



## Research article

## Measurement of lead complexation by humic acids and humic acid analogues using competitive ligand exchange

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## ABSTRACT

In soil and water, metal complexation by humic substances has been demonstrated to have great importance in determining the bioavailability of many trace metals including lead. The binding of lead by humic acids has important implications for lead toxicity, and remediation. In this study we demonstrate the use of 4-(2-pyridylazo)-resorcinol (PAR) as a competitive ligand for the purpose of determining sorption isotherms for  $Pb^{2+}$  on humic acids and humic acid analogs. Equilibration rates at pH 8 were very rapid and the sorption isotherms measured were fit to the Langmuir isotherm equation and values for the stability constants ( $K_{HA}$ ) and complexing site concentrations ( $L_t$ ) are reported. At a PAR concentration of 0.24 mM and humic acid concentrations of 25–200 mg/L, the mass normalized  $\log K_{HA}$  values for the humic substances ranged from 7.2 to 7.9, while the  $\log L_t$  values ranged from  $-2.8$  to  $-3.8$ . At lower PAR concentrations both  $K_{HA}$  and  $L_t$  tended to increase in magnitude indicating the role of the PAR concentration in establishing the measurement window for the interaction of  $Pb^{2+}$  with humic materials.

## 1. Introduction

The occurrence of lead (Pb) in air, water and soil remains a pervasive environmental problem and a potential health issue. Because lead has a long history of civil and industrial applications, including its use in plumbing, paint and fuel, it is widely distributed in the environment. A recent literature review has illustrated the continued wide dispersal of lead in soil, water and air, with concentrations in all media tending to be significantly higher in urban areas (Frank et al., 2019). The wide distribution of lead contamination persists despite heightened environmental awareness and the worldwide discontinued use of lead additives in gasoline. In this study we have focused our attention on characterizing lead binding by various humic acids in order to better understand the role of humic acids in controlling lead speciation. In the natural environment it is likely that other ligands would compete with humic acids and certainly other metals may compete with  $Pb^{2+}$  for humic acid binding sites. However, the purpose of this study was to focus on  $Pb^{2+}$  interaction with humic acids in the presence of a single well-defined competing ligand (PAR).

The nature of humic and fulvic acids remains a controversial topic because of their heterogeneous composition and diverse origins. Early studies envisioned humic and fulvic acids as high molecular weight

polyelectrolytes, while recent work indicates that humic acids are a collection of relatively low molecular mass components that form dynamic associations in solution that are a strong function of pH, ionic strength and concentration (Klučáková, 2018; Sutton and Sposito, 2005). Despite their ambiguous nature, it is clear that humic substances are polyelectrolytes that can play a major role in controlling chemical speciation of metals in water and soils, by interacting with metals principally through available carboxylic acid and phenolic functional groups which is enhanced by an electrostatic contribution.

The toxicity and bioavailability of lead in the environment is highly dependent on chemical speciation and is often considered in light of the Biotic Ligand Model (BLM). It is generally assumed that the free metal cation (i.e.  $Pb^{2+}$ ) concentration is an important index of toxicity for elements such as copper, lead and cadmium. The BLM provides a mechanistic paradigm for metal toxicity and utilizes metal speciation calculations to calculate the free ion activity and couples this calculation to uptake by a “biotic ligand”. For fish species the biotic ligand is the sodium or calcium protein channels of the fish gill surface. For other organisms the biotic ligand may not be well defined, but it is assumed the biotic ligand can be modeled in the same way (Mebane et al., 2020; Di Torro et al., 2001). Therefore, the need for elucidating the chemical speciation of metals including their interaction with humic acid in order

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to calibrate these types of models is of paramount importance (Markich et al., 2001; Peijnenburg et al., 2007). In many studies the formation of humic and fulvic acid complexes appeared to decrease heavy metal toxicity. However, Sánchez-Marín et al. (2007) observed increased toxicity for dissolved lead in the presence of humic acids, with two bioassays that involved marine invertebrates.

Various analytical approaches have been used to quantify the interaction of metals with humic materials. These methods include ultraviolet and visible spectroscopy, fluorescence spectroscopy, electrochemistry, and competitive ion exchange equilibrium with well characterized ion exchange resins (Schnitzer and Hansen, 1970; Baker and Khalili, 2005; Pandey et al., 2000; Brown et al., 1999; Pinheiro et al., 1994). García-Mina et al. (2004), García-Mina (2006) and Liu and Gonzalez (2000) utilized ultra-filtration methods to address speciation.

Competitive ligand approaches have been implemented using spectroscopic and electrochemical methods as well as selective solvent extractions. Whitby and van den Berg (2015) measured copper binding by humic materials in seawater by competition with salicyladoximine. The copper salicyladoximine complex was detected by cathodic stripping voltammetry (CSV). Fischer and van den Berg (2001) characterized lead complexation by natural organic matter in a lake by ligand competition with calcein-blue. Christl et al. (2005) utilized anodic stripping voltammetry (ASV), competitive ligand exchange and ion selective electrode (ISE) methods to study the interaction of lead and copper over a broad range of humic acid concentrations. Xue and Sunda (1997) compared competitive ligand exchange with CSV and an ISE for determining the speciation of  $\text{Cu}^{2+}$  in lake water. Logan et al. (1997) utilized ion selective  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  electrodes to measure complexation by peat and humic acids. Buffle et al. (1977) also investigated the complexation properties of humic and fulvic acids with lead and copper using an ISE. Tuschall and Brezonik (1983) compared continuous flow ultrafiltration, ISE and the use of salicylate as a competing ligand to address copper binding to natural organic matter in swamp water. Van Ginneken and Blust (2000) measured conditional stability constants for cadmium and humic acids using competitive ligand equilibration and solvent extraction. While Benoit et al. (2001) and Han and Gill (2005) used competitive ligand exchange with solvent extraction to address mercury binding by dissolved organic matter from the Florida everglades. Steinberg et al. (2020) utilized competition between 4-(2-pyridylazo) resorcinol (PAR) and humic substances to assess gadolinium ( $\text{Gd}^{3+}$ ) complexation.

