

Autoclaving Soil Samples Affects Algal-Available Phosphorus

Brandon H. Anderson and Frederick R. Magdoff*

ABSTRACT

Unwanted microbial interference in samples used for biological assays of P availability has routinely been eliminated by autoclaving samples before inoculation with algae. Twenty-three soils were selected to evaluate the relationship between algal growth in P-deficient solutions containing small quantities of soil and the level of P determined by a variety of tests used to evaluate P availability in soils and sediments. Soils were either autoclaved or not before addition to flasks containing P-starved algae in a nutrient solution without P. Compared to non-autoclaved samples, autoclaving soil resulted in approximately 60% more available P as estimated by increased algal growth. However, algal growth in the presence of autoclaved soil was highly correlated with growth in the presence of non-autoclaved samples. There was no consistent change in the correlations (r) between autoclaving or non-autoclaving samples in the relationships of algal numbers with P extracted by a number of soil tests. The effect of autoclaving soil on soluble P was also evaluated for a subset of six soils. Autoclaved soils had significantly greater concentrations of soluble P than non-autoclaved soils, with 78% more orthophosphate monoesters, 60% more orthophosphate diesters, and 54% more soluble inorganic P. Inhibition of algal growth may have occurred with two high-Zn soils that produced relatively low numbers of algae despite being very high in estimated available P by all extraction methods. Removing those samples from the calculations dramatically improved correlations between soil P measured by various methods and algal growth. With these two soils removed from calculations, algal growth with autoclaved soil was most highly correlated with Olsen P ($r = 0.95$), with other correlations as follows: Fe-oxide strip ($r = 0.80$), Mehlich 3 ($r = 0.75$), modified Morgan ($r = 0.61$), and Bray-Kurtz 1 ($r = 0.57$).

OVER THE LAST 40 years, there has been a growing concern over the effects of increasing aquatic phosphorus levels on the biology of surface waters. There is now a strong body of scientific evidence supporting the claim that excessive phosphorus (P) in fresh water systems results in eutrophic conditions. According to the USEPA, agriculture is the leading source of pollution in rivers and lakes in the United States (USEPA, 1995). With decreases in point sources of P, mainly by upgrading sewage treatment plants, more emphasis is being placed on abatement of nonpoint sources. This has led to experiments that directly measure and/or indirectly estimate the P pollution potential of runoff waters and sediments as well as soils that might erode from field.

Before performing experiments to assess P availability of environmental samples, traditionally lake water or sediments, unwanted microbial interference has routinely been eliminated by autoclaving samples before

biological assays with algae. The USEPA guideline for algal bioassays (Miller et al., 1978) has provided the basis for the experimental methodology of batch culture algal bioassays. These procedures, which include an autoclaving process to sterilize samples before algal inoculation, were originally drafted for use on lake water samples, not soil or sediment samples. These algal assay procedures have been recently applied to field runoff samples containing sediments. Autoclaving sediment samples before algal inoculation has become an accepted practice of sample preparation for algal assays (Sharpley et al., 1991; Sharpley, 1993a, 1993b, 1993c; Robinson et al., 1994). Preliminary studies of Sharpley et al. (1991) indicated that "autoclaving did not affect the amount of P extracted ... suggesting similar availability of P to algae before and after sterilization."

However, almost any drastic soil or sediment treatment may affect P dynamics of the sample. Many researchers have focused on the effects of drying on P chemistry (Bartlett and James, 1980; Haynes and Swift, 1985; McLaughlin et al., 1981; Payne and Rechcigl, 1989; Sims and Ellis, 1983; Raveh and Avnimelech, 1978). In addition to effects on inorganic P dynamics, drying increases solubility and dispersion of organic matter (Bartlett and James, 1980) and may influence soluble organic P compounds. Autoclaving a soil or sediment sample may produce similar effects on soil P chemistry as drying. Xie and MacKenzie (1990) reported that autoclaving soils resulted in the dissociation of organically bound Fe and the condensation of crystallized Fe and Al, resulting in less surface area and a reduction in P adsorption. They also described a decrease in surface tension from surface hydration of autoclaved soils, thereby blocking or occupying P adsorption sites.

