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Atrazine Degradation, Sorption and Bioconcentration in Water Systems

Duane C. Wolf

Ramon L. Jackson

Citation

Arkansas Water Resources Center

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PREPARED BY:

Duane C. Wolf
And
Ramon L. Jackson

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ARKANSAS WATER RESOURCES
UNIVERSITY OF ARKANSAS
112 OZARK HALL
FAYETTEVILLE, ARKANSAS 72701

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Duane C. Wolf

Ramon L. Jackson

University of Arkansas

Fayetteville

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Duane C. Wolf
Ramon L. Jackson

Arkansas Water Resources Research Center
University of Arkansas
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ABSTRACT

ATRAZINE DEGRADATION, SORPTION, AND BIOCONCENTRATION
IN WATER SYSTEMS

The herbicide atrazine is used extensively to control broadleaf and grass weeds in such crops as sorghum and corn. A small portion of the atrazine may be lost from the area of application by surface runoff and could enter a stream or lake. The objective of this study was to evaluate atrazine degradation, sorption, and bioconcentration in water-sediment systems. The results indicated that sediments with lower pH values and higher organic matter levels adsorbed higher levels of atrazine than sediments with neutral pH values and lower organic matter levels. Microbial decomposition of the herbicide was slow under the conditions of this study. Accumulation of atrazine by microorganisms in an aqueous system was demonstrated. The results indicated that the organic fraction of a water system may be the most important adsorption component. Data from this study will be useful in assessing the ramifications of herbicides in aquatic ecosystems and provide a better understanding of the reactions of herbicides in sediment-water systems.

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INTRODUCTION

Approximately 82% of the corn acreage and 73% of the sorghum acreage are located in the Mississippi River drainage area. The herbicide atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is used extensively for selective control of broadleaf and grass weeds in these crops (Fig. 1). Atrazine is manufactured by the Ciba-Geigy Corporation, Agricultural Division and is marketed under the trade name of AAtrex. Atrazine is a photosynthetic inhibitor that is absorbed through both roots and foliage and translocated acropetally in the xylem and accumulates in the apical meristems and leaves of plants (Ashton and Crafts, 1973). Atrazine sprays may be applied preplant, preemergence, or postemergence normally before weed seedlings reach a height of approximately 4 cm. Application rates of 2.2 to 4.5 kg/ha are required for selective control in most situations.

Atrazine is normally not found below a soil depth of 30 cm in detectable quantities even after prolonged useage (Hilton, 1974). Runoff from atrazine-treated fields contains measurable quantities of the herbicide. The amount of herbicide lost from nonpoint sources varies depending on soil, topography and hydrology of the area. Several studies have reported atrazine losses in the range of 18.0 - 1.2% of the total quantity applied, however, generally losses of less than 3% occurred (White et al., 1967; Hall et al., 1972; Ritter et al., 1974; Hall and Pawlus, 1973; Hall, 1974; Triplett et al., 1978). Wu 1980) reported 17 g/ha dissolved atrazine was discharged near the bottom of a drainage channel of a corn field watershed in Maryland.
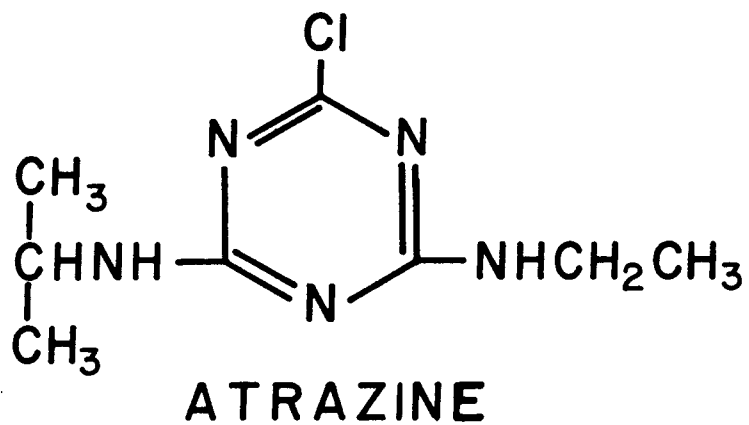
ATRAZINE

Chemical Name: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine
Common Name: Atrazine
Molecular Weight: 215.7 amu
Water solubility: 33 mg/l
pka: 1.85

Fig. 1 – Chemical structure and chemical properties of the herbicide
         atrazine.

Soil and sediment chemical and physical properties determine herbicide adsorption characteristics (Bailey and White, 1964). Hance (1974) reported that atrazine adsorption was reduced when the soil organic matter was removed by treatment with H_2O_2. Hays et al. (1968) showed that as the amount of soil organic matter increased, the amount of triazine adsorption also increased. Ladlie et al. (1976) reported that adsorption increased with decreasing soil pH for the triazine herbicide, metribuzin. Cobert et al. (1975) and Weber (1966) have also found that adsorption of atrazine is dependent on pH of the soil. They reported that atrazine adsorption decreased as pH increased from 5.2 to 8 in a study of ten soils.

Once the atrazine enters a water system, the partitioning of atrazine between the aqueous and solid phases of the water-sediment system largely determines the impact of the herbicide on water quality and aquatic ecosystems. Correll et al. (1977) have postulated about the importance of the suspended particulate phase of runoff water in relation to atrazine concentrations. More than 50 percent of the atrazine lost from a watershed was in the suspended particulate phase, which consisted of approximately 50 percent mineral and 50 percent organic materials. The majority of suspended particulates was probably the finer soil particles such as clays, which are more easily eroded. It is possible that the clays could either protect certain compounds or stimulate their decomposition. Moyer et al. (1972) reported that the presence of 7.5 - 38% clay in a soil slightly increased the persistence of atrazine. Montmorillonite has been shown to have no effect on the rate of atrazine hydrolysis in soils (Armstrong and Chesters, 1968). However, clays have been shown to have a stimulatory effect on the growth

and activity of soil bacteria and fungi (Filip, 1973). Clays have also been shown to stimulate microbial decomposition of various compounds such as aldehyde and benzene rings.

Once atrazine has entered the water system and is partitioned between the sediment and aqueous phase, degradation of atrazine will determine the length of time atrazine will persist. Atrazine may be converted to nonphytotoxic hydroxyatrazine by chemical hydrolysis which does not require the presence of a biological system (Armstrong et al., 1967). However, microbial degradation has been shown to be important in metabolism of the ethyl and isopropyl side chains of atrazine (Couch et al, 1965; Kaufman et al., 1965; Skipper et al., 1967). The addition of a microbial energy source such as glucose has been shown to increase the evolution of $^{14}CO_2$ from ring-labeled atrazine in a soil decomposition study (Wagner and Chahal, 1966). An increase in the temperature of incubation has also been shown to stimulate atrazine degradation (Roeth et al., 1969). Goswami and Green (1971) reported that chemical hydrolysis of atrazine to hydroxyatrazine increased the microbial clevage of the s-triazine ring which was a measure of microbial degradation. Apparently several microorganisms can degrade atrazine and utilize the ethylamino and/or isopropylamino side chains of the herbicide as carbon and nitrogen sources (Kaufman and Kearney, 1970).

In addition to microbial degradation of atrazine, it is possible that the compound could be adsorbed and/or absorbed and not degraded by the microorganisms in the sediment-water system. The bioaccumulation of the compound could play an important role in partitioning atrazine in the water system.

Since approximately one-half of the particulate material Correll et al. (1977) reported was organic, atrazine bioaccumulation by micro-organisms may have been partially responsible for the higher concentrations in this suspended phase. Grimes and Morrison (1975) have investigated the bioconcentration of chlorinated hydrocarbon insecticides by thirteen soil bacteria. They observed bioaccumulation of various insecticides with all the bacteria studied, and found that the degree of bioconcentration was inversely proportional to the water solubilities of the insecticides. Once sorption had occurred, the insecticides were not easily desorbed from the bacterial cells. They suggested that the pesticides were sorbed into the lipid material of cells, and that desorption may not occur even after cells died and lysed. Paris et al (1977) conducted bioconcentration studies of toxaphene by soil micro-organisms. They observed that cultures of autoclaved fungi, bacteria, and algae sorbed just as much toxaphene as viable cells of the same treatments, and thus, suggested that bioconcentration is not an active process. Percich and Lockwood (1978) found that mycelia of six living actinomycetes species accumulated between 900–4,300 µg atrazine/g dry mycelium. Autoclaved mycelium sorbed 100 µg atrazine/g dry mycelium during the same time period. Greater variability was observed with fungi. Living fungal mycelium bioaccumulated between 20–6,600 µg atrazine/g dry mycelium.

The research work reported above has been primarily related to soil systems and thus is of limited use in evaluating possible atrazine persistence and accumulation in a water system. Limited data are available for atrazine degradation and adsorption in water systems. Before the environmental consequences of possible herbicide addition to water

systems can be evaluated, research must be conducted to determine the fate of atrazine in a water system.

Specific objectives of this research were to:

1. Determine atrazine adsorption characteristics of sediments collected from various water systems and investigate the mechanism of adsorption.

2. Assess the magnitude of atrazine bioconcentration by several microorganisms found in water systems.

3. Quantitate atrazine degradation rates in sediment-water systems.

MATERIALS AND METHODS

Atrazine Adsorption by Sediments

Sample Collection: Sediment samples were collected with an Ekman Dredge at eight sample sites within Arkansas in September, 1980 (Fig. 2). Three sub-samples were obtained at each site, combined, air dried, and sieved to pass a 2 mm sieve.

Adsorption Measurements: The batch equilibrium procedure (Talbert and Fletchall, 1965) and liquid scintillation techniques were utilized to determine adsorption of ring ^{14}C labeled atrazine (2-chloro-4-(ethyl-amino)-6-(isopropylamino-$\underline{s}$-triazine). The ^{14}C atrazine had a specific activity of 50.1 µCi/mg and a purity of 99.6%. The atrazine was obtained from the Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC.