Many investigators have used ASV to measure trace metals in the environment and to probe the interaction of trace metals with humic materials. Botelho et al. (2001) measured Pb speciation in a polluted river using differential pulse anodic stripping voltammetry (DPASV). Greter et al. (1979) and Buffle and Greter (1979) made a detail analysis of the factors that influence the behavior of trace metals and humic and fulvic acids on a mercury electrode. Adsorption of surface-active substances like humic materials can complicate the interpretation of electrochemical methods, especially when higher concentrations (10–100 mg/L) are examined (Liu and Gonzalez, 2000). Padan et al. (2021) presented a modified ASV method that utilized triton x-100 to suppress the adsorption of humic acids onto the electrode.

Our previous investigation utilized differential pulse polarography (DPP) to measure the interaction between lead and humic acids and various humic acid analogs (Steinberg and Hodge 2020). In the presence of humic acids, the DPP peak maximum for reduction of  $\text{Pb}^{2+}$ , in the presence of humic acids or humic acid analogs, was shifted to more negative values when compared to humic free solutions. The shift was a function of the concentration of the humic acid or the humic analog. This electrochemical behavior was rationalized using the Lingane equation (Lingane, 1941). These measurements indicated that  $\text{Pb}^{2+}$  in aqueous solution was in rapid equilibrium with humic acids.

We investigated the interaction of  $\text{Gd}^{3+}$  with several organic ligands and with humic acid by ligand competition, using (PAR) (Steinberg et al., 2020). PAR forms brightly colored complexes with many metals that are

easily quantified by absorption spectroscopy. In the current work we have used a similar approach to elucidate and quantify the complexation of  $\text{Pb}^{2+}$  by humic acids and humic acid analogs using ligand competition with PAR.

In addition to commercially available humic materials, polyacrylic acid and tannic acid were also examined. Polyacrylic acid was used by Marinsky and co-workers to develop a polyelectrolyte model of humic acid (Marinsky and Ephraim, 1986). The interaction of metals with polyelectrolytes such as polyacrylic acid has been a subject of interest for many years. The interaction of  $\text{Cu}^{2+}$  with polyacrylate has been investigated by spectroscopic and polarographic methods (Wall and Gill, 1954).

Polyacrylate has been investigated by Gregor et al. (1955) using titration (pH) in the presence of  $\text{Cu}^{2+}$ . Their results show very strong interaction with  $\text{Cu}^{2+}$  and it was likely that carboxylates were participating in metal binding. They compare their polyacrylate results to glutaric acid which showed much weaker interaction. This observation implies that the electrostatic contribution to binding could be very important for metals interacting with polyelectrolytes. This electrostatic component of metal-humate interaction has been discussed in depth by Tipping (1998) and (2002). Esteban et al. (1990) investigated the behavior of  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  in polyelectrolytes using DPASV. These observations have implications for metal remediation in contaminated soils, as several workers have suggested that synthetic polyelectrolytes may be useful for removing metals from contaminated soils (Saur et al., 2004; Torres and DeVarennes, 1998). Similarly, humic acid soil amendments have been demonstrated to reduce metal uptake by plants (Rong et al., 2020).

We also examined the behavior of tannic acid with  $\text{Pb}^{2+}$ . Tannic acid is a naturally occurring polyelectrolyte. The phenolic structures of tannic acid (gallic acid units) are likely similar to structures that occur in humic materials. Tannins and tannic acids are natural products that occur in many foods and show strong interactions with metals. For example, foods high in tannic acid are known to inhibit iron adsorption (South and Miller, 1998). Cruz et al. (2000) used voltammetric analysis to investigate heavy metal binding by tannic acid. They demonstrated that  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  form labile complexes with tannic acid. Kraal et al. (2006) investigated the interaction of  $\text{Cu}^{2+}$  with tannic acid using an ISE. These results have implications for  $\text{Pb}^{2+}$  bioavailability in soil and water, as soil organic matter undoubtedly possesses some functionalities in common with tannic acid.

## 2. Materials and methods

### 2.1. Source materials and chemicals

Water was prepared by redistilling house deionized water using a glass still. All reagents used were of ACS grade or better and employed without further purification.  $\text{Pb}^{2+}$  standards were prepared by dilution of 1000 ppm atomic absorption standards purchased from VWR Scientific (Visalia, CA). A commercially available leonardite humic acid was obtained from Tera Vita (Lancaster, PA). This commercially available humic material is marketed as a soil amendment (Tera Vita product information). A second leonardite humic acid, Pahokee Peat humic acid, Elliot Soil Humic acid and Suwannee River NOM were obtained from the International Humic Substances Society (IHSS). The physical and chemical properties of these humic acids are listed on the IHSS website (<https://humic-substances.org/#products>). Size exclusion chromatography of these materials was discussed in our previous publication (Steinberg and Hodge, 2020). With the exception of the Elliot Soil humic acid, the mass averaged molecular weights were around 1000–2000. Number average molecular weights were considerably lower and indicate the presence of low molecular weight components in the humic acids. Sodium polyacrylate (NaPAA) with an estimated molecular weight of 5100 g/mol was purchased from Aldrich Chemical Company. Tannic Acid with a

molecular weight of 1701.23 g/mol was obtained from Alfa Aesar. The humic acids, tannic acid and polyacrylic acid were dissolved buffer using gentle sonication.

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased from Sigma Aldrich. Buffer concentrations were 0.1 M in all experiments. The buffer pH values were adjusted to 8. 6 M HCl and 10 M NaOH purchased from Baker were used to adjust pH. The pH of the solution was monitored and verified be within 0.02 pH units of the prescribed pH with a pH meter calibrated with NIST standard buffers. This HEPES buffer was used for all  $\text{Pb}^{2+}$ -binding experiments.

