The Effects Of Calcium Salts On The Growth And Uptake Of Phosphorus By Coontail

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ABSTRACT

Calcium salts are non-toxic substances proposed for use in phosphorus inactivation as a means of lake rehabilitation. Calcium salts ($CaCO_3$, $CaSO_4$, and both combined in a 1:1 ratio) were applied at 0, 7, 14, and 21 ppm in triplicate for each of twelve treatment combinations. Coontail (*Ceratophyllum demersum* L.) showed no response to these treatments, in either productivity or tissue concentrations of phosphorus. Thus, these salts, at the specified dosage level, would be ineffective in controlling this weed.

INTRODUCTION

Limnologists are frequently asked to recommend strategies for lake rehabilitation. Approaches have been many and varied and their effectiveness is highly dependent upon the specific situation in question (5).

One viable means, that has some universality, is the depletion of phosphorus. The reason for this attack on phosphorus is because it is a "key" nutrient (9), that is, a nutrient that can be controlled with current technological and financial resources and that may not be limiting at any 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 from allochthonous inputs (21). 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) (15), alum ($Al_2(SO_4)_3 \cdot 14H_2O$) (10), ferrous and ferric sulfate, cupric sulfate, and sodium aluminate ($NaAlO_3$) (1,8), 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 1970, Peterson et al. (16) implemented waste treatment technology by applying 200 mg per liter of 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 for lake restoration (7). 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 per liter. 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 (7) 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 (19). With specified temperatures, pH, and solubility products, effective treatment would require at least an order of magnitude increase in the dosage level of these salts to bring about a significant lowering of phosphorus due to hydroxyapatite formation and precipitation. What Stumm and Morgan's (19) theoretical equations fail to take into consideration is the complex array of interactions that occur in natural aquatic systems. 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. Such factors as multiple chemical species, coprecipitation, dissolved organics, absorption, adsorption, and flocculation are a few of these inherent qualities. As Otsuki and Wetzel (14) showed, coprecipitation of phosphates with carbonates can greatly increase the amount of phosphorus inactivation. Thus, there might be some beneficial effects of dosing calcite and gypsum 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 (21) 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. (13) on eelgrass (*Zostera marina* L.).

The objective of this study was to determine if calcium salts (carbonate and sulfate), at dosage levels of a maximum of 21 mg per liter lowered productivity or affected the

phosphorus tissue concentration of an aquatic macrophyte.

Coontail was chosen as the test organism because it is a non-rooted plant, thus eliminating the need for monitoring the sediments. Moreover, studies have shown that coontail's tissue concentration of phosphorus responds proportionally to available ambient phosphorus concentrations (11, 12). Thus measuring that element in the tissues would reveal the availability of that element within the water column.

METHODS

For 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 (6).

The experiment was undertaken in the fourth cell of the system of waste stabilization ponds at Belding, Michigan described by Bulthuis (3). It was an aerobic pond with transparency extending to the bottom in which macrophytes flourished. A roped enclosure, approximately 37 m^2 was tethered off in the middle of the pond, free of any macrophytic growth.

Thirty-six experimental units (three replications of each of the 12 treatment combinations) that consisted of 0.1 mm polyethylene bags (89.5 cm by 164.5 cm) were suspended from a 50-cm diameter, doughnut shaped styrofoam float. Each unit was filled with 240 liters of ambient water. Placed inside the poly-bag and suspended near mid-depth were two nylon-mesh bags holding coontail. Each unit was covered with a plexiglass plate and allowed to equilibrate for 48 hr before treatment.

Coontail was obtained from the experimental pond, subjected to a series of rinses in pondwater to remove invertebrate organisms and duckweed (*Lemna minor* L.), and placed within the nylon bags. Terminal portions, 18 cm in length, were selected, shaken, blotted, and wet-weighed into 30-g 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.

TABLE 1. TREATMENT COMBINATIONS AND EXPERIMENTAL UNIT NUMBERS.

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

At the time of treatment, the calcium compounds were weighed and placed in individually labeled plastic bags for transport to the field. The covers were removed from the experimental units, and water was 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. Although 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 by 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 surface, middle and bottom, respectively. 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 coontail 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 (2).

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 per liter $CaCO_3$.

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 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 fraction of the total phosphate which will react with the combined reagant after 10 minutes following its addition to the sample.

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 (ca 48 hr). 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 igniting a 100-mg sub-sample at 550 C in a muffle furnace for 4 hr. Tissue phosphorus was determined by wet ashing a 500-mg sub-sample with 10 ml of 1:1 HNO_3 and $HClO_4$ acid mixture in a Bethge distillation apparatus. The residue was diluted and neutralized and then colorimetrically analyzed, using the l-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 1:1 HNO_3 and HOH bath overnight and then triple 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.

The data that were collected can be divided into two components: those that were chemically related and those that had biological significance. There were no significant 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.

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).

TABLE 2. OVERALL MEANS FOR THOSE PARAMETERS NOT SHOWING SIGNIFICANT DIFFERENCES.

