ALLELOPATHIC EFFECT OF PENGUIN EXCREMENTS AND GUANOS ON THE GROWTH OF ANTARCTIC SOIL ALGAE

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Abstract: An experiment was carried out to ascertain the effect of substances in the soil of Adélie penguin rookery on the growth of algae isolated from the Antarctic soil. In the paper disc test, both of acetone and water extracts of guano soil gave an inhibitive effect on the algal growth. It was recognized that acrylic and oxalic acids were the algal growth inhibitors. The function of the oxalic acid as a new growth inhibitor of algae was examined by comparing it with that of acrylic acid.

1. Introduction

The activities of such sea birds as penguins, skuas and snow petrels conspicuously contribute to the eutrophication of lake waters and terrestrial soils in Antarctica (BOYD *et al.*, 1966; MATSUDA, 1968; GOLDMAN, 1970; LAMB, 1970; GOLDMAN *et al.*, 1972; TEDROW and UGOLINI, 1972; SAMSEL and PARKER, 1972; AKIYAMA *et al.*, 1981; PIETER *et al.*, 1983; TATUR and MYRCHA, 1983; ORCHARD and CORDEROY, 1983). Particularly the Adélie penguins play an important role in organic matter transfer between sea and ice-free areas.

BOYD *et al.* (1966) and BOYD (1967) recognized that the guano soil in Cape Royds, Ross Island contains such high level of nutrients as an amount of 2400 ppm of phosphorous and 6000 ppm of ammonia (values of Hellige-troug soil tester). These figures exceed those in the temperate regions. In this connection, HOSHIAI and MATSUDA (1979) reported that *Prasiola* and *Nostoc* vegetation developed not only around the presently occupied rookeries but also already abandoned rookeries in Ongulkalven. Furthermore, they pointed out that the distribution and occurrence of the terrestrial epipsamic microalgal biomass in terms of chlorophyll content of sandy soil, were clearly related to the past and present Adélie penguin rookeries.

In contrast, BOYD *et al.* (1966) and BOYD (1967) recognized that, despite of the eutrophic condition of Adélie penguin rookeries, bacteria and fungi are scarce in the rookery soils in comparison with the terrestrial soils of the both polar regions, and they assumed the presence of certain antibiotic substances in the rookery soil originated from penguin feces considering the results obtained by SIEBURTH (1960, 1963) who had demonstrated the antibiosis of acrylic acid, a three carbon unsaturated aliphatic acid produced by a common marine phytoplankton, *Phaeocystis pouchetii*, in the

Antarctic sea. This substance remains stable when the alga are eaten by the krill, *Euphausia*, and when consumed by the penguin, it becomes concentrated in the penguin's intestinal tract and excrement.

In this paper, the authors give the results of experimental tests to demonstrate the inhibitory effects of rookery soil on the growth of Antarctic soil algae and of the analytical identification of the inhibiting substances.

2. Materials and Methods

In the 1982–83 and 1983–84 austral summers, soil samples were obtained from Ongulkalven and East Ongul Island. Samples were kept in the sterilized petri dish in the frozen state at −20°C until analyses. They were used for measuring the chlorophyll content according to the standard method by UNESCO (1966) and for culturing tests as follows: dilutions of the soil samples were plated on BBM agar (BOLD, 1970) and were incubated at 20°C under illumination of *ca.* 2 k lux in a 12–12 h diurnal light-dark cycle.

Assumed antialgal activities were demonstrated by paper disc tests for both of acetone and water extracts of guano soils. The inhibiting materials were analyzed by means of a high performance liquid chromatography (HPLC; Shimazu LH-4 type with SCR-101H column; 7.9 mm×300 mm; flux rate 1.2 m*l*/min).

3. Results and Discussion

As shown in Table 1, the chlorophyll content of the soils obtained from the central part of both the present and the abandoned rookeries (NR-1, OR-1) was lower than that of the soils sampled in the surrounding area (NR-2, -3, -4, OR-2, -3, -4, -5). Besides, the chlorophyll maxima occur at the sites which are located about 10–30 meters or more distant from the boundary of a clearly guanized area of rookeries. The authors could not detect any of the terrestrial and subterranean microalgae by

Table 1. Terrestrial epipsamic algal chlorophyll in Antarctic sandy soils.

Soils	Chlorophyll contents (μg/g)	
Control site in East Ongul		
E-1	0.34	
E-2	0.75	Average=0.40
E-3	0.12	
Presently occupied rookery		
NR-1	0.35	
NR-2	8.42	Average=9.61
NR-3	28.13	
NR-4	1.52	
Abandoned rookery		
OR-1	2.34	
OR-2	7.43	
OR-3	18.03	Average=11.45
OR-4	28.39	
OR-5	1.08	

culturing tests on the soils obtained from the central part of both the present and the abandoned rookeries. However, algae such as *Stichococcus*, *Koliella*, *Klebsormidium*, *Chlorococcum*, *Tetracystis*, *Myrmecia*, *Dictyosphaerium*, *Pleurochloris*, *Botrydiopsis*, *Heterothrix* and *Heterococcus* are commonly recognized in cultures of the soils obtained from the surrounding area of both rookeries. These algae appeared in the soils collected from East Ongul Island, where the Adélie penguin rookery has not been formed.