The equilibrium solution was 0.01 $\underline{M}$ $CaCl_2$ amended with $10^{-4}\underline{M}$, 5×10^{-5} $\underline{M}$, 10^{-5} $\underline{M}$, 10^{-6} $\underline{M}$ and 10^{-7} $\underline{M}$ atrazine which were equivalent to 21.57, 10.79, 2.15, 0.22 and 0.02 µg/ml, respectively. In all tests, the equilibrium solution to sediment ratio was 10:1 and 2 g of sediment and 20 ml of solution was used. A preliminary study indicated that an
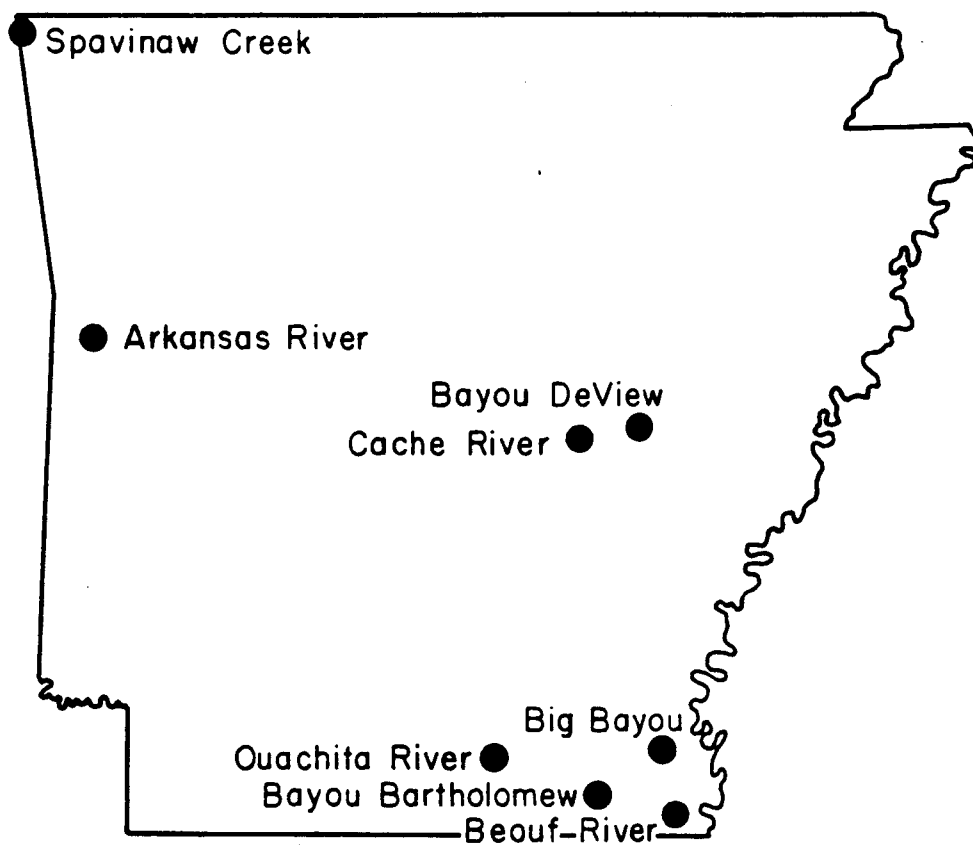
Fig. 2 - Sediment sample collection sites for the atrazine adsorption study. All samples were collected during September of 1980.

equilibrium time of 24 hrs was sufficient and all studies were analyzed after 24 hrs of horizontal shaking at low speed on a Eberback horizontal shaker. The $CaCl_2$ and atrazine solutions were sterilized by membrane filtration through membranes with a 0.45 µm pore diameter. The pH of the equilibrium solution was measured before and after the tests using a combination electrode and a Corning 135 pH ion meter. After shaking, 0.1 ml of the equilibrium solution was transferred by a Eppendoff pipette to 20 ml of a dioxane based scintillation cocktail containing 120 g naphthalene, 4 g 2,5-diphenyloxazole and 0.05 g 2,2-p-phenylenebis [5-phenyl-oxazole] per liter of p-dioxane (all chemicals, Eastman Chemical Co., scintillation grade).

Properties of Sediments: The physical and chemical properties of electrical conductivity (EC), cation exchange capacity (CEC), percent sand, silt, and clay, pH, total organic carbon (TOC), and extractable content of phosphorous (P), potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) were determined for each sediment.

The EC (µmho/cm) was measured by a Yellowsprings model 34 FL conductivity bridge with a 2:1 solution to sediment extract ratio. The ammonium saturation method (Chapman, 1965) was utilized to obtain CEC (meq/100 g) values. Percent TOC was determined by the dry combustion proceedure (Allison, 1965) and the hydrometer method (Day, 1965) was used to determine texture. Extractable P levels were determined on acid fluoride extracts using spectrophotometric techniques (Olsen and Dean, 1965). Extractable K, Ca, Na, Mg were determined on 1N ammonium acetate extracts in conjunction with flame spectrophotometry (Pratt, 1965).

Atrazine Degradation Studies

Sample Collection: Sediment samples were collected from Clear Creek, Washington Co., AR. The sample was passed through a 2 mm sieve and placed in 500 ml flasks each with a 20 g portion on a dry weight basis.

Incubation: Degradation rates were determined for concentrations of 1.0 μg/ml and 0.10 μg/ml ^{14}C ring and ethyl side chain labeled atrazine. A portion of the sediment samples with 1.0 μg/ml atrazine concentrations were treated with separate amendments of 100 mg of glucose or 100 mg of peptone.

The flasks were connected to a CO_2 collection apparatus at 25°C and were kept in constant motion on an orbital shaker at 150 revolutions/minute. Air was passed through a 2$\underline{N}$ KOH prescrubber allowing CO_2-free air to pass over the sediment. The CO_2 evolved from the sediment was collected in 25 ml of 0.5 $\underline{N}$ KOH. Total CO_2 evolution was determined by the method of Stotzky (1965). A 5 ml portion of the KOH was added to 10 ml 2$\underline{N}$ $BaCl_2$ and titrated with a standardized 0.5$\underline{N}$ HCl. The total CO_2 evolved was used to estimate total heterotrophic microbial activity.

Liquid scintillation techniques were used to determine the $^{14}CO_2$ produced. A 0.1-ml of the 0.5$\underline{N}$ KOH was added to 20 ml of scintillation solution and counted. Both CO_2 and $^{14}CO_2$ evolution were measured at intervals of 3, 10, 25, and 35 days. A minimum of two replications were performed for each concentration of ^{14}C ring-labeled and ^{14}C ethyl side chain-labeled atrazine.

Atrazine Bioconcentration

Organisms and Culture Techniques: Two bacteria, Bacillus cereus ATCC 1178 and Pseudomonas aeruginosa ATCC 14886 and two fungi Penicillium frequentans ATCC 10444 and Hendersonula toruloidea

(Martin et al., 1972) were incubated in 100 ml of liquid media at 24-26°C
for three days on an orbital shaker. The media selected allowed a maxi-
mum three day growth for the respective organisms. The media were for
B. cereus: beef extract 3.0 g/l and peptone 5 g/l; P. aeruginosa: tryptone
5 g/l, yeast extract 2.5 g/l and glucose 1.0 g/l; P. frequentans: and
H. torulodiea; glucose 10 g/l, K_2HPO_4 2g/l, peptone 0.8 g/l, yeast extract
0.2 g/l and 1 ml/l of trace element solution. The trace element solution
consisted of (per liter) 3.0 g $CaCl_2$; 0.4 g $CuSO_4$ $5H_2O$; 2.0 g $Fe(NH_4)_2$
$(SO_4)_2$ 12 H_2O; 1.0 g $ZnSO_4$ $7H_2O$; 1.0 g $MnSO_4$ H_2O; 0.1 g $CoCl_2$
$6H_2O$; 0.1 g H_3BO_3; and 0.05 g $(NH_4)_6$ Mo_7O_{24} $4H_2O$.

Bacterial cells and fungal mycelia were harvested by transfer to
pre-weighted tubes for centrifugation and three washes with sterile
H_2O. Bioconcentration was measured for both nonautoclaved and autoclaved
cells. For those tests conducted with autoclaved bacteria and fungi, the
harvested organisms were autoclaved for 1 hr at 1.4 bars (15 psi) and
121°C. After each test, the dry cell and dry mycelium weight were deter-
mined by drying to a constant weight. Atrazine bioconcentration was
determined by the batch equilibrium and liquid scintillation proce-
dures as described for the sediment studies. Equilibrium solution to
dry cell/mycelium weight varied from 2000:1 to 50:1.

Desorption measurements: After 24, 48 and 72 hrs, one half volume of the
equilibrium solution was decanted and this volume replaced by sterile 0.1$\underline{M}$
$CaCl_2$. After the respective 24 hr intervals, the equilibrium solution
was centrifuged and 0.10 ml was removed and placed in 20 ml of scintillation
fluid for the determination of ^{14}C atrazine having been desorbed. A
minimum of two replications were performed for each organism and each
treatment.

Statistical Methods

Sediment Analysis: The Freundlich equation (Travis and Etnier, 1981) was used to mathematically describe the atrazine adsorption and is presented in equation [1].

$$S = KC^N \qquad [1]$$

where S = amount of atrazine adsorped, μg/g

K = distribution adsorption coefficient

C = equilibrium concentration, μg/ml

N = constant

The K and N values were obtained for each sediment by plotting log C values (independent variables) against log S values (dependent variables) using linear regression techniques (Helwig and Council, 1979). In this regression technique, K values are the antilog of the y intercept. All log C and log S values at each atrazine concentration for all replications were combined into a single regression representing each sediment sample. In addition to using the Freundlich equation to describe adsorption, a multiple regression procedure (Helwig and Council, 1979) was utilized to evaluate the influence of sediment properties on atrazine adsorption. Correlation coefficients and the corresponding significance levels were established between the physical and chemical properties of the sediments and their respective K values.