Disodium ethylenediaminetetraacetic acid, trisodium nitrilotriacetic acid and disodium iminodiacetate monohydrate were purchased from Tokyo Chemical Industry (TCI). The hydration state of the disodium iminodiacetate was verified by titration with standardized HCl. Other low molecular weight organic acids were of analytical quality and used without any further purification. PAR was purchased from Sigma Aldrich. PAR solutions of 1.20 mM were prepared gravimetrically in the 0.10 M HEPES buffer.

## 2.2. Instrumentation

An Ocean Optics SD2000 fiber optic UV-VIS spectrophotometer with a DT-Mini 26 s deuterium tungsten light source was used to perform all measurements. A quartz cuvette with a 1.0 cm pathlength was used for all measurements. The cuvette holder for this instrument was magnetically stirred at 300 rpm using a magnetic stirrer equipped with a digital tachometer (VWR Scientific). The temperature of the cuvette was maintained at 25 °C with a circulating water bath (VWR Scientific).

## 2.3. Measurements

Measurement of the Pb-PAR response factors as performed as follows. A 2.400 mL volume of 0.100 M pH 8 HEPES buffer was added to the cuvette followed by 0.600 mL of the 1.2 mM PAR solution which is also made up in 0.1 M HEPES. The cuvette was allowed to equilibrate at 25 °C for 10 min and then a  $\text{Pb}^{2+}$  standard solution was added in 1.0–2.0  $\mu\text{L}$  aliquots using a micropipette. The mixture was allowed to equilibrate for 5 min in between additions, although a steady absorbance reading was generally achieved in less than 1 min. This addition resulted in a linear increase absorbance with  $\text{Pb}^{2+}$  concentration. The slope a plot of Pb-PAR absorbance at 510 nm against the concentration of  $\text{Pb}^{2+}$  corresponded to the absorptivity of the Pb-PAR complex and a response factor for Pb-PAR.

For ligand competition experiments a concentrated solution of the ligand (varied with ligand) was prepared in the buffer. The ligand was then added to the cuvette (in microliter volumes). Concentrations of the ligands were adjusted so as to limit the volume addition to less than 25  $\mu\text{L}$  in order to minimize dilution. Again, the solution was allowed to equilibrate for 5 min between each ligand addition. For the tannic acid and humic acid experiments a stock solution containing 100 mg/L to 500 mg/L of the polyelectrolyte was prepared in 0.100 M HEPES buffer. These stock solutions were then diluted with 0.100 M buffer to obtain various humic acid or polyelectrolyte concentrations in solution. A 2.400 mL volume of the humic (or tannic) acid solution was pipetted into the cuvette and mixed with 0.600 mL of the 1.2 mM PAR. After temperature equilibration the mixture was titrated with the  $\text{Pb}^{2+}$  stock solution as described above. The Pb-PAR complex was measured using the response factor determined during the calibration. It was assumed that the speciation of  $\text{Pb}^{2+}$  in these experiments was dominated by interaction with PAR and with the competing ligand. The decrease in Pb-PAR concentration in the presence of an added ligand was interpreted as  $\text{Pb}^{2+}$  binding by the competing ligand. Although 5 min was allowed between each  $\text{Pb}^{2+}$  addition, steady readings were achieved in less than 1 min.

Humic Acid  $\text{Pb}^{2+}$  sorption data were fit to the isotherm equations using CurveExpert Professional Software 2.6.5 (Hyams Development).

## 3. Results and discussion

The addition of  $\text{Pb}^{2+}$  to the HEPES buffered aqueous solution in the presence of  $2.4 \times 10^{-4}$  M PAR is illustrated in Figure 1. These linear plots were used to determine the response factor for PbPAR that was used for calculating the concentration of PbPAR.

In order to properly interpret the competition between PAR and the humic materials it was necessary to determine the stoichiometry of the  $\text{Pb}^{2+}$ -PAR complex. The method of continuous variations (Job's Plot) was performed as follows. A solution of  $1.20 \times 10^{-4}$  M solution of PAR in 0.100 M HEPES buffer, and a separate solution of  $1.20 \times 10^{-4}$  M solution of  $\text{Pb}^{2+}$  in 0.100 M HEPES were prepared. These two solutions were combined in various ratios and the PbPAR absorbance was measured for each combination. The results are illustrated in Figure 2 and indicate that the  $\text{Pb}^{2+}$  combines with PAR in a 1:1 ratio under the conditions used in our experiments.

A further confirmation of the stoichiometry of the PAR complex was obtained by the mole ratio method. The following two mole ratio experiments were performed with limiting concentrations of PAR in 0.1 M

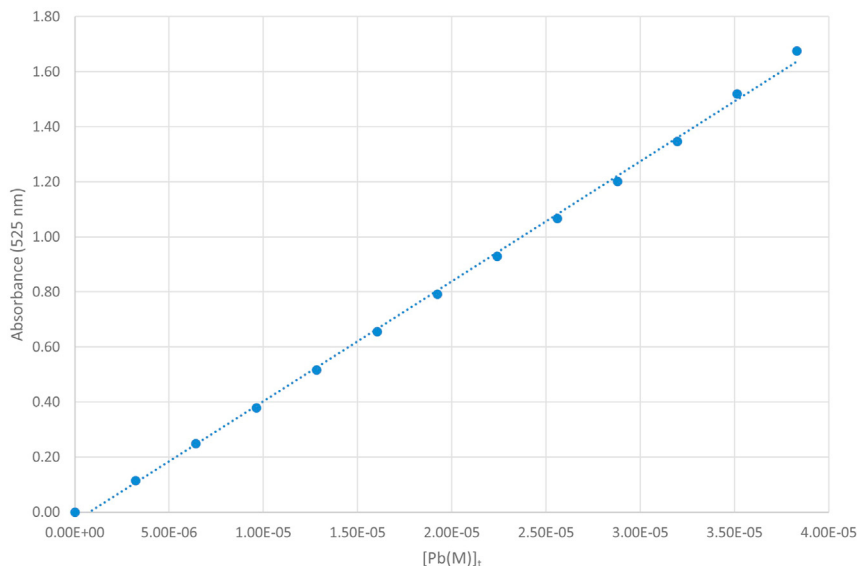


Figure 1. Calibration plot for the total  $\text{Pb}^{2+}$  ( $[\text{Pb}]_t$ ) added to the solution in the presence of 0.24 mM PAR.

