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Storage Procedures Affect pH, Electrical Conductivity, and Nutrient Concentrations of Pour-through Leachate from Pine Bark and Peat-based Substrates

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Abstract. **The pour-through (PT) method is used in greenhouse and nursery production to monitor nutrient availability in soilless substrates. Efficacy of this method is based on the assumption that chemical properties of extracted solutions remain stable from the moment of collection until analysis. Extracted substrate solution can be analyzed directly in the greenhouse or sent to laboratories for complete nutritional analysis; thus, proper sample preservation methods (e.g., filtration and low temperatures) are critical for reducing sample contamination or degradation during storage. However, evidence of how these preservation methods affect chemical characteristics of PT samples is limited. The objective of this study was to evaluate the effect of storage time, storage temperature, and filtration of PT samples on pH, electrical conductivity (EC), and nutrient concentrations from pine bark– and peat-based substrates. PT extracts were obtained from liquid-fertilized fallow pots of either 100% milled pine bark (Expt. 1) or a 4 sphagnum peat: 1 perlite (by volume) substrate (Expt. 2). Aliquots of PT extract were either filtered or nonfiltered and then stored in plastic bottles at −22, 4, or 20 °C. EC, pH, and nutrient concentrations were analyzed at 0, 1, 7, and 30 days after PT sample collection. EC and pH in PT extracts of peat and pine bark, respectively, changed 1 day after collection. Storage time had the greatest effect on nutrient concentrations of samples stored at 20 °C. However, at day 30, nutrient concentrations had also changed in samples stored at 4 and −22 °C. Analytes that fluctuated most in both experiments and across all preservation treatments were dissolved organic carbon, total dissolved nitrogen, NO_3^--N, and PO_4^{3-}-P, whereas Ca^{2+}, Mg^{2+}, and SO_4^{2-}-S were more stable in PT samples. This research suggests EC and pH should be analyzed immediately, whereas samples requiring nutrient analysis should be filtered immediately after collection, stored at 4 or −22 °C (preferably −22 °C), and analyzed within 7 days of collection.**

Nutrient management in greenhouse and nursery production is a key operational practice to ensure availability of nutrients in the substrate and their subsequent utilization for plant growth (Wright, 1986). Three primary methods for monitoring substrate nutritional status of container-grown crops are the saturated media extraction (SME) (Warncke, 1986), 2:1 extraction (Sonneveld and van den Ende, 1971), and PT methods (Wright, 1986). Among these, the most widely adopted by the nursery and greenhouse industries is the PT method, which is a nonintrusive water displacement technique used to monitor pH, EC, and available nutrient concentrations in soilless substrates (Torres et al., 2010). Advantages of the PT relative to SME and 2:1 methods are its nondestructive procedure, simple sample collection (i.e., application of deionized water to the substrate surface and collection of leachate for analysis), and direct interpretation of nondiluted measured analytes (Cavins et al., 2004).

PT extracts can be analyzed immediately in the greenhouse or sent to a laboratory for complete nutritional analysis. The usefulness of PT testing is based on the assumption that chemical properties (e.g., pH, EC, nutrient concentrations) of extracted solutions remain stable from the moment of collection until analysis; thus, steps should be taken to ensure sample integrity is preserved (Gardolinski et al., 2001). Preservation methods are necessary to minimize the physical, chemical, and biological processes that can alter the chemical characteristics of the sample during storage (Gardolinski et al., 2001). The efficacy of preservation methods for water samples depends on the sample matrix (e.g., groundwater, surface water, saline water, or wastewater), filtration, container material and size, storage temperature, and chemical addition (Matthiensen et al., 2013). Preservation methods have been developed for water samples from aquatic systems. However, little research has been done on storage protocols for PT water samples.

PT extracts commonly have pH values between 4.5 and 6.5. Target EC values are 0.5 to 1.5 $mS \cdot cm^{-1}$ for most nursery crops and 2.6 to 5.3 $mS \cdot cm^{-1}$ for most greenhouse crops (Bilderback et al., 2013; Nelson, 2012). These values differ from those measured in aquatic systems, which generally have a neutral pH and EC of less than 0.6 $mS \cdot cm^{-1}$ (Miller et al., 1988; Wieben et al., 2013). Nutrient concentrations in PT extracts are also higher than those found in natural waters. For example, N can range from 50 to 100 $mg \cdot L^{-1}$ in PT samples, whereas N concentrations in surface waters are often between 0.2 and 2.0 $mg \cdot L^{-1}$ (Bilderback et al., 2013; China Ministry of Environmental Protection, 2002). Chemical preservation methods are specific to each analytical technique (e.g., titration, spectroscopy, and chromatography); thus, a separate sample is needed for each analyte (Sliwka-Kaszyńska et al., 2003), which is unpractical when collecting PT samples due to the volume of leachate that would be required. In contrast to chemical preservation methods, physical methods (e.g., refrigerating or freezing) have the advantage of reflecting the original state of the matrix evaluated (Sliwka-Kaszyńska et al., 2003). Refrigerating (2 to 5 °C) and freezing (–20 °C) are the main alternatives to chemical preservation and are broadly applied as storage protocols for aqueous samples (Clementson and Wayte, 1992; Matthiensen et al., 2013). According to Matthiensen et al. (2013), freezing samples is more effective than other storage options because samples may be preserved for months to years. Nevertheless, freezing is not recommended for "hard water" (water containing high concentrations of Ca^{2+} and Mg^{2+}) samples because phosphate (PO_4^{3-}) can coprecipitate with calcite during thawing, resulting in an underestimation of PO_4^{3-} in the subsequent nutrient analysis (Johnson et al., 1975). Filtration is a preliminary treatment routinely applied in water analyses that separates particulate and

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dissolved phases (Matthiensen et al., 2013). PT samples may contain particulate material that could sorb soluble nutrient ions (e.g., dolomite sorption of PO_4^{3-}; Mangwandi et al., 2014; Yuan et al., 2015). Similarly, the effect of storage time on chemical parameters should be evaluated because chemical reactions (e.g., oxidation, reduction, and hydrolysis) can rapidly alter sample chemistry (Sliwka-Kaszyńska et al., 2003).

