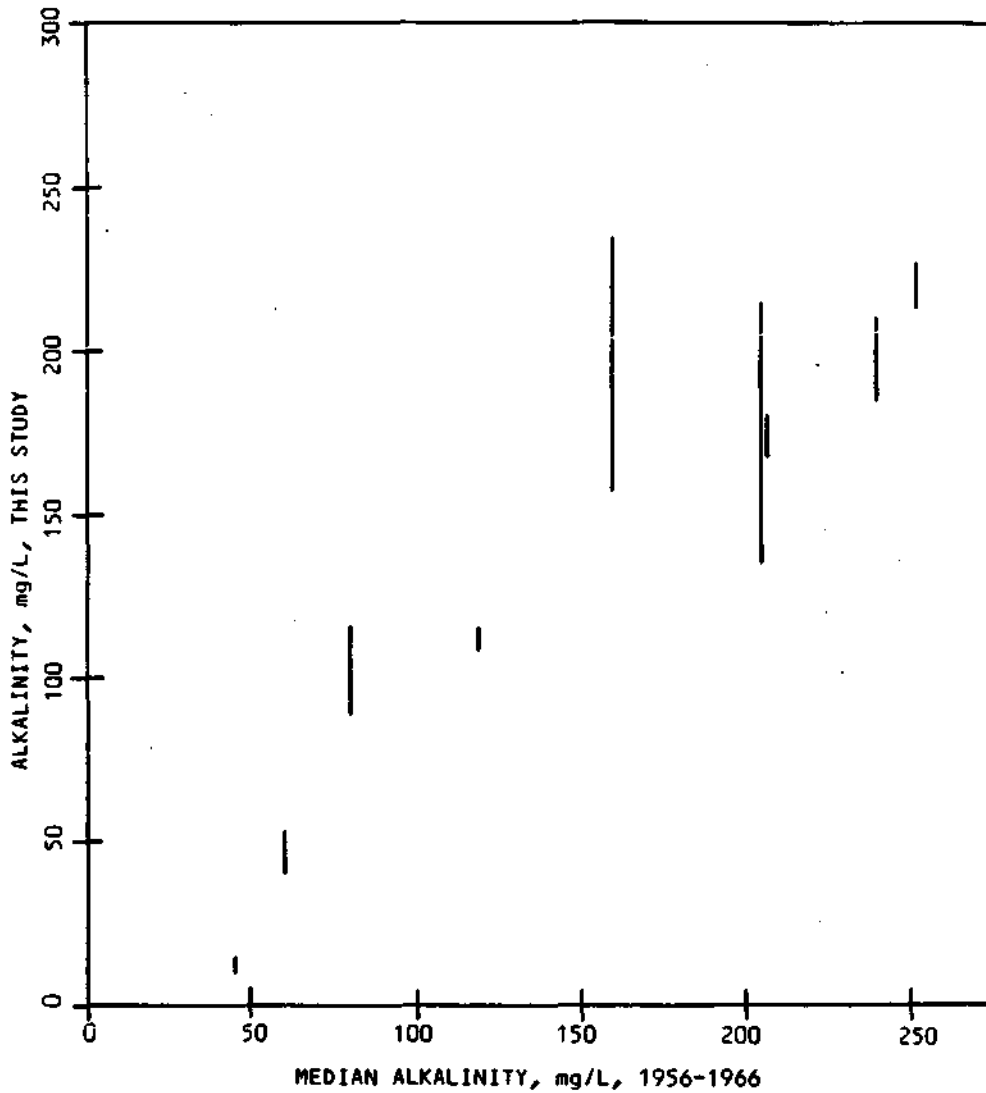


Fig. 2. Comparison of alkalinity of stations in 1956-1966 (Harmeson and Larson, 1969) and this study, in mg/L as CaCO₃-

Table 5. Duckweed Growth (Number of Fronds) in Water Samples, 11/7/84, Enriched with Different Levels of Plant Nutrients

	Fox River			sample	Lake Michigan			sample
			Mean	t-test			Mean	t-test
Control	51, 50, 55		52		38, 36, 38		37	
1X	56, 55, 60		57	2.32	64, 55, 67		62	6.73*
2X	58, 66, 59		61	3.06*	58, 70, 61		63	7.00*
3X	63, 59, 67		63	3.97*	81, 62, 85		76	5.43*

* 95% significance level (degrees of freedom, 4)

C. Controls

In view of the long study period, a fundamental question can be asked about the health and/or the reproducibility of the test specimens over time.

The health of the test specimens can be indicated by using results for the control samples. If the controls show extreme variation, the choice of the test specimens will be questionable. In this study, both water controls and sample controls were prepared in every test. Equal amounts of nutrients were added to both. Each experiment was conducted with triplicates for water control and 12 replicates for sample control. Fig. 3 pools all the results.

The mean number of duckweed fronds \pm standard deviations for water controls and sample controls were 65 ± 12 , and 73 ± 10 . The t-test showed that the difference in mean values between these two controls was highly significant ($CP < 0.01$). Obviously duckweed grew better in the sample controls than in the water controls. This is interesting in light of the fact that a total of 59 water samples were collected, encompassing a wide variation in water quality. Although equal amounts of plant nutrients were added to both types of controls, it is likely that the sample controls contained additional plant nutrients and/or unknown stimulants which promoted the growth of duckweed plants.

The results of this 19-month study provide guidelines for deciding whether results of future duckweed experiments should be accepted or rejected. As mentioned earlier, the number of fronds in the water and sample controls were 65 ± 12 and 73 ± 10 , respectively. The numbers of respective tests were 61 and 58. Taking 2 as the approximate t-value (degrees of freedom 60), the 95 percent confidence limits are respectively 41-89 and 53-93. In other words, any control test that gives values that are outliers of these ranges will be a cause of concern. The health of test specimens, environmental conditions, contamination, etc., all require close examination. During this study, there was only one instance where duckweed growth in water controls exceeded the upper limit (90 in comparison with 89). There were three instances where duckweed growth in sample controls exceeded the upper limit (95, 96, and 97 in comparison with 93). It is likely that additional plant nutrients were present in the water samples in these cases. There was no instance where the growth fell below the lower limit.

Because of the discrepancy between duckweed growth in water controls and sample controls, metal toxicity data were calculated only on the basis of sample control values.

D. Repeatability

The Skunk River sample collected on March 3, 1986, was tested twice for barium and chromium toxicity. The results in terms of percent inhibition of duckweed growth are shown in Figs. 4 and 5. The mean numbers of duckweed fronds \pm standard deviations for the

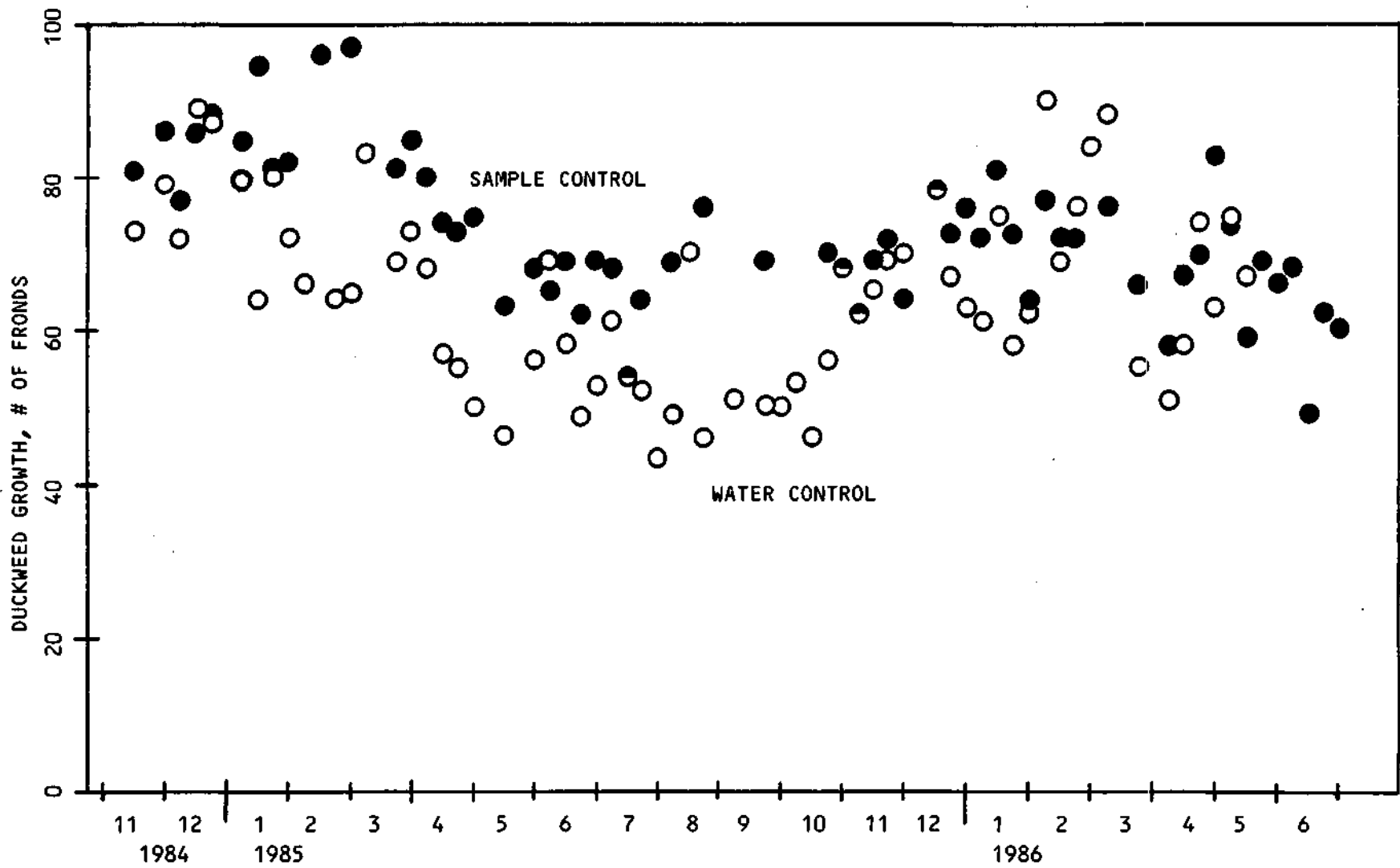


Fig. 3. Negative controls.

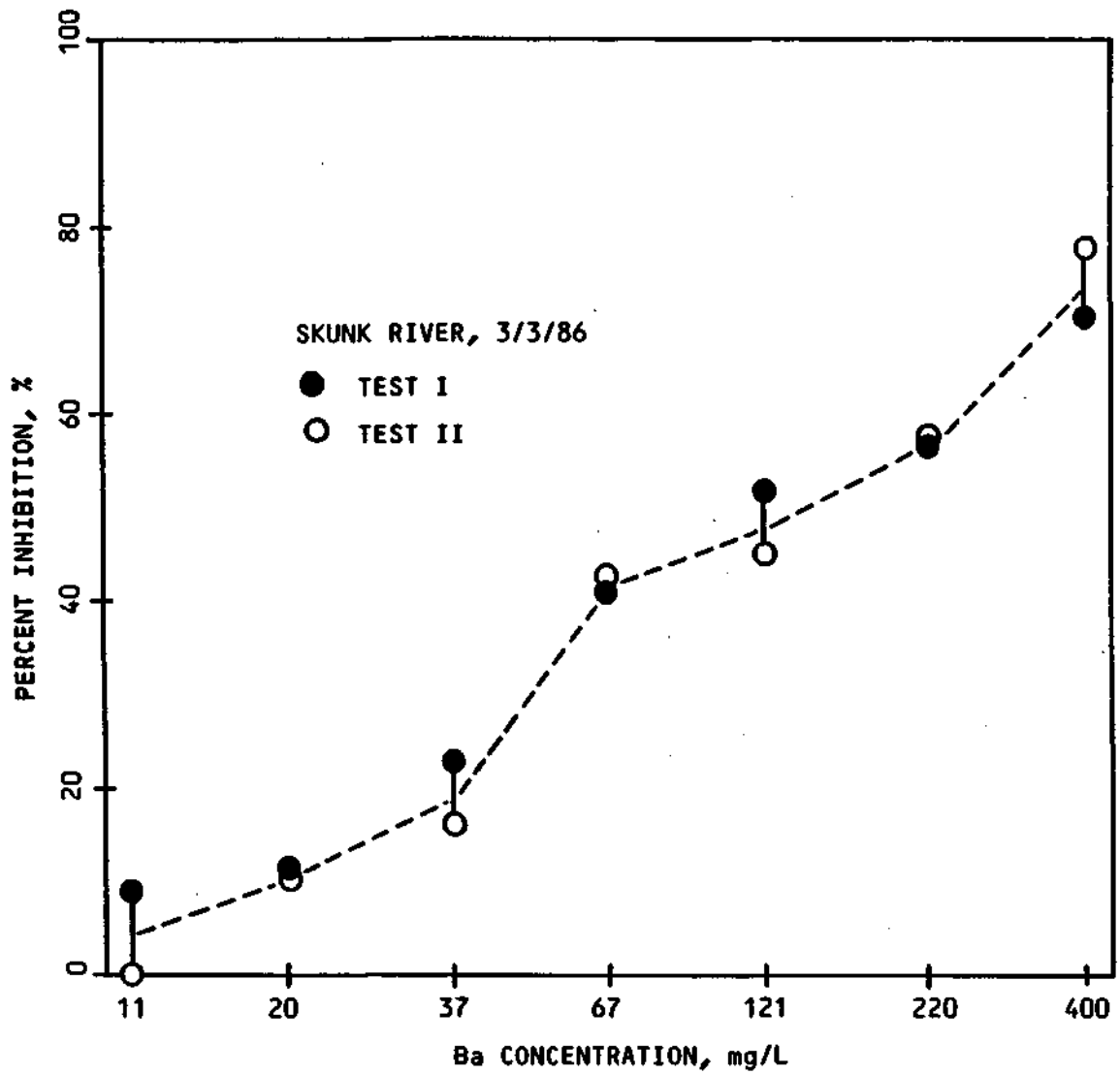


Fig. 4. Duplicate tests of enriched Skunk River water sample, 3/3/86, Ba toxicity.

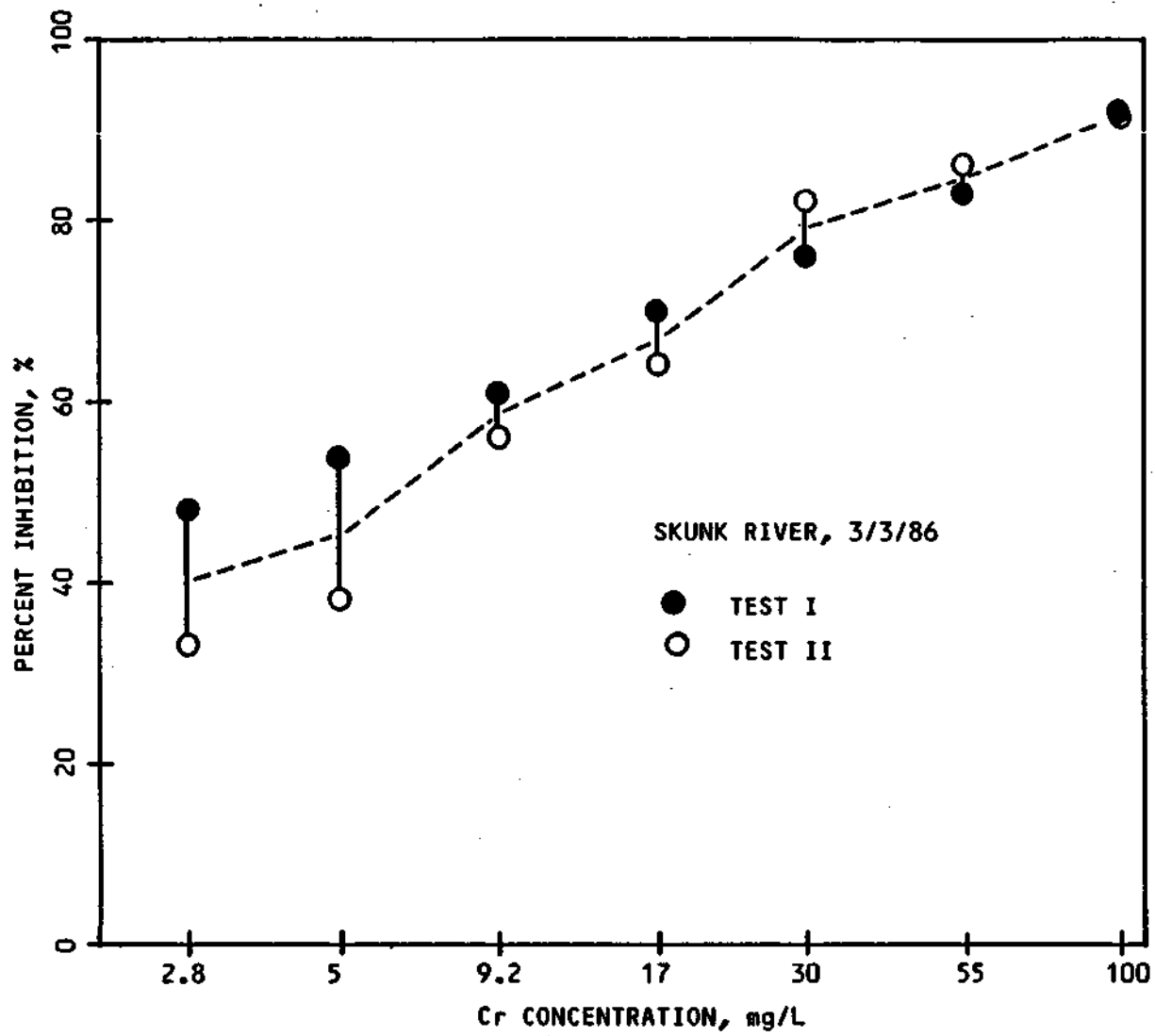


Fig. 5. Duplicate tests of enriched Skunk River water sample, 3/3/86, Cr toxicity.

water controls in Tests I and II were 63 ± 6 and 75 ± 3 (replication 3), respectively, while for the sample controls the mean numbers of duckweed fronds were 83 ± 7 and 74 ± 4 , respectively. The results in Figs. 4 and 5 indicated that there was some variation between the two tests; however, the variation was relatively minor.

