A Bioassay Using Common Duckweed To Evaluate The Release Of Available Phosphorus From Pond Sediments[1]

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ABSTRACT

Experiments were conducted to study the availability of phosphorus (P) released from pond sediments under aerobic and anaerobic conditions to common duckweed (*Lemna minor* L.). A bioassay technique using common duckweed to measure available P released from sediments was developed and the results compared to results of chemical assays. Common duckweed exposed to varying concentrations of P showed that parameters such as frond numbers, root length, frond diameter, and dry weight consistently reflected P concentrations in solution. Three sediments having relatively low, medium, and high NH_4F+ HCl-extractable P were incubated under aerobic and anaerobic conditions in a minus-P nutrient solution. Common duckweed cultured in solutions decanted from the high phosphorus sediment exhibited the greatest release of P as measured by the P content of the plants and the various growth responses. Much greater release of available P took place under anaerobic than under aerobic conditions. The levels of the chemically-measured P released from the sediments and the chemically-assayed P removed by duckweed from the incubated solutions showed no relationship to the extractable P of the sediments or the bioassay responses of the common duckweed. These findings illustrate the superiority of the bioassay over chemical methods in the evaluation of P availability. Information regarding P requirements of common duckweed was also obtained.

INTRODUCTION

The attention P has received in the past is attributed to its importance as one of the major causative factors in the eutrophication of lakes. Inorganic and biochemical reactions are responsible for a dynamic equilibrium of P exchange between the water, seston, sediment, and the various flora and fauna in a lake. Depending on specific environmental conditions, the sediment in a lake has been shown to act as either a reservoir or a trap for P (14). Common techniques for the evaluation of the P release phenomenon have generally involved the measurement of P concentrations in the supernatant waters using chemical methods (5, 8, 14) and radioisotopes (11). Radioisotope techniques, however, require highly specialized apparatus and chemical procedures often yield misleading information with regard to the biological relevance of the measured P concentrations (3, 12). Bioassay techniques such as the provisional algae assay procedure (2) are available for the analysis of biologically available P in waters but these are cumbersome, requiring rigidly defined experimental conditions and complex equipment.

In an attempt to provide a simple and straightforward method for the measurement of the release of biologically available P from freshwater sediments, a bioassay technique utilizing uptake and growth responses of common duckweed was designed. Comparisons were made between the amounts of chemically determined soluble P, the actual P uptake by common duckweed, and the release of P from sediments under aerobic and anaerobic conditions.

METHODS AND MATERIALS

Evaluation of Common Duckweed as the Bioassay Organism

A study was conducted to determine whether frond numbers, frond diameters, and root lengths of common duckweed could be used to estimate available P in solution. The experiment was conducted for a period of 4 weeks

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[1]Paper of the Journal Series, N. J. Agric. Expt. Sta., Cook College,

TABLE 1. COMPOSITION OF NUTRIENT SOLUTION USED IN GREENHOUSE EXPERIMENT.

Salt or element	Concentration
	M
$MgSO_4$	0.002
$Ca(NO_3)_2$	0.004
K_2SO_4	0.002
$(NH_4)_2SO_4$	0.005
	mg per liter
Fe as $FeSO_4$ + Na_2EDTA[a]	1.0
Zn as $ZnSO_4$	0.1
B as H_3BO_4	0.1
Cu as $CuSO_4$	0.1
Mo as Na_2MoO_4	0.01
Cl as $MnCl_2$	0.32
Mn as $MnCl_2$	0.25
P as KH_2PO_4	3.1, 0.31. 0.10, 0.031, 0.010, and 0.0031

[a] 6.7 mg/ml Na_2 EDTA was used to chelate 1 mg per liter of Fe.

during which common duckweed, previously maintained in nutrient solution for 21 days without P, were grown in each of the following concentrations of P:3.1, 0.31, 0.10, 0.031, 0.010, and 0.0031 mg per liter. Each treatment was replicated twice. The cultures were maintained in a greenhouse under late spring light and temperature conditions. A nutrient-flow system was used to maintain the assigned levels of P. The composition of the nutrient solution used is presented in Table 1.

After 4 weeks of growth, the fronds were counted, and frond diameters and root lengths measured. Dry weights were obtained and the P contents of the plants were determined using the method of Murphy and Riley (9) after oxidizing the tissue with perchloric and nitric acid.

Characterization of Sediments

Bottom sediments were sampled from man-made impoundments at Rutgers University Soils and Crops Research Center, Adelphia, New Jersey, on 30 January 1973. The samples were sieved to remove debris larger than 2 mm in size, and then dry matter, organic matter, and adsorbed P were determined. Adsorbed P was measured by extracting fresh sediment equivalent to 1 g dry weight with 25 ml of 0.03 *N* NH_4F in 0.025 *N* HCl (1). The modified method of Murphy and Riley (9) was used to measure the P in these extracts and such P will be hereafter referred to as Bray-extractable P.