HOSHIAI and MATSUDA (1979) recognized that the variation of standing crop of terrestrial epipsamic algal chlorophyll in the soil of Ongulkalven is clearly related to the contiguous relationship of algal habitat with the penguin rookeries. However, it also implies some interesting points, particularly as regards the microdistribution pattern of chlorophyll standing crop in the rookery and its surrounding area.

SIEBURTH (1960) showed the antibiotic activity of acrylic acid on bacteria and fungi such as *Pasteurella*, *Corynebacterium*, *Streptococcus*, *Staphylococcus*, *Escherichia*, *Candida* and so on, and he also suggested a possible presence of the antialgal property of this substance. Subsequently, SIEBURTH (1963, 1968) reported that this substance remains stable when the alga are eaten by *Euphausia* and when consumed by the penguin, the substance becomes concentrated in the intestinal tract and excrement of the penguin. He demonstrated biologically the antibiotic activities of penguin feces and guanos in the rookeries. However, he did not chemically identify that antibiotic substance in the rookery soils as the acrylic acid. The present results agree with those of SIEBURTH (1960, 1963) and BOYD (1967).

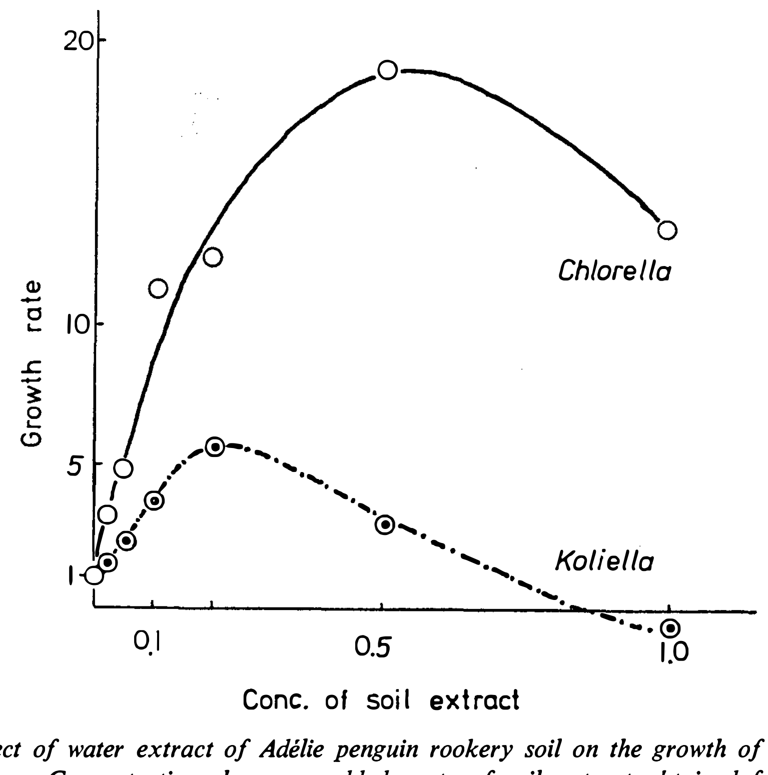

Fig. 1. Effect of water extract of Adélie penguin rookery soil on the growth of Antarctic soil algae. Concentration shows as added parts of soil extract obtained from 50 g soil treated in 150 ml distilled water.

Table 2. Antialgal activities of extracts of penguin rookery soil showing width (mm) of inhibition zone by paper disc test.

Test algae	Extracts	
	Acetone	Water
Koliella	1.0	
Stichococcus	0.5	
*Fritschiella**	4.0	
*Chlorella**	1.5	2.0
*Monoraphidium**	3.0	
*Monodus**	0.2	1.0
Bumilleria	7.0	

*Materials isolated from Japanese soil.

