Method Article

Determination of bioavailable phosphorus from water samples with low suspended sediment using an anion exchange resin method

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Abstract

Measurement of phosphorus in surface waters for eutrophication studies is common. Phosphorus is commonly adsorbed to suspended sediment, but can desorb under certain conditions. Only a fraction of measured total phosphorus is bioavailable to aquatic organisms like algae, and for assessment, monitoring, and regulatory applications, researchers need to understand what proportion of total phosphorus in mixed water-sediment samples is bioavailable. Ion exchange resins have high capability of taking up dissolved ions like phosphate ($\text{PO}_4^{3-}$) and a long history of use in examining bioavailable phosphorus in surface water runoff. Previous work using resins to quantify bioavailable phosphorus was undertaken in waters with high total suspended sediment (TSS) concentrations (200–2,240 mg TSS/L) and correspondingly high phosphorus. The work presented here was undertaken in watersheds where runoff TSS concentrations are an order of magnitude lower; since bioavailable phosphorus as low as a few micrograms per liter can be important to eutrophication, it was necessary to modify existing anion exchange resin methods for use in low TSS waters. Modifications of the original method and quality controls presented include:

- A passive method to concentrate low TSS samples;
- Elimination of P cross-contamination from reuse of anion exchange resin; and
- Comparison to the original method and reproducibility of results.

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Method details

Background

Phosphorus (P) is one of the principal nutrients controlling eutrophication in freshwaters and has been the focus of eutrophication research for decades [1]. Measurement of total P in surface waters as part of watershed studies is very common; when monitoring and assessing eutrophication of waterbodies, total P is included in virtually all projects undertaken by the author’s agency. Total P is closely associated with suspended sediment in flowing waters [2], and total P concentrations are often tightly correlated to total suspended sediment (TSS) concentrations or surrogates like turbidity [3,6]. However, only a fraction of total P is bioavailable to aquatic organisms like algae, bacteria, and aquatic vascular plants, and researchers often want to know what proportion of total P is actually bioavailable.

Surface freshwaters are usually a mix of water and suspended sediment (inorganic and organic) and methods have been developed to quantify the bioavailable P found in them; chemical fractionation protocols, algal bioassays, and ion exchange resin methods have all been used [5–10]. Each of the aforementioned methods has its strengths and weaknesses. Chemical fractionation protocols extract certain forms of P susceptible to attack by specific acids or bases; they are consistent, inexpensive, and fairly easy to do. But they may not necessarily represent bioavailable P [7,11]. Algal bioassays, using algae culture lines like Selenastrum capricornutum [8], probably come closest to quantifying all bioavailable P in a sample [12]. However, they are generally lengthy, complex, and expensive, and some researchers find the method may underestimate bioavailable P [4]. Ion exchange resin methods take advantage of a specific resin’s high capability of taking up certain dissolved ions. Anion exchange resin methods for P are not expensive analytically and the P they extract is strongly correlated with bioavailable P quantified via algal bioassay methods [9,13]. On the down side, anion exchange methods are fairly labor intensive and tend to yield low estimates of bioavailable P [13]. One positive aspect of anion exchange methods that will be important to some readers is that analysis of phosphorus samples generated during the procedure can be outsourced to commercial laboratories, making the method accessible to a wider array of researchers who may not have their own P-analyzing equipment.

Ion exchange resins have a high capability of taking up certain dissolved ions and this led to their early use for studying soil P availability to terrestrial plant roots [14]. For studies of P in aquatic systems, this characteristic is an added attraction because it tends to mimic P uptake by algae in the aquatic environment. For example, the author’s agency is currently studying a river that recently began to manifest large blooms of the filamentous algae Cladophora glomerata (blanket weed) during late spring runoff. P is important to Cladophora growth [15], and when long streamers of Cladophora dominate the stream bottom they can draw down the soluble P concentration in the river water. P uptake by algae can continue to the point where a river’s soluble P concentration is so low that P loosely adsorbed to suspended sediment in the river can desorb from the sediment particles [2]; this desorbed P can contribute to ongoing algal growth. Like the Cladophora, anion exchange resin draws down soluble P in a water sample to a low concentration and then proceeds to extract the loosely-adsorbed P off the sediment particles [13]. Quantification of the P loosely attached to this suspended sediment (“sediment-bound bioavailable P”) is of great interest because it represents a bioavailable fraction of total P which can contribute to eutrophication.
Table 1
Water quality of sites examined using the anion exchange resin method described in this article. Water samples were grab samples collected during spring runoff in 2019 and 2020. Range shown with average in parenthesis.