Based on the results of the Bray-extractable P analyses, three sediments were chosen for the study which contained relatively low, (L) medium (M), and high (H) levels of adsorbed P. Total P was determined on these sediments by digesting with a mixture of 20% $HClO_4$ and 80% HNO_3 and measuring P by the vanadomolybdophosphoric yellow technique (7).

Aerobic P Release and Its Availability to Common Duckweed

Triplicate subsamples equivalent to 50 g of dried material of each of the L, M, and H sediments were placed into wide-mouth, 3.78-liter glass jars. One liter of a tenfold dilution of the minus-P nutrient solution described in Table 1 was added to all the jars as well as to three jars containing no sediment; these were designated as blanks. The design therefore included three levels of P and one blank as treatments with three replications of each.

The pH was measured and each jar was then covered with plastic film. They were incubated at 20 C in complete darkness for 1 week. Aeration was maintained in each jar throughout this period. Following incubation, the dissolved oxygen (DO), temperature, and pH were measured, taking care not to disturb the sediments. Unfiltered aliquots of the incubated solutions were acid-digested, and, using the method of Murphy and Riley (9), the total P in the solutions was determined. Soluble P was determined by the method of Stephens (13) on aliquots of filtered solution. The solution from each incubation jar was drawn off, the volume measured and then transferred into a similar 3.78-liter jar which was to contain the common duckweed cultures. These were therefore designated as "culture jars."

Fresh, dilute minus-P solution was added to all the incubation jars and the pH was adjusted to 6.7 which was determined to be the value at the onset of incubations. The sediments were again allowed to incubate for 1 week and the whole cycle of operations was performed a total of three times at weekly intervals.

After the incubated solutions were transferred into culture jars, the concentrations of all the salts with the exception of KH_2PO_4 were raised to the levels of the full-strength solution in Table 1. All solutions were adjusted to a pH of 6.7, and 25 randomly selected common duckweed fronds from a 5 week-old culture maintained in the absence of P were inoculated into each jar. The cultures were covered with transparent plastic film and supplied with 3867 lux of continuous mixed fluorescent and incandescent light. Temperatures were maintained at 20 C and continuous aeration was provided. The plants were allowed to respond for 1 week, during which the cultures were periodically checked.

At the end of 1 week, the fronds were counted and aliquots of the solutions were analyzed for soluble and total residual P. The residual solutions were discarded and replaced with fresh solutions from the 2nd week's incubation, into which the same common duckweed plants were reinoculated.The plants were allowed to respond again for 1 week. A total of three such applications of incubated solutions were made to the common duckweed cultures.

At the termination of the last 7-day culture period, a final frond count was taken and the root lengths and frond diameters for each culture were determined. Total P and dry weights were determined on tissues dried at 100 C for 24 hrs. Phosphorus uptake was calculated from these values (hereafter referred to as P uptake) and also by taking the difference between the soluble P applied to the plants and the soluble P left in solution after the culture periods (the difference is hereafter referred to as $P_{sa} - P_{sr}$). Phosphorus uptake is the bioassay estimate of available P incorporated into common duckweed tissue and P_{sa}-P_{sr} is the measure

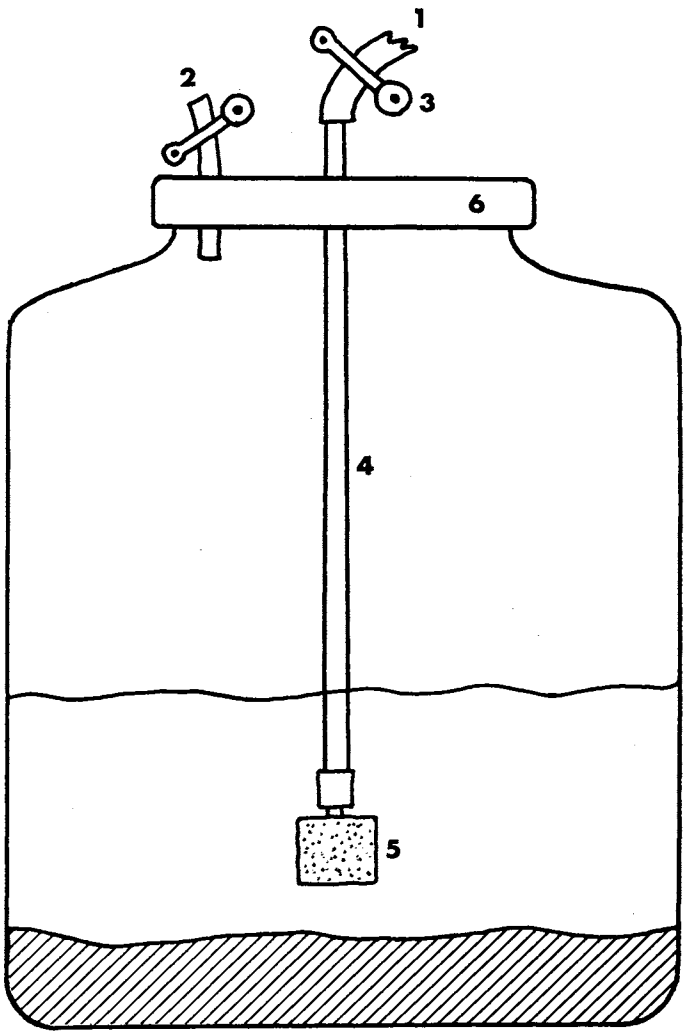

Figure 1. Anaerobic incubation jar: 1. Argon gas input (plastic tubing); 2. Gas exhaust; 3. Screw clamp; 4. Glass argon delivery tube; 5. Aeration stone; 6. Screw lid.

