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INTERACTION BETWEEN ESCHERICHIA COLI AND LUNAR FINES

Karl R. Johanson
Professor of Microbiology
Department of Biological Sciences
North Texas State University
Denton, Texas 76203

ABSTRACT

A sample of mature lunar fines (10084.151) was solubilized to a high degree (about 17 percent) by the chelating agent salicylic acid (0.01 M). The neutralized (pH adjusted to 7.0) leachate was found to inhibit the growth of Escherichia coli (ATCC 25922) in a minimal mineral salts glucose medium; however, the inhibition was somewhat less than that caused by neutralized salicylic acid alone. The presence of lunar fines in the minimal medium was highly stimulatory to growth of E. coli following an early inhibitory response. The bacterium survived less well in the lunar leachate than in distilled water, no doubt because of the salicylate. It was concluded that the sample of lunar soil tested has nutritional value to E. coli and that certain products of fermentation helped to solubilize the lunar soil.

INTRODUCTION

The notion of a manned lunar base has been considered exhaustively by engineers, geochemists, biochemists, psychologists, and other experts, by and large through conferences, workshops, and contracts sponsored by NASA (5,10,18-20). Starting in 1960 with the first one entitled "Moonlab" (8). The ultimate goal would appear to be CELSS, an acronym coined by NASA meaning a "controlled ecological life support system" (18-20). To establish a self-sufficient, regenerative closed environment fit for human habitation for long periods will require a careful selection of desired species of living forms, from microbes to crop plants to man. At the microbial level, there are almost countless candidate species representing various physiologic types, e.g., photosynthetic, chemosynthetic, autotrophic, heterotrophic, anaerobic, fermentative, oxidative, sulfur oxidizers, denitrifiers, nitrifiers, iron oxidizers, etc., etc. (1). Those microbial types known to play key roles in the cycles of the "major elements" (C, H, O, N, S, P) are certainly prime candidates for a CELSS.

The moon is an inhospitable environment for all Earth's creatures, from bacteria to all higher forms of life. It has no atmosphere, is virtually devoid of water, contains in the regolith ppm/ppb levels of nitrogen, hydrogen, and carbon (CO , CO_2 , CH_4) and contains no free oxygen, although there is an abundance of combined oxygen, largely metal oxides in the lower oxidation state--ferrous, not ferric, for example (9,14,17). Temperature extremes from approximately 120°C to -180°C , intense radiation, a diurnal cycle of two earth weeks each of night and day, and a force of gravity one-sixth that of earth impose further complications in establishing a CELSS on the moon (9,14).

While exceedingly small amounts of amino acids have been detected in lunar soil, unquestionably the moon is sterile, i.e., without life (9,10,14). No life could possibly thrive on the moon without a supply of free oxygen and without additional water, nitrogen, and carbon dioxide and without protection from radiation and temperature fluctuations. All of these inadequacies or deficiencies would be compensated by the CELSS. The engineering and technology required to achieve a self-perpetuating (or nearly so) controlled ecological life support system seems feasible. To establish a dynamically stable (homeostatic) ecosystem is central to the concept of CELSS and no doubt will be the most stubborn, if not tantalizing, obstacle to an ultimately successful CELSS on the moon.

THEORY

It is important to ascertain whether lunar soil (i) is toxic to some species of life; (ii) can be a source of nutrients; and (iii) can be leached and biochemically modified by living systems.

Inevitably, there will be introduced into a lunar man-made, regenerative environment a variety of microbial species which are normal members of the body's microflora. A prime example is Escherichia coli, long used as an indicator of fecal pollution and probably the bacterium most widely investigated by molecular biologists and geneticists. Indeed, its entire genome has been essentially mapped (2). Although some strains of E. coli are capable of causing disease in man and animal, its introduction into a CELSS would be unavoidable once the recycling system were operative, and thus its influence on lunar soil and vice versa should be assessed.

