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Ecological Characteristics of Some Algal Populations along Environmental Gradients of Zinc

Song, Seung-Dal

(Department of Biology, Kyungpook National University, Daegu)

亞鉛環境勾配에 의한 綠藻類個體群生長의 生態學的 特性

宋 承 達
(慶北大學校 文理科大學 生物學科)

ABSTRACT

The maximum relative growth rate of algae treated with Zinc was shown as follows: 15, 8, 6, 3 and −5% per day for the rather sensitive *Chlorella* sp. populations, or 14, 7, 5 and 4% per day for the *Pleurococcus* sp. populations, and 22, 20, 13, 9 and 7% per day for the more resistant *Scenedesmus spinosus* populations, respectively for the culture medium with 0, 1, 5, 10 and 20 ppm of Zinc treatment. With mixed cultures of *Chlorella* sp. and *Scenedesmus spinosus* populations, the growth of the *Chlorella* sp. population overcame that of the *S. spinosus* population from the cultures treated with relatively low concentration of Zinc. On the contrary, the population growth of the latter resistant species overcame that of the former sensitive species when the concentration of Zinc was above 5 ppm Zn of the medium. This paper describes the results of further investigations of the effects evaluated by direct cell counts method, optical density comparisons, oxygen production and consumption determinations and the measurements of the fate of Zinc treated in the solutions.

INTRODUCTION

It is becoming apparent that the environmental emissions of toxic heavy elements from mining operations, industries and automobiles are concentrating and affecting in the world's ecosystem.

Unfortunately in large areas, the concentration of these solid waste outputs dissolved in an ecosystem is considered to be sufficiently high to eradicate the biotic community or to change the composition of the ecosystem (Mitchell, 1974).

The toxicity of heavy metals to fresh water algae is of considerable biological and economic interest, and there are some literatures on the toxicity of heavy metal of Zinc to fresh water algae:Besch et al. (1972); Dooren, (1965); Monsi et al. (1976); Whitton (1968, 1970a, 1970b, 1972, 1973, 1975); and Williams and Mount (1965). Toxicity of Zinc is shown to the range of 1.7 mg/*l* for the lowest inhibitory concentration through 3.5 mg/*l* for the highest concentration tolerated. On the other hand, Zinc is generally known to be an important micro nutrient for metabolism being limiting factor. Price and Miller (1962) have examined their hypothesis that the growth of Zinc deficient *Euglena gracilis* (Klebs) is limited by the rate of respiration. However, much of the literature is fragmentary, and it is difficult to get from it the importance of

toxic substances in the stability and diversity of species of the ecosystem.

The aim of the present study was to measure mass-diversity change and to compare the toxicity resulting from various Zinc treatment to the growth characteristics of green algal populations with pure and mixed cultures. Three species, *Chlorella* sp., *Scenedesmus spinosus* and *Pleurococcus* sp. were chosen for more intensive study. These are preiominant unicellular algal that develop usually in sewage ponds.

MATERIALS AND METHODS

Strains of *Chlorella* sp., *Scenedesmus spinosus* and *Pleurococcus* sp. provided by the Laboratory of International Courses, Delft, were used through the experiment.

The experiments were carried out in a series of Zinc treated 100 m*l* erlenmeyer flasks with usual aseptic conditions (stoppered with cotton plugs) of shaking incubation (150 rpm). Cultivation took place at room temperature (28°C) and complete photoautotrophic growth being possible in light conditions of ca. 3000 lux with continuous illumination.

