An Innovative Approach to Estimate Bioavailable Phosphorus in Agricultural Runoff Using Iron Oxide-Impregnated Paper

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ABSTRACT

The transport of bioavailable phosphorus (BAP) in agricultural runoff can accelerate the eutrophication of receiving waters. Bioavailable P is comprised of dissolved P (DP) and bioavailable particulate phosphorus (BPP). Although several algal assays and chemical extractions have been proposed to estimate BAP, procedural and theoretical limitations have restricted their widespread use. This study evaluates the use of Fe oxide-impregnated paper strips (Fe-oxide strips) to estimate the potential BAP content of runoff from 20 agricultural watersheds in the Southern Plains during 1988 to 1990. In the proposed method, BAP and DP are determined by shaking 50 mL of unfiltered or filtered runoff, respectively, with one Fe-oxide strip for 16 h. Phosphorus is removed from the strip by 0.1 M H2SO4, and measured, with BPP calculated as the difference between BAP and DP. Growth of Selenastrum capricornutum was related (r2 = 0.63–0.96) to the BPP content of runoff sediment in 2- to 29-d incubations. Bioavailable PP estimated by the proposed strip method and more widely used NaOH extraction, closely followed a 1:1 relationship. Averaged for all runoff events, strip DP was 90 and 75% of DP determined by standard direct methods, for watersheds with and without tillage, respectively. The lower estimates of strip DP may be partially attributed to a greater transport of inorganic and condensed PP in runoff from no-till compared to tilled watersheds. Acting as a P sink, the strip method may have a stronger theoretical basis than chemical extraction in estimating algal-available P in agricultural runoff. In addition, the simplicity of the proposed Fe-oxide strip method will allow BAP estimation with limited resources and may facilitate evaluation of the impact of agricultural runoff on the biological productivity of receiving waters.

THE TRANSPORT in agricultural runoff of BAP as DP and BPP can accelerate the eutrophication of surface waters (Levine and Schindler, 1989; Krogstad and Lovstad, 1989). Dissolved P is comprised mostly of orthophosphate, which is usually recognized as being immediately available for uptake by algae (Peters, 1981; Walton and Lee, 1972). However, DP also includes varying amounts of organic and colloidal P forms, which are not immediately bioavailable (Lean, 1973; Rigler, 1968). A dynamic equilibrium also exists between DP and particulate P (PP) during transport in runoff. Thus, BAP includes DP and that portion of PP (BPP), entering equilibrium with DP in runoff. Bioavailable PP comprises a variable portion (10–90%) of PP transported in runoff as a function of management factors affecting soil loss and runoff volume (DePinto et al., 1981; Dorich et al., 1985; Sharpley et al., 1992). The varying amounts and bioavailability of DP and PP transported in different runoff events, stress the need for accurate BAP estimates to evaluate the impact of agricultural runoff on the biological productivity of surface waters.

The BAP content of runoff or sediment is measured by algal assay methods, which require from 70 to 100-d incubations (Lovstad and Krogstad, 1990; Miller et al., 1978). Therefore, more rapid acidic and basic chemical extractions have been used to estimate BAP on a routine basis (Dorich et al., 1980; Hegemann et al., 1983; Sharpley et al., 1991). However, the complexity of both assay and extraction methods often limit their use, especially in situations where limited resources restrict even simple analyses. Questions also have been raised as to the validity of relating the form or availability of P extracted by chemicals differing by more than 1 pH unit from runoff or sediment, to in situ bioavailability (Goltermann, 1988). Consequently, there is a need for methodology that will facilitate BAP estimation. One such method may be the use of Fe oxide-impregnated filter paper strips (Fe-oxide strips), which have been developed (Menon et al., 1989a,b) and successfully applied to the estimation of plant-available P in a wide range of soils and cropping situations (Menon et al., 1990; Sharpley, 1991).

This paper assesses the use of Fe-oxide strips to estimate the BAP content of runoff from 20 agricultural watersheds during a 3-yr period (1988–1990). Phosphorus loss from these watersheds, which included BAP estimated by NaOH extraction, has been reported by Sharpley et al. (1992).

MATERIALS AND METHODS

Watersheds

Location and management of 20 watersheds, representative of agricultural land use in the Southern Plains region of Oklahoma and Texas, is given in Table 1. More detailed information of tillage operations is given by Sharpley et al. (1992). The watersheds ranged in area from 1.6 to 5.6 ha, with annual rainfall averaging 540, 750, 900, and 600 mm at Bushland, El Reno, Ft. Cobb, and Woodward locations, respectively, over the 3 yr (1988–1990) for which data is presented. When applied, fertilizer P rates were determined by Bray-I P soil test recommendations (Bray and Kurtz, 1945).