E. Ba toxicity

i. In duckweed growth medium

Between January 1985 and January 1986, a total of seven tests were conducted to determine Ba toxicity to duckweed in the duckweed growth medium. The results of these tests are summarized in Fig. 6.

The maximum Ba concentration was 200 mg/L. With seven test solutions in a 45 percent dilution scale, the semi-log dose-response relationship is a sigmoid curve. The point of tangent is situated at approximately 55 percent inhibition of duckweed growth. The IC50 value was 25 mg/L with a 95 percent confidence limit of 18-33 mg/L.

According to the literature (Fig. 7), the certainty of dose-effect relationships is greater at the extreme high and extreme low concentration ranges, and lower in the middle range (Whyte and Burton, 1980). The empirical results in Fig. 6 suggest a higher degree of certainty, as suggested by the lower coefficient of variation, at the extreme high concentration range. For example, at 200 mg/L Ba concentration, the coefficient of variation was 3 percent. This is much smaller than the values of 48 and 21 percent at 0 and 33 mg/L concentrations, respectively.

ii. In water samples (nutrient-enriched)

Ba toxicity results in enriched water samples are expressed as IC50 values, in mg/L, in Table 6. The mean values range from 102 and 107 mg/L for Hayes Creek and Horseshoe Lake to values out of range in the test for Beaucoup Creek. In fact, the Beaucoup Creek samples taken on December 4, 1984 and March 7, 1985 exhibited 0 and 6 percent duckweed growth inhibition when 400 mg/L Ba was added, attesting to the strong detoxification capacity at this station.

The most extensive testing was conducted using the Illinois River samples. A total of 13 samples were tested and the IC50 results can be seen in Table 6. In six, or nearly half, of these samples 400 mg/L Ba did not cause over 50 percent inhibition, so that IC50's are given as >400 mg/L. The percentages of inhibition in these six samples were only 46, 37, 29, 25, 45, and 46.

The results in Table 6 indicate that, in general, lake water samples show a more consistent response to Ba toxicity than do river water samples. The IC50's in water samples from Horseshoe'

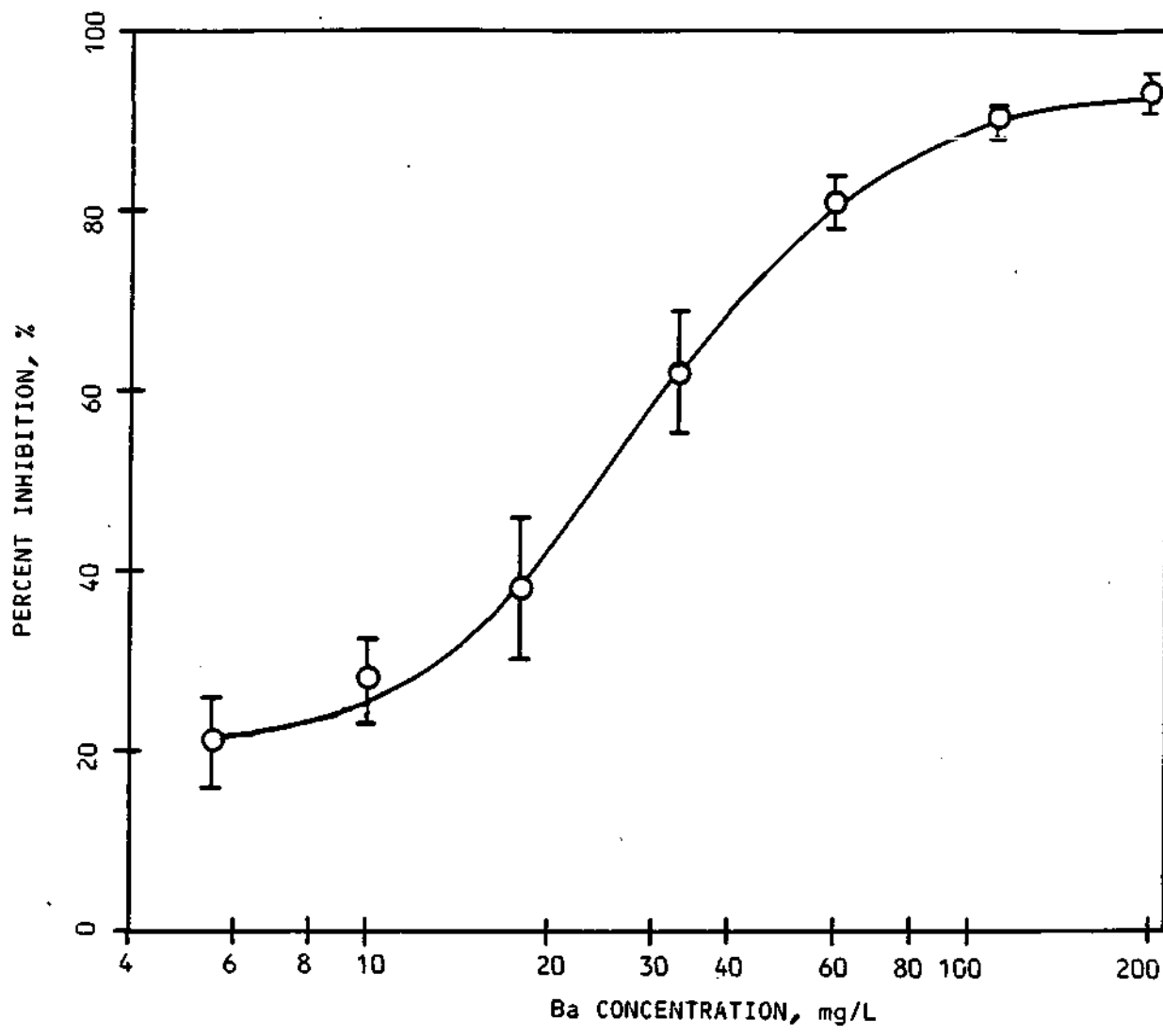


Fig. 6. Ba toxicity in duckweed growth medium, 7 tests.

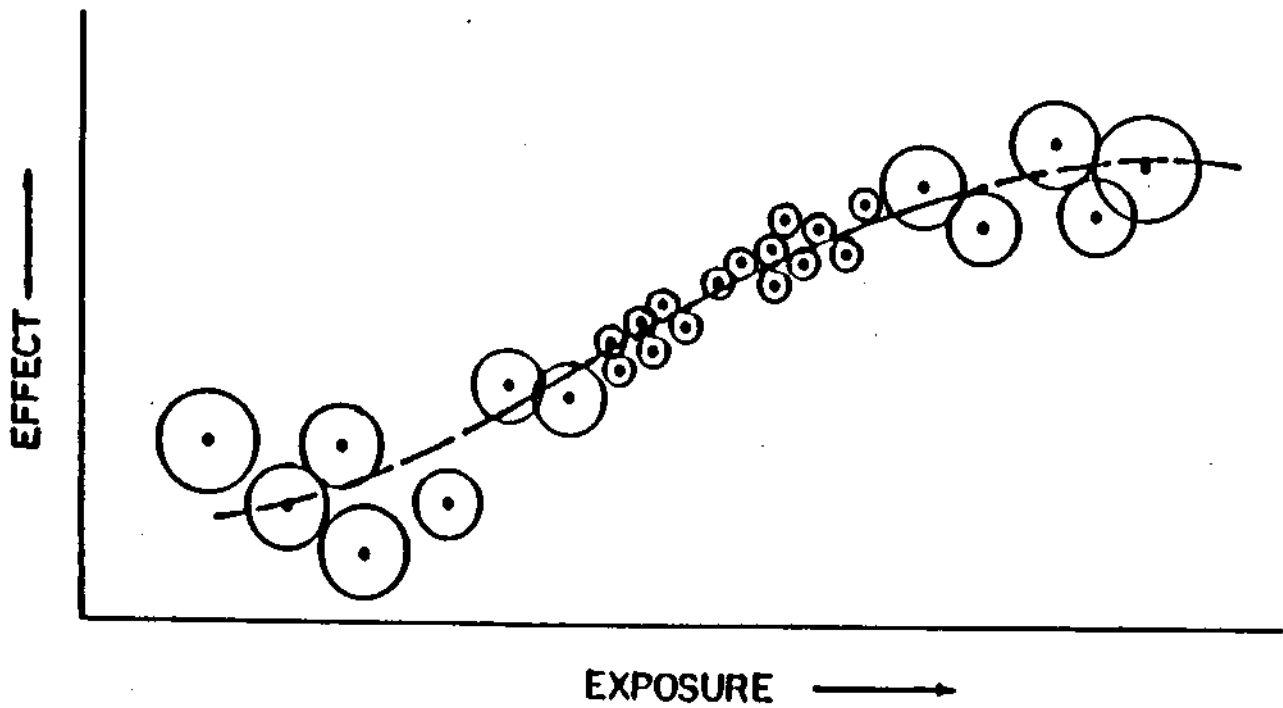


Fig. 7. Generalized exposure-effect curve, showing diameter of circles as degree of certainty about data points. From Whyte and Burton, 1980, p. 64. Reproduced by permission of SCOPE, #15, Paris, France.

Table 6. Ba Toxicity in Enriched Water Samples, in IC50's

	IC50, mg/L					Mean
1. Beaucoup Creek	>400	>400				
2. Embarras River	300	328				314
3. Fox R.	155	337				246
4. Hayes Cr.	73	130				102
5. Horseshoe Lake	155	58				107
6. Illinois R.	280	>400	MOO	>400	>400	
	>400	333	265	400	300	
	MOO	232	326			
7. LaMoine R.	310	MOO				
8. L. Michigan	121	135	104	118		120
9. Rend L.	189	194	143			175
10. Sangamon R.	330	MOO				
11. L. Geneva	148	149	113			137
12. Kankakee R.	328	365	372			355
13. Mississippi R.	151	52	173			125
14. Missouri R.	386	331	MOO			
15. Rock R.	156	113				135
16. Salt R.	61	104	143			103
17. Skunk R.	245	152				
18. Wabash R.	345	220	230			265

Lake, Lake Michigan, Rend Lake, and Lake Geneva all fell in a relatively narrow range with mean IC50's of 107, 120, 175, and 137 mg/L, respectively. In the river water samples, the mean IC50's varied from lows of 102 and 103 mg/L for Hayes Creek and Salt River to well over 400 mg/L for Beaucoup Creek.

The dose-response relationships for typical samples are depicted in Fig. 8. The relationship for the duckweed growth medium is also included for comparison. Obviously Ba toxicity in various natural waters fell between the extremes of that in Hayes Creek or Horseshoe Lake and that in Beaucoup Creek.

The water samples investigated in this study can be classified into three groups on the basis of Ba toxicity results. One possibility is to divide Ba IC50's into <150, 151-300, and >300 mg/L (Table 7). According to this scheme, seven sample stations were in the highly sensitive to Ba toxicity category, seven were in the less sensitive category, and the remaining four were in the moderately sensitive category. It is interesting to note that the stations at the Mississippi River at Fort Madison, Iowa, and Salt River at Route 79, Missouri, both had water samples that were highly sensitive to Ba toxicity, while the samples from the Missouri River at St. Charles, Missouri, were less sensitive to Ba toxicity.

A previous study (Wang, 1986C) showed that Ba toxicity in Illinois River water was controlled by the sulfate content of the water sample. Other water quality parameters such as suspended solids had little effect. Consequently, an attempt was made to correlate sulfate concentration and Ba IC50 values for all samples. All results, excluding those with IC50's greater than 400 mg/L, are pooled in Fig. 9. A linear relationship with a relatively high coefficient of determination was obtained, $R = 0.68$. This indicates that Ba toxicity in a water sample is controlled by sulfate concentration to a large extent. Factors other than sulfate content affected only 32 percent variation of Ba toxicity.

F. Cr toxicity

i. In duckweed growth medium

Cr toxicity in the duckweed growth medium was tested ten times. The results are plotted in Fig. 10. The semi-log plot of the dose-response relationship, is linear with near perfect correlation of determination, $R = 0.99$. The IC50 value was 16 mg/L and the 95 percent confidence limit was 9.2-17 mg/L.

ii. In Illinois River water (enriched)

Thirteen Illinois River water samples were taken between October 1984 and February 1986 and tested for Cr toxicity. The results are depicted in Fig. 11.

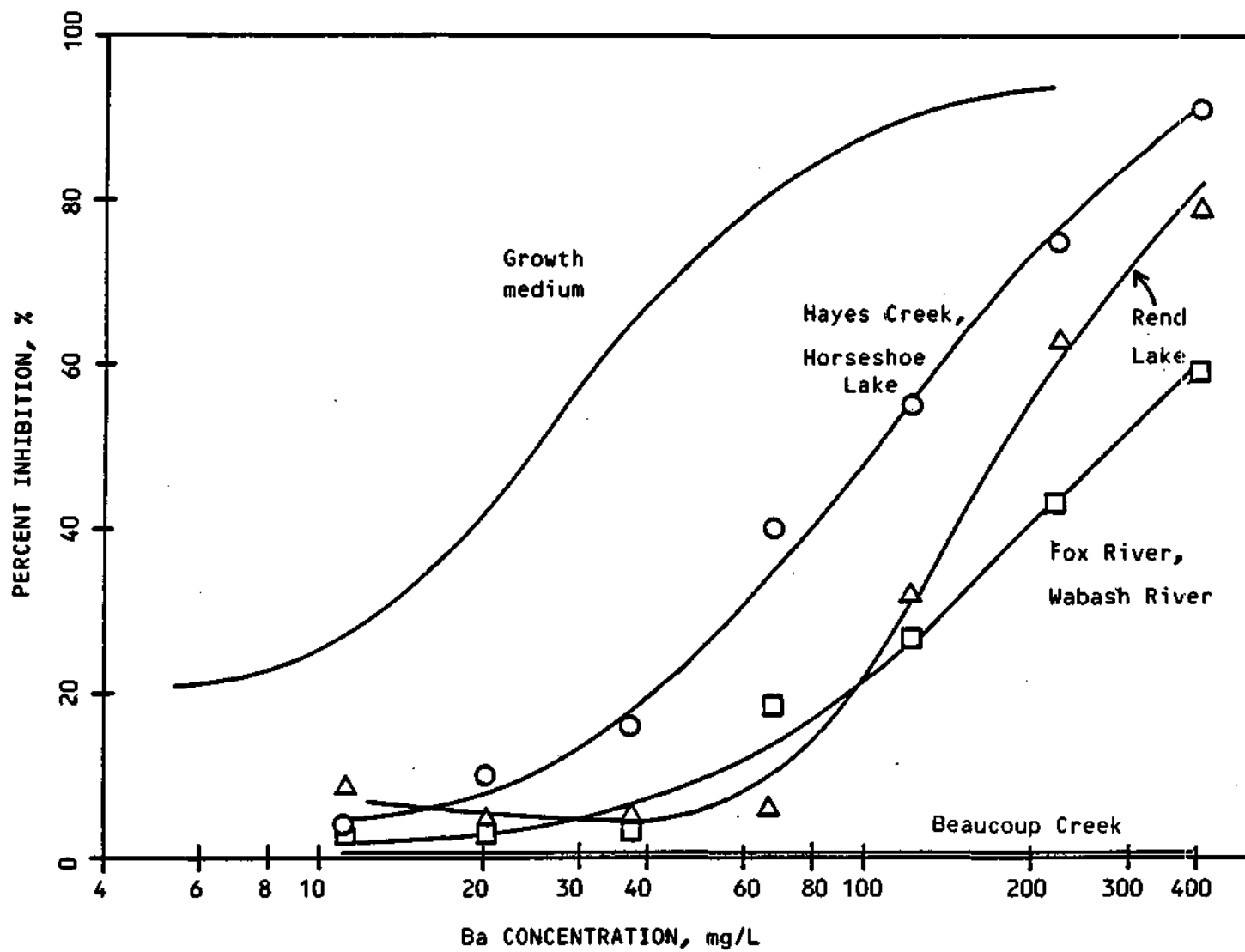


Fig. 8. Ba toxicity in various water samples (enriched).

