

THE ACUTE TOXICITIES OF ANTIMYCIN A
AND JUGLONE TO SELECTED AQUATIC ORGANISMS

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ABSTRACT

This study was performed to determine the toxicity of antimycin to aquatic populations, both pelagic and benthic. Test organisms included clams, crayfish, frogs, scuds and caddisflies. Lethal concentration 50 (LC50) values were found for varying water qualities and formulations of antimycin. Results indicated that antimycin was less toxic to these organisms, except for scuds, than to fish. Antimycin was toxic to scuds at piscicidal concentrations.

The toxicity of juglone to carp, green sunfish, channel catfish and phytoplankton was determined. The efficacy of juglone was measured in the presence of sediments and after exposure to ultra-violet and visible light.

Channel catfish were found to be the most sensitive to juglone, and carp the least sensitive. Both the presence of sediments and ultra-violet light lowered the toxicity of juglone to fish. Visible light showed an insignificant change in the efficacy of juglone. Growth of the phytoplankton was inhibited by concentrations of juglone that were toxic to fish.

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INTRODUCTION

The development of fish farming and the management of ponds and lakes on a "put and take" basis has brought about an increased need for chemical compounds which can be used to control fish populations. The two compounds used in the present study as fish toxicants were juglone (5-hydroxy-1, 4-naphthoquinone) and antimycin A. Both compounds are considered desirable because they are natural products, are highly toxic to fish at low concentrations, and are readily degradable.

Due to physical, chemical and biological characteristics of juglone, it was thought that juglone possessed the potential to become a registered fish toxicant. Thus, one of the objectives of this study was to acquire data which could be used to support the registration of juglone.

Skepticism on the part of some scientists has brought about concern as to the effect of antimycin A on non-target organisms. The main objective of research pertaining to antimycin was to produce data on the toxicity of antimycin to selected representatives of the aquatic ecosystem excluding fish.

The concentrations and forms of juglone which exist in solution can be affected by physical and chemical parameters. Therefore, an analytical procedure for determining the presence of juglone in the aquatic environment was derived. Visible and ultra-violet spectrum analyses

were performed to obtain a standard curve.

By use of stock solutions and serial dilutions, the toxicity of juglone to three species of fish was tested. The fish included carp, Cyprinus carpio, green sunfish, Lepomis cyanellus, and channel catfish, Ictalurus punctatus. The reduction in the standing crop of two species of green algae (Chlorella vulgaris and Chlamydomonas sp.) was also determined in the presence of juglone.

Together with toxicity studies, the effect of several physical parameters on juglone was tested in the laboratory. Parameters selected which would most likely effect the efficacy of juglone were ultra-violet light, visible light and three types of sediment.

Research involving antimycin included study of toxicity to selected aquatic organisms. Frogs, crayfish, clams, scuds, and caddisflies were all tested to determine their sensitivity to antimycin. Two formulations of antimycin were used including a liquid and a powder. However, the active antimycin in each formulation was being tested, not the formulation itself.

LITERATURE REVIEW

JUGLONE

Juglone (5-hydroxy-1, 4-napthoquinone) was first isolated from the walnut pericarp as a yellow, crystalline compound by Vogel and Reischauer in 1856. At the time, they called the compound nucin. Mylius (1885) reported that the walnut did not contain juglone, but contained reduced forms of juglone (α and β -hydrojuglone). Westfall et al. (1961) comments that even before this time, walnut hulls were a part of the folk history of the United States in that they were used successfully as both medicines and poisons.

Juglone can be extracted from three species of walnut: Juglans cinerea; J. nigrans; J. regia. The bark of the hickory, Carya ovata is also known to contain juglone. Stecker (1960) has synthetically produced juglone by the oxidation of 1,5-dihydroxynapthalene.

Considered both a chemically and biologically active compound, juglone has been the source of a considerable amount of research. Daghish (1950a) demonstrated that the compound most often existing in the walnut pericarp is the 5-glucoside of α -hydrojuglone. His results showed seasonal and tissue variation in the concentrations of hydrojuglone glucoside present. The highest concentrations

have been found in the winter buds and catkins. With this evidence at hand, he theorized that the hydrojuglone glucoside may have use in the oxidation-reduction systems associated with increased cell metabolism.

Daglish (1950b) has also shown that hydrojuglone glucoside readily undergoes hydrolysis to form α -hydrojuglone and glucose. Once this occurs, hydrojuglone is easily oxidized in aqueous media or in air to produce 1,4,5-naphthoquinone (Gries, 1943a). Prolonged exposure to air reduces juglone to α -hydrojuglone.

As a biological agent, juglone has shown toxicity to most organisms from bacteria to higher plants and mammals. According to Westfall et al. (1961), juglone is the only biologically active agent in the walnut.

Gries (1943a) has shown that juglone is toxic to both bacteria and fungi. Massey (1925) found that tomatoes would wilt and die when planted in close proximity to walnut trees. Davis (1928) and Gries (1943b) observed the toxicity of juglone to tomatoes and alfalfa; Bode (1958) found the same with tomatoes and tobacco. However, MacDaniels and Muenscher (1941), have revealed that juglone showed no inhibition to tomatoes.

Daglish (1950a) believes that these discrepancies are due to the compound present in the walnut. Only juglone shows toxic effects. MacDaniels and Muenscher (1941) speculated that soil texture, aeration and moisture may relate to the toxicity of juglone.

Gilbert et al. (1967) proved juglone in the bark of the hickory to be a deterrant to feeding by the elm bark beetle, Scolytus multistriatus. Fish were found to be sensitive to juglone in low concentrations by Marking (1970). Exposure of nine species of fish to juglone revealed that the 96 hour LC50 values ranged from 27 to 88 parts per billion. He also indicated that water temperature and water quality had little or no effect on the toxicity of juglone.

Research with mammals has shown that juglone acts as a depressant to mice, rats and rabbits (Westfall et al., 1961). After working with dogs, Boelkins et al. (1968) has found that ten mg. of juglone per kg. of body weight was fatal. Five mg. showed only histopathic changes in the liver and lungs. He concluded that juglone was toxic to cell membranes; therefore, increasing capillary permeability. Bhargava and Westfall (1968) have shown that juglone decreases tumor growth rates.

ANTIMYCIN

Antimycin is a nitrogenous, phenolic compound which was first isolated from the bacteria Streptomyces sp. The role of antimycin was found to be an antifungal antifubiotic. Dunshee et al. (1949), Strong (1956) and Lockwood et al. (1954) all isolated the antibiotic from Streptomyces sp. At least seven species of Streptomyces are known to produce antimycin.

In Japan, Watanebe et al. (1957) and Harada et al.

(1959) found another antibiotic similar to antimycin. Isolated from S. blastmyceticus, the new antibiotic was called blastmycin or antimycin A₃.

The isolation of this antibiotic led to the determination of the physical and chemical properties of antimycin. Dunshee et al. (1949), Strong (1956) and van Temelen et al. (1961) were instrumental in revealing the structural nature of antimycin. The empirical formula of antimycin was found to be C₂₈ H₄₀ N₂ O₉. The structural formula is indicated in figure 2.

According to Keitt et al. (1953), antimycin is relatively insoluble in water but is completely soluble in acetone, ethanol and chloroform. Although insoluble in water, antimycin will undergo hydrolytic degradation in alkaline waters. Research by van Temelen et al. (1961) has shown that the alkaline degradation of antimycin will form blastmycic acid plus antimycin lactone (figure 2). Another possible mode of alkaline degradation occurs at the hydroxy radical. Under alkaline conditions, the H⁺ ion goes into solution leaving an O⁻.