Biological tests such as growing algae exposed to soil, sediment, or runoff waters are very time consuming and expensive to perform. Simple tests to assess P pollution potential of soils and sediments are needed to help screen large numbers of samples so as to be able to focus on those that pose the greatest threat. There are a number of different types of soil extractants commonly used to estimate P availability to plants. A few of the more frequently used soil P tests include: Morgan (Morgan, 1941), modified Morgan (McIntosh, 1969), Mehlich 3 (Mehlich, 1984), Bray-Kurtz 1 (Bray and Kurtz, 1945), and Olsen (Olsen and Sommers, 1982). The amount of P extracted by many of the soil tests tends to be correlated with one another for soils of similar characteristics (Bates, 1990; Beegle and Oravec, 1990; Blanchar and Caldwell, 1964; Magdoff et al., 1999; Mallarino and Blackmer, 1992). Factors such as clay, Al, and the presence of carbonates have been used to help explain the large differences found between how well the various soil tests perform as estimates of plant-available P (Blanchar and Caldwell, 1964; Wolf et al., 1985).

Department of Plant and Soil Science, University of Vermont, 105 Carrigan Drive, Burlington, VT 05405. Received 24 Jan. 2005. *Corresponding author (fmagdoff@uvm.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Magdoff et al. (1999) found that P extracted with a modified Morgan solution (pH 4.8) was correlated with the water-extractable P and the equilibrium P concentration of a soil, as well as plant growth in greenhouse experiments, supporting the assumption that the modified Morgan's solution is a good extractant for estimating plant-available P. However, it is not known if P removed by this or another routinely used soil extractant is correlated with growth of algae exposed to soils. It seems logical that P removed by extractants that are correlated with agricultural plant response could also be correlated with changes in algal growth when eroded soil in runoff enters rivers and lakes.

A number of tests have been developed specifically to assess P availability of water or sediment samples. Sediment-bound P extracted with 0.1 M NaOH was one of the first chemical extractants used to relate sediment-bound P with algae growth. Sagher (1976) used 0.1 M NaOH (1000:1 solution to sediment ratio) to extract sediment-bound P. Results from this study show a significant correlation between extracted P and algae growth. However, Sagher cautioned readers that P extracted by NaOH may underestimate the availability of recently applied calcium-phosphate fertilizers and overestimate the availability from soils with large quantities of iron and aluminum oxides. Sharpley et al. (1991) derived a method for estimating bioavailable P from Menon et al. (1989), who used iron oxide-impregnated filter paper (Fe-oxide strip) to estimate the plant-available P in a variety of soils. Phosphorus extracted using the modified Fe-oxide-strip method has been found to be well correlated with algal growth response for sediment samples (Sharpley et al., 1991; Sharpley, 1993a, 1993b, 1993c; Robinson et al., 1994). The Fe-oxide-strip method is argued to have a better theoretical basis for estimating algal-available P compared to other chemical extraction

procedures because it functions as a P sink, mimicking the assimilation of P by algae. Although more convenient to use than an algal bioassay, P analysis using the Fe-oxide-strip method is not routinely performed in soil testing laboratories. The need to prepare the strips and the extra care in recovery of P associated with the strips means that this test is more time consuming and complicated to perform than conventional soil tests.

Regardless of which test is used to estimate soil or sediment bioavailable (algal-available) P, whether autoclaving influences P availability when soil test levels are compared to algal growth is an important issue. The purpose of this study was to determine whether autoclaving soil influences P availability to algae and the relationship between algal growth and soil test P. As part of the study, we wanted to evaluate the relationship between algal growth and P levels determined by a variety of soil tests, including P soil tests routinely used for soil fertility evaluation as well as selected other tests proposed specifically for environmental P assessment.