Studies comparing storage protocols for analyzing nutrients from natural waters (e.g., soil solutions, surface water, and seawater) concluded that the appropriate storage protocol depends on both the sample matrix and the measured analyte (Avanzino and Kennedy, 1993; Fellman et al., 2008; Gardolinski et al., 2001; Haygarth et al., 1995; Wong et al., 2017). No research has addressed the storage of PT extracts from soilless substrates used in greenhouse and nursery production as a matrix. Accordingly, the objective of this study was to evaluate the effect of storage duration, storage temperature, and filtration before storage on pH, EC, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and nutrient ion concentrations of PT samples of pine bark– and peat-based substrates extracts.

Materials and Methods

Experimental design. For pH and EC, the treatment design was a 4 × 2 × 3 factorial that included storage duration (0, 1, 7, and 30 d after collection), filtration (filtered and nonfiltered before storage), and storage air temperature [20 °C (room temperature; RM), 4 °C (refrigerator; RF), and –22 °C (freezer; FZ)]. Because the analytical techniques used for all other measured analytes required filtration before analysis, it was not a preservation treatment for samples analyzed at day 0, and nonfiltered samples were filtered on the day of analysis. Therefore, the treatment design for measured variables except pH and EC was a 3 × 2 × 3 augmented factorial that included storage duration (0, 1, 7, and 30 d after collection), filtration (filtered and nonfiltered before storage), and storage air temperature (RM, RF, and FZ). These factorial combinations of treatments were compared with filtered samples analyzed on day 0 (control). There were seven replicates per treatment combination.

Sample preparation. Milled pine bark (screened to <1.27 cm; T.H. Blue Inc., Eagle Springs, NC), with 37 ± 0.02% SD air space, 79 ± 0.02% SD total porosity, and 0.16 ± 0.002 SD g·cm^{-3} bulk density (n = 3), was amended with 2.97 kg·m^{-3} pulverized dolomite (95.0% $CaCO_3$ equivalent, 21.6% Ca, 10.0% Mg; Soil Doctor, Atlanta, GA) and 0.89 kg·m^{-3} granular micronutrient fertilizer (6.0Ca–3.0Mg–12.0S–0.1B–1.0Cu–17.0Fe–2.5Mn–0.1Mo–1.0Zn; Micromax, Everris, Dublin, OH) on 21 Oct. 2019. Pulverized dolomite had 100%, 95%, 80%, and 70% passing through 2.00-, 0.84-, 0.25-, and 0.15-mm mesh screens. Substrate was mixed using a mortar mixer (Model WM-90S; Multiquip, Cypress, CA).

Twenty 19-L containers (PT-5S; Nursery Supplies Inc., Chambersburg, PA) were filled with the amended pine bark and irrigated with tap water until leaching on the day of potting and again12 h before fertilization. On 30 Oct. 2019 (day 0), each pine bark–filled container was fertilized by handpouring a solution of ammonium nitrate (NH_4NO_3) and monopotassium phosphate (KH_2PO_4) onto the substrate surface until leaching. The fertilizer solution contained 23 mg·L^{-1} N, 16 mg·L^{-1} P, and 21 mg·L^{-1} K. PT extractions (Wright, 1986) were performed 60 min after fertilization. Containers were elevated 2.5 cm above a shallow saucer to collect leachate, and 600 mL of deionized water was applied evenly to the substrate surface. After 20 min of drainage, ≈500 mL leachate was collected from each container, combined into an 18.9-L bucket, and taken to the laboratory. While the leachate was being stirred with a magnetic stir bar, 75-mL aliquots were transferred to 125-mL rectangular plastic bottles (high-density polyethylene; Thermo Scientific, Rochester, NY). Half of the samples were filtered using 0.45-µm polyvinylidene fluoride (PVDF) membranes (Durapore; MilliporeSigma, Burlington, MA) on a vacuum filtration system. Filtered and nonfiltered samples were stored in the previously described rectangular bottles either on a laboratory benchtop at 20 °C, in a refrigerator at 4 °C, or in a freezer at –22 °C.

Sample analysis. Samples were analyzed on day 0, 1, 7, or 30 after collection. Contents within each sample bottle were analyzed only once to avoid freeze–thaw–freeze cycles. Samples stored at –22 °C (FZ) were thawed using a water bath before analysis. Filtered samples were analyzed for pH, EC, DOC, TDN, and nutrient ions (NO_3^--N, PO_4^{3-}-P, K^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-}-S). Nonfiltered samples were analyzed for pH and EC, then subsequently filtered on the day of analysis before analyzing for DOC, TDN, and nutrient ions. A 5-mL aliquot of each sample was transferred into a 15-mL conical tube for pH and EC analyses. Temperature-corrected pH was measured using a benchtop meter (S470 SevenExcellence; Mettler Toledo, Columbus, OH) with an Expert Pro-ISM pH electrode (Mettler Toledo). EC was analyzed with a conductivity meter (S230 SevenCompact; Mettler Toledo) and a 741-ISM electrode. A 25-mL aliquot was analyzed for TDN and DOC using a total N measurement unit and a total organic carbon (C) analyzer (TNM-L ROHS and TOC-L CSN; Shimadzu Scientific Instruments, Columbia, MD). A 10-mL aliquot was analyzed for nutrient ion concentrations using an ion chromatography (IC) system (Dionex ICS-6000; Thermo Scientific, Madison, WI). The IC system used 2 × 250-mm (i.d. × length) anion- and cation-exchange columns (AS19, CS12A, respectively, Thermo Scientific) at 35 °C and an autosampler (Autoselect Polyvial 074228; Thermo Scientific).