The major soil types at the Bushland, El Reno, Ft. Cobb, and Woodward locations are Pullman clay loam (fine, mixed, thermic Torrertic Paleustolls), Kirkland silt loam (fine, mixed, thermic Udertic Paleustoll), Cobb fine sandy loam (fine-loamy, mixed, thermic Udic Haplustals), and Woodward loam (coarsely, mixed, thermic Typic Ustochopts), respectively.

Watershed runoff volume was measured using precalibrated flumes equipped with water level recorders, with 5 to 15 samples collected automatically during each runoff event. The samples were composited in proportion to flow, to provide a single representative flow-weighted sample, which was stored at 277 K until analysis. Aliquots of runoff samples were centrifuged (266 km s-1 for 5 min, 27 100 g) and filtered (0.45 μm). Dissolved P and total DP (TDP) were determined on filtered runoff samples with and without acid ammonium per sulfate digestion, respectively. Total P (TP) was determined following perchloric acid digestion of unfiltered runoff samples (Olsen and Sommers, 1982). Particulate P was calculated as the difference between TP and TDP. Bioavailable P concent-

Abbreviations: BAP, bioavailable phosphorus; DP, dissolved phosphorus; BPP, bioavailable particulate phosphorus; PP, particulate phosphorus; TDP, total dissolved phosphorus; TP, total phosphorus.
tation of runoff was determined using Fe-oxide strips as detailed below in the “Proposed Method.” In all cases, P was determined on neutralized filtered extracts by the colorimetric method of Murphy and Riley (1962). Suspended sediment concentration of runoff was determined in duplicate as the difference in weight of 250-mL aliquots of unfiltered and filtered (0.45 μm) runoff samples after evaporation (378 K) to dryness.

**Algal Assay**

*Selenastrum capricornutum,* acquired from Carolina Biological Supply Co., Burlington, NC, were cultured in Provisional Algal Assay Procedure (PAAP) medium (Miller et al., 1978) with fluorescent and incandescent light at an intensity of 90 W m⁻². At the stationary growth phase, *S. capricornutum* cells were rinsed in P-free PAAP medium and incubated in light at 295 K until the culture began to yellow (15 d), indicating the onset of P deficiency in the cells. Cells were incubated for an additional 5 d prior to inoculation.

Runoff samples from four events in May and June, 1986 and three events in May and June 1987 were combined and sediment concentrated by centrifugation and decantation. The sediment concentration of each slurry was determined by evaporation (378 K) to dryness.

Two milliliters of sediment slurry (approximately 0.1 g sediment) was added to 57 mL of P-free PAAP medium in 250-mL Erlenmeyer flasks. The suspension was inoculated with P-deficient *S. capricornutum* to attain cell densities of 2 × 10⁶ for 2- and 15-d incubations and 5 × 10⁴ cells mL⁻¹ for 29-d incubations, at 90 W m⁻² of light intensity at 295 K. Duplicate flasks of each treatment were prepared and shaken twice daily. After incubation, three subsamples were removed from each flask and cell counts were made with an improved Neubauer hemacytometer (American Scientific Products, Grand Prairie, TX). Eight replicate counts were made per flask. Detailed information on the physical and chemical properties of these sediment slurries were reported by Sharpley et al. (1991).

