MUTAGENIC AND LETHAL EFFECT OF SURFACE WATER IN MISSISSIPPI'S RIVERS AND LAKES

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INTRODUCTION

The aquatic systems in the world have been polluted in various degrees with toxic organic and inorganic chemicals following years of civil activity, industrialization, war, and agrichemical usage (Callahan et al. 1979; Edward et al. 1979; Bakre et al.1990, Rehana et al. 1996). Civil activities along the river have released huge amounts of waste into the river. Old pollutants resurfaced from increased combustion of fossil fuels for transportation, heating, and power generation. Agrichemicals and war industries added varieties of new xenobiotics including insecticides to the environments. Our previous study indicated that lower stream of Mississippi River had been polluted with various concentrations of selenium and mercury (Yang et al. 1994).

Our current study intends to focus further on the genotoxic effect of pollutants in the surface waters of rivers and lakes in Mississippi. The first river to be examined in Mississippi is the Mississippi River, one of the major rivers going through most active industrial, agricultural, and populated areas in the U.S.A. The second river, Pearl River, is a local river of Mississippi going through less industrialized rural area. The third river, Tombigbee Waterway is important for transportation for Ohio, Tennessee, Mississippi, Alabama, and Gulf of Mexico. Most of the reservoirs or lakes in Mississippi are located in the rural areas of the state. Study of those rivers and lakes with different backgrounds and functions will help our better understanding of problems in our aquatic environments.

This study intends to assay water samples at three sequentially diluted levels so that best fit regression and the correlation factor (or coefficient) of data can be obtained. The sign and number of the correlation factor is used for assessing the phase difference in the genotoxicity. And the number of bacterial colony formation will be evaluated proportionally to the degree of toxicity in the mutagenic phase and inversely to that in the lethal phase.

MATERIALS AND METHODS

Salmonella mutagenesis test strain TA98 was obtained from Dr. Ames's Laboratory from the University of California at Berkeley. Mutagenic assay was performed according to the method of Ames et al (1977). The test strain, TA98 was streak cultured in MG agar plate (1.5% agar, 2% dextrose, 0.00978% $MgSO_4$, 0.183% citric acid,1% K_2PO_4, and 0.229% $NaHNH_4PO_4$) with the addition of histidine (260 µM), biotin (3 µM) and ampicillin (25 µg/ml) for maintenance. For the preparation of frozen stocks, liquid culture of TA98 was made by shaking cultures of a single colony of tester strain to early stationary phase in 40 ml of the Oxoid Nutrient broth No.2. The 40 ml culture was mixed with 7 ml glycerol and dispensed 1 ml per microtube for storage at -80°C in a deep freezer. For the mutagenecity test, one volume of frozen stocks was diluted into 80 volumes of fresh Nutrient broth No. 2 for culture in a shaker incubator. Following 5 hours of incubation at 30°C and 200 rpm in a shaker incubator, mid-logarithmic phase of the growth was obtained for the bacterial culture to be used for the assay. The serial dilutions were made by mixing 600 µl of bacterial culture with 600 µl of sample (original sample without dilution); 600 µl of culture with 300 µl of sample and 300 µl of distilled water (2 times diluted sample); 600 µl of culture with 150 µl of sample and 450 µl of distilled water (4-times diluted sample). The mixtures of bacterial culture and samples (or standards) in glass test tubes (12 x 100 mm) were pre-incubated at room temperature for 30 minutes after mixing the culture and samples by vortex for 20 seconds. Thereafter, 3 ml of salts-buffer solution containing 0.1 M sodium phosphate buffer (pH 7.4), 33 mM KCl, and 8 mM $MgCl_2$ was added to each tube for 20 seconds vortex. The final mixtures were dispensed 0.7 ml per tube into 5 tubes which contained 2 ml of warm top agar (0.7% agar, 0.54% sodium chloride, 0.5 mM histidine and 0.5 mM biotin) and kept at 50°C in an incubator. Immediately after the addition of assay material into tubes containing warm top agar, the mixture was vortexed vigorously for 30 seconds. The mixture was used for coating a new layer of agar on the top of MG agar plate containing exactly 30 ml of solidified MG agar in it. One hour after coating, the MG plates were placed in an incubator for

