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ENVIRONMENTAL MUTAGENESIS: WATER STUDIES

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ABSTRACT

The "Blue Cotton" method was used to separate mutagens from water samples from rivers and esteros around Manila. The Rec assay was used to determined direct DNA damaging capacity. Ames test was employed to study mutagenicity potential before metabolic activation. Genetoxicity after metabolic activation was determined by the Host-mediated assay while chromosome breaking effects were investigated using the micronucleus test.

Water samples from Pasig river, Las Pinas river, Marikina river, Navotas river, and Zapote river did not possess direct DNA damaging potential. These did not exhibit mutagenic activity before and after metabolic activation. These did not possess chromosome breaking effects.

Water samples from Malabon river, (near Federico St., Isla de Cocoma), Paranaque river (across MIA road), San Juan river (across P. Sanchez St.) and Taguig river (along Gen. Luna St., Sta. Ana, Peteros) were mutagenic before metabolic activation. However, the mutagenicity of the water samples from Paranaque and Taguig rivers disappeared after metabolic activation. Water samples from Malabon river and San Juan river were genotoxic before and after metabolic activation. None of the river water samples around Manila exhibited chromosome breaking effects. Water samples frm 20 esteros did not exhibit direct DNA damaging effects and were not mutagenic before and after metabolic activation. However, chromosome breaking effects were observed from water samples of Estero de Magdalena, Estero de Uli-Uli, Estero de Tripa de Galina and estero de San Lazaro.

Introduction

Mutagen pollution in our rivers and esteros should be of great concern. Some of these mutagens can be transformed to atmosphere genetoxins that will not only affect the populace near these rivers and esteros but also those of distant places.

Mutagens are substances that alter the structure of DNA, the genetic material of the living cells. Mutagenic effects on somatic cells can induce cancer while mutagenic effects on germ cells can lead to genetic disorders that can be transmitted

from one generation to the next. Mutagenic effects of cells during organogenesis can induce physical defects.

Materials and Methods

Blue Cotton was purchased from Funakoshi Pharmaceuticals, Tokyo Japan. *Salmonella typhimurium*, TA 100 was a gift from Dr. Bruce N. Ames, University of California, Berkeley. *Bacillus subtilis* (Rec^+ and Rec^- strains) were obtained from the National Institute of Genetics, Mishima, Japan). *Salmonella typhimurium* His G 46, was given by Dr. Masaaki Meriya, of the National Institute of Toxicology, Tokyo, Japan. Fetal calf serum was obtained from Grand Island Biological Supply, New York.

The Blue Cotton method (1) was used to separate mutagens from water samples that were obtained from rivers and esteros around Metro Manila. Blue cotton is an adsorbent cotton bearing covalently linked trisulfo-copper-pthalocyanine residues which adsorb polycyclic aromatic hydrocarbons and heterocyclic amines. The adsorbed materials were eluted from Blue Cotton using methanol-ammonium hydroxide mixture (50:1). After the solvent was evaporated, the mutagenic material was taken up in dimethylsulfoxide. The extract was used for Rec assay, Ames test, Host-mediated assay and micronucleus test.

Rec assay (2) was used to study the direct DNA damaging potential of the test systems, while Ames test (3) was employed to study the mutagenicity potential before metabolic activation. Mutagenicity after metabolic activation was studied using the Host-mediated assay (4) and chromosome breaking effects were determined by the micronucleus test (5).

Results and Discussion

Table 1 shows the location of different sampling places of the rivers around Metro Manila. Table 2 shows that the water samples from Pasig river and other rivers around Metro Manila did not exhibit direct DNA damaging potential. No zones of inhibition were observed even with Rec– strain of Bacillus subtilis which does not possess a recombination repair system.

From Table 3, mutagenicity before metabolic activation is exhibited by water samples from Malabon river (near Federico St., Isla de Cocomo), Paranaque river (across MIA road), San Juan river (across P. Sanchez St.) and Taguig river (along Gen. Luna street, Sta. Ana, Pateros). These samples possibly contain base-pair mutagens before metabolic activation. *Salmonella typhimurium* TA 100 is easily reverted to the wild type by base-pair mutagens because it does not contain the excision repair system and it contains and R plasmid which makes it more sensitive to test systems.