of soluble P removed from solution as determined chemically.

Anaerobic P Release and its Availability to Common Duckweed

Twelve 3.78 liter jars were prepared containing sediment and minus-P solution using the same experimental procedure and design as in the aerobic study. Anoxic conditions were attained by constructing an airtight assembly as shown in Figure 1 for each jar and purging the solutions for 1.5 hr with argon. The jars were then sealed and allowed in incubate. After 1 week of incubation, DO, temperature, and pH were determined. Aliquots of water were removed and later analyzed for soluble and total P. From this point on, the procedure was identical to the aerobic study with the exceptions that the second incubation and culture periods were 2 weeks long and the third, 1 week long and that these incubations were maintained anoxic.

Response of Common Duckweed to Fixed Amounts of P

Based on data obtained from chemical determinations, the average total amount of soluble P released from each anaerobic treatment was calculated. A range of soluble P levels was then chosen which encompassed the amounts released from the sediments. This range included 0.00, 0.01, 0.05, and 0.10 mg P as KH_2PO_4 incorporated into the nutrient solution specified in Table 1. Each of these amounts was made available to common duckweed in three equal applications distributed over a period of 4 weeks using the same schedule as for the cultures of the anaerobic study. The residual P in the solutions and the plant responses were evaluated in the same manner.

RESULTS AND DISCUSSION

Evaluation of Common Duckweed as the Bioassay Organism

Total frond numbers, frond diameters, dry weights, root lengths, and P content of common duckweed grown at six levels of P in the greenhouse are presented in Table 2. Growth as measured by the number of fronds and dry weights increased at P concentrations greater than 0.031 mg per liter. The 0.031 mg per liter P level represents a critical concentration above which growth was most dramatic. This indicates that the level of P in common duckweed tissues above which rapid growth can take place is near 0.15%. The minimum concentration of P associated with maximum growth, otherwise referred to as the "critical value" by Gerloff and Krombholz (6) was found to be 0.65%. The "critical value" found in this study was found to be lower, somewhere between .15% and .22%. Root lengths decreased with increasing P in solution from 45.8 mm to 19.5 mm at the 0.0031 and 3.1 mg per liter

TABLE 2. TOTAL FROND NUMBER, DIAMETER, DRY WEIGHT, ROOT LENGTH AND P CONTENT OF COMMON DUCKWEED AT VARIOUS CONCENTRATIONS OF P IN NUTRIENT SOLUTIONS[a].

Concentration of P (mg per liter)	Total no. of fronds	Frond diam. (mm)	Dry weight (g)	Root length (mm)	Tissue P (%)
3.1	4753 a	4.21 a	0.9030 a	19.5 d	1.90 a
0.31	4851 a	4.11 a	0.6978 b	29.8 cd	0.64 b
0.10	1262 b	4.37 a	0.2710 c	51.9 a	0.22 c
0.031	405 c	3.44 b	0.0797 d	48.8 ab	0.15 d
0.010	158 c	2.64 c	0.0398 d	38.4 bc	0.12 d
0.0031	124 c	2.42 c	0.0349 d	45.8 ab	0.04 e

[a] Means within each column followed by the same letters are not significantly different at the 0.05 level as determined by Duncan's Multiple Range Test.

TABLE 3. CHARACTERISTICS OF THREE SEDIMENTS USED IN AEROBIC AND ANAEROBIC INCUBATION.

Parameters	Sediments[a] L	M	H
Solids (%)	30.1	15.1	21.6
Bray-extractable P			
Fresh sediment (ppm)	35.0	122.5	270.0
Dried sediment (ppm)	13.8	65.0	115.0
Total P (%)	0.55	0.25	0.17

[a] L, M and H are low, medium and high phosphorus sediments respectively.

P concentrations. These observations are consistent with those reported by Pirson and Gollner (10). The findings of this first study indicated that easily measurable growth responses of common duckweed were found to reflect increasing concentrations of P in nutrient solutions.