incubation at 37°C. The position of plates inverted to upside-down position 24 hours later for continuation of incubation. The number of colonies (revertants) formed by reverse mutation were recorded 48 hours after incubation. Mean numbers and standard deviations of colonies for each sample dilution were calculated from 5 plates. A mean number and a standard deviation were calculated from 5 plates for representation of data at each concentration level. Three mean numbers obtained from the test of three sequentially diluted samples or standards were used for calculation of best fit regression (linear, exponential, logarithmic or power regression) and correlation coefficient for each sample or standards according to the computer software in the HP 48GX graphic calculator. The sign and the two-digit number of the correlation factor (F) obtained by magnification of the correlation coefficient (γ) with a factor of 10. The preliminary data obtained for mutagenic effect of chromium trioxide at sequentially diluted concentrations from 200 mM to 6.25 mM were listed on Table 1 and Figure 1. The responses of TA98 to the increasing concentration of chromium trioxide were used to evaluate the genotoxic effect of the chemical to TA98 at different phases (mutagenic or lethal phases). For a practical approach to the genotoxic effect, the response of TA98 was also divided into three stages (mutagenic, intermediate, or lethal stages) according to the sign and number of the correlation factor. It was evaluated as mutagenic with F-number between +8.5 to +10.0 (with a significant level of $\alpha = 0.10$), and prominently mutagenic with F-number between +9 to +10.0 (with a significant level of $\alpha = 0.07$ in t-test). Similarly, it was evaluated as lethal with F-number between -8.5 to -10.0, and prominently lethal with F-number between -8.5 to -10.0. Intermediate stage was set between +8.5 to -8.5. Using this model of classification, the sequentially diluted chromium trioxide solutions in Table 1 were evaluated from 3 neighboring concentration levels. Similarly the response of test bacteria to three sequentially diluted water samples from the Mississippi River were evaluated (Table 2). The responses of test bacteria to water sample from Pearl River and Tombigbee waterway were also examined in Table 3. Finally, responses of test bacteria to surface waters from lakes or reservoirs were evaluated in Table 4.

RESULTS

The quality of surface water with regard to its genotoxic effect was tested at various locations in the Mississippi River and summarized in Table 2. Minus 10 (Prominent lethal stage; P.L.S) was graded for the water quality at Memphis. After passing the rural area in Tunica, the water quality improved. The surface water of Mississippi River at Greenville was evaluated to be at Plus 10 (Prominent mutagenic stage; P.M.S.). Nevertheless, the water quality was worse at Vicksburg again to the grade of Minus 10 (P.L.S.). Following improvement of water quality at the rural area, Plus 7.2 (Early intermediate stage; E.M.S) was the grade for Natchez and Plus 9.0 (Prominent mutagenic stage; P.M.S.) was the grade for St. Francisville. The water quality degraded again to prominent lethal stage (P.L.S.) at Baton Rouge (Minus 10) and New Orleans (Minus 10). The quality of water improved again at Belle Chase with the grade of Minus 3.2 (Late intermediate stage ; L.I.S.) and degraded again at the Marine Transportation with the grade of Minus 9.1 (Prominent lethal stage).

The genotoxic effect of surface water at various locations in Pearl River was assayed and evaluated in Table 3. The surface water at Edinburg (upper Pearl River) was evaluated to be at Minus 7.2 (Late intermediate stage) which is slightly better than Minus 8.1 (Late intermediate stage) at Ross Barnett Reservoir. Soon after coming out from the Reservoir to the Jackson city near Flowood, the quality of the surface water of Pearl River at Jackson greatly deteriorated to the level of Minus 10, (Prominent lethal stage; P.L.S.). This is the worst water quality which we had examined for Pearl River. However the quality of the surface water was greatly improved after flowing through the rural area of Monticello (Plus 3.9; Early intermediate stage) and Foxworth (Minus 3.7; Late intermediate stage). Near the outlet of Pearl River, the water quality of surface water at East Pearl River was graded as Minus 6.7 (Late intermediate stage). Near another outlet at West Pearl, the water quality of surface water for Pearl River was graded as Plus 10 (Prominent mutagenic stage), the best quality of water which we can see in Pearl River.

