Effect of Several Clay Minerals and Humic Acid on the Survival of *Klebsiella aerogenes* Exposed to Ultraviolet Irradiation [1]

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The effect of various clay minerals and humic acid on the survival of *Klebsiella aerogenes* exposed to ultraviolet (UV) irradiation was investigated. A protective effect was observed and found to depend on the specific light absorption and light scattering properties of the clay minerals and the humic acid used. The higher the specific absorption, the better was the survival of *K. aerogenes* after UV irradiation. Bacterial survival was lower in clays saturated with divalent cations (Ca, Zn) than in those homoionic to monovalent cations (K).

Clay minerals are hydrous aluminosilicate particles, the properties of which are related to their colloidal nature (25). Because of their occurrence in soils, streams, and other water bodies such as lakes and fish ponds, clay minerals are closely associated with microorganisms in their natural habitat. The effects of clay minerals on microorganisms are not easily demonstrated under field conditions (21, 22). However, many laboratory studies have elucidated certain aspects of the nature of the interaction between microorganisms, especially bacteria, and clay minerals. This interaction may have two effects on the behavior of microorganisms: (i) an effect on microbial metabolism (11, 19, 20, 23, 24), and (ii) an effect on the surface properties of the microbial cells (9, 12, 13, 15, 18). The latter effect has a great ecological significance in the survival of bacteria under adverse conditions such as high temperatures and desiccation (12, 14).

The optical properties of several clay minerals in aqueous suspensions have been studied in both the visible and ultraviolet (UV) light wavelengths (1, 2, 7, 25). In the UV range, the light absorbance is the sum of both scattering and specific absorption, and the proportion of these two components depends on factors such as clay mineral type, clay concentration, and particle size. Factors such as exchangeable cation, electrolyte concentration, and method of

preparation influence the particle size, which has a decisive effect on the light scattering of the clay suspensions (10). Perhaps more than clay minerals, organic molecules are found in the close vicinity of microbial cells in soils and other natural habitats. Little is known about the role of these organic molecules in the resistance of the microbial cells to adverse environmental conditions, one of the reasons being the great variability of the organic molecules. Thus, when considering organic molecules in soils or lake bottoms, it is practically impossible to consider one chemical compound. Instead, a fraction of the organic matter is considered, this fraction being defined according to its method of preparation. Of the several fractions of soil organic matter which have been studied, humic acid is one of the most important (17).

The purpose of this study was to investigate the influence of clay minerals and humic acid on the survival of *Klebsiella aerogenes* under a lethal dose of UV irradiation.

MATERIALS AND METHODS

Clay minerals. The clay minerals used in these experiments were supplied by Ward's National Science Establishment Inc., New York. They were described by Kerr et al. (8). The relevant characteristics of these clays are described in Table 1. Samples of montmorillonite no. 25 were passed through a cation exchange resin saturated with K to produce K-clay. Other samples were converted to Ca and Zn-montmorillonite by leaching with a 1 N chloride

solution of the corresponding cation. Free electrolytes were removed from the clay suspensions by leaching with distilled water and centrifugation. Aqueous suspensions, prepared from crude samples of nontronite, hectorite, dickite, kaolinite, and halloysite were centifugated to obtain clay particles less than 2 μm (Jackson, M. L. 1956. Soil chemical analysis. Advanced course. Published by M. L. Jackson). The absorbance of the clay suspensions was determined in a Cary model 15 recording spectrophotometer within the range of 200 to 400 nm (Fig. 1A and 1B).

Humic acids. The method of preparation of the humic acids, produced by Aldrich Chemical Co., Milwaukee, Wis., is described by Narkis et al. (16). Their absorbance spectra are shown in Fig. 1C.

Bacterium. *K. aerogenes* $K_{12}A_3$ was isolated and defined by Duguid (4) and by Wilkinson et al. (27). The bacteria were grown for 18 hr at 37 C on nutrient agar (Difco) or on a rich sugar-agar medium (3) consisting of: 2% agar (Difco), 0.2% NaCl, 0.1% K_2SO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 1% $CaCO_3$, 0.0001%

$FeSO_4$, 0.1% peptone (Difco), and 1% sucrose. Bacterial cells were washed twice with saline, by centrifugation, and then suspended in saline to yield a final concentration of 5×10^3 to 10^4 cells/ml. Samples (0.5 ml) were mixed with 4 ml of autoclaved clay suspensions; the mixtures were placed in open petri dishes and irradiated for 5 min. The thickness of the mixture in the petri dish was 5 mm.