Escherichia coli is a Gram-negative heterotroph which can derive all of its nutritional needs from simple inorganic compounds, except for carbon. It can utilize a variety of low molecular weight organic compounds as sources of carbon and energy. It will grow well in the absence of molecular oxygen, but also can utilize O_2 as an electron receptor; thus, it is a facultative aerobe. In the presence of fermentable carbohydrate, e.g., glucose, E. coli produces lactic, succinic, acetic, and formic acids plus ethanol. Most of the formate ends up as CO_2 and H_2 through catalysis by the formic hydrogenlyase enzyme complex. However, in the presence of an abundant oxygen supply, E. coli will grow most vigorously and few fermentative end products will accumulate; most of the glucose will thus be metabolized stoichiometrically into carbon dioxide and water. Under ideal conditions, E. coli is one of the most rapidly growing microbes known. E. coli is moderately tolerant of environmental change, although it does not produce spores. It will grow in a temperature range of approximately 10 to 42°C and in a pH range of approximately 5 to 8.

This study was designed principally to measure the effects of lunar leachates and of lunar soil on the growth and survival of E. coli. While chemical analyses of the leachate and of the spent culture medium containing lunar fines were intended, and would have provided some helpful information, they will have to await subsequent experiments that may emerge from this one.

METHODS

I. Bacterium and Culture Medium

A well-characterized strain of Escherichia coli (ATCC 25922) was obtained from the Microbiology Laboratory, Division of Life Sciences, NASA. It was grown on a brain heart infusion agar slant. The growth on the slant was suspended in 1 ml sterile distilled water and constituted the inoculum for 75 ml of a mineral salts glucose (MSG) medium contained in a 125-ml Erlenmeyer flask. The composition of the medium is listed in Table 1.

The inoculated MSG broth was incubated at 35°C and the cells were recovered 18 hours later by centrifugation and washing in sterile distilled water. Growth was not abundant in this minimal MSG medium, so the final cell suspension was concentrated 15-fold by resuspending the cells in 5 ml distilled water. One drop from a sterile 1-ml serologic pipette constituted the inoculum for each tube in the experiment.

II. Lunar Soil Leachate

Based upon the success of Keller and Huang (7) in partially solubilizing an Apollo 12 lunar dust (12070.128), salicylic acid was selected as the solvent. To 50 ml of .01 M salicylic acid in a small polypropylene container was added 0.11476 g of lunar soil (10084.151).

A plastic-coated stirring bar was placed in the vessel, a cap inserted and the contents heated in a boiling water bath on a hot plate-magnetic stirrer for eight hours. After cooling overnight, the vessel was stirred and heated for a second eight-hour period. For two more successive days, the lunar material was stirred at room temperature on the stirrer.

The leachate with the undissolved fines was filtered through a sterile membrane filter (0.45 μ m) and aseptically transferred to a sterile 50-ml flask. A portion of the leachate was adjusted to pH 7.01 with 1 N NaOH for incorporation into the culture medium.

Chemical analysis (2,9) of lunar soil 10084 has revealed its principal constituents to be SiO_2 (41.0%), FeO (16.2%), Al_2O_3 (12.8%), CaO (12.4%), MgO (9.2%), TiO_2 (7.3%); lesser amounts of MnO (0.22%), Na_2O (0.38%) and K_2O (0.15%) were found with ppm amounts of Ba, Co, Ni, Sc, Ce, and some ten other trace elements. There is no information on its content of sulfur or phosphorus. It is a "mature" soil (Is/FeO ratio of 78.0) (9), meaning that it had been long exposed to solar winds and micrometeorites.

In order to determine the amount of fines solubilized by the chelating solution, a measured volume was pipetted onto a tared watch glass. The sample was dried at 110°C overnight and weighed, thereby obtaining the weight of dissolved solids, including the salicylic acid. An alternative method consisted of heating a measured volume of leachate in a porcelain crucible placed in a muffle furnace at 700°C for a couple of hours, weighing before and after. The latter method would combust completely the salicylic acid and required a small correction resulting from the oxidation of FeO to Fe_2O_3 .

The leachate possessed an intense red-brown color, probably because of the iron complexed with the organic acid. Its absorption spectrum was measured over the range of 400 to 700 nm. Upon addition of the leachate to the culture medium, the color disappeared.

