number of healthy plants were obtained and washed thoroughly with sterile water. They were then immersed for an instant, one at a time, in 95 per cent. alcohol followed by one minute in $HgCl_2$ solution 1:1,000 and then rinsed in several changes of sterile acid phosphate solution. From this the plants were transferred to Knop's solution plus 0.5 per cent. of dextrose. In this manner six or eight pure cultures were obtained. It is suggested that this method may overcome the difficulties mentioned by Saeger[5] in connection with the direct disinfection of the fronds.

Experiments to determine the relation of manganese to the growth of Lemna were made on solutions freed from manganese in two ways. In the first case the chemicals used in the preparation of Knop's solution were recrystallized three times from conductivity water. In the other case manganese was removed by absorption on calcium phosphate at pH 8.0 as in the work with Chlorella. This latter solution which contained dextrose was acidified and its calcium content increased by adding to it a sterile solution of recrystallized calcium nitrate and potassium hydrogen phosphate. This procedure was resorted to because of the difficulty encountered in removing manganese from glucose by recrystallization. Known amounts of iron (1:500,000) were added to each culture from a solution prepared from recrystallized ferric alum and recrystallized sodium citrate. For plus manganese cultures manganese sulphate was added to give a concentration of Mn of 1:5,000,000.

In the first experiments without dextrose the controls showed no development of fronds and no root formation while with manganese 1:500,000 there was good development of green fronds and healthy root growth. In fact the fronds planted in the control flasks finally became chlorotic and died. In table I the results of such an experiment are given.

TABLE I

THE NECESSITY OF MANGANESE FOR THE GROWTH OF *Lemna minor* (WITHOUT DEXTROSE)

	Green fronds (54 days)
+ Mn	127 (5 cultures)
− Mn	9 (5 cultures)

In the later experiments the presence of dextrose (0.5 per cent.) made it possible to obtain results in a shorter time. The summarized data from one of these experiments is given in Table II.

The necessity of manganese for Lemna is clearly shown in the above data both with and without iron being added. In the latter experiment after the second transfer the plants without manganese showed no development at all and finally died. The plants with both iron and manganese were very healthy in appearance with large deep green fronds. Those with manganese and without iron were smaller and chlorotic. Since the dry weights of the plants agree closely with the counts of the number of fronds these data are not given here. An interesting occurrence is the development of definite symptoms of manganese deficiency consisting of depressed root formation and of light brownish to white necrotic areas on the fronds in the case of those cultures without manganese.

TABLE II

THE NECESSITY OF MANGANESE FOR THE GROWTH OF *Lemna minor* (WITH DEXTROSE)

Treatment	Per cent. increase in no. of fronds in 21 days	Per cent. increase in 15 days after transferring one plant to another set of culture solutions
− Fe − Mn	222 (5)*	0 (5)
− Fe + Mn	769 (5)	810 (5)
+ Fe − Mn	90 (5)	0 (5)
+ Fe + Mn	843 (5)	1160 (5)

* The figures in parenthesis refer to the number of cultures in each case.

I believe that these experiments which have been repeated a number of times with the same result show conclusively that manganese is an essential element for Lemna and suggest that it is undoubtedly necessary for the growth of all green plants.

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[5] Albert Saeger, "A Method of Obtaining Pure Cultures of *Spirodella polyrhiza*," *Bull. Torrey Bot. Club* 57: 117–122, 1930.

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