METHODS AND MATERIALS

Soil Preparation and Analysis

Twenty-three representative agricultural soils from the Champlain Valley of Vermont were selected for this study (Table 1). These are subsamples from the same larger sample of soils that were subsampled for Experiment 3 described by Magdoff et al. (1999). These soils represent a wide range in P availability and P-fixing capacity, as indicated by extractable Al (Magdoff et al., 1999). In preparation for the algal assay, soils were dried at 55°C and passed through a 2-mm sieve and P was extracted with 1.25 M NH₄OAc at pH 4.8 (modified Morgan solution) and a 1:500 soil to solution ratio with 0.1 M NaOH (Sharpley et al., 1991). Mehlich-3 P (0.2 M CH₃COOH, 0.25 M NH₄NO₃, 0.015 M NH₄F, and 0.001 M EDTA with a 1:10 soil to solution ratio by volume) was determined by the

Table 1. Vermont soil test results for 23 soils used in algal bioassays.†

| Number | Series | Al | Zn | P | pH _‡ |
|--------|----------------------------------------------------------------------------------|-------|-----|------|-----------------|
| | | | | | |
| 1§ | Colton (sandy-skeletal, isotic, frigid Typic Haplorthod) | 48.5 | 4.9 | 36.9 | 6.4 |
| 2 | Hogansburg (coarse-loamy, mixed, semiactive, frigid Aquic Eutrudept) | 6.2 | 3.3 | 64.6 | 7.2 |
| 3 | Malone (coarse-loamy, mixed, active, nonacid, frigid Aeric Epiaquept) | 3.5 | 3.2 | 50.5 | 7.4 |
| 4 | Hogansburg (coarse-loamy, mixed, semiactive, frigid Aquic Eutrudept) | 12.2 | 1.2 | 20.6 | 6.6 |
| 5 | Plainfield (mixed, mesic Typic Udipsamment) | 12.4 | 5.0 | 64.7 | 5.9 |
| 6§ | Malone (coarse-loamy, mixed, active, nonacid, frigid Aeric Epiaquept) | 144.0 | 0.9 | 1.4 | 5.6 |
| 7 | Raynham (coarse-silty, mixed, active, nonacid, mesic Aeric Epiaquept) | 49.8 | 0.8 | 4.9 | 6.1 |
| 8§ | Georgia (coarse-loamy, mixed, semiactive, mesic Aquic Dystric Eutrudepts) | 16.4 | 1.3 | 2.8 | 6.3 |
| 9§ | Malone (coarse-loamy, mixed, active, nonacid, frigid Aeric Epiaquept) | 71.3 | 1.3 | 2.2 | 5.5 |
| 10 | Covington (very-fine, illitic, mesic Mollic Epiaqualf) | 18.3 | 1.3 | 13.7 | 7.2 |
| 11 | Scantic (fine, illitic, nonacid, frigid Typic Epiaquept) | 99.5 | 2.7 | 3.6 | 4.9 |
| 12 | Winooski (coarse-silty, mixed, active, nonacid, mesic Aquic Udifluent) | 31.1 | 1.3 | 10.0 | 5.8 |
| 13§ | Scantic (fine, illitic, nonacid, frigid Typic Epiaquept) | 17.6 | 1.7 | 3.3 | 6.1 |
| 14 | Adjidaumo (fine, mixed, active, nonacid, frigid Mollic Endoaquepts) | 49.2 | 1.6 | 19.8 | 5.7 |
| 15 | Plainfield (mixed, mesic Typic Udipsamment) | 125.2 | 0.7 | 3.4 | 5.1 |
| 16 | Muskellunge (fine, mixed, active, frigid Aeric Epiaqualf) | 30.0 | 1.4 | 3.9 | 6.2 |
| 17 | Peru (coarse-loamy, isotic, frigid Aquic Haplorthod) | 193.5 | 2.4 | 4.5 | 4.9 |
| 18 | Kingsbury (very-fine, illitic, mesic Aeric Epiaqualf) | 41.3 | 2.1 | 1.2 | 5.7 |
| 19 | Munson (coarse-silty over clayey, mixed, active, nonacid, mesic Aeric Epiaquept) | 9.2 | 1.2 | 5.0 | 6.6 |
| 20 | Windsor (mixed, mesic Typic Udipsamment) | 39.7 | 3.2 | 15.1 | 6.8 |
| 21 | Adjidaumo (fine, mixed, active, nonacid, frigid Mollic Endoaquepts) | 12.1 | 1.2 | 4.5 | 7.3 |
| 22 | Kingsbury (very-fine, illitic, mesic Aeric Epiaqualf) | 48.3 | 1.5 | 2.7 | 6.4 |
| 23§ | Vergennes (very-fine, mixed, active, mesic Glossaquic Hapludalf) | 31.0 | 1.3 | 4.0 | 5.7 |

† Elements extracted using modified Morgan's solution.