Bioconcentration Analysis: The Freundlich equation was used to calculate atrazine adsorption on the four microorganisms which were both nonautoclaved and autoclaved. All log C and log S values at each atrazine concentration for all replications were combined into a single regression line representing each nonautoclaved and autoclaved microorganism. Differences in adsorption capacities of the microorganisms were compared by

mean separation of the respective K values using the Duncans multiple

range test.

RESULTS AND DISCUSSION

Atrazine Adsorption by Sediments

The sediment samples collected had a range in percent total organic
carbon from 0.01% to 2.17% (Table 1). The pH values ranged from a low
of 5.6 to a high value of 7.7. The CEC values were all low with the
highest value being 6.7 meq/100g. The three sediment samples with the
highest CEC values also contained the highest percent total organic
carbon and/or percent clay. Six of the eight sediment samples contained
less than 7 percent clay and as a result of their coarse texture they
contained low concentrations of K^+, Ca^{+2}, Mg^{+2}, and Na^+. All samples
contained relatively low levels of extractable phosphorus.

Freundlich adsorption isotherms for eight Arkansas sediment samples
were prepared (Figs. 3 and 4). The isotherms were derived by plotting
the amount of atrazine adsorbed, S, against the atrazine concentration in
the equilibrium solution, C, for each sediment.

The isotherms all exhibited slopes, N, approximating 1.0. All co-
efficients of determination were greater than 0.86. Of the eight river
sediments studied, the K values ranged from 0.61 for sediment from Bayou
DeView to 4.20 for sediment collected from the Cache River. The remaining
six sediments had a narrow K value range of 0.97 to 2.14. The Cache
River sediment had the highest K value and also had the highest percentage
of total organic carbon and a relatively low pH (Table 1). Previous
studies by Obrigawitch et al. (1981), Biggar et al. (1978) Karickhoff
et al. (1979), Carringer et al. (1975) and Savage (1976) have shown high
positive correlations between the amount of organic matter in soils and
their absorptive capacity toward a variety of organic pesticides. Brown
and Flagg (1981) studied atrazine adsorption by the silt fraction of a

Table 1. Physical and chemical properties of the sediments studied.

Sediment Location	TOC	pH	EC	CEC	Texture			P	K	Ca	Na	Mg
	-%-		μmho/cm	meq/100g	% clay	% sand	% silt	----------- μg/g -----------				
Arkansas River	0.01	7.6	170	0.3	1.1	98.9	0.0	9	60	225	98	35
Bayou Bartholomew	0.68	7.7	190	4.6	17.5	27.4	55.1	43	105	2175	58	225
Bayou DeView	0.04	6.7	170	0.9	0.3	99.7	0.0	9	58	350	100	58
Big Bayou	1.00	6.3	310	5.5	18.9	57.8	23.3	29	100	1050	125	243
Boeuf Bayou	0.04	5.6	120	3.4	4.5	76.5	19.0	11	90	1350	88	325
Cache River	2.17	5.8	110	6.7	6.4	26.0	67.3	25	175	1525	150	375
Ouachita River	0.05	6.7	180	0.7	3.2	83.7	13.1	17	45	400	125	58
Spavinaw Creek	0.45	7.7	170	2.8	4.6	59.7	35.7	23	120	1850	220	35

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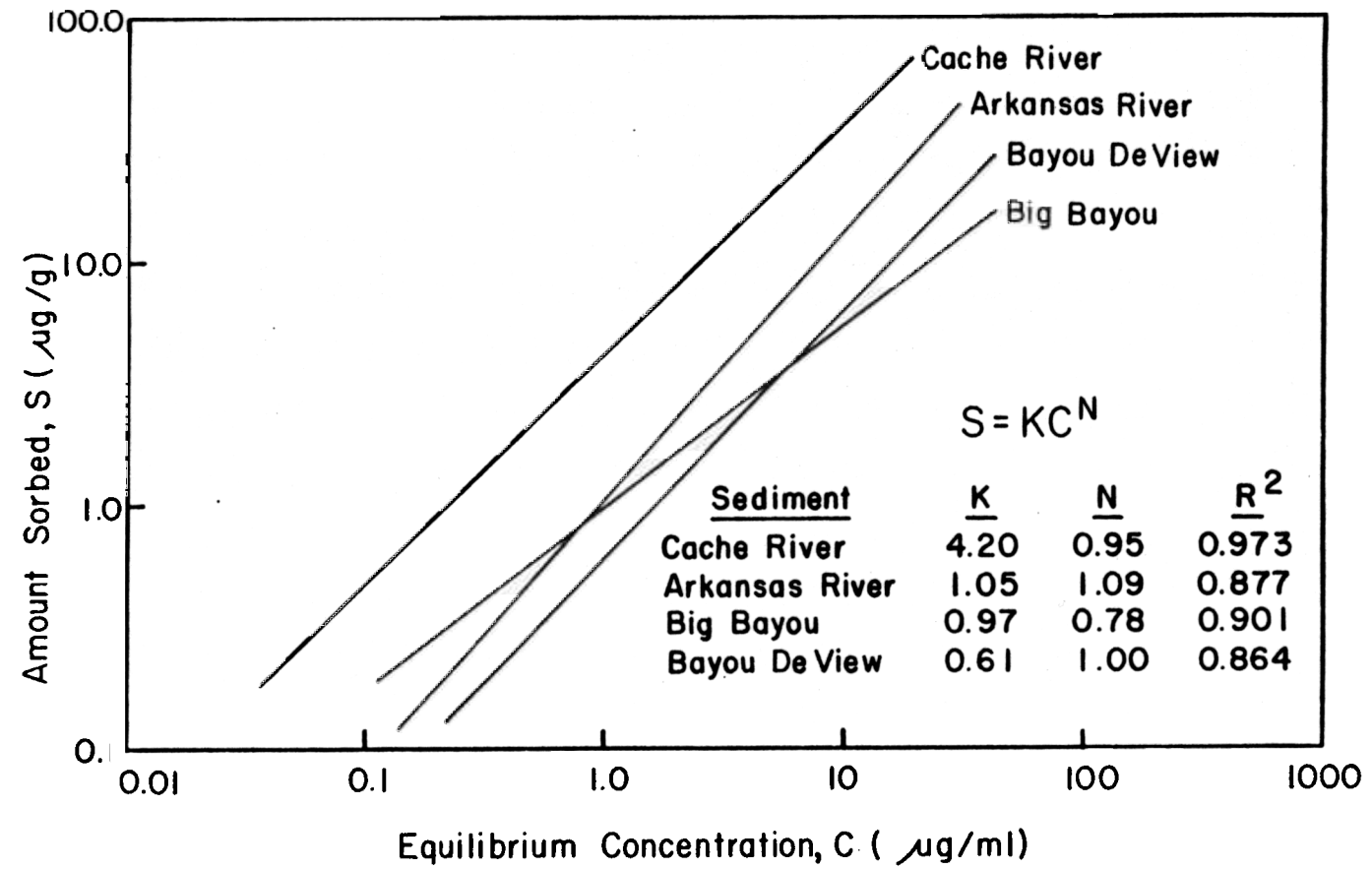
Fig. 3 - Freundlich adsorption isotherms for atrazine adsorption by four sediments.
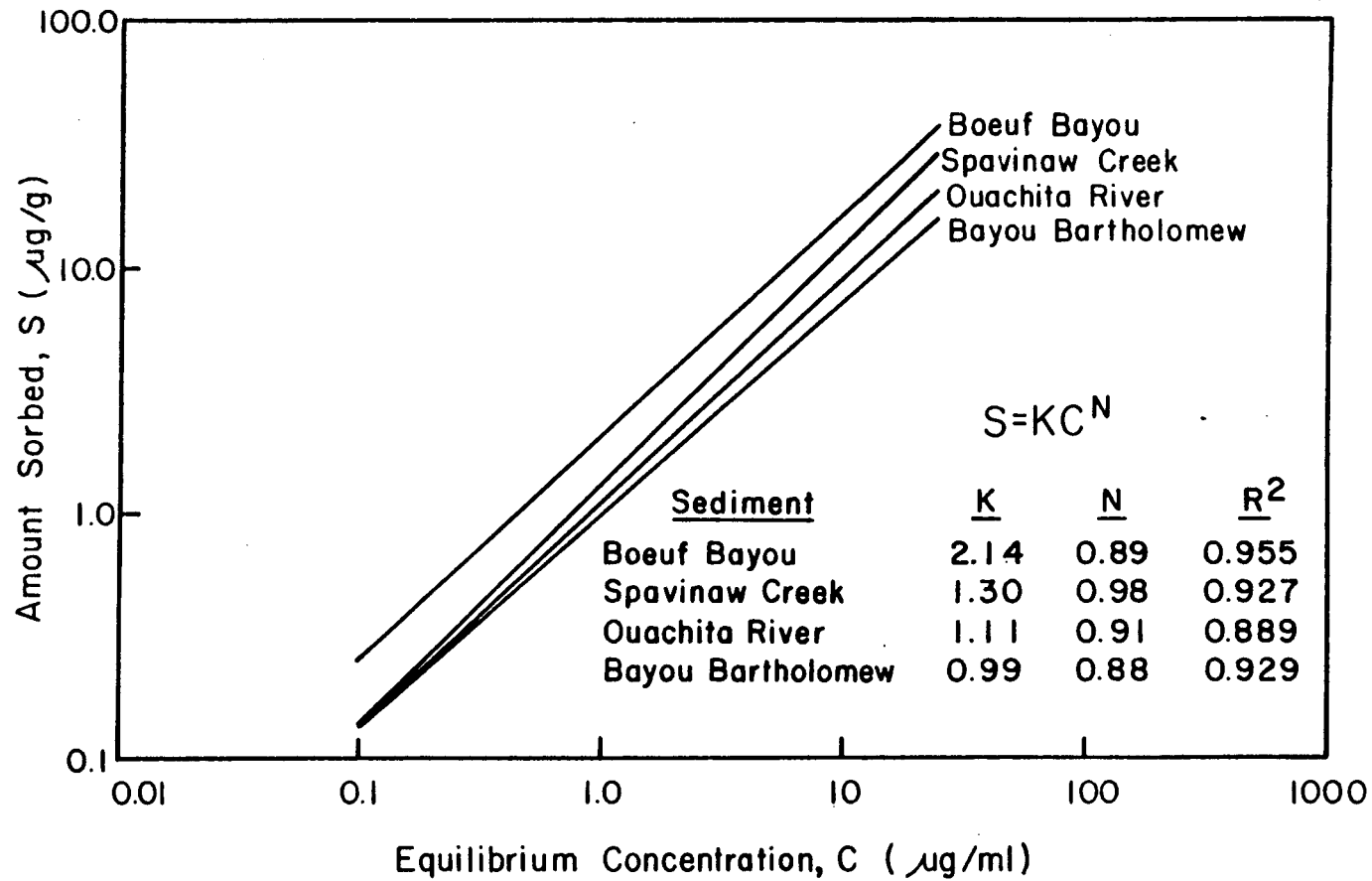
Fig. 4 — Freundlich adsorption isotherms for atrazine adsorption by four sediments.

