THE EFFECTS OF CALCIUM SALTS ON THE GROWTH AND UPTAKE OF PHOSPHORUS BY CERATOPHYLLUM DEMERSUM

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ABSTRACT

THE EFFECTS OF CALCIUM SALTS ON THE GROWTH AND UPTAKE OF PHOSPHORUS BY CERATOPHYLLUM DEMERSUM

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Certain non-toxic substances have been proposed for phosphorus inactivation as a means of lake rehabilitation. Calcium salts are thought to be such substances.

The calcium salts ($CaCO_3$, $CaSO_4$ and both combined in a 1:1 ratio) were dosed at 0, 7, 14 and 21 ppm in triplicate for each of twelve treatment combinations. The effects these doses had on the aquatic macrophyte, *Ceratophyllum demersum* were measured.

The plant showed no response to these dosages, in either productivity or tissue concentrations of phosphorus. Thus, these salts, at the specified dosage levels, would be ineffective in controlling this weed.

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INTRODUCTION

Limnologists are frequently asked to recommend strategies for lake rehabilitation. Approaches have been many and varied and as Dunst, *et al.* (1974) concluded, it is highly dependent upon the specific situation in question.

One viable means, that has some universality, is nutrient depletion of phosphorus. The reason for this attack on phosphorus is because it is a "key" nutrient (Lee, 1973); that is, a nutrient that can be controlled with current technological and financial resources and that may not be limiting at that particular point in time, but can be made limiting through specified control efforts. It has been shown that biological productivity is directly coupled to phosphorus cycling in relation to allochthonous inputs (Wetzel, 1975). Ideally, if one could lower the loading rate, productivity would decline. Many times this is an infeasible approach, since a diffuse source of phosphorus is enriching the lake. In certain of these cases, phosphorus might be most readily removed from the system by inactivation once it is within the lake basin.

Extrapolation from successful waste treatment technology would indicate that lime (CaO, slaked or $Ca(OH)_2$, unslaked) (Owen, 1953; Buzzell and Sawyer, 1967; and Schmid and McKinney, 1969), alum ($Al_2(SO_4)_3 \cdot 14H_2O$) (Malhotra, *et al.*, 1964), ferrous and ferric sulfate, cupric sulfate and sodium aluminate ($NaAlO_3$) (Lea, *et al.*, 1954;

Albertson and Sherwood, 1969) are all chemicals that provide feasible means of reducing phosphorus concentrations in water. However, all but alum, have some inherent problems associated with *in situ* applications at dosage levels that would have a significant effect. Drastic pH alterations due to the addition of lime, toxicity of cupric sulfate and cost of sodium aluminate are examples.

In May of 1970, Peterson, *et al.* (1973) implemented waste treatment technology by applying 200 mg/l alum to Horseshoe Lake, Wisconsin. The results were an overall improvement in water quality reflected by a decrease in total phosphorus concentrations, improved dissolved oxygen levels ostensibly associated with reduced autochthonous production and increased Secchi disc readings.

At about this same time another non-toxic phosphorus inactivating chemical was being employed by Laing (1974) for lake restoration. Technical information regarding this material was scant during this period of patent development. It was reported to be a calcium based compound that was dosed at approximately 15 mg/l. Its remedial effects were purported to be lowered phosphorus concentrations and concomitant reductions in algal and macrophytic productivities. These reported responses provided the impetus for this research.

A priori, assuming the compound was either $CaCO_3$ (calcite) or $CaSO_4 \cdot 2H_2O$ (gypsum), Laing's dosage levels would be ineffective in producing a noticeable phosphorus reduction. This observation is based on values obtained from using equations from Stumm and Morgan (1970). With specified temperatures, pH and solubility products effective treatment would require at least a magnitude of difference between the alkalinity level and dosage level of those salts to have a