Table 7. Classification of Ba Toxicity in Various Enriched Water Samples

	Ba IC50 mg/L	Stations
Highly sensitive	<150	Hayes Creek, Horseshoe Lake, L. Michigan L. Geneva, Mississippi River, Rock R., Salt R.
Moderately sensitive	151-300	Fox R., Rend L., Skunk R., Wabash R.
Less sensitive	>300	Beaucoup C., Embarras R., Illinois R., LaMoine R., Sangamon R., Kankakee R., Missouri R.

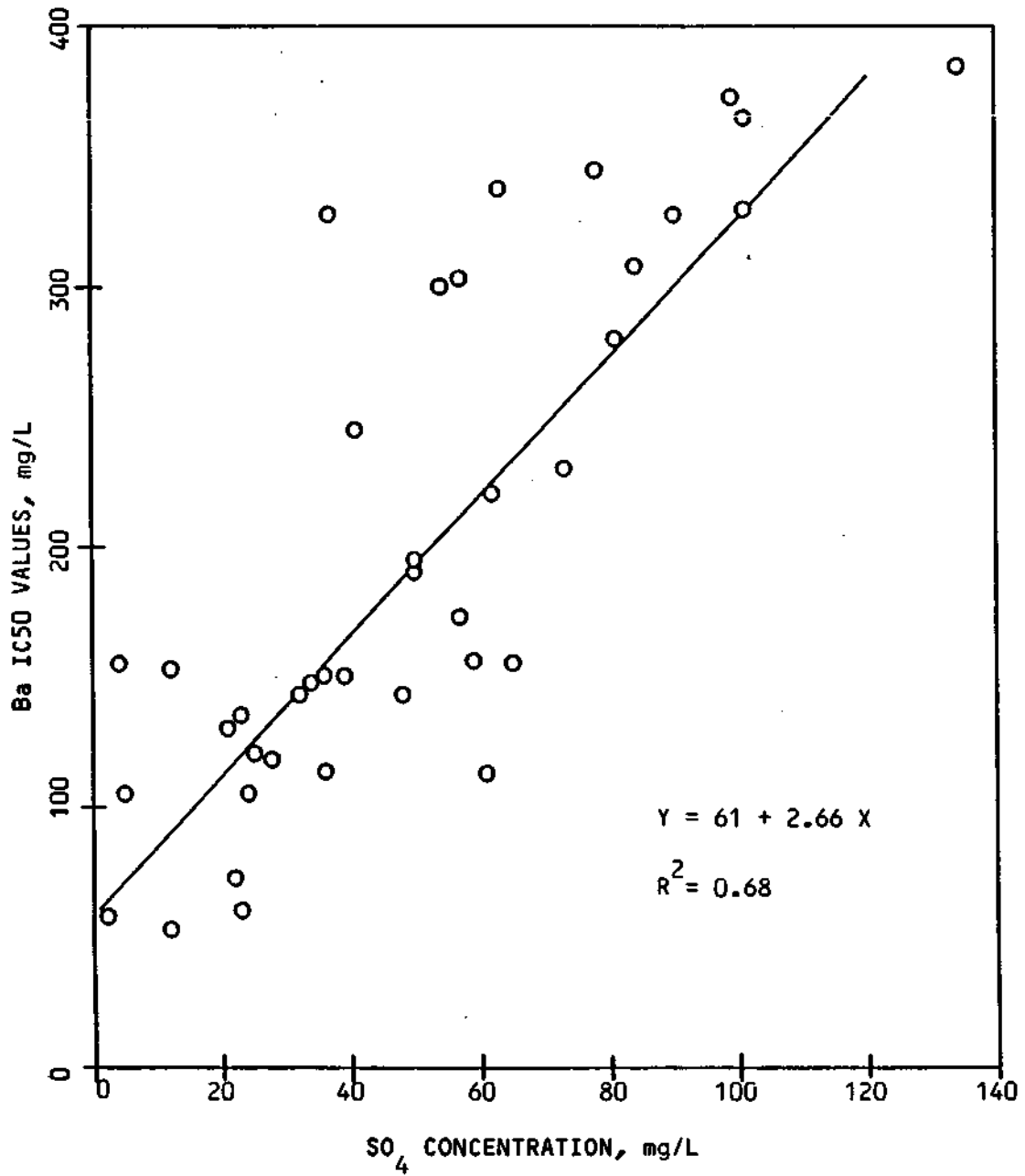


Fig. 9. Ba toxicity (IC50's) and SO₄ concentration relationship.

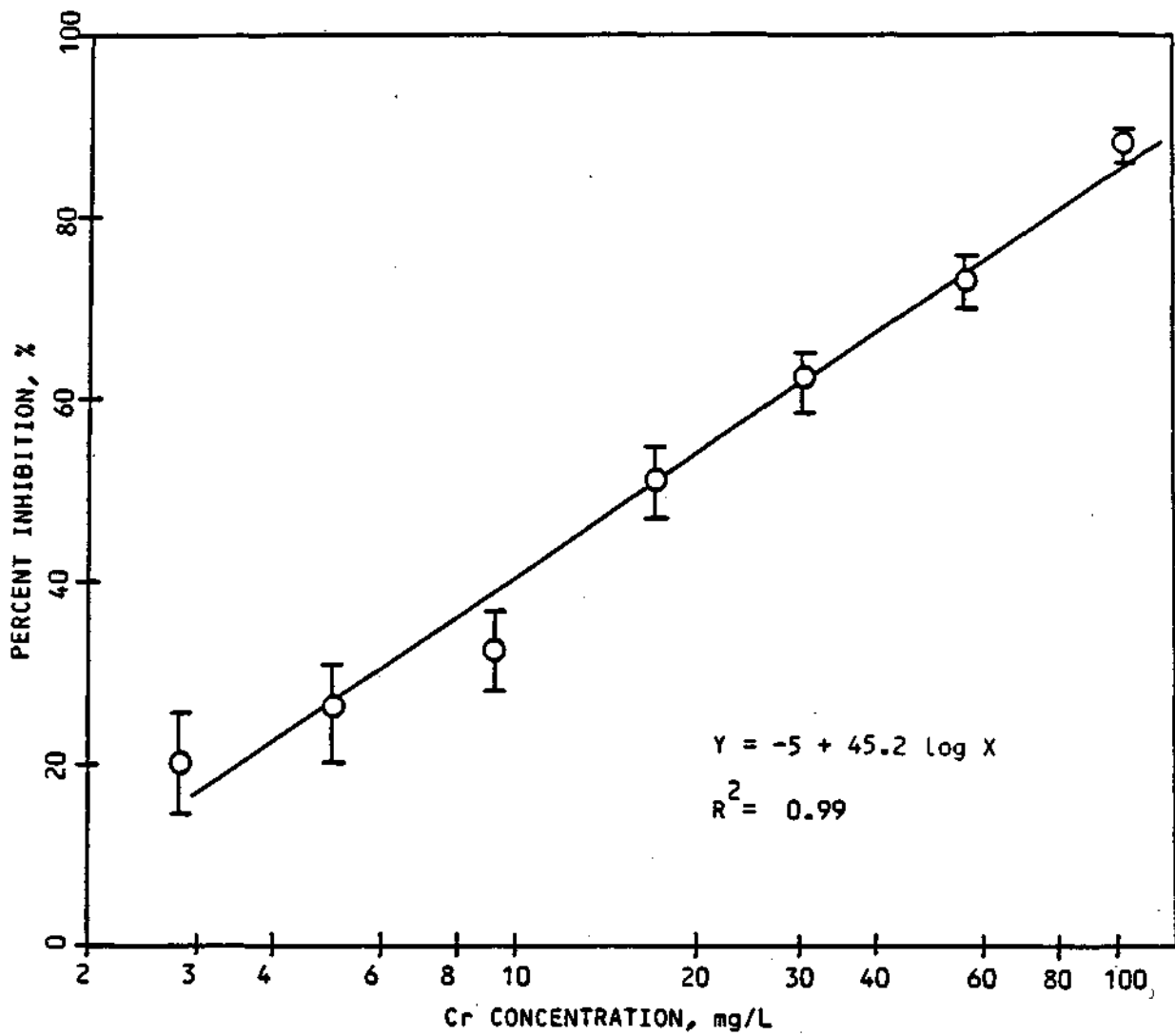


Fig. 10. Cr toxicity in duckweed growth medium, 10 tests.

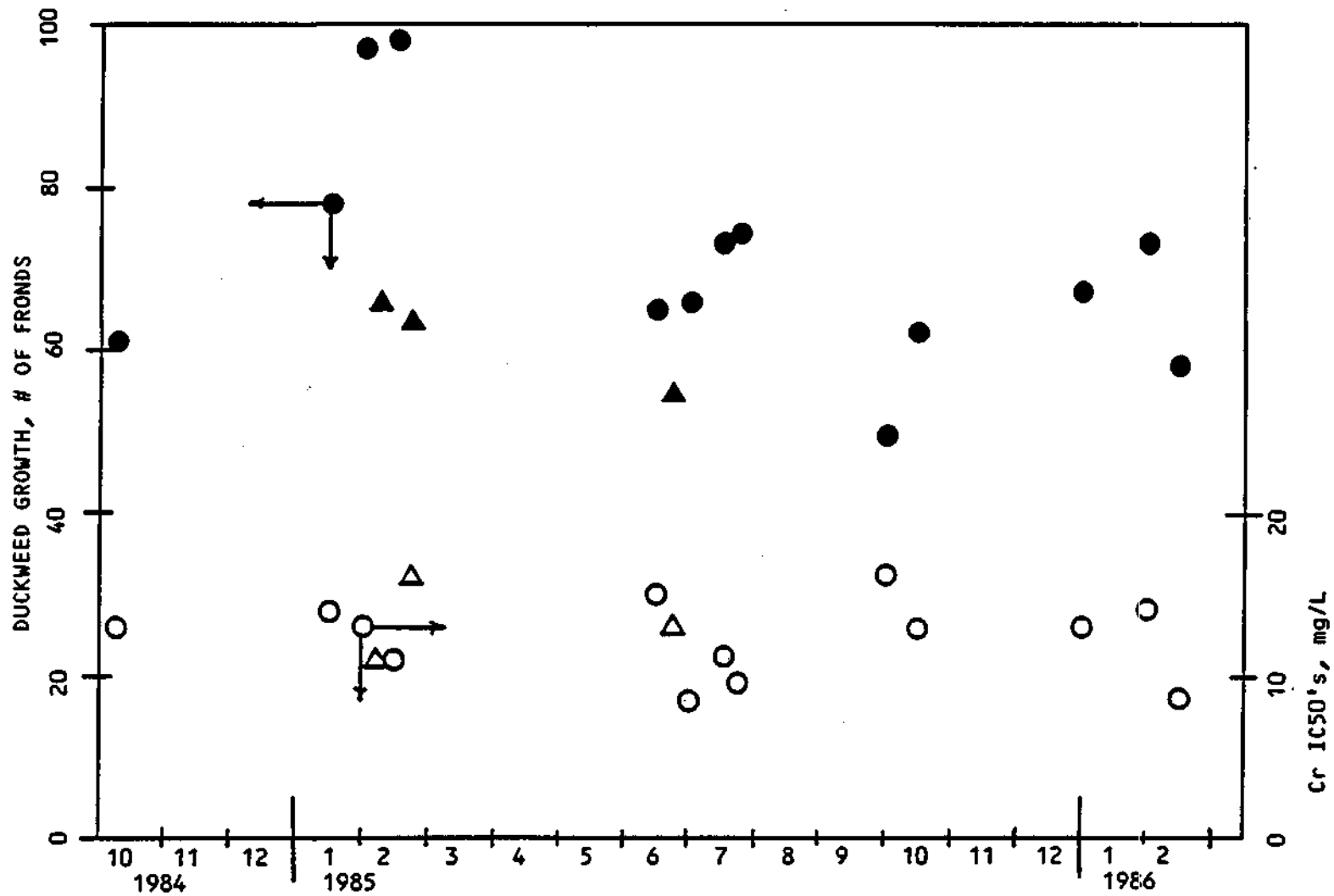


Fig. 11. Duckweed growth in water controls (▲) and in Illinois River sample controls (●) and Cr toxicity in duckweed growth medium (△) and in Illinois River samples (○).

In this figure, the IC50's of individual samples are given as open circles, while the duckweed net growth values of the sample controls are given as solid circles. The results of three Cr toxicity tests during this period that used duckweed growth medium are also shown for comparison. (The remaining tests using duckweed growth medium were conducted outside of this time frame and are shown in Fig. 13). The IC50 values for the duckweed growth medium and the duckweed net growth in the water controls are shown as open triangles and solid triangles, respectively. The mean and standard deviation of the Cr IC50's in the Illinois River samples are 12 ± 3 mg/L. This mean value of 13 samples compares favorably with that in the duckweed growth medium, 16 mg/L, from 10 tests.

There appears to be no relationship between IC50 values and duckweed growth.

From the combined results of all Illinois River water samples, a dose-response relationship can be shown (Fig. 12). As in Fig. 10, a linear semi-log relationship was obtained. The regression analysis indicated that it is highly correlated, with $R^2 = 0.99$. The regression equations are $Y = -5 + 45.2 \log X$ for the duckweed growth medium (Fig. 10) and $Y = -4 + 50.6 \log X$ for the Illinois River water samples. They are strikingly similar.

iii. In all water samples (enriched)

Table 8 summarizes Cr toxicity values in terms of IC50's in all water samples. The results show that they are in a relatively narrow range, 3.5 - 29 mg/L, with a variation of less than one order of magnitude. The mean IC50 value is 12 mg/L and the standard deviation is 4.7 mg/L. The coefficient of variation is 41 percent.

Many concurrent tests were conducted using seven Cr concentrations in duckweed growth medium and in water samples. The water samples were from ten stations: stations 11 through 18 (Table 4), Lake Michigan, and the Illinois River. The results are presented in Fig. 13. With the diverse water quality in the samples used in this study, it is interesting to note that a linear relationship existed between the Cr toxicity in the ambient waters and in the growth medium. The correlation of determination was very high, $R = 0.88$.

When the results for all the water samples are combined, the generalized dose-response relationship of Cr toxicity to duckweed growth can be depicted as in Fig. 14. This diagram can be considered the general relationship for Cr toxicity to duckweed plants. The mean IC50 value was 11 mg/L and the 95 percent confidence limit was 9.2-17 mg/L.

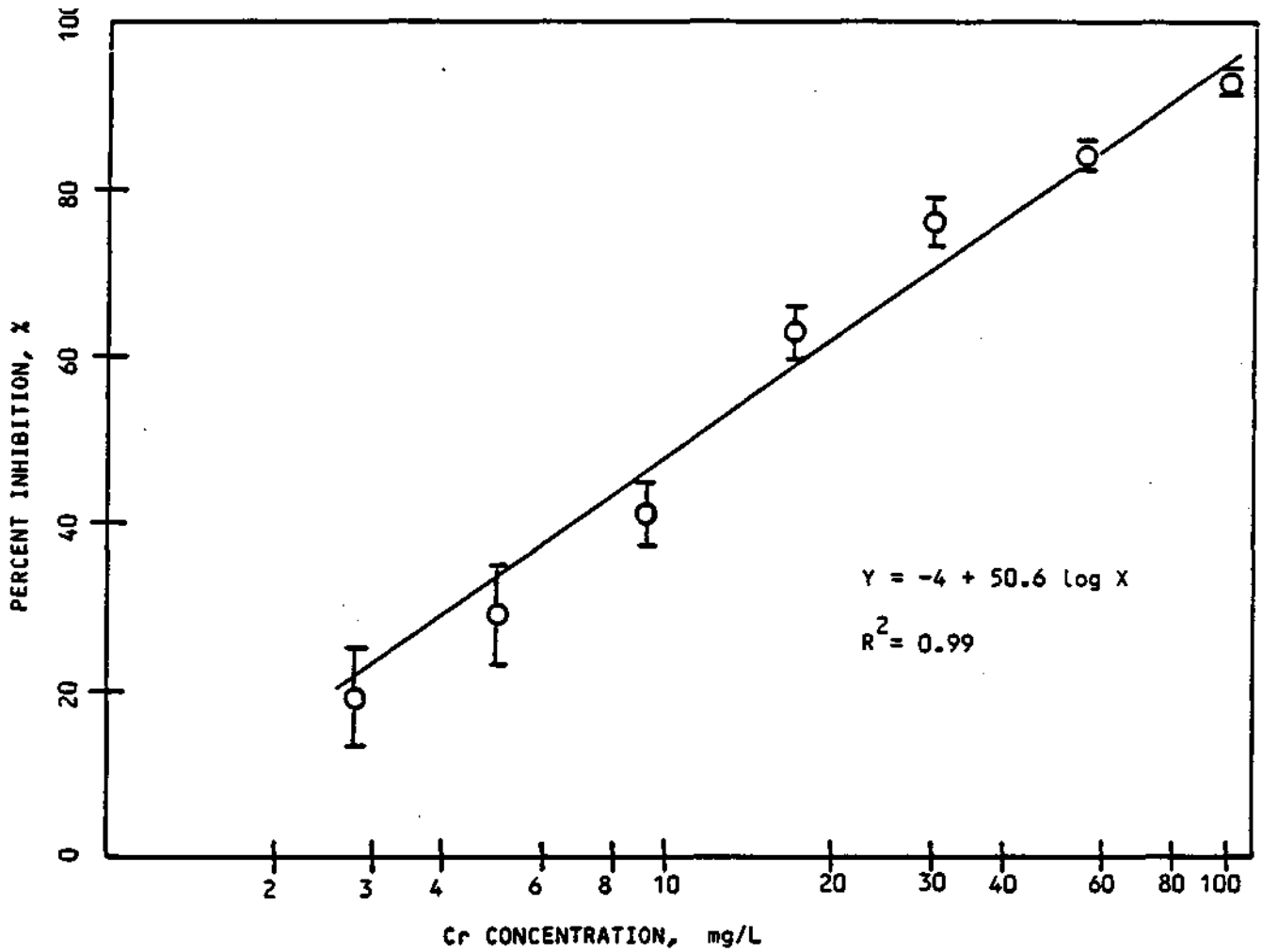


Fig. 12. Cr toxicity in enriched Illinois River water samples, 13 tests.