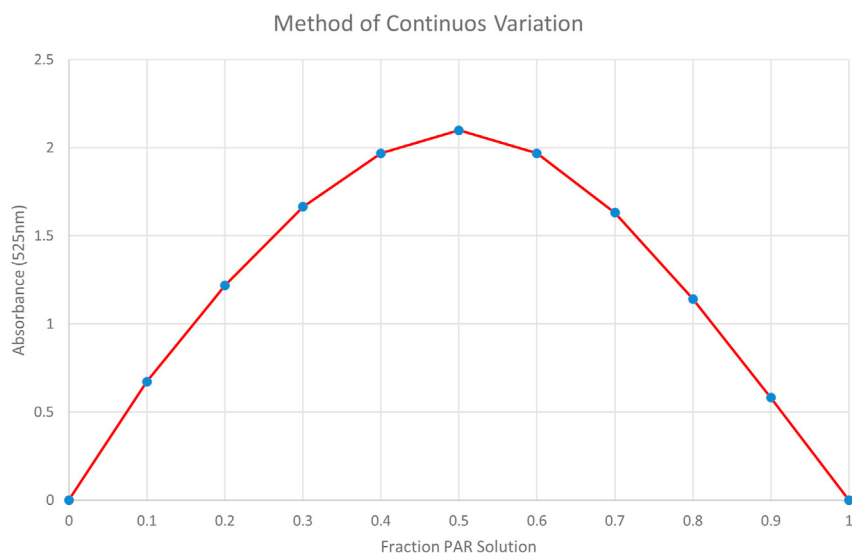


Figure 2. Results for the method of continuous variation indicating a 1:1 ratio of Pb and PAR in the complex.

HEPES pH 8. The concentration of  $Pb^{2+}$  was incrementally increased into a solution of 10  $\mu M$  or 16  $\mu M$  PAR while the absorbance of the PbPAR complex was monitored. The results are illustrated in Figure 3 and show that the absorbance levels off when the  $Pb^{2+}$  to PAR ratio is 1. This result is consistent with the method of continuous variation and indicate that the 1:1 Pb:PAR complex predominates under the experimental conditions used in this study.

Measurement of ligand competition for  $Pb^{2+}$  between PAR with several small molecules with well characterized  $Pb^{2+}$  stability constants was performed in order measure the conditional stability constant for  $Pb^{2+}$  with PAR under our experimental conditions. Experiments with oxalate, citrate, ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA) as competitive ligands were performed. In all cases, it was confirmed that the complexes of  $Pb^{2+}$  with these ligands did not absorb light at 510 nm. The important objective was to find a ligand with an well-established  $Pb^{2+}$  stability

constant to compete against PAR. The competitive ligand exchange would then be used to assign a stability constant for the  $Pb^{2+}$ -PAR complex. With a conditional  $Pb^{2+}$ -PAR stability constant established, competition experiments with humic and humic analogs can be performed and  $Pb^{2+}$  complexation by these materials can be assessed. The results of these competition experiments allow the determination of  $Pb^{2+}$  binding constants with competitive small molecules or the determination of sorption isotherms for  $Pb^{2+}$  with humic acids or other polyelectrolytes.

EDTA and NTA both easily displace PAR from  $Pb^{2+}$ . These results are not shown, however both of these ligand form strong complexes with  $Pb^{2+}$  and effectively prevent the formation of the Pb-PAR complex at pH 8. Thus, both of these ligands are too strong to effectively estimate a Pb-PAR stability constant. Stability constants for  $Pb^{2+}$  with EDTA and NTA were estimated from the constants provided in the Visual Minteq software (<https://vminteq.lwr.kth.se/>). These stability constants were corrected to an ionic strength of 0.10, using the Davies equation. The

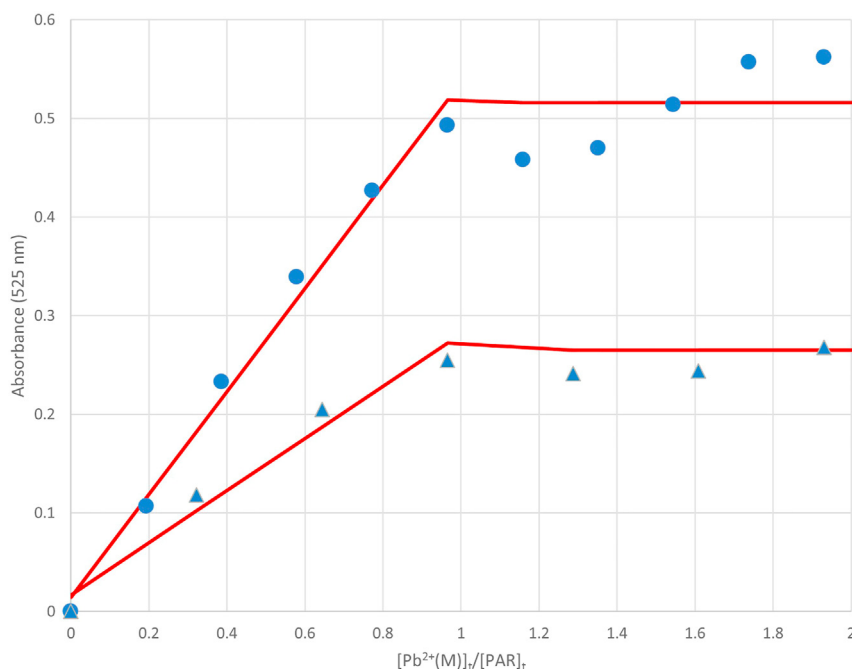
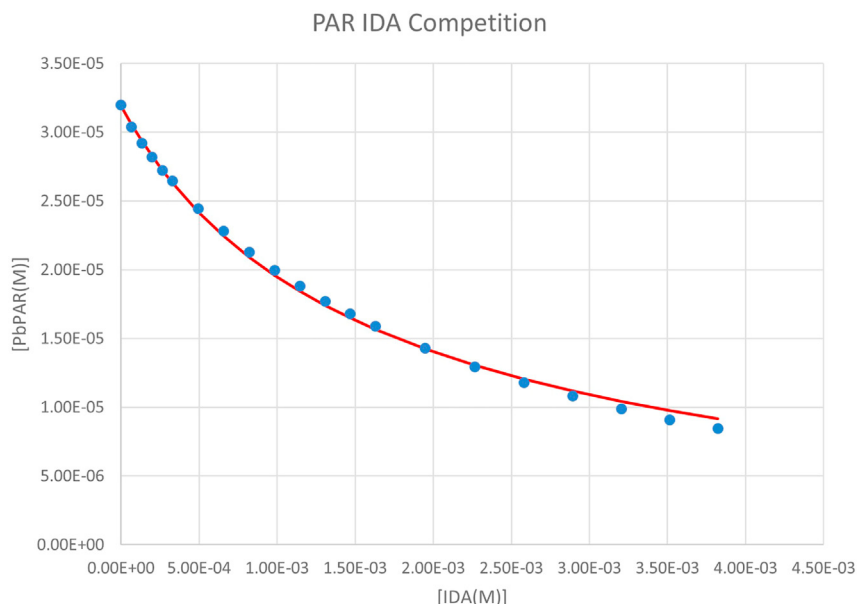


