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## Turbidimetric Determination of Anionic Polyacrylamide in Low Carbon Soil Extracts

Jihoon Kang, Tyler D. Sowers, Owen W. Duckworth, Aziz Amoozegar, Joshua L. Heitman, and Richard A. McLaughlin\*

Concerns over runoff water quality from agricultural lands and construction sites have led to the development of improved erosion control practices, including application of polyacrylamide (PAM). We developed a quick and reliable method for quantifying PAM in soil extracts at low carbon content by using a turbidimetric reagent, Hyamine 1622. Three high-molecular weight anionic PAMs differing in charge density (7, 20, and 50 mol%) and five water matrices, deionized (DI) water and extracts from four different soils, were used to construct PAM calibration curves by reacting PAM solutions with hyamine and measuring turbidity development from the PAM–hyamine complex. The PAM calibration curve with DI water showed a strong linear relationship ( $r^2 = 0.99$ ), and the sensitivity (slope) of calibration curves increased with increasing PAM charge density with a detection limit of 0.4 to 0.9 mg L<sup>-1</sup>. Identical tests with soil extracts showed the sensitivity of the hyamine method was dependent on the properties of the soil extract, primarily organic carbon concentration. Although the method was effective in mineral soils, the highest charge density PAM yielded a more reliable linear relationship ( $r^2 > 0.97$ ) and lowest detection limit (0.3 to 1.2 mg L<sup>-1</sup>), compared with those of the lower charge density PAMs (0.7 to 23 mg L<sup>-1</sup>). Our results suggest that the hyamine test could be an efficient method for quantifying PAM in environmental soil water samples as long as the organic carbon in the sample is low, such as in subsurface soil material often exposed at construction sites.

THE USE OF polyacrylamide (PAM) has gained wide acceptance for reducing soil erosion and improving runoff water quality in agricultural fields and construction sites. The primary functions of PAM application are two-fold: (i) a flocculating agent for suspended materials in aqueous suspension and (ii) an aggregating agent for soil conditioning. Application of PAM for land management has proven to be effective and economical in stabilizing soil structure, reducing soil erosion, and improving runoff water quality (Hayes et al., 2005; Sojka et al., 2007). As its use has become more widespread, determination of PAM concentration in soil water (e.g., runoff water, groundwater, soil solution) has also become increasingly important to better understand the fate and transport of PAM in the environment (Lu and Wu, 2003).

Polyacrylamide is a broad class of synthetic organic polymers formed by the polymerization of acrylamide (AMD) units, and it can be synthesized to have specific chemical properties, including net charge (anionic, neutral, or cationic), charge density (% hydrolysis), and molecular weight. Cationic PAMs are usually avoided in environmental applications due to their potential aquatic toxicity (Barvenik, 1994). High-molecular weight, linear, anionic PAMs are used for soil and water conservation purposes as they adsorb to soil irreversibly through linkages between the anionic groups and the negatively charged soil constituents by exchangeable cation bridges (Seybold, 1994). Whereas PAM is stable and nontoxic, the residual AMD monomers raise concerns as a neurotoxin and a skin irritant (Rudén, 2004). Commercial-grade PAMs for water treatment have <0.05% AMD monomer, which is considered to be a safe level when used at low concentrations (Barvenik, 1994; Sojka et al., 2007). Nonetheless, potential concerns highlight the need for reliable analytical methods for the quantification of PAM in environmental samples.

Lu and Wu (2003) reviewed the existing analytical methods for quantifying aqueous PAM concentration. They emphasized that any method for quantifying PAM in soil waters should be sensitive at low concentrations (<1 mg L<sup>-1</sup>) and insensitive to

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**Abbreviations:** AMD, acrylamide; CPM, Coastal Plain muck; CPS, Coastal Plain sand; DI, deionized; EC, electrical conductivity; NTU, nephelometric turbidity units; OM, organic matter; PAM, polyacrylamide; PCL, Piedmont clay loam; PSL, Piedmont sandy loam; TOC, total organic carbon.

