# MINERAL REQUIREMENTS OF *LEMNA MINOR*

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## Introduction

The use of *Lemna minor* Link as a test organism for the micronutrient requirements of the higher plants was undertaken by the writer as a check on similar data with the fungus *Aspergillus niger* v. Tiegh. It was possible to demonstrate that the macro- and micronutrient needs of both were similar, except that Lemna also required calcium and boron (**1**). The mineral and acidity requirements of Lemna were identical with those of other higher green plants.

In Lemna cultures with *fixed, minimal quantities of salts,* the ratio of yield to total macronutrient salts was 0.9, a value termed "efficiency of salt utilization" by the writer. This corresponded to a total of 1,113 mg. of macronutrients per gram of yield, each salt of the nutrient solution having been adjusted to be at the experimental minimum for maximum yield. Addition of 0.5 per cent. sucrose, nevertheless, increased the ratio to 2.3 though the quantity of salts remained unaltered. The macronutrient salt requirement was thereby decreased to 434 mg. per gram of yield. Aspergillus, however, reaches an efficiency of 9.6, which is equivalent to 106 mg. salts per gram. The lower efficiency of Lemna may be intrinsic but might be only a reflection of secondary requirements, such as buffering of the nutrient solution and insufficient energy (low photosynthesis) for efficient absorption.

An effort has been made to obtain an improvement in the results previously reported. Numerical data on the effects of copper are also included.

## Methods

*Lemna minor* Link was grown on 50 ml. of nutrient solution in 200-ml. Erlenmeyer flasks under aseptic conditions. Illumination was continuous at 500 foot-candles supplied by 3,500° C. white fluorescent lamps. The temperature was 25° ± C. Photosynthesis is the limiting factor for growth under these conditions (**1**). Only quartzware was used. Nutrient solutions were prepared with water twice redistilled in quartz and standard reagent chemicals having a purity sufficient for chemical analyses. Micronutrients, however, were almost spectroscopically pure chlorides. The sucrose contained 0.0011 per cent. ash. Each experiment was started with a single plant (having two fronds) per flask. The roots usually varied from 2 to 5 mm. in length. Duration of growth was 13, 14, or 27 days with continuous illumination. No change was made in the solutions of the individual flasks for the duration of the experiments. The plants were dried at 103–5° C. for 3 to 4 hours before weighing.

## Results

### Effects of micronutrient deficiencies

The effects of micronutrient deficiencies on yields are shown in table I. Selected reagent chemicals were used in various combinations. The solutions used were adjusted to minimum salt content for maximum yield. Sharp diminutions in growth were readily obtained on omission of iron, manganese, molybdenum, and boron. Results with zinc, copper, and gallium were less clear cut.

TABLE I

Effects of micronutrient deficiencies on growth of Lemna with selected reagent chemicals

| Element omitted | Selected reagent chemicals* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 days pH = 4.17 | | | 13 days pH = 4.06 | | | 13 days pH = 4.10 | | | 27 days pH = 4.30 | | |
| | Yield | Relative yield | Appearance† | Yield | Relative yield | Appearance† | Yield | Relative yield | Appearance† | Yield | Relative yield | Appearance† |
| | *mg.* | % | | *mg.* | % | | *mg.* | % | | *mg.* | % | |
| None | 13.2 | 100.0 | 4,M | 15.6 | 100.0 | 4,M | 14.0 | 100.0 | 4,M | 60.1 | 100.0 | 4,M |
| Fe | 2.5 | 18.9 | 1,S | 3.3 | 21.2 | 2,S | 2.1 | 15.0 | 1,T | 3.9 | 6.5 | 1,S |
| Zn | 12.1 | 91.7 | 4,M | 13.3 | 85.3 | 4,M | 11.7 | 83.6 | 4,M | 44.7 | 74.4 | 2,M |
| Cu | 10.6 | 80.3 | 4,M | 13.9 | 89.1 | 4,M | 7.8 | 55.7 | 4,M | 34.8 | 57.9 | 4,M |
| Mn | 3.1 | 23.5 | 2,T | 2.9 | 18.6 | 2,S | 1.7 | 12.1 | 1,T | 2.5 | 4.2 | 1,T |
| Mo | 4.3 | 32.6 | 2,M | 3.2 | 20.5 | 2,S | 3.1 | 22.1 | 2,S | 40.5 | 67.4 | 4,M |
| Ga | 12.2 | 92.4 | 4,M | 15.4 | 98.7 | 4,M | 8.9 | 63.7 | 4,M | 55.7 | 92.7 | 3,M |
| B | 5.4 | 40.9 | 5,T | 9.2 | 59.0 | 5,S | 5.2 | 37.1 | 5,S | 43.4 | 72.2 | 5,S |

* First 3 experiments: Water, 1 liter; $KNO_3$, 50; $KH_2PO_4$, 50; $MgSO_4 \cdot 7H_2O$, 30; Fe, 0.20; Zn, 0.05; Cu, 0.01; Mn, 0.05; Mo, 0.02; Ga, 0.02; B, 0.02; Ca, 4.0 mg. Last experiment: Water, 1 liter; $KNO_3$, 300; KCl, 50; $KH_2PO_4$, 70; $MgSO_4 \cdot 7H_2O$, 45; Fe, 0.40; Zn, 0.10; Cu, 0.02; Mn, 0.20; Mo, 0.06; Ga, 0.04; B. 0.06; Ca, 8.0 mg.