Figure 3. Mole Ratio Method Results for: ● 17  $\mu M$  PAR, ▲ 10  $\mu M$  PAR. These results support a 1:1 Pb to PAR ratio in the complex.



**Figure 4.** The concentration of PbPAR was reduced upon introduction of IDA at pH 8 in 0.100 HEPES buffer. The red curve shows the best fit of Eq. (1) to the data.

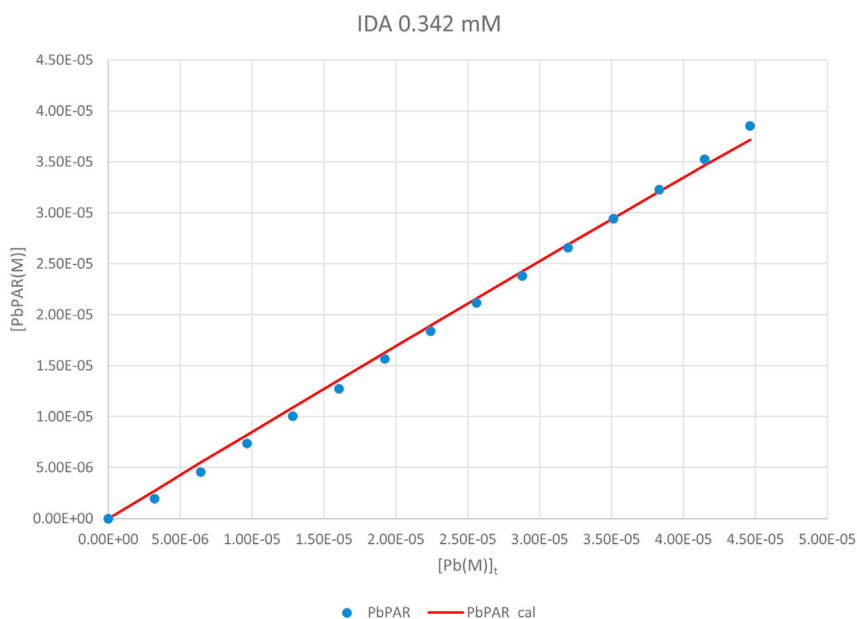
conditional stability constant at pH 8 were estimated to be  $\sim 10^{15.6}$  for EDTA and  $\sim 10^{12.6}$  for NTA.

Our measurements indicated that IDA effectively competes with PAR for the  $\text{Pb}^{2+}$  ion. However, the stability of Pb-IDA is much closer to the stability of the Pb-PAR constant. For our study, the stability constant for the complex between  $\text{Pb}^{2+}$  and IDA was estimated from the results of Anderegg (1964). The thermodynamic stability constant reported the 1:1 complex for  $\text{Pb}^{2+}$ -IDA, by Anderegg (1964), was corrected for pH and ionic strength. The ionic strength correction was performed with the Davies equation. The  $\log K_{\text{IDA}}$  estimated is 5.98. Ligand competition experiments between IDA and PAR were performed and used to assign conditional stability constant at pH 8 in 0.1 M HEPES to the Pb-PAR interaction. The results were fit to Eq. (1) which assume that the IDA and PAR lead complex dominate  $\text{Pb}^{2+}$  speciation under these experimental conditions. In addition, we assumed that no mixed  $\text{Pb}^{2+}$ -IDA-PAR complexes were formed.

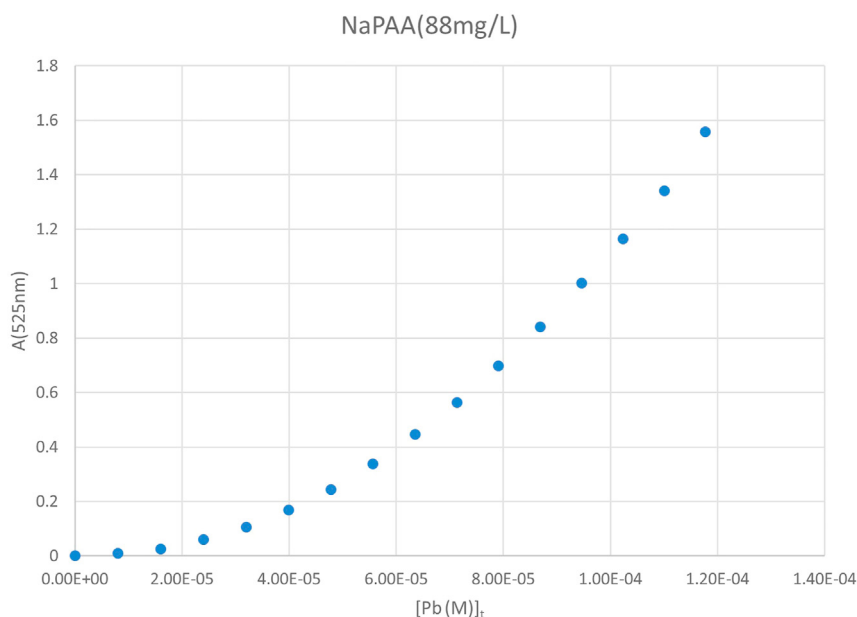
$$[\text{PbPAR}] = \frac{K_{\text{PAR}}[\text{PAR}][\text{Pb}]_{\text{tot}}}{K_{\text{PAR}}[\text{PAR}] + K_{\text{IDA}}[\text{IDA}]} \quad (1)$$