‡ pH measured in 0.01 M CaCl₂.

§ Six soils selected for evaluation of autoclaving on soluble P. These soils represent greatest differences in algal growth response for autoclave and non-autoclave pretreatment.

Soil Testing Laboratory of the North Carolina Department of Agriculture's Agronomic Division and Bray-Kurtz P (0.025 M HCl in 0.03 M NH_4F , 1:10 soil to solution ratio) and Olsen P (0.5 M NaHCO_3 , 1:25 soil to solution ratio) by the University of Nebraska Soil Testing Laboratory. Phosphorus was also determined by the iron oxide-impregnated-strip method (STR; Sharpley, 1993a, 1993b) and total P by microwave digestion (USEPA, 1986).

Algal Assay

Selenastrum capricornutum obtained from the University of Texas was cultured in 100 mL of complete algal nutrient growth medium (Miller et al., 1978) in 250-mL Erlenmeyer flasks under continuous light ($250 \mu\text{E m}^{-2} \text{s}^{-1}$) until cells reached a stationary growth phase (10 d). Thirty milliliters of nutrient solution containing algae at stationary growth phase was centrifuged at $1000 \times g$, supernate was discarded, and one quarter of the cells were transferred into 100 mL of P-free nutrient growth medium in a 250-mL Erlenmeyer flask and incubated in a growth chamber at a constant temperature of 23°C , light intensity of $110 \mu\text{E m}^{-2} \text{s}^{-1}$, and humidity of 70%. Severe phosphorus deficiency was estimated to occur when cells began to yellow (about 7 d). Cells were allowed to grow for an additional 3 d to ensure that the culture was P starved.

Three replicates of each soil were prepared by adding 20 mg of homogeneous soil to 50 mL of P-free nutrient growth medium in separate 250-mL Erlenmeyer flasks, that were then capped with foam stoppers and autoclaved at 394 K and 104 Pa for 20 min. Three replicates were prepared in the same way but were not autoclaved. Flasks were inoculated with P-starved cells to give an initial flask concentration of 3×10^4 cells mL^{-1} and placed in a growth chamber at a constant temperature of 23°C , 70% relative humidity, and light intensity of $110 \mu\text{E m}^{-2} \text{s}^{-1}$ and stirred gently each day for 14 d. On the 14th day of incubation, flasks were stirred and 1 mL of the culture was taken and preserved with 200 μL of M3 fixative solution (Clesceri et al., 1998) and refrigerated at 5°C in 3-mL capped vials. Cells were visually counted using an improved Neubauer hemacytometer. Algal cell numbers discussed below represent the average of three replicates.

Phosphorus Release by Autoclaving Soil

Substantial differences in cell density between autoclaved and non-autoclaved soils in the experiment described above had indicated differences in algal-available P. Of the original 23 soils, the six that showed the greatest differences (Soils 1, 6, 8, 9, 13, and 23 in Table 1) were selected to evaluate organic and inorganic P release into solution by autoclaving. Dry samples of each soil (10 g) were added to each of six plastic bottles containing 40 mL of distilled water and the bottles were then capped and shaken at 60 rpm on a platform shaker for 1 h. Samples were transferred to 250-mL Erlenmeyer flasks and capped with foam stoppers. Three reps of each soil were autoclaved as described above while three reps were not autoclaved. A 30-mL subsample of the soil slurry mixture was centrifuged at $8000 \times g$ for 15 min and then filtered with a $0.45\text{-}\mu\text{m}$ syringe filter. Algal cell numbers discussed below represent the average of three replicates.