The experiment was repeated using the previously described methodology with the following modifications. A substrate comprising 80% sphagnum peat (Sun Gro Horticulture, Agawam, MA) and 20% perlite (Therm-O-Rock East, Inc., New Eagle, PA) (by volume) with 10% ± 0.27% SD air space, 91% ± 1.46% SD total porosity, and 0.11 ± 0.001 SD g·cm^{-3} bulk density (n = 3), was amended with 4.75 kg·m^{-3} pulverized dolomite on 17 Jan. 2020. Twenty-seven 19-L containers were filled with the substrate and irrigated with tap water until leaching on the day of potting. Substrate was fertilized 1 d

Table 1. pH and electrical conductivity (EC) in filtered (F) or nonfiltered (NF) pour-through water samples from a pine bark substrate[z] (Expt. 1). Samples (n = 7) were analyzed immediately after collection (control; day 0) or after being stored at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) for 1, 7, or 30 d.

		pH		EC (mS·cm^{-1})	
Storage temp	Time (d)	F	NF	F	NF
Control	0	6.81***	6.57	0.93	0.89
RM	1	6.89***	6.81***	0.85*	0.85
RF	1	6.92***	6.85***	0.88	0.88
FZ	1	6.88***	6.86***	0.88	0.87
RM	7	6.44**	6.50	0.87	0.88
RF	7	6.88***	6.75***	0.87	0.88
FZ	7	7.06***	6.95***	0.84**	0.87
RM	30	6.17***	6.22***	0.87	0.87
RF	30	6.62	6.63	0.86	0.86
FZ	30	7.06***	6.94***	0.85	0.86

[z]Pine bark was amended with 2.97 kg·m^{-3} dolomitic limestone and 0.89 kg·m^{-3} micronutrient fertilizer (Micromax) and liquid fertilized with 23, 16, and 21 mg·L^{-1} N, P, and K, respectively.

*, **, ***Significantly different from the control (NF at day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate-N (NO_3^--N), and phosphate-phosphorus (PO_4^{3-}-P) concentrations in pour-through water samples from a pine bark substrate[z] (Expt. 1). Samples (n = 7) were filtered and analyzed immediately after collection (control; day 0) or stored as filtered (F) or nonfiltered (NF) at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) and analyzed 1, 7, or 30 d after collection.

		DOC ($mg·L^{-1}$)		TDN ($mg·L^{-1}$)		NO_3^--N ($mg·L^{-1}$)		PO_4^{3-}-P ($mg·L^{-1}$)	
Storage temp	Time (d)	F	NF	F	NF	F	NF	F	NF
Control	0	70.5		3.58		2.15		1.32	
RM	1	65.4	67.1	3.56	3.62	2.13	2.09	1.32	1.31
RF	1	65.7	66.9	3.47	3.56	2.10	2.08	1.31	1.32
FZ	1	66.4	64.7**	3.49	3.52	2.09	2.10	1.32	1.32
RM	7	63.1***	61.4***	3.35***	3.25***	2.02**	1.99***	1.31	1.32
RF	7	66.7	67.4	3.43*	3.55	2.16	2.14	1.31	1.31
FZ	7	66.2	67.7	3.38**	3.44*	2.10	2.04**	1.32	1.31
RM	30	47.5***	43.6***	2.58***	2.46***	1.30***	1.27***	1.23***	1.18***
RF	30	65.8	64.6**	3.36***	3.50	2.14	2.09	1.38***	1.33
FZ	30	65.9	61.6***	3.41**	3.34***	2.13	2.13	1.32	1.33

[z]Pine bark was amended with 2.97 $kg·m^{-3}$ dolomitic limestone and 0.89 $kg·m^{-3}$ micronutrient fertilizer (Micromax) and liquid fertilized with 23, 16, and 21 $mg·L^{-1}$ N, P, and K, respectively.
*, **, ***Significantly different from the control (day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulfate-sulfur (SO_4^{2-}-S) concentrations in pour-through water samples from a pine bark substrate[z] (Expt. 1). Samples (n = 7) were filtered and analyzed immediately after collection (control; day 0) or stored as filtered (F) or nonfiltered (NF) at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) and analyzed 1, 7, or 30 d after collection.

		K^+ ($mg·L^{-1}$)		Ca^{2+} ($mg·L^{-1}$)		Mg^{2+} ($mg·L^{-1}$)		SO_4^{2-}-S ($mg·L^{-1}$)	
Storage temp	Time (d)	F	NF	F	NF	F	NF	F	NF
Control	0	55.0		31.4		38.8		63.7	
RM	1	47.4	45.7**	30.9	29.4*	38.0	36.1**	62.6	60.3**
RF	1	46.9	45.3**	30.4	29.2**	37.4	35.8**	61.6	60.1**
FZ	1	46.7	46.0*	29.7	29.7	36.9	36.5*	60.9*	60.7**
RM	7	47.5	47.1	30.6	30.8	37.5	37.6	61.6	62.1
RF	7	47.9	46.3	32.2	30.3	39.4	37.0	64.0	61.1*
FZ	7	46.6	44.6***	30.0	27.9***	36.7*	34.1***	60.7**	57.5***
RM	30	48.9	48.8	32.5	32.8	37.9	38.6	61.6	61.9
RF	30	49.7	48.0	33.1	31.9	38.9	37.4	62.1	60.3**
FZ	30	49.3	48.6	33.1	33.3*	39.0	39.2	62.2	62.1

[z]Pine bark was amended with 2.97 $kg·m^{-3}$ dolomitic limestone and 0.89 $kg·m^{-3}$ micronutrient fertilizer (Micromax) and liquid fertilized with 23, 16, and 21 $mg·L^{-1}$ N, P, and K, respectively.
*, **, ***Significantly different from the control (day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

before and on 21 Jan. 2020 (day 0) by slowly hand-pouring a 20N–4.4P–16.6K fertilizer solution (Jack's Professional 20–10–20 General Purpose; JR Peters Inc., Allentown, PA) until leaching. The fertilizer solution contained (in $mg·L^{-1}$) 100 N, 21.82 P, 83.02 K, 75 Mg, 0.034 B, 0.018 Cu, 0.250 Fe, 0.125 Mn, 0.005 Mo, and 0.013 Zn. PT extractions were performed as previously described. While the leachate was stirring, 100-mL aliquots were transferred to rectangular plastic bottles. Due to the large quantity of particulate matter in the leachate, filtering was facilitated by first coarse-filtering solutions through a 47-mm-diameter glass microfiber filter (GF/F; Whatman, Maidstone, UK) before passing them through a 0.45-µm PVDF membrane. Samples were stored and analyzed following the same methodology. Samples stored at –22 and 4 °C were brought to room temperature using a water bath before analyses.