### Table 1. Watersheds characteristics for 1988 to 1990.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Management</th>
<th>Area</th>
<th>Fertilizer P applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ha</td>
<td>kg ha⁻¹ yr⁻¹</td>
</tr>
<tr>
<td><strong>Bushland, TX</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10A</td>
<td>No-till wheat (Triticum aestivum)</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>B11A</td>
<td>Austivum L-sorghum</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>B12A</td>
<td>Sorghum bicolor (L.) Moench</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>B10B</td>
<td>Reduced-till wheat-</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>sorghum-fallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B11B</td>
<td></td>
<td>2.6</td>
<td>0</td>
</tr>
<tr>
<td>B12B</td>
<td></td>
<td>2.6</td>
<td>0</td>
</tr>
<tr>
<td><strong>El Reno, OK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>Native grass†</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>E2</td>
<td>Native grass</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>E3</td>
<td>Native grass</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>E4</td>
<td>Native grass</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>E5</td>
<td>Conventional till wheat</td>
<td>1.6</td>
<td>12</td>
</tr>
<tr>
<td>E6</td>
<td>Conventional till wheat</td>
<td>1.6</td>
<td>13</td>
</tr>
<tr>
<td>E7</td>
<td>No-till wheat</td>
<td>1.6</td>
<td>13</td>
</tr>
<tr>
<td>E8</td>
<td>Conventional till wheat</td>
<td>1.6</td>
<td>13</td>
</tr>
<tr>
<td><strong>Ft. Cobb, OK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>Peanut (Arachis hypogena L.)</td>
<td>2.6</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>-grain sorghum rotation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td>2.1</td>
<td>18</td>
</tr>
<tr>
<td><strong>Woodward, OK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>Native grass</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>W2</td>
<td>Native grass</td>
<td>5.6</td>
<td>0</td>
</tr>
<tr>
<td>W3</td>
<td>Native grass</td>
<td>2.7</td>
<td>23</td>
</tr>
<tr>
<td>W4</td>
<td>Native grass</td>
<td>2.9</td>
<td>23</td>
</tr>
</tbody>
</table>

† Native grass is a mixture of big *(Andropogon geradii* Vitman) and little *(Schizachyrium scoparium* (Michx.) Nash) bluestems; tall dropseed *(Sporobolus drummondi* (trim) Vasey-Fernald); side oat grama *(Bouteloua arripendula* (Michx.) Torr.); and hairy grama *(Bouteloua hirsuta* Lag.).

### Preparation of Iron Oxide–Impregnated Paper Strips

Iron-oxide strips were prepared by immersing filter-paper circles (15-cm diam., Whatman no. 541) in a solution containing 10 g FeCl₃ · 6 H₂O in 100 mL distilled water. The paper circles were air dried and immersed in 2.7 M NH₄OH solution to convert FeCl₃ to Fe oxide. Immersion in NH₄OH was carried out as rapidly as possible to avoid uneven oxide deposition on the paper (Lin et al., 1991). After the paper circles were air dried, they were cut into strips 10 by 2 cm and stored for subsequent use.

The effect of shaking period on the extraction of P from unfiltered runoff by Fe-oxide strips was determined by shaking individual strips with 50 mL of unfiltered runoff for 30 min., 1, 2, 4, 8, and 16 h. In addition, the efficacy of DP measurement by the Fe-oxide strips was evaluated by shaking individual strips with 50 mL of standard solutions of varying P concentration (P added as KH₂PO₄) for 16 h. The amount of P retained on the strip was determined as described below. All treatments were duplicated.

### Proposed Paper Strip Method

The BAP concentration of runoff was estimated by shaking one Fe-oxide strip with 50 mL of unfiltered runoff for 16 h on an end-over-end shaker (30 revolutions min⁻¹) at 298 K. The strip was then removed, rinsed free of adhering soil particles, and dried. Phosphorus retained on the strip (BAP) was removed by shaking the strip end-over-end with 40 mL of 0.1 M H₂SO₄ for 1 h, and following neutralization was measured by the method of Murphy and Riley (1962), Bioavailable PP was calculated as the difference between BAP and DP.

### RESULTS AND DISCUSSION

#### Method Development

The effect of contact time on the extraction of P from runoff sediment was determined by shaking individual Fe-oxide strips with sediment slurry from C2, E6, W2, and W4 watersheds, diluted with distilled water to 20 g L⁻¹ (solution/soil ratio of 50:1). The amount of P retained on the strip increased rapidly with shaking time (Fig. 1). Within 4 h, P retained on the strip was on average 87% of that for the 16-h extraction, which increased to 97% after 8 h (Fig. 1). A similar pattern of P removal with time by strips was observed at sediment

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Fig. 1. Strip P content of runoff sediment from four watersheds as a function of shaking time using a 50:1 water to sediment ratio.
concentrations ranging from 1 to 40 g L\(^{-1}\) \((1000-25:1)\) (A.N. Sharpley, data not presented). The range in sediment concentration of the extracting medium used \((1-40 g L^{-1})\) was within the concentration range observed for individual runoff events \((0.01-31.41 g L^{-1})\) from the Southern Plains watersheds over the 1988 to 1990 period.