Derse and Strong (1963) agree with these data. They believe that complete degradation of 10 ppb antimycin occurs in seven days. Marking and Dawson (1971), however, demonstrated that the time of degradation is dependent on the pH. The biological half-life of antimycin at pH 6 and 6.5 is 310 hours, whereas at pH 10, it is reduced to 1.5 hours. They also found that antimycin is deactivated

more readily at higher temperatures.

Antimycin is one of the few compounds whose biological activity is known. Strong (1956) reports that antimycin is a respiratory inhibitor. Specifically, antimycin prevents the transfer of electrons from cytochrome b to cytochrome c on the electron transport system. Schoettger and Svendsen (1970) demonstrated that in vivo, the respiration of the brain was more sensitive than either the liver or kidney.

The toxicity of antimycin to most organisms has been well established. Antimycin appears to be non-toxic to bacteria, but is extremely toxic to fungi. Walker et al. (1964) found that antimycin was highly toxic to fish. They found that three members of the family Ictaluridae were most resistant to antimycin. The LC50 of these fish averaged near 50 ppb. They also reported that plankton, aquatic plants, benthic fauna, salamanders, tadpoles and turtles were not harmed by piscicidal concentrations. Gilderhus (1969) agrees that concentrations which were toxic to fish were not detrimental to nontarget fauna.

METHODS AND MATERIALS

The juglone used in these experiments was obtained from Aldrich Chemicals, Inc. of Milwaukee, Wisconsin. Approximately 98% pure, the juglone was considered to be analytical grade. Since juglone is relatively insoluble in water, equal volumes of acetone and water were used to place juglone in solution.

Research pertaining to antimycin was performed with two formulations of antimycin. Antimycin powder was approximately 95.5% active antimycin. Antimycin liquid was used in the form of Liquid Fintrol Concentrate^{R1} (10% active antimycin). Both forms of antimycin were produced by Ayerst Laboratories Inc., New York, New York. All the stock solutions of antimycin were made up to be 100% active antimycin.

Antimycin, like juglone, is relatively insoluble in water. However, antimycin is also unstable in water. Stock solutions containing antimycin were obtained by using acetone as the sole solvent. All forms of antimycin were refrigerated, due to the fact that antimycin is susceptible

^{R1} Registered Trademark - Ayerst Laboratories Inc., New York, New York.

to degradation at higher temperatures and in the presence of light. It was also suggested that the stock solutions be made up no sooner than 24 hours before a test.

Stock solutions of antimycin and juglone were made using the following relationship:

0.1 gm. of solute per 100 ml. of solvent. A one ml. aliquot of this stock solution introduced into one liter of water in the bioassay vessel produced a concentration of one part per million. To avoid excessive error, these additions to the vessel were made such that no more than five ml. of the stock solution were added per liter of water.

WATER CHEMISTRY

All water used for the bioassays was reconstituted distilled water. Reconstituted water was classified into four types: very soft; standard; hard; very hard (Table 1). Periodic water analyses (EDTA hardness, pH, total alkalinity) were run to assure the correct composition of the water.

All the reconstituted water was mixed in two 200 liter containers with inert plastic liners. Salts were added in varying amounts to achieve the desired water quality. Prior to use, the water was aerated for several hours. A water temperature of 13° C was maintained throughout the duration of this study.

BIOASSAY PROCEDURES

Static bioassay procedures used were similar to those

of Lennon and Walker (1964). Several modifications were made due to the facilities at hand.

All testing was performed in one and five gallon glass jars. The volume of water contained in each vessel was dependent on specimen size. To prevent overloading, no more than one gm. of test organisms was used per liter of water; therefore, one, ten, and fifteen liter vessel sizes were employed.

Test organisms were added to the vessels and allowed to acclimate for 24 hours. At this time, the toxicant was introduced in appropriate amounts. Observations of mortality were then made at 1, 3, 6, 24, 48, 72, and 96 hours. Dead test organisms were removed after each observation and incinerated.

After testing, the vessels were filled with tap water containing 25 gm. of charcoal. The jars were allowed to stand several days before dumping. The jars were washed with Deox^{R2} fortified with hydrochloric acid, tap water, and distilled water prior to reuse.

EFFICACY STUDIES WITH JUGLONE

Spectrophotometric analysis of water for the presence of juglone was done with a Gilford spectrophotometer (Model 240). The visible and ultra-violet wavelengths were scanned for

^{R2} Registered Trademark - National Chemsearch Laboratories, Irving, Texas.

a maximum absorbance peak.

The uptake of juglone by sediments was tested by adding silt, sand, and organic clay separately. Two-hundred gm. of sediment, 500 ml. of water and five ppm juglone were added to an Erlenmeyer flask. All flasks were maintained at 12° C. For each sediment type, three static flasks and three agitated flasks were maintained. The contents of each flask were analyzed for juglone at 8 and 48 hours.

The toxicity of juglone to fish was also tested in the presence of a organic sediment. In the lab, the sediment was blended, autoclaved and dried. Fifty gm. of sediment were added to ten liters of water. The sediment was allowed to settle for 24 hours. Fish were added and standard bioassay procedures were employed.

The efficacy of juglone was also tested after exposure to visible and ultra-violet light. Stock solutions of juglone were made such that a ten ml. aliquot contained 180 ppb when added to ten liters of water. The ten ml. aliquots were added to Petri dishes and were covered with saran wrap. Control dishes were covered with aluminum foil. The light sources were a General Electric UV Sunlamp (1000 lux) and a 300 watt incandescent clear bulb.

After the 96 hours had elapsed, the contents of each Petri dish were emptied into separate bioassay vessels. Standard bioassay procedures were then employed. The degree of degradation was determined by the toxicity of the juglone in each Petri dish.

The toxicity of juglone to two species of green algae was determined. Chlorella vulgaris and Chlamydomas sp. in pure culture, were grown on Bold's Basal medium for ten days. The cultures were placed in an environmental chamber in a 12 hour light and dark cycle at 23° C.

One ml. of culture, appropriate amounts of toxicant, and 200 ml. of Bold's Basal medium were added to a 500 ml. Erlenmeyer flask. The new cultures were incubated for eight days at 23° C on a 12 hour light and dark cycle. After eight days, the cultures were centrifuged for five minutes at 5700G. The centrifugate was dried, weighed and ashed. Reduction in standing crop was expressed as mg. ash free dry weight.

TEST ORGANISMS

The test organisms in this study included carp, Cyprinus carpio, green sunfish, Lepomis cyanellus, channel catfish, Ictalurus punctatus, leopard frog, Rana pipiens, crayfish, Orconectes immunis, clams, Lampsilis recta, scuds, Gammarus limnaeus, and caddisfly, Hesperophylax sp. The fish were obtained from the Fish Control Laboratory in La Crosse, Wisconsin. Frogs, clams and crayfish were purchased from Nasco, Inc., Fort Atkinson, Wisconsin. Scuds and caddisfly larvae were collected from Coon Valley Creek, Vernon County, Wisconsin. Procedures for handling the fish were those of Hunn et al. (1968). Food was withheld from the fish 96 hours before the test, and the fish were allowed to acclimate to the reconstituted water for 24 hours before the test. To

prevent overloading and for valid statistical analyses, ten fish were added to each jar. If more than 10% of the control died, the test was invalidated.

Twenty scuds were used in each jar. Ten organisms of all other species were employed.

STATISTICAL ANALYSES

Toxicology data obtained in this study were analyzed according to the methods of Litchfield and Wilcoxon (1949). Using log-probit graph paper, a linear relationship was obtained between dosage and mortality. From this regression line, values were obtained to determine a chi-square value, and this single value insured the validity of the test.

Also from the regression line, the LC_{16} , LC_{50} , and LC_{84} were obtained. With the use of computer, the 95% confidence intervals were calculated for 24 and 96 hour readings.