Statistical analysis. Data were subjected to analysis of variance. Storage treatments were compared with the control, defined as the nonfiltered (pH and EC) or filtered (DOC, TDN, and nutrient ions) samples analyzed on the day of collection (day 0), using Dunnett's test in JMP Pro 14 (SAS Institute Inc., Cary, NC). Correlations were assessed using Pearson's correlation coefficient (r) to aid in interpreting results.

Results and Discussion

Expt. 1: Pine bark substrate. On day 0, the pH of the control was 6.57, and filtering increased it by 0.24 units (Table 1). On day 1, in all storage and filtration conditions, pH was up to 0.35 units higher than the control. By day 7, pH of nonfiltered samples in RM was similar to the control, whereas filtered samples were lower than the control. Samples stored in RF or FZ, regardless of filtration, had a higher pH than the control. At day 30 of storage, samples stored at RM had lower pH than the control, whereas pH of those stored at RF and FZ was similar to or higher than the control, respectively. The decrease in pH in RM from day 1 to day 30 may have been due to carbonic acid (H_2CO_3) formation because the samples equilibrated with carbon dioxide (CO_2) respired by microbes as well as CO_2 trapped in the headspace above the sample. Water in contact with CO_2 produces carbonic acid, a weak acid that can reduce pH of aqueous solutions (Toews et al., 1995). Decomposition of dissolved organic compounds or suspended solids from the bark substrate in RM samples might also have reduced pH because decomposition of pine wood and bark releases CO_2 (Allison, 1965). Organic substances in samples stored in RF and FZ likely decomposed slower than those in RM, resulting in more stable pH over time. We observed an increase in pH due to vacuum filtration, whereas others have shown that vacuum filtration decreased or had no effect on pH (Cavins et al., 2004; Lang, 1996; Van Lierop, 1990). The reason pH increased from vacuum filtration in our study is unclear. The maximum recommended storage time for determining pH of water samples is 2 h (American Public Health Association, 1992; Environmental Protection Agency, 1987) or 6 h (ISO, 2012). Our conclusions generally agree with these storage recommendations; pH of nonfiltered PT samples should be measured the day of collection because pH had increased by as much as 0.35 units by day 1 of storage.

EC of the control was 0.89 $mS·cm^{-1}$ and was generally unaffected by filtration, storage temperature, and storage time (Table 1). The only two exceptions were filtered RM at day 1 and filtered FZ at day 7, with 5% and 6% lower EC values than the control, respectively.

Dissolved organic C of the control was 70.5 $mg·L^{-1}$ (Table 2). Samples analyzed at day 1 had similar concentrations to the control, except for nonfiltered FZ, which was lower. At day 7, DOC in RF and FZ was similar to the control, while RM had up to 13% lower concentrations. On day 30, DOC in RM samples had decreased by up to 38%, whereas filtered RF and FZ samples were

similar to the control and nonfiltered RF and FZ samples were slightly (<13%) lower. Lower DOC in RM compared with RF and FZ samples may have been due to greater microbial activity and corresponding DOC degradation at the higher temperature storage (Wangersky, 1993). In the current study, DOC had a moderate, positive correlation with pH ($r = 0.67$; $P < 0.0001$). Humic acid solubility has been shown to be positively related to pH (Kipton et al., 1992); therefore, changes in sample pH and corresponding solubility of humic acid, a component of DOC, may partially explain the observed instability of DOC during storage. Sinsabaugh et al. (1986) reported decreased DOC due to coagulation facilitated by iron sulfate, which was present in the micronutrient fertilizer used in our study and may also have contributed to decreased DOC concentrations over time. Dissolved organic C in nonfiltered FZ samples decreased 8% and 13% by days 1 and 30, respectively. Similarly, Spencer et al. (2007) observed a 10% decrease in DOC after freezing surface water samples. Giesy and Briese (1978) stated that freezing water samples with high DOC concentrations (i.e., >5 mg·L^{-1}) can reduce DOC due to humic substance aggregation. For DOC determination, filtration immediately after collection combined with RF and FZ provided the most stable concentrations.

Total dissolved N of the control was 3.58 mg·L^{-1} (Table 2). On day 1, TDN concentrations had not changed relative to the control, regardless of filtration and storage temperature. By day 7, treatments stored at RM, RF, and FZ had lower TDN than the control, except in nonfiltered RF samples. Similarly, Kotlash and Chessman (1998) found that N losses in RM can occur after 2 d. Samples stored at RM, filtered RF, and FZ at day 30 had lower TDN concentrations than the control, which might have been due to volatilization, denitrification, or microbial uptake (Vymazal, 2007). Total dissolved N in nonfiltered samples stored in RF remained similar throughout the experiment, providing evidence that refrigeration is an effective preservation method when measuring TDN (Fishman et al., 1986). Likewise, Yorks and McHale (2000) found N concentrations were stable for 8 weeks when soil water samples were stored at 2 to 4 °C.