Table 1. Location and designation of river water samples

Location	*Sample Designation*
Pasig River	
Barangay Napindan, Taytay, Rizal	R-I-A
Along Rembo St., at Malapad na Bato	R-I-B
Along EDSA	R-I-C
Across Panadero St.	R-I-D
Across Quezon Bridge	R-I-F
Across Jones Bridge, Sta. Cruz, Manila	R-I-G
Across Roxas Bridge, Intramuros, Manila	R-I-H
Las Pinas River	
Across Quirino, Avenue, Las Pinas	R-II-A
Malabon River	
Across Gov. W. Pascual Ave.	R-III-A
Marikina River	
Along Bonifacio Ave.	R-IV-A
Across Marcos Highway, Tanong, Marikina	R-IV-B
Across Ortigas Ave., At Rosario	R-IV-C
Navotas River	
Across Capt. E. Cruz St., Flores, Navotas	R-V-A
Near Navotas Market	R-V-B
Paranaque River	
Across MIA Road	R-VI-A
Across Imelda Ave.	R-VI-B
San Juan River	
Across N. Domingo St., San Juan	R-VII-A
Across P. Sanchez St., San Juan	R-VII-B
Across Kampal St., Bacood	R-VII-C
Taguig River	
Along Gen. Luna St., Sta. Ana, Pateros	R-VIII-A
Mouth of the River	R-VIII-B
Zapote River	
At Trinidad St., San Isidro Subd.	R-IX-A
Near Pulang Lupa, Las Pinas	R-IX-B

Table 2. DNA damaging potential of river water samples

	Zones of Inhibition (mm)	
	Rec$^+$	*Rec*$^-$
Benzo(a)pyrene, positive control	17.37 ± 1.25	22.15 ± 2.24
DMSO, negative control	0	0
Distilled water	0	0
R-I-A	0	0
R-I-B	0	0
R-I-C	0	0
R-I-D	0	0
R-I-E	0	0
R-I-F	0	0
R-I-G	0	0
R-I-H	0	0
R-II-A	0	0
R-III-A	0	0
R-III-B	0	0
R-IV-A	0	0
R-IV-B	0	0
R-IV-C	0	0
R-V-A	0	0
R-V-B	0	0
R-Vl-A	0	0
R-VI-B	0	0
R-VII-A	0	0
R-VII-B	0	0
R-VII-C	0	0
R-VIII-A	0	0
R-VIll-B	0	0
R-IX-A	0	0
R-lX-B	0	0

The mutagenic activity of water samples from Paranaque river and Taguig river disappeared after metabolic activation as shown in Table 4. The genotoxic activity of water samples from Malabon and San Juan rivers was still exhibited after metabolic activation. There is a possibility for some mutagens to be transformed to non-mutagens after metabolism or some pro-mutagens to be transformed to mutagens after metabolism.

No chromosome breaking effects were observed from the water samples from different rivers (Table 5) inspite of the fact that some samples exhibited mutagenic activity after metabolic activation. It is possible that the mutagens produced after metabolism possess a lifetime short enough to allow them to reach the bene marrow

Table 3. Mutagenicity potential before metabolic activation of river water samples

	Rivertants per plate TA 100
Benzo(a)pyrene, positive control	TNTC
DMSO, negative control	31.41 ± 1.56
R-I-A	28.22 ± 3.12
R-I-B	27.16 ± 1.67
R-I-C	33.19 ± 3.12
R-I-D	25.77 ± 1.43
R-I-E	32.45 ± 1.89
R-I-F	29.87 ± 2.11
R-I-G	32.45 ± 2.09
R-I-H	30.99 ± 1.67
R-II-A	34.29 ± 5.22
R-III-A	31.86 ± 4.32
R-III-B	69.34 ± 3.12
R-IV-A	38.11 ± 2.67
R-IV-B	31.67 ± 3.11
R-IV-C	29.98 ± 2.65
R-V-A	39.11 ± 3.62
R-V-B	39.32 ± 2.65
R-VI-A	80.43 ± 2.11
R-VI-B	24.72 ± 4.23
R-VII-A	28.45 ± 2.22
R-VII-B	78.22 ± 1.47
R-VII-C	31.12 ± 3.22
R-VIII-A	69.41 ± 3.25
R-VIII-B	38.25 ± 2.89
R-IX-A	39.92 ± 4.11
R-IX-B	38.77 ± 3.26

Table 4. Mutagenicity potential after metabolic activation of river water samples

	Mutation Frequency of S. typhimurium His G 46
Benzo(a)pyrene, positive control	16.24 ± 4.22
DMSO, negative control	1.01 ± 0.12
Distilled water	0.71 ± 0.05
R-I-A	0.95 ± 0.02
R-I-B	1.18 ± 0.13
R-I-C	0.87 ± 0.05