RESULTS

The maximum absorbance peak for juglone occurred at 264nm. Ultra-violet spectrum analyses proved more fruitful than visible spectrum analyses since absorbancy was unaffected by the pH of the solution. A standard curve was obtained by using known concentrations of juglone analyzed at 264nm (Figure 3). A linear relationship was obtained when concentration vs. absorbancy was plotted on semi-log paper. Although the plot remains linear, the slope changes above and below 500 ppb. Concentrations below 20 ppb produced erratic results.

TOXICITY OF JUGLONE TO FISH

The toxicity of juglone to three species of fish was determined by using the static bioassay (Table 2). Of the three, channel fish, Ictalurus punctatus appeared to be the most sensitive to juglone. The LC50 values were 47.0 and 40.0 ppb at 24 and 96 hours respectively. Carp, Cyprinus carpio were the most resistant. The LC50 values for 24 and 96 hours in standard water were 57.0 and 53.0 respectively. The differences in sensitivity however, were insignificant.

The LC50 values were somewhat greater in the hard or alkaline waters for all species (Table 2). Although the difference appeared to be appreciable, it can be

considered significant. Therefore, the efficacy of juglone is not changed by alkaline or high pH waters.

ABSORPTION OF JUGLONE BY SEDIMENT

Reduction in the concentration of juglone was found to occur in all three flasks and with each sediment type (Figures 4, 5, and 6). The flasks containing the organic sediment showed the most reduction (Figure 6).

Little or no reduction in the concentration of juglone occurred after eight hours. However, after 48 hours, the concentration of juglone was reduced to half in the static vessels and approximately one-fifth in the agitated samples. Reduction was even noted in the aged or control flasks.

Not only was the concentration of juglone reduced in the presence of a sediment, but the toxicity of juglone to fish was greatly reduced (Table 3). The concentrations needed to kill carp and green sunfish nearly doubled in the presence of an organic sediment. The LC50 for carp at 96 hours without sediment was 72.0 ppb and this was raised to 117.0 ppb with sediment. The values for green sunfish were 53.0 ppb and 107 ppb respectively. Twenty-four hour readings were similar (Table 3).

OXIDATION OF JUGLONE BY LIGHT

Exposure of juglone to ultra-violet light drastically reduced the efficacy of juglone (Figure 7). Solutions exposed to ultra-violet light for 36 and 58 hours possessed no killing power. Solutions exposed for 1, 8, and 16 hours

were quite toxic to carp at 24 hours.

The control solutions of juglone remained toxic to carp after 58 hours of aging. However, aging did reduce the toxicity of juglone (Figure 7). Visible light had an insignificant effect on the toxicity of juglone (Figure 7). The line representing exposure to visible light was nearly parallel to the control.

TOXICITY OF JUGLONE TO PHYTOPLANKTON

Chlamydomas sp. and Chlorella vulgaris were extremely sensitive to juglone (Figure 8). Concentrations of juglone below ten ppb were non-toxic to algae. However, concentrations of 75 ppb reduced the standing crop by 50%. A complete reduction was noted with concentrations approaching 150 ppb.

TOXICITY OF ANTIMYCIN

Scuds, Gammarus limnaeus, were found to be most sensitive to antimycin (Table 4). The LC50 for 96 hours was approximately 500 parts per trillion. This value coincided with piscicidal concentrations (Figure 9).

The caddisfly, Hesperophylax sp., was much more sensitive at 96 hours than at 24 hours (Table 4 and 5). The average LC50 at 96 hours was approximately ten ppb. Crayfish, Orconectes immunis, and clams, Lampsilis recta, were found to be completely resistant to antimycin (Tables 4 and 5). Three test runs produced similar results for both organisms. Concentrations up to five ppm were found to

be non-toxic to clams and crayfish.

The only aquatic vertebrate tested was the leopard frog, Rana pipiens. Concentrations producing a LC50 were similar to those of the caddisfly. At 24 hours, the LC50 averaged near 45 ppb and at 96 hours, ten ppb.

DISCUSSION

On the basis of this study, juglone appeared to be toxic to three representative genera at concentrations less than 150 ppb. These data agree with that of Marking (1970) who tested nine species of fish. Time and water quality have little effect on the toxicity of juglone to fish. Marking (1970) also states that juglone is degraded after seven days in alkaline water. However, this degradation does not take place before elimination of target organisms. Thus, juglone appears to be more promising than antimycin as a fish toxicant because it is relatively unaffected by temperature and alkalinity but is readily degradable. Also, channel catfish are highly sensitive to juglone. Further research is needed to determine the effect of juglone on the aquatic environment.

Once juglone is added to water, the fate of this compound is determined by the presence of sediment and exposure to ultra-violet light. Sediment studies have revealed that juglone is readily adsorbed onto the surface of sediment, regardless of composition, thus accounting for the reduction in concentration of juglone. Agitation of the vessel exposed more of the surface of the sediment; therefore, greater adsorption occurred.

Control flasks also showed some reduction in the concentration of juglone. This deduction can be explained

by adsorption of juglone to the sides of the glass vessel. This bound juglone was recovered by rinsing with acetone. However, a small quantity of juglone was still unaccounted for. It was thought that this juglone had undergone oxidation in the aqueous media.

Adsorption of juglone to the surface of sediments accounts the reduced toxicity to fish. Movement of the fish in the bioassay vessel suspended the sediment, thus increasing the adsorptive powers.

Adsorption of juglone by the sediment can also be controlled by the amount of oxygen present. It is well known that adsorption proceeds at a higher rate with oxidized sediment. Adsorption may not take place at all in the oxygen depleted waters of the hypolimnion.

Juglone was also shown to exhibit extreme toxicity to Chlorella vulgaris and Chlamydomonas sp. Fish-killing concentrations were shown to have a herbicidal effect on the algae. This data is important in determining the usefulness and fate of juglone. Incorporation of juglone into algal cells will reduce the concentration in solution. It is suspected that higher concentrations of juglone are needed to kill fish in natural waters than what was previously been reported.

Extended exposure to ultra-violet light reduces efficacy of juglone to Cyprinus carpio. It is suspected that juglone is oxidized by ultra-violet light. This oxidation, coupled with the spontaneous oxidation in

aqueous media, causes a considerable depletion in concentration; therefore, the toxicity of juglone is reduced.

Control flasks again demonstrated reduction in the efficacy of juglone. These results agree with those of Gries (1943a). Visible light caused an insignificant change in the efficacy of juglone.

The differences between visible and ultra-violet light cannot be readily explained. However, it is thought that the ultra-violet light increases the chemical activity of juglone.

Results obtained from the antimycin research proved to be erratic. Antimycin was toxic to Rana pipiens and Hesperophylax sp., but not in piscicidal concentrations. Gilderhus (1969) and Walker et al. (1964) both agree that antimycin is non-toxic to non-target organisms. Gilderhus, however, reports partial mortality of scuds exposed to concentrations of antimycin near 4.0 ppb. This is in conflict to the results obtained on Gammarus limnaerus. This discrepancy could be explained by the fact that the results of Gilderhus were obtained from a field test.

The most striking results were those obtained from Oronectis immunis and Lampsilis recta. Calculated concentrations of five ppm antimycin produced no effect. Although acetone was used as a carrier to place antimycin in solution, it was thought that concentrations of antimycin in the vessel did not exceed one or two ppm. This agreed with Keitt et al. (1953) who demonstrated that

antimycin is readily insoluble in water.

The mechanism of toxicity to the organisms tested has not been clearly determined. Derse and Strong (1963) demonstrated that antimycin was taken up at the gills and eventually inhibited respiration. Thus, differences in gill structure may cause differences in uptake of antimycin.

Strong (1956) reports that some higher animals can bypass the electron transport system, and therefore, be unaffected by antimycin. Cheah (1957) found that the cestode, Moniezia expansa possessed a branched electron transport system. Inhibition by antimycin in one system can be overcome. These two theories may perhaps be an explanation for the resistance of clams and crayfish to antimycin.