Organic forms of soluble P were determined using the enzyme assay as described by Turner et al. (2002). Alkaline phosphatase-phosphomonoesterase (P-4252; Sigma, St. Louis, MO) and phosphodiesterase I (P-4506; Sigma) were dissolved in 0.1 M Tris-HCl buffers containing 2 mM MgCl_2 (activator of enzymes). The enzyme assay mixture consisted of 4.5 mL of filtered soil extract, 0.25 mL of 0.1 M NaN_3 (to prevent micro-

bial interference), and 0.25 mL of enzyme solution (one of two enzymes in buffer, either mono- or di-phosphoesterase, and one check containing only Tris-HCl to determine soluble inorganic P). The final 5-mL mixture was incubated at 37°C in sealed glass test tubes for 16 h overnight. After the 16-h incubation, samples were analyzed for P using the molybdate-stannous chloride method. Inorganic P was also determined in samples prepared as described for organic P, but without the addition of a phosphatase enzyme. Organic P hydrolyzed by the monoesterase and diesterase enzymes and inorganic P (no enzyme) were determined colorimetrically with a spectrophotometer set at 660 nm. The amount of monoester P hydrolyzed was determined by subtracting the amount of molybdate-reactive P (inorganic P) present without addition of an enzyme from the amount of molybdate-reactive P present following reaction with monoesterase. Likewise, the amount of diester P hydrolyzed was determined by subtracting the amount of molybdate-reactive P (inorganic P) present without addition of an enzyme from the amount of molybdate-reactive P present following reaction with diesterase. Statistical comparisons were made using the general linear model procedure (SAS Institute, 1990) with the probability value set at ≤ 0.05 .

RESULTS AND DISCUSSION

Autoclaving Effects on Soil Phosphorus Availability

Flasks containing autoclaved soil were generally greener and had greater algal densities than flasks containing non-autoclaved soils (Fig. 1). For most soils, differences in chlorophyll color and cell density between algal assay flasks containing autoclaved soils and non-autoclaved soils were easily detected visually. Mean algal densities in flasks containing two autoclaved soils were lower, and those for two more soils were only slightly greater than in flasks containing non-autoclaved soils (near the 1:1 line in Fig. 1). However, for the set of 23 soil slurries, mean algal cell densities for the autoclaved samples were significantly greater than non-autoclaved samples with 3.8×10^6 and 2.4×10^6 cells mL^{-1} , respectively ($p < 0.0001$). Assuming no algal inhibition or stimulation due to other factors influenced by autoclaving, the average difference represents 58% greater [$(3.8 \times 10^6 - 2.4 \times 10^6) / 2.4 \times 10^6$] P availability following autoclaving compared with non-autoclaving.

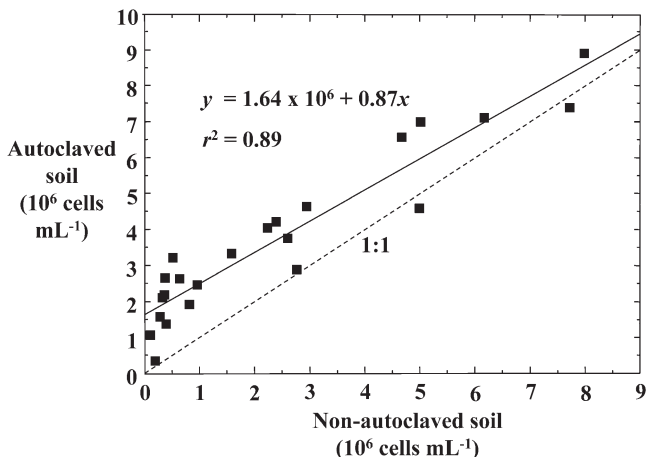


Fig. 1. Algal growth in P-free solution with and without soil pretreatment by autoclaving.