where  $K_{\text{PAR}}$  is the conditional  $\text{Pb}^{2+}$ -PAR stability constant and  $K_{\text{IDA}}$  is the conditional  $\text{Pb}^{2+}$ -IDA stability constant.

For the purpose of establishing the conditional  $\text{Pb}^{2+}$ -PAR stability constant, the concentration of the  $\text{Pb}^{2+}$ -PAR complex  $[\text{PbPAR}]$  was calculated from the response factor that was determined in the presence of excess PAR and in the absence of any competing ligand. For both Figures 4 and 5, the concentration of PAR ( $[\text{PAR}]$ ) was calculated from the difference between the total concentration of PAR in the solution and the measured concentration of PbPAR. It was assumed that the difference between total Pb ( $[\text{Pb}]_{\text{t}}$ ) and  $[\text{PbPAR}]$  is the concentration of PbIDA ( $[\text{PbIDA}]$ ). The value for  $[\text{PbIDA}]$  was subtracted from the total IDA concentration to determine  $[\text{IDA}]$  for the measurement. The  $K_{\text{PAR}}$  constant adapted for this study was the  $10^{6.84}$ . This stability constant is



**Figure 5.** PbPAR formation in the presence of 0.342 mM IDA and a PAR concentration was 0.240 mM. Competition between IDA and PAR for lead reduced the formation of PbPAR.



**Figure 6.** Addition of  $Pb^{2+}$  to a sodium polyacrylate solution (88 mg/L) in HEPES pH 8 in the presence of 0.24 mM PAR. The formation of PbPAR is clearly inhibited by the polyelectrolyte.

specific for the conditions used in this study and was used to calculate sorption isotherms for  $Pb^{2+}$  with humic materials.

The best fit curve for an example of a competition experiment is shown below in Figure 4. In this experiment IDA was titrated into a solution containing 32  $\mu M$   $Pb^{2+}$  and 0.24 mM PAR. The best fit of Eq. (1) yields a value of  $\log K_{PAR}$  of 6.84.

We did some additional experiments to explore IDA competition with PAR and to confirm the  $Pb^{2+}$ -PAR stability constant. In these experiments IDA was added to a solution of PAR in HEPES pH 8 buffer.  $Pb^{2+}$  was then titrated into the solution. An example of the results from one of these experiments is shown in Figure 5. The best fit curve in Figure 5 is consistent with a  $\log K_{PAR}$  of 6.88 which is very close to the value calculated using the results from Figure 4. Experiments with other IDA concentrations yielded similar results.

We also examined the possibility of using of oxalate and citrate to compete with PAR for  $Pb^{2+}$ . The concentrations of oxalate and citrate needed for effective competition were too high to be useful in this context, as we could not maintain a reasonably constant ionic strength in our buffer with these two competing ligands. The results of these experiments are not shown.

All of the humic and humic acid analog data we obtained were fit to a Langmuir type isotherm (Eq. (2)). This simple isotherm assumes a finite number of equal energy sites. The majority of binding sites on humic acids likely consist of carboxylic acid or phenolic moieties or a combination of both. The general premise of a finite number of equal energy sites is unrealistic for humic acids, however the use of the Langmuir isotherm for adsorption of metals onto natural organic matter does have some history and support in the environmental chemistry literature (van den Berg, 1982a; van den Berg, 1982b; van den Berg, 1984; Gerringa et al., 1995; Li et al., 2015; Du et al., 2015; Shaker and Albishri, 2014). The choice of the Langmuir isotherm, however, does not imply monodentate associations of  $Pb^{2+}$  with humic acid binding sites (Pinheiro et al., 1994). Multidentate interactions can also be compatible with the Langmuir isotherm.

$$[PbHA] = \frac{K_{HA}L_t[Pb^{2+}]}{1 + K_{HA}[Pb^{2+}]} \quad (2)$$

In Eq. (2),  $K_{HA}$  is the conditional  $Pb^{2+}$  humic acid stability constant and  $L_t$  is the conditional total complexation site concentration and  $[Pb^{2+}]$

is the concentration of lead as the hydrated cation or as labile inorganic complexes. This concentration is conditional for the pH 8 0.100 M HEPES buffer used for these experiments.

All of our results were obtained in the HEPES buffer, at pH 8, and all of these ligand competition experiments were conducted under conditions where most (>99%) of the  $Pb^{2+}$  is either bound by the humate or humate analog or by the PAR.

The following equilibrium was also operative (Eq. (3)).

**Table 1.** Summary of Langmuir Isotherm fitting parameter obtained with 0.24 mM PAR. The symbols are as defined in Eq. (2).