significant lowering of phosphorus due to hydroxyapatite formation and precipitation. What Stumm and Morgan and other theoretical equations fail to take into consideration are the complex array of interactions that come into play in natural aquatic environs. The theoretical considerations are based on straight stoichiometric relations of one or two species within a container of distilled water. Thus a multitude of interactions and synergisms that are inherent in the natural waters are ignored. Things such as multiple chemical species, coprecipitation, dissolved organics, adsorption, absorption and flocculation are a few. As Otsuki and Wetzel (1972) showed, coprecipitation of phosphates with carbonates can greatly increase the amount of phosphorus inactivation. Thus, there might be some beneficial effects of dosing $CaCO_3$ and $CaSO_4 \cdot 2H_2O$ at low levels. The efficacy of these dosing rates over time, is dependent on the reducing strength of the environment in which resulting compounds settle and their resultant availability to rooted aquatic macrophytes. The work of Solski cited in Wetzel (1975) has shown that plants are effective phosphorus pumps and can recycle the element quite appreciably. This has been further substantiated by the work of McRoy, *et al.* (1972) on eelgrass (*Zostera marina* L.).

The hypothesis then to be tested in this study is: do calcium salts (carbonate and sulfate), at dosage levels of maximally 21 mg/l, lower productivity or effect the phosphorus tissue concentration of an aquatic macrophyte. *Ceratophyllum demersum* was chosen as the test organism because it is a non-rooted plant, thus eliminating the need for monitoring the sediments, and from the work of McNabb, *et al.* (1972)

and McNabb and Tierney (1972), *C. demersum* was shown to respond proportionally to available phosphorus as reflected in tissue concentrations of that element.

METHODS

For stringent statistical analysis a factorial experiment was chosen that was of a split-plot, repeat measurement design. The general model for the three fixed factors, A (compound type), B (dosage level) and C (time) was taken from Gill and Hafs (1971).

The experiment was undertaken in the fourth cell of the system of waste stabilization ponds at Belding, Michigan described by Bulthuis (1973). A roped enclosure, approximately twenty feet square, was tethered off in the middle of the pond, free of any macrophytic growths.

Thirty-six experimental units (3 replications of each of the twelve treatment combinations) that consisted of 4-mill polyethylene bag (89.54 cm X 164.47 cm) were suspended from a 50 cm diameter, doughnut shaped styrofoam float. Each unit was filled with 240 liters of ambient water. Placed inside of the poly-bag and suspended near mid-depth were two nylon-mesh bags holding *Ceratophyllum demersum*. Each unit was covered with a plexiglass plate and allowed to equilibrate for 48 hours before treatment.

Ceratophyllum demersum that was placed within the nylon bags was obtained from the experimental pond and subjected to a series of rinses in pond-water to remove invertebrate organisms and *Lemna minor*. Terminal portions, 18 cm in length, were selected, shaken, blotted and wet-weighed into 30 gram portions. These were placed within the nylon-mesh bags, which were large enough to facilitate a quadrupling

in biomass without crowding effects. Eighty-five wet-weighed plant packets were made, of which 13 were randomly selected and brought back to the laboratory for analysis. The remaining 72 were randomly assigned, two at a time, to the poly-bag units. Table 1 depicts the treatment combinations. Each of the twelve treatment combinations were triplicated.

Table 1. Treatment combinations and experimental unit numbers.

		FACTOR A (COMPOUND TYPE)		
		All $CaCO_3$	$CaCO_3$:$CaSO_4$ (1:1)	All $CaSO_4$
FACTOR B (DOSAGE LEVEL)	0 mg/l	- - - - - - - - Controls - - - - - - - - Experimental units 1-9		
	7 mg/l	10-12	13-15	16-18
	14 mg/l	19-21	22-24	25-27
	21 mg/l	28-30	31-33	34-36

At the time of treatment, the compounds were weighed and placed in individually labeled plastic bags for transport to the field. The covers were removed from the experimental units, water withdrawn from the unit and mixed with a randomly selected treatment compound to form a slurry. This slurry was then slowly introduced into the unit with stirring, after which the units were covered and labeled as to treatment combination. Though no compound was added to bags 1 through 9, they were subjected to the same mechanical treatment.