Table 8. Cr Toxicity in Enriched Water Samples, in IC50's

	IC50, mg/L					Mean, mg/L
1. Beaucoup Creek	16	13				15
2. Embarras River	7.3	6.4				6.9
3. Fox R.	13	12				13
4. Hayes C.	10	11				11
5. Horseshoe Lake	14	9.2				12
6. Illinois R.	12	14	13	11	15	
	14	5.5	8.3	8.4	11	
	9.6	16	13			12
7. LaMoine R.	9.6	10				10
8. L. Michigan	6.8	9.2	16	23	11	13
9. Rend L.	29		21		13	21
10. Sangamon R.	8.7	7.5				8
11. L. Geneva	14	7.9	16			13
12. Kankakee R.	16	6.3	14			12
13. Mississippi R.	10	5.5	6.5			7.3
14. Missouri R.	8.9	4.5	13			8.3
15. Rock R.	12	8.8	19			13
16. Salt R.	13	3.5	8.2			8.2
17. Skunk R.	6.8	7.5				7.2
18. Wabash R.	18	8.3	12			13

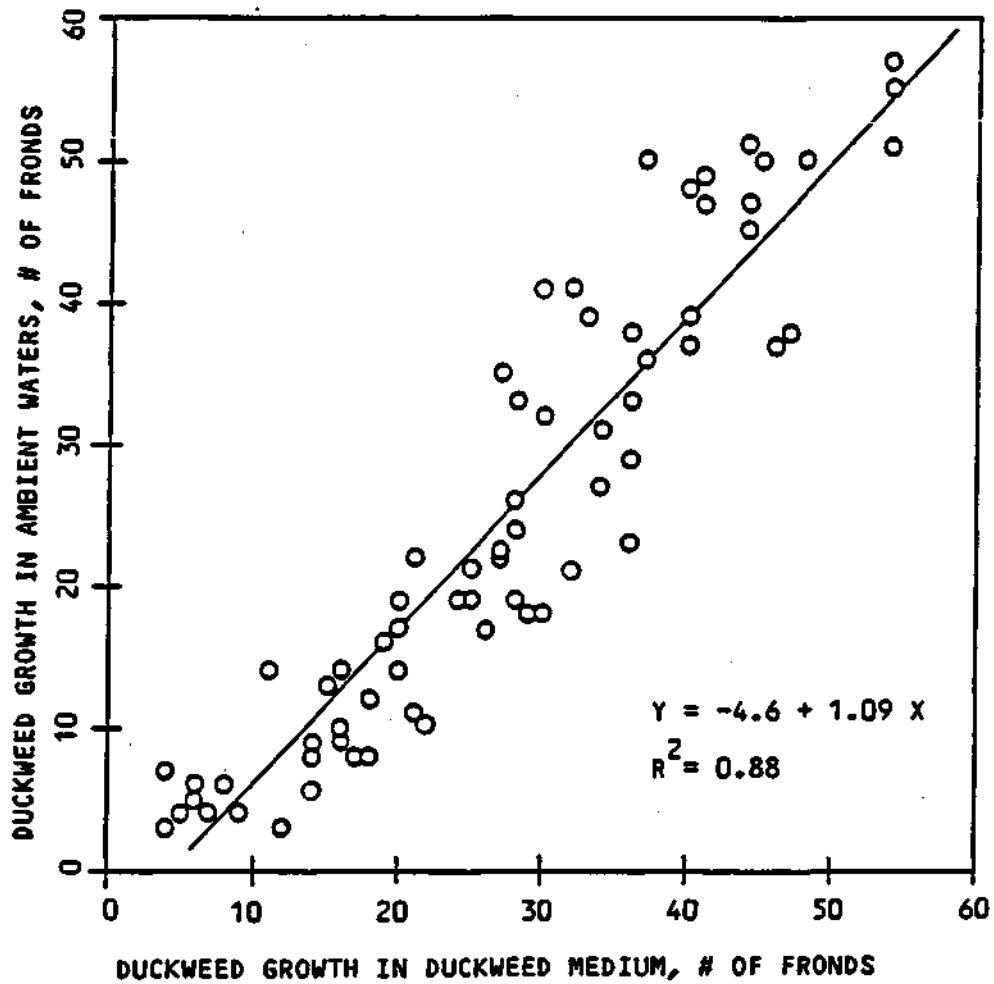


Fig. 13. Duckweed growth in growth medium and 10 surface waters (enriched) containing Cr ion.

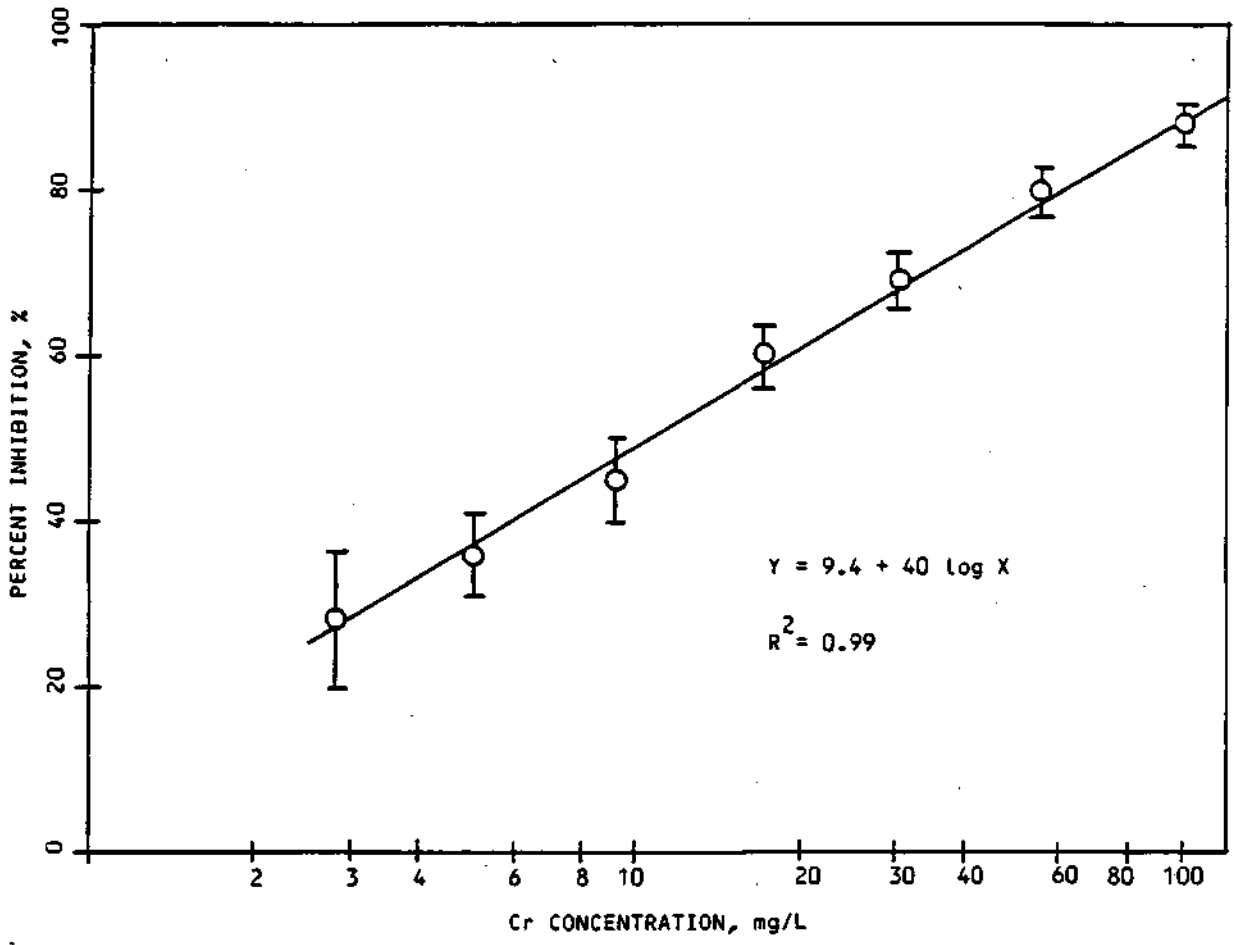


Fig. 14. Generalized Cr concentration-effect relationship in 18 surface waters (enriched).

G. Ni toxicity

i . In duckweed growth medium

Ni toxicity to duckweed using the duckweed growth medium was tested seven times. The results are summarized in Fig. 15. The dose-response relationship appears to be a sigmoid pattern, with the point of tangent situated at approximately 0.46 mg/L Ni concentration and 60 percent growth inhibition. The highest Ni concentration used in the growth medium tests was 5 mg/L. In comparison, 20 mg/L Ni was the maximum concentration used for the water samples. The composite Ni toxicity data give an IC50 value of 0.33 mg/L Ni with a 95 percent confidence limit of 0.25 to 0.46 mg/L.

i i . In Hayes Creek and Horseshoe Lake samples (enriched)

Among all the ambient water samples, Hayes Creek and Horseshoe Lake showed the most sensitive response to Ni toxicity. For example, the IC50's were 0.36 and 0.21 mg/L for these two stations, respectively, while the other 16 stations pooled together showed mean and standard deviation values of 2.5 ± 0.83 mg/L. Consequently, results for these two stations were treated separately.

The results for four water samples from these two stations are given in Table 9. The highest Ni concentration was set at 5 mg/L, the same as that used in the growth medium experiments. An exception was the Hayes Creek sample taken December 4, 1984, in which a maximum concentration of 20 mg/L was tested. Because these two stations behaved strikingly similarly, the results were combined.

The dose-response relationship is nearly linear in the semi-log plot in Fig. 16. This feature is somewhat different from that of using the growth medium (Fig. 15). An interesting point is that at 0.14 mg/L Ni concentration, the percent duckweed growth inhibition was 29 ± 10 percent and 40 ± 8 percent respectively for the growth medium and the Hayes Creek-Horseshoe Lake samples. A t-test indicated, however, that the difference was insignificant.

i i i . In Illinois River water (enriched)

There were a total of 12 Illinois River water samples tested for the effect- of Ni toxicity. The samples were taken from October 1984 until February 1986. The results of the Ni toxicity tests are given in Table 10. These results indicate that the Ni toxicity to duckweed was in a relatively narrow range. The maximum and minimum IC50 values were 4.4 and 0.97 mg/L, respectively. The mean value of the IC50's was 2.8 with a standard deviation of 0.97 mg/L.

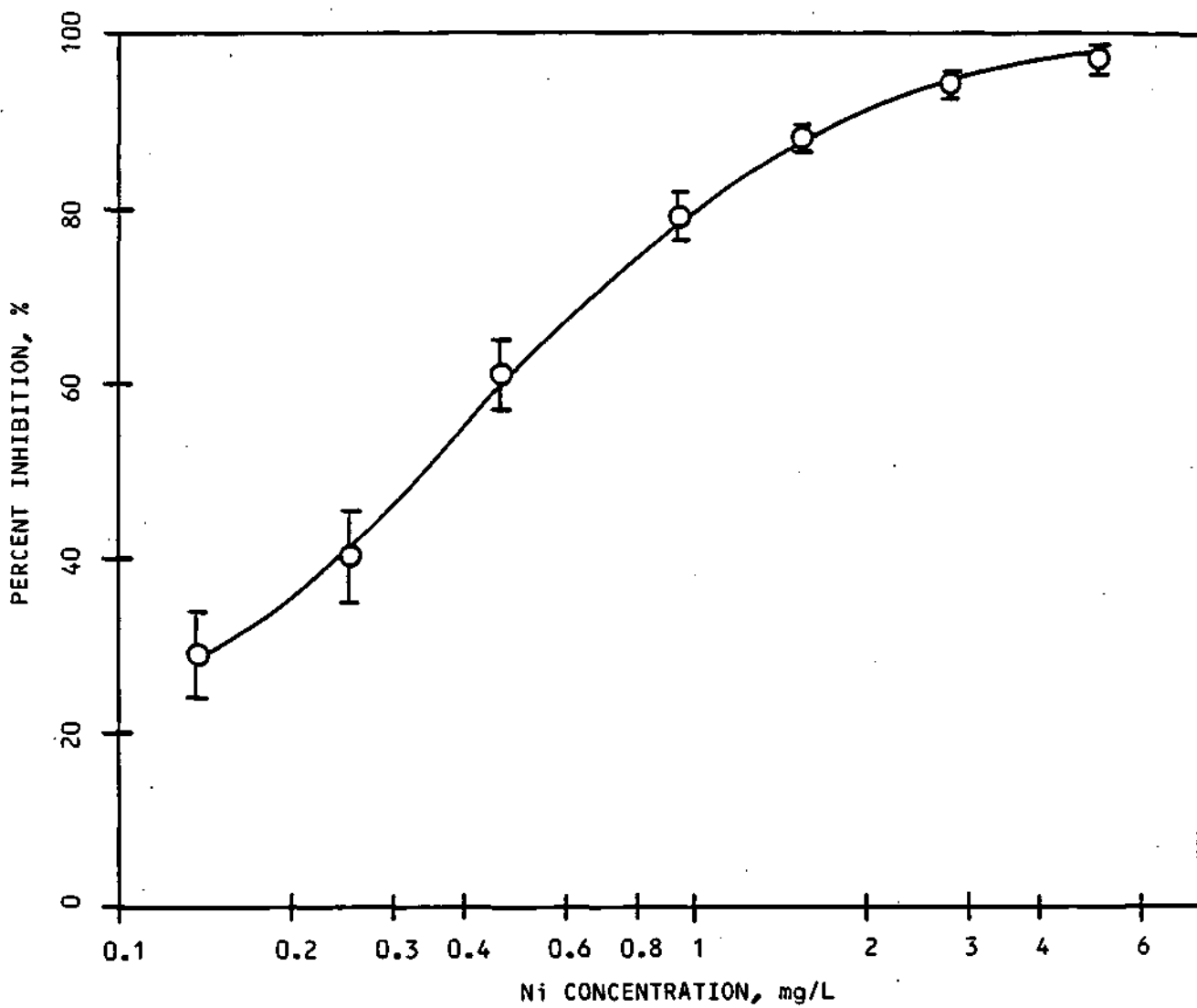


Fig. 15. Ni toxicity in duckweed growth medium, 7 tests.

