TABLE II THE EFFECT OF CONCENTRATION OF MANGANESE ON THE GROWTH OF CHLORELLA SP. pH 8.0

Concentration	0	1 to	1 to	1 to	1 to	1 to
manganese		5,000,000	1,000,000	500,000	100,000	50,000
Dry weight mgs. av	1.4(4)*	52.9(4)	53.7(4)	48.1(4)	36.2(3)	11.9(3)

^{*} The figures in parenthesis refer to the number of cultures included in the average.

growth without added manganese at pH 7.0 may be due either to the difficulty of completely removing the manganese impurity or to the greater ionization of the manganese at this reaction.

In Table II the toxicity of manganese is also shown as the amount added is increased. It should be stated that to each culture of the above experiments was added 0.1 mg of iron and 0.04 gms of sodium citrate, and therefore soluble iron which is essential for this organism⁵ was not a limiting factor. An important point in connection with these experiments is that the alkaline limit for the growth of this species as reported by Wann and Hopkins⁶ must now be extended to higher pH values since pH 8.0 is very close to the limiting reaction reported by them. Other experiments show that, when manganese is added to manganese-free cultures which have shown no development of the organism in two weeks, growth then begins. The cells with which the cultures were inoculated were not dead but were unable to develop without manganese. I have also found that manganese will not replace iron—both are essential.

In most of the literature on manganese an explanation of its action has not been attempted. The present writer wishes to suggest that manganese functions physiologically in an indirect manner by its action on the state of oxidation of iron. In other words, manganese tends to control the ratio [Fe++]: [Fe+++] in the culture or in the cell. Experiments in vitro have shown that the reduction of ferric iron to ferrous which is brought about slowly by sodium citrate tends to be prevented by the presence of manganese. For example, a solution of ferric chloride and sodium citrate on being allowed to stand in a stoppered flask lost its original greenish-yellow color after some time. A similar solution which contained manganese did not change color. On testing them the first one showed only a slight test for ferric iron and a strong test for ferrous iron, and the second solution showed just the reverse.

Culture experiments with yeast also indicate that the reduction of the iron by the yeast organism tends to be prevented by the presence of manganese. Further, oxidation-reduction potential measurements on culture solutions containing ferric iron and sodium citrate show that when manganese is added a higher potential is developed.

On this basis it is believed that not only the necessity of manganese but its toxicity can be explained. In the first case, sufficient manganese must be present to insure the reoxidation of the iron after its reduction by the organism. In the second case, a large amount of the element either results in too high a concentration of ferric ions or prevents its reduction by the organism. Different species may be expected to vary in their relation to manganese depending on the reducing power of their cells.

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⁵ E. F. Hopkins and F. B. Wann, "Iron Requirement for Chlorella,' Bot. Gaz., 84: 407-427, 1927.

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