Tracer Experiments on the Effect of Micronutrients on the Growth of *Porphyra* Plants–II

Manganese–54 and zinc–65 Assimilation in Relation to Environmental Factors

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Introduction

Studies on the absorption of micronutrients by *Porphyra* plants are difficult if appropriate methods for culturing *Porphyra* and reliable standards for judging a plant's nutrient-deficiency symptom are not established. Furthermore, the studies of this kind find difficulties in obtaining totally pure nutrient salts and in eliminating contaminants unexpectedly introduced during culture experiments.

The author used radioisotopes since 1966 to investigate the micronutrient requirement of *Porphyra* plants in relation to environmental factors. The present paper deals with the result of manganese-54 and zinc-65 assimilation experiments under various conditions. Manganese and zinc are trace metals essential for the growth of *Porphyra* plants Manganese has relation to oxidative enzymes and zinc to oxidizing-reducing reactions and photosynthesis.

The present experiments were carried out on the assumption that the *Porphyra* plant assimilates manganese and zinc regardless of their chemical types, whether they are stable or unstable, colloidal or ionic in form. And most of manganese and zinc in the medium were considered chelated and ionized owing to the presence of Na_2EDTA.

Materials and Methods

The plant mainly used in the experiments was *Porphyra leucosticta* THURET collected at Woods Hole in 1965 and maintained in the laboratory through monospores using artificial sea water. From a proper portion of its thallus about two months old, a round piece was cut off with a leaf-puncher. The punched pieces of the thallus, 8 mm in diameter were put in the vessels containing artificial sea water and at 8–10°C, 9 hour day light, and 3,000 lux.

Five to ten of pieces of the thallus thus preserved, were put into a 100 ml Erlenmeyer flask containing artificial sea water, and the flasks were placed in a case kept at a constant temperature and light intensity. Then a proper amount of manganese-54 or zinc-65 was added into the flasks and they were covered with a cotton stopper. The flasks were shaken at times to expose all the thallus pieces uniformly to the light. After a lapse of a certain time, the thallus pieces were taken out, fixed in 5–10% formalin in sea

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water, and dried on paper as done for herbarium specimens.

The radio-activity measurement of the samples was mainly carried out in a well-type gamma ray NaI scintillation counter. The dried thallus pieces on paper were brought to the counter plate and all measurements were made at room temperature (20±2°C), recording either the time passed until the counts reach 10,000 in total or the number of counts per 5 minutes. No account was taken of isotope effect.

Results

I. Manganese-54 assimilation in relation to environmental factors

1. Temperature

The rate of assimilation was observed at various temperatures, namely at 8–9°C, 18–19°C, and 27–28°C, under the light intensity fixed at 3,000 lux. The R.I. was put into the medium as much as 5 μCi per 50 ml for one hour. Eeffct of temperature upon the assimilation rate was not observed in the range of the temperatures examined.

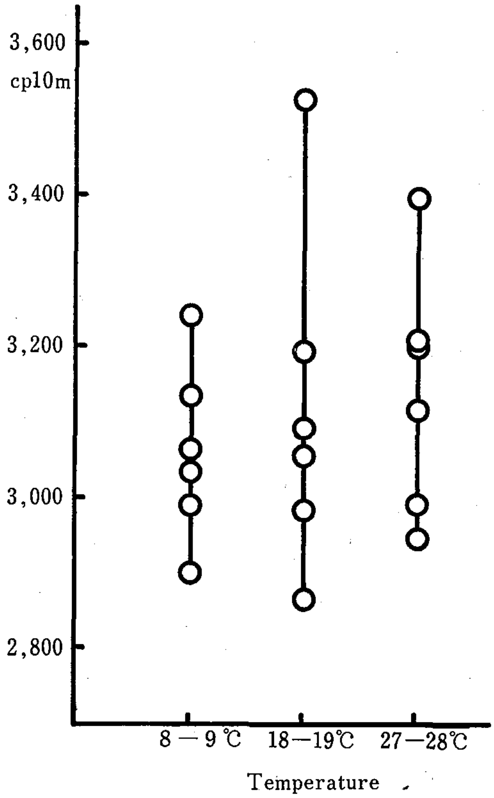

Fig. 1. Manganese-54 assimilation in relation to temperature.

2. Light intensity

The rate of assimilation was observed at various light intensities, namely at 0 lux, 1,000 lux, 3,000 lux and 6,000 lux, under the temperature fixed at 9–10°C. The R.I. was put into the medium as much as 2 μCi per 50 ml for 24 hours. Effect of light intensity upon the assimilation rate was not observed in the range of the light intensities examined.