Table 4 (Continued)

	Mutation Frequency of S. typhimurium His G 46
R-I-D	0.71 ± 0.04
R-I-E	0.51 ± 0.07
R-I-F	1.07 ± 0.07
R-I-G	0.92 ± 0.04
R-I-H	1.97 ± 0.09
R-II-A	1.15 ± 0.03
R-III-A	1.00 ± 0.07
R-III-B	5.28 ± 0.97
R-IV-A	1.25 ± 0.03
R-IV-B	1.26 ± 0.07
R-IV-C	0.76 ± 0.02
R-V-A	2.49 ± 0.09
R-V-B	0.76 ± 0.05
R-VI-A	0.62 ± 0.05
R-VI-B	1.02 ± 0.06
R-VII-A	1.19 ± 0.09
R-VII-B	9.67 ± 0.94
R-VII-C	8.71 ± 2.43
R-VIII-A	0.96 ± 0.05
R-VIII-B	1.05 ± 0.04
R-IX-A	0.36 ± 0.01
R-IX-B	0.82 ± 0.08

Table 5. Chromosome breaking effects of river water samples

	No. of micronucleated polychromatic erythrocytes per thousand
Benzo(a)pyrene, positive control	18.45 ± 1.83
DMSO, negative control	2.66 ± 0.04
Distilled water	1.44 ± 0.05
R-I-A	1.55 ± 0.02
R-I-B	2.22 ± 0.07
R-I-C	1.55 ± 0.06
R-I-D	2.33 ± 0.08
R-I-E	2.32 ± 0.09
R-I-F	2.99 ± 0.86
R-I-G	2.54 ± 0.39
R-I-H	2.55 ± 0.16

Table 5 (Continued)

	No. of micronucleated polychromatic erythrocytes per thousand
R-II-A	0.77 ± 0.03
R-III-A	0.83 ± 0.24
R-III-B	1.33 ± 0.09
R-IV-A	0.49 ± 0.03
R-IV-B	0.88 ± 0.03
R-IV-C	0.53 ± 0.01
R-V-A	1.44 ± 0.07
R-V-B	0.83 ± 0.06
R-VI-A	1.22 ± 0.09
R-VI-B	1.22 ± .0.05
R-VII-A	1.33 ± 0.06
R-VII-B	0.55 ± 0.09
R-VII-C	1.31 ± 0.09
R-VIII-A	0.66 ± 0.05
R-VIII-B	1.28 ± 0.34
R-IX-A	0.99 ± 0.07
R-IX-B	1.22 ± 0.12

cells. Had they reached the bone marrow cells, they could have fragmented the chromatin material and after telephase some fragments could be left behind resulting in the formation of micronucleated cells. In all the samples examined, the formation of micronucleated polychromatic erythrocytes was not significant.

Water samples from the different esteros did not exhibit direct DNA damaging potential (Table 6). Mutagenicity before (Table 7) and after (Table 8) metabolic activation were not observed. However, water samples from Estero de Magdalena, Estero de San Lazaro, Estero de Tripa de Gallina and Estero de Uli-Uli exhibited chromosome breaking effects. The rest of the water samples from the other esteros were not clastogenic.

Table 6. Direct DNA damaging potential of water samples from esteros around Metro Manila

	Zone of inhibition (mm)	
	Rec$^+$	*Rec*$^-$
4-nitroquinoline oxide, positive control	18.72 ± 2.89	26.51 ± 3.16
DMSO, negative control	0	0
Estero de Balete	0	0

Table 6 (Continued)

	Zone of inhibition (mm)	
	Rec^{+}	*Rec*$^{-}$
Estero de Binondo	0	0
Estero de Concordia	0	0
Dilain Creek	0	0
Ermitano Creek	0	0
Junction of esteros of Magdalen, Tutuban and La Reina	0	0
Estero de La Reina	0	0
Estero de Magdalena	0	0
May Atlas Creek	0	0
Estero de Maypajo	0	0
Estero de Pandacan	0	0
Estero de San Lazaro	0	0
Estero de San Miguel	0	0
Sunog Apog Creek	0	0
Estero de Tan Que	0	0
Estero de Tripa de Gallina	0	0
Estero de Tutuban	0	0
Estero de Uli-Uli	0	0
Estero de Valencia	0	0
Estero de Vitas	0	0

Table 7. Mutagenicity potential before metabolic activation of water samples from esteros in Metro Manila