The medium used was da Silva complete mineral medium. The basal medium consisted of : 496 ppm $NaNO_3$, 39 ppm K_2HPO_4, 75 ppm $MgSO_4 \cdot 7H_2O$, 36 ppm $CaCl_2 \cdot 2H_2O$, 20 ppm Na_2CO_3, 58 ppm $Na_2SiO_3 \cdot 9H_2O$, 0.6 ppm $FeSO_4$, 0.6 ppm citric acid, 1 ppm EDTA, 1.14 ppm H_3BO_3, 0.72 ppm $MnCl_2 \cdot 4H_2O$, 80 ppb $ZnSO_4 \cdot 7H_2O$, 32 ppb $CuSO_4 \cdot 5H_2O$, 19.6 ppb $Co(NO_3)_2$, 7.2 ppb $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. The pH of the solution was adjusted to 8.0. Sterilization was effected by autoclave at 120°C for 30 minutes. As under this culture medium the growth rate was rather low by preliminary experiment, comparisons with two other media (modified Ogawa and New Algal Assay Medium, NAAM) were made during the periods (Bhumiratana, 1973; Fogg, 1965; Golterman, 1975; Lewin, 1962; Nomita and Fogg, 1966; Toerien, 1973).

The observations were made by the addition of the requisite amounts of concentrated solution of $ZnSO_4 \cdot 7H_2O$ for control (0), 1, 5, 10 and 20 ppm Zinc, immediately prior to the addition of the algae and continued until the cultures were 2 weeks old. The algal growth under conditions of Zinc treatments was estimated by direct cell counts on days; 0, 2, 4, 6, 8, 10 and 12 days after inoculation. Counts were made in duplicate for every duplicated treatment with the aid of a haemacytometer. Through the experiments, the coefficient of variation for each measurement was shown to the range of 25−35%.

For the exponential phase of growth, relative

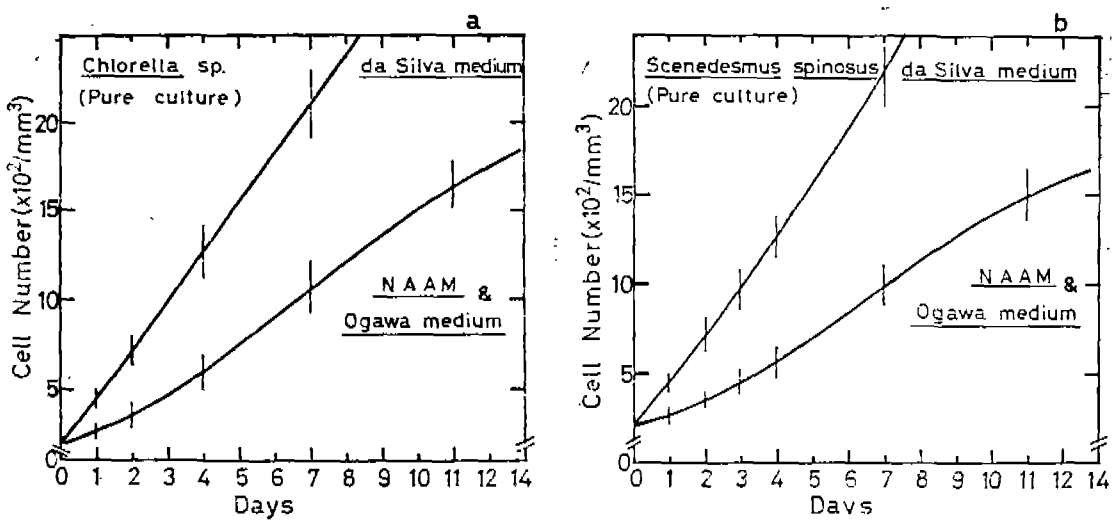

Fig.1. Comparisons of algal growth with three different cultures: da Silva, NAAM and Ogawa modified media. a; Growth of *Chlorella* sp. population, b; growth of *Scenesmus spinosus* population.

growth constants per day were evaluated. And at the end of growth, comparisons were made for determinations of optical density and oxygen production/consumption by Winkler method and Zinc concentration of the cultures by dithizone method (Sandell, 1959).

RESULTS AND DISCUSSION

1. Effects of medium on the algal growth

Comparisons of algal growth cultivated with three different cultures are summarized in Fig. 1a and b. As these strains of algae were long adapted to da Silva medium during the stock periods, the population growth rate with this medium was faster than those with modified Ogawa and NAAM cultures, though the maximum growth rate is considered rather to be low than predicted growth. However, it must be realized that maximum growth rates of 15% per day for *Chlorella* sp. population and 22% per day for *Scenedesmus spinosus* population were determined from data obtained every 48 hours (see 2and 3); therefore, accurate calculation of the growth rates can be expected because the time intervals between measurements were adequate. As a consequences, in this experiment we analyzed the algal growth by the da Silva medium.