freshwater sediment and found a K value of 7.07. Atrazine partition co-
efficient between octanol and water was highly correlated with organic
carbon in the silt. Rao and Davidson (1979) stated that adsorption of
pesticides such as atrazine, was correlated to a greater extent to the
amount of organic matter present in a soil rather than different soils.
In a study of 25 soils, Walker and Crawford (1970) also reported a
linear correlation between atrazine adsorption and the soil organic C
content. However, Karickhoff et al. (1979) suggested that the humic
materials associated with sand were less efficient in adsorption of
methoxychlor than humic materials associated with the silt and clay
fractions.

Colbert et al. (1975) reported that atrazine adsorption decreased in
10 soils as the pH increased from 5.2 up to 8.0. The correlation co-
efficient between pH and atrazine adsorption was -0.47. Adams and
Pritchard (1977) found that atrazine phytotoxicity decreased as soil pH
decreased and organic matter increased. The decrease in phytotoxcity
was attributed to increased atrazine adsorption and hydrolysis.

Correlations between atrazine adsorption, as indicated by K values,
and sediment properties listed in Table 1 were evaluated (Helwig and
Council, 1979). The correlation coefficient between the Freundlich K
value and TOC was 0.733 which was significant at the 0.05 level (Table 2).
The correlation coefficient between K and pH was -0.624 and was signifi-
cant at the 0.10 level. Colbert et al. (1975) reported a correlation co-
efficient between atrazine adsorption and soil pH of -0.47 for soil pH
values of less than or equal to 8.0. Correlation coefficients for TOC
and CEC, percent sand, and percent silt were 0.867, -0.808, and 0.824,
respectively. In general, CEC and percent silt were not thought to be

Table 2. Simple correlations between sediment properties and atrazine adsorption or K values.

	Sand	Silt	Clay	pH	TOC
K		0.631 *		-0.624 *	0.733 **
EC			0.657 *		
CEC	-0.884 ***	0.835 ***	0.693 *		0.867 ***
Sand		-0.977 ***	-0.676 *		-0.808 **
Silt					0.824 **

*, **, *** Significant at the 0.10, 0.05 and 0.01 levels, respectively.

important in atrazine adsorption in the pH range of the sediments tested, but rather, their influence on TOC was concomitant.

A stepwise multiple regression analysis was performed and the resulting equation which related atrazine adsorption to sediment properties is given in equation 2.

$$K = 5.240-0.620 \ (pH) + 0.977(TOC) \qquad [2]$$

where

TOC is expressed as a percentage

pH is expressed as units

The coefficient of determination or R^2 value for the above relationship was 0.69 and was significant at the 0.05 level.

An evaluation of atrazine adsorption as determined by Freundlich K and N values can be calculated any one of three ways. The traditional method was used and involved the generation of one line using all data points and the values are given in Table 3. A second method was used to calculate K and N values which used a method of averaging the individual K and N values for each of two replications and was designated as unweighted. A third method used a weighting technique and was designated as weighted. As can be seen from Table 3, the three methods compared favorably for K values for such sites as Boeuf Bayou and Spavinaw Creek, but in other cases the three methods yielded large differences in K values with the greatest difference found for the Ouachita River sample.

Both the weighted and unweighted methods of calculating N values showed that none of the values were significantly different from 1.00. The coefficients of determination for both weighted and the one line methods were all greater than 0.86.

Table 3. Comparison of three methods of calculating Freundlich K and N values and coefficients of determination (R^2) for atrazine adsorption by eight sediments.

Sediment	K			N			R^2		
	One Line	Unweighted	Weighted	One Line	Unweighted	Weighted	One Line	Repli-cations	Unweighted
Arkansas River	1.05[1]	0.90b[2]	0.83b[2]	1.09[1]	1.03[3]	1.03[3]	0.877	1 2	0.973 0.864
Bayou Bartholomew	0.99	0.89b	0.89b	0.88	0.92	0.92	0.929	1 2	0.974 0.932
Bayou DeView	0.61	0.66b	0.66b	1.00	0.98	0.99	0.864	1 2	0.883 0.950
Big Bayou	0.97	0.91b	0.87b	0.78	0.80	0.81	0.901	1 2	0.969 0.899
Boeuf Bayou	2.14	2.14ab	2.16ab	0.89	0.89	0.88	0.955	1 2	0.953 0.965
Cache River	4.20	4.18a	4.42a	0.95	0.96	0.94	0.973	1 2	0.982 0.992
Ouachita River	1.11	1.30ab	1.61b	0.91	0.89	0.90	0.889	1 2	0.944 0.989
Spavinaw Creek	1.30	1.31ab	1.28b	0.98	0.98	0.97	0.927	1 2	0.951 0.960

[1] The K and N values were generated by using all data points to produce one line and thus it was not possible to make a statistical comparison among samples.

[2] Values within a column followed by the same letter are not significantly different at the 0.05 level.

[3] None of the values in the column were significantly different from each other or 1.0 at the 0.05 level.

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From these data, it can be concluded that sediments with a relatively low pH and high TOC would adsorb the largest quantity of atrazine.

Atrazine Degradation Studies

The presence of either 0.1 or 1.0 µg/ml of atrazine in a sediment-water system did not affect microbial activity as measured by CO_2 evolution (Table 4). Other researchers have reported that the addition of atrazine to soil resulted in an increase, a decrease, or had no effect on CO_2 production (Kaiser et al., 1970)

Due to technical problems, it was not possible to determine total CO_2 production at each sample time. In general, CO_2 production was greatest during the initial 10 days of the incubation and in most cases the highest rate of CO_2 evolution occurred during the first 3 days. Atrazine did not appear to have any appreciable influence on CO_2 production by the microbial population of the sediment studied. The rate of CO_2 production for the sediment amended with 0.1 or 1.0 µg atrazine/ml or 0 atrazine were similar during the study. The addition of organic amendments such as glucose or peptone resulted in a substantial increase in CO_2 evolution as these easily degraded materials were utilized by the microbial population.

The degradation rate of ^{14}C-labeled atrazine during the 35 day incubation was slow with a maximum rate of 5.2% for side-chain labeled atrazine added at a rate of 1.0 µg/ml (Table 5). In all cases, the side-chain labeled atrazine was recovered as $^{14}CO_2$ at a greater rate than the ring-labeled material. The accumulated ^{14}C recovered as $^{14}CO_2$ during the incubation was higher than previous work reported by Wolf and Martin (1975) who reported only 0.02% of the ^{14}C from ring-labeled atrazine recovered as $^{14}CO_2$ during the initial 32 days of a saturated soil incubation study.

Table 4. Respiration rate of sediment samples amended with two levels of
          atrazine and incubated 35 days.

Atrazine Concentration	Position of ^{14}C-Label	Amend-ment	Incubation Time (days)			
			3	10	25	35
--- μg/ml ---			--- mg C as CO_2/20g dry sediment/day ---			
0.1	chain	--	0.80	1.54	MD[1/]	MD
0.1	ring	--	1.83	1.96	1.36	0.64
1.0	chain	--	0.40	1.94	0.32	0.51
1.0	ring	--	1.60	2.07	1.47	0.57
1.0	chain	glucose	9.27	MD	MD	MD
1.0	chain	peptone	7.07	4.20	MD	0.37
1.0	ring	glucose	8.97	5.82	MD	0.52
1.0	ring	peptone	9.87	3.39	0.80	0.71
0	--	--	0.07	1.93	1.10	0.54

[1/] Indicates missing data.

Table 5. Percent of ^{14}C recovered as $^{14}CO_2$ from two rates of ring or side chain-labeled atrazine added to a sediment-water system.

Atrazine Concentration	Position of ^{14}C-Label	Amend-ment	Incubation Time (days)			
			3	10	25	35
--- μg/ml ---		--- accumulative % ^{14}C recovered as $^{14}CO_2$---				
0.1	chain	--	0.0	0.0	3.8	3.8
0.1	ring	--	0.0	0.0	0.9	2.3
1.0	chain	--	0.6	1.0	4.9	5.2
1.0	ring	--	0.6	0.6	2.0	3.2
1.0	chain	glucose	2.8	2.9	3.0	3.2
1.0	chain	peptone	1.1	1.1	3.1	3.8
1.0	ring	glucose	0.7	0.7	2.1	2.1
1.0	ring	peptone	0.0	0.6	2.3	2.4

The addition of the readily available carbon and energy sources of glucose and peptone had no apparent influence on the rate of atrazine decomposition which agrees with previous work reported by Wolf and Martin (1975) but does not agree with work by Wagner and Chahal (1966) and McCormick and Hiltbold (1965).