interferences such as dissolved salts and organic matter (OM). The analytical methods for aqueous PAM are generally based on (i) the chemical properties of amide groups in PAM assessed by N-bromination of the amides (Scoggins and Miller, 1979; Lu and Wu, 2001); (ii) the physical properties of the PAM solution determined by viscosity measurement (Jungreis, 1981), flocculation-based methods (Lentz et al., 1996), or size exclusion chromatography (Hunt et al., 1988); (iii) the combined physical and chemical properties determined by turbidimetric methods (Allison et al., 1987) or polarography (Smith-Palmer et al., 1988); and (iv) methods based on total organic carbon (TOC) concentration and radioactive labeling (Ben-Hur et al., 1992; Entry et al., 2008). Most of these methods are complicated procedures that may require expensive laboratory equipment. The TOC measurement is considered to be the simplest method, but it is not selective for PAM as the analytical signal responds to all dissolved organic substances (Lu and Wu, 2003). An alternate and equally simple analytical procedure based on turbidimetry may provide an improved method to quantify PAM in solution with higher selectivity, if the interferences from salts and OM are minimal (Taylor and Nasr-El-Din, 1994).

The turbidimetric method for quantifying PAM uses a reagent that reacts with AMD subunits in anionic PAMs to produce insoluble colloids that remain suspended in solution, giving rise to turbidity. Hyamine 1622 (benzethonium chloride; hereafter referred to as hyamine) is the most commonly used turbidimetric reagent for anionic PAMs (Michaels and Morelos, 1955; Crummett and Hummel, 1963; Wimberley and Jordan, 1971; Allison et al., 1987). When a large anionic polyelectrolyte reacts with a large cationic molecule such as hyamine, optical density determined by a turbidimeter or a spectrophotometer is proportional to the concentration of PAM in solution. Previous studies determined that the methods based on hyamine had detection limits of 0.5 to 1 mg L<sup>-1</sup> for dissolved PAM in either deionized (DI) water or oil-field brines (Crummett and Hummel, 1963; Allison et al., 1987; Taylor and Nasr-El-Din, 1994). These results suggest that the hyamine test may be useful for quantifying PAM in environmental soil water samples.

The objective of this study was to evaluate the hyamine test for determining PAM concentration in soil extract samples. We first established PAM calibration curves based on measured turbidity after the addition of hyamine in DI water matrix. Then we evaluated the hyamine test in the same manner for PAM detection in four extracts of soils with differing physical and chemical properties. The results are discussed in terms of the test's analytical sensitivity and detection limit in quantifying PAM in the soil extracts and its overall applicability to soil water samples.

## Materials and Methods

### Polyacrylamide Materials

Three high-molecular weight (15–16 Mg mol<sup>-1</sup>) anionic PAMs (Superfloc, Kemira Chemicals, Inc.) with a range of charge density were selected for study (Table 1). Stock solutions of PAM (1.0 g L<sup>-1</sup>) were prepared by adding granular PAM to DI water and stirring for at least 24 h at room temperature. A 3.5% w/v hyamine solution was prepared by dissolving 3.5 g

**Table 1. Properties of anionic polyacrylamide (PAM) formulations used in this study.**

PAM	Molecular weight	Charge density
	Mg mol <sup>-1</sup>	mol%
A150	16	50
A120	15	20
A100	15	7

of benzethonium chloride (Hyamine 1622; Acros Organics) in 100 mL of DI water.

### Soil Extract Preparation

Four North Carolina soils, a Piedmont clay loam (PCL), a Piedmont sandy loam (PSL), a Coastal Plain sand (CPS), and a Coastal Plain muck (CPM), were used to represent a range of textures and organic matter contents (Table 2). Three were mineral soils with high clay (PCL), high silt (PSL), or high sand (CPS) contents, and one was mineral-organic soil with a high OM content (CPM). The CPM was surface soil (0–15 cm) from an agricultural field, and the other three were fill soil materials from construction sites (surface and subsurface mixed). All soil materials were air-dried and passed through a 2-mm sieve. Soil extracts were obtained by equilibrating each soil with DI water for 24 h at 1:20 soil-to-solution ratio in an end-to-end shaker. After equilibration, the suspensions were centrifuged and the supernatant was decanted for use in experiments and further analysis. The soil extracts were analyzed for Al, Fe, Ca, Mg, K, and Na by inductively coupled plasma atomic emission spectroscopy (PerkinElmer), TOC with a Shimadzu carbon analyzer (Shimadzu Corp.), and electrical conductivity (EC) with an EC Tester (Oakton Instruments). The results are presented in Table 3.