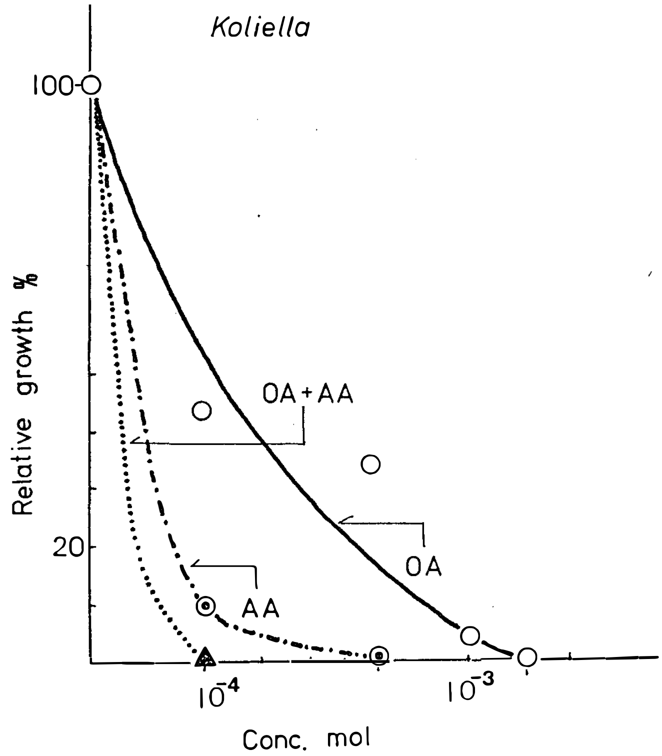

Fig. 2. Inhibitory effects of acrylic acid and oxalic acid on the growth of Antarctic Koliella *(AA: acrylic acid, OA: oxalic acid).*

The highly concentrated water extract of rookery soil inhibited algal growth, whereas the diluted extract accelerated the growth of algae (Fig. 1). The paper disc test on both acetone and water extracts of guano soils gave an evident inhibitive effect on the algal growth (Table 2). The acrylic acid was detected in rookery soil extract by means of HPLC, and a large quantity of oxalic acid was also recognized (Fig. 3). These two organic acids, either coexisting or occurring separately, inhibited the growth of *Koliella* (Fig. 2).

The present result supports the previous hypothesis on the antibiotic activity of Adélie penguin rookery soil (SIEBURTH, 1963, 1968; BOYD *et al.*, 1966 and BOYD, 1967). It is a new finding that the oxalic acid was another inhibitor of algal growth

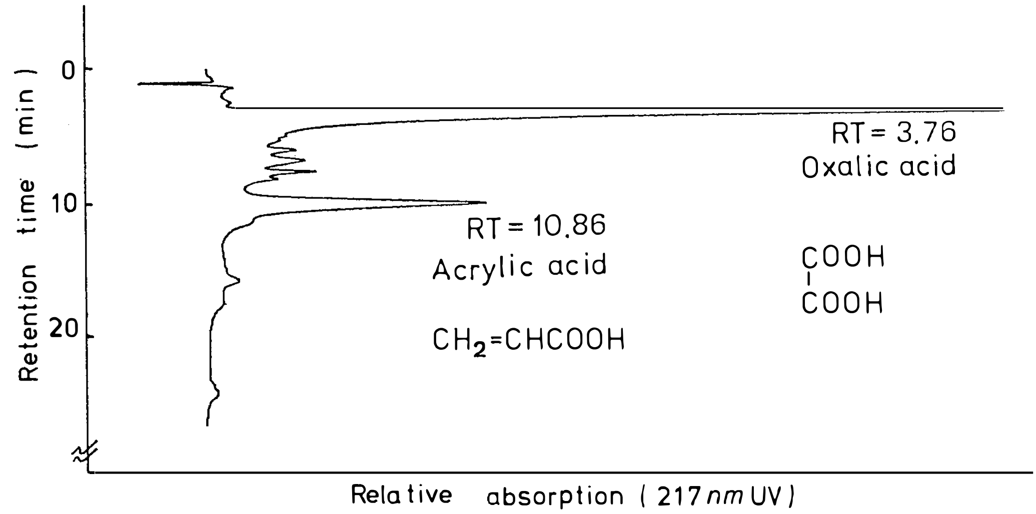

Fig. 3. HPLC data of acrylic acid and oxalic acid in the water extract of penguin rookery soil obtained from Ongulkalven, Antarctica.

as well as acrylic acid in the Adélie penguin rookery soils. On the origin of oxalic acid in rookery soils, it may be suggested that this substance is an end-product through the decomposition of penguin feces by soil microbes (ASAI, 1968), particularly in such a cold environment in Antarctica. However, further studies are needed to discuss the origin of oxalic acid.

Although the penguins play an important role in organic transfer between sea and coastal ice-free area in Antarctica, there are two paradoxical effects of penguin excrements on the soil microbes, namely the provision of not only nutrient substances but also growth-inhibiting substances at the same time. It is very interesting ecologically that there are two types of algal growth inhibiting, allelopathic substances in rookery soils. Namely, in the case of acrylic acid, it is produced by marine algal plankton and this substance is transported from sea to coastal ice-free area through the food-chain. In contrast, in the case of oxalic acid, it is situated in the different position from that of acrylic acid in the material cycle in the Antarctic ecosystem, as an end-product of the decomposition flow in the terrestrial environment of Antarctica.

Acknowledgments

The authors are indebted to Prof. O. HIRAYAMA of Shimane University for his kind cooperation in the course of detection of these organic substances in rookery soils.

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(Received February 5, 1986; Revised manuscript received June 2, 1986)