Table 1. Relative growth rates of *Chlorella* sp. populations in pure cultures treated with various concentrations of Zinc

Treatment ppm	Days, % per day				
	0—2	2—5	5—7	7—10	10—12
Control	15	10	14	10	4
1	8	7	8	5	2
5	6	3	3	2	1
10	3	2	2	1	0
20	−5	−5	−8	−6	−2

Table 2. Relative growth rates of *Scenedesmus spinosus* populations in pure cultures treated with various concentrations of Zinc

Treatment ppm	Days, % per day				
	0—2	2—5	5—7	7—10	10—12
Control	22	15	12	8	2
1	20	11	9	8	2
5	13	8	7	6	3
10	9	9	5	5	4
20	6	7	4	3	3

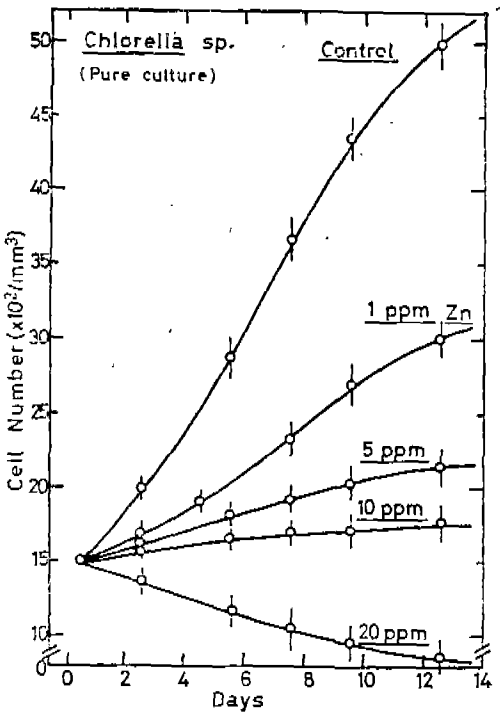

Fig. 2. Growth Patterns of pure cultured *Chlorella* sp. populations treated with various concentrations of Zinc.

2. Effects of Zinc on the growth of pure *Chlorella* sp. population

Fig. 2 presents the pattern of the algal growth of pure cultured *Chlorella* sp. population treated with various concentrations of Zinc during the periods. The growth inhibitions of pure cultured *Chlorella* sp. population are showing clear relationships to the Zinc concentrations. The growth rate constants (bicmass cells/ cell·day) of each treatment during the periods are summarized in Table 1. The maximum relative growth rates for the control (0), 1, 5, 10 and 20 ppm of Zinc treatment were 15, 8, 6, 3 and −5% per day, respectively. In the higher concentrations of Zinc medium the growth ceased and the population decreased. As a result, the *Chlorella* sp. population seems rather sensitive to the Zinc treatment of higher concentration.

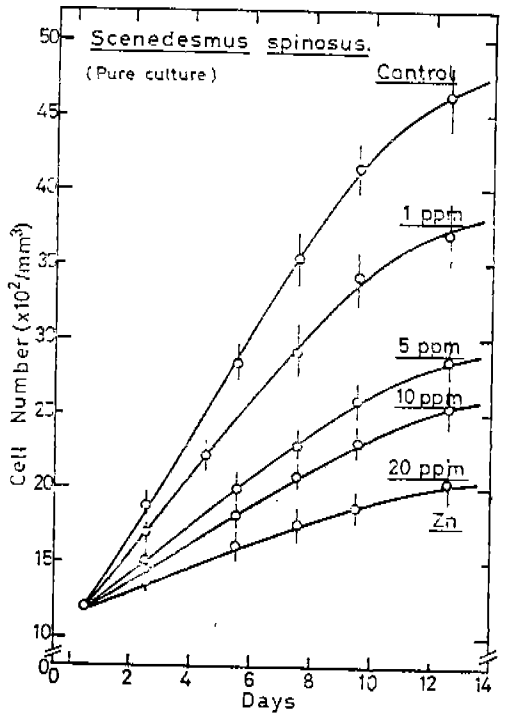

Fig. 3. Growth patterns of pure cultured *Scenedesmus spinosus* populations treated with various concentrations of Zinc.