### Turbidimetric Method Using Hyamine (Hyamine Test)

Solutions containing a range of PAM concentrations (0.5, 1, 2.5, 5, 10, 25, and 50 mg L<sup>-1</sup>) were prepared in either DI water or soil extract. A 10-mL aliquot of PAM solution at each concentration was transferred to a 15-mL conical tube and 100 µL of hyamine solution was then added to the tube. The sample was mixed by shaking for 10 s. After a 5-min reaction (waiting) time, the turbidity was measured using a portable turbidimeter (LaMotte Company) in nephelometric turbidity units (NTU). Our preliminary results in DI water indicated that PAM

**Table 2. Selected soil properties of Piedmont clay loam (PCL), Piedmont sandy loam (PSL), Coastal Plain sand (CPS), and Coastal Plain muck (CPM).**

Property	Soil material			
	PCL	PSL	CPS	CPM
Texture	clay loam	sandy loam	sand	sandy loam
Sand† (g kg <sup>-1</sup> )	412	524	900	685
Silt† (g kg <sup>-1</sup> )	219	339	50	285
Clay† (g kg <sup>-1</sup> )	369	137	50	30
pH‡	5.3	5.1	7.1	4.4
Organic matter§ (g kg <sup>-1</sup> )	9.5	4.4	1.5	143.6

† Particle size analysis by hydrometer method.

‡ pH by a glass electrode at 1:1 soil-to-solution ratio.

§ Organic matter by loss-on-ignition.

concentrations  $\leq 0.5 \text{ mg L}^{-1}$  did not yield enough turbidity on hyamine addition to be distinguished from blank solutions.

## Polyacrylamide Calibration Curve and Method Validation

Using the turbidity readings obtained by the hyamine–PAM reaction, PAM calibration curves (plots of PAM concentration vs. turbidity) were constructed individually as a factorial combination of three anionic PAMs, five solution compositions (DI water and four soil extracts), and seven concentration levels (0.5, 1, 2.5, 5, 10, 25, and  $50 \text{ mg L}^{-1}$ ). All combinations were performed in triplicate and the mean values were used to develop the PAM calibration curves.

For the analytical method validation, we determined signal detection limit, minimum detectable concentration ( $C_{\text{md}}$ ), linearity, and sensitivity. Signal detection limit ( $y_{\text{dl}}$ ) is the highest analytical signal (NTU) when replicates of a sample containing no analyte are tested, and it was determined by (Harris, 2007)

$$y_{\text{dl}} = y_{\text{blank}} + 3\sigma \quad [1]$$

where  $y_{\text{blank}}$  is the mean turbidity signal (NTU) of blank solutions containing hyamine but no PAM ( $n = 8$ ) and  $\sigma$  is the standard deviation ( $n = 8$ ) of turbidity in low PAM concentration samples ( $0.5 \text{ mg L}^{-1}$ ). Assuming a Gaussian distribution of the raw analytical signals from blank samples, the  $y_{\text{dl}}$  represents 99% of the observed values and the remaining 1% represents a response that could be produced by a sample containing a very low concentration of analyte (Armbruster and Pry, 2008). The corrected signal ( $y_{\text{sample}} - y_{\text{blank}}$ ) was used to construct a PAM calibration curve by