Although removal of P by the Fe-oxide strip was essentially complete after 8 h, an extraction of 16 h (i.e., overnight), is recommended for analytical convenience. A similar kinetic pattern of P retention by Fe-oxide strips shaken with Dutch \((\text{Van der Zee et al., 1987})\), Taiwan \((\text{Lin et al., 1991})\), and U.S. soils \((\text{Menon et al., 1990})\) also was found, with retention essentially complete within 8 to 10 h.

The uniformity of BPP estimation by Fe-oxide strips was investigated within and between separately prepared batches of strips. Within the same batch, little variation was found in the amount of P removed by 10 individual strips from E6 and W2 sediment slurry at a 50:1 solution/sediment ratio and 16 h shaking \((\text{Table 2})\). Under the same extracting conditions, similar amounts of P also were removed from C2 and W3 sediment by individual strips from 10 separately prepared batches \((\text{Table 2})\). The standard deviation for both within and between batches, was less than 3% of the mean, which indicates that Fe-oxide strips can provide a highly reproducible estimate of available P.

The growth of \(S. \text{capricornutum}\) was related to the P content of runoff sediment, as determined by Fe-oxide strips \((\text{Fig. 2})\). The relationship was significant when algae were incubated with runoff sediment as the sole source of P for 2- \((P > 0.01)\), 15- \((P > 0.001)\), and 29- \((P > 0.001)\) d. The amount of DP added to the algal incubations in the 2 mL sediment slurry \((1.2 \times 10^{-5} \text{ mg P})\) was small compared with strip P levels \((4-23 \times 10^{-2} \text{ mg P})\). Thus, strip P provides an estimate of the potential BPP content of runoff sediment that may become available for uptake by \(S. \text{capricornutum}\). Caution must be exercised, however, in relating BAP estimates by the Fe-oxide strip or any other incubation method, which estimate P bioavailability under optimum conditions, to a quantitative BAP value in surface waters.

### Comparative Assessment

#### Dissolved Phosphorus

The recovery of P by Fe-oxide strips during 16-h shaking was linearly related to the DP concentration of standard solutions \((P \text{ added as } KH_2PO_4)\), containing up to 3.0 mg P L\(^{-1}\) \((\text{Fig. 3})\). The range in DP covers that found in runoff from the study watersheds \((0.01-2.32 \text{ mg L}^{-1})\) during 1988 to 1990. Overall, the strips were 91% efficient in removing P from solution as represented by the slope of the P added-strip P relationship of Fig. 3. A similar relationship \((y = 0.91x + 0.46, r^2 = 0.99)\) between P retained on Fe-oxide strips shaken for 16 h in solutions of DP concentration up to 0.05 mg L\(^{-1}\) was also found by Menon \((1993)\).

Strip P concentration was determined on filtered runoff samples collected between 1988 and 1990 and compared with DP values \((\text{Fig. 4})\). Strip P and DP concentrations were closely related \((P > 0.001)\). However, the recovery of DP in filtered runoff by the Fe-oxide differed with watershed management \((\text{Table 1})\), as represented by the slope of the DP-strip P regression \((\text{Fig. 4})\). In fact, regression slope was significantly greater \((P > 0.05, \text{using analysis of variance})\) for runoff from the tilled compared to no-till and native grass watersheds. Thus, the recovery of DP in runoff as strip P was greater from tilled than no-till and native grass watersheds \((\text{Fig. 4})\).

The difference in estimation of DP by the Fe-oxide strip method with watershed tillage, may be due in part to the potentially greater transport of hydrolyzable organic and condensed P compounds, resulting from the increased vegetative cover of no-till and native grass compared to tilled watersheds \((\text{Krogstad and Lovstad, 1991; Langdale et al., 1985; Logan, 1982; Timmons and Holt, 1977; Wendt and Corey, 1980})\). The standard procedure to separate liquid and particulate phases of runoff is by filtration through a 0.45-μm pore diameter membrane filter \((\text{APHA, 1975})\). However, some fine colloidal materials less than 0.45 μm may pass through the

### Table 2. Phosphorus removed by 10 strips of the same and different production batches from watershed runoff sediment.

<table>
<thead>
<tr>
<th>Strip P</th>
<th>Same production batch</th>
<th>Different production batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E6</td>
<td>W2</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>W3</td>
</tr>
<tr>
<td>Mean</td>
<td>101</td>
<td>251</td>
</tr>
<tr>
<td>SD</td>
<td>± 2.0</td>
<td>± 2.4</td>
</tr>
</tbody>
</table>

### Figure 2. Relationship between the bioavailable strip P content of runoff sediment and P-starved \(S. \text{capricornutum}\) growth during 2-, 15-, and 29-d incubations.
filter and be hydrolyzed or dissolved by the strong acidic medium of the molybdate colorimetric procedure of Murphy and Riley (1962). Thus, dissolved orthophosphate, which is immediately available for algal uptake, may be overestimated, particularly at low DP concentrations (Rigler, 1966; Tarapchak and Rubitschun, 1981).