A sampling device was constructed that would allow sampling from a unit with minimum disturbance to the water column. It consisted of a board (56 cm X 9 cm) with three holes in it that would receive PVC tubing of equal diameter but differing lengths. The lengths were 15 cm, 53 cm, and 102 cm and labeled respectively, surface, middle and bottom. Poly-tubing was used to connect each PVC tube to the import side of a small electric pump. The export side had a tube that transported the water to an appropriately labeled sample bottle.

For each sampling time, enough water per depth per bag was withdrawn to allow for the following analyses in the laboratory: total alkalinity, phenolphthalein alkalinity, total hardness, orthophosphate and total phosphate. One of the mesh bags containing *C. demersum* was removed, the contents rinsed in pond-water, placed in labeled poly-bags and transported back to the laboratory. Dry weights, ash-free dry weights and tissue concentrations of phosphorus were obtained from these. *In situ* measurements of temperature and pH were made.

The chemical analyses for total alkalinity, phenolphthalein alkalinity, total hardness, orthophosphate and total phosphate were performed to specifications outlined in Standard Methods for the Examination of Water and Wastewater (Anon., 1973).

Total alkalinity was measured with bromcresol green-methyl red indicator (endpoint = pH 4.5), while phenolphthalein alkalinity was measured at an endpoint of pH 8.3. Total hardness was analyzed with HexaVer^{R} Hardness Titrant and ManVer^{R} Hardness Indicator-2, prepared by Hach Chemical Company. These measurements were expressed as mg/l (ppm) $CaCO_3$.

Phosphorus analyses were done by colorimetry using the 1-ascorbic acid-single reagent method with absorbancies read at 880 nm on a Bausch and Lomb Spectronic-20. Matched cuvettes having a light pathway length of 1.5 cm were used. Values were determined by comparison to standard curves and expressed as ppm PO_4-P. The data points comprising a standard curve were based on known concentrations of potassium dihydrogen phosphate (KH_2PO_4) that were subjected to the same procedures and dilutions as the samples.

Total phosphorus was measured after digestion, with persulfate oxidation carried out in 125 ml Erlenmeyer flask, the contents of which were brought to boiling on a hot plate. Following cooling and neutralization (pH 7.3), each sample was rediluted to the original volume of 100 ml. The orthophosphate values represent that faction of the total phosphate which will react with the combined reagent after 10 minutes following its addition to the sample.

Well washed plant material was returned from the field in poly-bags, placed in a forced draft drying oven and dried at 80°C until a constant weight was obtained (circa 48 hours). The entire sample was then homogenized in a micro-Wiley mill using a 40 mesh screen and placed back into the drying oven. Ash-free dry weights were obtained by taking a 100 mg sub-sample and igniting at 550°C in a muffle furnace for 4 hours. Tissue phosphorus was determined by taking a 500 mg sub-sample and wet ashing with 10 ml of 1 to 1 HNO_3 and $HClO_4$ acid mixture in a Bethge distillation apparatus. The residue was diluted and neutralized and then colorimetrically analyzed, using the 1-ascorbic acid-single reagent method.

Glassware for the phosphorus analyses was cleaned with sulfuric acid-dichromate solution, followed by a wash and rinse in hot, 30% (v/v) HCl and finalized by triple rinses in deionized distilled water. All other glassware, except the Bethge distillation apparatus, was cleaned by the same procedure, but deleting the hot HCl bath. The Bethge distillation apparatus was cleaned by soaking in a 1 to 1 HNO_3 and HOH bath overnight and then tripled rinsed with deionized distilled water.

RESULTS

Appropriate statistical tests were derived from the general model. These tests were based on mean squares of the appropriate factors, interactions and error terms. Initially, due to the factorial nature of the experiment, significant interactions were examined (Snedecor and Cochran, 1967).

The data that were collected can be divided into two components; those that were chemically related and those that have biological significance. There were no measurable differences (at alpha = 0.05) between surface, middle and bottom waters for any of the chemical components. Thus, those data for the experimental units were pooled. Table 2 represents those chemical parameters which were tested for mean factor differences and found not to be significantly different (at alpha = 0.05). They are therefore expressed as overall means ± standard error.

Table 2. Overall means for those parameters not showing significant differences.