Table 9. Ni Toxicity in Enriched Hayes Creek and Horseshoe Lake Samples, in Percent Growth Inhibition

	Ni concentration, mg/L						
	0.14	0.25	0.46	0.83	1.51	2.75	5
Hayes Creek							
12/4/84	-	-	49	60	76	88	99
3/7/85	49	58	69	77	80	90	95
Horseshoe Lake							
3/19/85	32	52	55	68	74	83	86
8/13/85	40	60	78	84	95	98	100
Mean	40	57	63	72	81	90	95
SD	8.5	4	13	10	10	6	6

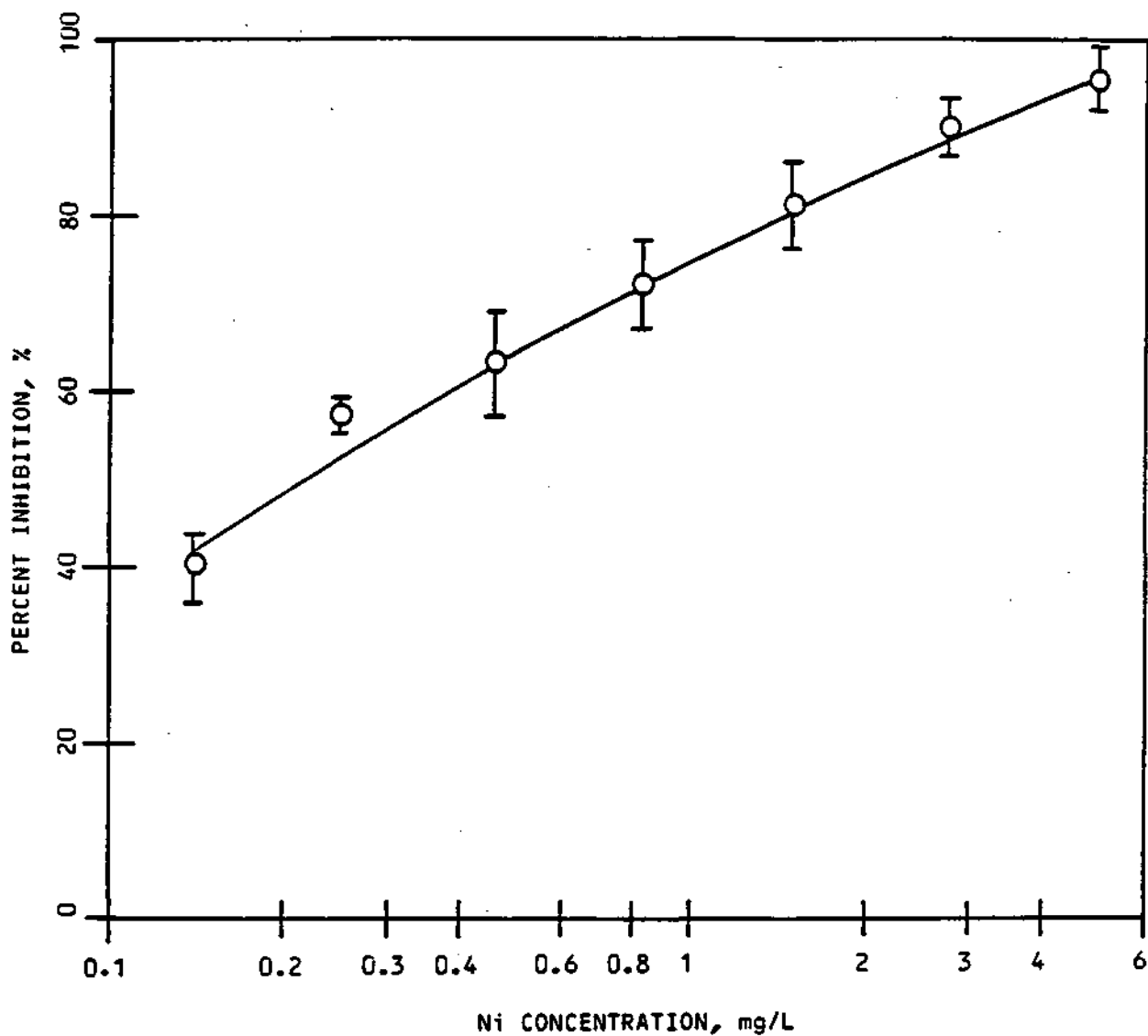


Fig. 16. Ni toxicity in enriched Hayes Creek and Horseshoe Lake samples, 4 tests.

Table 10. Ni Toxicity in Enriched Illinois River Water Samples,
in Percent Growth Inhibition

Sample dates	Ni concentration. mg/L							IC50 mg/L	95% C.L.
	0.55	1.0	1.8	3.3	6.1	11	20		
10/8/84	17	12	22	43	53	78	95	3.5	3.3-6.1
1/21/85	15	24	23	38	64	85	91	4.4	3.3-6.1
2/4	15	15	37	51	68	79	87	3.2	1.8-3.3
2/18	25	30	45	58	70	77	87	2.3	1.8-3.3
7/1	17	19	30	53	73	87	94	2.8	1.8-3.3
7/15	33	23	29	41	65	80	97	3.9	3.3-6.1
7/29	35	38	48	66	75	89	89	1.9	1.8-3.3
9/16	31	51	54	72	79	95	100	0.97	0.55-1.0
10/7	14	12	30	66	84	91	98	2.4	1.8-3.3
1/13/86	4	4	27	43	67	77	80	3.7	3.3-6.1
2/3	14	24	35	48	80	92	97	3.1	3.3-6.1
2/17	30	30	47	61	82	85	94	2.0	1.8-3.3

The Ni toxicity results are plotted in Fig. 17, along with the duckweed net growth results for the control tests. As with the Cr results (Fig. 11), the IC50's were independent of the duckweed growth in the sample controls. Unlike with the Cr results, Ni toxicity to duckweed was very much reduced in the Illinois River water samples in comparison with that in the growth medium. The former was 2.8 mg/L expressed as the Ni IC50 value, while the latter was 0.33 mg/L. The attenuation of Ni toxicity in the Illinois River was thus greater than 8-fold.

The dose-response relationship of the Ni ion in the Illinois River water samples is presented in Fig. 18. A typical sigmoid curve is obtained. The point of tangent was situated at approximately the 3.8 mg/L Ni concentration where duckweed growth inhibition was 55 percent.

iv. In all water samples (enriched)

Ni toxicity in all the water samples is summarized in Table 11. The results show that Ni toxicity in all water samples is in a very narrow range. Excluding the results for Hayes Creek and Horseshoe Lake samples, the mean value for the remaining 16 stations was 2.5 mg/L with a standard deviation of 0.83 mg/L. If these two stations are also included, the corresponding overall values were 2.2 and 1.0 mg/L.

Even though Ni toxicity results appeared to be in a relatively narrow range, the sample can be classified into three groups as in Table 12. The samples which are highly sensitive to Ni toxicity include those from Hayes Creek and Horseshoe Lake. These samples showed a toxic response nearly identical to that of the growth medium, with Ni IC50 values substantially below 1 mg/L. The Illinois River, Missouri River, Rock River, and Wabash River all attenuated Ni toxicity greatly (IC50's > 3 mg/L), causing Ni toxicity much less than in the growth medium. The samples from the great majority of stations (IC50's 1-3 mg/L) moderately attenuated Ni toxicity.

Combining Ni toxicity results from the 16 stations yields a generalized dose-response relationship as presented in Fig. 19. This relationship is useful as an estimate of Ni toxicity in ambient waters, except in the extremely soft, clean, forested streams, for which the relationship in Fig. 16 is more appropriate.

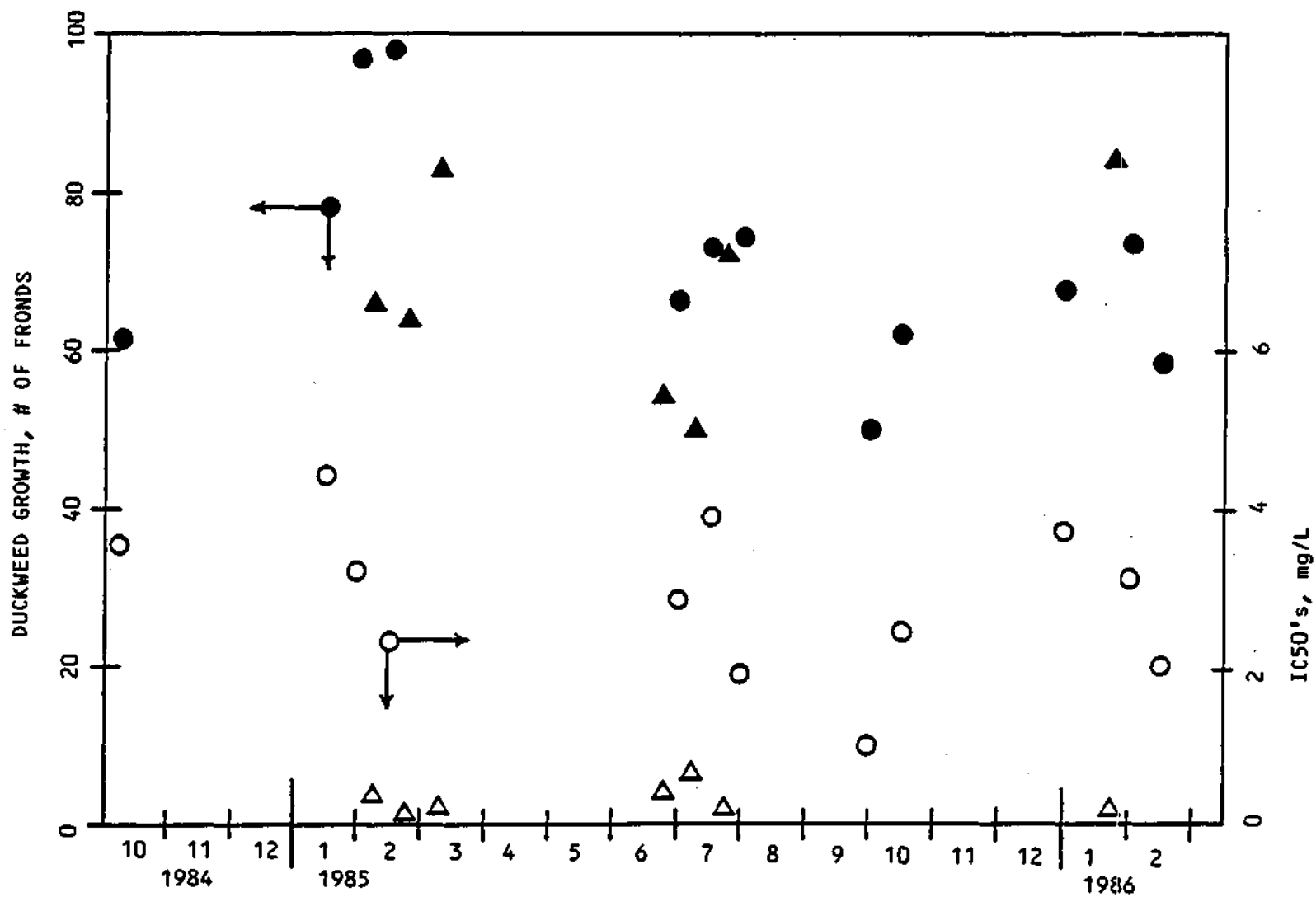


Fig. 17. Duckweed growth in water controls (▲) and in Illinois River sample controls (●) and Ni toxicity in duckweed growth medium (△) and in Illinois River samples (○).

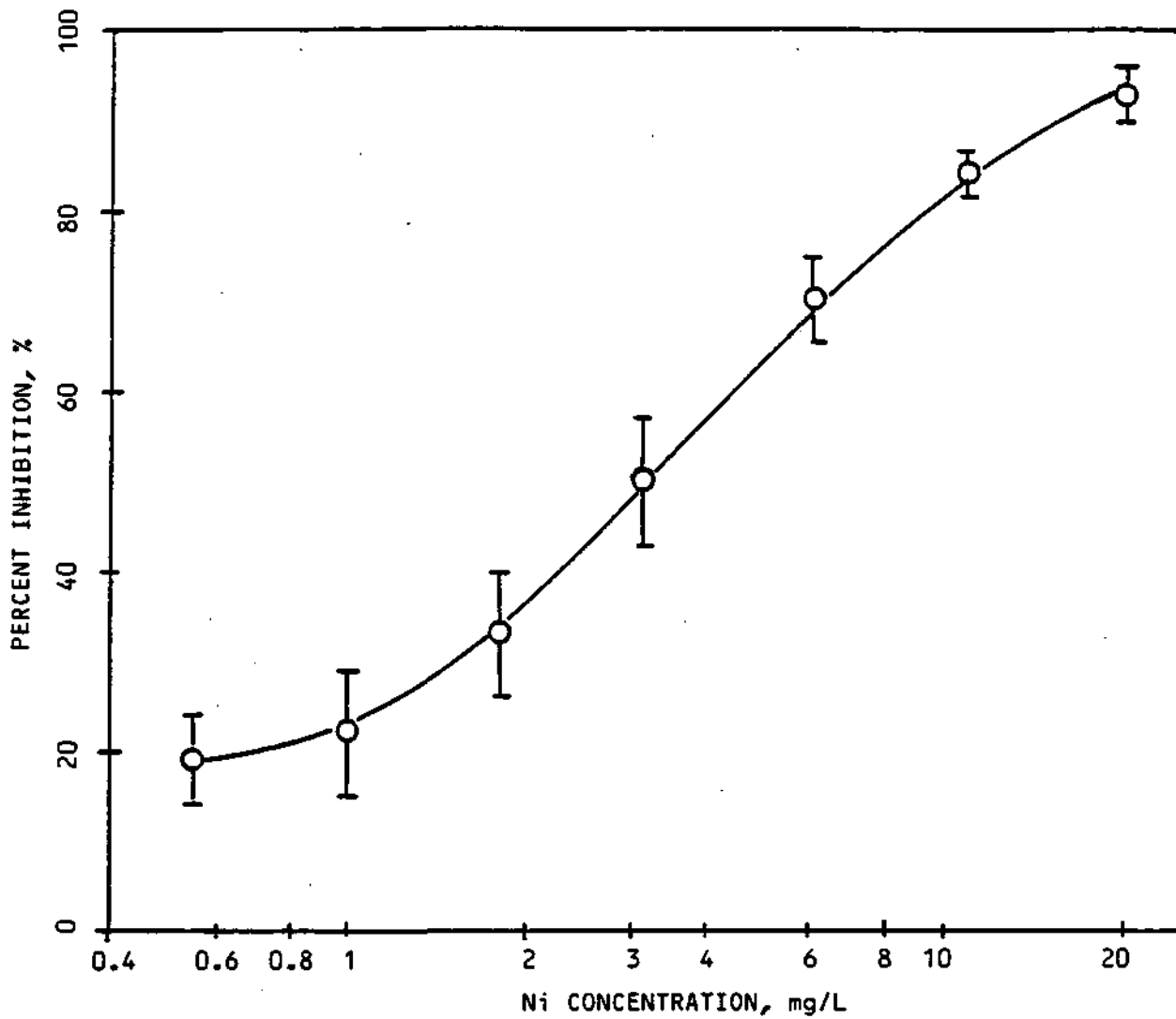


Fig. 18. Ni toxicity in enriched Illinois River water samples, 12 tests.

Table 11. Ni Toxicity in Enriched Water Samples, in IC50's

	IC50. mg/L					Mean, mg/L
1. Beaucoup Creek	3.8	1.8				2.8
2. Embarras River	2.6	2.3				2.5
3. Fox R.	2.5	2.0				2.3
4. Hayes C.	0.56	0.15				0.36
5. Horseshoe Lake	0.24	0.18				0.21
6. Illinois R.	3.5	4.4	3.2	2.3	3.9	
	5.7	3.7	3.1	5.0	2.8	
	1.9	0.97	2.4			3.1
7. LaMoine R.	2.9	1.3				2.1
8. L. Michigan	1.3	1.2	0.77	1.1	1.1	1.1
9. Rend L.	2.1	1.2	0.64			1.3
10. Sangamon R.	3.8	1.7				2.7
11. L. Geneva	1.8	0.85	4.6			2.4
12. Kankakee R.	1.9	1.6	3.7			2.4
13. Mississippi R.	1.9	0.79	2.2			1.6
14. Missouri R.	2.5	1.5	8.1			4.0
15. Rock R.	3.8	2.6	4.7			3.7
16. Salt R.	1.6	1.5	1.7			1.6
17. Skunk R.	3.1	1.5				2.3
18. Wabash R.	1.9	2.5	5.7			3.4
			(16 stations)		C18 stations)	
			2.51 ± 0.83		2.2 ± 1.0	

Table 12. Classification of Ni Toxicity in Various Water Samples

	Ni IC50 mg/L	
Highly sensitive	<1	Hayes Creek, Horseshoe Lake
Moderately sensitive	1-3	Beaucoup C, Embarras R., Fox R., LaMoine R., L. Michigan, Rend L., Sangamon R., L. Geneva, Kankakee R., Mississippi R., Salt R., Skunk R.
Less sensitive	>3	Illinois R., Missouri R., Rock R., Wabash R.