Table 2. Mean P values for soluble orthophosphate monoesters, orthophosphate diesters, and inorganic P in the water extracts of six soils with and without autoclaving.

| P form | Autoclaved | Non-autoclaved | Difference (autoclaved – non-autoclaved) | P |
|-------------|------------|----------------|------------------------------------------|--------|
| Monoester P | 0.506 | 0.285 | 0.220 | 0.003 |
| Diester P | 1.193 | 0.745 | 0.448 | <0.001 |
| Inorganic P | 1.490 | 0.966 | 0.523 | <0.001 |

A subset of six soils, representing the greatest algal growth response to the autoclave pretreatment, was selected to determine the effect of autoclaving on soluble P. Autoclaved soil samples had significantly greater amounts of soluble P, with 78% more orthophosphate monoesters, 60% more orthophosphate diesters, and 54% more soluble inorganic P compared with non-autoclaved samples (Table 2). A “biological effect,” such as a reduction of the quantity of P available to algae by competition or antagonism from native soil organisms, may have contributed to reduced algal numbers in the absence of autoclaving. However, the increases in soluble P with autoclaving (54–78% increases for the different fractions measured) are in the same range as the increased growth of algae with autoclaving (about 60%). This indicates that the greater growth of algae following autoclaving was probably mainly caused by additional available P.

These results of increased soluble P and algal growth with autoclaving suggest that the physical disruption by the high temperature and pressure of the autoclave treatment converted P in complex organic compounds and microbial cells into smaller organic subunits and to inorganic orthophosphate as well as release of inorganic P by killed microbial cells. The greater availability of small soluble organic compounds may have allowed for the greater formation of enzyme–substrate complexes with added phosphatase enzymes. As a survival mechanism, algae excrete high concentrations of phosphatase enzymes under P-starved growth conditions (Jansson et al., 1988). Thus, it is likely that P-starved algal cultures used to inoculate samples in this experiment contained substantial concentrations of phosphatase enzymes. The enzymatic hydrolysis of soluble organic P would provide a greater pool of orthophosphate for algal assimilation than release of inorganic P alone.

All soil samples were dried at 55°C before algal inoculation so that accurate sample weights of 20 mg soil could be measured. A different algal response to autoclaving soil sample soil test P might have occurred if field-moist soil samples had been used instead of dry samples. However, it is likely that the drying process

produced a similar effect on soil P availability as determined for the autoclave treatment, only to a lesser extent. Drying soils has been found to increase extractable P (Haynes and Swift, 1985; Bartlett and James, 1980) and increase the solubility and dispersibility of soil organic matter (Raveh and Avnimelech, 1978; Bartlett and James, 1980).

While not indicated in our studies, indigenous microorganisms might compete for the available P in nonsterilized samples and thereby suppress the algal growth response in the assay. In a comparison of sterilization methods for water samples, Filip and Middlebrooks (1975) concluded that ultraviolet light treatments would be a better sterilization pretreatment because of only minimal changes in the chemical characteristic of the water sample. They reported that the autoclave pretreatment of water samples raised the pH, causing precipitation of salts and the loss of CO₂. Although the only effect of ultraviolet light exposure was the oxidation of organic and inorganic nitrogen, this is not an important issue with a P algal bioassay because the nutrient media provides all nutrients (except P) in sufficient amounts. Further research needs to be done on soil and sediment sterilization techniques for algal bioassays so that samples can be effectively sterilized and physical and chemical side effects of the treatment do not interfere with the availability of P.

Algal Growth and Soil Test Phosphorus

Linear regression correlations between algal growth and soil test P (Table 3) were substantially improved after Soils 1 and 5 were removed from statistical analysis. All soil test measurements were consistently very high in soil P for Soils 1 and 5; however, algal growth response was very low (Fig. 2). One possible explanation is the presence of an inhibitory substance or organism. Because the effect occurred in both autoclaved and non-autoclaved samples, the effect was probably not caused by competition or antagonism from another organism. Soils 1 and 5, have coarse textures and contained the two highest levels of Zn extracted by modified Morgan's

Table 3. Intercept, slope, and correlation coefficient for linear regression models of soil test autoclaved and non-autoclaved soil slurry samples (mg kg⁻¹) with algal cell numbers (cells mL⁻¹). Soils 1 and 5 were removed from analysis.†