III. Effects of Lunar Leachate on E. coli

A. In mineral salts glucose broth

The neutralized sterile leachate was diluted serially 1:2 in sterile distilled water to yield a dilution series starting with 1:2 and ending with 1:128. Sterile screw-cap 10 x 100 mm test tubes were employed. An equal volume (2.5 ml) of sterile double-strength MSG medium was aseptically added to each tube resulting in a total volume in each tube of 5 ml.

Each tube, except for the MSG control tube, was inoculated with one drop of the freshly-washed suspension of E. coli and incubated at room temperature (about 22°C). At intervals the amount of growth was recorded by means of spectrophotometric (Bausch and Lomb, Spectronic 170) absorbance readings at 500 nm wavelength.

As a control, neutralized, sterilized 0.1 M salicylic acid was serially diluted and double-strength MSG broth was pipetted into each tube which also received one drop of the E. coli suspension.

B. In aqueous dilutions

The leachate was serially diluted in sterile distilled water as described in Part A above. After inoculation with a drop of E. coli suspension, a loopful was streaked periodically on nutrient agar Petri plates to check for viability. The control was a tube containing sterile distilled water.

IV. Effects of Lunar Fines on E. coli

A. In mineral salts glucose broth

Five ml single strength MSG broth was pipetted into each of three 10 x 100 mm screw-cap test tubes containing measured quantities of fines (50.94 mg, 20.42 mg, and 11.48 mg). The tubes were autoclaved at 121°C for 15 minutes. Upon cooling to room temperature, spectrophotometric readings were made and each tube was inoculated with one drop of the freshly-washed suspension of E. coli. Periodically thereafter, growth was measured in the spectrophotometer.

B. On agar plates

A suspension of E. coli from a recently seeded brain heart infusion slant was diluted in sterile distilled water. One drop of this suspension was spread evenly over the surface of a plate of nutrient agar by means of a sterile glass spreader.

Three small clumps of moon fines were placed upon the inoculated plate and the plate was incubated at room temperature for two days. The plate was examined intermittently for any signs of stimulation or inhibition of growth around the fines.

RESULTS

I. Leachate

It was not possible to achieve a constant dry weight value employing the low temperature oven (110°C), probably because of the tendency for salicylic acid to sublime slowly. The results from ashing revealed the 50 ml of solution to contain .0198 g solids. Thus, of the 0.14476 g suspended in the salicylic acid solution, 17.25% was solubilized.

The absorption spectrum revealed a peak at or near 475 nm (Figure 1).

II. Effects of Lunar Leachate on the Growth of E. coli

Tables 2 and 3 and Figure 2 clearly reveal the inhibitory effect of the solvent 0.01 M salicylate on growth of the test bacterium. A dilution of 1:128 of the leachate solution (equivalent to 7.8×10^{-5} M salicylic acid) was slightly inhibitory. Note that E. coli grew better in dilutions of the leachate than in comparable dilutions of the neutralized salicylate. The increase in growth after 180 hours is probably a response, in part, to autolytic products from the cells serving as nutrients.

III. Effects of Lunar Fines on the Growth of E. coli

Table 4 and Figure 3 show that while the presence of fines (not leachate) in the culture medium was inhibitory to growth of E. coli early in the incubation period (< 20 hours), the maximal growth achieved was greater in the presence of each of the three levels of fines than in the control culture. In fact, the stimulus was bimodal, with peaks at 24 hours and again at 216 hours of incubation, the latter being substantially greater than the former. Another interesting feature of these data is that the highest amount of fines (50.9 mg)

resulted in growth greater than the control all the way past the first peak at 24 hours; the lesser amounts of fines became inhibitory after the initial peak but later became stimulatory (216 hours).

IV. Toxicity of Leachate to E. coli

Undiluted leachate and its dilutions up to 1:64 were demonstrably toxic to E. coli. At approximately the eighth day, there were few if any viable bacteria present in either the undiluted or 1:2 dilution of leachate. In retrospect, a parallel study should have been made with neutralized salicylic acid.