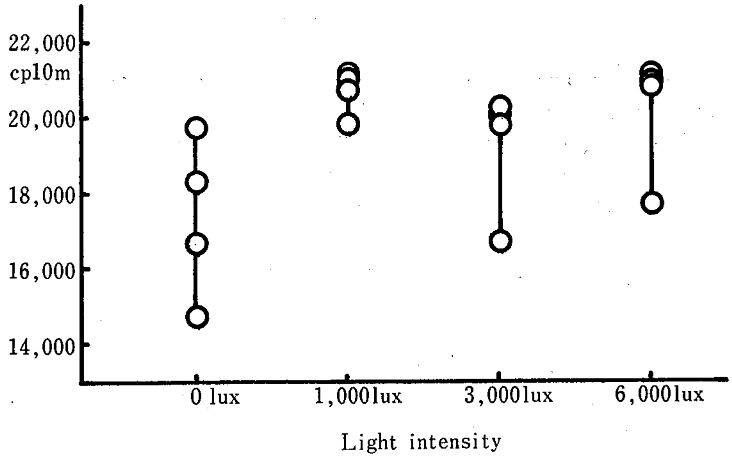

Fig. 2. Manganese-54 assimilation in relation to light intensity.

3. **Chlorinity**

The rate of assimilation was observed at various chlorinities, namely at 15.16‰, 12.13‰ and 7.58‰, under the temperature fixed at 10°C and the light intensity at

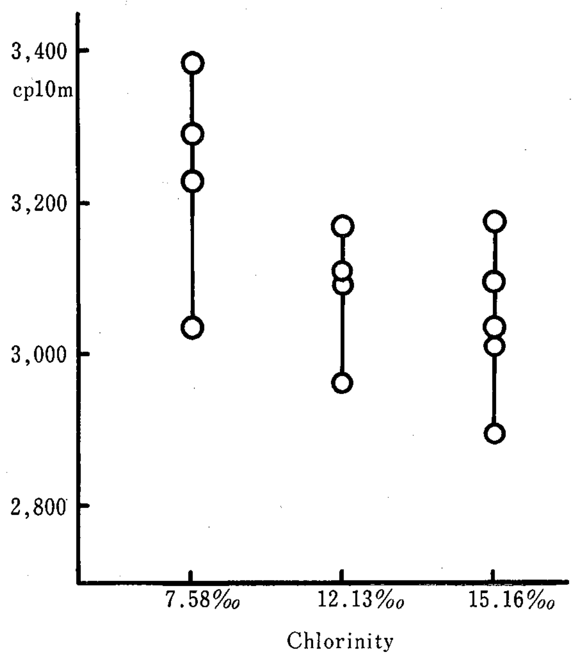

Fig. 3. Manganese-54 assimilation in relation to chlorinity.

3,000 lux. The R.I. was put into the medium as much as 5 μCi per 50 ml for 3 hours. Effect of chlorinity upon the assimilation rate was not observed in the range of the chlorinities examined.

4. pH

Effect upon the assimilation rate of various pH degrees, namely pH 7, 7.5, 8, 8.5 and 9 was investigated under the temperature fixed at 9–10°C and the light intensity at 3,000 lux. The R. I. was put into the medium as much as 2 μCi per 50 ml for 24 hours. The assimilation rate accelerated at pH between 7.5 and 8.5, but retarded at pH below 7.5 or over 8.5.

5. Na_2EDTA concentrations

The rate of assimilation was observed at various concentraions of Na_2EDTA, namely at 0.1 mg%, 0.3 mg%, 1 mg%, 3 mg% and 10 mg%, under the temperature fixed at 10°C and the light intensity at 3,000 lux. The R.I. was put into the medium as much as 5 μCi per 50 ml for one hour. The rate decreased with the increase of Na_2 EDTA concentrations examined.

II. Zinc-65 assimilation in relation to environmental factors

1. Temperature

The rate of assimilation was observed at various temperatures, namely at 8–9°C, 18–19°C and 27–28°C under the light intensity fixed at 3,000 lux. The R. I. was put into the medium as much as 10 μCi per 50 ml for one hour. Effect of the temperature upon the assimilation rate was not observed in the range of the temperatures examined.