It should be noted that the rate of atrazine degradation was low in all cases in the incubation. The low values were at the detection limits of the available instrumentation and thus the error associated with each number may be large. In the sediment-water system used in the present study, the data indicated that atrazine degradation would be slow.

Atrazine Bioconcentration

The adsorption capacity of the bacteria and fungi studied increased as the concentration of atrazine in solution increased. Freundlich adsorption isotherms (Figs. 5-8) were derived by plotting the amount of atrazine adsorbed (S) against the concentration of atrazine in the equilibrium solution (C) for each organism in both the non-autoclaved and autoclaved condition. The adsorption isotherms were linear in nature with a slope, N, ranging from 1.09 to 0.74 with an overall mean of 0.95. Coefficients of determination, R^2, were above 0.80 for all adsorption isotherms. The Freundlich distribution adsorption coefficients or K values were used as indices for comparing the amount of atrazine adsorbed by the two bacteria, Bacillus cereus (Gram +) and Pseudomonas aeruginosa (Gram -) and the two fungi, Penicillium frequentans and Hendersonula toruloidea.

The K values for all organisms tested both non-autoclaved and autoclaved were much higher than the K values obtained from the eight Arkansas sediment samples tested.

The Freundlich K values for both the bacteria increased over three-

Fig. 5 - Freundlich isotherms for atrazine adsorption by autoclaved and nonautoclaved cells of the bacterium B. cereus.
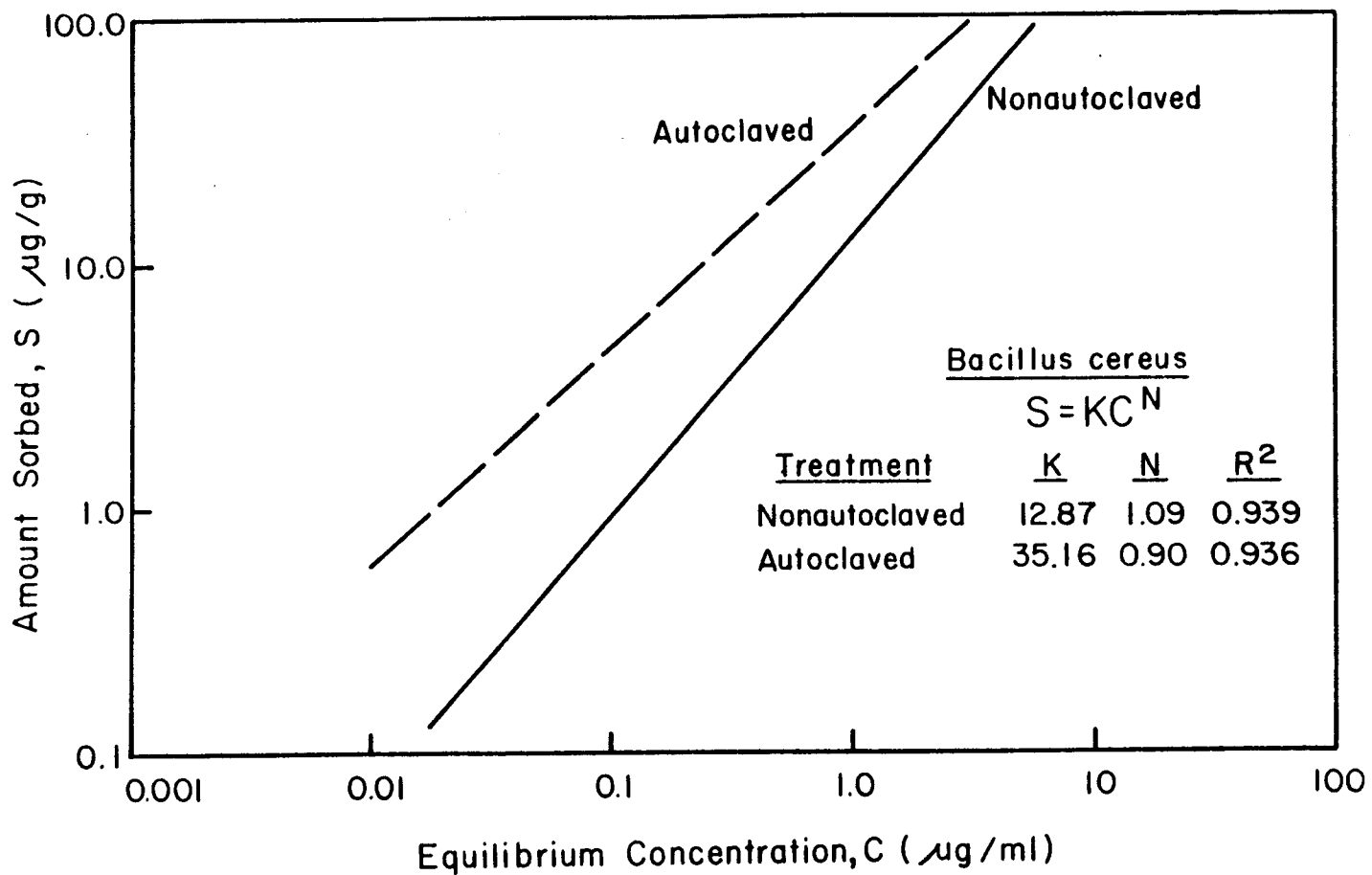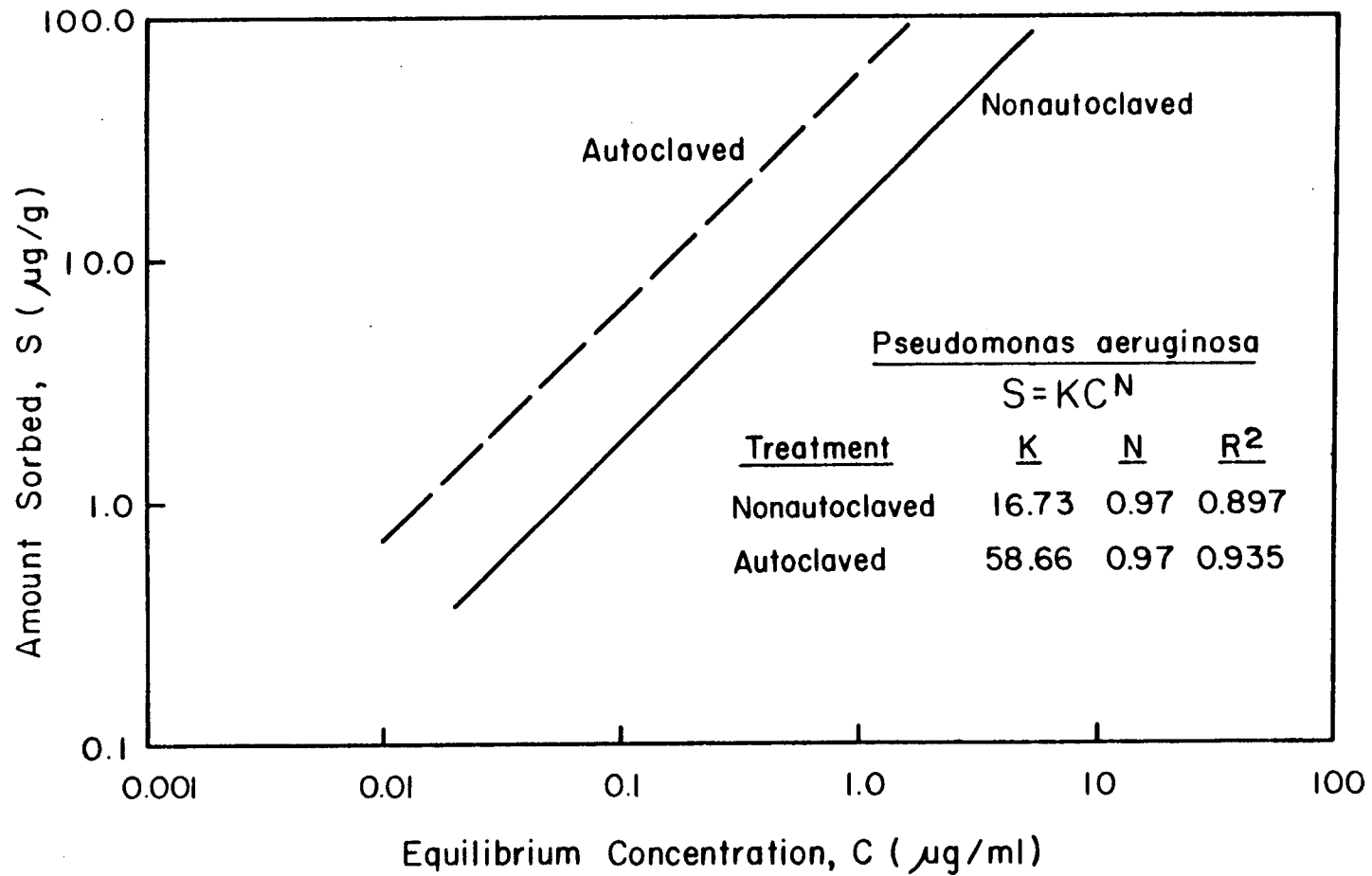
Fig. 6 – Freundlich isotherms for atrazine adsorption by autoclaved and nonautoclaved cells of the bacterium P. aeruginosa.
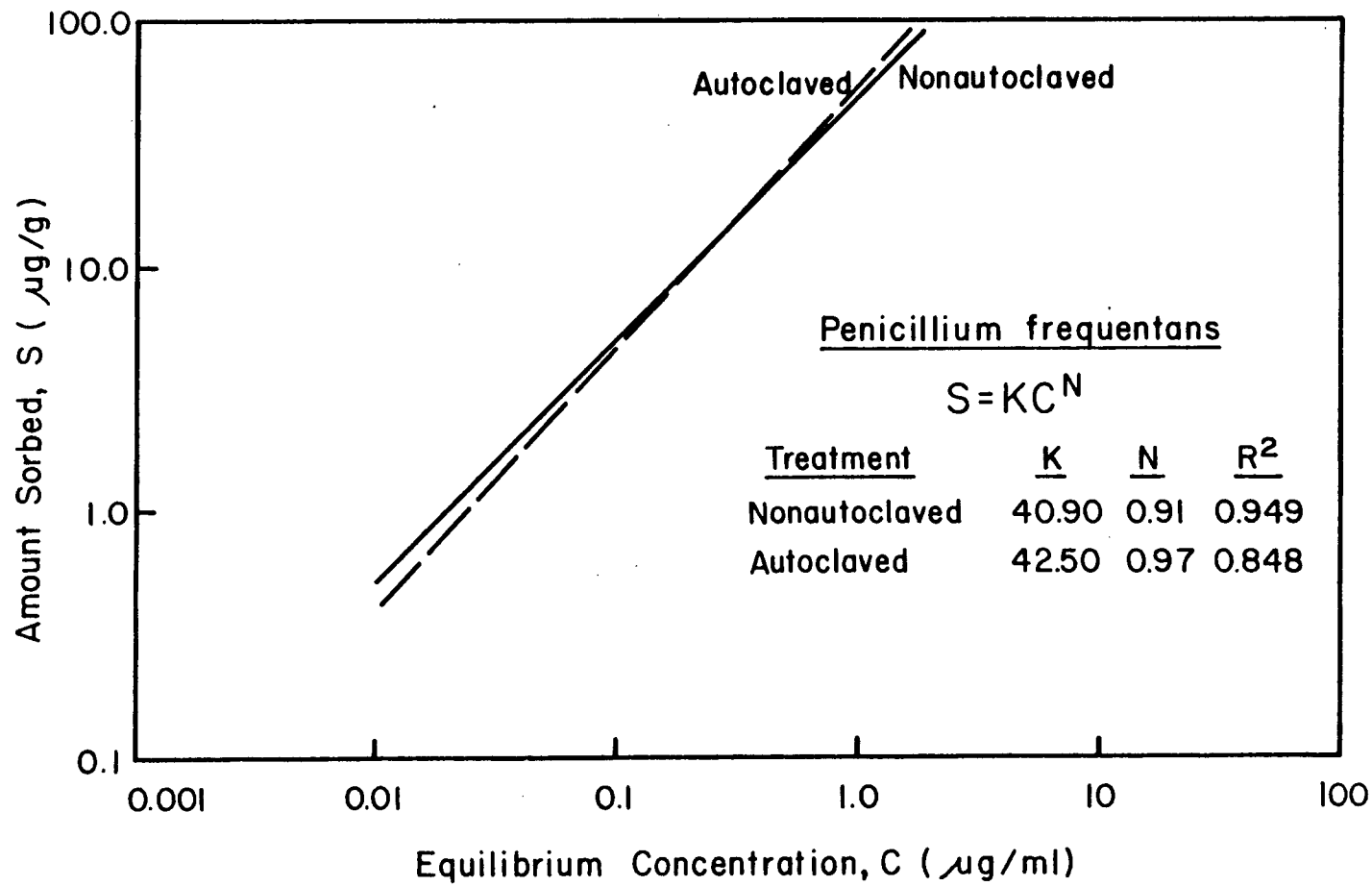
Fig. 7 - Freundlich isotherms for atrazine adsorption by autoclaved and nonautoclaved mycelia of the fungus P. frequentans.
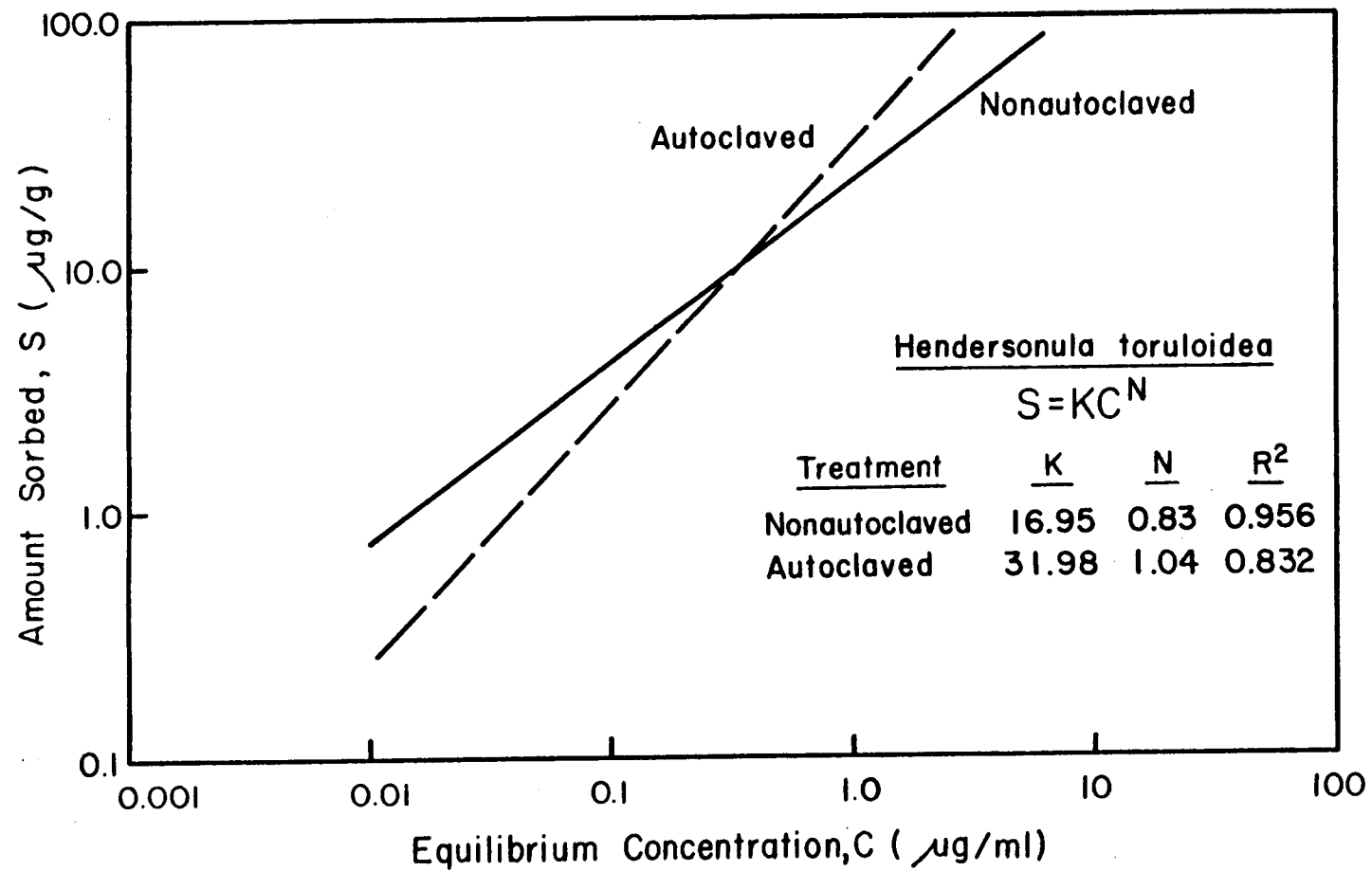
Fig. 8 Freundlich isotherms for atrazine adsorption by autoclaved and nonautoclaved mycelia of the fungi H. toruloidea.

