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Ames' Mutagenic Activity in Recycled Water from an Israeli Water Reclamation Project

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Scarcity of adequate amounts of rainfall in arid zones around the world as well as the increasing demand for water by industrialized nations have created the need for the development of various technological methods for the reclamation of water for human and agricultural uses. These approaches include desalinization of brackish water and purification of sewage by natural eutrophication. A project using eutrophication of sewage water has recently reached the final stages of development at a major facility located south of Tel Aviv, Israel (IDELOVITCH et al. 1976).

This project involves the siphoning of a portion of sewage outflow from the Tel Aviv area into a series of fanshaped holding ponds where the water becomes microbiologically and chemically purified due to heavy algae overgrowth. The bulk of the algae are then precipitated by the addition of magnesium hydroxide. The partially clarified water is returned to a second series of polishing ponds where residual algae and insoluble magnesium oxide continue to settle out, as well as ammonia diffuses from the water's surface resulting in the pH returning towards neutrality. From the outlet of the polishing ponds, the considerably purified water is pumped to nearby sand dunes where it is allowed to percolate through the sand for final purification and storage in underground tables. Presently, this water is being used solely for agricultural purposes.

One of the major areas of concern in the release of this water for direct human consumption involves the possibility of the presence of carcinogens. For example, polycylic aromatic hydrocarbons have recently been identified in sewage lagoons and lakes (ROSE & HARSHBARGER 1977). To test the water from the purification facility for the presence of either known or unknown carcinogens is extremely expensive and time-consuming. Recently, a short term in vitro assay for potential carcinogens has been developed (AMES et al. 1975). This test depends upon the back-mutation (reversion) of histidine auxotrophs of Salmonella typhimurium. A high correlation has been shown to exist between mutagens in this system and mammalian carcinogens (MCCANN et al. 1975, MCCANN & AMES 1976). The test has the further advantage of assessing the role of mammalian host factors, such as metabolism, on the activation of mutagens/carcinogens, thus allowing detection of pro-mutagens/pro-carcinogens. This assay has recently been used to determine the carcinogenic

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potential of the water obtained from the Mississippi River (POLEN et al. 1977) and from treated waste water (SAXENA & SCHWARTZ 1979).

Preliminary tests performed with unconcentrated water from the Israeli facility suggested the presence of low levels of pro-mutagens/pro-carcinogens (SHUVAL et al. 1977). Testing unconcentrated water directly in the Salmonella system has the disadvantage that only mutagens/carcinogens present in relatively high concentrations can be detected due to sample volume limitation. However, recently, various non-polar resins have been used to absorb and concentrate drugs and their metabolites from urine (MULE et al. 1971, COX & LEVIN 1975) and organic compounds from sea water and fresh water (RILEY & TAYLOR 1965, LAWRENCE & TOSINE 1976). Those columns have the added advantage of not allowing histidine to appear in the final concentrate in amounts that would interfere with the assay for mutagenicity (YAMASAKI & AMES 1977).

In this study, we collected samples of unconcentrated water from two sites in the polishing ponds and from a well located adjacent to the polishing ponds. In addition, a non-polar resin (XAD-2) was used to concentrate potential mutagenic materials in the water collected from the polishing pond at the terminal end of the reclamation project. The water samples and the concentrates were then tested by the Ames assay system.

MATERIALS AND METHODS

Water Samples. A water sample was drawn from a well (identified as Well #5 on a map of the facility) into which water has percolated through the ground from the final polishing ponds. Water was also taken directly from the polishing ponds (identified as sampling site 10-11 on the map). The water was immediately transported to the laboratory where each of the samples mentioned above was centrifuged and the clarified water filtered through a 0.45μm sterile membrane. The water was then stored at 4°C until assayed.

In a subsequent series of experiments, water was taken from site 18 located at the end of the polishing ponds and stored at 4°C until it was flown to our laboratory in Baltimore where it was received within 24 h of having been drawn from the pond. It was filtered through Whatman No. 1 filter paper.

Concentration of Samples on XAD-2 Resin. XAD-2 resin was washed several times by mixing and decanting with ten volumes of acetone followed by absolute methanol and distilled water. It was then stored at 4°C. Glass columns (2 x 20 cm) were loaded with 18.5 cm^3 of washed XAD-2 resin. Filtered water samples up to 5 L were loaded on the column and the flow rate adjusted to 4-5 mL/min. All operations were performed at room temperature. The column was washed with 300 mL of distilled water. After the

wash, nitrogen was introduced into the top of the column for a few seconds to force the bulk of the aqueous phase into the column material.

The absorbed materials were then eluted with 200 mL acetone. The acetone and residual aqueous phase from the column were evaporated at 60°C with a flash evaporator under vacuum. After complete drying (1-1.5 h) dimethyl sulfoxide (DMSO) was added to dissolve the acetone residue.

In each experiment a control XAD-2 column was prepared and loaded with a volume of distilled water that was equal to the test sample and that had been filtered through Whatman No. 1 filter paper. The column was eluted with acetone under conditions identical to that of the test column.