2. Light intensity

The rate of assimilation was observed at various light intensities, namely at 0 lux, 1,000 lux, 3,000 lux and 6,000 lux, under the temperature fixed at 9–10°C. The R.I. was put into the medium as much as 20 μCi per 50 ml for 18 hours. Effect of light intensity upon the assimilation rate was not observed in the range of the light intensities examined.

3. Chlorinity

The rate of assimilation was observed at various chlorinities, namely at 15.16‰, 12.13‰ and 7.58‰, under the temperature fixed at 10°C and the light intensity at 3,000 lux. The R. I. was put into the medium as much as 10 μCi per 50 ml for 3 hours. Effect of the chlorinity upon the rate was not observed in the range of the chlorinities examined.

4. pH

Effect upon the assimilation rate of various pH degrees, namely pH 7, 7.5, 8, 8.5 and 9 was investigated under the temperature fixed at 9–10°C and the light intensity at 3,000 lux. The R.I. was put into the medium as much as 20 μCi per 50 ml for 18 hours. Effect of pH change upon the rate was not observed in the range of the pH

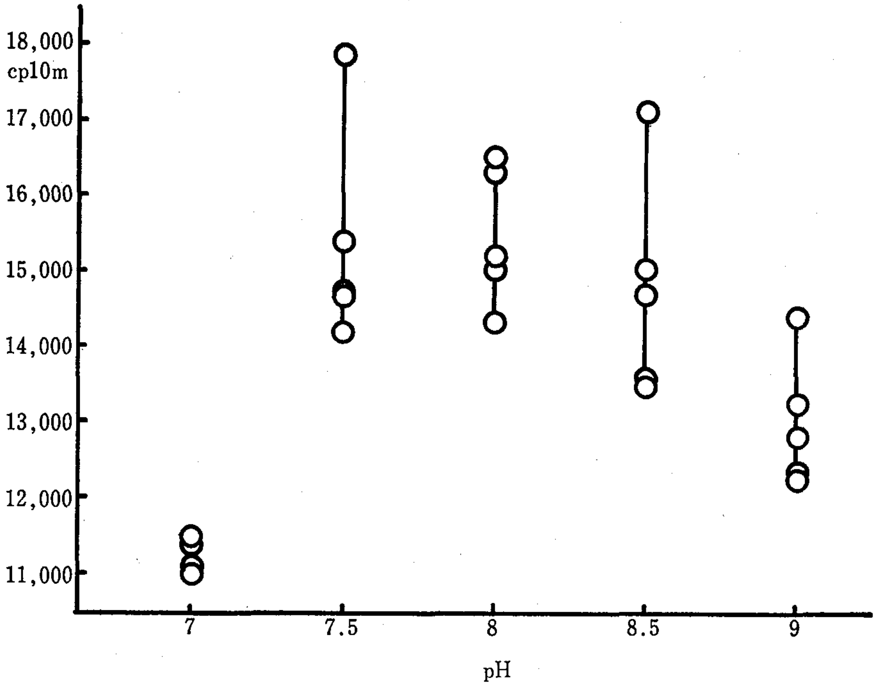

Fig. 4. Manganese-54 assimilation in relation to pH.