The toxicity of antimycin was reduced in hard water. This agrees with Marking and Dawson (1972). Alkaline degradation of antimycin in pH 8 waters is well established (van Temelen et al., 1961).

Toxicity studies with antimycin revealed two facts. One, both formulations of antimycin produced similar results. Two, it was noted from all the values that the LC50 was raised in hard water; antimycin was less toxic in hard water.

SUMMARY AND CONCLUSIONS

1. Juglone was determined to be an effective fish toxicant for Cyprinus carpio, Lepomis cyanellus, and Ictalurus punctatus. Juglone showed no species specificity.
2. Juglone can be readily adsorbed onto the surface of sand, silt and organic sediment particles. Agitation increases the rate of adsorption.
3. The toxicity of juglone to Cyprinus carpio and Lepomis cyanellus was reduced in the presence of an organic sediment.
4. Photooxidation of juglone occurs when exposed to ultra-violet light for more than 35 hours. Visible light did not effect juglone. Juglone does undergo spontaneous oxidation and concentration was reduced over a time period of 96 hours.
5. The efficacy of juglone to carp was effected by exposure to ultra-violet light.
6. Concentrations of juglone exceeding 100 ppb substantially reduced the standing crop of Chlorella vulgaris and Chlamydomas sp.
7. Concentrations of antimycin used to eradicate fish will also kill Gammarus limnaeus. Antimycin is toxic to this nontarget organism is piscicidal concentrations.
8. Antimycin was also toxic to Hesperophlax sp. and Rana pipens, but not in fish-killing concentrations. Antimycin was not toxic to Orconectes immunis or Lampsilis recta at concentrations of 5 ppm.
9. Antimycin degrades in alkaline waters; therefore, the LC50 values for hard water were higher than those

in standard water.

10. Antimycin powder and Liquid Fintrol Concentrate produced similar results to five organisms in standard and hard waters.

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Table 1. Characteristics of reconstituted waters used in static bioassays.

Water type	Salts added in mg per liter				pH range	Total hardness Expressed as ppm CaCO ₃
	NaHCO ₃	CaSO ₄	MgSO ₄	KCL		
Soft	12	7.5	7.5	0.5	6.4-6.8	10 - 13
Standard	48	30.0	30.0	2.0	7.2-7.6	40 - 48
Hard	192	120.0	120.0	8.0	7.6-8.0	160 - 180
Very Hard	384	240.0	240.0	16.0	8.0-8.4	280 - 320

Table 2. Toxicity of juglone to three species of fish at different water hardnesses and 13° C.

Species	Water hardness	LC50 and 95-percent confidence intervals in ppb at	
		24 hours	96 hours
Carp <u>Cyprinus carpio</u>	Soft	55.0 40.9 - 73.9	-
Carp	Standard	94.0 76.2 - 115.9	76.0 57.6 - 100.0
Carp	Standard	85.0 77.7 - 92.9	85.0 77.7 - 92.9
Carp	Hard	130.0 99.6 - 169.5	106.0 85.9 - 130.8
Carp	Very Hard	110.0 86.3 - 140.0	110.0 86.3 - 140.1
Channel catfish <u>Ictalurus punctatus</u>	Standard	47.0 42.6 - 51.7	40.0 36.9 - 45.1
Green sunfish <u>Lepomis cyanellus</u>	Soft	57.0 52.1 - 62.3	52.0 47.6 - 56.8
Green sunfish	Standard	57.0 54.2 - 59.9	53.0 48.9 - 57.4
Green sunfish	Hard	-	58.0 55.2 - 61.0
Green sunfish	Very Hard	62.0 57.5 - 66.8	56.0 52.8 - 59.4

Table 3. Toxicity of juglone to fish with and without organic sediment (50g/10 L) (standard reconstituted water, 13^o C.

LC-50 and 95-percent confidence intervals in ppb at

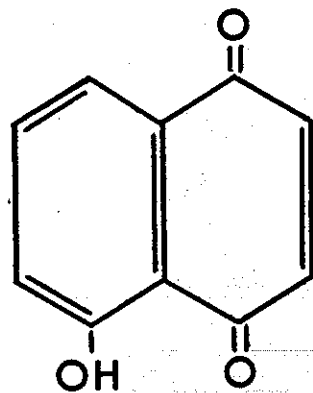
	24 hours		96 hours	
	With sediment	w/o sediment	w/ sediment	w/o sediment
Carp	108.0 104 - 113	79.0 63 - 97	117.0 109 - 125	72.0 57 - 94
Green Sunfish	109.0 99 - 119	57.0 54 - 60	107.0 99 - 115	53.0 49 - 57

Table 4. Toxicity of antimycin A (Liquid Fintrol Concentrate) to selected aquatic organisms. (13° C)

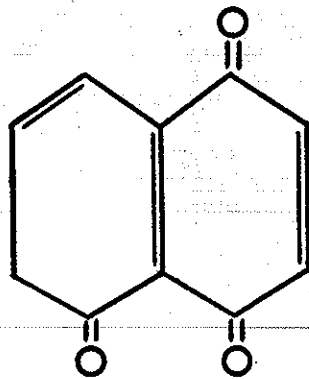
Species	Water hardness	LC50 and 95-percent confidence intervals in ppb at	
		24 hours	96 hours
Caddisfly <u>Hesperophylax</u> sp.	Standard	45.0 27.9 - 72.5	13.0 .8.0 - 21.0
Caddisfly	Hard	550.0 297.8 - 1015.8	14.6 10.3 - 20.7
Clams <u>Lampsilis recta</u>		Non-toxic at 5.0 parts per million	
Crayfish <u>Orconectes immunis</u>		Non-toxic at 5.0 parts per million	
Leopard Frog <u>Rana pipens</u>	Standard	48.0 32.2 - 71.4	3.8 2.6 - 5.6
Leopard frog	Hard	59.0 48.1 - 72.4	11.7 8.9 - 15.4
Scud <u>Gammarus limnaeus</u>	Standard	2.9 2.1 - 4.0	0.63 0.44 - 0.90
Scud	Hard	9.2 6.7 - 12.6	1.4 0.99 - 1.96

Table 5. Toxicity of antimycin A (antimycin powder-95.5%) to selected aquatic organisms. (13° C)

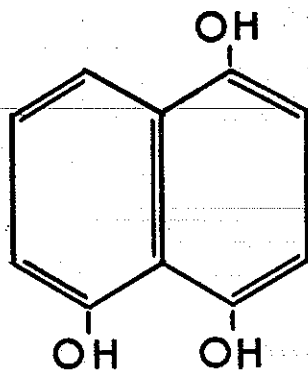
Species	Water hardness	LC50 and 95-percent confidence intervals in ppb at	
		24 hours	96 hours
Caddisfly <u>Hesperophylax</u> sp.	Standard	50.5 32.0 - 79.5	5.9 5.0 - 7.0
Caddisfly	Hard	175.0 107.2 - 285.6	7.1 6.4 - 7.8
Clams <u>Lampsilis recta</u>		Non-toxic at 5.0 parts per million	
Crayfish <u>Orconectes immunis</u>		Non-toxic at 5.0 parts per million	
Leopard frog <u>Rana pipeins</u>	Standard	32.5 23.4 - 45.1	8.5 6.6 - 10.9
Leopard frog	Hard	44.0 37.8 - 51.2	9.9 8.8 - 11.1
Scud <u>Gammarus limnaeus</u>	Standard	2.9 2.1 - 4.0	0.43 0.29 - 0.62
Scud	Hard	5.6 3.8 - 7.9	0.32 0.19 - 0.52