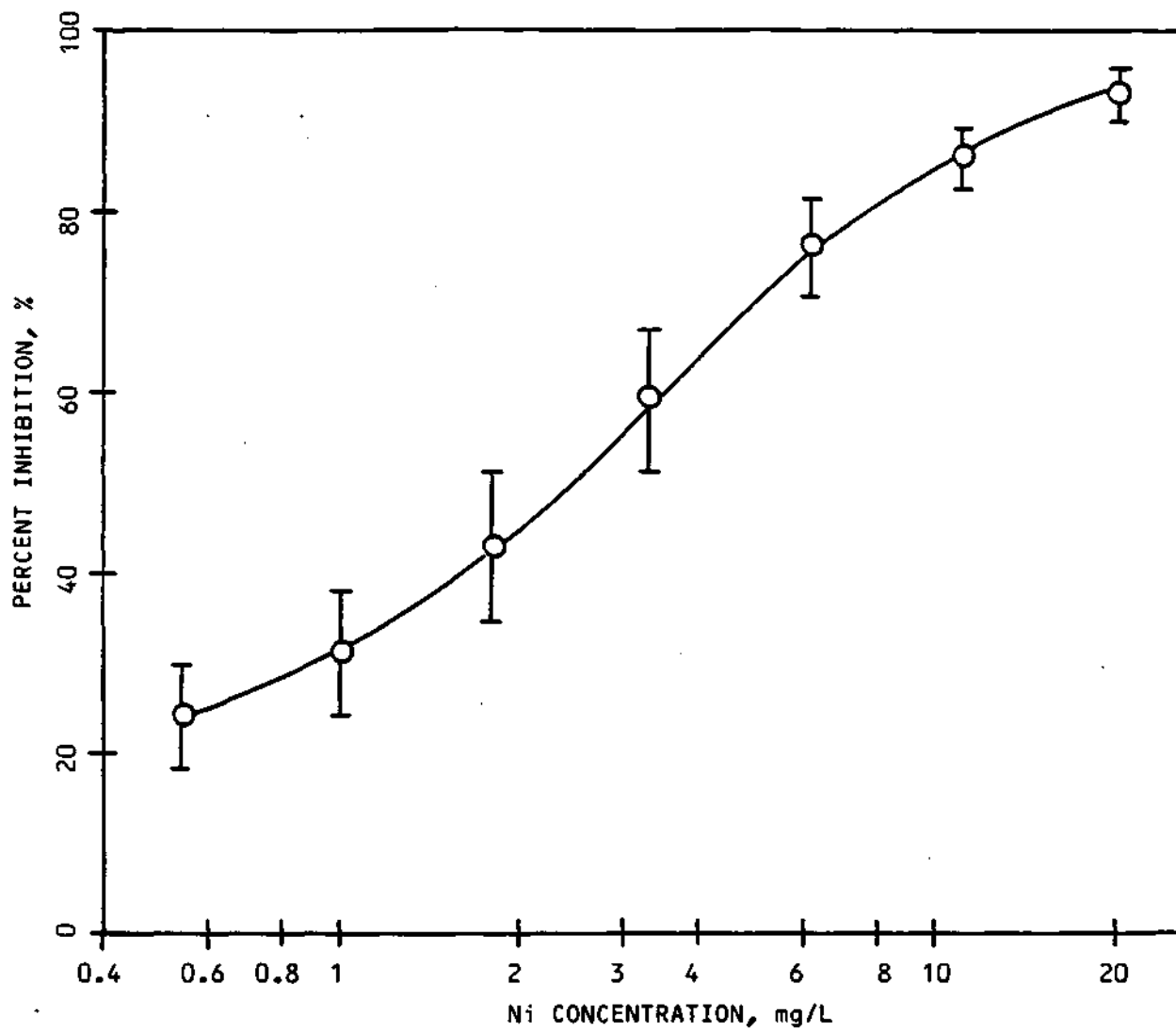


Fig. 19. Ni concentration-effect relationship in 16 surface water samples (enriched).

6. Discussion

Current water quality criteria rely primarily on values derived from laboratory studies. For example, the Rules and Regulations. State of Illinois, listed the standards for the chemical constituents Ba, Cr (VI), and Ni for secondary contact and indigenous aquatic organisms as 5, 0.3 and 1.0 mg/L, respectively, all in total form (State of Illinois, 1986). A catch-all section is provided which states that "Any substance toxic to aquatic life not listed in Section 302.407 shall not exceed one half of the 96-hour median tolerance limit (96-hour TLM) for native fish and essential fish food organisms." There are three important issues here.

A. Importance of aquatic vegetation

A healthy environment is one in which organisms are in a balanced and dynamic state. In that state, plant life as a producer provides oxygen and organic substances, while animals and microorganisms consume, decompose, and recycle these substances back to plant life, making a complete cycle. In this tripartite ecosystem, each part (plants, animals, and microorganisms) has an essential role to play. An emphasis on any one part without adequately addressing the other part(s) is unlikely to lead to a healthy environment (Wang, 1984). The Illinois River offers an interesting example.

The Illinois River and its tributaries are an important asset to the State of Illinois as a major water resource. The annual per-acre yield of fish from the river declined drastically in the Illinois River between the 1950's and 1970's, while that in the Upper Mississippi River actually increased slightly (Sparks and Sandusky, 1981):

<u>Illinois River</u>	<u>lbs/acre</u>	<u>Upper Mississippi River</u>	<u>Ibs/acre</u>
1950-1959	38.1	1953-1962	26.3
1973-1980	3.7	1973-1977	29.2

The decline has been speculated to be due to fish food decline. In the meantime, Bellrose et al. (1979) noted a general decline of aquatic vegetation in the bottom lakes of the river. They pointed out four important factors affecting the food sources of waterfowl: 1) fluctuating water levels, 2) turbidity, 3) water depth, and 4) competition by other plants.

In a recent news story (Peoria Journal Star, April 23, 1986, page D10), large fish kills involving smallmouth bass, green sunfish, and other species were reported. The suspected pollutant was a herbicide, Sonalan. Because herbicides are commonly 3 to 4 orders of magnitude more toxic to plants than to animals (Bishop and Perry, 1981), is it not logical to suspect that the phytotoxicity has done untold damages to aquatic vegetation? With the magnitudes of herbicides (millions of pounds annually) applied by farmers and homeowners, is it unlikely that the aquatic

vegetation declines in the face of this chronic herbicide exposure? It is not likely that the loss of aquatic vegetation by itself, and more likely in combination with other factors, causes fish decline, waterfowl decrease, and water quality deterioration manifested by erosion, siltation, and other ill effects? On the basis of this deduction, is it not an inadequate approach to protect the aquatic environment by protecting only "fish and essential fish food organisms"?

B. Site-specific water quality criteria

The current water quality criteria, as mentioned previously, rely on single values (State of Illinois, 1986) or maximum and average concentrations (U.S. Environmental Protection Agency, 1982). The results of this research project, however, point out another area of concern.

Water quality varies from one station to another and from one time to another. Under these dynamic circumstances, the toxicity of a substance may be modified and altered accordingly. The modification of toxicity, furthermore, is toxicant-specific. On the basis of the experimental results, Ba, Cr, and Ni toxicities in ambient waters can be summarized as follows:

- Ba - Its phytotoxicity is the least among the three metals and is influenced the most by the water quality of the test samples.
- Cr - Its phytotoxicity is moderate among the three metals and is influenced the least by the water quality of the test samples.
- Ni - Its phytotoxicity is the greatest among the three metals and is influenced moderately by the water quality of the test samples.

Consequently, the Cr water quality criterion may be adopted universally for all surface waters. The Ba criterion C5.0 mg/L can be substantially moderated according to water quality. The Ni criterion C1.0 mg/L as stated in the Rules and Regulations (State of Illinois, 1986) is extremely inadequate. At this concentration, a 30 percent growth inhibition of aquatic vegetation (based on duckweed as the test organism) can be expected in almost all surface waters (Fig. 19), and 70 percent inhibition can be expected in extremely soft waters (Fig. 16).

C. Reference toxicant

For conducting biological toxicity tests, it is essential to ascertain the health and suitability of test organisms. The common practice is to include a control test in which the experimental condition is identical to the test condition, except that the test substance(s) is absent. This control is also called negative control. In this study, both water controls and sample

controls were used. However, the negative control, measuring only the test organism under favorable conditions, is not sufficient. A complete approach is to add a second control (also known as positive control) so that the test organism can also be observed under unfavorable conditions.

Both organic and inorganic compounds have been studied as potential reference toxicants. They include pentachlorophenoI , Guthion-R, phenol, picloram, sodium dodecyl sulfate, dehydroabiatic acid, sodium chloride, cadmium, and chromate (Davis and Hoos, 1975; Adelman and Smith, 1976; Fogels and Sprague, 1977; Threader and Houston, 1983; Lewis and Weber, 1985; Jop et al. in press). Jop et al. (in press) mention the advantages of using the chromate ion as high purity, accuracy and ease of determination, and the like.

A factor which has received little attention is the suitability of a reference compound. This factor becomes important when the compound is intended for ambient waters or complex mixtures. For example, antibiotics and sodium chloride were effective in inhibiting oxygen uptake in sediment samples. They lost effectiveness, however, in 30 to 40 h (Wang, 1980). As another example, when duckweed was used as a test specimen it was found that Cd toxicity in a river water sample was ameliorated by both dissolved and particulate fractions in river water (Wang, 1986C). The Ba ion, on the other hand, was affected only by the dissolved fraction of the river water samples. In other words, the toxic responses of Ba and Cd ions varied according to the water quality of a given sample. In the same study, the Cr ion was found to be nearly identical in the growth medium and the river water.

The additional results as presented in Figs. 10 through 14 support the contention that Cr can be the universal reference toxicant for biological toxicity tests using surface waters.

References

- Adelman, I.R. and Smith, L.L. 1976. Fathead minnows (*Pimephalas promelas*) and goldfish (*Carassins auratus*) as standard fish in bioassays and their reaction to potential reference toxicants. Jour. Fish. Res. Board Can. 33, 209-214.
- Ahsanullah, M. and Florence, T.M. 1984. Toxicity of copper to the marine amphipod *Allorchestes compressa* in the presence of water- and lipid-soluble ligands. Marine Biol. 84, 41-45.
- Ahsanullah, M., Mobley, M.C., and Negilski, D.S. 1984. Accumulation of cadmium from contaminated water and sediment by the shrimp *Callianassa australiensis*. Marine Biol. 82, 191-197.
- Ajmal, M. and Khan, A.U. 1984. Effect of water hardness on the toxicity of cadmium to micro-organisms. Water Res. 18, 1487-1491.
- Anderson, P.D. and Weber, L.J. 1976. The multiple toxicity of certain heavy metals: Additive actions and interactions. Workshop on Toxicity to Biota of Metal Forms in Natural Water IJC, Great Lakes Research Advisory Board, Windsor, On, Ca. 325 p.
- Anderson, P.D. and Weber, L.J. 1977. The toxicity to aquatic populations of mixtures containing certain heavy metals. Symposium Proceedings on International Conference on Heavy Metals in the Environment, Toronto, Canada, Vol. II, 933-953.
- Andrew, R.W. 1976. Toxicity relationships to copper forms in natural waters. In Workshop on Toxicity to Biota of Metal Forms in Natural Water. Intl. Joint Comm., Duluth, MN, 329 p-
- Andrew, R.W., Biesinger, K.E., and Glass, G.E. 1977. Effects of inorganic complexing on the toxicity of copper to *Daohnia magna*. Water Res. 11, 309-315.
- Antonovics, J., Bradshaw, A.D., and Turner, R.G. 1971. Heavy metal tolerance in plants. Adv. Ecol. Res. 7, 1-85.
- Attar, E.N. and Maly, E.J. 1982. Acute toxicity of cadmium, zinc, and cadmium/zinc mixtures to *Daphnia magna*. Arch. Environ. Contam. Toxicol. 11, 291-296.
- Aulio, K. and Salin, M. 1982. Enrichment of copper, zinc, manganese, and iron in five species of pondweeds (*Potamogeton* spp.). Bull. Environ. Cont. Am. Toxicol. 29, 320-325.
- Babich, H., Devanas, M.A., and Stotzky, G. 1985. Review. The mediation of mutagenicity and clastogenicity of heavy metals

- by physicochemical factors. Environ. Res. 37, 253-286.
- Babich, H. and Stotzky, G. 1978. Toxicity of zinc to fungi, bacteria, and coliphages: Influence of chloride ions. Appl. Environ. Microbiol. 36, 906-914.
- Babich, H. and Stotzky, G. 1979. Abiotic factors affecting the toxicity of lead to fungi. Appl. Environ. Microbiol. 38, 506-513.
- Babich, H. and Stotzky, G. 1982A. Nickel toxicity to microbes: Effect of pH and implications for acid rain. Environ. Res. 29, 335-350.
- Babich, H. and Stotzky, G. 1982B. Nickel toxicity to fungus: Influence of environmental factors. Ecotoxicol. Environ. Saf. 6, 577-589.
- Babich, H. and Stotzky, G. 1983. Temperature, pH, salinity, hardness, and particulates mediate nickel toxicity to eubacteria, and anctiomycete, and yeasts in lake, simulated estuarine, and sea waters. Aq. Toxicol. 3, 195-208.
- Babich, H. and Stotzky, G. 1985. Heavy metal toxicity to microbe-mediated ecologic processes: A review and potential application to regulatory policies. Environ. Res. 36, 111-137.
- Bartlett, L., Rabe, F.W., and Funk, W.H. 1974. Effects of copper, zinc, and cadmium on Selenastrum capricornutum. Water Res. 8, 179-185.
- Baudo, R. 1981. Chemical speciation of trace elements in the aquatic environments: a literature review. Memorie dell' Istituto Italiano di Idrobiologia 38, 463-492.
- Bellrose, F.C., Paveglio, F.L., and Steffeck, D.W. 1979. Waterfowl populations and the changing environment of the Illinois River Valley. Illinois Natural History Bull. Vol. 32, Article 1, 54 p.
- Benson, W.H. and Birge, W.J. 1985. Heavy metal tolerance and metallothionein induction in fathead minnows: Results from field and laboratory investigations. Environ. Toxicol. Chem. 4, 209-217.
- Bentley-Mowat, J.A. and Reid, S.M. 1977. Survival of marine phytoplankton in high concentration of heavy metals and uptake of copper. Jour. Expl. Marine Biol. Ecol. 26, 249-264.
- Biesinger, K.E., Andrew, R.W., and Arthur, J.W. 1974. Chronic toxicity of NTU (nitrilotriacetate) and metal-NTA complexes to Daohnia magna. Jour. Fish. Res. Board Can. 31, 486-490.
- Birge, W.J., Black, J.A., Hobson, J.F., Westerman, A.G., and

- Short, T.M. 1984. Toxicological studies on aquatic contaminants originating from coal production and utilization: the induction of tolerance to silver in laboratory populations of fish and chronic toxicity of nickel to fish early life stages. Research Report 151. Univ. of Kentucky, Water Resour. Res. Instl. 27 p.
- Bishop, W.E. and Perry, R.L. 1981. Development and evaluation of a flow-through growth inhibition test with duckweed (Lemna minor). ASTM STP 737, Amer. Soc. Testing & Materials, 421-435.
- Borgmann, U. 1980. Interactive effects of metals in mixtures on biomass production kinetics of freshwater copepods. Can. Jour. Fish. Aq. Sci. 37, 1295-1302.
- Borgmann, U. and Charlton, C.C. 1984. Copper complexation and toxicity to Daohnia in natural water. J. Great Lakes Res. 10, 393-398.
- Borgmann, U. and Ralph, K.M. 1983. Complexation and toxicity of copper and the free metal bioassay technique. Water Res. 17, 1697-1703.
- Braek, Q.S., Jensen, A., and Mohus, A. 1976. Heavy metal tolerance of marine phytoplankton. III. Combined effects of copper and zinc ions on culture of four common species. Jour. Exp. Marine Biol. Ecol. 25, 37-50.
- Braginskii, L.P. and Shcherban, E.P. 1978. Acute toxicity of heavy metals to aquatic invertebrates at different temperatures. Hydrobiol. Jour. 14, 78-82.
- Brinkhurst, R.O., Chapman, P.M., and Farrell, M.A. 1982. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aq. Toxicol. 2, 47-67.
- Brkovic-Popovic, I. and Popovic, M. 1977. Effects of heavy metals on survival and respiration rate of Tubificid worms: Part 1 - Effects on survival. Environ. Poll. 13, 65-72.
- Brown, V.M., Shaw, T.L., and Shurben, D.G. 1974. Aspects of water quality and the toxicity of copper to rainbow trout. Water Res. 8, 797-803.
- Brunge, W.A., Geckler, J.R., and Gast, M. 1976. Acute and chronic toxicity of copper to the fathead minnow in a surface water of variable quality. Water Res. 10, 37-43.
- Bryant, V., McLusky, D.S., Roddie, K., and Newbery, D.M. 1984. Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates (Corophium volutator, Macoma balthica, Nereis diyersicolor). Marine Ecol-Progress Series 20, 137-149.
- Buckley, J.A. 1983. Complexation of copper in the effluent of a

- sewage treatment plant and an estimate of its influence on toxicity to coho salmon. *Water Res.* 17, 1929-1934.
- Cairns, J. Jr. (Ed.) 1985. *Multispecies toxicity testing*. Pergamon Press, N.Y. 261 p.
- Cairns, J. Jr., Heath, A.G., and Parker, B.C. 1975A. The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia* 47, 135-171.
- Cairns, J. Jr., Heath, A.G., and Parker, B.C. 1975B. Temperature influence on chemical toxicity to aquatic organisms. *Jour. Water Poll. Cont. Fed.* 47, 267-280.
- Calamari, O. and Marchetti, R. 1973. The toxicity of mixtures of metals and surfactants to rainbow trout (Salmo gairdneri Rich.). *Water Res.* 7, 1453-1464.
- Calamari, D., Marchetti, R., and Vailati, G. 1980. Influence of water hardness on cadmium toxicity to Salmo gairdneri Rich. *Water Res.* 14, 1421-1426.
- Canterford, D.R. and Canterford, G.S. 1980. Toxicity of heavy metals to the marine diatom Ditylum brightwellii (West) Grunow: correlation between toxicity and metal speciation. *Jour. Marine Biol. Assoc.* 60, 227-242.
- Chapman, G., and Dunlop, S. 1981. Detoxification of zinc and cadmium by the freshwater protozoan *Tetrahemena pyroformis*. I. The effect of water hardness. *Environ. Res.* 26, 81-86.
- Chapman, P.M., Farrell, M.A., and Brinkhurst, R.O. 1982A. Effects of species interactions on the survival and respiration of Limnodrilus hoffmerteri and Tubifer tubifer (Oligochaeta Tubificidae) exposed to various pollutants and environmental factors. *Water Res.* 16, 1405-1408.
- Chapman, P.M., Farrell, M.A., and Brinkhurst, R.O. 1982B. Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. *Aq. Toxicol.* 2, 47-67, *ibid*, 69-78.
- Cherry, D.S., Guthrie, R.K., and Singleton, F.L. 1977. Aquatic bacterial populations and heavy metals. II. Influence of chemical content of aquatic environments on bacterial uptake of chemical elements. *Water Res.* II, 643-646.
- Christenson, E.R., Scherfig, J., and Dison, P.S. 1979. Effects of manganese, copper, and lead on Selenastrum capricornutum and Chlorella stigmatophora. *Water Res.* 13, 79-92.
- Chynoweth, D.P., Black, J.A., and Mancy, K.H. 1976. Effects of organic pollutants on copper toxicity to fish. *Workshop on Toxicity to Biota of Metal Forms in Natural Water*, IJC, Great Lakes Res. Advisory Board, Windsor, ON, Can. 325 p.