Assay of Mutagenicity. Histidine-dependent strains of S. typhimurium TA 98 (a frame-shift mutant) and TA 100 (a base substitution mutant) were obtained from Dr. Bruce Ames of the University of California. The method is essentially that of AMES et al. 1975 with some modifications. Biotin (15 µg/plate) and L-histidine (50 µg/plate) were included in the bottom agar phase (15 mL). The amount of glucose in the bottom agar was 100 mg/plate. The top agar phase consisted of 0.75% Bacto agar in Vogel-Bonner medium. Bacterial suspensions of TA 98 and TA 100 were incubated at 37°C for 16 h. An aliquot of the culture, containing about 10^8 cells, was then added to a sterile test tube at 45°C containing soft agar and an aliquot of either unconcentrated water or of concentrate dissolved in DMSO. The contents of the tubes were then rapidly poured over an agar plate which contained the bottom agar. The plates were incubated for 48 h at 37°C and the number of colonies per plate was counted. All plates were done in duplicate. During these experiments, a known mutagen and carcinogen, hycanthone, was used as a positive control. To test for the metabolic activation of pro-mutagen/pro-carcinogens, a liver extract (S-9) was prepared from Aroclor 1254-induced rats and 50 µL was incorporated with the sample in the petri plate. Lucanthone, which is known to be nonmutagenic to TA 98 and TA 100, but can be metabolized by the S-9 fraction to the active mutagen, hycanthone, was used to control for the adequacy of the S-9 activation step.

Assay of Toxicity. TA 98 and TA 100 cultures were grown up overnight in complete nutrient broth. The cultures were diluted to about 10^3 organisms/mL in nutrient broth and 0.1 mL of the dilution (containing about 100 cells) was added to a sterile tube at 45°C containing nutrient agar and an aliquot of DMSO alone or DMSO containing the test or control extracts. The plates were incubated at 37°C and the colonies were counted after 24 h. The data are calculated as the percent of viable cells in test or control plates relative to that with DMSO alone.

RESULTS

In Table 1 it can be seen that unconcentrated well water at volumes of 0.5 and 1.0 mL produced no significant increase in the reversion of TA 98 in the absence of S9 activation (Experiment 1) or in the presence of S9 activation (Experiment 2). In TA 100, the same well water at volumes up to 1.0 mL produced no significant change in reversion rate in the absence of S9 (Experiments 1 and 2) but, in the presence of S9 (Experiment 2) there was a small but definite increase in the rate of mutation that was seen with both 0.5 and 1.0 mL of water.

Also shown in Table 1 are the data for unconcentrated water drawn from one of the polishing ponds. Once again there was no observable effect on TA 98 either in the presence or absence of S9 while in TA 100 the addition of S9 resulted in a small increase in mutation rate.

Since the experiments on the water from both the well and the polishing pond suggested the presence of low levels of pro-mutagens/pro-carcinogens that could be activated by factors present in mammalian liver, additional experiments were performed on water that was concentrated by absorption of non-polar organic material on XAD-2 resin. Following concentration, aliquots equivalent to up to 500 mL of water could be tested in the Ames assay. Assays of the toxicity of these concentrates on the test organisms revealed that aliquots containing the equivalent of up to 500 mL of water had no significant toxic effect on TA 98 and TA 100 (data not shown).

In Figure 1 are shown the dose-response curves for varying amounts of water concentrate on TA 98 in the presence or absence of S9 supplementation. The extracts from the control column produced no significant change in reversion rate either in the absence or presence of S9. The test extracts, however, produced a slight, but dose-related increase in reversion rate that was greatly magnified following S9 activation.

In Figure 2 are shown the comparable data for TA 100. The control extracts resulted in no systematic, dose-related changes in reversion rate either in the absence or presence of S9. The test extracts, likewise produced no dose-related changes in the absence of S9. However, in the presence of S9, the test extracts resulted in significant, dose-related increases in the reversion of TA 100.

DISCUSSION

These results reveal the presence in recycled water of low levels of one or more pro-mutagens that require activation by mammalian host factors prior to exhibiting mutagenic activity. The data indicate an absence of a response to unconcentrated water in one tester strain (TA 98) with a positive response in a second

TABLE 1

Mutagenic Assay of Unconcentrated Water
from the Israeli Recycling Facility

Additions Per Plate and Amount		S9 Present:+ or Absent: -	Number of Revertant Colonies Minus Background[a] TA 98	TA 100
Experiment 1				
Hycanthone	15.0 µg	-	133	328
Well Water[c]	0.5 mL	-	0	15
Well Water[c]	1.0 mL	-	0	5
Experiment 2				
Hycanthone	30.0 µg	-	367	-[b]
Lucanthone	30.0 µg	-	3	-
Lucanthone	30.0 µg	+	142	-
Hycanthone	15.0 µg	-	-	470
Lucanthone	15.0 µg	-	-	110
Lucanthone	15.0 µg	+	-	205
Well Water[c]	0.5 mL	-	-	0
Well Water[c]	0.5 mL	+	-	100
Well Water[c]	1.0 mL	-	3	40
Well Water[c]	1.0 mL	+	0	100
Experiment 3				
Hycanthone	30.0 µg	-	460	394
Lucanthone	30.0 µg	-	11	0
Lucanthone	30.0 µg	+	139	109
Pond Water[d]	0.5 mL	-	1	0
Pond Water[d]	0.5 mL	+	4	32
Pond Water[d]	1.0 mL	-	3	0
Pond Water[d]	1.0 mL	+	2	40

a: Background levels: Ta 98: in absence of S9 = 14-22; in presence of S9 = 52-63. TA 100: in absence of S9 = 137-399; in presence of S9 = 389-410.