Although Walton and Lee (1972) found that DP was essentially all available using standard bioassay procedures, several investigators have reported from 50 to 95% DP was actually bioavailable (Lean, 1973; Nurnberg and Peters, 1984; Rigler, 1968; Tarapchak et al., 1982). This range in percentage bioavailability of DP is similar to the overall recovery of DP (90% for tilled, and 75% for no-till and native grass, Fig. 4), by the Fe-oxide strips in the present study.

Although Fe-oxide strips will adsorb both orthophosphate and colloidal or dissolved organic P compounds from a water sample (Ognalaga et al., 1992), P adsorption capacities of the Fe-oxide strips and acidity of the strip eluant, indicate that under Southern Plains runoff conditions, the effect of organic P adsorption by the strip on BAP estimates will be negligible. For example, a 10-by 2-cm Fe-oxide strip has a specific surface area of 1.5 cm² as determined by N₂ gas adsorption and would retain 6.72 mg of Fe (Menon et al., 1989a). This amount of Fe would adsorb up to 0.56 mg P. If a 50-mL runoff sample is shaken with a strip, as in the proposed method, the P adsorption capacity of the strip would not be exceeded unless runoff P concentration was greater than 11.16 mg L⁻¹. The maximum TP concentration of runoff from the 20 Southern Plains watersheds was only 7.91 mg L⁻¹ between 1988 and 1990. Consequently, under these conditions organic P should not decrease the efficiency of orthophosphate adsorption by the Fe-oxide strips.

If TP concentrations exceed 11.16 mg L⁻¹, a smaller sample aliquot should be used. It is also likely that the 0.1 M H₂SO₄ Fe-oxide strip eluant (pH 1.0) will not hydrolyze any additional organic P compared to the 0.23 M H₂SO₄ Murphy and Riley reagent in the color development flask (pH 0.8).

**Particulate Phosphorus.** The BPP concentration of runoff determined by the proposed Fe-oxide strip method was compared with estimates determined by 0.1 M NaOH extraction of unfiltered runoff (Sharpley et al., 1991). Strip BPP was calculated as the strip P concentration of unfiltered minus filtered runoff samples. Similar estimates of the BPP concentration of runoff were determined by both methods for all watersheds (Fig. 5). Although NaOH extraction has been used by several researchers to estimate potential BPP (Butkus et al., 1988; Dorich et al., 1985; Engle and Sarnelle, 1990; Sagher et al., 1975), the strip method may be used as an alternative procedure. The Fe-oxide strip procedure may alleviate some of the potential reproducibility problems associated with the high solution/soil ratio (500:1) and dilution factor of the NaOH method.

**CONCLUSIONS**

The amount of P removed from a 50-mL sample of unfiltered or filtered runoff by one Fe-oxide strip, during overnight end-over-end shaking, can be used as a con-
venient and interference-free method to routinely estimate the BAP concentration of agricultural runoff. As the strips act as a P-sink, they simulate P removal from sediment-water samples by algae. In as much, the Fe-

oxide strip method may have a stronger theoretical justification for its use over chemical extractants in estimating BAP.

As the acid-molybdenum blue method (Murphy and Riley, 1962) is widely accepted as the standard procedure to determine the DP concentration of water samples (APHA, 1975; USEPA, 1979) and is rapid and relatively interference free, use of the strip method is not recommended as a general replacement for DP determination. However, the strip method may be advantageous for BAP measurement in situations where laboratory facilities are minimal. For example, prepared Fe-oxide strips may be sent to a field location and BAP measured using only a 100- to 500-mL bottle in which a strip and runoff sample is shaken overnight. The strip may then be dried and returned to an analytical laboratory for P removal and measurement. This also would avoid potential problems with P transformations during sample storage and shipping (USEPA, 1979). Further, strip P development with the use of prepackaged color reagents may allow P determination in the field by comparison with a standard color chart.

Use of Fe-oxide strips may facilitate estimation of the potential bioavailability of P transported in agricultural runoff and may thereby improve assessment of the resultant impact on the biological productivity of receiving water bodies.

REFERENCES