Nitrate-N (NO_3^--N) on day 0 was 2.15 mg·L^{-1} and remained stable through day 1 for all treatments (Table 2). Compared with the control, NO_3^--N stored at RM decreased 7% by day 7 and 40% by day 30 when averaged across filtered and nonfiltered samples. Total dissolved N and NO_3^--N had a strong, positive correlation ($r = 0.92$; $P < 0.0001$), and NO_3^--N was between 50% and 64% of TDN. Accordingly, changes in TDN were likely due to changes in NO_3^--N. Samples were likely microbially enriched because of the high nutrient concentrations (Kotlash and Chessman, 1998). As a result, processes mediated by microorganisms, such as denitrification, might have proceeded faster in samples stored at RM compared with those stored at RF and FZ, thus decreasing NO_3^--N concentrations (Burghate and Ingole, 2013; Kotlash and Chessman, 1998). Nitrate-N in RF and FZ samples was stable throughout the experiment (30 d), except in nonfiltered FZ on day 7, in which NO_3^--N decreased. This contrasts with a study by Yorks and McHale (2000), which found that NO_3^--N decreased slightly in soil water samples stored in refrigeration for 7 or 21 d.

Phosphate-P (PO_4^{3-}-P) concentration was 1.32 mg·L^{-1} at day 0 (Table 2) and remained stable through day 7 regardless of filtration or storage temperature. PO_4^{3-}-P decreased (<11%) by day 30 in samples stored at RM, probably due to algal or bacterial uptake, the formation of insoluble phosphate precipitates (e.g., $MnHPO_4$ and $Ca_5[PO_4]_3OH$), or both (Gardolinski et al., 2001; Lambert et al., 1992; Shreckhise et al., 2019). PO_4^{3-}-P concentrations generally remained stable for the duration of the experiment (30 d) when stored at RF or FZ; filtered RF was an exception, with a 5% higher PO_4^{3-}-P concentration than the control. In other studies, refrigeration preserved filtered reactive phosphorus (FRP) in surface water samples for 28 d (Gardolinski et al., 2001), and freezing preserved FRP concentrations for 4 to 8 years (Avanzino and Kennedy, 1993). This study, in agreement, averaged only a 2% changes in PO_4^{3-}-P across all treatments, with a maximum decrease of 11% by day 30 in nonfiltered samples stored at RM temperature.

Potassium concentration in the control was 55.0 mg·L^{-1} (Table 3). The higher PT K^+ concentration compared with that supplied by the fertilizer solution (i.e., 21 mg·L^{-1}) was likely contributed by the pine bark substrate (Koch, 1972). At day 1, K^+ in filtered samples stored at RM, RF, and FZ were similar to the control, whereas nonfiltered samples had lower K^+ concentrations. At day 7, K^+ was similar to the control except in nonfiltered FZ samples in which concentrations were lower. By day 30, K^+ remained unchanged compared with the initial concentration in all storage temperatures and filtration treatments. In general, K^+ concentrations decreased minimally (i.e., <10 mg·L^{-1}), which agrees with Bull et al. (1994), who found that K^+ loss was small (4.5% decrease) for surface water samples stored at RM for more than 90 d. Filtration before storage was more consistent across all timepoints evaluated and therefore would be a slightly more effective preservation pretreatment for K^+ determination.

Calcium concentration in the control was 31.4 mg·L^{-1} and was stable in filtered samples over the course of the experiment (30 d) regardless of storage temperature (Table 3). On day 1, nonfiltered samples stored at RM or RF had ≈2 mg·L^{-1} lower Ca^{2+} concentrations than the control. Nonfiltered, FZ samples had lower Ca^{2+} concentrations than the control at day 7 but higher than the control at day 30. A previous study found that storing water samples in RM and FZ decreased Ca^{2+}, whereas RF increased it (Bull et al., 1994). However, Ca^{2+} concentrations in the current study showed no clear pattern. Considering the recommended range for Ca^{2+} concentrations in PT extracts is 20 to 40 mg·L^{-1} (Bilderback et al., 2013), the 3.55 mg·L^{-1} (i.e., 11%) decrease relative to the control observed in nonfiltered FZ samples would not likely alter the interpretation from a substrate fertility standpoint.

Magnesium concentration for the control at day 0 was 38.8 mg·L^{-1} (Table 3). At day 1, Mg^{2+} concentrations in nonfiltered samples had decreased regardless of storage temperature, whereas filtered samples were similar to the control. On day 7, filtered and nonfiltered samples stored in FZ had up to 12% lower Mg^{2+} concentrations, whereas samples stored in RM and RF were similar to the control. On day 30, Mg^{2+} in all samples was equivalent to the control, regardless of storage temperature and filtration treatment. Similarly, Bull et al. (1994) reported that Mg^{2+} in surface water samples showed no clear pattern during storage. However, because Mg^{2+} concentrations were generally stable in the filtered samples, our results suggest that filtration is an adequate pretreatment for storing PT samples with respect to Mg^{2+} concentration.

Sulfate-S (SO_4^{2-}-S) in the control was 63.7 mg·L^{-1} (Table 3). On day 1, SO_4^{2-}-S in nonfiltered samples in all storage temperatures and filtered FZ samples was lower than the control, whereas filtered samples stored at RM or RF had similar concentrations to the control. On day 7, filtered and nonfiltered samples stored at RM and filtered samples at RF were similar to the control, whereas nonfiltered RF and FZ samples, regardless of filtration, were lower than the control. On day 30, SO_4^{2-}-S was similar to the control in all but nonfiltered RF samples. Regardless of treatment, changes in SO_4^{2-}-S were minor, with concentrations generally within 5% of the control. The only exception was nonfiltered samples at day 7 stored at FZ (10% lower than the control). In a study that evaluated SO_4^{2-}-S in natural waters, filtrating resulted in stable SO_4^{2-}-S concentrations during storage, but the effect of storage temperature on SO_4^{2-}-S was unclear (Bull et al., 1994). Our results generally agree with this study in that SO_4^{2-}-S was similar to the control in samples filtered before storage in RM or FZ.