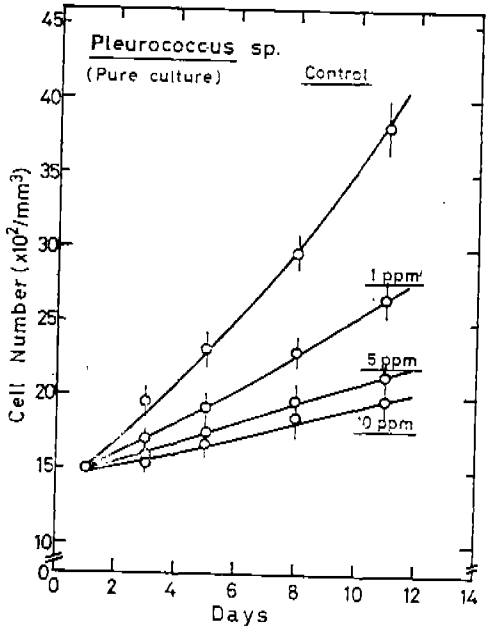

Fig. 4. Growth patterns of pure cultured *Pleurococcus* populations treated with various concentrations of Zinc.

Table 3. Relative growth rates of *Pleurococcus* sp. populations in pure cultures treated with various concentrations of Zinc

Treatment ppm	Days, % per day			
	0—2	2—5	5—7	7—10
Control	14	9	8	7
1	7	6	4	3
5	5	4	4	2
10	4	3	3	2

Table 4. Relative growth rates of *Chlorella* sp. and *Scenedesmus spinosus* populations in mixed cultures treated with various concentrations of Zinc

Treatment ppm		Days, % per day				
		0—2	2—5	5—7	7—10	10—12
Chlorella, sp	0	14	10	12	4	3
	5	9	10	5	3	3
	10	7	6	5	2	1
	20	−4	−4	−5	−4	1
Scenedesmus spinosus	0	19	10	10	4	0
	5	12	9	8	6	1
	10	9	7	6	6	2
	20	5	4	3	7	1

3. Effects of Zinc on the growth of pure *Scenedesmus spinosus* population

Growth pattern of the pure cultured *S. spinosus* population with various Zinc treatment during the periods is compared in Fig. 3. The growth rate constants of each treatment during the periods are presented in Table 2. The maximum relative growth rates for the control culture (0), 1, 5, 10 and 20 ppm of Zinc treatment were 22, 20, 13, 9 and 7% per day, respectively. Comparisons of the growth characteristics of *S. spinosus* population with the data for the growth of *Chlorella* sp. population indicated that the former seems rather resistant to Zinc treatment of higher concentration.

4. Effects of Zinc on the growth of pure *Pleurococcus* sp. population

The pattern of the algal growth of the pure cultured *Pleurococcus* sp. population treated with various Zinc concentrations and the growth rate cons-