Parameter	Mean ± Standard error
Total hardness (mg/l $CaCO_3$)	199.72 ± 2.14
Phenolphthalein alkalinity (mg/l $CaCO_3$)	25.60 ± 1.01
Total alkalinity (mg/l $CaCO_3$)	156.62 ± 2.03
Orthophosphate (mg/l PO_4-P)	1.017 ± 0.047
Temperature	22.30 ± 0.30
pH	8.90

For each factor, appropriate statistical tests were utilized. For factor A (compound type), the means were tested by Tukey's HSD (Honestly Significant Difference) method. Factor B (dosage level) means were compared by Dunnett's test and factor C (time) means were compared using Student's-t test. In the case of orthophosphate, though there were no significant differences, there was an apparent depression of that substance with increasing dosage levels (Figure 1).

Total phosphorus presented a different case, for in that instance, there was a significant interaction (at alpha = 0.05) between the dosage level and time. Therefore, treatment combination means (BC means) were analyzed using Dunnett's test. Since there were no differences between compound types (factor A), the BC means were averaged over all compound types. For time-1 (T_1) there were no significant differences, but T_2 had significant differences (at alpha = 0.05) between the controls (0 ppm) and dosage levels of 14 and 21 ppm.

The biological parameters were derived from the response of *Ceratophyllum demersum*. The ash-free dry weights increased linearly over time (Figure 2). A doubling of biomass took approximately 16 days. The percent tissue concentration of phosphorus, based on previously sited statistical analyses, showed no differences between compound types or dosage levels, and had an overall mean of 2.1%.