Expt. 2: Peat-based substrate. The pH of the control on day 0 was 6.79 and filtering increased pH by 0.28 units when analyzed on the same day (Table 4), a similar response to that observed in Expt. 1. By day 1, filtered RF and nonfiltered FZ had lower pH, whereas the other treatments had similar pH values to the control. The pH of samples at day 7 was similar to the control, regardless of filtration and storage condition. On day 30, pH was equivalent to the control in all storage conditions except filtered RM samples (which was 1.15 units lower). As discussed for Expt. 1, this reduction in pH may have been due to CO_2 release and subsequent carbonic acid formation during microbial decomposition of dissolved organic compounds (Wang et al.,

Table 4. pH and electrical conductivity (EC) in filtered (F) or nonfiltered (NF) pour-through water samples from a peat-based substrate[z] (Expt. 2). Samples (n = 7) were analyzed immediately after collection (control; day 0) or after being stored at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) for 1, 7, or 30 d.

		pH		EC ($mS·cm^{-1}$)	
Storage temp	Time (d)	F	NF	F	NF
Control	0	7.07**	6.79	0.83	0.84
RM	1	6.64	6.60	0.77*	0.75***
RF	1	6.56*	6.59	0.73***	0.73***
FZ	1	6.59	6.56**	0.75***	0.77**
RM	7	6.59	6.73	0.76**	0.77**
RF	7	6.67	6.80	0.76**	0.76**
FZ	7	6.78	6.81	0.78*	0.76**
RM	30	5.64***	6.62	0.62***	0.70***
RF	30	6.78	6.87	0.64***	0.64***
FZ	30	6.94	6.87	0.64***	0.63***

[z]Peat-based substrate was amended with 4.75 $kg·m^{-3}$ dolomitic limestone and liquid fertilized with 100, 21.82, and 83.02 $mg·L^{-1}$ N, P, and K, respectively.
*, **, ***Significantly different from the control (NF at day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5. Dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate-N (NO_3^--N), and phosphate-phosphorus (PO_4^{3-}-P) concentrations in pour-through water samples from a peat-based substrate[z] (Expt. 2). Samples (n = 7) were filtered and analyzed immediately after collection (control; day 0) or stored as filtered (F) or nonfiltered (NF) at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) and analyzed 1, 7, or 30 d after collection.

		DOC ($mg·L^{-1}$)		TDN ($mg·L^{-1}$)		NO_3^--N ($mg·L^{-1}$)		PO_4^{3-}-P ($mg·L^{-1}$)	
Storage temp	Time (d)	F	NF	F	NF	F	NF	F	NF
Control	0	222.7		29.2		17.7		4.25	
RM	1	219.4	211.7**	29.7	27.7**	17.1	17.8	3.99*	3.71***
RF	1	229.2	220.0	30.0	28.4	16.9	15.9	3.96**	3.80***
FZ	1	224.0	212.2**	29.6	27.9*	16.7	16.5	3.98*	3.90**
RM	7	192.7***	196.1***	30.0	28.6	14.9*	9.9***	4.00*	4.18
RF	7	205.3***	205.4***	28.9	28.2	18.0	15.8	4.03	3.76***
FZ	7	217.5	209.4***	29.6	28.4	18.5	15.9	4.34	3.73***
RM	30	177.2***	156.1***	28.2	24.6***	19.5	16.4	3.74***	3.84***
RF	30	203.1***	191.8***	29.0	25.5***	15.8	17.0	3.99*	3.67***
FZ	30	226.4	210.6***	28.7	27.1***	18.1	15.1	4.04	3.63***

[z]Peat-based substrate was amended with 4.75 $kg·m^{-3}$ dolomitic limestone and liquid fertilized with 100, 21.82, and 83.02 $mg·L^{-1}$ N, P, and K, respectively.
*, **, ***Significantly different from the control (day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

2013). Microbial activity and concomitant carbonic acid production are limited in lower temperatures (Wang et al., 2013). Generally, pH varied more in pine bark samples (≈0.28 units averaged across all treatments) than in sphagnum peat samples (≈0.19 units), as indicated by more significant differences detected in samples derived from pine bark. Nonetheless, pH stability in Expt. 2 was improved by RF or FZ.

EC of the control was 0.84 $mS·cm^{-1}$, which was similar to the EC of filtered samples at day 0 (Table 4). After day 0, EC was consistently between 8% and 26% lower than the control value and numerically decreased with time. These results contrast those from Expt. 1, during which EC in pine bark leachate was generally the same as in the control. EC in Expt. 2 had a moderate, positive correlation with K^+ (r = 0.50; P = 0.0002), Mg^{2+} (r = 0.49; P = 0.0002), and Ca^{2+} (r = 0.67; P < 0.0001), all of which have been shown to strongly influence EC in natural waters (McCleskey et al., 2012). Changing concentrations of these nutrient ions may therefore have had a role in the decreasing EC in Expt. 2. However, we cannot rule out the possible impact on EC of other ions that were likely present in samples by not measured in this study (e.g., Cl^-, HCO_3^-, CO_3^{2-}, Fe^{2+}, etc.).

Dissolved organic C in the control was 222.7 $mg·L^{-1}$ (Table 5). At day 1, DOC in all filtered treatments and in nonfiltered RF was similar to the control, whereas nonfiltered samples at RM and FZ had decreased slightly. Dissolved organic C decreased at days 7 and 30 in filtered and nonfiltered samples stored at either RM or RF. Dissolved organic C deviated furthest from the control in nonfiltered samples on day 30, with decreases of 30% in RM, 14% in RF, and 5% in FZ. The high concentration of DOC, relative to bark in Expt. 1, can be attributed to C accumulation in peat, as decomposed peats have been shown to contain high DOC (Boron et al., 1987; Kern et al., 2017). A lower lignin content in peat (5% to 40%; Boron et al., 1987) compared with bark (40% to 55%; Pan et al., 2013) may also have resulted in higher DOC in Expt. 2 since lignin is resistant to microbial breakdown and thus retards decomposition (Berg and Staaf, 1981; Godshalk and Wetzel, 1978). Unlike in Expt. 1, DOC concentrations in PT samples from peat decreased over time in filtered RF samples. This decrease may be linked to the higher concentration of DOC, which resulted in a higher decomposition rate even in samples stored in RF. On the basis of results from both experiments, pine bark– and peat-based PT extracts collected for DOC analysis should be filtered immediately and either analyzed within 1 d of collection or stored in FZ for later analysis.