$$y_{\text{sample}} - y_{\text{blank}} = m \times C \quad [2]$$

where  $y_{\text{sample}}$  is the mean turbidity signal (NTU) of PAM solutions reacted with hyamine ( $n = 3$ ),  $m$  is the slope of the linear calibration curve, which is a measure for the sensitivity of the calibration curve, and  $C$  is PAM concentration ( $\text{mg L}^{-1}$ ) in the sample. Data were subjected to regression analysis using the least squares method. For each regression model, coefficient of determination ( $r^2$ ) and residual standard deviation (RSD) were estimated to assess the linearity and deviation of the data from the fitted regression line by using the data analysis package within Microsoft Excel 2010 (Kuss, 2003). Significance of the linear regression model was determined with  $p$  value for the  $F$  test in the analysis of variance. The  $C_{\text{md}}$ , often called as detection limit, was obtained by substituting  $y_{\text{dl}}$  from Eq. [1] for  $y_{\text{sample}}$  in Eq. [2]:

$$C_{\text{md}} = 3\sigma / m \quad [3]$$

## Results and Discussion

### Polyacrylamide Calibration Curves in Deionized Water Matrix

All anionic PAMs yielded strong linear relationships between turbidity and PAM concentration with the  $r^2 \geq 0.99$  (Fig. 1; Table 4). As seen in the increasing slopes, the sensitivity of the hyamine test increased with increasing charge density of the anionic PAMs. The  $C_{\text{md}}$  for the highest charge density

Table 3. Selected chemical properties of soil extracts.

Property	Soil extract†			
	PCL	PSL	CPS	CPM
EC‡ ( $\mu\text{S/cm}$ )	17	13	46	80
TOC§ ( $\text{mg L}^{-1}$ )	1.9	2.9	4.2	60
Al ( $\text{mg L}^{-1}$ )	< 0.01	3.00	3.00	2.30
Fe ( $\text{mg L}^{-1}$ )	< 0.01	1.50	1.90	0.31
Ca ( $\text{mg kg}^{-1}$ )	1.20	0.08	7.00	5.30
Mg ( $\text{mg kg}^{-1}$ )	0.48	0.20	0.44	1.70
K ( $\text{mg kg}^{-1}$ )	0.19	0.50	0.63	3.60
Na ( $\text{mg kg}^{-1}$ )	0.49	2.00	0.34	0.92

† PCL, Piedmont clay loam; PSL, Piedmont sandy loam; CPS, Coastal Plain sand; CPM, Coastal Plain muck.

‡ Electrical conductivity.

§ Total organic carbon.

PAM ( $0.4 \text{ mg L}^{-1}$ ) was less than half that of the PAM with the lowest charge density ( $0.9 \text{ mg L}^{-1}$ ). Our results are thus in agreement with Allison et al. (1987), who found similar dependence of sensitivity on charge density of anionic PAMs in brine solutions.

### Polyacrylamide Calibration Curves in Soil Extracts

The background turbidity in soil extracts (i.e., with no PAM added and before the hyamine addition) varied by soil materials. For instance, PCL extract had the lowest background turbidity ( $2.2 \pm 0.3 \text{ NTU}$ ) whereas those of other

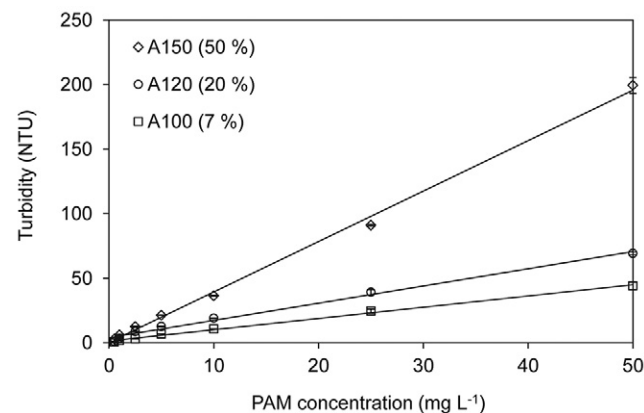


Fig. 1. Polyacrylamide (PAM) calibration curves in deionized water. Charge density (mol%) for each PAM formulation is shown in parenthesis. Error bars are the standard error of the three replicates. NTU, nephelometric turbidity units.

Table 4. Hyamine method analytical parameters in deionized water for each polyacrylamide (PAM) formulation.