fold when the samples were autoclaved. For the two fungi, the K values were only slightly greater in the autoclaved compared to the nonautoclaved fungi. For the nonautoclaved samples, atrazine adsorption was greatest for the fungus P. frequentans and least for the bacterium B. cereus. When the samples were autoclaved, the largest K value was for the bacterium P. aeruginosa and least for the fungus H. toruloidea.

As with the sediment samples, three methods were used to derive Freundlich K and N values (Table 6). Percich and Lockwood (1978) reported that actinomycete and fungal mycelia accumulated atrazine from water concentrations up to 87-fold and 132-fold, respectively, over that in the ambient medium.

The high adsorption or K values for the test organisms were not the result of metabolic activity since the K values of the autoclaved organisms were greater than the K values obtained from the viable organisms. Paris and Lewis (1976) autoclaved bacteria and fungi and performed adsorption tests with the chlorinated hydrocarbon insecticide methoxychlor. They found that the autoclaved cells sorbed as much or more of the pesticide than the viable cells, which indicated that the adsorption was not the result of a metabolically active process. Paris et al. (1977) reached the same conclusion using another chlorinated hydrocarbon insecticide, toxaphene, in adsorption tests with autoclaved bacteria and fungi.

Atrazine is not a highly lipid soluble pesticide like toxaphene and methoxychlor and, therefore, would not be expected to be as strongly adsorbed to bacterial cell wall surfaces that are rich in lipoprotein. It would appear that the electrostatic nature of the microbial cell wall surface could be largely responsible for the high amount of atrazine adsorption. Most bacterial cells carry a negative surface charge at

Table 6. Comparison of three methods of calculating Freundlich K and N values and coefficients of determination (R^2) for atrazine by four microorganisms in the nonautoclaved (NA) and autoclaved (A) condition.