| Stream site              | TSS (mg/L) | Specific Conductivity (µS/cm) | Total P (µg/L) |
|--------------------------|------------|-------------------------------|----------------|
| North Fork Smith River   | 4–23 (12)  | 250–388 (324)                 | 27–108 (60)    |
| South Fork Smith River   | 4–33 (11)  | 506–833 (684)                 | 39–93 (59)     |
| Newlan Creek             | 16–121 (53)| 436–470 (447)                 | 29–129 (70)    |
| Camas Creek              | 5–101 (23) | 163–324 (277)                 | 32–174 (60)    |
| Benton Gulch             | 23–79 (46) | 558–636 (595)                 | 57–195 (108)   |
| Beaver Creek             | 3–61 (18)  | 366–494 (429)                 | 24–56 (37)     |
| Smith River @ Camp Baker | 12–122 (51)| 228–473 (369)                 | 38–172 (83)    |
| Sheep Creek              | 5–13 (9)   | 127–224 (173)                 | 7–48 (22)      |

So far, ion exchange methods have only been extensively used in situations where high concentrations of suspended sediment are the norm. The lowest TSS concentration reported in [6], the method modified in this article, is 200 mg/L; most of those authors’ samples averaged around 765 mg TSS/L and higher concentration samples were in the 1700–2240 mg TSS/L range. These TSS concentrations are far in excess of TSS in the study sites currently under investigation (Table 1), streams which are located in an agricultural valley dominated by Quaternary alluvium consisting of gravel, sand, silt, clay, and undifferentiated alluvial soils [16,17]. Since bioavailable P as low as a few micrograms per liter can be important to eutrophication [18], there is a need to modify existing anion exchange methods to allow for their use in low TSS waters. In this article the method of Uusitalo et al. [6] has been modified and is referred to herein as the original method.

To operate at the low TSS concentrations in Table 1, it was necessary to modify the procedures of the original method [6] and carry out a number of quality assurance tests and studies to ensure the modified method could function at low TSS concentrations. Key changes and quality assurance/quality control actions addressed in this article include:

- How to passively concentrate suspended sediment so that higher TSS and detectable P concentrations can be achieved;
- How to eliminate low-level P contamination of samples caused by reuse of anion exchange resin and bags;
- Development of mass-balance equations to compute sediment-bound bioavailable P concentration in a sample;
- Comparison of results between the original method and the modified method;
- Determination of percent recovery and a method detection limit for the modified method;
- Determination of relative percent difference of split sample duplicates;
- Review and discussion of possible matrix interferences; and
- Working with provider laboratories to identify the best analytical protocol for running your submitted P samples.

Preparation of anion exchange resin (AER) bags

Nylon mesh biopsy bags (Cancer Diagnostics Inc., part No. BIO3045WM) are filled with 1 g of DOWEX 1 × 8 strongly basic anion exchange resin (AER). Bags are folded over at the top and sealed using an ordinary clothing iron and small strips of iron-on mending fabric (Dritz part No. 55,120–70), being careful to avoid melting any holes in the nylon bag during the sealing process. Fold and seal the bags opposite of their seams to ensure a 3-dimensional space is formed for the resin to circulate in (Fig. 1). AER resin comes in the chloride form and must be converted to the bicarbonate form before use [5,6,14]. To convert the resin, place the completed AER bags into a glass beaker containing 0.5 M NaHCO₃ solution in an amount equal to 100 mL per bag. Stir them from time to time for ½ hour, after which the process is repeated in a fresh 0.5 M NaHCO₃ solution for another half hour. This is followed by three washings with laboratory grade deionized (DI) water; the bags are then stored in the last portion of DI water used. The NaHCO₃ solution from the second treatment can be stored and
used for the first treatment of a subsequent batch of AER bags. AER bags should only be used one time for one sample and then discarded.

**Phosphorus extraction procedure**

All glassware and plasticware are washed with non-phosphorus containing dish soap, rinsed five times with tap water, rinsed with 10% HCl from a squirt bottle, and then rinsed three times with DI water. DI water was laboratory grade and all chemicals were reagent grade.