	PAM		
	A150	A120	A100
Minimum detectable signal $y_{\text{dl}}$ (NTU)†	2.3	1.8	1.4
Minimum detectable concentration $C_{\text{md}}$ ( $\text{mg L}^{-1}$ )	0.4	0.8	0.9
Sensitivity (regression slope $m \pm \text{SE}$ , $\text{NTU L mg}^{-1}$ )	$3.91 \pm 0.06$	$1.44 \pm 0.07$	$0.90 \pm 0.02$
Coefficient of determination ( $r^2$ )	0.99***	0.99***	0.99***
Residual standard deviation (NTU)	3.74	4.02	1.47

\*\*\* Statistical significance of regression model at the 0.001 level.

† NTU, nephelometric turbidity units.

**Table 5. Turbidity (mean ± SD) of blank samples in deionized (DI) water and soil extracts before and after hyamine addition.**

Hyamine	Turbidity				
	DI water	Soil extract†			
		PCL	PSL	CPS	CPM
			NTU		
Before	0.1 ± 0.2	2.2 ± 0.3	11.7 ± 0.3	8.3 ± 0.4	10.5 ± 2.2
After	0.6 ± 0.3	2.7 ± 0.4	22.5 ± 1.6	25.9 ± 0.9	298 ± 35

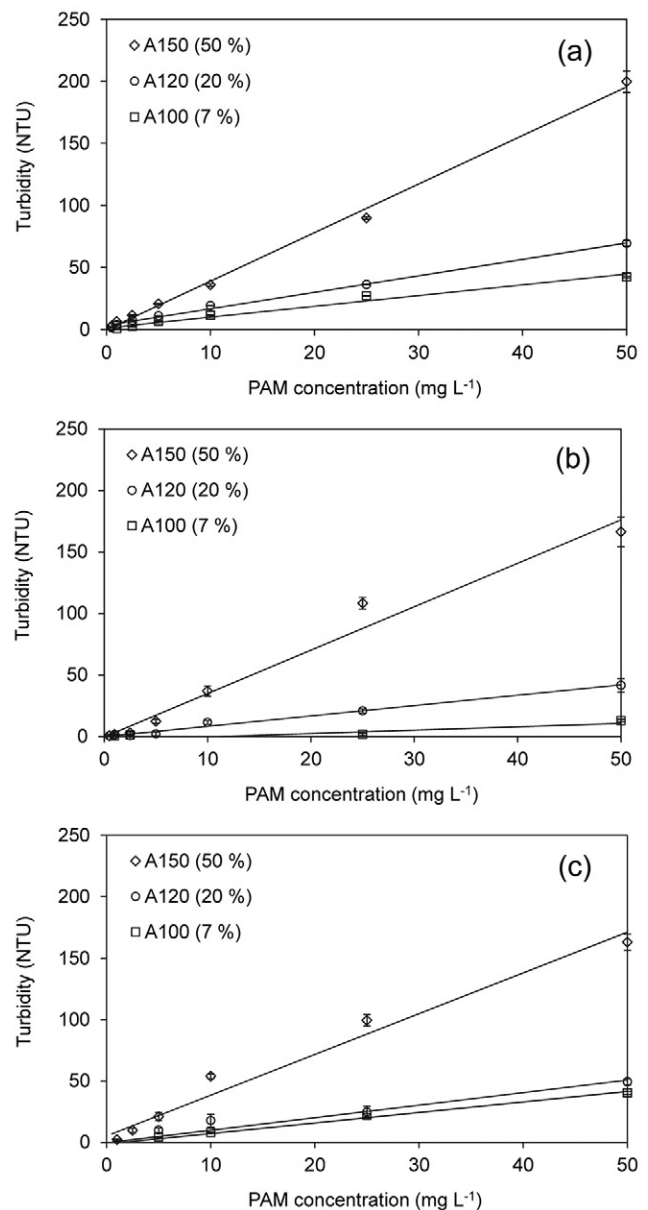
† PCL, Piedmont clay loam; PSL, Piedmont sandy loam; CPS, Coastal Plain sand; CPM, Coastal Plain muck.