Water quality of several larger reservoirs or lakes in Mississippi was evaluated in Table 4. The mutagenic condition of Tombigbee Lake was found to be the best with the grade of Plus 8.9 (Prominent mutagenic phase; P.M.S.). Both Enid Lake and Grenada Reservoir were evaluated as Minus 2.7 (Late intermediate stage) and Minus 7.6 (Late intermediate stage) respectively in their grades of water quality. The water quality at Enid Lake appeared to be better than that at Grenada Reservoir. On the other hand, water quality at Ross Barnett Reservoir and Pontacella Lake were evaluated as Minus 8.1 (Late intermediate stage) and Plus 8.6 (Early intermediate stage) respectively. The water quality at Pontachella Lake appeared to be much better than that at Ross Barnett Reservoir in its genotoxic effect. The quality of surface water at Sandis Lake was evaluated as Minus 10 (Prominent lethal stage), the worst water quality in the class of lakes and reservoir in the state.

DISCUSSION

Two different phases (mutagenic and lethal phases) and three different stages (mutagenic, intermediate, and lethal stages) were proposed in the current study for classification of bacterial responses to different concentrations of genotoxic chemical, chromium trioxide (Table 1 and Figure 1). The bell-shaped curve in the number of colony formation in response to the increased concentration of the mutagenic chemical is the commonly observed phenomena in the genotoxic reaction. The reaction curve may be modified somewhat in the mixed contamination with several chemicals. In such occasion, the formation of several peak heights in intermediate phase may complicate the simplicity of the reaction curve. Except the complicated intermediate stage, the initial mutagenic stage and the final lethal stage in the mixed chemical pollutions may not be so different from those initial mutagenic and final lethal stages in a single chemical pollution. Three stages of classification for grading of genotoxic environment appears to be not only useful but also effective for evaluation of mixed contamination with several genotoxic chemicals. It is simple, practical and clear cut in its evaluation of the genotoxic environment in the surface water of rivers and lakes (Tables 2, 3, and 4).

In the cities of Baton Rouge and New Orleans where big oil refineries and other petrochemical industries are very active, the waste materials from the industries might have contaminated the surface water of rivers causing high incidence of genotoxicity and carcinogenesis. However, in both Memphis and Jackson, the genotoxic effect of the surface water was increased due to the higher population of the cities. Among all smaller cities or towns in Mississippi, Vicksburg appears to have genotoxicity of the river water unparallel to such size of city in population. It is possible that the casino industries bring in many peoples to cause pollution in the city. It is also possible that this city may still have the genotoxic damages of explosive chemicals left from the previous civil war. It is well documented that nitro benzene and other nitrated aromatic compounds are mutagenic and carcinogenic. (Watanabe et al. 1989; U.S. EPA 1978).

In spite of the increased genotoxic effect in the surface water of the river near the big city, the reduced genotoxic effect in the surface water of the river near the small town or rural area was recognized in Mississippi River and also in Pearl River. It is apparent that both rivers have remediation capability of the genotoxic materials in their river water environment. The remediation effect is particularly clear in a river like the Pearl River which has more rural areas along the lower stream than in the upper stream. It is well demonstrated that some enzymes of microorganisms, algae, and water plants are capable of metabolizing many genotoxic chemicals. In addition, some antioxidants and binding chemicals are also produced from the microorganisms, algae or water plants, and are capable for neutralizing such genotoxic chemicals in the water.