JUGLONE
5-HYDROXY-1,4-NAPTHOQUINONE

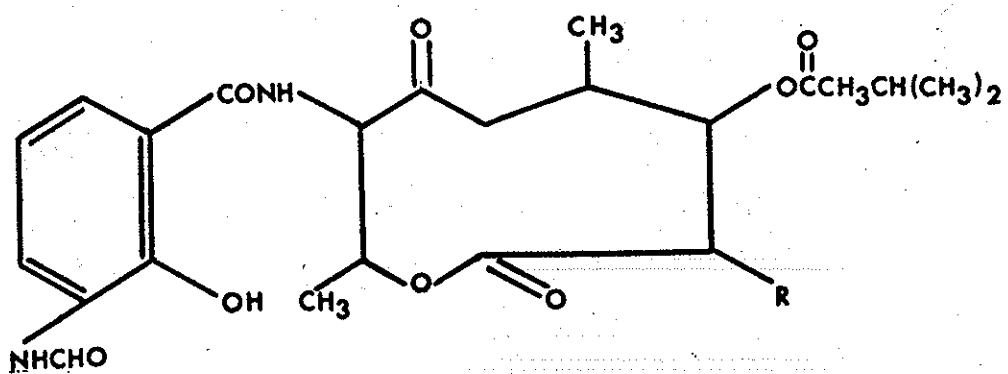


OXIDIZED JUGLONE
1,4,5-NAPTHOQUINONE



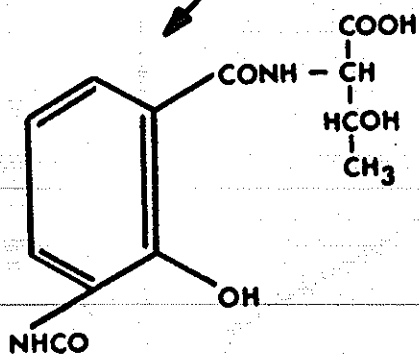
REDUCED JUGLONE
1,4,5-TRIHIDROXYNAPHTHALENE

Figure 1. Three common forms of juglone found in the walnut, soil or in solution.

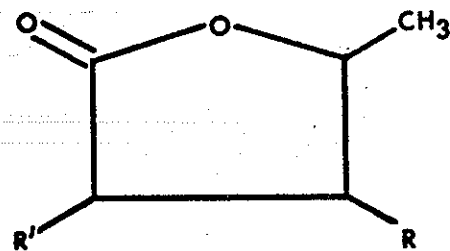


Antimycin A

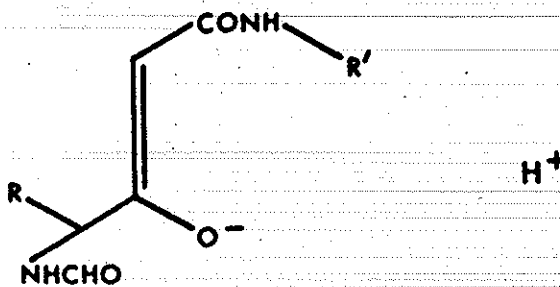
undergoes
hydrolytic cleavage
to form



Blastmycic acid



Antimycin lactone



possible site of ionization under alkaline conditions

Figure 2. Structure of antimycin A and possible modes of alkaline degradation.