| Soil test P | Autoclaved | | | Non-autoclaved | | |
|-------------------|------------------------|-----------------------|------|------------------------|-----------------------|------|
| | Intercept | Slope | r | Intercept | Slope | r |
| NaOH P | 1.3 × 10 ⁶ | 1.0 × 10 ⁴ | 0.52 | -1.0 × 10 ⁶ | 1.4 × 10 ⁴ | 0.67 |
| Fe-oxide-strip P | 6.7 × 10 ⁵ | 9.2 × 10 ⁴ | 0.80 | -8.0 × 10 ⁵ | 9.4 × 10 ⁴ | 0.76 |
| Modified-Morgan P | 2.7 × 10 ⁶ | 1.2 × 10 ⁴ | 0.61 | 1.3 × 10 ⁶ | 1.3 × 10 ⁵ | 0.58 |
| Bray-Kurtz-1 P | 2.2 × 10 ⁶ | 2.9 × 10 ⁴ | 0.58 | 4.7 × 10 ⁵ | 3.4 × 10 ⁴ | 0.64 |
| Olsen P | 3.1 × 10 ⁵ | 8.6 × 10 ⁴ | 0.95 | -1.3 × 10 ⁶ | 9.2 × 10 ⁴ | 0.95 |
| Mehlich-3 P | 7.8 × 10 ⁵ | 3.3 × 10 ⁴ | 0.74 | -9.7 × 10 ⁵ | 3.6 × 10 ⁴ | 0.78 |
| Total P | -4.0 × 10 ⁶ | 7.2 × 10 ³ | 0.73 | -4.6 × 10 ⁶ | 6.5 × 10 ³ | 0.61 |

† All regressions statistically significant at the 0.05 probability level.

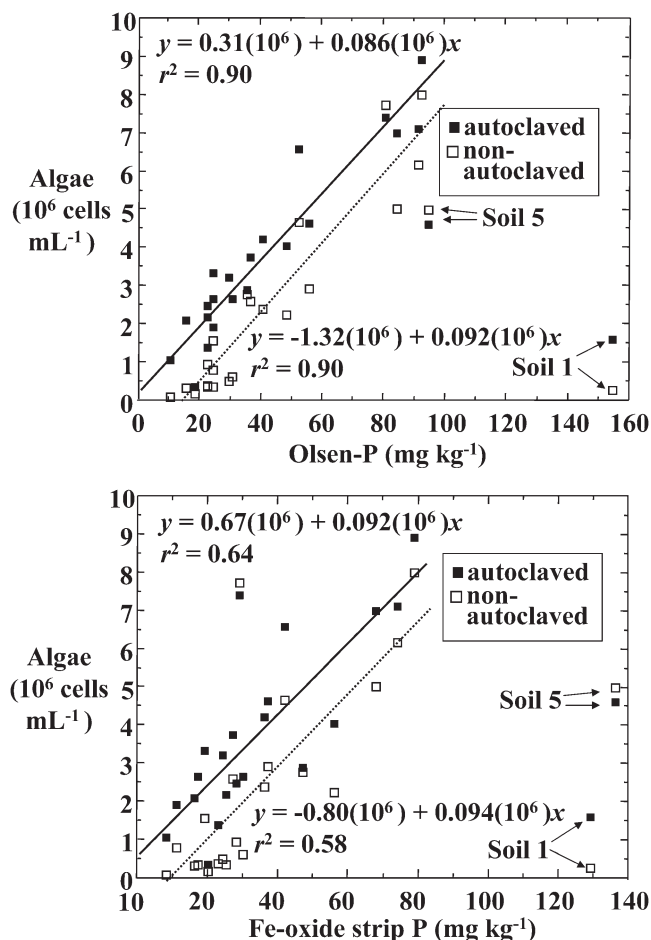


Fig. 2. Algal growth with soil prepared with and without autoclave pretreatment and P extracted by Olsen (top) and Fe-oxide-strip (bottom) methods (Soils 1 and 5 removed from analysis).

solution (4.9 and 5.0 mg Zn kg⁻¹ soil, respectively). To put these extractable Zn levels in perspective, they are greater than 98% of more than 6000 soils tested by the University of Vermont's Agricultural and Environmental Testing Laboratory in 1999. The relatively high concentrations of this known algicide (Greenfield, 1942; Parry and Hayward, 1973), with extractable Zn in Soils 1 and 5 approximately 50% higher than in the next highest soil, might be responsible for inhibiting the growth of algae in these very high P soils (Fig. 2, top). Removing these soils from the analysis substantially improved the correlations of algal growth with all of the P tests. For example, the correlation for algal growth with the remaining 21 non-autoclaved soil samples increased from 0.33 to 0.90 for Olsen P, from 0.19 to 0.58 for Fe-oxide-strip P, from 0.09 to 0.41 for Bray-Kurtz-1 P, and from 0.29 to 0.34 for modified Morgan P.