CONCLUSION

Genotoxic effects of sample waters at three sequentially diluted water levels were assayed with Ames test for measuring their effects to the numbers of colony formation. The concentration of each sample (fixed X-variables) and the number of revertants (free Y-variables) corresponding to that were used for calculation of correlation factor (converted from the correlation coefficient) at best fit regression. Following the 10 times magnification of the correlation coefficient for the correlation factor, the sign and the two-digit number of the correlation factor were used for evaluation of the status of genotoxic environment in the river water or in the lake water. According to these evaluations, the genotoxic environments of the surface water were compared with each other on their stages of genotoxicity in addition to their intensity to cause reverse mutation. The surface water of rivers (Mississippi River, Pearl River, and Tombigbee Waterway), lakes and reservoirs in Mississippi and neighboring states were contaminated with various degrees of genotoxic pollutants, particularly in the big cities of Memphis, Baton Rouge, and New Orleans. However, medium sized cities, such as Jackson (Flowood) was also seriously polluted in the Pearl River. When the river goes through other rural area, the remediation of river takes place so that the toxic water will be cleaned into better genotoxic level. In general, the genotoxic environments of the lakes were found to be better than the neighboring Mississippi River in Mississippi.

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This work is dedicated to the heroic death of Dr. Ye Shengji at Taipei 49 years ago.

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Figure 1. Mutagenic effect of Chromium Trioxide on TA98 at various concentration levels.