Parameter	Mean ± Standard error
Total hardness (ppm $CaCO_3$)	199.7 ± 2.1
Phenolphthalein alkalinity (ppm $CaCO_3$)	25.6 ± 1.0
Total alkalinity (ppm $CaCO_3$)	156.6 ± 2.0
Orthophosphate (ppm PO_4-P)	1.02 ± 0.05
Temperature	22.3 ± 0.3
pH	8.9

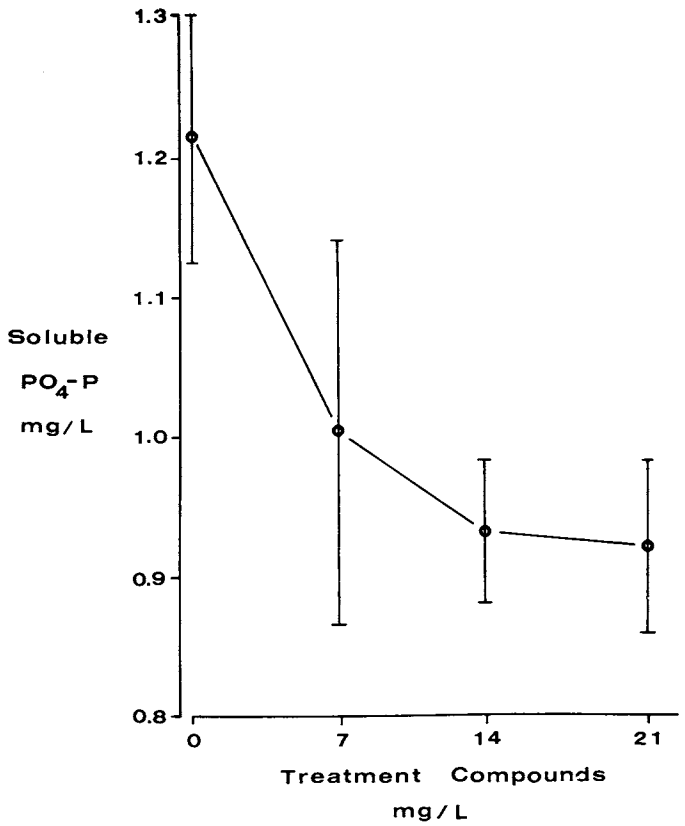

Figure 1. The response of ambient orthophosphate to increasing dosage with treatment compounds ($CaCO_3$, $CaCO_3$:$CaSO_4$, $CaSO_4$). Means ± one standard error are shown.

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 coontail. 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 cited statistical analyses, showed no differences between compound types or dosage levels, and had an overall mean of 2.1%.

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 minimum reported in McNabb and Tierney (11). Regarding orthophosphate, which showed no significant differences, McNabb and Tierney's (11) predictive response of tissue concentration reflecting ambient concentrations is correct; for there

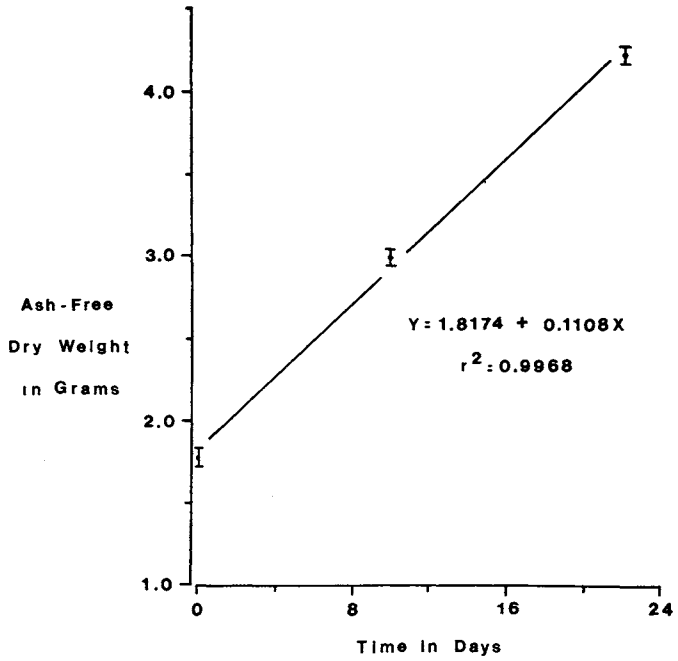

Figure 2. The rate of growth of *Ceratophyllum demersum* in all enclosures over the experimental interval. Means ± one standard error are shown. Biomass doubling time was approximately 16 days.

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 (18). 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 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%) and inert ingredients (2.0%).[1] 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 needed on Horseshoe Lake, Wisconsin (16) for a significant treatment effect, namely 1.3%, one sees that this component of Laing's compound would seem to have little impact on phosphorus reduction. Essentially then the compound is calcium sulfate. Accepting this assumption as being valid, comparisons between his data and mine might be made. Laing (7), Trent and McArthur (20) and Laing and Adams each talk of the efficacy of this compound, maximally dosed at 20 ppm, in reducing productivity by means of phosphorus inactivation. Laing and Adams mention cases in which there is immediate die-off of coontail after treatment with only 10 ppm of their compound.[1] Their proposal that this is due to phosphorus inactivation is highly improbable based on my findings. One possible explanation for this discrepancy is that the compound does not lower productivity by phosphorus inactivation but by some other, as yet, undetermined mechanism.

[1]Laing, R. L. and A. M. Adams. 1975. A study of the efficacy of Clean-Flo Lake Cleanser(TM) in controlling aquatic plants in three aerated Minnesota lakes. Clean-Flo Laboratories, Inc., Hopkins, Minn. Mimeo. 67 pp.

CONCLUSION

The hypothesis that calcium salts (carbonate and sulfate), at maximal dosage levels of 21 ppm, lower productivity of coontail 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 the use of calcium compounds at these dosage levels should not be used as a means of lake rehabilitation.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to Professor Clarence D. McNabb for providing guidance during this study.

This project was supported in part by funds provided by the United States Environmental Protection Agency Training Grant (T-900331) and the Michigan Agricultural Experiment Station at Michigan State University.

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