A 2-L HDPE bottle is filled with sample in the field, placed on ice, returned to the laboratory ASAP, and placed in the refrigerator (analysis ideally starts within 24 h of collection). In the laboratory the capped 2-L container is inverted by hand until the contents are a uniform water-sediment mix and then a 50–100 mL subsample is collected mid-container using a 60-cm³ syringe. The subsample is then passed through a Millex HA 0.45 μm syringe filter into a HDPE sample bottle and frozen, for later analysis of soluble reactive phosphorus (SRP$_{\text{initial}}$). The entire remaining sample’s volume (“Volume A”) is then accurately measured in a large volumetric cylinder, its temperature recorded, and then the sample is returned to the 2-L HDPE bottle and allowed to settle overnight or longer in the refrigerator. The purpose of this last step is to concentrate the suspended sediment.

After settling, the clear supernatant is decanted off using a peristaltic pump and a piece of Masterflex silicon tubing, making sure not to stir up the sediment at the bottom and making sure to record the volume of supernatant removed (“Volume B”). Do not remove supernatant that is not clear; shining a flashlight into the 2-L bottle during this process aids in identifying the demarcation point between supernatant and concentrate. After separation, obtain a 50–100 mL subsample of the supernatant, filter it (0.45 μm), place it in a HDPE bottle, and freeze it (SRP$_{\text{supernatent}}$). Well-settled supernatant should only require one or perhaps one and part of a second 0.45 μm syringe filter to collect the SRP$_{\text{supernatent}}$ sample; if more than two filters are needed settling is not complete and TSS and P are probably being lost to the supernatant.

The remaining sample (comprising sample water and the concentrated sediment) is shaken by hand and left at room temperature for another 10–24 h, during which time additional hand shaking may occur if practicable. “Volume C” is the volume remaining after the supernatant was removed but before the SRP$_{\text{concentrate}}$ sample was collected, and can be computed by simple difference, Volume A minus Volume B.

After the 10–24 h wait, shake by hand the concentrated sample until it is uniform and withdraw a 50–100 mL subsample, filter it (0.45 μm), and freeze it (SRP$_{\text{concentrate}}$) as for the previous subsamples. (This may take a number of 0.45 μm syringe filters.) It is important to assure the water and sediment remain well mixed during the subsampling process. The remaining sample is then processed as follows.
Shake by hand the remaining sample and quickly transfer 40 mL of it to a 50 mL centrifuge tube. A wide-orifice (ca. 5 mm) suction-bulbed pipette works well for this purpose. Again, it is important to assure the sample is well mixed and that the 40 mL subsample is representative of the well mixed sample. Put one AER bag into the centrifuge tube, cap, and shake overnight (19–20 h, 30 rpm) on an end-over-end shaker at room temperature (Fig. 2). This process extracts P from the water and sediment of the sample.

After 19–20 h, remove the AER bag (wear gloves) and set it aside for the moment in a clean glass beaker. Discard the liquid sample from the centrifuge tube and triple rinse the tube and cap with DI water, and shake dry. Rinse the AER bag with DI water to remove any attached sediment until it is clean (usually 2–3 times), fill the centrifuge tube with 39 mL of 0.5 M NaCl solution, place the AER bag back in the centrifuge tube, and cap. Shake the tube on the end-over-end shaker for four hours (room temperature). (The abundant chloride ions compete for exchange sites on the resin which releases the extracted P back into solution.) Next, remove the AER bag from the tube and discard, add 1 mL of 6 M HCl to the salt solution in the tube, and let the tube stand overnight (room temperature, no shaking) with the cap slightly loose. The cap is left loose to allow CO₂ to escape; CO₂ is generated from the acidification of residual HCO₃ ions, and this CO₂ could later disturb P analysis. Finally, transfer the contents of the centrifuge tube to a HDPE bottle (no 0.45 μm filtration is necessary) and freeze it (SRPₐᵉʳ).