- Clubb, R.W., Gaufin, A.R., and Lords, J.L. 1975. Synergism between dissolved oxygen and cadmium toxicity in five species of aquatic insects. *Environ. Res.* 9, 285-289.
- Dave, G. 1985. The influence of pH on the toxicity of aluminum, cadmium, and iron to eggs and larvae of the zebrafish, Brachydanio rerio. *Ecotoxicol. and Environ. Saf.* 10, 253-267.
- Davis, J.C. and Hoos, R.A.W. 1975. Use of sodium pentachlorophenate and dehydroabiatic acid as reference toxicants for salmoid bioassays. *J. Fish. Res. Board Can.* 32, 411-416.
- Demayo, A., Reeder, S.W., and Taylor, M.C. 1980. Guidelines for surface water quality. Vol. 1. Inorganic chemical substances. Lead. Inland Water Directorate, Water Quality Branch, Ottawa, Canada, 43 pp.
- Dongmann, G., and Nuernberg, H.W. 1982. Observations with Thalassiosira rotula (Meunier) on the toxicity and accumulation of cadmium and nickel. *Ecotoxicol. Environ. Saf.* 6, 535-544.
- Duncan, D.A. and Klaverkamp, J.F. 1983. Tolerance and resistance to cadmium in white suckers (Catostomus commersoni) previously exposed to cadmium, mercury, zinc, or selenium. *Can. J. Fish. Aqu. Sci.*, Vol. 40, 128-138.
- Eaton, J.G. 1973. Chronic toxicity of a copper, cadmium, and zinc mixture to the fathead minnow (Pimephales promelas Rafinesque). *Water Res.* 7, 1723-1736.
- Eaton, J.G. 1974. Chronic cadmium toxicity to the bluegill (Leoomis macrochirus Rafinesque). *Trans. Amer. Fish. Soc.* 103, 729-735.
- EIFAC (European Inland Fisheries Advisory Commission). 1980. Water quality criteria for European freshwater fish. Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water. FAO, Rome, EIFAC Technical Paper (International) No. 37, 58 p.
- Engler, R.M., Folsom, B.L., and Lee, C.R. 1982. Availability and plant uptake of heavy metals from contaminated dredge material placed in flooded and upland disposal environments. *Environ. Intl.* 7, 65-71.
- Erickson, S.J. 1972. Toxicity of copper to a marine diatom in unenriched inshore seawater. *J. Phycol.* 8, 318-323.
- Ernst, W.H.O. 1975. Physiology of heavy metal resistance in plants. Symposium Proceedings on International Conference on Heavy Metals in the Environment. Toronto, Ca., Vol. II, 121-136.

- Finlayson, B.J. and Verrue, K.M. 1982. Toxicities of copper, zinc, and cadmium mixtures to juvenile Chinook salmon. Trans. Am. Fish. Soc. 111, 645-650.
- Florence, T.M. 1983. Trace element speciation and aquatic toxicology. Trends in Analyt. Chm. 2, 162-166.
- Fogels, A. and Sprague, J.B. 1977. Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Water Res. 11.811-817.
- Foster, P.L. 1982. Species associations and metal contents of algae from rivers polluted by heavy metals. Freshwater Biol. 12, 17-39.
- Frank, P.M. and Robertson, P.B. 1979. The influence of salinity on toxicity of cadmium and chromium to the blue crab, Callinectes sapidus. Bull. Environ. Contam. Toxicol. 21, 74-76.
- Franzin, W.G. and McFarlane, G.A. 1980. An analysis of the aquatic macrophyte, Myriophyllum exalbescens, as an indicator of metal contamination of aquatic ecosystems near a base-metal smelter. Bull. Environ. Contam. Toxicol. 24, 597-605.
- Frazier, J.M. 1976. The dynamics of metals in the American oyster, Crassostrea virginica. II. Environmental effects. Chesapeake Sci. 17, 188-197.
- Fuhrer, J. 1983. Phytotoxic effects of cadmium in leaf segments of Avena sativa L., and the protective role of calcium. Experientia, 39, 525-526.
- George, S.G. and Coombs, T.L. 1977. Effects of high stability iron-complexes on the kinetics of iron accumulation and excretion in Mytilus edulis (L.) Jour. Expl. Marine Biol. Ecol. 28, 133-140.
- Gipps, J.F. and B.A.W. Collier. 1982. Effect of some nutrient cations on uptake of cadmium by Chlorella pyrenoidosa. Aust. Jour. Mar. Freshw. Res. 33, 979-987.
- Graney, R.L., Cherry, D.S., and Cairns, J. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (Corbicula Fluminea) in laboratory artificial streams. Water Res. 18, 833-842.
- Gray, J.S. 1976. The effects of salinity, temperature, and mercury on mortality of the Trocophore larvae of Seroula vermicularis L. (Annelida: polychaeta). Jour. Exp. Marine Biol. Ecol. 23, 127-134.
- Gregory, P.P.G. and Bradshaw, A.D. 1965. Heavy metal tolerance in populations of Agrostis tenuis Sibth and other grasses. New Phytol. 64, 131-43.

- Hallas, L.E., Thayer, J.S., and Cooney, J.J. 1982. Factors affecting the toxic effects of tin on estuarine microorganisms. *Appl. Environ. Microbiol.* 44, 193-197.
- Hardy, J.T., Schmidt, R.L., and Apts, C.W. 1981. Marine sediment and interstitial water: Effects on bioavailability of cadmium to gills of the clam Protothaca staminea. *Bull. Environ. Contam. Toxicol.* 27, 798-805.
- Hargraves, J.W. and Whitton, B.A. 1976. Effect of pH on tolerance of Hormidium rivulare to zinc and copper. *Oecologia* 26, 235-243.
- Harmeson, R.H. and Larson, T.E. 1969. Quality of surface water in Illinois, 1956-1966. *Illinois State Water Survey, Bulletin* 54, Urbana, IL, 184 p.
- Harrison, F.L. 1983. Detoxification of environmental copper in the common mussel. *Energy Technology Review.* 9-19.
- Hart, K.M. and Cairns, J. Jr. 1984. Maintenance of structural integrity in freshwater protozoan communities under stress. *Hydrobiologia* 198, 171-180.
- Hartman, W.A. and Martin, D.B. 1984. Effect of suspended bentonite clay on the acute toxicity of glyphosate to Daphnia pulex and Lemna minor. *Bull. Environ. Contam. Toxicol.* 33, 355-361.
- Hodson, P.W. and Sprague, J.B. 1975. Temperature induced changes in acute toxicity of zinc to Atlantic salmon (Salmo salar). *J. Fish. Res. Board Can.* 32, 1-10.
- Hongve, D., Skogheim, O.K., Hinder, A., and Abrahamsen, H. 1980. Effects of heavy metals in combination with NTA, humic acid, and suspended sediment on natural phytoplankton photosynthesis. *Bull. Environ. Contam. Toxicol.* 25, 594-600.
- Hooper, F.F. 1969. Eutrophication indices and their relation to other indices of ecosystem change. In "Eutrophication: Causes, consequences, correctives." *National Acad. Sci.* Washington, D.C. 225-235,
- Howarth, R.W. and Sprague, J.B. 1978. Copper lethality to rainbow trout in waters of various hardness and pH. *Water Res.* 12, 455-462.
- Huckabee, J.W. and Griffith, N.A. 1974. Toxicity of mercury and selenium to the eggs of Carp (Cyprinus carpio). *Trans. Am. Fish. Soc.* 103, 822-824.
- Hutchinson, T.C. and Czyska, H. 1975. Heavy metal toxicity and synergism to floating aquatic weeds. *Verh. Internat. Verein. Limnol.* 19, 2102-2111.
- Hutchinson, T.C. and Stokes, P.M. 1975. Heavy metal toxicity and

- algal bioassays. In Water Quality Parameters, ASTM STP 573, Amer. Soc. Testing Materials, 320-343.
- Jackim, E., Morrison, G., and Steele, G. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. *Marine Biol.* 40, 303-308.
- Jana, S. and Choudhuri, M.A. 1984. Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plants. *Water, Air, Soil Poll.* 21, 351-357.
- Johnels, A.G., Westermarck, T., Berg, W., Persson, P.I., and Sjostrand, B. 1967. Pike (Esox Lucius L.) and some other aquatic organisms in Sweden as indicator of mercury contamination in the environment. *Oikos* 18, 323-333.
- Jones, A.K. and McLean, R.O. 1975. Studies of tolerance to heavy metals in the flora of the rivers, Ystwyth and Clarach, Wales. *Freshwater Biol.* 5, 431-444.
- Jones, L.H., Jones, N.V., and Radlett, A.J. 1976. Some effects of salinity on the toxicity of copper to the polychaete Nereis diversicolor. *Estuarine Coastal Marine Sci.* 4, 107-111.
- Jop, K.M., Rodgers, J.H., Dern, P.B., and Dickson, K.L. In press. Use of hexavalent chromium as a reference toxicant in aquatic toxicity tests. ASTM STP.
- Karbe, L., Antonacapsulos, N., and Shnier, C. 1975. The influence of water quality on accumulation of heavy metals in aquatic organisms. *Verh. Internat. Verein. Limnol.* 19, 2094-2101.
- Khummongkol, D., Canterford, G.S., and Fryer, C. 1982. Accumulation of heavy metals in unicellular algae. *Biotechnol. Bioengin.* 24, 2643-2660.
- Kinkade, M.L. and Erdman, H.E. 1975. The influence of hardness components (Ca^{2+} and Mg^{2+}) in water on the uptake and concentration of cadmium in a simulated freshwater ecosystem. *Environ. Res.* 10, 308-313.
- Kraft, K.J. and Sypniewski, R.H. 1981. Effect of sediment copper on the distribution of benthic macro-invertebrates in the Keweenaw Waterway. *Jour. Great Lakes Res.* 7, 258-263.
- Kuwabara, J.S. 1985. Phosphorus-zinc interactive effects on growth by Selenastrum capricornutum (Chlorophyta). *Environ. Sci. Tech.* 19, 417-421.
- Laegreid, M., Alstad, J., Klaveness, D., and Selp, H.M. 1983. Seasonal variation of cadmium toxicity toward the alga Selenastrum capricornutum Printz in two lakes with different humus content. *Environ. Sci. Tech.* 17, 357-361.
- LeBlanc, G.A. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. *Environ. Toxicol. Chem.*

3, 47-60.

- LeBlanc, G.A. and Surprenant, D.C. 1984. Influence of mineral salts on fecundity of the water flea (Daphnia magna) and the implications on toxicity testing of industrial wastewater. *Hydrobiologia* 108, 25-31.
- Lemke, A.E. 1981. Interlaboratory comparison: Acute testing set. U.S. Environmental Protection Agency EPA-600/S3-81-005.
- Les, A., and Walker, R.W. 1984. Toxicity and binding of copper, zinc, and cadmium by the blue-green alga, Chroococcus parisi. *Water, Air, Soil Poll.* 23, 129-139.
- Lewis, P.A. and Weber, C.I. 1985. A study of the reliability of *Daphnia* acute toxicity tests, ASTM STP 854, Amer. Soc. Testing Materials, 73-86.
- Lewis, T. and McIntosh, A. 1984. Accumulation of the trace elements lead and zinc by Asellus communis at three different pH levels. NTIS PB84-202514, 21 p.
- Lindestrom, L. 1980. The ability of aquatic organisms to adapt to heavy metals. *Votten* 36, 322-324.
- MacLeod, J.C. and Pessah, E. 1973. Temperature effects on mercury accumulation, toxicity and metabolic rate of rainbow trout (Salmo gairdneri). *J. Fish Res. Board Can.* 30, 485-492.
- Marshall, J.S., Mullinger, D.L., and Parker, J.I. 1981. Combined effects of cadmium and zinc on a Lake Michigan zooplankton community. *J. Great Lakes Res.* 7, 215-223.
- Mayes, R.A., McIntosh, A.W., and Anderson, V.L. 1977. Uptake of cadmium and lead by a rooted aquatic macrophyte (Elodea canadensis). *Ecol.* 58, 1176-1180.
- McNaughton, S.J., Folsom, T.C., Lee, T., Park, F., Price, C, Roeder, D., Schmitz, J., and Stockwell, C. 1974. Heavy metal tolerance in *Typha latifolia* without the evolution of tolerant races. *Ecol.* 55, 1163-1165.
- Merlani, M. and Pozzi, G. 1977. Lead and freshwater fishes: Part I lead accumulation and water pH. *Environ. Poll.* 12, 167-172.
- Michnowicz, C.J. and Weaks, T.E. 1984. Effects of pH on toxicity of As, Cr, Cu, Ni, and Zn to Selenastrum cannicornutum Printz. *Hydrobiologia* 118, 299-305.
- Middaugh, D.P., Davis, W.R., and Yoakum, R.L. 1975. The exposure of larval fish, Leiostromus xanthurus. to environmental stress following sublethal cadmium exposure. *Contributions Mar. Sci.* 19, 13-19.
- Morris, O.P. and Russel, G. 1973. Effect of chelation on toxicity

- of copper. Marine Poll. Bull. 4, 159-160.
- Muramoto, S. 1980. Effect of complexans (EDTA, NTA and DTPA) on the exposure to high concentrations of cadmium, copper, zinc, and lead. Bull. Environ. Contam. Toxicol. 25, 941-946.
- Muramoto, S. and Oki, Y. 1984. Influence of anionic surface-active agents on the uptake of the heavy metals by water hyacinth. Bull. Environ. Contam. Toxicol. 33, 444-450.
- Nakada, M, Fukaya. K., Takashita, S., and Wada, Y. 1979. The accumulation of heavy metals in the submerged plant (Elodea nallallii). Bull. Environ. Contam. Toxicol. 22, 21-27.
- Nasu, Y. and Kugimoto, M. 1981. Lemna (duckweed) as an indicator of water pollution. I. The sensitivity of Lemna paucicostata to heavy metals. Arch. Environ. Contam. Toxicol. 10, 159-169.
- Nasu, Y., Kugimoto, M., Tanaka, O., and Takimoto, A. 1983. Comparative studies on the absorption of cadmium and copper in Lemna paucicostata. Environ. Poll. (Series A) 32, 201-209.
- National Research Council. 1972. Water quality criteria. EPA-R3-73-033, 594 p.
- Nay, J.J. and Van Hassel, J.H. 1983. Sources of variability in accumulation of heavy metals. Arch. Environ. Contam. Toxicol. 12, 701-706.
- Nebeker, A.V., Savonen, C, and Stevens, D.G. 1985. Sensitivity of rainbow trout early life stages to nickel chloride. Environ. Toxicol. Chem. 4, 233-239.
- Negilski, D.S. 1976. Acute toxicity of zinc, cadmium, and chromium to the marine fishes, yellow-eye mullet (Aldrichetta forsteri C. and V.) and small mouth hardhead (Atherinasoma microstoma Whitley). Aust. Jour. Marine Freshwater Res. 27, 137-149.
- Nishikawa, K. and Tabata, K. 1969. Studies on the toxicity of heavy metals to aquatic animals and the factors decreasing toxicity. III. On the low toxicity of some heavy metal complexes to aquatic animals. Bull. Tokai Reg. Fish, Res. Lab. 58, 233-242.
- Oakden, J.M., Oliver, J.S., and Flegal, A.R. 1984. EDTA chelation and zinc antagonism with cadmium in sediment: Effects on the behavior and mortality of two infaunal amphipods. Marine Biol. 84, 125-130.
- O'Keefe, D.H., Hardy, J.K., and Rao, R.H. 1984. Cadmium uptake by the water hyacinth: Effects of solution factors. Environ. Poll. (Series A) 34, 133-147.
- Ozoh, P.T.E. 1979. Malformation and inhibitory tendencies induced