Characterization of Sediments

Some characteristics of the three sediments used in the incubations are presented in Table 3. The treatment designations of L, M, and H represented 35.0, 122.5, and 270.0 ppm Bray-extractable P in the fresh sediment. Oven drying of the sediments had the effect of lowering the extractable P levels. Therefore, sediments were kept wet from time of collection until extraction. The total P data

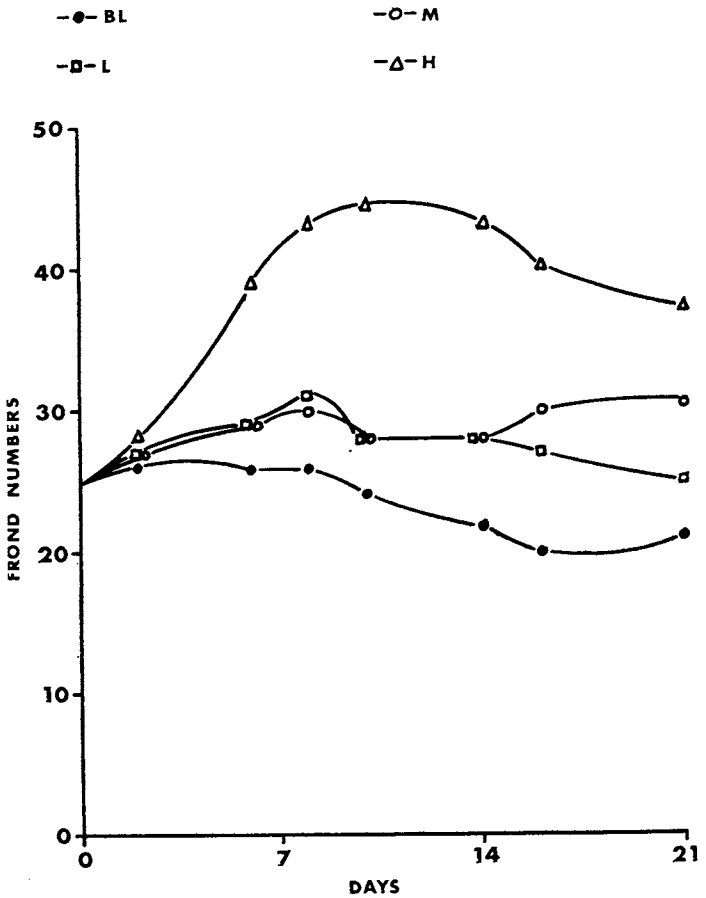

Figure 2. Growth curves of common duckweed in response to P released from the three pond sediments incubated under aerobic conditions.

showed an inverse relationship with the Bray-extractable P data.

Growth Responses of Common Duckweed to Aerobic Release of P

At the conclusion of the experiment, greater numbers of fronds were found in the H treatments than in the M, L, and blank (Bl) treatments as shown in Figure 2[2]. A count of 21 fronds in the blank was less than 31 and 37 fronds in the M and H treatments, respectively. The average dry weight of the tissue in the H treatment was greater than in the other treatments and the frond diameter of 2.8 mm in this treatment was also greater than the others. The root lengths were found to increase from 23.4 mm to 31.1 mm to 46.5 mm in treatments Bl, M, and H, respectively. The root lengths in the L treatment were not statistically different from the blanks. These trends can be seen in Figure 3.

These findings indicate that greater amounts of available P were released from the sediment having the highest level of Bray-extractable P.

Growth Responses of Common Duckweed to Anaerobic Release of P.

The growth responses of common duckweed to the P released during anaerobic conditions from the three sediments followed similar trends but were greater in magnitude that the responses observed in the aerobic study. Figure 4 shows an average of 196 fronds produced in H treatment which is greater than the numbers produced in the other treatments. Although frond counts did not show differences between the Bl, L, and M treatments, the dry weights increased from 8.9 mg to 13.4 mg to 66.0 mg in the L, M, and H treatments, respectively. Root length and frond diameter showed increases between the Bl treatment, and the L and M treatments, and the H treatment. These trends are illustrated in Figure 3. As in the aerobic study, the results indicate that readily measurable growth responses reflected the increasing concentrations of Bray-extratable P in the sediments.

Although statistical analysis could not be used to compare the various responses of the two experiments because of an additional week of incubation in the anaerobic experiment, it is readily apparent from Figures 2, 3, and 4 that growth was always greater, when comparing the same sediments, in the anaerobic experiment. This implies that a greater release of available P occurred under anaerobic conditions.

Growth Responses of Common Duckweed to Fixed Levels of P

A range of soluble P levels comparable to the range of P levels released in the anaerobic incubations was applied to common duckweed in three equal increments over a 4-week period. Increases from 18 to 26 fronds at the 0.00 and 0.01 mg P treatment to 94 at the 0.05 mg P

[2]Significance determined at the 0.05 level according to Duncan's Multiple Range Test.