Of the seven soil test P measurements, Olsen P provided the highest correlation for the relationship between soil test P and algal growth response (Table 3). Part of the explanation for the high correlation between Olsen P and algal growth may be that the pH of algal growth solution, buffered at pH 7.5 with NaHCO₃, is much closer chemically to the Olsen extract of 0.5 M NaHCO₃ at pH 8.5 than the other extractants. Phospho-

rus released from soil with the NaHCO₃ extract (Olsen and Sommers, 1982) may best resemble a soil's algal-available P when placed in algal growth nutrient solution prepared according to Miller et al. (1978). However, eutrophic surface water can vary in pH, and values ranging from pH 6.0 to 10.0 had no adverse effects on maximum algal growth of *S. capricornutum* in algal assay (Miller et al., 1978).

Relationships between algal-available P and either Fe-oxide-strip P (Fig. 2, bottom) in this experiment are not as well correlated as found by others (Sharpley et al., 1991; Sharpley, 1993a, 1993b, 1993c; Robinson et al., 1994). This may be a result of differences in soils and sampling methods. For example, the majority of algal P assays/Fe-oxide-strip studies have been performed on a relatively small group of runoff samples collected from 20 watersheds from four locations, representing a total of only four soil types (Sharpley et al., 1991; Sharpley, 1993a, 1993b, 1993c; Robinson et al., 1994). In contrast, our study of 23 agricultural soils represented a wide diversity of properties such as texture, pH, and extractable P. A second difference is that we used dried soil samples (20 mg) instead of collected sediment samples that were not dried before algal inoculation. In addition, the process of runoff and erosion may also dilute chemicals or compounds residing in a bulk soil sample that might cause algal inhibition or stimulation.

CONCLUSIONS

Autoclaving sediment samples has become the standard sterilization pretreatment for algal bioassays. We found more P available for algal assimilation, as indicated by greater growth with autoclaved samples, in 21 of 23 autoclaved soils. In a subset of six soils, monoester, diester, and soluble inorganic P in autoclaved soil samples were higher than when samples were not autoclaved. However, algal growth in flasks containing autoclaved soils was well correlated with growth with non-autoclaved soils. Additionally, correlations between the various soil tests evaluated and algal growth were similar with autoclaved and non-autoclaved samples. Thus, although autoclaving samples did result in higher P and greater algal growth, autoclaving does not appear to greatly affect the P contribution of a soil, relative to other soils, to algal growth. Thus, autoclaving may be used to rank the potential relative contributions of soils and/or sediments to algal-available P.

Chemical compounds causing algal inhibition were suspected in 2 of the 23 soils because of their very high soil test P and very low algal growth response. When these very high P-high Zn soils were omitted there was a large increase in correlations between the remaining 21 soil test P levels and algal numbers, to 0.90 for Olsen P and to 0.58 for Fe-oxide-strip P. Of the soil P tests correlated with algal growth, Olsen P provided the highest linear correlation ($r = 0.95$), with P extracted by modified Morgan ($r = 0.61$), Fe-oxide strip ($r = 0.80$), and Bray-Kurtz 1 ($r = 0.57$), and Mehlich 3 ($r = 0.75$) also significantly correlated with algal growth. Correlations between algal growth response with either the

Fe-oxide-strip P or 0.1 M NaOH methods were not as well correlated as reported by others. Sampling differences (soil in our study vs. sediment), a wide range of soil chemical and physical properties used in our study, and algal inhibition or stimulation may have resulted weaker correlations. Routine soil P tests may be used for estimating the potential contribution of P to aquatic systems following erosion events. However, other properties, such as the presence of an algaeicide or competing and/or antagonistic organisms (in non-autoclaved samples), may influence the contribution of soil P to accelerated growth of algae.

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