Organism	K			N			R^2		
	One Line	Unweighted	Weighted	One Line	Unweighted	Weighted	One Line	Repli-cations	Unweighted
B. cereus (NA)	12.87[1]	13.45[2]	12.40	1.09	1.11[2]	1.08	0.939	1	0.994
								2	0.887
								3	0.991
B. cereus (A)	35.16	35.18	33.47	0.90	0.95	0.94	0.936	1	0.984
								2	0.846
								3	0.993
								4	0.998
P. aeruginosa (NA)	16.73	15.30	19.16	0.97	0.98	0.97	0.897	1	0.909
								2	0.984
								3	0.978
								4	0.974
P. aeruginosa (A)	58.66	59.86	55.91	0.97	0.98	1.00	0.950	1	0.997
								2	0.958
								3	0.964
								4	0.979
								5	0.982
								6	0.994
P. frequentans (NA)	40.90	38.51	40.86	0.91	0.96	0.94	0.949	1	0.965
								2	0.993
								3	0.982
								4	0.981

Table 6. Comparison of three methods of calculating Freundlich K and N values and coefficients of determination (R^2) for atrazine by four microorganisms in the nonautoclaved (NA) and autoclaved (A) condition (cont).

Organism	K One Line	K Unweighted	K Weighted	N One Line	N Unweighted	N Weighted	R^2 One Line	R^2 Repli- cations	R^2 Unweighted
P. frequentans (A)	42.50	30.69	39.91	0.97	0.96	0.92	0.848	1	0.962
								2	0.852
								3	0.963
								4	0.999
H. toruloidea (NA)	16.74	16.95	17.47	0.82	0.83	0.82	0.956	1	0.966
								2	0.989
H. toruloidea (A)	31.98	32.74	41.67	1.04	1.05	1.03	0.832	1	0.977
								2	0.995
								3	0.930

[1] The K and N values were determined by all data points to generate one regression line and it was; therefore, impossible to make a statistical comparison among samples.

[2] None of the values in the column were significantly different at the 0.05 level.

neutral ph. Also, negatively charged carboxyl sites and positively charged amino sites may be prevalent on the cell wall (Umbreit, 1976). Through hydrogen bonding or van der Waals forces, atrazine may be attached to such areas on bacterial and fungal cell walls. A possible mechanism of adsorption is presented in Fig. 9.

Autoclaving would cause bacterial cells and fungal mycelia to lyse and thus release any soluble cell contents which would result in a greater surface area exposed for possible adsorption sites per unit weight of cell material. The autoclaved K values for the four test organisms were greater than the nonautoclaved K values in support of this hypothesis. The K values for the autoclaved bacteria and fungi also appeared to indicate that the bacteria may be more susceptible to cellular damage by autoclaving due to their individual cellular nature.

The amount of atrazine adsorbed to the bacteria was calculated on a molecule per given surface area. Scanning electron photomicrographs were prepared of samples from broth cultures of B. cereus and P. aeruginosa at magnifications ranging from 4.5×10^3 to 6.5×10^3. Average cell length and width were determined from the photomicrographs. Buchanan and Gibbons (1975), report the average length for B. cereus to be $3.0 - 5.0$ μm with an average width of $1.0 - 1.2$ μm. For P. aeruginosa they reported an average length of 1.5 μm and an average width of 0.5 μm. The cultures photographed and measured in the present study, had an average length of 3.98 μm and an average width of 0.93 μm for B. cereus. P. aeruginosa had an average length of 1.13 μm and average width of 0.41 μm. Surface area (SA) was determined for the rod shaped bacteria using equation [3].

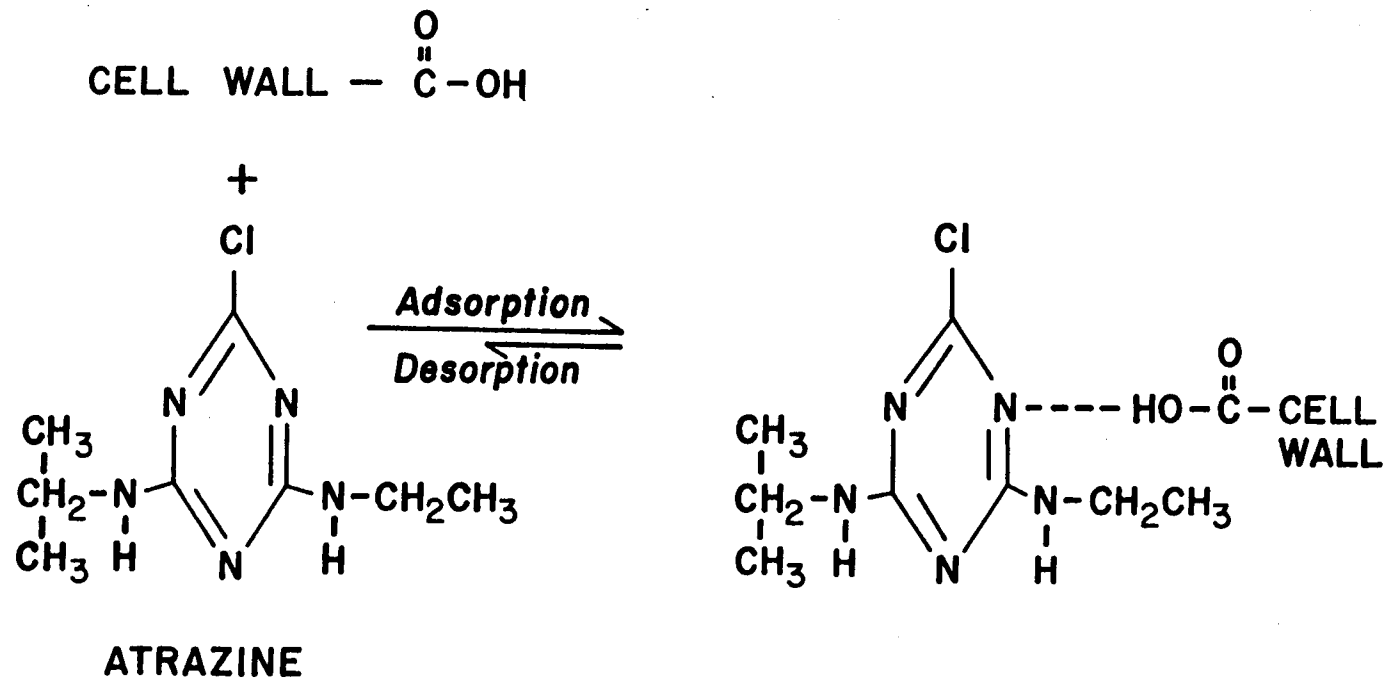$$SA = 2\pi R^2 + l\pi d \qquad [3]$$

where

Fig. 9 Possible mechanism for the adsorption of atrazine by cell walls of microorganisms.

R = cell radius

l = cell length

d = diameter of cell

The SA for a <u>B. cereus</u> cell was calculated to be 12.99 μm^2 while <u>P. aeruginosa</u> SA per cell was 1.72 μm^2.

The number of cells/g dry weight were determined by the spread plate technique. At the time of harvest, <u>B. cereus</u> had grown to 4.5 x 10^{10} cells/g and <u>P. aeruginosa</u> was at 4.8 x 10^{12} cells/g. The Freundlich K values were expressed in units of g of atrazine adsorbed/g of dry cell weight. As an example of the calculations involved, if one used a 100 mg as a typical value of dry cell weight per test and 2.7909 x 10^{15} as the number of molecules/ g atrazine, the number of atrazine molecules adsorbed per m^2 cell surface could be calculated using equation [4].

$$\begin{array}{l} \text{No. molecules atrazine} \\ \text{adsorbed per } \mu m^2 \text{ cell} \\ \text{surface} \end{array} = \frac{\text{(No. molecules atrazine/ } \mu g) \text{ (K)}}{\text{(cell no./0.10 g) (surface area/cell)}} \quad [4]$$

The relative amounts of molecular atrazine adsorbed per μm^2 by <u>B. cereus</u> and <u>P. aeruginosa</u> both nonautoclaved and autoclaved are given in Table 7.

Considering atrazine adsorption to bacteria as a nonmetabolic process, those bacteria with the largest surface area per unit dry weight would act as the greatest atrazine adsorptive sink given the same K values. Due to difficulties in calculating surface area of fungal mycelia, similar adsorption calculations could not be completed for the fungi.

Results from the bioconcentration research indicated that nonautoclaved and autoclaved bacteria and fungi adsorb the herbicide atrazine.

Table 7. Atrazine adsorption per surface area for two bacteria.

Bacterium	Nonautoclaved	Autoclaved
	-- molecules of atrazine/ μm^2 of cell surface --	
B. cereus	6.14×10^4	1.68×10^5
P. aeruginosa	5.66×10^3	1.98×10^4

CONCLUSIONS

Atrazine is an important herbicide in the corn and sorghum producing areas. Loss of atrazine in runoff from atrazine-treated fields has generally been shown to be low. Once the atrazine enters a sediment-water system, the herbicide is partitioned between the solid and the aqueous phase. Sediments with lower pH and higher total organic carbon levels would be expected to adsorb higher amounts of atrazine than sediments with higher pH and lower organic carbon levels. The greater the amount of atrazine adsorbed by the sediment, the lower the level of atrazine in the aqueous phase.

Microbial degradation of atrazine in the sediment-water system used in this study was slow. The chain-labeled atrazine appeared to be degraded to CO_2 at a higher rate than the ring-labeled material. Addition of supplemental carbon sources in the form of glucose or peptone did not appear to influence the rate of atrazine degradation.

Bioconcentration of atrazine by fungi and bacteria was demonstrated. The results indicated that the organic component of the suspended particulate matter in a water system may be an active adsorptive site.

LITERATURE CITED

1. Adams, R. S. Jr., and D. J. Pritchard. 1977. Influence of soil pH on the phytotoxicity of three s-triazine herbicide. Agron. J. 69:820-824.