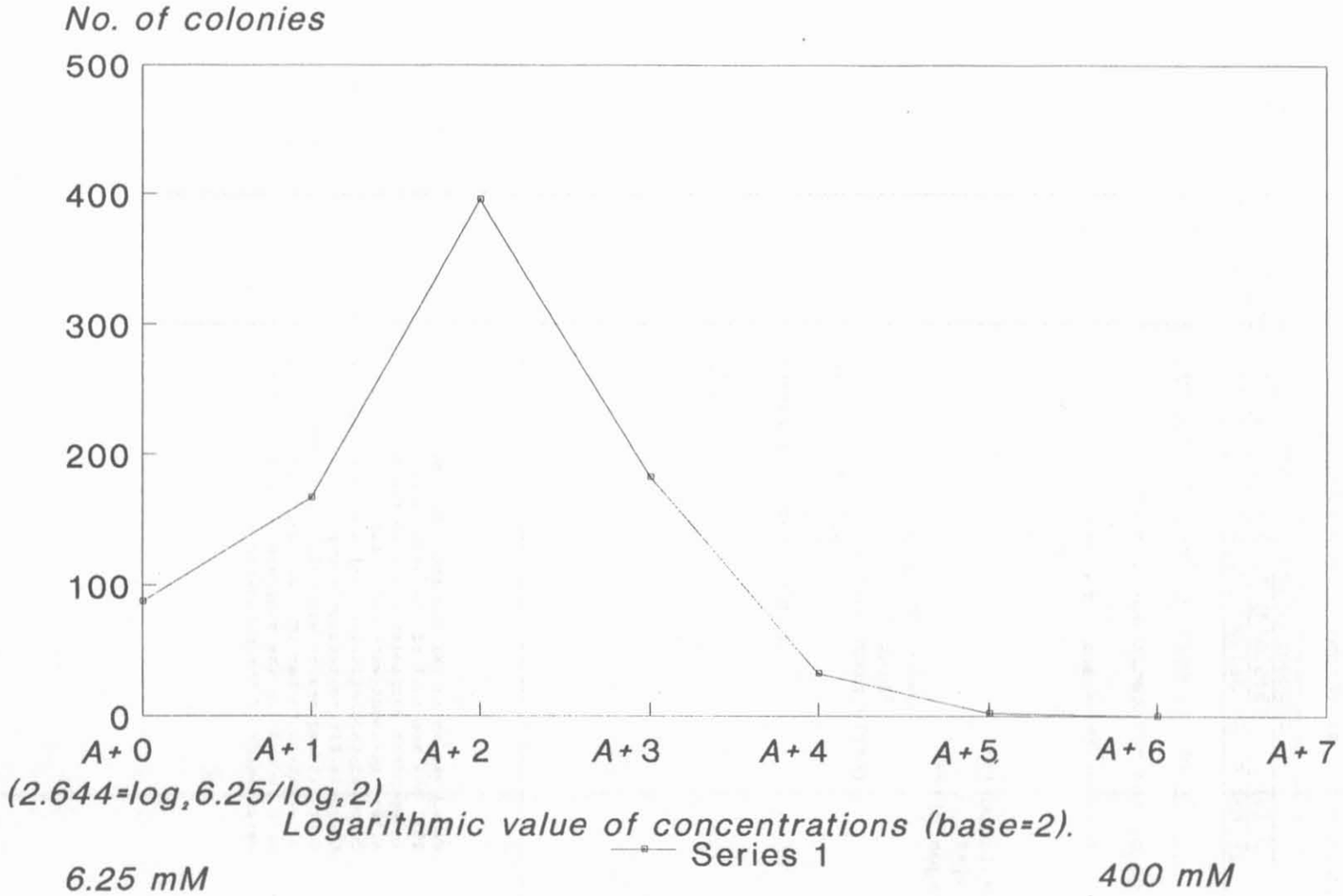
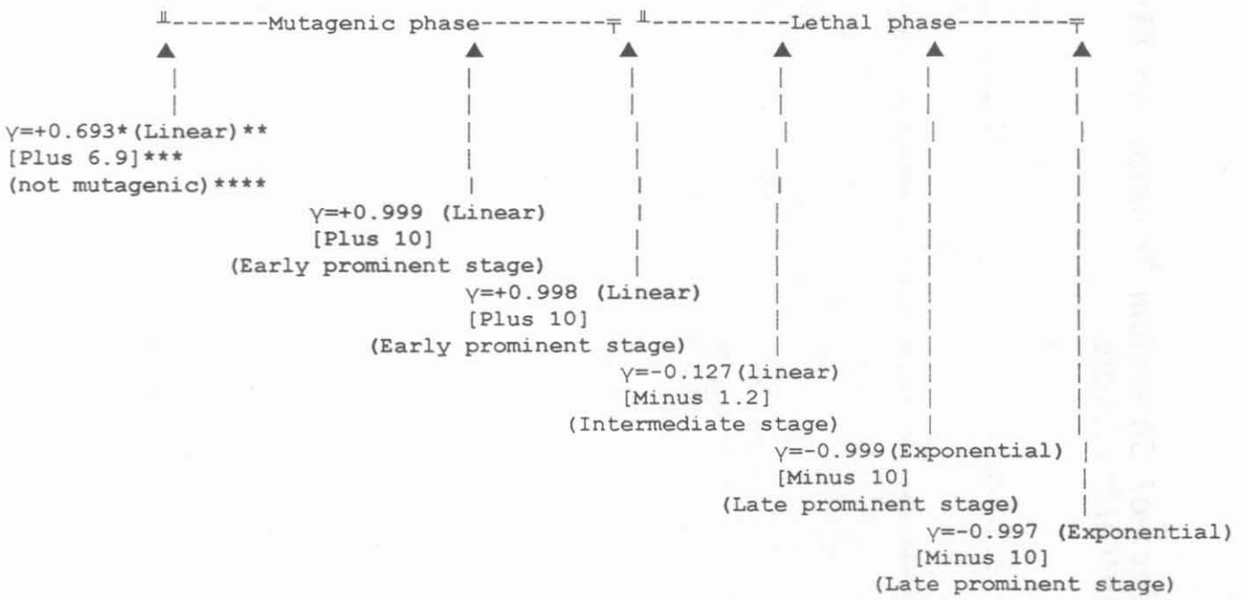


Table 1. Quantitative and descriptive evaluations of genotoxicity at various concentration of chromium trioxide.