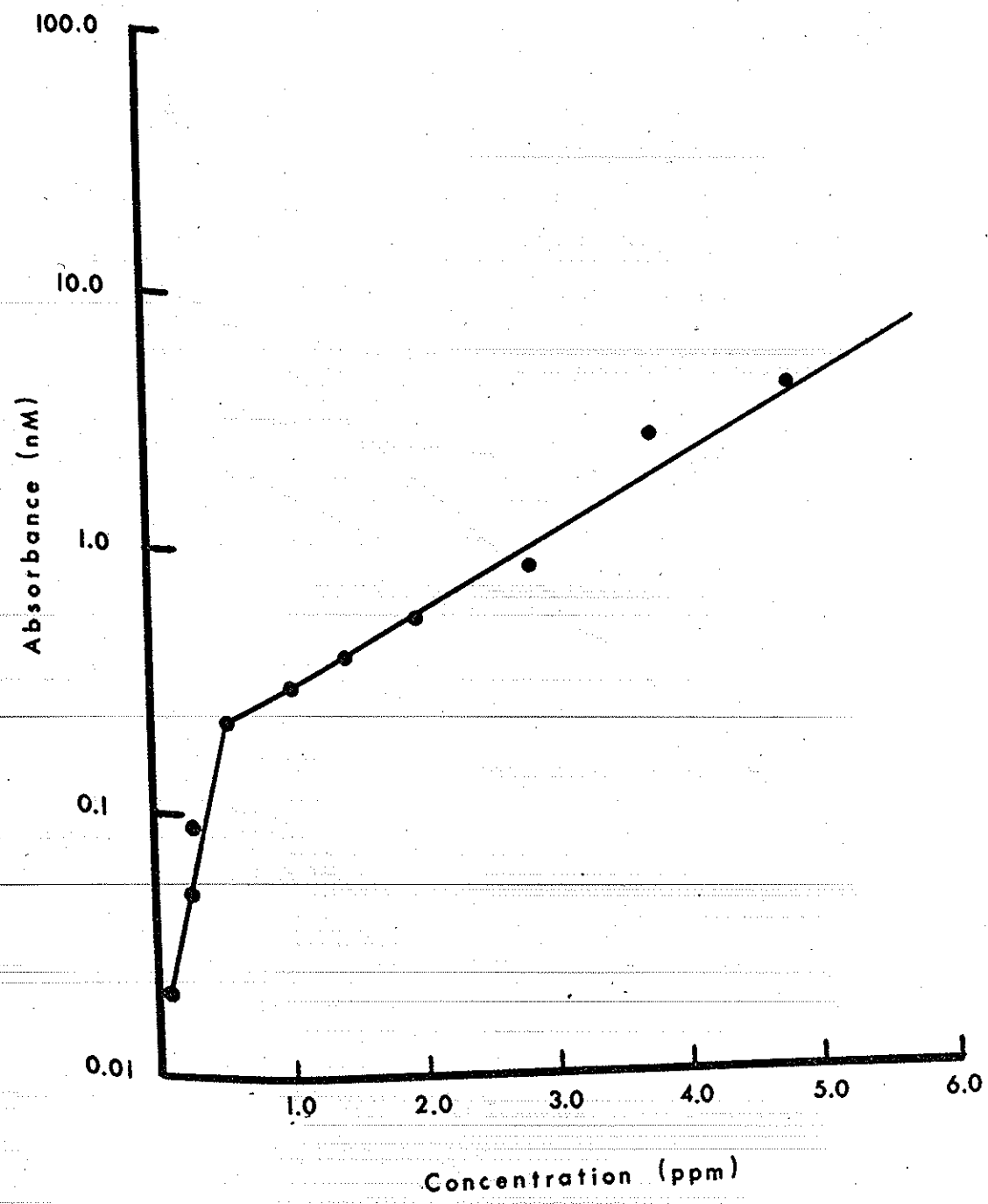


Figure 3. Absorbance (264 nM) vs. concentration of juglone

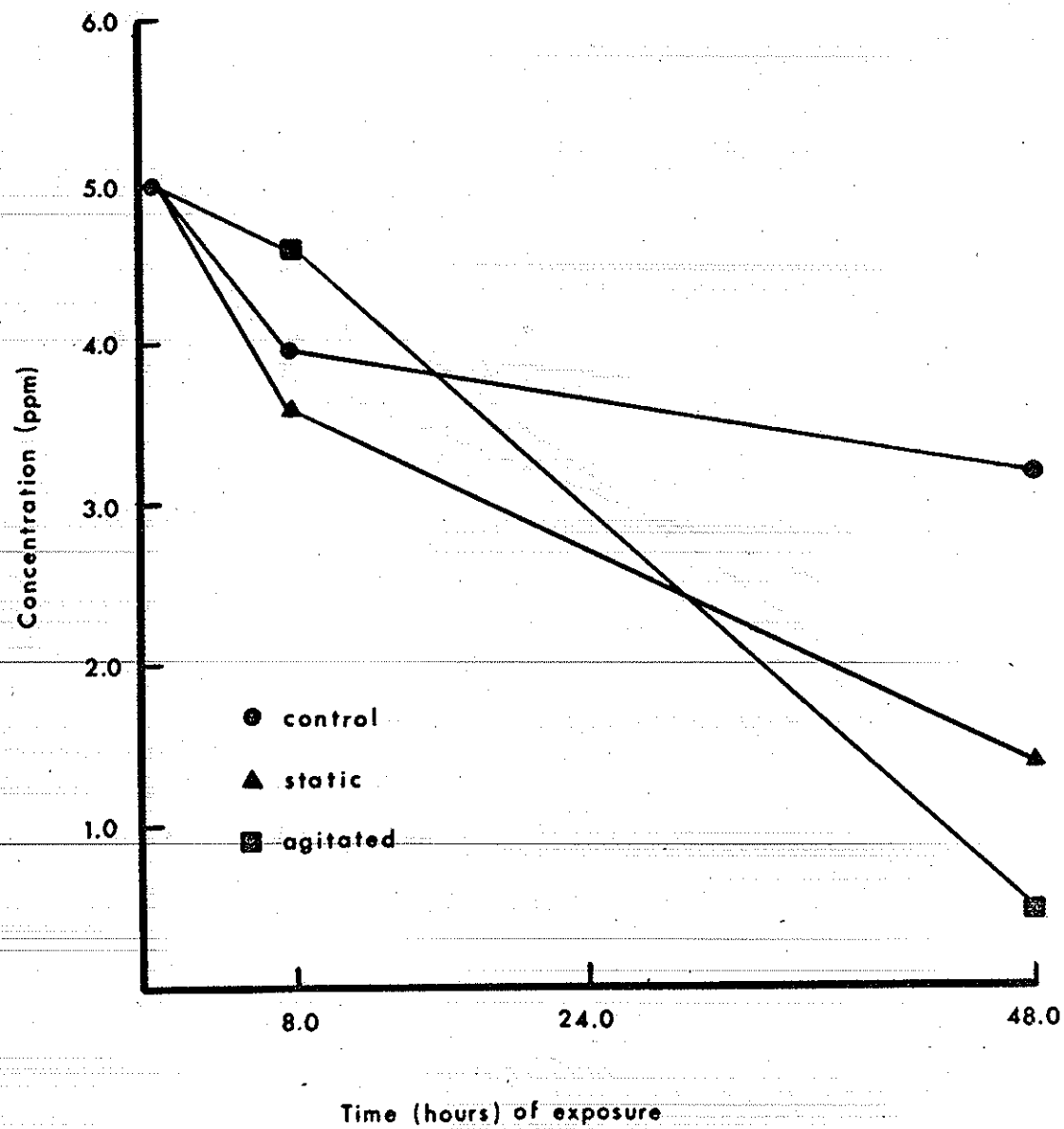


Figure 4. Reduction in the concentration of juglone in the presence of an organic sediment.

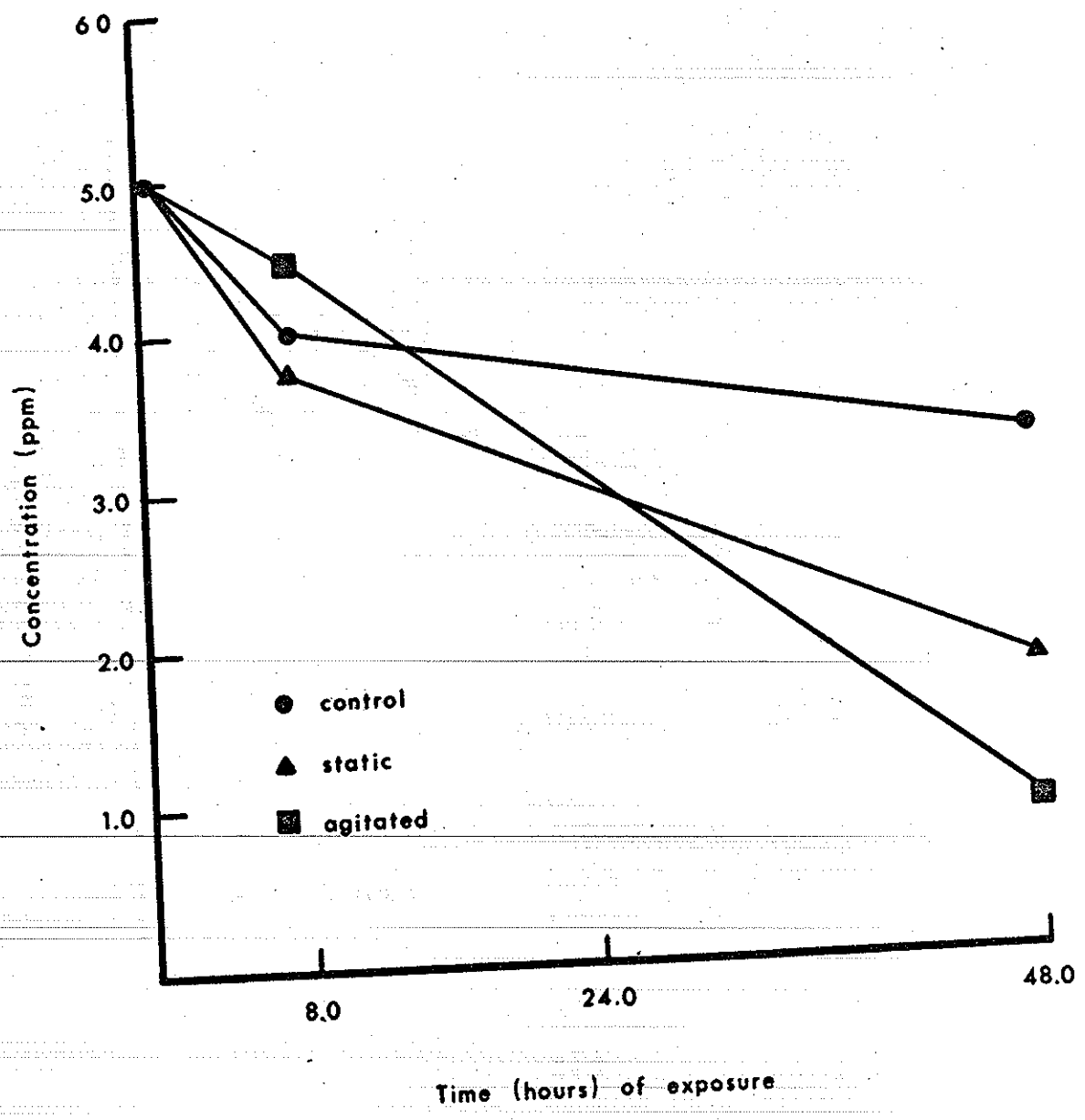


Figure 5. Reduction in the concentration of juglone in the presence of sand.