The SRP samples may be run inhouse if so equipped, or provided to a qualified external laboratory (the author did the latter). SRP samples (aka dissolved molybdate-reactive P [6]), were analyzed using EPA Method 365.1 [19], by the Montana Department of Health and Human Services Environmental Laboratory (DPHHS lab, Helena, MT) via flow injection analysis (Lachat Quikchem Automated Ion Analyzer). The DPHHS lab’s lower reporting limit for SRP is 1.0 μg/L. Optimal use of the Lachat was determined in consultation with laboratory chemists using SRP samples provided single-blind to the laboratory; these samples were prepared with K₂HPO₄ dried at 105 °C and weighed on a Denver Instrument APX 60 balance (readability 0.1 mg). Phosphorus solutions were volumetrically diluted to prepare different replicate concentrations, and each replicate was then processed via the AER extraction process described earlier. Working with the DPHHS lab, it was concluded that brackish integration [20] provides the most accurate results for all samples, and was critically important for accuracy of the SRPₐᵉʳ samples since they are in a 0.5 M NaCl solution.
Equations

For each sample four SRP concentrations (μg/L) have been obtained: SRP\text{\textsubscript{INITIAL}}, SRP\text{\textsubscript{SUPERNATANT}}, SRP\text{\textsubscript{CONCENTRATE}}, and SRP\text{\textsubscript{AER}}. To derive sediment-bound bioavailable P, a mass balance is used to account for any changes in the particle P-solute P relationship that may have occurred during sample processing.

Note that Volume C is the same for both SRP\text{\textsubscript{CONCENTRATE}} and SRP\text{\textsubscript{AER}}. Also note that different sample volumes have been measured at different temperatures. For Volume A and Volume B this is 2–4 deg C, whereas Volume C is taken at laboratory temperature (about 20 deg C). To assure all volumes are normalized to laboratory temperature, apply a coefficient of volume expansion for water. The equation is: \( \Delta V = \beta V_0 \Delta T \); where \( \Delta V \) = change in volume (mL), \( \beta \approx 0.00013 \) for water in this temperature range, \( V_0 \) is the Volume A or B water volume (milliliters, at 4 deg C), and \( \Delta T \) = difference between 4 deg C and the laboratory’s temperature. The computed additional volumes are added, accordingly, to the cold-measured volumes of Volumes A and B. An Excel spreadsheet has been provided in Supplementary material with these equations (and the equations below) embedded in it.

Sediment-bound bioavailable P (in μg/L) is calculated using the following equations. Eq. (1) has four parts whose purpose is to convert the four concentration-based SRP values to mass units.

\[
\text{SRP}\text{\textsubscript{INITIAL}}(\mu g/L) \times \text{Volume A (L)} = \mu g \ P\text{\textsubscript{INITIAL}} \tag{1a}
\]

\[
\text{SRP}\text{\textsubscript{SUPERNATANT}}(\mu g/L) \times \text{Volume B (L)} = \mu g \ P\text{\textsubscript{SUPERNATANT}} \tag{1b}
\]

\[
\text{SRP}\text{\textsubscript{CONCENTRATE}}(\mu g/L) \times \text{Volume C (L)} = \mu g \ P\text{\textsubscript{CONCENTRATE}} \tag{1c}
\]

\[
\text{SRP}\text{\textsubscript{AER}}(\mu g/L) \times \text{Volume C (L)} = \mu g \ P\text{\textsubscript{AER}} \tag{1d}
\]

Then:

\[
(\mu g \ P\text{\textsubscript{SUPERNATANT}} + \mu g \ P\text{\textsubscript{CONCENTRATE}}) - \mu g \ P\text{\textsubscript{INITIAL}} = X_1(\mu g \ P) \tag{2}
\]

\[
\mu g \ P\text{\textsubscript{AER}} + (X_1 - \mu g \ P\text{\textsubscript{CONCENTRATE}}) = X_2(\mu g \ P) \tag{3}
\]

\[
X_2(\mu g \ P) \div \text{volume A (L)} = \text{sediment–bound Bioavailable P (μg/L)} \tag{4}
\]

Eq. (2) accounts for any changes in the mass of P, plus or minus, resulting from the settling and separation process. Eq. (3) provides the mass of P extracted from the sediment particles, and Eq. (4) provides the concentration of sediment-bound P extracted off the sediment particles. To compute total bioavailable P in the sample (P that was dissolved in the water and P extracted from particles), use Eq. (5).

\[
\text{SRP}\text{\textsubscript{INITIAL}}(\mu g/L) + \text{sediment–bound Bioavailable P (μg/L)} = \text{Total Bioavailable P (μg/L)} \tag{5}
\]