Specimen	mg/L	$\log K_{HA}$	$L_t$ (mol/L)	r (correlation coefficient)
TV	200	7.617	7.68E-05	0.996
	100	7.299	7.87E-05	0.994
	50	7.382	3.80E-05	0.990
	25	7.614	1.53E-05	0.994
	12.5	7.407	1.56E-05	0.989
Pahokee	200	7.687	1.14E-04	0.999
	100	7.778	5.01E-05	0.997
	50	7.831	2.98E-05	0.991
	25	7.680	2.29E-05	0.989
IHSS Leo	200	7.230	1.64E-04	0.997
	100	7.565	5.25E-05	0.999
	50	7.670	3.09E-05	0.992
	25	7.593	2.36E-05	0.991
Elliot	200	7.993	9.61E-05	0.991
	100	7.917	5.00E-05	0.996
	50	8.111	1.78E-05	0.977
	25	7.526	2.18E-05	0.928
Suwanee	400	7.655	4.58E-05	0.997
	200	7.644	1.93E-05	0.959
	100	7.220	2.60E-05	0.993
NaPAA	344	8.920	1.94E-04	0.989
	88	8.570	8.25E-05	0.990
	44	8.430	3.97E-05	0.993
	22	8.630	1.73E-05	0.996



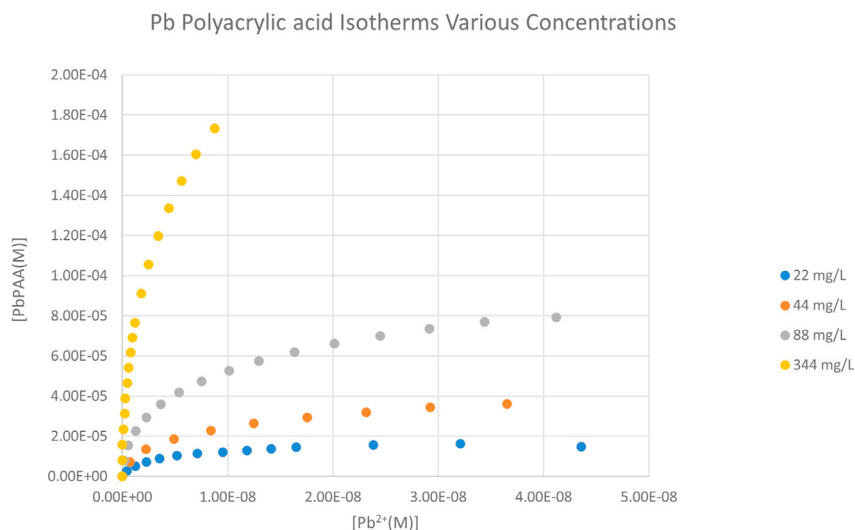


Figure 7. Sorption isotherms form  $Pb^{2+}$  on NaPAA in the presence of 0.24 mM PAR.  $[PbPAA]$  and  $[Pb^{2+}]$  are reported as in mol/L (M).

$$K_{PAR} = \frac{[PbPAR]}{[Pb^{2+}][PAR]} \tag{3}$$

In these experiments the difference between the total Pb concentration ( $[Pb]_t$ ) and  $[PbPAR]$  was assumed to be the humic acid bound Pb ( $[PbHA]$ ). With a value for  $K_{PAR}$  and a measured concentration of  $PbPAR$  and the concentration of unbound PAR, it was possible to calculate the concentration of uncomplexed  $Pb^{2+}$  in the solution using Eq. (4).

$$[Pb^{2+}] = \frac{[PbPAR]}{K_{PAR}[PAR]} \tag{4}$$

The calculated  $[Pb^{2+}]$  was plotted against  $[PbHA]$ . This resulting curve represents the sorption isotherm for  $Pb^{2+}$  by the humic acid sample. In some applications a linearized version of the Langmuir isotherm was employed, and the observation of nonlinearity with this version of the Langmuir isotherm has been used to infer more than one type of sorption site (van den Berg, 1982a; van den Berg, 1982b; van den Berg, 1984; Gerringa et al., 1995). Linearized versions of the Langmuir isotherm were also plotted (presented below) and we did observe the usual curvature that has been reported in other humic and fulvic acid studies. However, we believe that non-linear fitting of the Langmuir

equation gave a superior representation of the data (Gerringa et al., 1995).

In the presence of humic acid or a polyelectrolyte a plot of absorbance against the total lead concentration deviated from linearity as a result of the suppression of the  $PbPAR$  absorption at 525 nm. As an example, the results presented in Figure 6 show the absorbance of  $PbPAR$  (0.24 mM PAR) with increasing total  $Pb^{2+}$  in the presence of 88 mg/L sodium polyacrylate (NaPAA).

Similar observations were made for tannic acid and the various humic substances we investigated. Experiments were performed at several different concentrations for all of these materials. Using the response factor for  $PbPAR$  and the calculated concentration of uncomplex PAR along with the  $Pb^{2+}$ -PAR stability constant of  $10^{6.84}$ , adsorption isotherms were derived from this data. The best-fit parameters for the Langmuir isotherms are summarized in Table 1 for the humic acids and humic acid analogs examined in this study.

Examples of sorption isotherms for the NaPAA, tannic acid and two different humic acids are presented in Figures 7, 8, 9, and 10.

When the sorption site concentration determined by fitting the Langmuir isotherm to the data are plotted against mass concentrations of NaPAA and the various humic acids, there is a clear correlation

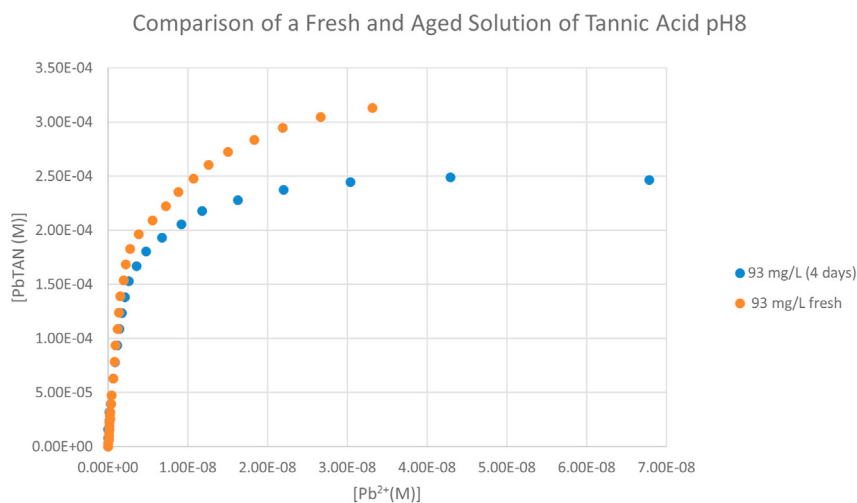


Figure 8. Absorption of  $Pb^{2+}$  on tannic acid. Results are shown for a freshly prepared and a four-day old solution of tannic acid.  $[PbTAN]$  and  $[Pb^{2+}]$  are reported as in mol/L (M).