2. Allison, L. E. 1965. Organic Carbon. In C. A. Black (ed.) Methods of soil analysis. Agronomy 9:1367-1378. Am. Soc. of Agron., Madison, Wis.

3. Armstrong, D. E., G. Chesters, and R. F. Harris. 1967. Atrazine hydrolysis in soil. Soil Sci. Soc. Amer. Proc. 31:61-66.

4. Armstrong, D. E. and G. Chesters. 1968. Adsorption catalyzed chemical hydrolysis of atrazine. Environ. Sci. Technol. 2:683-689.

5. Ashton, F. M., and A. S. Crafts. 1973. Mode of action of herbicides. John Wiley and Sons, New York. p. 310-346.

6. Bailey, G. W. and J. L. White. 1964. Review of adsorption and desorption of organic pesticides by soil colloids with implications concerning pesticide bioactivity. J. Agri. Food Chem. 12:324-331.

7. Biggar, J. W., U. Mingelgrin, and M. W. Cheung. 1978. Equilibrium and kinetics of adsorption of picloram and parathion with soils. J. Agric. Food Chem. 26:1306-1312.

8. Brown, D. S. and E. W. Flagg. 1981. Empirical predication of organic pollutant sorption in natural sediments. J. Environ. Qual. 10: 382-386.

9. Buchannan, R. E. and N. E. Gibbons. 1975. Bergey's manual of determinative bacteriology. Williams and Wilkins Co., Baltimore, MD.

10. Carringer, R. D., J. B. Weber, and T. J. Monaco. 1975. Adsorption-desorption of selected pesticides by organic matter and montmorillonite. J. Agric. Food Chem. 23:568-572.

11. Chapman, H. D. 1965. Cation-exchange capacity. In C. A. Black (ed.) Methods of soil analysis. Agronomy 9:891-900. Am. Soc. of Agron., Madison, Wis.

12. Colbert, F. O., V. V. Volk, and A. P. Appleby. 1975. Sorption of atrazine, terbutryn, and GS-14254 on natural and lime-amended soils. Weed Sci. 23:390-394.

13. Correll, L., T. Wu, J. W. Pierce, and M. A. Faust. 1977. Rural nonpoint pollution studies in Maryland. EPA Report #904/9-77-001.

14. Couch, R. W., J. V. Gramlich, D. E. Davis, and H. H. Funderburk, Jr. 1965. The metabolism of atrazine and simazine by soil fungi. Proc. Southern Weed Conf., Dallas, TX. 18:623-631.

15. Day, P. R. 1965. Particle fractionation and particle-size analysis. In C. A. Black (ed.) Methods of soil analysis. Agronomy 9:545-567. Am. Soc. of Agron., Madison, Wis.

16. Filip, Z. 1973. Clay minerals as a factor influencing the biochemical activity of soil microorganisms. Folia Microbiol. 18:56-74.

17. Goswami, K. P., and R. E. Green. 1971. Microbial degradation of the herbicide atrazine and its 2-hydroxy analog in submerged soils. Environ. Sci. Technol. 5:426-429.

18. Grimes, D. J. and S. M. Morrison. 1975. Bacterial bioconcentration of chlorinated hydrocarbon insecticides form aqueous systems. Microbial Ecology. 2:43-59.

19. Hall, J. K. 1974. Erosional losses of s-triazine herbicides. J. Environ. Qual. 3:174-179.

20. Hall, J. K. and M. Pawlus. 1973. Runoff and soil losses on Hagerstown silty clay loam: Effect of herbicide treatment. J. Soil Water Conserv. 28:73-76.

21. Hall, J. K., M. Pawlus, and R. E. Higgins. 1972. Losses of atrazine in runoff water and soil sediment. J. Environ. Qual. 1:172-176.

22. Hance, R. J. 1974. Soil organic matter and the adsorption and decomposition of the herbicides atrazine and linuron. Soil Biol. biochem. 6:39-42.

23. Hays, M. H. B., M. Stacey, and J. M. Thompson. 1968. Adsorption of s-triazine herbicides by soil organic matter preparations. p. 75-90. In Isotopes and radiation in soil organic matter studies. Vienna.

24. Helwig, J. T., and K. A. Council. 1979. SAS User's Guide. SAS Institute Inc., Raleigh, NC.

25. Hilton, J. L. 1974. Herbicide handbook of the weed sicnece society of America. Weed Sci. Soc. Am., Champaign, IL.

26. Kaiser, P., J. Pochon, and R. Cassivi. 1970. Effect of triazine herbicides on soil microorganisms. Residue Rev. 32:211-233.

27. Karickhoff, S. W., D. S. Brown, and T. A. Scott. 1979. Sorption of hydropholic pollutants on natural sediments. Water Research 13:241-248.

28. Kaufman, D. D., and P. C. Kearney. 1970. Microbial degradation of triazine herbicides. Residue Rev. 32:235-265.

29. Kaufman, D. D., P. C. Kearney, and T. J. Sheets. 1965. Microbial degradation of simazine. J. Agr. Food Chem. 13:238-241.

30. Ladlie, J. S., W. F. Meggitt, and D. Penner. 1976. Effect of soil pH on microbial degradation, adsorption and mobility of metribuzin. Weed Sci. 24:477-481.

31. Martin, J. P., K. Haider, and D. Wolf. 1972. Synthesis of phenols and phenolic polymers by Hendersonula toruloidea in relation to humic acid formation. Soil Sci. Soc. Amer. Proc. 36:311-315.

32. McCormick, L. L., and A. E. Hiltbold. 1965. Microbiological decomposition of atrazine and diuron in soil. Weeds 14:77-82.

33. Moyer, J. R., R. J. Hance, and C. E. McKone. 1972. The effects of adsorbants on the rate of degradation of herbicides incubated with soil. Soil Biol. Biochem. 4:307-311.

34. Obrigawitch, T., F. M. Hons, J. R. Abernathy, and J. R. Gipson. 1981. Adsorption, desorption, and mobility of metoalachlor in soils. Weed Sci. 29:332-336.

35. Olsen, S. R. and L. A. Dean. 1965. Phosphorus. In C. A. Black (ed.) Methods of soil analysis. Agronomy 9:1035-1048. Am. Soc. of Agron., Madison, Wis.

36. Paris, D. F. and D. L. Lewis. 1976. Accumulation of methoxychlor by microorganisms isolated from aqueous systems. Bull. Environ. Toxicology. 15:24-32.

37. Paris, D. F., D. L. Lewis and J. T. Barnett. 1977. Bioconcentration of toxaphene by microorganisms. Bull. Environ. Toxicology. 17:564-572.

38. Percich, J. A. and J. L. Lockwood. 1978. Interactions of atrazine with soil microorganisms: Population changes and accumulation. Can. J. Microbiol. 24:1145-1152.

39. Pratt, P. F. 1965. Digestion with hydrofluoric and perchloric acids for total potassium and sodium. In C. A. Black (ed.) Methods of soil analysis. Agronomy 9:1019-1034. Am. Soc. of Agron., Madison, Wis.

40. Rao, P. S. C. and J. M. Davidson. 1979. Adsorption and movement of selected pesticides at high concentrations in soils. Water Research 13:375-380.

41. Ritter, W. F., H. P. Johnson, W. F. Lovely, and M. Molnau. 1974. Atrazine, propachlor, and diazinon residues on small agricultural watersheds. Environ. Sci. Technol. 8:38-42.

42. Roeth, F. W., T. L. Lavy and O. C. Burnside. 1969. Atrazine degradation in two soil profiles. Weed Sci. 17:202-205.

43. Savage, K. E. 1976. Adsorption and mobility of metribuzin in soil. Weed Sci. 24:525-528.

44. Skipper, H. D., C. M. Gilmour, and W. R. Furtick. 1967. Microbial versus chemical degradation of atrazine in soils. Soil Sci. Soc. Amer. Proc. 31:653-656.

45. Stotzky, G. 1965. Microbial Respiration. In C. A. Black (ed) Methods of soil analysis. Agronomy 9:1550-1569. Am. Soc. of Agron., Madison, Wis.

46. Talbert, R. E. and O. H. Fletchall. 1965. The adsorption of some s-triazines in soils. Weeds: 13:46-52.

47. Travis, C. C. and Elizabeth L. Etnier. 1981. A survey of sorption relationships for reactive solutes in soil. J. Environ. Qual. 10:8-17.

48. Triplett, G. B., Jr., B. J. Conner, and W. M. Edwards. 1978. Atrazine and simazine concentrations and transport in runoff water from conventional and no-tillage corn watersheds. J. Environ. Qual. 7:77-83.

49. Umbreit, W. W. 1976. Essentials of bacterial physiology. Burgess Publishing Company, Minneapolis. pp. 7-9.

50. Wagner, G. H., and K. S. Chahal. 1966. Decomposition of carbon-14 labeled atrazine in soil samples from Sanborn Field. Soil Sci. Soc. Amer. Proc. 30:752-754.

51. Walker, A. and D. V. Crawford. 1970. Diffusion coefficients for two triazine herbicides in six soils. Weed Res. 10:126-132.

52. Weber, J. B. 1966. Molecular structure and pH effect on the adsorption of 13 s-triazine compounds on montmorillonite clay. Am. Mineral. 51:1657-1670.

53. White, A. W., A. P. Barnett, B. G. Wright and J. H. Holloday. 1967. Atrazine losses from fallow land caused by runoff and erosion. Environ. Sci. Technol. 1:740-744.

54. Wolf, D. C. and J. P. Martin. 1975. Microbial decomposition of ring-^{14}C atrazine, cyanuric acid, and 2-chloro-4,6-diamino s-triazine. J. Environ. Qual. 4:134-139.

55. Wu, T. L. 1980. Dissipation of the herbicides atrazine and alachlor in a Maryland corn field. J. Environ. Qual. 9:459-465.