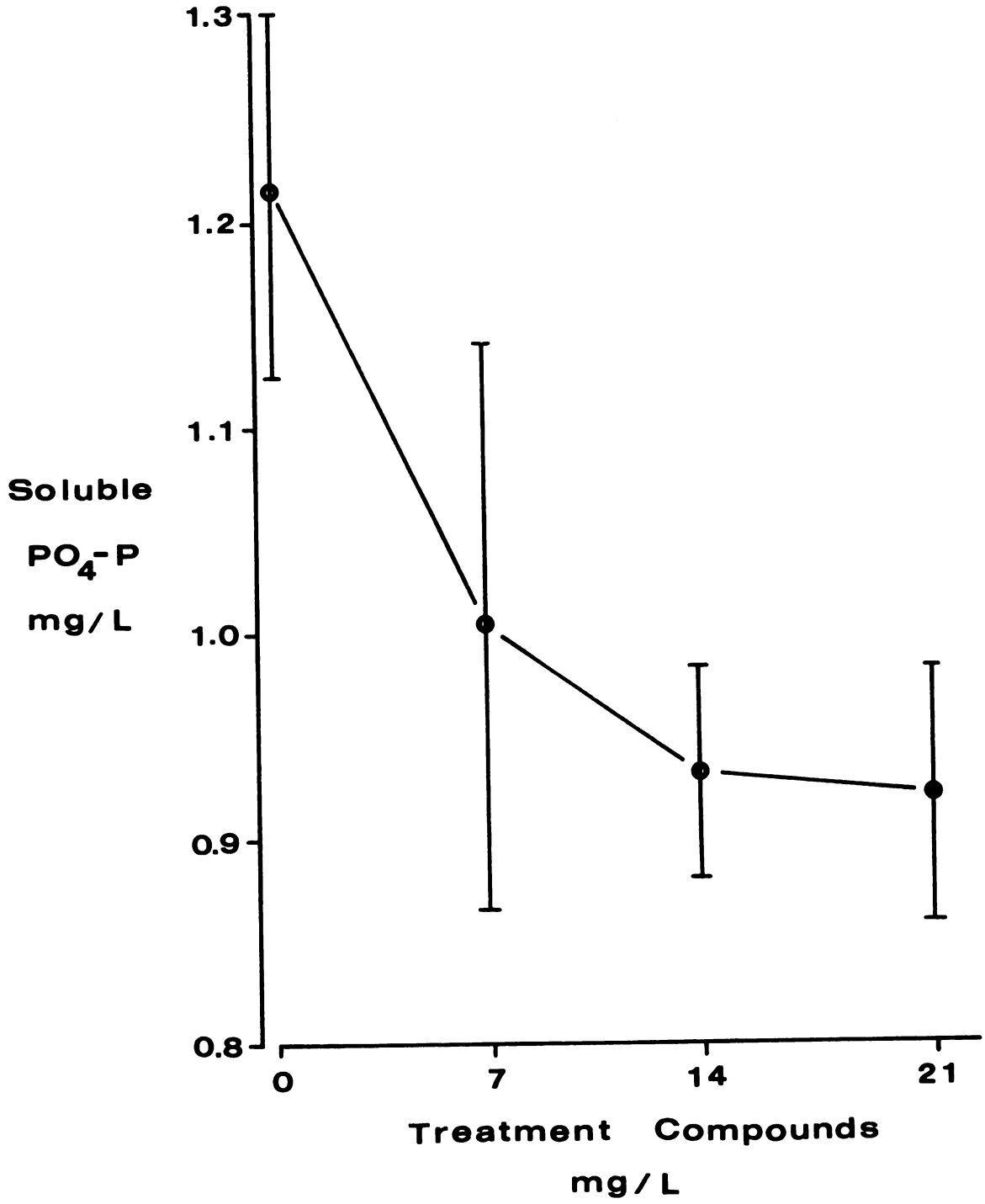

Figure 1. The response of ambient orthophosphate to increasing dosage with treatment compounds ($CaCO_3$, $CaCO_3$:$CaSO_4$, $CaSO_4$). Means ± one standard error are shown.