UV source and irradiation conditions. A far UV source (Mineralight U.V.S., San Gabriel, Calif.) which emits, almost exclusively, radiations at a wavelength of 254 nm, was placed at a distance of 13 cm from the bacterial suspensions. At this distance, the dose rate throughout the suspension was 12 ergs per mm^2 per sec.

Viability of the irradiated cells was determined by plating samples of the mixture on nutrient agar, incubating at 37 C for 20 hr, and counting the colonies.

RESULTS AND DISCUSSION

The optical properties of the clay minerals

TABLE 1. *Some properties of the clay minerals*

Clay type	Particle size (μm)	Ion to which the clay is homoionic	Origin of the clay
Montmorillonite	2	K, Ca, Zn	Montmorillonite, no. 25; John C. Lane Tract; Upton, Wy.
Nontronite	2	Natural[a]	Nontronite, H-33a; Garfield, Spokane, Wash.
Hectorite	2	Natural	Hectorite, no. 34; Hector, Calif.
Kaolinite	2	Natural	Kaolinite, no. 2; Birch Pit, Ga.
Halloysite	2	Natural	Halloysite, no. 29; Wagon Wheel, Cap, Colo.
Dickite	2	Natural	Dickite, no. 15a; San Juanito Chihuhahua, Mexico

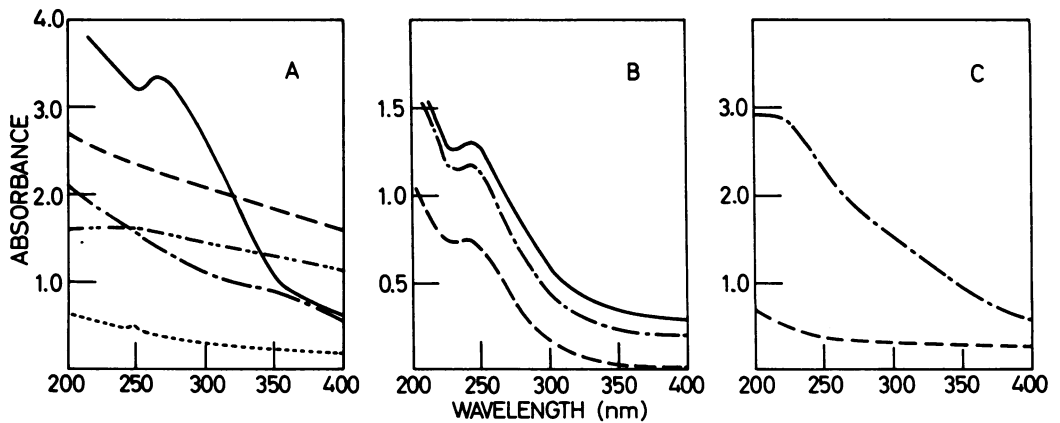[a] The clay was used without saturation with any cation.

FIG. 1. *Absorbance spectra of the clay and humic acid suspensions (250 ppm) at wavelengths from 200 to 400 nm. A:* (———) *nontronite,* (– – –) *dickite,* (–·–·–) *halloysite,* (–· ··–··) *kaolinite,* (· · · ·) *hectorite. B:* (———) *potassium-montmorillonite,* (–·· –··–) *calcium-montmorillonite,* (———) *zinc-montmorillonite. C:* (–··–··–) *humic acid at pH 7.2,* (– – –) *humic acid at pH 5.7.*