Method validation

Sample contamination caused by reuse of AER bags: The original method [6] calls for reuse of AER bags after re-conversion to the bicarbonate form per techniques in Preparation of anion exchange resin (AER) bags (see above). But later work showed that AER bags used 15 times or more cause progressively worsening P contamination in subsequent samples receiving the used bags [13]. At the very beginning of the project, bags were reused per the original method. However, measurement precision samples (DI water blanks) run with used bags showed, after four bag re-uses, up to 16 μg P/L contamination in the SRP\text{\textsubscript{AER}} Samples; doubling the NaHCO\textsubscript{3} preparation process did not remedy the issue. To better understand the problem, tests were undertaken to determine how quickly and to what degree P cross-contamination occurs. Anion exchange resin contamination was tested by placing
resin that had been used once for field samples in new nylon bags which were then sealed (three replicates). Nylon bag & sealing strip contamination was tested by opening bags which had been used once for field samples, replacing the old resin with new, and then resealing the bags (four replicates). These QC test samples were then processed via the AER extraction method using DI water only. Trace contamination (3–4 μg/L in the SRP_{AER} samples) was observed among some (not all) replicates for both the used resin and the used nylon bags. This indicates contamination begins to occur after only one use and may be sourced to either resin or bags. In light of earlier research [13], and these findings, it was concluded that resin and bags must only be used once in low TSS water samples as described in the protocols above.

Relative percent difference (RPD) of sample duplicates: The AER extraction method has steps for which uncareful collection of a subsample has the potential to alter the sediment-water ratio and cause error in the measurement of sediment-bound bioavailable P. For example, if the 40 mL transferred to the centrifuge tube is not done quickly and carefully (before sediments can resettle) and is not representative of well-mixed water and sediment, errors will occur. Method reproducibility was analyzed by taking individual stream samples and splitting the water-sediment concentrate remaining after the supernatant was removed, and running the remaining steps of the process independently on each split (main, duplicate). Duplicate RPD ranged from 0 to 18% (Table 2) which is considered good since, ideally, duplicate water samples should have RPDs <20% [21].

Percent recovery and quality control check of different Lots of anion exchange resin: Percent recovery of SRP_{AER} samples was evaluated based on standard solutions of K₂HPO₄ prepared as described earlier and measured by the DPHHS lab; samples were provided to the DPHHS lab single-blind. Recovery for SRP_{AER} samples (n = 17) ranged from 90 to 100% (average: 92%) over a range of standard solutions from 9 to 92 μg P/L. In addition, the DPHHS lab routinely carries out matrix spikes on provided samples to check for potential matrix interferences. They carried out matrix spikes on 13 SRP samples from seven of the stream sites and reported percent recoveries of 92–107% (within the laboratory's ±10% limit). (Note, however, that the DPHHS lab's matrix spikes evaluate their SRP analytical method, but do not evaluate matrix interferences that might affect the anion exchange resin during the extraction and release phases carried out under the current method.)

During method development a problem arose that is worth mentioning. In the first year of the study, percent recoveries of standard K₂HPO₄ solutions ranged from 90 to 100%. At the end of the first field season a new Lot of anion exchange resin was purchased and used to carry out post-season QC using standard K₂HPO₄ solutions. These QC samples came back with abnormally low concentrations and calculated recoveries in the 33–88% range. Through process of elimination the problem was identified as the new Lot of anion exchange resin. The Lot with poor recovery had a light brown color whereas the original Lot was a light blonde color. A third Lot of resin was purchased (also light blonde like the original Lot) and, using it, QC samples again came back from the DPHHS lab in expected recovery ranges. Based on this experience, it is recommended that all Lots of anion exchange resin first be tested with standard K₂HPO₄ solutions to ensure SRP_{AER} recoveries are within acceptable ranges prior to proceeding with field samples.

Comparison of the original method vs. the current method: The original method is for water samples with high sediment concentrations [6]. The original method's ability to quantify sediment-bound bioavailable P in low TSS samples was compared to the current method by running both methods

Table 2
Relative percent difference in SRP_{AER} concentrations between splits of the same sample.