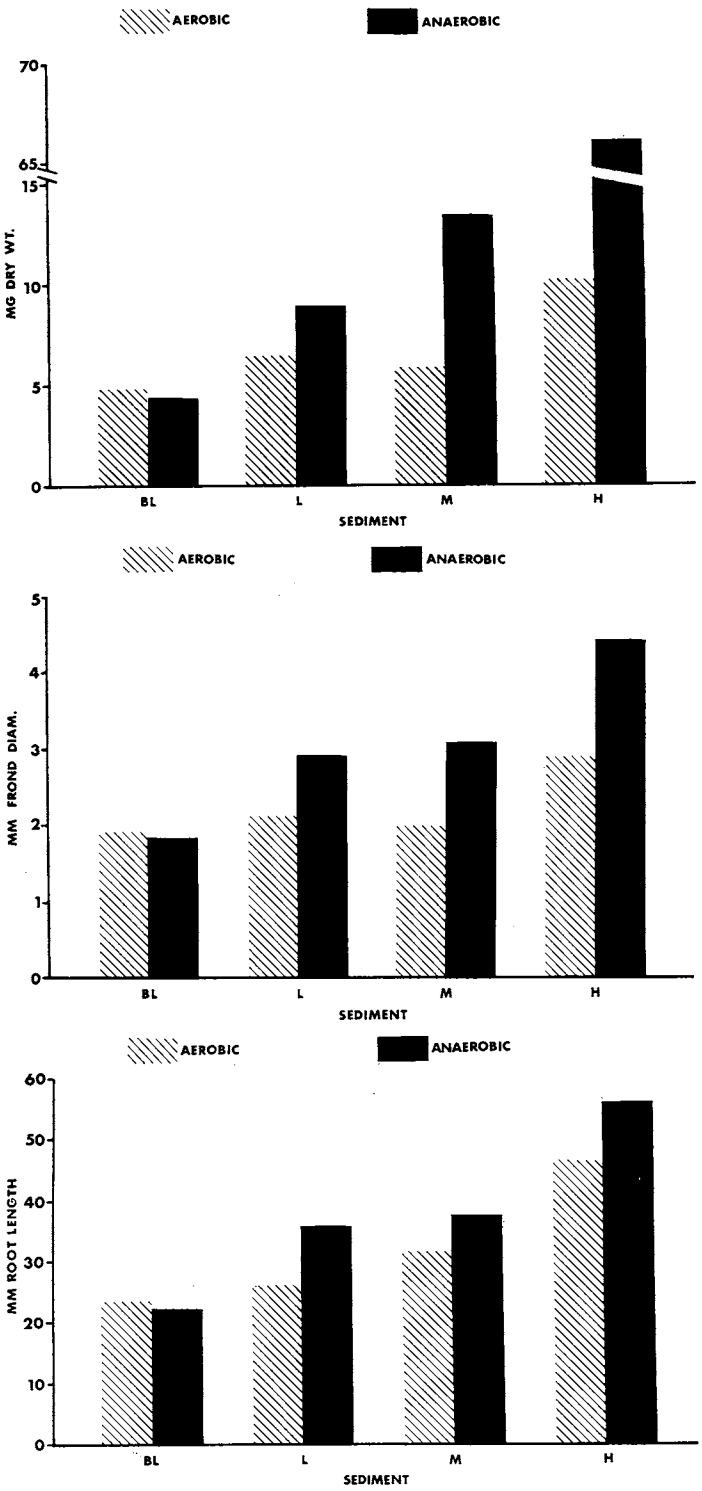

Figure 3. Oven-dried weight (A), frond diameter (B), and root length (C) responses of common duckweed to P released from the three pond sediments incubated under aerobic and anaerobic conditions.

treatment and to 396 at the 0.10 mg P treatment were found after 4 weeks (see Figure 5). Table 4 shows that the same trend is indicated by the dry weights, root lengths, and frond diameters. These data indicate that 0.01 mg P applied as KH_2PO_4 over a 4-week period was not sufficient to promote any measurable growth in common duckweed