and humic acid used in this study are given in Fig. 1. The absorbance peaks of montmorillonite (Fig. 1B) and hectorite (Fig. 1A) were at a 245 nm (the peak of hectorite was too small to be seen at the scale used in Fig. 1A), whereas that of nontronite was at 260 nm (Fig. 1A). Halloysite, dickite, and kaolinite had very small specific absorption in the UV range; their absorbance was mainly due to light scattering. A semiquantitative estimate of the specific light absorption of the clay suspensions at 254 nm (the predominant wavelength of the UV source) was made by the method of Banin and Lahav (2). Thus, ΔA_{254}, which is proportional to the specific light absorption, is the difference between the measured absorbance, A, at 254 nm and the value, at the same wavelength, found from extrapolation of the straight line in the log A versus log λ plot. The relevant optical properties of the clay minerals under study are given in Table 2. The differences in the values of ΔA_{254} of the montmorillonite clay minerals are related to the number of unit layers (silicate sheets) in a particle, this number being greatest in calcium-montmorillonite and smallest in the potassium-montmorillonite suspension (2).

Preliminary experiments showed that, whereas a 30-sec exposure to UV was enough for zero survival of the pure bacterial suspension, nontronite and montmorillonite clay minerals had a significant protective effect on the bacteria. In a typical experiment, where potassium-montmorillonite was compared to halloysite (Fig. 2), a significant difference was apparent even after 1 min of exposure to UV irradiation. Nontronite, a clay mineral with a high specific absorption in the UV range (Y. Chen, Master's thesis, Faculty of Agriculture, The Hebrew University, Rehovot, 1970), had

the highest protective effect (Fig. 3). In general, the protective effect was correlated with specific absorption in the UV range, i.e., to the value of ΔA_{254} (Fig. 3, Table 3). Our results with kaolinite, dickite, halloysite, and hectorite, which have negligible specific absorption in the UV range and no protective effect on the bacteria, are consistent with this view.

The high specific absorption of nontronite was related to its high content of trivalent iron (6). A $Fe_2(SO_4)_3$ solution, which had a similar specific absorption in the UV range, also had a significant protective effect on the cells (Table 3). A possible role of iron compounds in protecting potential extraterrestrial microorganisms against UV radiation has been suggested (26).

The saturating cation also had an influence on the survival of the bacteria. Consequently, the survival of $K.$ $aerogenes$ in montmorillonite homoionic to K, was higher than in montmorillonite homoionic to Ca or Zn (Table 3). This suggests that montmorillonite homoionic to divalent cations scatters more light and, therefore, has a lower specific absorption than montmorillonite homoionic to monovalent cations (1, 2).

In addition to clay minerals, the influence of humic acid was investigated. Because of their larger particle size, the clay minerals are better light scatterers than the humic acid. The light absorbance of the clay suspensions in the UV range is the sum of scattering and specific absorption, whereas that of humic acid is mainly due to specific absorption. Because of this difference the ΔA_{254} was not calculated for the humic acid. The specific absorption of the humic acid at pH 7.2 was much higher than at pH 5.7 (Fig. 1C). In a 0.3% humic acid solution, the survival of $K.$ $aerogenes$ was as high

TABLE 2. Optical spectra of the clay minerals[a]

Clay type	Absorbance (A) at				ΔA_{254}[b]
	700 nm	400 nm	254 nm	225 nm	
Nontronite	0.17	0.67	3.14	3.51	1.10
Potassium-montmorillonite	0.002	0.026	0.64	0.64	0.43
Calcium-montmorillonite	0.02	0.14	0.95	1.0	0.27
Zinc-montmorillonite	0.17	0.46	1.39	1.45	0.35
Hectorite	0.12	0.22	0.43	0.53	0.07
Dickite	0.77	1.7	2.3	2.5	-0.93
Kaolinite	0.33	0.64	0.98	1.0	-0.11
Halloysite	0.17	0.62	1.5	1.8	-0.27
$Fe_2(SO_4)_3$	0.03	0.44	2.2	3.3	

[a] Clay concentration = 250 μg/ml.