† Color is rated from 0 to 5 (normal green); and size of fronds designated as L = large, M = medium, S = small, T = tiny.

Symptoms of micronutrient deficiency followed a parallel course. Normal plants had large green fronds with flat, rounded apices and long, light green roots. With insufficient iron, the plants remained adherent; the fronds were tiny with distorted apices and displayed a diffuse chlorosis, usually progressing from apex to base of frond. Roots were short, light green, and sometimes distorted. Deficiencies in zinc, gallium, and particularly copper were sporadically accompanied by a general diffuse chlorosis of fronds very similar to that shown in absence of iron.

In the absence of manganese the plants remained adherent and had very small fronds with sharply defined white margins and elongated blotches giving a green "trident effect" on all but the base, and occasional red spots. Roots were short, twisted, and dark green. Omission of molybdenum also resulted in adherent plants with small fronds, many of which had acute up-

turned apices. Chlorosis was diffuse with white blotches merging into green and often accompanied by narrow marginal streaks of dark green. The roots were long, twisted, and very dark green. Non-addition of boron led to plants markedly adherent, having tiny and very dark green fronds with occasional acute apices and red spots. Roots were very short, dark green, and kinky.

### Nutrient solution purification

Table II contains experiments illustrating the effects of nutrient solution purification (**1**) on micronutrient deficiencies. Improved results were obtained with iron, zinc, and copper as compared to unpurified solutions composed of selected reagents. Results with gallium were slightly better when calcium phosphate was used instead of calcium carbonate. Details of this process will be found in the publication cited above. The removal to a greater or lesser extent of residual traces of iron, zinc, copper, etc., impurities from the reagent chemicals is accomplished by neutralizing the complete nutrient solution with an excess of $CaCO_3$, $CaHPO_4$, CaO, or the corresponding magnesium salts, in the presence of excess phosphate. The sediment formed is an important factor in the treatment, since it serves also as an adsorbent for micronutrient impurities. The clear solution is used and the sludge rejected.

These solutions contained: water, 1 liter; $KNO_3$, 400; $K_2HPO_4$, 440; $MgSO_4 \cdot 7H_2O$, 350; $CaCO_3$, 250; and $CaHPO_4 \cdot 2H_2O$, 750 mg. After heating in a steamer for 20 minutes, the solutions were filtered cold, and 2.0–2.5 ml. of N/1 hydrochloric acid added. Micronutrients per liter then added were: Fe, 0.40; Zn, 0.04; Cu, 0.01; Mn, 0.075; Mo, 0.02; Ga, 0.02; B, 0.04 mg.

### Efficiency of salt utilization

Previous work (**1**) had indicated that addition of sucrose to a Lemna culture resulted in an increase in growth even though each nutrient component was presumably at the experimental minimum for maximum normal growth. The optimum macronutrient mixture of 32.5 mg. salts that was essential for a yield of 29.2 mg. without sucrose sufficed for the production of 74.9 mg. on addition of 0.5 per cent. sucrose. This is equivalent to a decrease in salt requirements for growth with an increase in level of carbon nutrition and is, therefore, referred to as an increase in "efficiency of salt utilization." The corresponding ratios 29.2/32.5, or 0.9; and 74.9/32.5, or 2.3 are reciprocals of salts per unit of yield.

Salt utilization ratios with Lemna have been given further attention in table III. The data summarized should suffice to demonstrate each of several points. Normal growth is dependent on quantity ratios of nutrients irrespective to total quantity. Available sugar is closely correlated with total quantity of salts required for growth. Increased salt utilization efficiency with increased carbon nutrition takes place with chlorotic as well as normal plants.