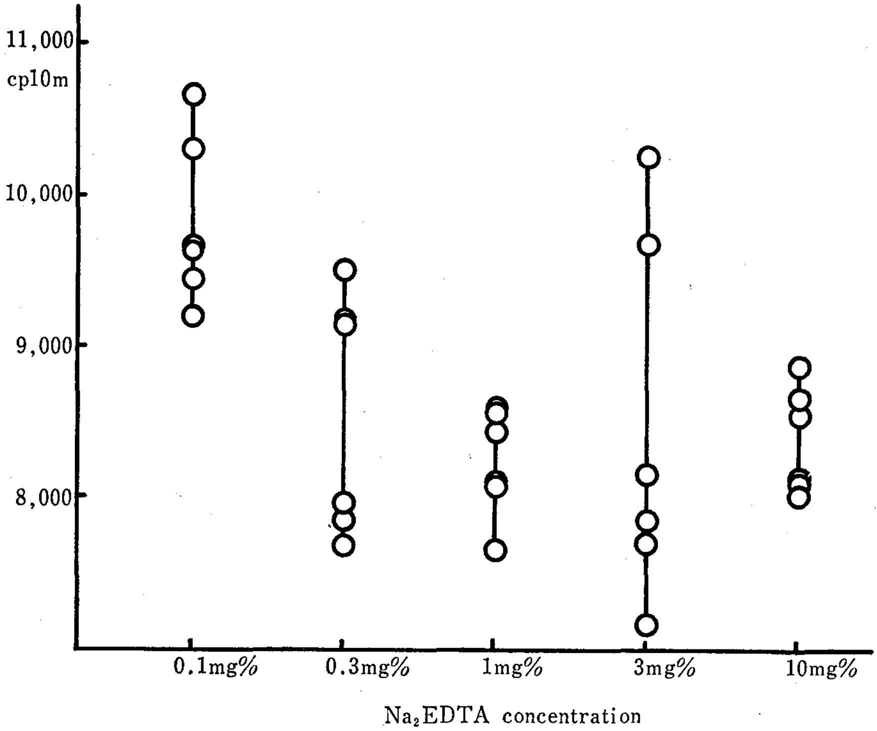

Fig. 5. Manganese-54 assimilation in relation to Na_2EDTA concentration.

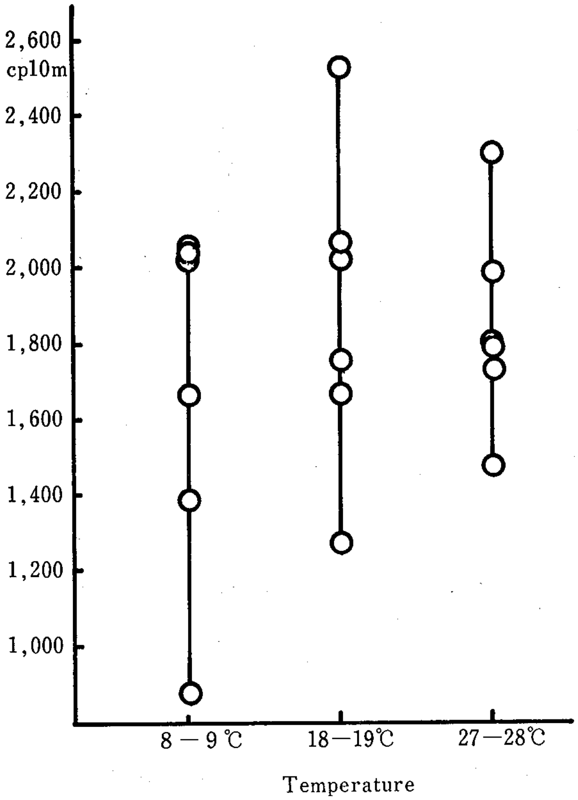

Fig. 6. Zinc-65 assimilation in relation to temperature.

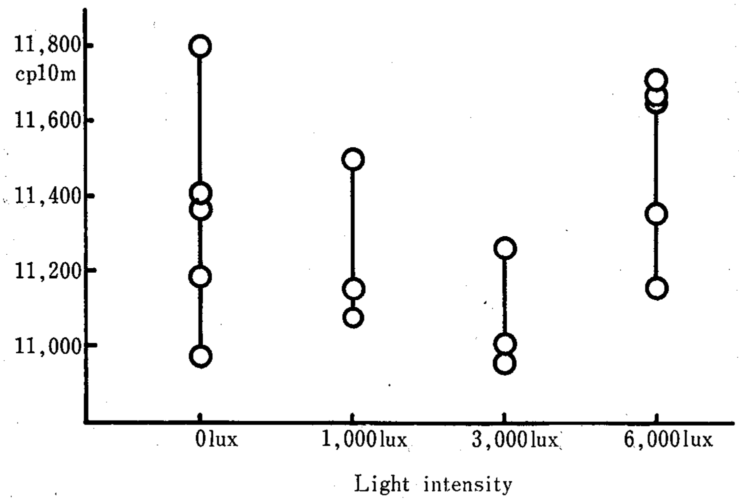

Fig. 7. Zinc-65 assimilation in relation to light intensity.

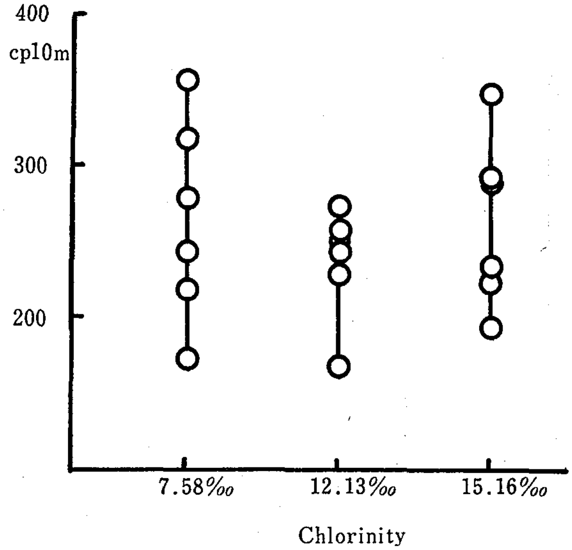

Fig. 8. Zinc-65 assimilation in relation to chlorinity.