	Revertants per plate TA 100
Methylmethane sulfonate, positive control	TNTC
DMSO, negative control	31.41 ± 1.56
Estero de Balete	28.66 ± 3.22
Estero de Binondo	31.12 ± 1.45
Estero de Concordia	29.54 ± 4.11
Dilain Creek	34.11 ± 1.89
Ermitano Creek	31.17 ± 2.11
Junction of esteros de Magdalena, Tutuban and La Reina	29.87 ± 3.21
Estero de la Reina	33.11 ± 2.87
Estero de Magdalena	29.15 ± 1.37

Table 7 (Continued)

	Revertants per plate TA 100
May Atlas Creek	34.11 ± 1.66
Estero de Maypajo	22.87 ± 4.67
Estero de Pandacan	36.32 ± 2.64
Estero de San Lazaro	35.21 ± 2.65
Estero de San Miguel	28.54 ± 3.89
Sunog Apog Creek	28.76 ± 4.54
Estero de Tan Que	32.12 ± 2.36
Estero de Tripa de Gallina	27.65 ± 3.42
Estero de Tutuban	31.18 ± 3.11
Estero de Uli-Uli	32.78 ± 4.11
Estero de Valencia	23.89 ± 6.77
Estero de Vitas	32.87 ± 3.67

TNTC – Too numerous to count.

Table 8. Mutagenicity potential after metabolic activation of water samples from esteros around Metro Manila

	Mutation frequency of S. typhimurium His G 46
Benzo(a)pyrene, positive control	13.43 ± 1.36
DMSO, negative control	0.69 ± 0.05
Estero de Balete	0.64 ± 0.06
Estero de Binondo	0.62 ± 0.07
Estero de Concordia	1.12 ± 0.08
Dilain Creek	0.56 ± 0.03
Ermitano Creek	0.57 ± 0.03
Junction of Esteros de Magdalena, Tutuban and La Reina	0.64 ± 0.06
Estero dela Reina	1.16 ± 0.09
Estero de Magdalena	0.67 ± 0.05
May Atlas Creek	0.68 ± 0.10
Estero de Maypajo	0.52 ± 0.04
Estero de Pandacan	0.52 ± 0.10
Estero de San Lazaro	0.62 ± 0.04
Estero de San Miguel	0.49 ± 0.07
Sunog Apog Creek	0.49 ± 0.01

Table 8 (Continued)

	Mutation frequency of S. typhimurium His G 46
Estero de Tan Que	0.73 ± 0.08
Estero de Tripa de Gallina	0.81 ± 0.06
Estero de Tutuban	0.46 ± 0.06
Estero de Uli-Uli	0.66 ± 0.05
Estero de Vitas	0.69 ± 0.05
Estero de Valencia	0.22 ± 0.02

Table 9. Chromosome breaking potential of water samples from esteros around Metro Manila

	No. of micronucleated polychromatic erythrocytes per thousand
Benzo(a)pyrene, positive control	14.99 ± 1.28
DMSO, negative control	1.55 ± 0.19
Estero de Balete	2.33 ± 0.32
Estero de Binondo	1.81 ± 0.15
Estero de Concordia	2.44 ± 0.11
Dilain Creek	1.44 ± 0.19
Ermitano Creek	1.55 ± 0.05
Junction of Esteros de Magdalena, Tutuban and La Reina	2.21 ± 0.07
Estero de la Reina	2.99 ± 0.18
Estero de Magdalena	4.11 ± 0.54
May Atlas Creek	2.21 ± 0.50
Estero de Maypajo	1.88 ± 0.06
Estero de Pandacan	2.84 ± 0.17
Estero de San Lazaro	5.41 ± 0.23
Estero de San Miguel	1.24 ± 0.11
Estero de Tan Que	1.55 ± 0.69
Sunog Apog Creek	2.86 ± 0.51
Estero de Tripa de Gallina	4.81 ± 0.62
Estero de Tutuban	2.77 ± 0.38
Estero de Uli-Uli	5.33 ± 0.10
Estero de Valencia	1.99 ± 0.13
Estero de Vitas	1.89 ± 0.11

Conclusion

Water samples from Malabon River, San Juan River, Paranaque River, and Taguig River exhibited genotoxic activity before metabolic activation but after metabolic activation the genotoxic activity of samples from Paranaque and Taguig Rivers disappeared.

Water samples from Malabon river and San Juan river were genotoxic before and after metabolic activation.

None of the river water samples around Metro Manila exhibited chromosome breaking effects.

Water samples from 20 esteros did not exhibit mutagenic activity before and after metabolic activation but chromosome breaking effects were observed in some of them.

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