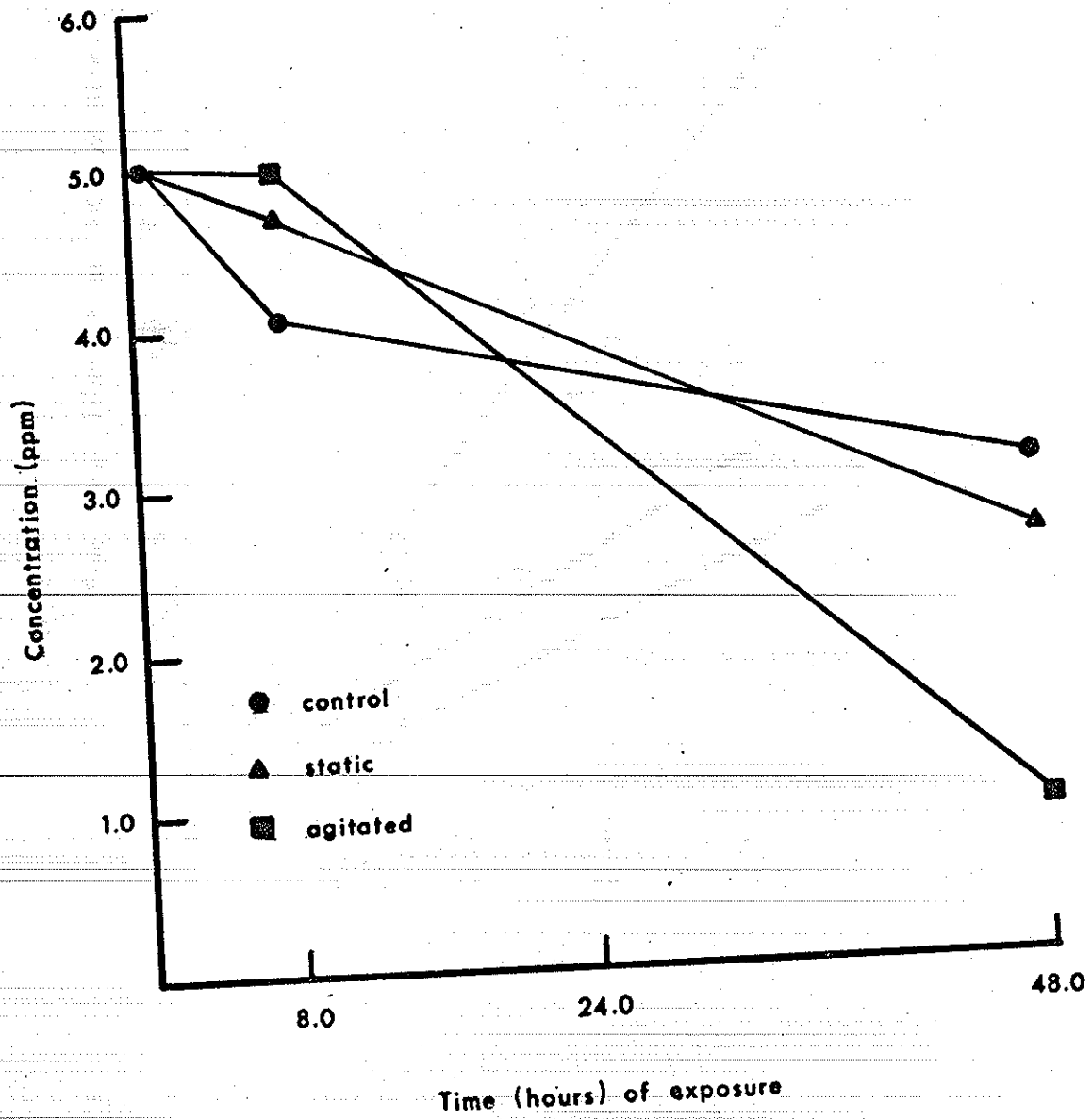


Figure 6. Reduction in the concentration of juglone in the presence of silt.

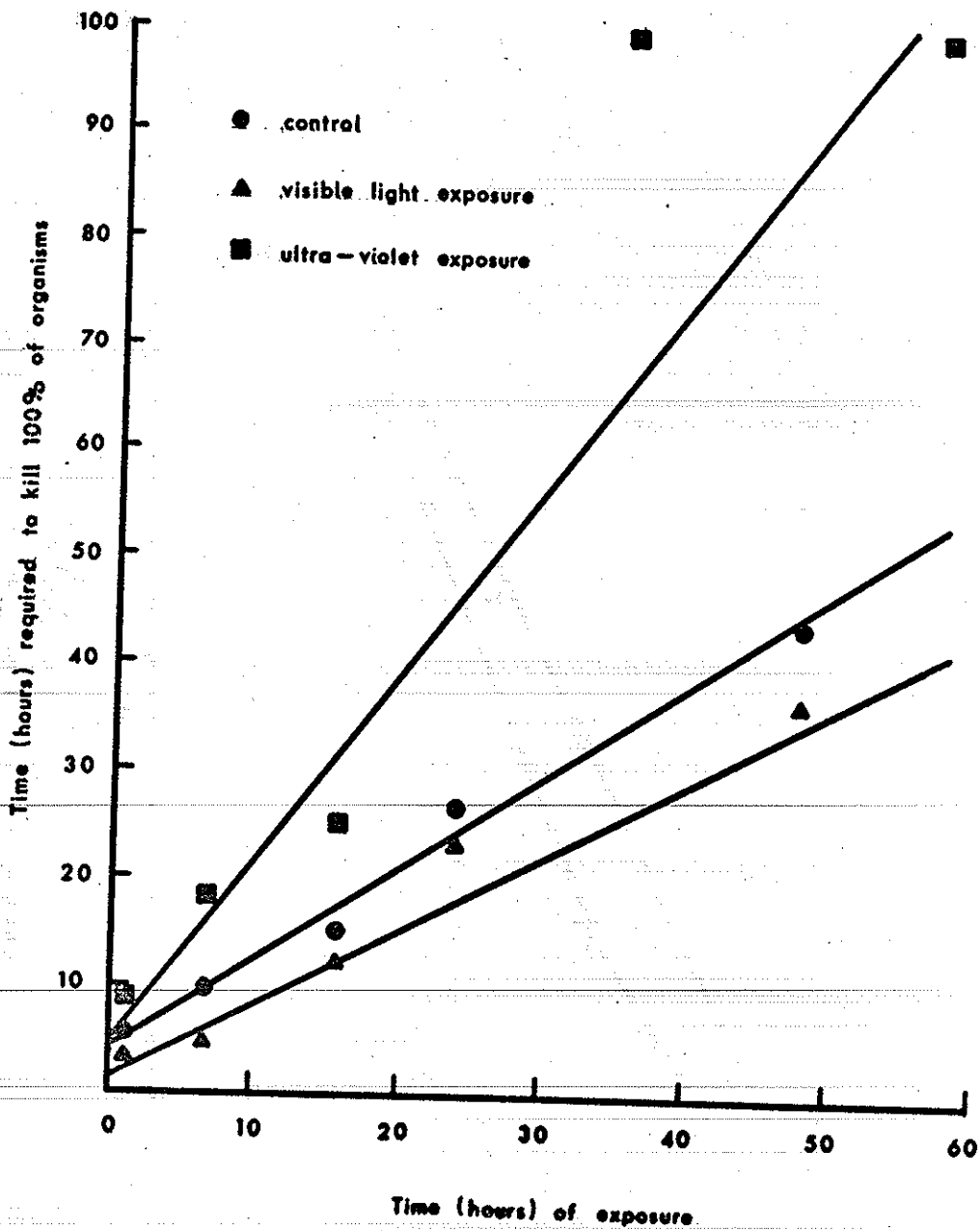


Figure 7. Time required to kill carp, Cyprinus carpio, with aged and light-exposed solutions of juglone (original concentration - 180 ppb).