Total dissolved N in the control was 29.2 $mg·L^{-1}$ and concentrations remained stable over time if filtered before storage, regardless of storage temperature (Table 5). By day 1, nonfiltered samples stored at RM and FZ had lower TDN concentrations relative to the control, whereas RF samples were similar. At day 7, TDN in nonfiltered samples was similar to the control at all storage temperatures. In nonfiltered samples at day 30, TDN had decreased by 5 $mg·L^{-1}$ (16%) in RM, 4 $mg·L^{-1}$ (13%) in RF, and 2 $mg·L^{-1}$ (7%) in FZ. Chemical and biological processes that can reduce TDN in water samples stored at RM (i.e., volatilization, denitrification, and microbial uptake) are dependent on N concentration (Kotlash and Chessman, 1998). Thus, the high concentration of TDN in Expt. 2 PT samples compared with those found in Expt. 1 could explain the decreases observed in nonfiltered samples in all storage temperatures.

Nitrate-N concentration in the control was 17.7 $mg·L^{-1}$ and, similar to Expt. 1, was stable over time in filtered or nonfiltered RF and FZ samples (Table 5). The only treatments with lower NO_3^--N than the control were filtered and nonfiltered samples at day 7 stored at RM, which had 16% and 44% lower NO_3^--N concentrations, respectively. Nitrate losses may have been due to biological denitrification. Levels of denitrification increase with increasing temperature due to greater activity of denitrifying organisms and faster enzyme reaction rates (Burghate and Ingole, 2013; Holtan-Hartwig et al., 2002; Saleh-Lakha et al., 2009). In contrast to pine bark

Table 6. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulfate-sulfur (SO_4^{2-}-S) concentrations in pour-through water samples from a peat-based substrate[z] (Expt. 2). Samples (n = 7) were filtered and analyzed immediately after collection (control; day 0) or stored as filtered (F) or nonfiltered (NF) at 20 °C (room temperature; RM), 4 °C (refrigerator; RF), or –22 °C (freezer; FZ) and analyzed 1, 7, or 30 d after collection.

		K^+ (mg·L^{-1})		Ca^{2+} (mg·L^{-1})		Mg^{2+} (mg·L^{-1})		SO_4^{2-}-S (mg·L^{-1})	
Storage temp	Time (d)	F	NF	F	NF	F	NF	F	NF
Control	0	2.86		27.4		42.5		12.3	
RM	1	2.71	2.52***	28.8**	28.8**	44.6**	44.4*	13.2***	12.8
RF	1	2.69*	2.63**	29.2***	28.5*	44.9**	44.0	13.1***	12.7
FZ	1	2.72	2.71	29.0**	28.6*	44.8**	44.0	13.1**	12.8
RM	7	2.99	3.15***	29.0**	29.4***	44.9**	45.2***	13.1**	13.0**
RF	7	3.19***	3.06**	28.0	27.9	44.0	43.8	12.6	12.5
FZ	7	3.17***	3.00	29.3***	28.1	45.4***	43.5	12.9**	12.4
RM	30	2.56***	2.76	25.4***	25.8**	42.0	42.5	13.1**	13.1**
RF	30	2.64**	2.59***	26.0**	25.1***	42.9	41.6	12.5	12.0
FZ	30	2.70*	2.83	26.4	25.5***	43.1	42.0	12.5	11.8

[z]Peat-based substrate was amended with 4.75 kg·m^{-3} dolomitic limestone and liquid fertilized with 100, 21.82, and 83.02 mg·L^{-1} N, P, and K, respectively.
*, **, ***Significantly different from the control (day 0) by Dunnett's test at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 7. Recommended protocols for storing leachate pour-through (PT) extracts from pine bark– and peat-based substrates that will ultimately be analyzed for pH, electrical conductivity (EC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and nutrient ion concentrations [nitrate-N (NO_3^--N), phosphate-phosphorus (PO_4^{3-}-P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulfate-sulfur (SO_4^{2-}-S)].

Analyte	Filtration before storage	Recommended storage temp[z]	Maximum recommended storage time
pH	Not required	RF	Same day
EC	Not required	RF	Same day
DOC	Yes	FZ	7 d
TDN	Yes	RF, FZ	7 d
NO_3^--N	Yes	RF, FZ	7 d
PO_4^{3-}-P	Yes	RF, FZ	30 d
K^+	Yes[y]	RF, FZ	30 d
Ca^{2+}	Yes	RF, FZ	30 d
Mg^{2+}	Yes	RF	30 d
SO_4^{2-}-S	Yes[y]	RF, FZ	30 d

[z]RF (refrigerator; 4 °C); FZ (freezer, –22 °C).
[y]Effective for PT samples from pine bark substrate but was not as effective for those from peat-based substrate.

extracts from Expt. 1, changes in TDN in Expt. 2 were not correlated with changes in NO_3^--N ($r = –0.03$; $P = 0.7022$).

PO_4^{3-}-P in the control was 4.25 mg·L^{-1} (Table 5). On day 1, PO_4^{3-}-P was an average of 0.36 mg·L^{-1} lower (8% decrease) in all samples, regardless of storage temperature and filtration. At day 7, nonfiltered RM, filtered RF, and filtered FZ had equivalent PO_4^{3-}-P concentrations to those from day 0, whereas all other treatments were lower. At the end of the experiment (day 30), PO_4^{3-}-P had decreased by up to 15%, except in filtered samples stored at FZ, which were similar to the control. Others have shown that PO_4^{3-}-P in surface waters was stable in RF and FZ (Avanzino and Kennedy, 1993; Gardolinski et al., 2001). In contrast to Expt. 1, PO_4^{3-}-P showed no clear pattern under different storage conditions; however, filtering combined with FZ was the most stable storage protocol evaluated for samples stored up to 30 d.