	Concentrations of chromium trioxide; mM (%)						
X:	0 (0)	6.25(0.063)	12.5(0.125)	25(0.125)	50(0.25)	100(1.0)	200(2.0)
Y:	18.6±4.0	87.5±4.3	166.3±8.7	395.7±89.4	182.3±9.6	32.4±9.6	2.3±0.6

Colony numbers of TA98 formed; Mean±standard deviation

Evaluation of the mutagenic environment at different concentration levels



X: Fixed variables (X) for the the concentrations of the test sample were set as 25%, 50% and 100% of the water sample.

Y: Independent variables (Y) were the numbers of colony formed for the test bactia after treatment with water sample.

*: Correlation coefficient (*) was calculated between two variables (X and Y) under the best fit regression curve.

**: Best fit regression was calculated according to the Hewlett Packard HP 48GX calculator.

***: Correlation factor (F) was calculated by multiplication of correlation coefficent with 10 and use the sign and two digitnumber of the product to express that .

****: Description of the mutagenic environment based on the correlation factor (F).

Table 2. Mutagenic effect of surface water at various locations in Mississippi River.

X:	Sample Concent.			(%)Corr. C.(Y)*	Corr. F. (F).***
	25	50	100	(best fit reg)**	(Des. Eval.)****
Y:					
Memphis(1)	21.3±6.2	17.5±6.8	13.4±5.0	-0.999(log)	Minus 10(P.L.S.)
Greenville(2)	17.3±3.9	17.8±5.0	19.6±7.0	+0.994(Exp)	Plus 10(P.M.S.)
Vicksburg(2)	23.0±8.1	20.0±5.4	13.5±1.3	-0.999(Lin)	Minus 10(P.L.S.)
Natchez(1)	12.5±2.4	16.8±4.3	16.6±5.9	+0.721(Lin)	Plus 7.2(E.I.S.)
St.Francisville	14.0±4.1	15.3±3.8	15.8±5.6	+0.904(Lin)	Plus 9.0(P.M.S.)
Baton Rouge(1)	21.3±6.2	17.3±5.1	15.0±5.5	-0.998(Pow)	Minus 10(P.L.S.)
New Orleans(1)	17.0±3.5	15.0±3.6	9.6±3.0	-0.997(Lin)	Minus 10(P.L.S.)
Belle Chase(1)	14.0±3.5	21.5±6.1	13.0±2.3	-0.321(Exp)	Minus 3.2(E.I.S.)
Marine Tran(1)	24.3±5.6	17.8±3.8	17.2±5.6	-0.907(Pow)	Minus 9.1(P.L.S.)
Distilled water	17.8±3.5	17.3±1.0	18.4±4.5	+0.693(Lin)	Plus 6.9(stand.)
Daunomycin (5 µg/ml)	227±75	166±63	55±18	-0.999(Lin)	Minus 10(stand.)

X: Fixed variables (X) for the the concentrations of the test sample were set as 25%, 50% and 100% of the water sample.

Y: Independent variables (Y) were the numbers of colony formed for the test bactia after treatment with water sample.

*: Correlation coefficient (*) was calculated between two variables (X and Y) under the best fit regression curve.

**: Best fit regression was calculated according to the Hewlett Packard HP 48GX calculator.

***: Correlation factor (F) was calculated by multiplication of correlation coefficent with 10 and use the sign and two digitnumber of the product to express that .

****: Description of the mutagenic environment based on the correlation factor (F).

Table 3. Mutagenic effect of surface water at various locations in Pearl River and in Tombigbee Waterway.