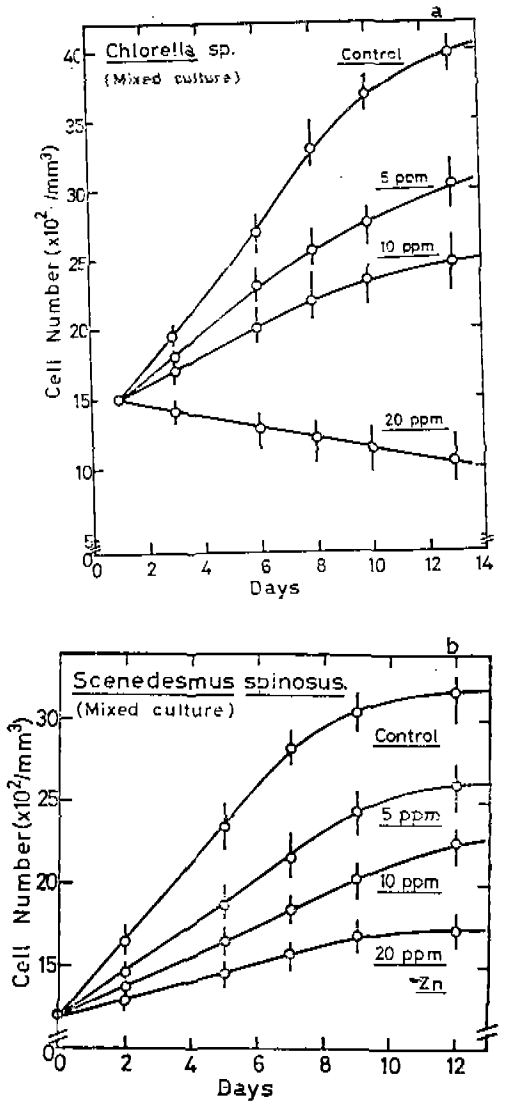

Fig. 5. Algal population growth in mixed cultures treated with various concentrations of Zinc: *a; Chlorella* sp. populations, *b; Scenedesmus spinosus* populations.

tants of each treated population during the periods are shown in Fig. 4 and Table 3, respectively. The maximum relative growth rates for the control culture (0), 1, 5 and 10 ppm of Zinc treatment were 14, 7, 5 and 4% per day, respectively. As a consequences, the more Zinc present in the medium the more the population decreased showing apparent sensitivity to Zinc.

5. Effects of Zinc on the growth of mixed populations

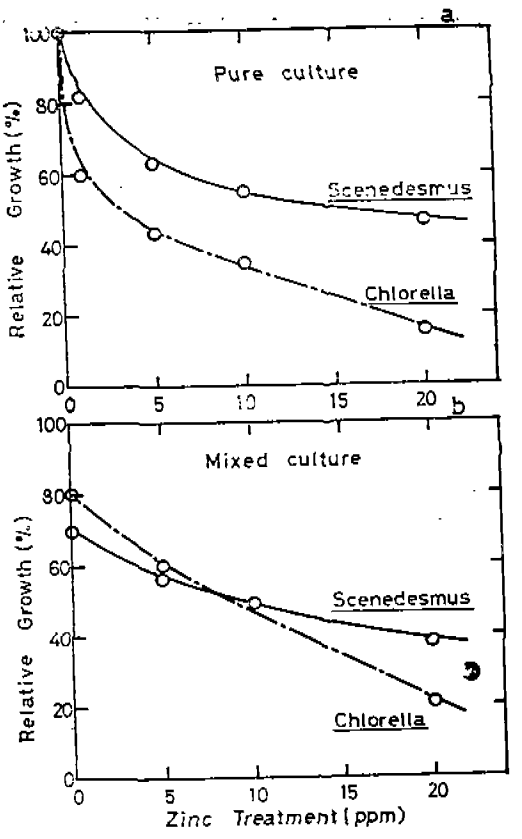

Fig. 6. Relative effects of Zinc treatments on the growth of *Chlorella* sp. and *Scenedesmus spinosus* populations. *a;* Pure cultures, *b;* mixed cultures.

In Fig. 5a and b and Table 4, the pattern of the algal growth of mixed populations of *Chlorella* sp. and *S. spinosus* treated with various concentrations of Zinc and the growth rate constants of each treated population during the periods were summarized. The maximum relative growth rates for the control culture (0), 5, 10 and 20 ppm of Zinc treatment were 14, 10, 7 and −4% per day for *Chlorella* sp. population and 19, 12, 9 and 7% per day for *S. spinosus* population, respectively. With this mixed cultures, the toxicity of Zinc on each population was different from that of pure cultures; i.e. *Chlorella* sp. population showed less sensitivity than with pure culture.

Fig. 6 a and b show the relative effects of Zinc treatment on the growth of *Chlorella* sp. and *S. spinosus* population for pure and mixed cultures. Here the relative effects mean the percentage of

each algal growth of various Zinc treatments to each algal growth of control culture at the end of growth. During the periods, *Chlorella* sp. population showed more sensitivity to Zinc treatment in pure culture, while with mixed culture they showed apparently less sensitivity.