Potassium concentration on day 0 was 2.86 mg·L^{-1} (Table 6). At day 1, filtered RM and filtered and nonfiltered samples stored at FZ had equivalent K^+ concentrations to the control, whereas nonfiltered RM and RF, regardless of filtration, were 9% lower. At day 7, K^+ concentrations in all but filtered RM and nonfiltered FZ samples were ≈0.28 mg·L^{-1} higher (10%) than the control. On day 30, K^+ was similar to the control only in nonfiltered RM and nonfiltered FZ, whereas the remaining samples had decreased K^+. Despite these significant differences, K^+ concentrations were consistently within 12% of the control; as such, K^+ losses during storage would not likely change the interpretation of a PT nutrient analysis. Not filtering combined with FZ was the most effective preservation method. Lower K^+ concentrations in PT extracts compared with the fertilizer solution (i.e., 83.0 mg·L^{-1}) may have been due to peat adsorption of K^+ on cation exchange sites. Peat has a high cation exchange capacity (50 to 160 cmol·L^{-1}) (Puustjarvi and Robertson, 1975), which facilitates adsorption of dissolved solids such as metals and polar organic molecules (Brown et al., 2000; Yahya and Rosebi, 2010).

Calcium in the control was 27.4 mg·L^{-1} (Table 6). By day 1, Ca^{2+} had increased by an average of 1.40 mg·L^{-1} (5%) in all storage temperatures, regardless of filtration. On day 7, Ca^{2+} had increased in nonfiltered and filtered samples at RM and filtered samples at FZ, whereas nonfiltered RF and FZ samples had similar concentrations to the control. At day 30, samples in all storage temperatures had ≈2 mg·L^{-1} (7%) lower Ca^{2+} concentrations than the control, except in filtered FZ, which was not affected by storage time. Calcium concentrations in this study varied more than those from Expt. 1, suggesting Ca^{2+} is less stable in extracts from a peat-based compared with a pine bark–based substrate over time. Precipitation of Ca^{2+} compounds such as hydroxyapatite [$Ca_5(PO_4)_3OH$] may have been partially responsible for lower Ca^{2+} concentrations than the control at day 30 (Shreckhise et al., 2019). As was also discussed for Expt. 1, these changes in Ca^{2+} are not likely horticulturally important because of their small magnitude (i.e., <2.4 mg·L^{-1} in all treatments) relative to target concentrations in PT extracts of greenhouse substrates (>200 mg·L^{-1} Ca) (Cavins et al., 2008).

Magnesium in the control was 42.5 mg·L^{-1} (Table 6). On day 1, filtered samples in all storage temperatures and nonfiltered samples at RM had ≈2 mg·L^{-1} higher Mg^{2+} concentrations than the control. By day 7, Mg^{2+} had increased by up to 2.75 mg·L^{-1} (6%) in samples at RM, regardless of filtration, and 3 mg·L^{-1} (7%) in filtered samples at FZ. Magnesium concentrations at day 7 in RF were not different from the control. By day 30, Mg^{2+} concentrations were equivalent to the control in all samples, regardless of storage temperature and filtration. These results differ from the decrease in Mg^{2+} observed in Expt. 1. The increase in Expt. 2 compared with Expt. 1 may be related to the inclusion of perlite in the substrate, as perlite has been shown to increase water soluble Mg^{2+} concentrations (Silber et al., 2010). Bull et al. (1994), likewise, found higher Mg^{2+} concentrations in surface water samples stored for 21 d relative to those measured on the day of collection. However, they concluded that changes in cations, including Mg^{2+}, during storage were generally minor.

SO_4^{2-}-S concentration in the control was 12.3 mg·L^{-1} (Table 6). On day 1, filtered samples, regardless of storage temperature, had up to 7% higher SO_4^{2-}-S than the day 0 control, whereas nonfiltered samples did not change. By day 7, SO_4^{2-}-S in filtered and nonfiltered samples at RM and filtered samples at FZ had increased, whereas SO_4^{2-}-S in the other samples were unchanged. On day 30, regardless of filtration, samples stored at RM had ≈6% higher SO_4^{2-}-S than the control, whereas samples at RF and FZ were equivalent to the control. These results concur with Bull et al. (1994), who observed an increase (<26%) in SO_4^{2-}-S in untreated water samples stored at RM temperature at 21 d of storage. Bull et al. (1994) also concluded that filtration improved stability of SO_4^{2-}-S during storage, which agrees with results from Expt. 1. However, filtration did not stabilize SO_4^{2-}-S concentrations in PT samples from peat-based substrate. Changes in SO_4^{2-}-S concentrations were small (<7% increase), suggesting that storage would not likely affect interpretation when performing nutrient analysis on PT samples.

Conclusions

PT extract storage protocols that were effective in both experiments are summarized for each analyte in Table 7. EC and pH in PT extracts of peat and pine bark, respectively, changed within 1 d of collection; thus, we recommend pH and EC be analyzed immediately. This can be readily accomplished using commercially available pH and EC meters that are easy to operate and rugged enough to be used in greenhouse and nursery conditions. The analytes that fluctuated most (i.e., >15%) in at least one of the experiments after 30 d of storage were DOC, TDN, NO_3^--N, and PO_4^{3-}-P, for which we recommend PT samples be filtered immediately after collection, stored at refrigeration or freezing temperatures (preferably freezing), and analyzed within 7 d from the time of collection. Calcium, Mg^{2+}, and SO_4^{2-}-S were generally stable in both experiments at day 30 of storage, remaining within 10% initial concentrations; however, filtration and low temperature storage minimized fluctuations in concentrations of these nutrient ions. Further research should investigate the effects of storage protocols on pH, EC, and nutrient ions of acidic PT extracts.

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