X:	Sample Concent.			(%)Corr. C.(Y)*	Corr. F. (F).***
	25	50	100	(best fit reg)**	(Des. eval.)****
Y:					
Edinburg(1)	17.3±3.3	14.5±4.4	15.2±2.4	-0.720(Log)	Minus 7.2(L.I.S.)
Ross Barnett R.	17.3±3.9	18.0±3.6	15.8±2.9	-0.805(Exp)	Minus 8.1(L.I.S.)
Jackson(F1)	16.3±2.9	15.5±3.3	14.2±2.6	-0.999(Exp)	Minus 10(P.L.S.)
Jackson(F2)	19.5±3.1	18.8±6.5	17.8±5.0	-0.997(Exp)	Minus 10(P.L.S.)
Monticello(1)	12.3±4.5	15.3±2.3	13.4±6.7	+0.389(Pow)	Plus 3.9(E.I.S.)
Foxworth(1)	10.0±3.5	15.3±3.3	9.0±1.6	-0.369(Exp)	Minus 3.7(L.I.S.)
East P. & Hwy95	13.0±2.7	14.0±7.0	12.0±2.8	-0.671(Exp)	Minus 6.7(L.I.S.)
West P. & Hwy95	12.8±1.7	14.0±2.8	15.0±3.4	+0.998(Log)	Plus 10(P.M.S.)
Tombigbee Wwy	17.1±4.2	19.5±3.1	13.2±2.8	-0.772(Exp)	Minus 7.7(L.I.S.)
Tombigbee Lake	10.6±4.2	9.4±7.0	16.8±4.2	+0.885(Lin)	Plus 8.9(M.S.)
Distilled water	17.8±3.5	17.3±1.0	18.4±4.5	+0.693(Lin)	Plus 6.9(stand.)
Daunomycin (5 µg/ml)	227±75	166±63	55±18	-0.999(Lin)	Minus 10(stand.)

X: Fixed variables (X) for the the concentrations of the test sample were set as 25%, 50% and 100% of the water sample.
Y: Independent variables (Y) were the numbers of colony formed for the test bactia after treatment with water sample.
*: Correlation coefficient (*) was calculated between two variables (X and Y) under the best fit regression curve.
**: Best fit regression was calculated according to the Hewlett Packard HP 48GX calculator.
***: Correlation factor (F) was calculated by multiplication of correlation coefficent with 10 and use the sign and two digitnumber of the product to express that .
****: Description of the mutagenic environment based on the correlation factor (F).

Table 4. Mutagenic effect of surface water at various lakes and reservoirs in Mississippi.

X:	Sample Concent.			(%)Corr. C.(Y)*	Corr. F. (F).***
	25	50	100	(best fit reg)**	(Des. Eval.)****
Y:					
Sardis L.	17.3±3.3	14.5±4.4	11.8±2.9	-0.999(Log)	Minus 10(P.L.S.)
Enid L.	14.3±4.6	15.3±3.3	14.2±3.9	-0.271(Exp)	Minus 2.7(L.I.S.)
Grenada R.	13.8±9.0	15.0±7.8	12.0±1.7	-0.756(Exp)	Minus 7.6(L.I.S.)
Ross Barnett R.	17.3±3.9	18.0±3.6	15.8±2.9	-0.805(Exp)	Minus 8.1(L.I.S.)
Pontachella L.	13.5±5.6	12.0±2.6	19.6±5.9	-0.867(Lin)	Plus 8.6(M.S.)
Tombigbee Wwy	17.1±4.2	19.5±3.1	13.2±2.8	-0.772(Exp)	Minus 7.7(L.I.S.)
Tombigbee Lake	10.6±4.2	9.4±7.0	16.8±4.2	+0.885(Lin)	Plus 8.8(M.S.)
Distilled water	17.8±3.5	17.3±1.0	18.4±4.5	+0.693(Lin)	Plus 6.9(stand.)
Daunomycin (5 µg/ml)	227±75	166±63	55±18	-0.999(Lin)	Minus 10(stand.)

X: Fixed variables (X) for the the concentrations of the test sample were set as 25%, 50% and 100% of the water sample.

Y: Independent variables (Y) were the numbers of colony formed for the test bactia after treatment with water sample.

*: Correlation coefficient (*) was calculated between two variables (X and Y) under the best fit regression curve.

**: Best fit regression was calculated according to the Hewlett Packard HP 48GX calculator.

***: Correlation factor (F) was calculated by multiplication of correlation coefficent with 10 and use the sign and two digitnumber of the product to express that .

****: Description of the mutagenic environment based on the correlation factor (F).