However, it is quite interesting from the ecological point of views that with mixed culture, when treated with low toxic gradient of Zinc the *Chlorella* sp. population overcame *S. spinosus* population, but with heavy treatment of Zinc the latter over came the former.

6. Changes of optical density

As comparisons of chlorophyll contents of algal population with concentrations of Zinc in the medium, Fig. 7 shows the results of the changes of optical density (663nm) at the end of maximum growth. It was also clear that the optical density of *Chlorella* sp. culture showed the lowest population content to the Zinc concentrations indicating the highest sensitivity, while that of *S. spinosus* culture showed the highest population content to the Zinc treatment indicating the most resistant growth. With mixed cultures of above two species, the optical densities showed the medium population contents.

7. Oxygen production and consumption of Zinc treated algal population

In order to compare productivity and respiration

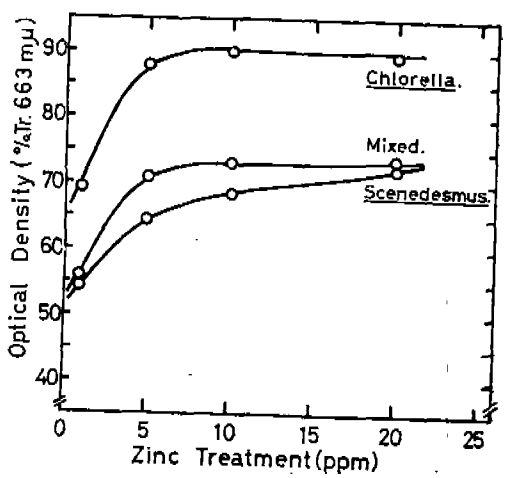

Fig. 7. Comparisons of chlorophyll contents of each algal population treated with various concentrations of Zinc.

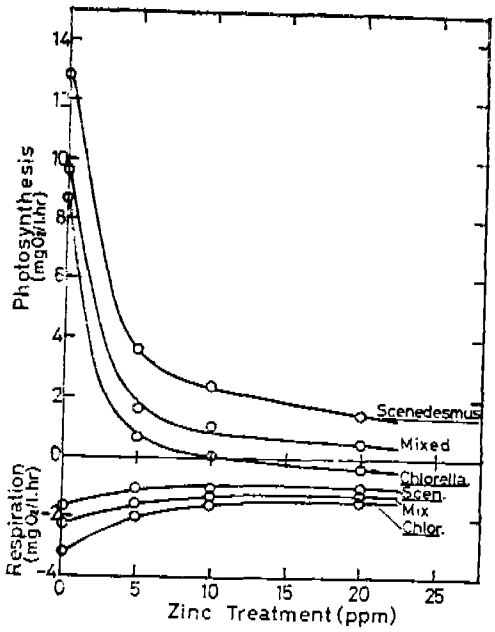

Fig. 8. Photosynthesis and respiration of the algal populations treated with various Zinc concentrations (measured by Winkler method).

of Zinc treated populations of *Chlorella* sp. and *S. spinosus*, it was necessary to establish Winkler method. The results for oxygen production (photosynthesis) and consumption (respiration) of the algal populations treated with various Zinc concentrations at the end of maximum growth were shown in Fig. 8. The production of oxygen was at a low level in the heavy Zinc treated populations of *Chlorella* sp. and *S. spinosus*, i.e. ca. 15—20% of the net control rates. However, the amount of oxygen production by *S. spinosus* population was larger than that of *Chlorella* sp. population in each Zinc treatment culture. While, the amount of oxygen consumption (respiration) of *Chlorella* sp. population was larger than that of *S. spinosus* population. And with mixed cultures, the values of oxygen production and consumption were in between of two values of above two species with pure cultures. When the growth rates of algal population treated with Zinc were 20—30% of the control growth in Fig. 2 and 3, the rates of oxygen uptake were appeared 50—60% of the control rates. It is also considerable to review Wilson's observation (1959) of good growth rates with respiratory rates no higher than the