soil extracts ranged from 8.3 to 11.7 NTU (Table 5). The relatively higher background turbidity in PSL and CPS than PCL was most likely attributed to the difference in chemical/mineralogical composition, including differences in clay-sized fraction (McLaughlin and Bartholomew, 2007) and increase in the TOC in the suspension (Lu and Wu, 2001). Upon addition of hyamine, PSL and CPS extracts in the absence of PAM yielded two- to threefold greater readings in turbidity compared with their background turbidity. The extreme case was observed with the CPM blank solution, which yielded 30-fold greater turbidity development compared with its background turbidity (Table 5). High TOC in the CPM extract ( $60 \text{ mg L}^{-1}$ ) likely reacted with hyamine in a manner analogous to PAM, producing high turbidity, which could interfere with PAM detection. This result implied that the hyamine test is only useful in solutions with relatively low organic carbon, as discussed below more in detail. The sensitivity (as shown by the slope  $m$ ) of the hyamine test varied not only with the charge density of PAMs but also with the type of mineral soil extract (PCL, PSL, and CPS) (Fig. 2; Table 6). For example,  $m$  for A150 PAM ranged from 3.47 to 3.90 ( $\text{NTU L mg}^{-1}$ );  $m$  for the PCL was the greatest and comparable to the  $m$  in DI water ( $3.91 \text{ NTU L mg}^{-1}$ ). As seen in trials in DI water (Fig. 1), the sensitivity of the hyamine method in soil extracts decreased with decreasing charge density. The method sensitivity in PSL and CPS was diminished compared with that of DI water, particularly with A120 and A100 PAMs. By comparing the values across soil extracts, it appears the high charge density PAM (A150) was least affected by soil extract types, having a consistently low  $C_{\text{md}}$  value of 0.3 to  $1.2 \text{ mg L}^{-1}$ .

There was no evidence of a turbidity response to increasing PAM concentrations in the CPM extract (Fig. 3). High blank turbidity in the CPM extract developed from reaction of hyamine with preexisting organic carbon (Table 5) overwhelmed the turbidity response derived from dissolved PAM. The soil extracts from mineral soils all contained  $<5 \text{ mg L}^{-1}$  TOC, whereas the CPM extract had  $60 \text{ mg L}^{-1}$  TOC (Table 3). Furthermore, the blank turbidity caused by hyamine in mineral soil extracts (2.7–25.9 NTU) trended with measured TOC in the extracts (Table 5), suggesting that there is a maximum TOC concentration beyond which interference from TOC renders the test not useful. One approach to handling higher TOC from organic-rich soils would be to dilute the extract, which we did with the CPM extract (Fig. 4). Once the dilution reached 25 times, or  $2.4 \text{ mg L}^{-1}$  TOC (i.e., 25 times diluted from  $60 \text{ mg L}^{-1}$ ), the CPM + hyamine solution yielded  $<20 \text{ NTU}$ , similar to the other soils. However, this would raise the  $C_{\text{md}}$  by the same factor, which may be unacceptable for applications needing greater sensitivity.

## Conclusions

Rapid, simple, and inexpensive determination of PAM in solution could be a very useful tool for determining its fate in environmental applications. Our results suggest that the turbidimetric method by hyamine is relatively simple and efficient in determining PAM concentration in soil extracts



**Fig. 2. Polyacrylamide (PAM) calibration curves in soil extracts: (a) Piedmont clay loam; (b) Piedmont sandy loam; and (c) Coastal Plain sand. Charge density (mol%) for each PAM is shown in parenthesis. Error bars are the standard error of the three replicates. NTU, nephelometric turbidity units.**