[b] ΔA_{254} is the difference between the measured value of A at 254 nm and the value found from extrapolation of the straight line of log A plotted against log λ (λ = wavelength).
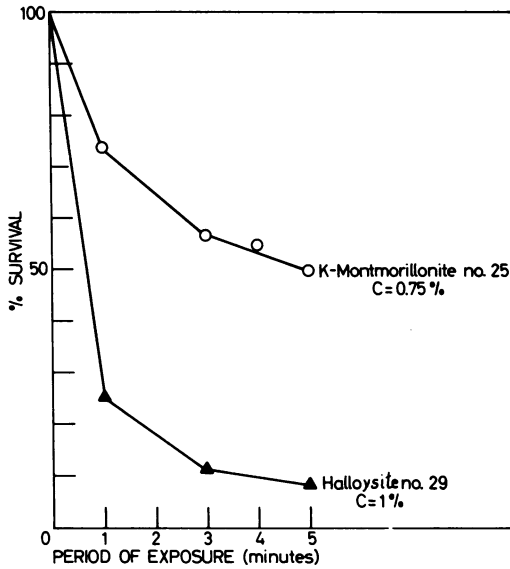
FIG. 2. *Survival of K. aerogenes exposed to UV irradiation in the presence of different types of clay minerals. (C refers to clay concentration, w/v.)*
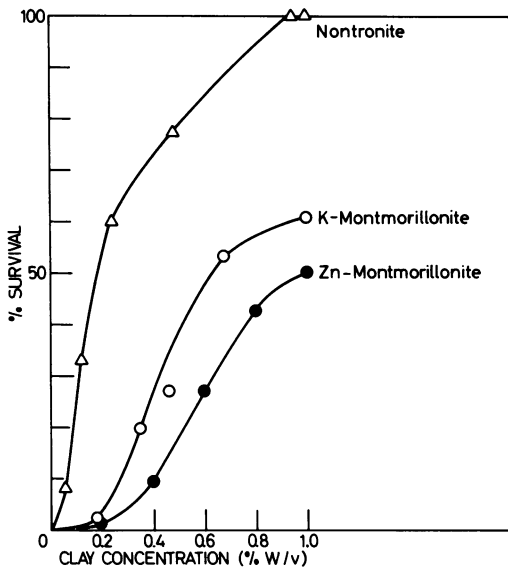
TABLE 3. *Survival of Klebsiella aerogenes exposed to ultraviolet radiations for 5 min in the presence of various clay minerals*

Clay type	Per cent viability	
	Clay concn = 0.3%	Clay concn = 0.6%
No clay	0	0
Nontronite	65 A[a]	84 a[a]
Potassium-montmorillonite	20 B	58 b
Calcium-montmorillonite	10 C	40 c
Zinc-montmorillonite	11 C	27 d
Hectorite	0 D	0 e
Dickite	0	
Kaolinite	0	0
Halloysite	0	0
$Fe_2(SO_4)_3$.....................	80 E	

[a] a–e, A–E mean values with different small and capital letters are significantly different at $P < 0.05$.

FIG. 3. *Effect of concentration of three clays on the survival of K. aerogenes exposed to UV irradiation for 5 min.*
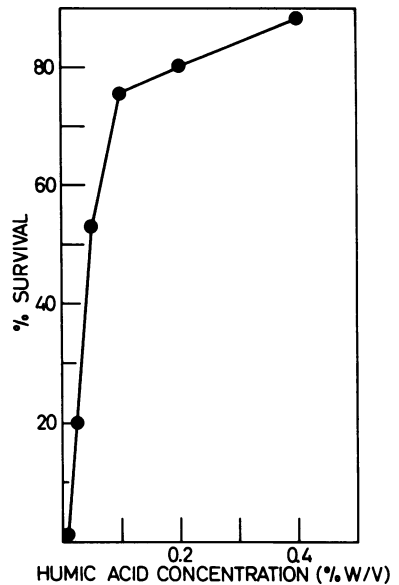

FIG. 4. *Effect of concentration of humic acid on the survival of K. aerogenes exposed to UV irradiation for 5 min.*

as 72% at pH 7.2, whereas at pH 5.7 only 11% survived. The effect of the concentration of humic acid on the survival of *K. aerogenes* is shown in Fig. 4. On the basis of suspension concentration the humic acid was more efficient in protecting the bacteria from UV radiations than any of the clay minerals studied.

In conclusion, bacterial survival was directly correlated with the specific absorption of the clay minerals and the humic acid under study, rather than with their absorbance.

interest during the course of this investigation. Thanks are due to N. Narkis (Sanitary Engineering Laboratories, Technion, Haifa, Israel) for providing the humic acid samples. The technical assistance of Roumia Gouvrin is gratefully acknowledged.

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