An intensity of 500 foot-candles is known to be sub-optimal for growth

## TABLE II

EFFECT OF NUTRIENT SOLUTION PURIFICATION ON RESPONSES OF LEMNA TO MICRONUTRIENT DEFICIENCIES (14 DAYS)

| Element omitted | $CaCO_3$ purification | | | | | | $CaHPO_4 \cdot 2H_2O$ purification | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sucrose 0.1% pH = 4.01 | | | No sucrose pH = 4.05 | | | Sucrose 0.1% pH = 4.32 | | | No sucrose pH = 3.97 | | | Filtered through 5 cm. $CaHPO_4 \cdot 2H_2O$, no sucrose pH = 4.01 | | |
| | Yield | Relative yield | Appearance* | Yield | Relative yield | Appearance* | Yield | Relative yield | Appearance* | Yield | Relative yield | Appearance* | Yield | Relative yield | Appearance* |
| | *mg.* | % | | *mg.* | % | | *mg.* | % | | *mg.* | % | | *mg.* | % | |
| None | 44.7 | 100.0 | 4, M | 15.8 | 100.0 | 4, L | 29.7 | 100.0 | 4, L | 22.0 | 100.0 | 4, L | 16.1 | 100.0 | 4, L |
| Fe | 1.8 | 4.0 | 1, S | 0.6 | 3.8 | 1, T | 1.5 | 5.1 | 1, S | 1.2 | 5.5 | 2, S | 1.2 | 7.4 | 2, S |
| Zn | 21.2 | 47.4 | 4, L | 7.6 | 48.1 | 4, M | 22.1 | 74.4 | 4, L | 11.4 | 51.8 | 4, L | 10.2 | 63.0 | 4, L |
| Cu | 16.0 | 35.8 | 4, L | 10.6 | 67.1 | 4, M | 24.3 | 81.8 | 3, L | 12.4 | 56.4 | 4, L | 12.6 | 77.8 | 4, L |
| Mn | 3.3 | 7.4 | 2, S | 1.0 | 6.3 | 1, T | 5.3 | 17.8 | 2, S | 1.3 | 5.9 | 2, T | 0.8 | 4.9 | 1, S |
| Mo | 27.2 | 60.9 | 4, L | 6.1 | 38.6 | 4, M | 10.1 | 34.0 | 4, L | 7.5 | 34.1 | 4, T | 14.7 | 90.7 | 4, L |
| Ga | 42.1 | 94.2 | 3, L | 15.2 | 96.2 | 3, M | 25.9 | 87.2 | 4, L | 16.6 | 75.5 | 4, L | 13.1 | 80.9 | 4, L |
| B | 16.5 | 36.9 | 5, S | 12.1 | 76.6 | 4, M | 27.2 | 91.6 | 4, L | 12.0 | 54.5 | 5, S | 12.9 | 79.6 | 4, L |

* See footnote, table I.

of Lemna (1) which requires 1500 foot-candles. Air enriched with $CO_2$ is ineffective in increasing yields except in experiments of over 2 weeks duration when the leaf surface increases to the point where air diffusion through the cotton plugs of the flasks becomes inadequate. Light is the limiting factor for growth during the early stages of the experiment and carbon dioxide during the later stages. Sucrose was effective at all stages in increasing growth, whereas manipulation of macro- and micronutrients was ineffective, as were also addition of trace elements or vitamins. The action of

## TABLE III

EFFICIENCIES OF SALT UTILIZATION (YIELD/TOTAL AVAILABLE MACRONUTRIENT SALTS) OF *Lemna minor* GROWN FOR 27 DAYS AT 25° C. WITH 500 FOOT-CANDLES OF FLUORESCENT LIGHT

| NUMBER | MILLIGRAMS PER CULTURE OF 50 ML. | | | | MILLIGRAMS YIELD PER CULTURE | SUPPLEMENT | YIELD DIVIDED BY TOTAL AVAILABLE SALTS |
|---|---|---|---|---|---|---|---|
| | $KNO_3$ | $KH_2PO_4$ | $MgSO_4 \cdot 7H_2O$ | TOTAL SALT | | | |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | | |
| 1* | 15.0† | 3.5 | 2.25 | 23.25 | 61.0 | ..... | 2.6 |
| 2 | 17.5 | 10.0 | 5.0 | 32.5 | 63.2 | ..... | 1.9 |
| 3 | 7.5‡ | 3.25 | 2.0 | 15.75 | 47.6 | ..... | 3.0 |
| 4 | 7.5‡ | 3.25 | 2.0 | 15.75 | 191.0 | 0.5% sucrose | 12.1 |
| 5 | 4.5 | 3.0 | 2.0 | 9.5 | 47.8 | ..... | 5.0 |
| 6 | 4.5 | 3.0 | 2.0 | 9.5 | 60.8 | $CO_2$ | 7.2 |
| 7 | 4.5 | 3.0 | 2.0 | 9.5 | 85.6 | *A. niger* vapors | 9.0 |
| 8 | 4.5 | 3.0 | 2.0 | 9.5 | 106.4 | *A. niger* vapors | 11.2 |
| 9 | 6.0‡ | 3.25 | 2.0 | 14.25 | 40.6 | ..... | 2.9 |
| 10 | 6.0‡ | 3.25 | 2.0 | 14.25 | 83.5 | 0.1% sucrose | 5.9 |
| 11 | 6.0‡ | 3.25 | 2.0 | 14.25 | 134.1 | 0.2% sucrose | 9.4 |
| 12 | 6.0‡ | 3.25 | 2.0 | 14.25 | 173.6 | 0.3% sucrose | 12.2 |
| 13 | 6.0‡ | 3.25 | 2.0 | 14.25 | 198.6 | 0.4% sucrose | 13.9 |
| 14 | 6.0‡ | 3.25 | 2.0 | 14.25 | 222.7 | 0.5% sucrose | 15.6 |
| 15 | 6.0‡ | 3.25 | 2.0 | 14.25 | 232.5 | 0.6% sucrose | 16.3 |