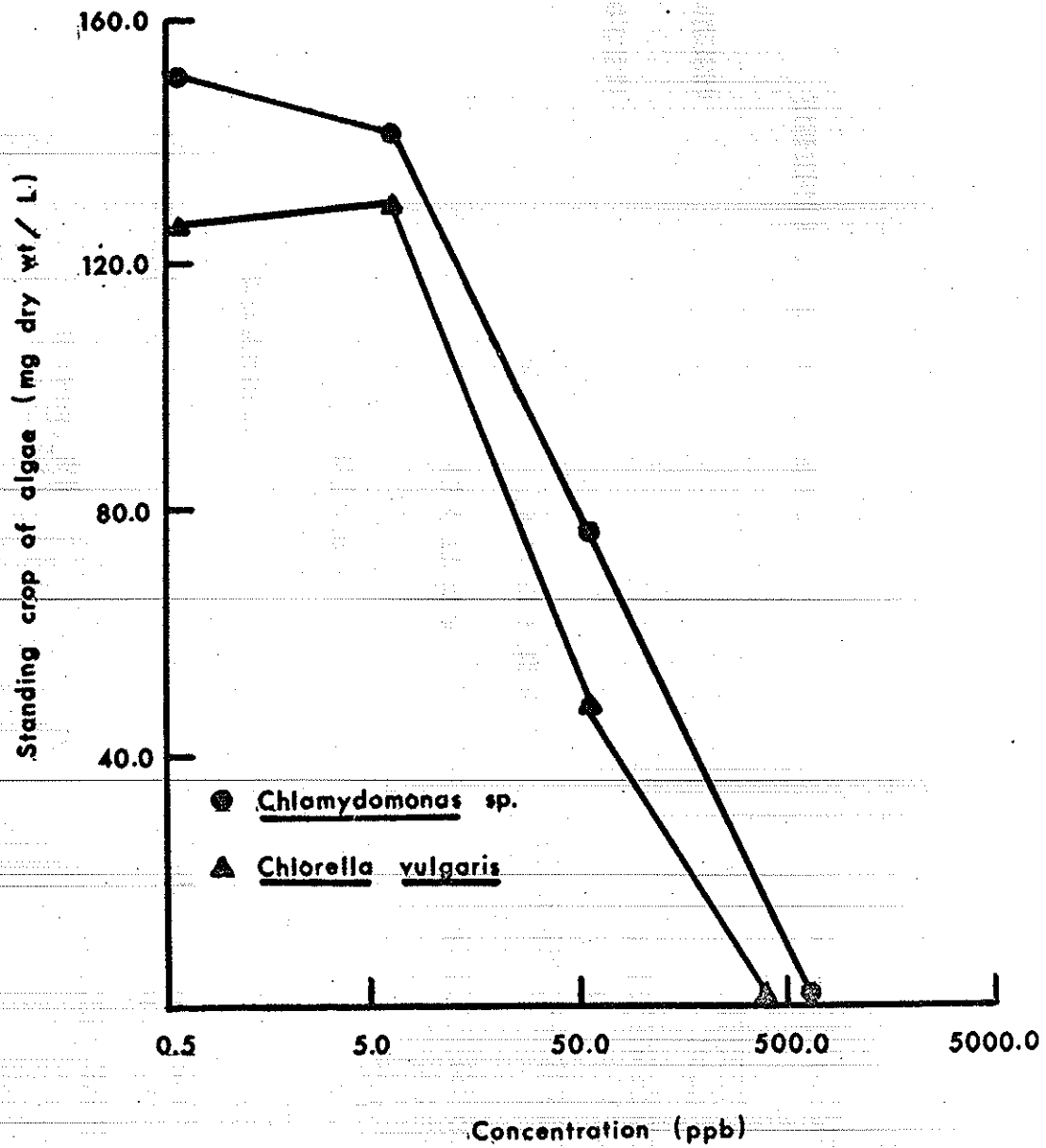


Figure 8. Toxicity of juglone to two species of green algae.

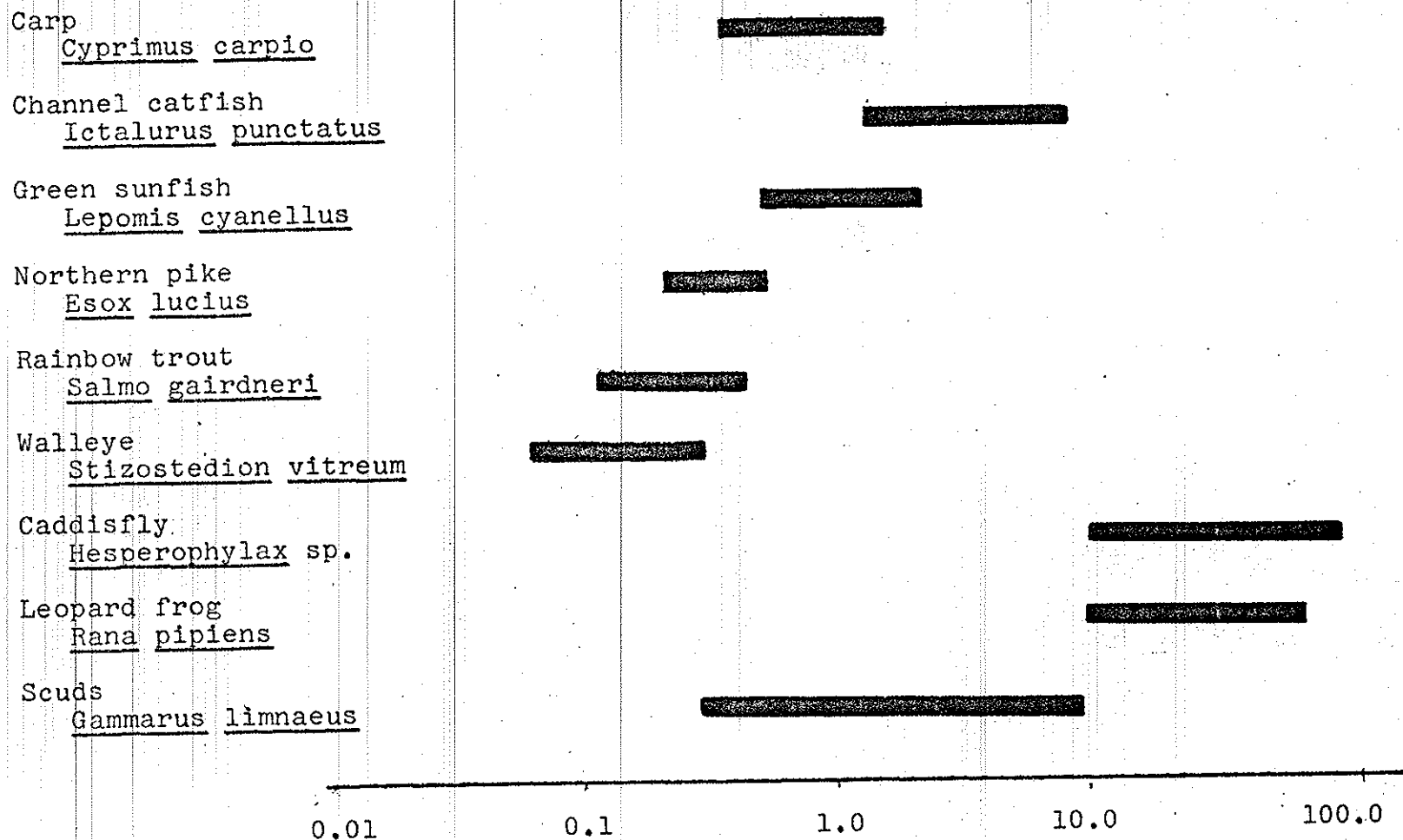


Figure 9. Concentrations of antimycin A (ppb) which span the ranges between the EC₅₀ and EC₁₀₀ for selected aquatic organisms. Responses taken at 24 hours and 13° C. (Data on fish reprinted from Lennon, et. al. Investigations in Fish Control, Circular 186.)

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