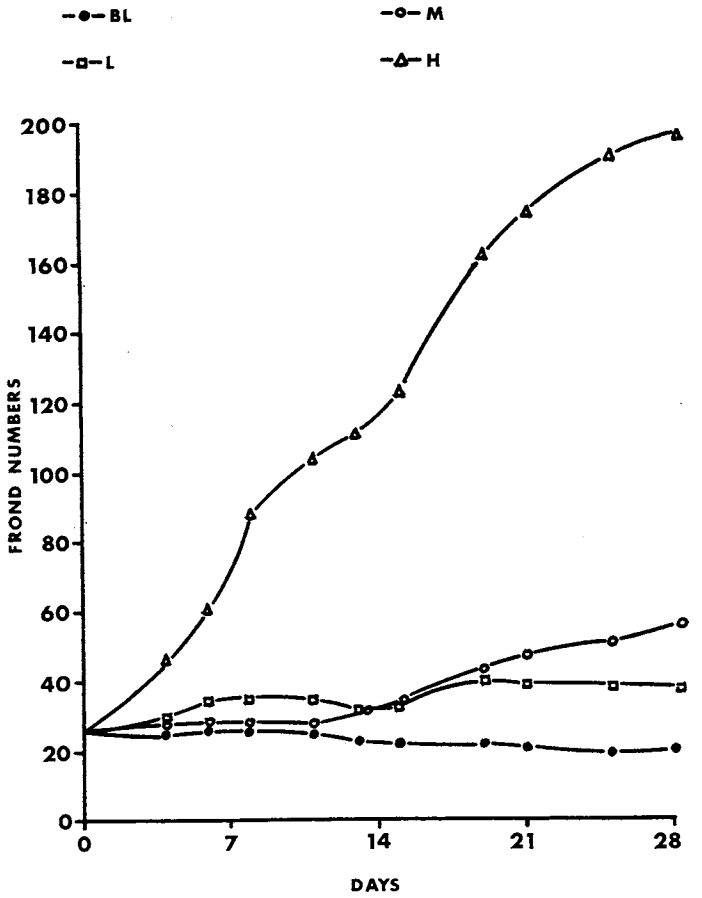

Figure 4. Growth curves of common duckweed in response to P released from the three pond sediments incubated under anaerobic conditions.

and that a tremendous increase in growth was stimulated by increasing the P from 0.05 to 0.10 mg. With these amounts of P in solution, the plant tissues were found to contain 0.036 and 0.053% P which indicates that the minimum concentration of P in the tissues necessary for growth to occur lies in this range.

The increase in root lengths as the total amount of P applied was increased from 0.00 to 0.10 mg P is attributed to the fact that the available P levels were just above those required to promote growth and therefore stimulate root elongation. This same phenomenon was responsible for the root elongation in the aerobic and anaerobic experiments. These and the findings of the preliminary experiment indicate that as the available P is increased from levels needed for growth stimulation, root elongation occurs until higher P levels serve to reduce root length.

TABLE 4. RESPONSE OF COMMON DUCKWEED TO FOUR LEVELS OF P.

Parameter	Concentration of P (mg)[a]			
	0.00	0.01	0.05	0.10
Dry weight (mg)	2.3 a	3.0 a	11.3 b	54.4 c
Root length (mm)	13.8 a	14.2 a	19.9 b	24.1 c
Frond diameter (mm)	1.8 a	1.9 a	2.7 b	3.4 c

[a] Means in each horizontal row followed by the same letters are not significantly different at the 0.05 level as determined by Duncan's Multiple Range Test. Each value is the average of three replications.

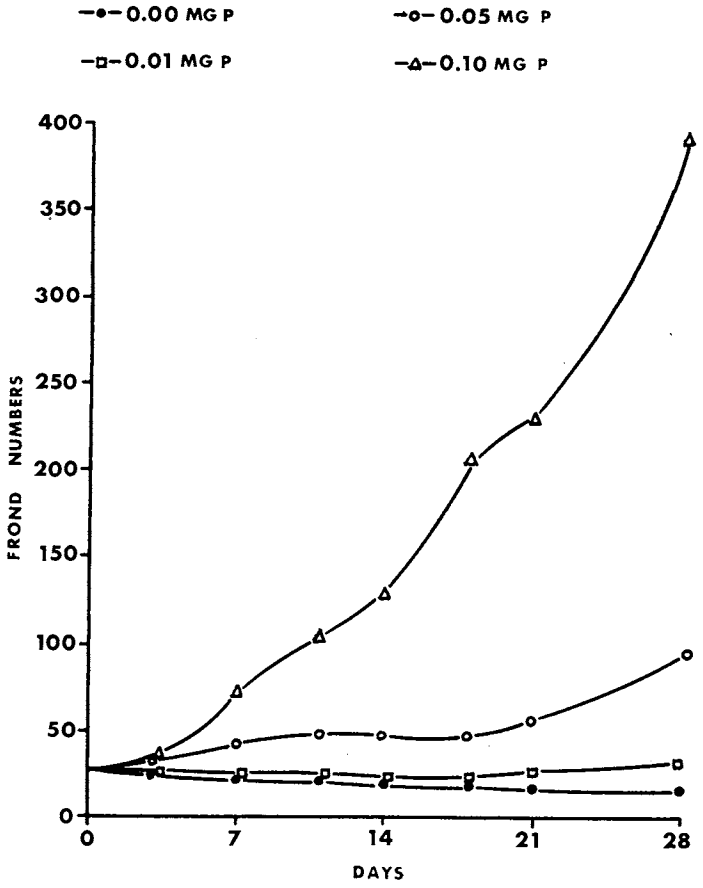

Figure 5. Growth curves of common duckweed in response to four levels of P applied over a 4-week period.