| Stream site                      | Field sample TSS concentration (mg/L) | SRP_{AER} (μg/L) Main | SRP_{AER} (μg/L) Duplicate | Relative% difference |
|----------------------------------|--------------------------------------|------------------------|----------------------------|----------------------|
| North Fork Smith River           | 11.5                                  | 21                     | 18                         | 15.4%                |
| Camas Creek                      | 15.5                                  | 16                     | 18                         | 11.8%                |
| Benton Gulch                     | 78.5                                  | 18                     | 15                         | 18.2%                |
| Beaver Creek                     | 61.0                                  | 11                     | 11                         | 0.0%                 |
| Smith River @ Camp Baker         | 63.5                                  | 32                     | 29                         | 9.8%                 |
side by side (Table 3). Five sites with varying TSS were tested; no sediment concentrating step was undertaken for the original method (it equates to SRP_{AER} minus SRP_{INITIAL}). Readers will note that most results for both methods are negative. Slight negative values from Eq. (4) are fairly common and indicate there is no measurable P to be extracted from particles; small analytical variations in the four SRP samples can then produce a negative result in the mass balance calculation. Note also, per the previous subsection, that percent recovery for SRP_{AER} samples is (on average) <100%; this alone would result in slight negative results. For a frame of reference, standard K_{2}HPO_{4} solution spikes and measurement precision samples (DI water blanks), neither of which have sediment, demonstrate a range of sediment-bound bioavailable P values from 0.1 to −4.2 μg/L (average: −0.98 μg/L). Keeping this methodological “noise” in mind, note that the original method provides negative results well below these bounds whereas the current method is essentially within these bounds, and in one stream measurable sediment-bound P was observed using the current method (Table 3). These findings indicate that the concentrating step of the current method allows quantification of sediment-bound bioavailable P which would otherwise not have been detectable using the original method.

**Testing supernatant for lost TSS and phosphorus:** Supernatant removed during sample processing was checked to ascertain if sediment (and associated P) was being lost from field samples due to incomplete settling or poor separation from the concentrate at the bottom of the 2-L bottle. Supernatant from all eight study sites was checked, some of them multiple times. TSS was determined via standard methods [22], while extractable P in supernatant was determined by placing 40 mL of supernatant in a centrifuge tube, adding an AER bag, and carrying the sample through the remaining steps of the AER extraction process. TSS concentrations in the supernatant were low (0–4 mg/L, average 1.8 mg/L), but in 18% of cases AER-extracted P from the supernatant (μg/L) exceeded the SRP_{SUPERNATENT} concentration; in one case this was substantial (SRP_{SUPERNATENT} =18 μg/L, AER-extracted P from the supernatant = 27 μg/L). Based on these findings the recommendations were developed regarding the number of 0.45 μm syringe filters one should expect to use when collecting the SRP_{SUPERNATENT} sample (see Phosphorus extraction procedure above).

**Determination of method detection limit (MDL) for the AER extraction method:** The AER extraction MDL was determined per [23]. Theoretically, an SRP_{AER} result should be equal to or greater than its corresponding SRP_{CONCENTRATE} sample; this is because the resin should extract all the soluble P from the water in the sample plus any additional P it extracts off the sediment particles. An initial review of stream samples processed using the AER extraction method showed it was fairly common to observe SRP_{CONCENTRATE} samples with concentrations in the 3–13 μg/L range but the corresponding SRP_{AER} samples were below the DPHHS lab’s reporting limit of 1.0 μg P/L. Assuming for these cases that the sediment particles had no P to release, the data suggested that once available P fell below ~10–13 μg/L, the AER extraction method is incapable of detecting it. Therefore, standard K_{2}HPO_{4} solution was used to make 11 μg/L replicates and 45 μg/L replicate spikes to compute the MDL based on [23].

### Table 3

| Stream site      | Original Method | This Method | Field sample TSS concentration (mg/L) | TSS concentration achieved in concentrate (mg/L) |
|------------------|-----------------|-------------|---------------------------------------|-----------------------------------------------|
|                  | Sediment-bound Bioavailable P (μg/L) | Sediment-bound Bioavailable P (μg/L) |                        |                                             |
| North Fork Smith River | −5             | 0.2         | 11.5                                  | 41.7                                          |
| Camas Creek      | −10             | −2.4        | 15.5                                  | 59.2                                          |
| Benton Gulch     | −5              | −0.5        | 78.5                                  | 266.6                                         |
| Beaver Creek     | −7              | −3.5        | 61.0                                  | 246.5                                         |
| Smith River @ Camp Baker | −13    | −4.6        | 63.5                                  | 228.5                                         |

Comparison of calculated sediment-bound bioavailable P between the original method [6] and the modified method presented in this article. The last column shows the TSS concentration achieved in the processed sample after the supernatant was removed.
batches of replicate samples were prepared on three different days and provide to the DPHHS lab. The MDL of the AER extraction method equals 13 μg/L (and is based on the MDLs; [23]). In terms of applying data flags to samples, if an SRP AER result is ≥13 μg/L it would not be flagged as below detection. If the SRP AER result is <13 μg/L but its corresponding SRP Concentrate is ≥13 μg/L, matrix interference is suggested but the sample would not be flagged as below detection. If the SRP AER result is <13 μg/L and its corresponding SRP Concentrate is <13 μg/L, it would be flagged as below detection.