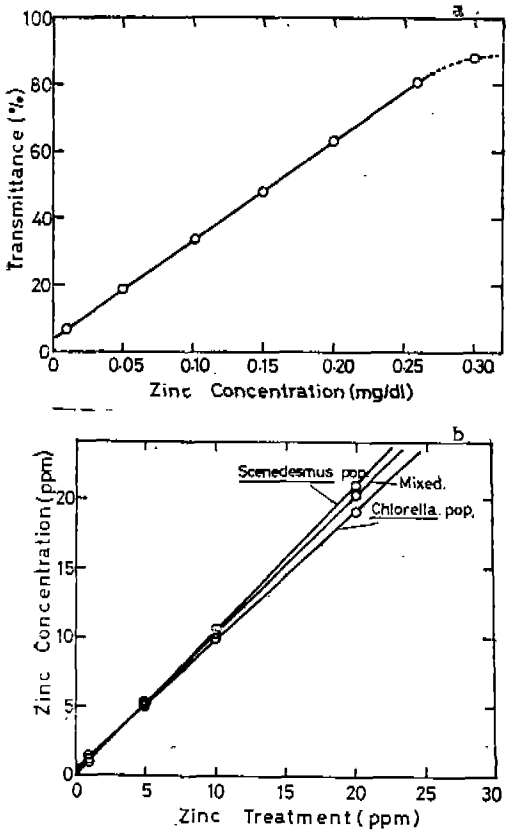

Fig. 9. a; Standard curve of dithizone method. b; Zinc concentrations remaining in pure and mixed cultures of *Chlorella* sp. and *Scenedesmus spinosus* populations treated with various concentrations of Zinc.

endogenous rate. This also speaks against a respiratory lesion limiting growth in excessive Zinc.

8. Fate of Zinc

The Zinc concentration of the culture media of each treatment was measured by the dithizone method (620nm unreacted dithizone) to prove about the fate of Zinc in the solution. Fig. 9a and b show respectively the standard curve of dithizone method and the Zinc remaining in each treated culture of algal population. But it is doubtful from these results whether the *Chlorella* sp. population absorbed more Zinc than the *S. spinosus* population did. However, the Zinc concentration of the culture of *S. spinosus* population showed somewhat larger than that of the *Chlorella* sp. population at the end of algal growth. It seems that the conditions of the algal population may have a considerable effects on the test results. The fact that the medium used in the present experiments is relatively rich in nutrients such as phosphate may well mask effects that would show in a less favourable environment.

It is also considered to be useful to do further experiments to prove this fate of Zinc in the culture solution during the growing periods.

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摘 要

數種의 綠藻類個體群에 對한 混合競爭培養 및 純粹培養을 通하여, 亞鉛의 環境汚染度에 따른 毒性의 影響과 個體群의 生長解析 및 生態的 特性을 比較檢討하였다. 五段階의 亞鉛汚染處理區(0, 1, 5, 10, 20ppm)에 對해서 各純粹培養個體群의 最大相對生長速度는 *Chlorella* sp. 個體群이 各各 15, 8, 6, 3, 및 −5% per day이고, *Scenedesmus spinosus* 個體群이 各各 22, 20 13, 9 및 7% per day이며, *Pleurococcus* sp.個體群이 各各 14, 7, 5 및 4% per day로서, *Scenedesmus spinosus* 個體群이 가장 높은 抵抗性을 보였다. 그리고 이들 個體群의 混合培養에서는 *Chlorella* sp. 個體群이 低濃度의 亞鉛汚染區에서 *S. spinosus* 個體群에 對해 優占種으로 나타났으나, 5 ppm 以上의 高濃度의 汚染區에서는 後者가 前者에 對해 優占種으로 나타나는 生態的特性을 보였다. 그리고 이들 個體群의 亞鉛環境勾配에 對한 生理的 反應, 葉綠素含有量의 變化, 光合成 및 呼吸量의 變化와 亞鉛의 吸收등에 對해서 檢討하였다.

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