V. Effects of Leachate and Lunar Fines Sprinkled on Agar Seeded with E. coli

Amounts of 20.9, 14.2, and 5.6 mg of lunar fines placed upon freshly-seeded nutrient agar failed to produce a noticeable effect upon the subsequent growth of E. coli.

One drop of leachate produced a circle within which there was no growth of the bacterium. The leachate preparation in which the pH was adjusted to 7.0, however, definitely stimulated growth as indicated by a circular area within which the agar medium was more opaque than the background.

CONCLUSIONS

It can be concluded that the lunar soil sample tested stimulates the growth of E. coli in a minimal mineral salts glucose culture medium. The chelator, salicylic acid, was toxic to E. coli; the leachate partially reversed this effect, however. The growth enhancement from the lunar fines is probably a nutritional response to unidentified trace elements or possibly to one or more major elements in the specimen.

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INGREDIENT (wt./liter)	(g)	CONCENTRATION (ppm)
Glucose	2.0 g	2,000
$(\text{NH}_4)_2 \text{SO}_4$	1.0 g	1,000
K_2HPO_4	1.0 g	1,000
KH_2PO_4	0.5 g	500
NaCl	0.5 g	500
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 g	100
CaCl_2	1.0 mg	1
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 mg	0.5
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.25 mg	0.25
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.1 mg	0.1
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.1 mg	0.1
EDTA*	50.0 mg	50

*Ethylene diamine tetraacetic acid (a chelator) was included in the trace element solution (Ca, Fe, Mn, Zn, Co) which was filter-sterilized as a concentrate. Glucose was sterilized by filtration as were the remaining salts.

Table 1. Mineral Salts Glucose Broth for Escherichia coli.

Diln. of Leachate 17	HOURS OF INCUBATION						
	24	41	48	70	185	209	
1:2	.062	.086	.076	.076'	.062	.066	.077
1:4	.076	.094	.054	.087	.076	.077	.085
1:8	.105	.115	.099	.096	.090	.091	.102
1:16	.143	.147	.128	.122	.115		.113
1:32*	.081	.144	.135	.111	.091	.099	.109
1:64	.198	.191	.173	.172	.151	.144	.179
1:128	.209	.207	.175	.177	.169	.168	.191
Control	.219	.229	.203	.189	.204	.181	.223

Incubation was at room temperature (22°C). The data are spectrophotometric readings which have been adjusted by deducting the zero time readings.

*This tube may be invalid since it was misplaced while taking the 17-hour readings and shortly found in another test tube rack with several similar tubes.

Table 2. Growth of Escherichia coli in a Mineral Salts Glucose Medium Containing Dilutions of Moon Leachate.

Diln. of .01 M Salicylic Acid	HOURS OF INCUBATION						
	17	24	41	48	70	185	209
1:2	.028	.060	.055	.055	.045	.033	.040
1:4	.057	.079	.073	.069	.069	.63	.066
1:8	.073	.096	.086	.080	.080	.69	.085
1:16	.099	.138	.112	.108	.103	.091	.101
1:32	.125	.147	.136	.135	.158		.119
1:64	.152	.180	.168	.166	.132	.159	.175
Control	.219	.229	.203	.189	.204	.181	.223

See note under Table 2

Table 3. Growth of Escherichia coli in a Mineral Salts Glucose Medium Containing Dilutions of Salicylic Acid.

Amount Moon Fines	HOURS OF INCUBATION						
	17	24	41	48	70	185	216
50.94 mg	.160	.260	.238	—	.208	.308	.345
20.42 mg	.144	.246	.181	—	.142	.186	.255
11.48 mg	.157	.237	.192	—	.152	.162	.277
0	.219	.229	.203	.189	.204	.181	.230

See note under Table 2.