- to Brachydanio rerio (Hamilton-Buchanan) eggs and larvae due to exposures in low concentrations of lead and copper ions. Bull. Environ. Contam. Toxicol. 21, 668-675.
- Pesch, C.E. 1979. Influence of three sediment types on copper toxicity to the polychaete Neanthes arenaceodentata. Mar. Biol. 52, 237-245.
- Pesch, C.E. and Morgan, D. 1978. Influence of sediment in copper toxicity tests with the polychaete Neanthes arenaceodentata. Water Res. 12, 747-751.
- Phelps, H.L. 1979. Cadmium sorption in estuarine mud-type sediment and the accumulation of cadmium in the soft-shell clam, Mya arenaria. Estuaries 2, 40-44.
- Piccardi, E.B. and Clauser, M. 1983. Absorption of copper by Iris pseudacorus. Water, Air, Soil Poll. 19, 185-192.
- Poston, T.M., Hanf, R.W., and Simmons, M.A. 1984. Toxicity of uranium to Daphnia magna. Water, Air, Soil Poll. 22, 289-298.
- Prasad, P.V.D. and Prasad, P.S.D. 1982. Effect of cadmium, lead, and nickel on three freshwater green algae. Water, Air, Soil Poll. 17, 263-268.
- Rai, L.C. and Khatoniar, N. 1980. Response of Chlorella to mercury pollution. Indian Jour. Environ. Health 22, 113-123.
- Reeder, S.W., Demayo, A., and Taylor, M.C. 1979. Guidelines for surface water quality. Vol. 1. Inorganic chemical substances. Nickel. Inland Water Directorate, Water Quality Branch, Ottawa, Canada. 19 p.
- Rehboldt, R., Menapage, L.W., Nerrie, B., and Alessandrello, D. 1972. The effect of increased temperature upon the toxicity of some heavy metal ions. Bull. Environ. Contam. Toxicol. 8, 91-96.
- Reish, D.J., Martin, J.M., Piltz, F.M., and Ward, J.Q. 1976. The effects of heavy metals on laboratory populations of two polychaetes with comparisons to the water quality conditions and standards in southern California marine waters. Water Res. 10, 299-302.
- Remacle, J., Houba, C, and Ninae, J. 1982. Cadmium fate in bacterial microcosms. Water, Air, Soil Poll. 18, 455-465.
- Riedel, G.F. 1984. Influence of salinity and sulfate on the toxicity of chromium (VI) to the estuarine diatom Thalassiosira pseudonana. Jour. Phycol. 20, 496-500.
- Robinson, J.W., and Deano, P.M. 1985. Synergistic effects on acidity and aluminum on fish (Golden Shiners) in Louisiana. Louisiana State Univ., Baton Rouge, Dept. of Chemistry. Jour.

of Environ. Sciences and Health, A20, 193-204.

- Roch, M. and Maly, E.J. 1979. The relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (Salmo gairdneri) and the influence of temperature on toxicity. J. Fish Res. Board Can. 36, 1297-1303.
- Rodgers, J.H., Cherry, D.S., and Guthrie, R.K. 1978. Cycling of elements in duckweed (Lemna perousilla) in an ash settling basin and swamp drainage system. Water Res. 12, 765-770.
- Rudd, J.W.M. and Turner, M.A. 1983A. The English-Wabigoon River system. V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. Jour. Fish. Aq. Sci. 40, 2251-2259.
- Rudd, J.W.M. and Turner, M.A. 1983B. The English-Wabigoon river system. III. Selenium in lake enclosures, its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Can. Jour. Fish. Aq. Sci. 40, 2228-2240.
- Ruthven, J.A. and Cairns, J. Jr. 1973. Response of freshwater protozoan artificial communities to metals. J. Protozool. 20. 127-135.
- Sanders, J.G. 1979. Effects of arsenic speciation and phosphorus concentration on arsenic inhibition of Skeletonema costatum (Bacillariophyceae). Jour. Phycol. 15, 424-428.
- Schecher, W.D., and Driscoll, C.T. 1985. Interactions of copper and lead with Nostoc muscorum. Water, Air, Soil Poll. 24, 85-101.
- Schierup, H.H. and Larsen, V.J. 1981. Macrophyte cycling of zinc, copper, lead, and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in Phraomites australias (Cau) Trin. Aq. Botany 11, 197-210.
- Seto, M., Takahashi, Y., Ushijima, T., and Tazaki, T. 1979. Chlorotic death of Lemna gibba by cadmium in different concentrations of nutritional minerals. Japanese Jour. Limnol. 40, 61-65.
- Singh, S.P., and Yadava, V. 1984. Cadmium induced inhibition of ammonium and phosphate uptake in Anacystis nidulans: Interaction with other divalent cations. Jour. Gen. Appt. Microbiol. 30, 79-86.
- Slonim, C.B., and Slonim, A.R. 1973. Effect of water hardness on the tolerance of the guppy to beryllium sulfate. Bull. Environ. Contam. Toxicol. 10, 295-301.
- Smith, A.L., Green, R.H., and Lutz, A. 1975. Uptake of mercury by freshwater clams (Family Unionidae). Jour. Fish. Res. Board Can. 32, 1297-1303.

- Smock, L.A. 1983A. The influence of feeding habits on whole-body metal concentrations in aquatic insects. *Freshwater Biol.* 13. 301-311.
- Smock, L.A. 1983B. Relationships between metal concentrations and organism size in aquatic insects. *Freshwater Biol.* 13, 313-321.
- Sparks, R.E. and Sandusky, M.J. 1981. Fish food decline - Illinois River and Mississippi River. Final Report, 3-291-R, Illinois Department of Conservation, Springfield, IL, 63 p.
- Spear, P.A. and Pierce, R.C. 1979. Copper in the aquatic environment: chemistry, distribution, and toxicology. National Research Council of Canada, Ottawa, Ont. NRCC Report 16454. 227 pp.
- Spehar, R.L., and Carlson, A.R. 1984. Derivation of site-specific water quality criteria for cadmium and the St. Louis River basin. NTIS PB84-153196, 52 p.
- Spehar, R.L., Leonard, E.N., and Defoe, D.L. 1978. Chronic effects of cadmium and zinc mixtures on flagfish (Jordanelia floridae). *Trans. Am. Fish. Soc.* 107, 354-360.
- Sprague, J.B. 1968. Promising anti-pollutant: Chelating agent NTA protects fish from copper and zinc. *Nature* 220, 1345-1346.
- Standard Methods. 1985. Standard methods for the examination of waters and wastewaters. American Public Health Association, Washington D.C. 1134 p.
- State of Illinois. 1986. Rules and regulations, Title 35: Environmental protection. Subtitle C: Water pollution, Chapter 1. Pollution Control Board, 44 p.
- Stauber, J.S. and Florence, T.M. 1985. The influence of iron on copper toxicity to the marine diatom, Nitzschia closterium (Ehrenberg) W. Smith. *Aquat. Toxicol.* 6, 297-305.
- Stendahl, D.H. and Sprague, J.B. 1982. Effects of water hardness and pH on vanadium lethality to rainbow trout. *Water Res.* 16, 1479-1488.
- Stephan, C.E. 1977. Methods for calculating an LC50. ASTM STP 634, Amer. Soc. Testing Materials, Philadelphia, PA, p. 65-84.
- Stephenson, R.R. 1983. Effect of water hardness, water temperature, and size of the test organism on the susceptibility of the freshwater shrimp, Gammarus pulex (L.) to toxicants. *Bull. Environ. Contam. Toxicol.* 31, 459-466.
- Stockner, J.Q. and Antia, N.J. 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients, and pollutants - A warning. *Jour. Fish. Res. Board Can.* 33,

2089-2096.

- Stokes, P.M. 1975A. Adaptation of green algae to high levels of copper and nickel in aquatic environments. Symposium Proceedings on International Conference on Heavy Metals in the Environment, Toronto, Ca. Vol. II, 137-154.
- Stokes, P. 1975B. Uptake and accumulation of copper and nickel by metal tolerant strains of Scenedesmus. Verh. Internat. Verein. Limnol. 19, 2128-2137.
- Stokes, P. and Hutchinson, C. 1976. Copper toxicity to phytoplankton, as affected by organic ligands, other cations and inherent tolerance of algae to copper. Workshop on Toxicity to Biota of Metal Forms in Natural Water IJC, Great Lakes Research Advisory Board, Windsor, ON, Can. 325 p.
- Stokes, P.M., Hutchinson, T.C., and Krauter, K. 1973. Heavy metal tolerance in algae isolated from contaminated lakes near Sudbury, Ontario. Can. J. Bot. 51, 2155-2168.
- Storch, T.A. 1977. Iron-algae interaction as a factor in Lake Erie water quality. Sea Grant Report, Environ. Resources Center, State U. of N.Y. at Fredonia, N.Y.
- Stotzky, G. 1980. Physicochemical factors that affect the toxicity of heavy metals to microbes in aquatic systems. Aquatic Microbial Ecology (ed. Colwell, Foster, and Ahearn). Univ. Maryland, College Park, MD, 181-203.
- Sugiura, K., Goto, M., and Kurihara, Y. 1982. Effect of copper stress on an aquatic microcosm: a holistic study. Environ. Res. 27, 307-315.
- Sunda, W.G., Engel, D.W., and Thuotte, R.M. 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp, Palaemonetes pugio: Importance of free cadmium ion. Environ. Sci. Tech. 12, 409-413.
- Sunda, W. and Guillard, R.R.L. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. Jour. Mar. Res. 34, 511-529.
- Sunda, W.G. and Lewis, J.M. 1978. Effect of complexation by natural organic ligands on the toxicity of copper to a unicellular alga, Monochrysis lutheri. Limnol. Oceanog. 23, 870-876.
- Sykora, J.L., Synak, M., and Smith, E.V. 1972. Effect of lime-neutralized iron hydroxide suspensions on juvenile brook trout (Salvelinas fontinalis. Mitchill). Water Res. 6, 935-950.
- Taylor, D. 1982. A review of the lethal and sub-lethal effects of cadmium on aquatic life. Cadmium, 81, Cadmium Association, London, 75-81.

- Taylor, D. 1983. The significance of the accumulation of cadmium by aquatic organisms. *Ecotoxicol. Environ. Saf.* 7, 33-42.
- Taylor, G.J. and Crowder, A.A. 1983. Uptake and accumulation of heavy metals by Typha latifolia in wetlands of the Sudbury, Ontario region. *Can. Jour. Bot.* 61, 63-73.
- Taylor, M.C. and Demayo, A. 1980. Guidelines for surface water quality, Vol. 1, Inorganic chemical substances. Zinc. Inland Water Directorate, Water Quality Branch, Ottawa, Canada, 59 P-
- Thorp, V.J. and Lake, P.S. 1974. Toxicity bioassays of cadmium on selected freshwater invertebrates and the interaction of cadmium and zinc on the freshwater shrimp, Paratva tasmaniensis Fiek. *Aust. J. Mar. Freshwater Res.* 25, 97-104.
- Threader, R.W. and Houston, A.H. 1983. Use of NaCl as a reference toxicant for goldfish. *J. Fish. Aquat. Sci.* 40, 89-92.
- Thurman, D.A. 1981. Mechanism of metal tolerance in higher plants. *Effects of Heavy Metal Pollution on Plants*. Vol. 2, Metals in the Environment. N.W. Lepp (ed.) *Appl. Sci.*, London, p. 239-257.
- Truhaut, R., Ferard, J.F., and Jonany, J.M. 1980. Cadmium IC₅₀ determinations on Chlorella vulgaris involving different parameters. *Ecotoxicol. Environ. Saf.* 4, 215-223.
- Turner, M.A. and Rudd, J.W.M. 1983. The English-Wabigoon river system. II. Suppression of mercury and selenium bioaccumulation by suspended and bottom sediments. *Can. Jour. Fish. Aq. Sci.* 40, 2218-2227.
- U.S. Environmental Protection Agency. 1982. Water quality standard handbook. Draft. Chapter 3: Guidelines for deriving site-specific water quality criteria for the protection of aquatic life and its uses, p. 3-1 to 3-41, with Appendix.
- Van Weers, A.W. 1975. The effect of temperature on the uptake and retention of Co60 and Zn65 by the common shrimp Crangon crangon CD. In *Combined Effects of Radioactive, Chemical and Thermal Releases to the Environment*, Symposium Proc, Stockholm, p. 35-49.
- Van der Werff, M. and Pruyt, M.J. 1982. Long-term effects of heavy metals on aquatic plants. *Chemosphere* 11, 727-739.
- Varini, P.G., Baudo, R., Galanti, G., and Guglielmucci, G. 1981. Toxicity of chemical forms of copper and chromium. Part II. Acute mortality of Daphnia magna Straus. *Memorie dell'Istituto Italiano di Idrobiologia* 39, 263-280.
- Verma, S.R., Chand, R., and Tonk, I.P. 1985. Effects of environmental and biological variables on the toxicity of

- mercuric chloride. *Water, Air, Soil Poll.* 25, 243-248.
- Vinot, H. and Larpent, J.P. 1984. Water pollution by uranium ore treatment works. *Hydrobiologia* 112, 125-129.
- Wang, H.K. and Wood, J.M. 1984. Bioaccumulation of nickel by algae. *Environ. Sci. Tech.* 18, 106-109.
- Wang, W. 1980. Fractionation of sediment oxygen demand. *Water Res.* 14, 603-612.
- Wang, W. 1983. The acute effects of iron and EDTA on oxygen production by algal communities. *Environ. Intl.* 9, 307-312.
- Wang, W. 1984. Editorial: Use of aquatic plants for ecotoxicology. *Environ. Intl.* 10(3), i-iii.
- Wang, W. 1985. Interaction of iron and zinc toxicity on algal community. *ASTM STP 854*, Amer. Soc. Testing Materials, 187-201.
- Wang, W. 1986A. Acclimation and response of algal communities from different sources to zinc toxicity. *Water, Air, Soil Poll.* 28, 335-349.
- Wang, W. 1986B. Toxicity tests of aquatic pollutants by using common duckweed. *Environ. Poll. (CB)*, 11, 1-14.
- Wang, W. 1986C. The effect of river water on phytotoxicity of barium, cadmium, and chromium ions. *Environ. Poll. (CB)* 11, 193-204.
- Wang, W. 1986D. Proposed new standard guide for conducting static toxicity tests with duckweed. *American Society for Testing & Materials. Draft*, 32 p.
- Weis, J.S. 1980. Effect of zinc on regeneration in the fiddler crab, *Uca pugilator* and its interactions with methylmercury and cadmium. *Marine Environ. Res.* 3, 249-255.
- Welsh, R.P.H. and Denny, P. 1980. The uptake of lead and copper by submerged aquatic macrophytes in two England lakes. *Jour. Ecology* 68, 443-455.
- Whitton, B.A., Say, P.J., and Jupp, B.P. 1982. Accumulation of zinc, cadmium, and lead by the aquatic liverwort *Scapania*. *Environ. Poll. (B)*, 3, 299-316.
- Whyte, A.V. and Burton, I. (ed.) 1980. *Environmental Risk Assessment*. SCOPE Report 15, Wiley & Sons, 157 p.
- Wilson, R.C.H. 1972. Prediction of copper toxicity in receiving waters. *Jour. Fish. Res. Board Can.* 29, 1500-1502.
- Wilson, W.B. and Freeberg, L.R. 1980. Toxicity of metals to marine phytoplankton cultures. *NTIS EPA-600/3-80-025*, 92 p.

- Winner, R.W. 1984. Toxicity and bioaccumulation of cadmium and copper as affected by humic acid. *Aq. Toxicol.* 5, 267-274.
- Wong, P.T.S., Chau, Y.K., and Luxon, P.L. 1978. Toxicity of a mixture of metals on freshwater algae. *Jour. Fish. Res. Board Can.* 35; 479-481.
- Wong, S.L. 1985. Algal assay evaluation of trace contaminants in surface water using the nonionic surfactant, Tiston X-100. *Aq. Toxicol.* 6, 115-131.
- Wong, S.L. and Beaver, J.L. 1980. Algal bioassays to determine toxicity of metal mixtures. *Hydrobiologia* 74, 199-208.
- Wood, J.M. and Wang, H.-K. 1983. Microbial resistance to heavy metals. *Environ. Sci. Tech.* 12, 582A-590A.
- Zitko, V., Carson, W.V., and Carson, W.G. 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic salmon in the presence of humic acid by cupric ion electrode. *Bull. Environ. Contam. Toxicol.* 10, 265-271.