PHOSPHORUS ANALYSES

Figure 6 and 7 show that chemical analyses of the total P released from the sediments were not useful in predicting the amount of P available to the common duckweed (presented as P uptake). A relationship between the total

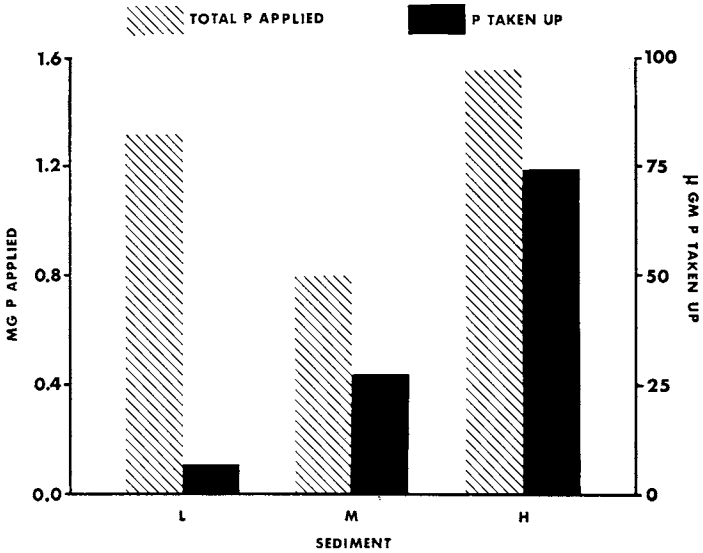

Figure 6. Total P applied to common duckweed from three periods of aerobic incubation of the three sediments and its uptake by the plants.

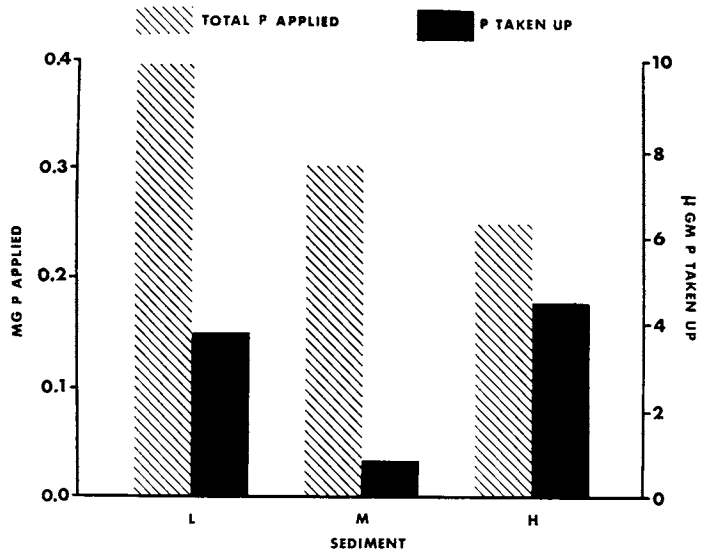

Figure 7. Total P applied to common duckweed from three periods of anaerobic incubation of the three sediments and its uptake by the plants.

P applied (P released from sediments as determined by chemical means) and the relative levels of Bray-extractable P was not found in either the aerobic or anaerobic experiments.

Figures 8 and 9 illustrate that there is no relationship between soluble P applied (P released from sediments), the P taken up, and the Bray-extractable P of the sediments. The chemically estimated soluble P was not useful in predicting the P available to the plants. This is especially evident in the anaerobic experiment where a definite increasing trend was observed in growth responses and P uptake as the Bray-extractable P in the sediments was increased, while the chemically measured soluble P applied did not follow such a trend.

The P uptake as measured chemically $(P_{sa}\text{-}P_{sr})^3$ was

$^3P_{sa}$ = soluble applied; P_{sr} = soluble P recovered after exposure to common duckweed.

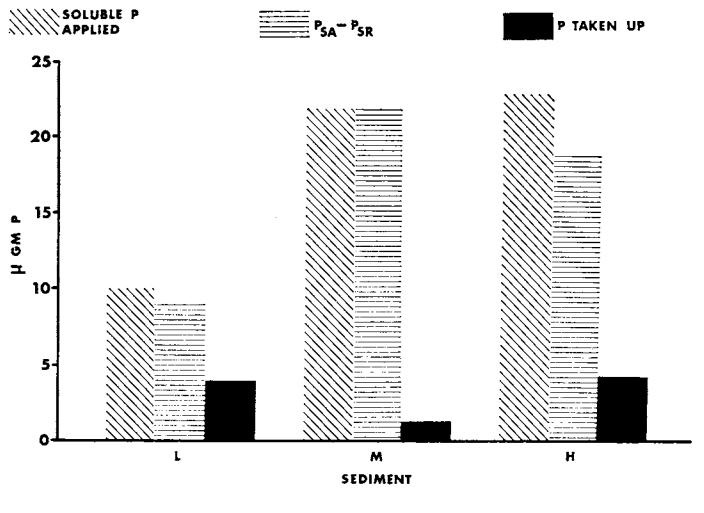

Figure 8. Soluble P applied, P_{sa}-P_{sr}, and P incorporated into common duckweed growing in solutions from aerobic incubation of the three sediments.