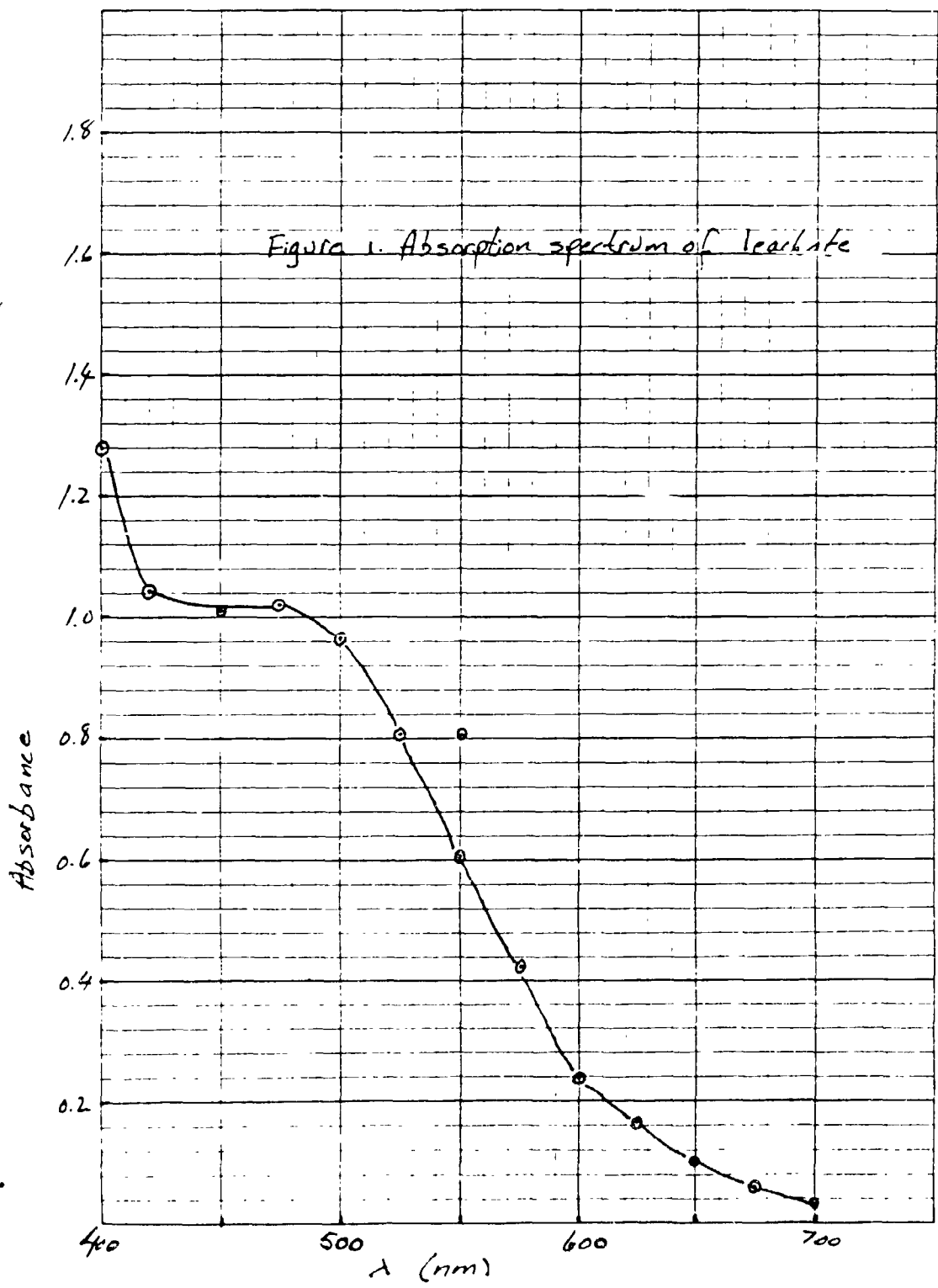
Table 4. Growth of Escherichia coli in a Mineral Salts Glucose Medium Containing Moon Fines (10084).