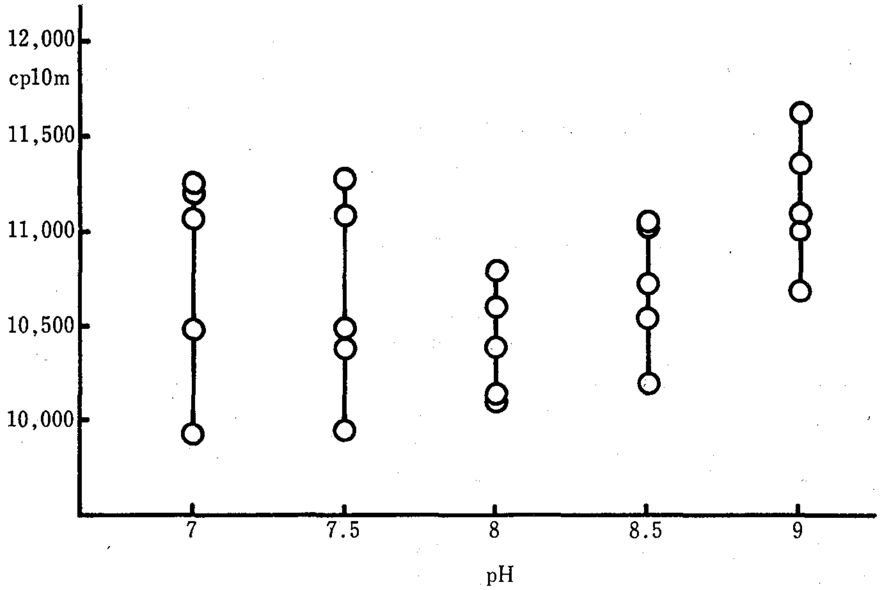

Fig. 9. Zinc-65 assimilation in relation to pH.

degrees examined.

5. **Na_2EDTA concentrations**

The rate of assimilation was observed at various concentrations of Na_2EDTA, namely at 0.1 mg%, 0.3 mg%, 1 mg%, 3 mg% and 10 mg%, under the temperature fixed at 10°C and the light intensity at 3,000 lux. The R.I. was put into the medium as much as 5 μCi per 50 ml for one hour. The rate decreased with the increase of Na_2EDTA concentrations examined.

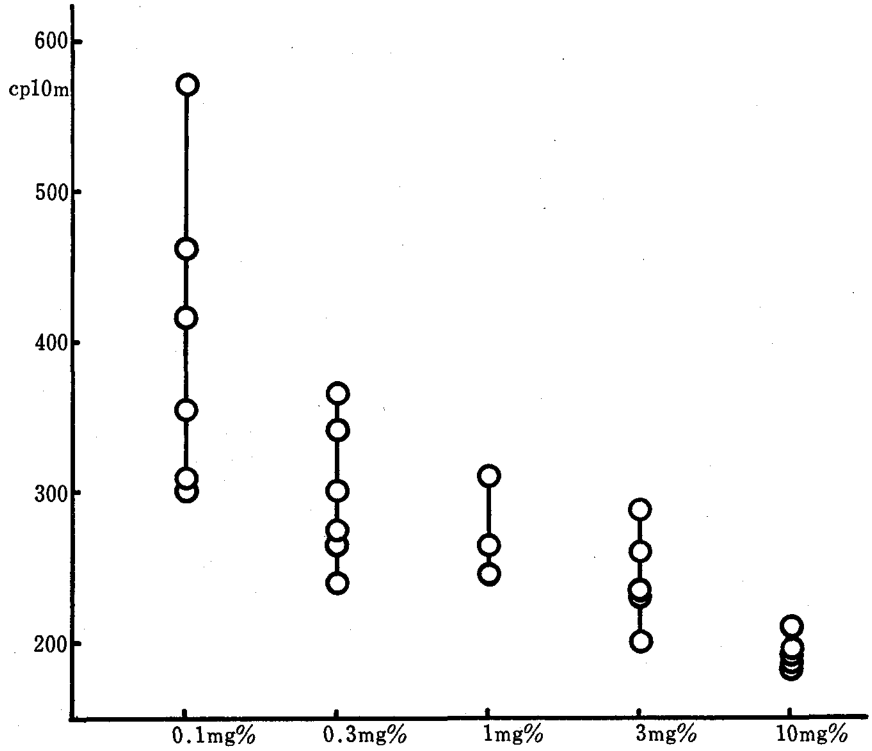

Fig. 10. Zinc-65 assimilation in relation to Na_2EDTA concentration.