* Experimentally determined to contain a minimal quantity of each salt for maximum normal growth under the specific environmental conditions employed. All other cultures were chlorotic. Micronutrient concentrations as in table I, last experiment.

† Plus 2.5 mg. KCl or $KNO_3$ per culture.

‡ Plus 3.0 mg. $Ca(NO_3)_2 \cdot 4H_2O$ per culture.

sucrose is, therefore, assumed to depend on an increase in the level of carbon nutrition of Lemna.

The minimal quantities of macronutrient salts required for maximum normal growth are shown in experiment 1. The yield was 61 mg. per culture and required 23.25 mg. of macronutrients; i.e., a ratio of 2.6. Neither an increase (#2) nor a decrease (#3) in individual or total components of the solution increased yield but did cause chlorosis. Addition of sucrose (#4) brought about a 3-fold increase in yield and a 4-fold increase in salt utilization ratio without eliminating chlorosis. Experiments 5 to 8 illustrate similar increases in salt utilization ratios with $CO_2$ and with vapors from cultures of *Aspergillus niger* v. Tiegh. The remaining experiments show the effect of increasing quantities of sucrose on yield and utilization ratios

despite the fact that nutrition was abnormal and the plants chlorotic. Sucrose caused a 6-fold increase in yield despite chlorosis.

These data would indicate, therefore, that nutrient salts required for growth of Lemna are, for reasons unknown, far in excess of the plant's actual requirements for formation of tissue. Usually 38.5 per cent. of salts (1/2.6) must be provided on the basis of anticipated yield. With sucrose, 6.1 per cent. of salts (1/16.3) sufficed. This value approaches that mentioned by PFEFFER in his treatise of plant physiology, where he points out that a minimal salt solution for the oat plant should require only about 2.5 per cent. salts per unit of yield. This computation was based on the experimental optimum for each element.

Though it has been possible to approach this value with Lemna, the problem remains of doing so without causing chlorosis. The causes of these chloroses were sought in experiments with macronutrient salts and trace elements, but unsuccessfully. The further addition of $KNO_3$ [also $Ca(NO_3)_2$ or KCl] prevented chlorosis under these conditions but only in association with marked decreases in efficiency of salt utilization.

## Discussion

Improved results with micronutrient deficiencies were obtained through selection of nutrient salts and their use in minimum quantities. Decreases in growth due to their omission from the nutrient solution were accompanied as a rule by specific symptoms in the case of iron, manganese, molybdenum, and boron. Symptoms of zinc, copper, and gallium deficiency were sporadic and consisted in uniform chloroses of older fronds.

The salt utilization studies with Lemna have been based on the use of limited quantities. That is to say, the quantities initially supplied were used at the experimental minima for maximum normal growth and were not replenished to compensate for depletion. Quantity ratios and total quantity were found to be important factors for growth under these conditions (**2**). An indefinite and variable proportion of the total salts supplied did not appear to function primarily in the elaboration of tissue by the plant. The apparent increase in effectiveness of the salt supply in maintaining growth on addition of sugar would indicate this to be so and also that maximum efficiency of salt utilization would be reached at the optimum carbon nutrition level of the plant. Hindrance of salt absorption because of low carbon nutrition level and buffer requirements may be factors in higher mineral needs. The carbon nutrition level, therefore, appears to be the determining factor for total salt requirements in the case of Lemna as well as Aspergillus (**2**).

## Summary

*Lemna minor* Link was grown in aseptic culture for 13, 14, or 27 days at 25° C. with continuous light (500 foot-candles, fluorescent lamps) using fixed, limited quantities of nutrients. The effects of deficiencies in micronutrients are described. Efficiency in salt utilization was found to be depen-

dent on the carbon nutrition level of the plant and reached a maximum value of 16.3 (yield/salt) or the equivalent of 6.1 per cent. salts for each unit of yield. Quantity and quantity ratios of nutrient salts were important factors in growth.

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