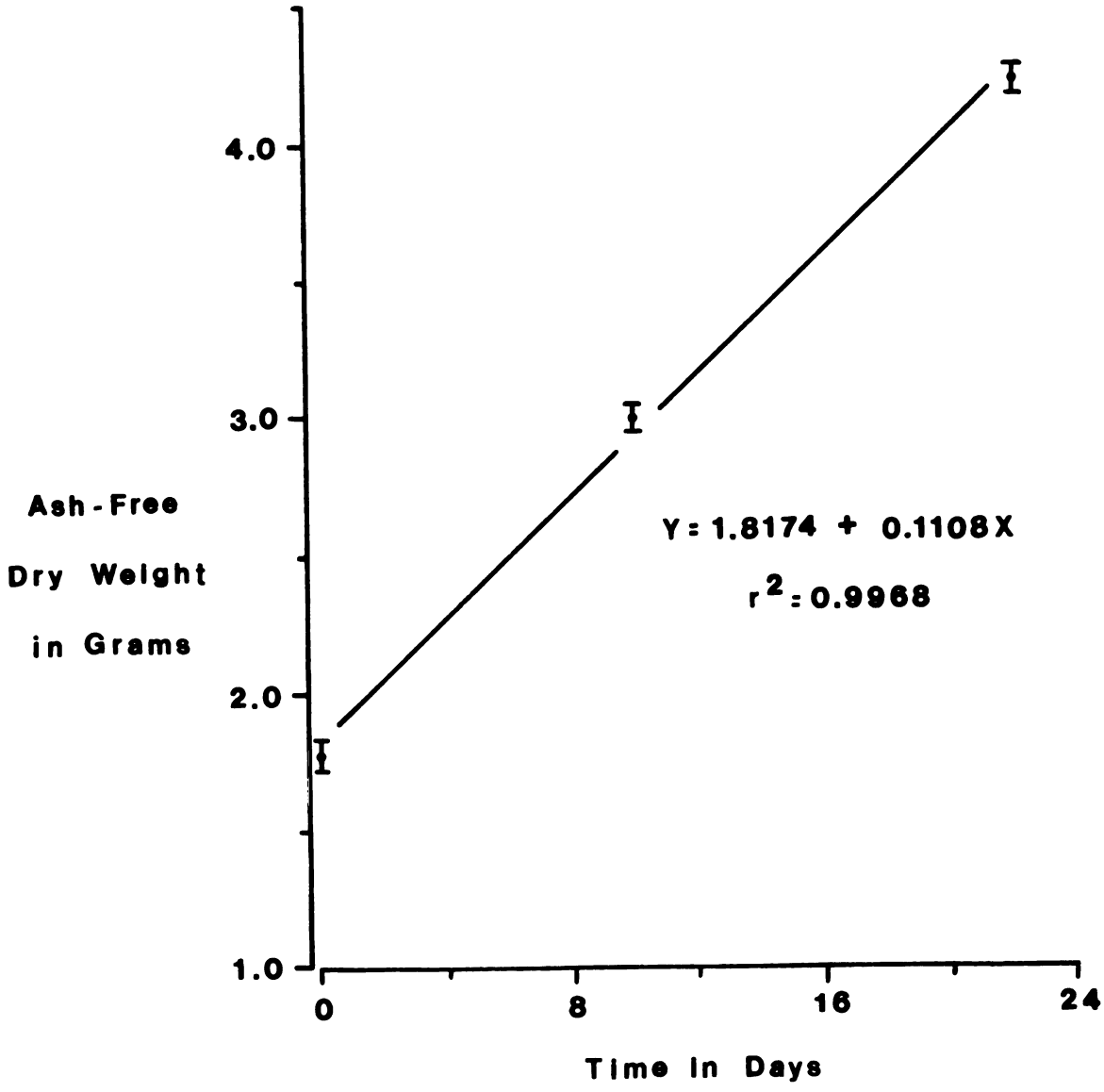

Figure 2. The rate of growth of *Ceratophyllum demersum* in all enclosures over the experimental interval. Means ± one standard error are shown. Biomass doubling time was approximately 16 days.

DISCUSSION

The hypothesis to which this study was directed would have to be rejected on the basis of the data that were obtained. Productivity of the aquatic macrophyte was not reduced. It displayed a doubling time close to the minima reported in McNabb and Tierney (1972). Regarding orthophosphate, which showed no significant differences, McNabb and Tierney's (1972) predictive response is correct, for there was no concomitant difference in the percent tissue phosphorus. The total phosphorus which showed a significant decline, is of no consequence, since this is an unavailable resource to the plant (Sculthorpe, 1967). Therefore, no matter what was transpiring in the ambient waters in relation to phosphorus, the plant responded as if it was constant.

Since the onset and subsequent completion of this experiment, Laing and Adams (1975) have revealed the various components of their chemical, which on a percent weight basis are the following:

Active ingredients	
Calcium sulfate	80.0%
Aluminum sulfate . . .	17.0%
Boric acid	1.0%
Inert ingredients	2.0%

Taking Laing's average dose as 15 ppm and using 17% by weight as aluminum sulfate, the dosage level of that compound is 2.55 ppm. If this is expressed as a percentage of the value (200 ppm aluminum sulfate)

that was used on Horseshoe Lake, Wisconsin (Peterson, *et al.*, 1973) for a significant treatment effect, namely 1.3%, one sees that this component of Laing's compound would have little impact on phosphorus reduction. Essentially then the compound is functionally calcium sulfate. This would facilitate direct comparison between his data and mine. Laing (1974), Trent and McArthur (1974) and Laing and Adams (1975) each talk of the efficacy of this compound, maximally dosed at 20 ppm, in reducing productivity by means of phosphorus inactivation. Laing and Adams (1975) mention cases in which there is immediate die-off of *Ceratophyllum demersum* after treatment with only 10 ppm of their compound. Their proposal that this is due to phosphorus inactivation is highly improbable based on my findings. The only possible explanation for this discrepancy, is that the compound does not lower productivity by phosphorus inactivation but by some other, as yet, undetermined mechanism.

CONCLUSION

The hypothesis that calcium salts (carbonate and sulfate), at maximal dosage levels of 21 ppm, lower productivity of *Ceratophyllum demersum* has been rejected on the basis of the data obtained. Not only was there not a reduction in productivity, but the percent tissue phosphorus concentrations were unaltered. The data indicate that Laing's compound can not be used as an effective means of lake rehabilitation.

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