**Final considerations**

Phosphorus contamination caused by used AER bags necessitated a means of quickly fabricating bags so that a new AER bag could be used for each sample. The very original protocol called for sewing the bags shut [24], but clearly this is impractical for the current method. Loading the nylon mesh biopsy bags with resin and then sealing them with iron-on strips is fairly fast, and the technique causes no P contamination based on the DI water blanks. Researchers should continue to search for faster and more efficient methods of holding the anion exchange resin in the centrifuge tube incubation vessels.

After the initial SRP sample is taken and after the settling process and removal of the supernatant, the method calls for an additional 10–24 h wait prior to the next processing step. This was included to ensure the SRP concentration in the remaining concentrate has largely stabilized. Upon separation of the supernatant and the concentrate, SRP concentration in the supernatant may not be the same as in the concentrated water-sediment solution at the bottom [25]. In the concentrate, it can take 10–20 h for SRP to come to equilibrium with the concentrated sediment particles and manifest a new, stable solute concentration (see Fig. (5) in [2]). Overall, the modifications to the original method require the sample to spend a considerable amount of time in the 2-L bottle, and loss of P to the walls of the container is a concern [22]. But good % recovery (90–101%) of SRP Concentrate samples relative to the K2HPO4 standard solutions they were taken from indicates the total wait time is not causing substantial loss of P in this manner.

Among the eight study sites (Table 1) and over the course of two spring runoff periods, four sites had measurable sediment-bound bioavailable P, three had no sediment-bound bioavailable P (South Fork Smith River, Newlan and Beaver creeks), and one (Sheep Creek) had samples all below the MDL. It cannot be ruled out that unknown matrix interferences may, in some circumstances, diminish the ability of the anion exchange resin to absorb and/or release P. Inorganic solute concentrations (as indicated by specific conductance; Table 1) in the study sites are far below concentrations that might inhibit the resin [13]. But organic ions may also interact with ion-exchange resins although less is known about this [26]. South Fork Smith River was heavily influenced by groundwater and wetland soils and often had water samples with a distinct yellow-brown color from tannins. All of the site’s sediment-bound bioavailable P concentrations were negative, and several were much more negative than the lower “noise” boundary of −4.2 μg/L discussed earlier; an organic ion matrix interference may be at play here. Some kind of matrix interference is also seen in the original method samples in (Table 3), evidenced by their much more negative results compared to the corresponding modified method samples. The study sites have low TSS (Table 1) and low sediment-to-water ratios; this clearly caused problems for the original method. To minimize any water-based matrix interferences, the evidence suggests it is best to raise the sediment-to-water ratio as high as possible (via settling and decanting off of the supernatant) as is done in the modified method presented here.

The mass balance in the modified method assumes all SRP is bioavailable. While largely true, it is not strictly the case in all circumstances [27]. P extracted by anion exchange resin (here, the SRP AER sample) has been shown to be essentially all bioavailable [9,13]. But if the other samples in the process (SRP Initial; SRP Supernatant; SRP Concentrate) have exaggerated bioavailable P concentrations then the resulting sediment-bound bioavailable P will be underestimated by the mass balance. It is probably safe to conclude that, like other researchers have found [13], the modified method presented in this article produces a conservative (low) estimate of sediment-bound bioavailable P in a water sample.
Selecting the most appropriate method

Below is provided a decision tree to help readers decide if the original method, the modified method in this article, or perhaps some other method is most appropriate for evaluating sediment-bound bioavailable P in their study sites.

Direct submission or co-submission

This paper is a direct submission. Co-submissions are papers that have been submitted alongside an original research paper accepted for publication by another Elsevier journal.

Declaration of Conflict Interests

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

An Excel spreadsheet is provided which includes all the equations presented in this article. It has marked input cells so that users can easily input their data and determine the sediment-bound bioavailable P concentration of their sample.

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.mex.2021.101343.

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