Discussion

The rate of manganese-54 and zinc-65 assimilation by *Porphyra* plants was not affected by the changes of temperature, light intensity and chlorinity. The change of pH affected the assimilation rate of manganese-54 but not that of zinc-65. The amounts of manganese and zinc required for the growth of *Porphyra* plants are not surely known. The concentrations of manganese and zinc generally used in culture experiments of *Porphyra* are 0.04 mg% and 0.005 mg% respectively when 1 mg% of Na_2EDTA, a chelate substance, is present. The ratio between the concentration of Na_2EDTA and the total amount of trace metals is from 3: 1 to 1: 1 in the culture media used. Metal deficiency in the plant body occurs when the rate is over 3: 1, and metal precipitation occurs to cause toxicity when the rate is less than 1: 1. The rate of the micronutrients assimilation, in the present experiment, decreased with the increase of Na_2EDTA concentration. From this fact, it is assumed that there was neither metal deficiency nor toxicity within the limits examined. Therefore the result of so far reported culture experiments, which gave the favorable ratio of chelate as lying between 3: 1, and 1: 1 was confirmed again. From the results of the preceding and the present researches, it may be concluded that the behaviors of trace metals differ with their nature.

The change in assimilation rate of micronutrients under various environmental conditions was judged by the figures. To make certain of the truth of the judgement, the analysis of variance in one-way classifications was practised. In the case of Mn-54 under various conditions of temperature, light intensity and chlorinity, the null hypothesis is accepted, since a variance ratio is less than an F value even the level of 5% significauce. However, under various conditions of pH and Na_2EDTA, it is rejected, because a variance ratio is greater than an F value even the level of 1% significance. In the case of Zn-65 under various conditions of temperature, light intensity, chlorinity and pH, the null hypothesis is accepted, since a variance ratio is less than an F value even the level of 5% significance. However, under various conditions of Na_2EDTA, it is rejected, because a variance ratio is less than an F value even the level of 1% significance. Thus the results judged by the figures were in accord with those calculated by the analysis of variance in one-way classification.

In order to solve the question whether the change of assimilation rate depends on absorption by the plant body or adsorption upon the plant surface, experiments using punched pieces of thallus killed with 5% formalin sea water were carried out in the same way. As a result, the assimilation rate was very slow and its amount was almost equivalent to that of the back ground.

The maximum amount of manganese-54 assimilation in artificial sea water, at 9–10°C, pH 8.2 and 3,000 lux, was 0.00135 μg per 50.24 mm^2 thallus piece, and that of zinc-65 assimilation in artificial sea water, at 9–10°C, pH 9 and 3,000 lux, was 0,0043 μg per 50.24 mm^2 thallus piece.

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Summary

This paper deals with a research carried out to know how much amounts of manganese-54 and zinc-65 are assimilated by *Porphyra* plants under various environmental conditions. The results obtained are as follows:

1. The main factor which affects the rate of manganese-54 and zinc-65 assimilations is the concentration of these stable isotopes themselves.

2. The manganese-54 assimilation is also affected by pH.

3. The maximum amount of manganese-54 assimilation in artificial sea water, at 9–10°C, pH 8.2 and 3,000 lux, was 0,00135 μg per 50.24 mm^2 thallus piece.

4. The maximum amount of zinc-65 assimilation in artificial sea water at 9–10°C, pH 9 and 3,000 lux, was 0,0043 μg per 50.24 mm^2 thallus piece.

5. The amount of manganese-54 or zinc-65 assimilation by *Porphyra* plants is so widely different with plant individuals or with portions of one and the same individual that the factors which were proved by the present experiments to be favorable for obtaining the maximum assimilations are not necessarily indisputable.

Reference

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2) ———., 1970: Reports on *Porphyra leucosticta* THURET. Bull. Jap. Soc. Phycol., 18 (3), 164–166.