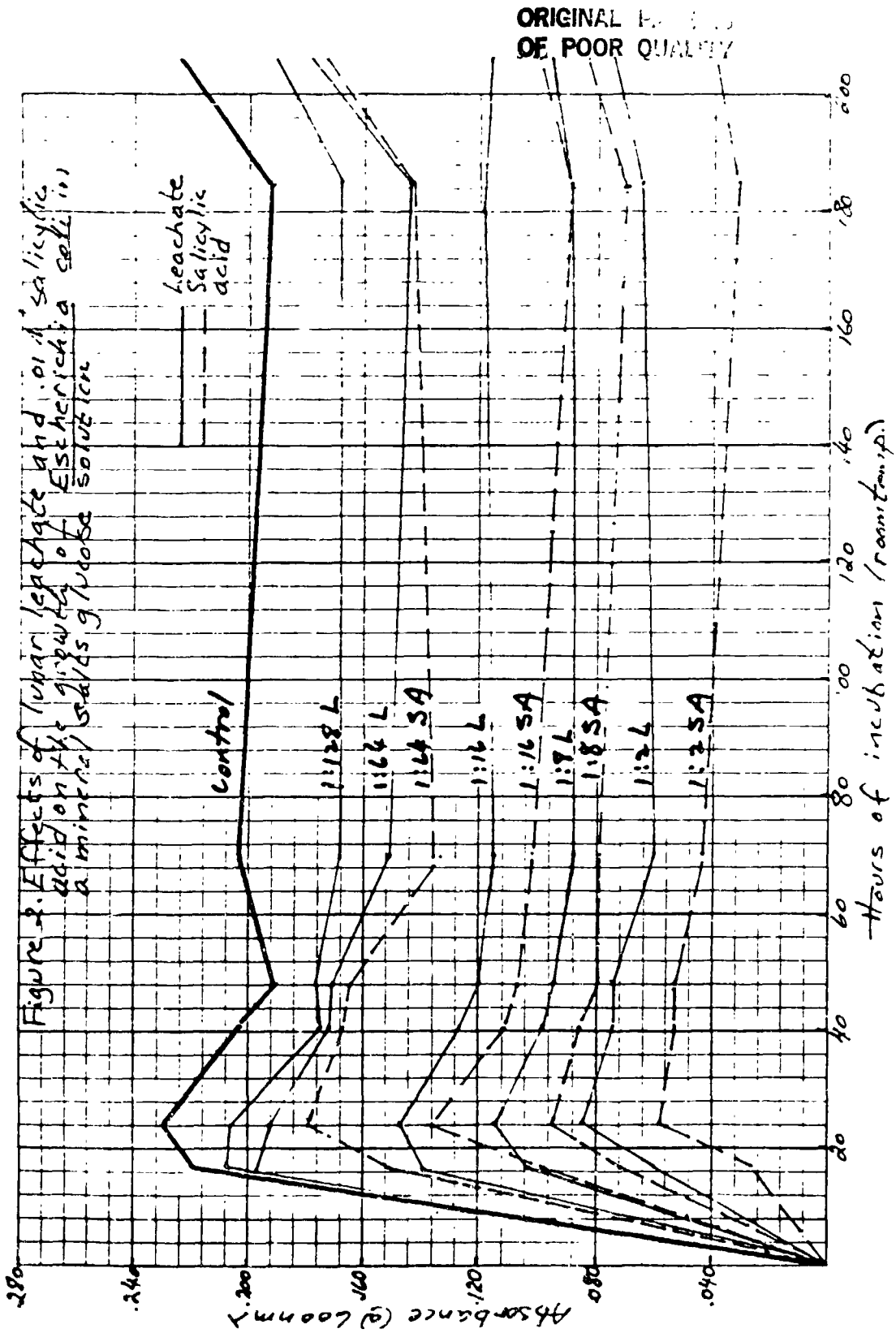
Hours of Dist. Incub.	Water	Undil.	1:2	1:4	1:8	1:16	1:32	1:64
24	++++	++	++	++	+++	+++	+++	++++
42	++++	+	++	++	++	+++	+++	+++
85	++++	-	-	+	+	+	++	++

++++ = dense growth (innumerable colonies).

+ = light growth (a few colonies).

Table 5. Survival of E. coli in Aqueous Dilutions of Neutralized Leachate.





ORIGINAL P. ...
OF POOR QUALITY