**Table 6. Hyamine method analytical parameters in soil extracts for each polyacrylamide (PAM) formulation.**

PAM formulation	Piedmont clay loam			Piedmont sandy loam			Coastal Plain sand		
	A150	A120	A100	A150	A120	A100	A150	A120	A100
Minimum detectable signal $y_{dl}$ (NTU)†	3.9	3.7	4.8	24.2	25.8	27.5	30.0	33.2	33.1
Minimum detectable concentration $C_{md}$ (mg L <sup>-1</sup> )	0.3	0.7	2.4	0.5	4.0	22.7	1.2	7.2	8.8
Sensitivity (regression slope $m \pm SE$ , NTU L mg <sup>-1</sup> )	$3.90 \pm 0.07$	$1.42 \pm 0.06$	$0.90 \pm 0.04$	$3.52 \pm 0.17$	$0.84 \pm 0.03$	$0.18 \pm 0.08$	$3.47 \pm 0.18$	$1.01 \pm 0.08$	$0.82 \pm 0.03$
Coefficient of determination ( $r^2$ )	0.99***	0.99***	0.98***	0.99***	0.99***	0.44*	0.98***	0.96***	0.99***
Residual standard deviation (NTU)	4.14	3.15	2.53	9.82	1.63	4.81	10.4	4.90	1.94

\* Statistical significance of regression model at the 0.05 level.

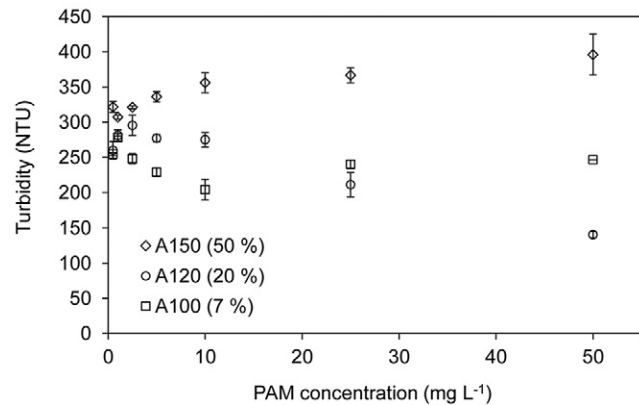
\*\*\* Statistical significance of regression model at the 0.001 level.

† NTU, nephelometric turbidity units.

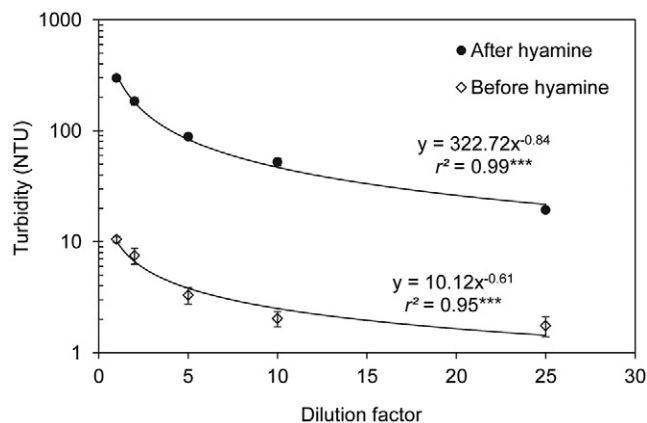
with low organic carbon (<5 mg TOC L<sup>-1</sup>). The hyamine test was not sensitive to PAM in the soil extracts with higher TOC (60 mg L<sup>-1</sup>).

The results have significant implications for the application of this method for determining PAM in soil water samples. Because dissolved organic carbon appears to be a problematic interference, the method may be poorly suited to use for runoff samples containing high organic carbon,

such as those found in agricultural settings. In contrast, the method has higher sensitivity and lower detection limit in extracts from mineral soils. This suggests that the method may be well suited for the quantification of PAM in runoff or leachate from construction sites, which often have low OM subsoils exposed at the surface. Because of the dependence of the signal on soil types, developing PAM calibration curves for the soil and anionic PAM of interest using hyamine test will be necessary for the determination of PAM in soil water samples from impacted areas.



**Fig. 3. Polyacrylamide (PAM) calibration data in the Coastal Plain muck extract for three PAMs (A150, A120, and A100). Charge density (mol%) for each PAM is shown in parenthesis. Error bars are the standard error of the three replicates. NTU, nephelometric turbidity units.**



**Fig. 4. Turbidity development in Coastal Plain muck extract affected by dilution before and after hyamine addition. Error bars are the standard error of the three replicates. Note a logarithmic scale on the Y axis. NTU, nephelometric turbidity units.**

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