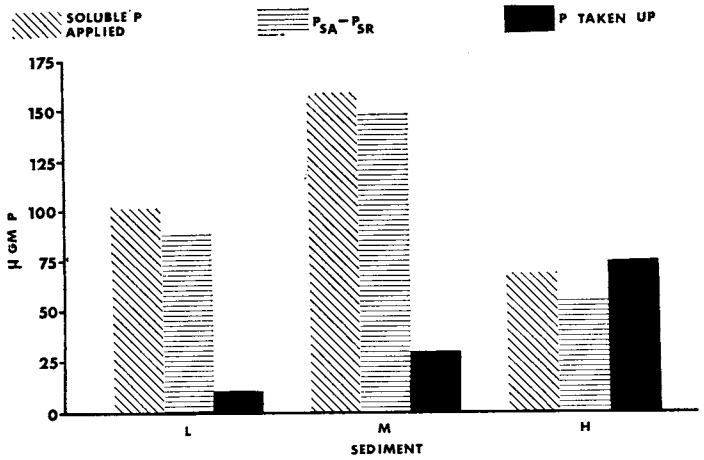

Figure 9. Soluble P applied, P_{sa}-P_{sr} and P incorporated into common duckweed growing in solutions from anaerobic incubation of the three sediments.

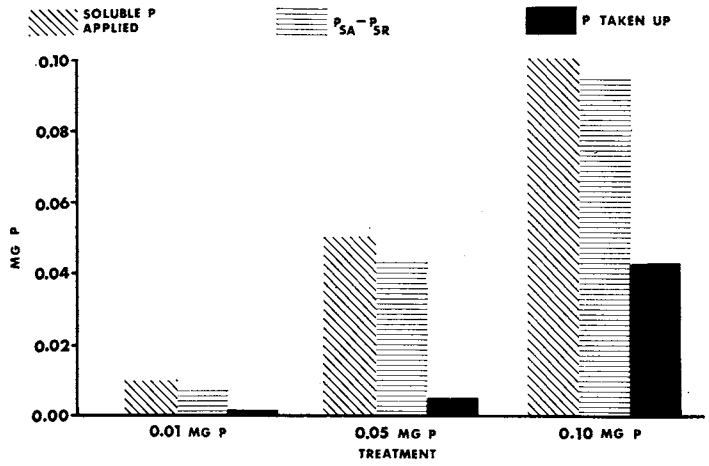

Figure 10. Soluble P applied, P_{sa}-P_{sr} and P incorporated in common duckweed growing in solutions to which 0.01, 0.05, and 0.10 mg P were added over a 4-week period.

found to be an overestimation of the values obtained by tissue analysis (P taken up, Figures 8, 9, and 10). Treatment H of the anaerobic experiment was an exception to this observation.

Such observations support the finding of Rigler (11, 12) and Chamberlain and Shapiro (3) which showed that the chemical analyses for soluble P were generally an overestimate of the true values for available P and were highly dependent on the species of P compounds present in solution. Further support for this observation can be found in Figure 11. Here, a comparison of the P applied as KH_2PO_4 and as P-containing compounds released anaerobically from the three sediments indicates that KH_2PO_4 was a more readily available form than the P from the L and M sediments. The P released from sediment H, however, was found to be nearly three times more available to the plants than the equivalent amount of P supplied as KH_2PO_4.

Such findings indicate that soluble and total P data obtained by chemical methods have possibly been misinterpreted in past studies when predicting biological phenomena based on such data. This could have been

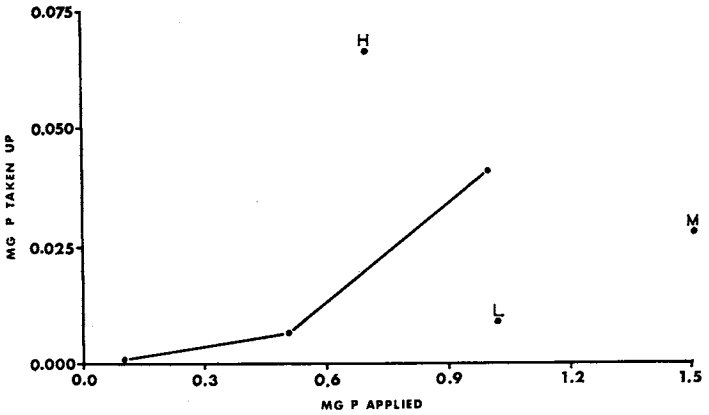

Figure 11. Comparison of P uptake by common duckweed from solutions containing 0.01, 0.05, and 0.10 mg P to the uptake of P applied to common duckweed from solutions of anaerobic incubation.

possible especially when levels of soluble P measured with chemical reagents have been equated with the amounts biologically available (3, 12). The bioassay technique developed in this study eliminates the risk of making such misleading correlations and provides a simple and direct method to estimate available P to aquatic plants.

These studies have also served to illustrate that common duckweed can be utilized as an effective biological assay organism for P studies. This plant produces readily quantifiable responses in a reasonable time, utilizing simple procedures. Its applicability to other nutrient assays would seem worthwhile exploring.

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