b. Assay not done under these condition

c. Drawn from Well #5

d. Drawn from Polishing Pond Site 10-11

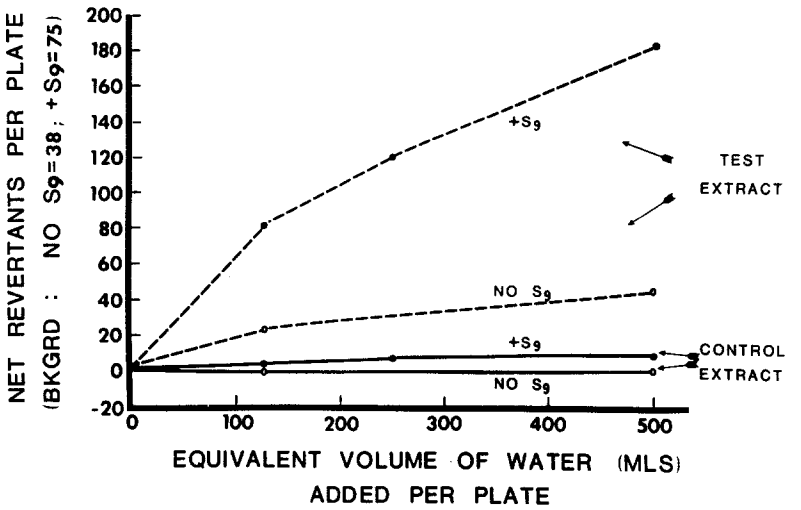

Fig. 1 - Mutagenic effect of concentrated water on TA 98.

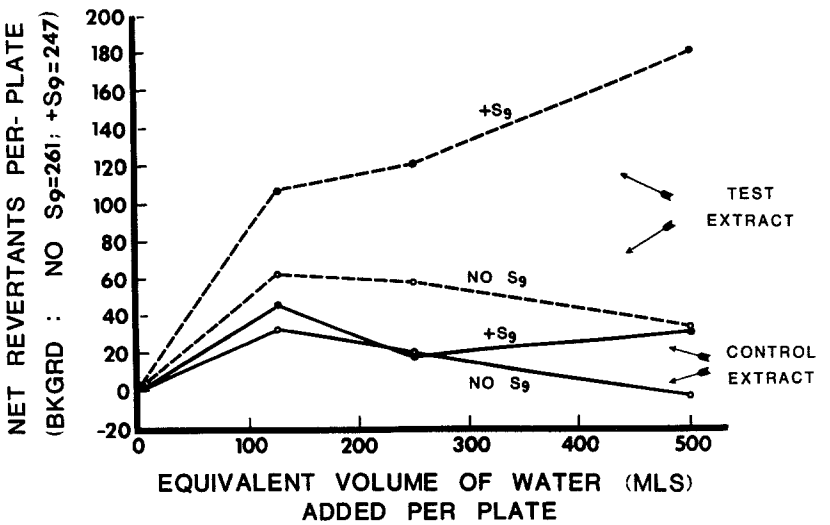

Fig. 2 - Mutagenic effect of concentrated water on TA 100.

strain (TA 100). This is coupled with a marked response in both of these strains to concentrated extracts from the water. These results suggest either a difference in dose-response relationship between the two strains for a single pro-mutagen or, more likely, the presence of multiple pro-mutagens, each with their own independent dose-response relationships. With regard to the latter case, it should be emphasized that the water samples were drawn at different periods of time and from different sites at the facility, giving rise to the possibility of differences in the type and amounts of pro-mutagens present in the water dependent upon time and site of sampling. One fact remains clear, however, and that is the demonstration of pro-mutagenic activity in various water samples drawn from the recycling facility. The source of this lipophilic pro-mutagenic activity may be directly from industrial or agricultural pollutants or indirectly from microbial action on the myriad organic chemicals present in the oxidation ponds (LINDMAR 1978).

To the extent that mutagens in a bacterial assay system might reflect the presence of potential human carcinogens, the data suggest the need for a thorough investigation of the nature and concentration of the organic materials in recycled water and an evaluation of their potential toxicologic and carcinogenic hazard either singly or in combination. These studies could be expected to lead to an altered and improved technology for treatment of recycled water that would be suitable for human consumption. The importance of such studies is emphasized by a recent report showing a correlation between cancer mortality and the degree of use of Mississippi River water for drinking water (PAGE et al. 1976).

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