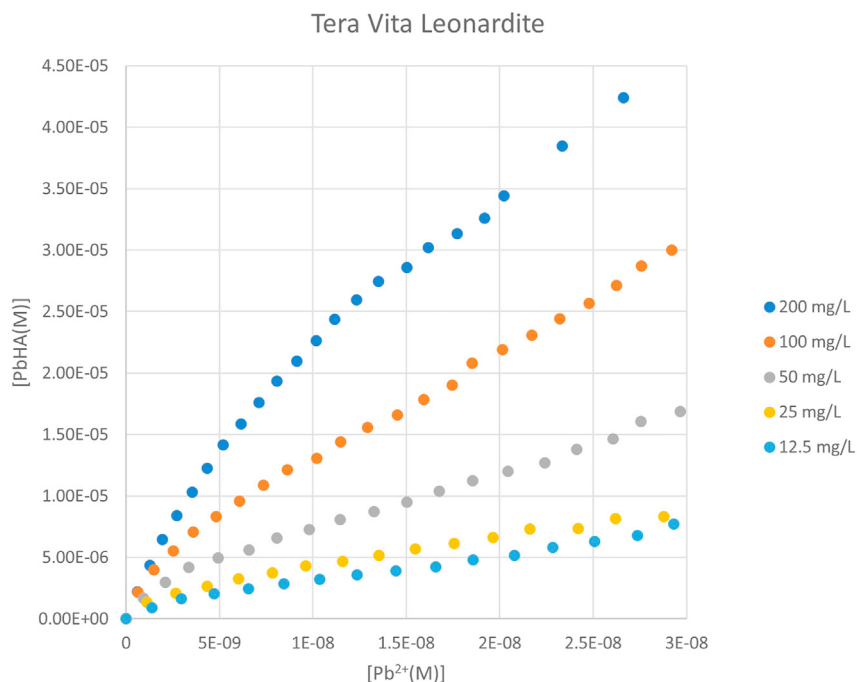


Figure 9. Tera Vita Leonardite isotherms measured in the presence of 0.24 mM PAR for various concentrations of the humic acid. [PbHA] and [Pb<sup>2+</sup>] are reported as in mol/L (M).

(Figure 11). However, it is also apparent that only a fraction of the available carboxylic acid groups can bind Pb<sup>2+</sup>. For example, for NaPAA a 344 mg/L solution contains approximately 3.7 mM of carboxylic acid groups, however the Langmuir isotherm fit indicates a ligand concentration of about 0.2 mM. Even if these sites represent bidentate metal associations only about 10% of the available carboxylic acid groups are utilized to complex Pb<sup>2+</sup>. For NaPAA the calculated stability constant is remarkably consistent between the four experiments (Table 1). Similar correlations for complexation or adsorption site concentrations (L<sub>t</sub>) with mass concentrations were observed for Terra Vita, Pahokee, IHSS Leonardite and Elliot humic acids and the data for these materials are also

plotted in Figure 11. Similarly, the values of L<sub>t</sub> for these materials are significantly lower than the reported sum of carboxylic and phenolic functional groups by the IHSS.

Lead complexation experiments were performed with tannic acid. Tannic acid has been described as a polygalloyl glucose or polygalloyl quinic acid esters with a variable number of galloyl moieties per tannic molecule. Tannic acids are plant components and as a result, the phenolic features of tannic acid are likely to contribute to humic acid formation in soils. Tannic has a very high affinity for Pb<sup>2+</sup>. In Figure 8 the Pb<sup>2+</sup> isotherms for a fresh tannic acid solution and a four-day old tannic acid solution are compared. The original concentration of tannic acid was 93

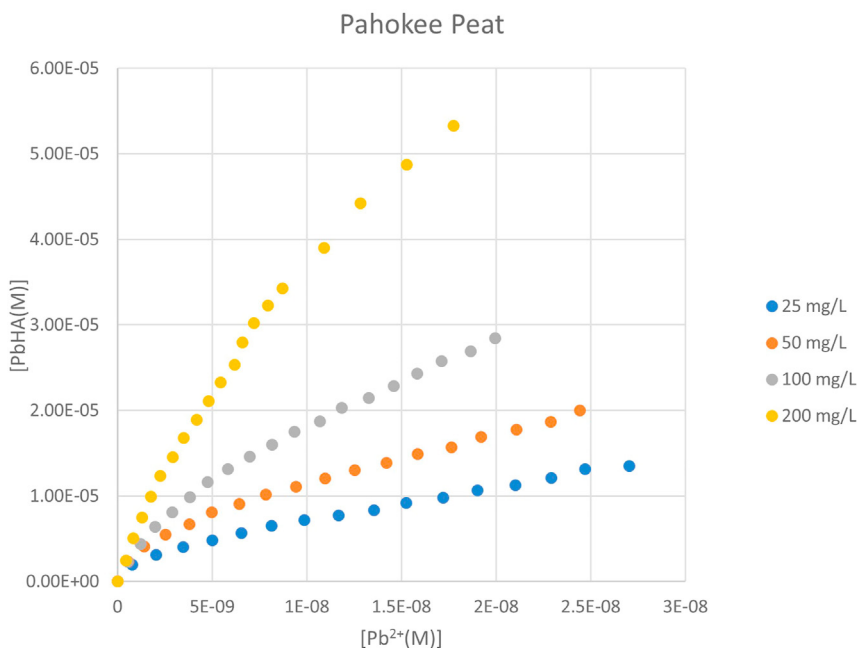
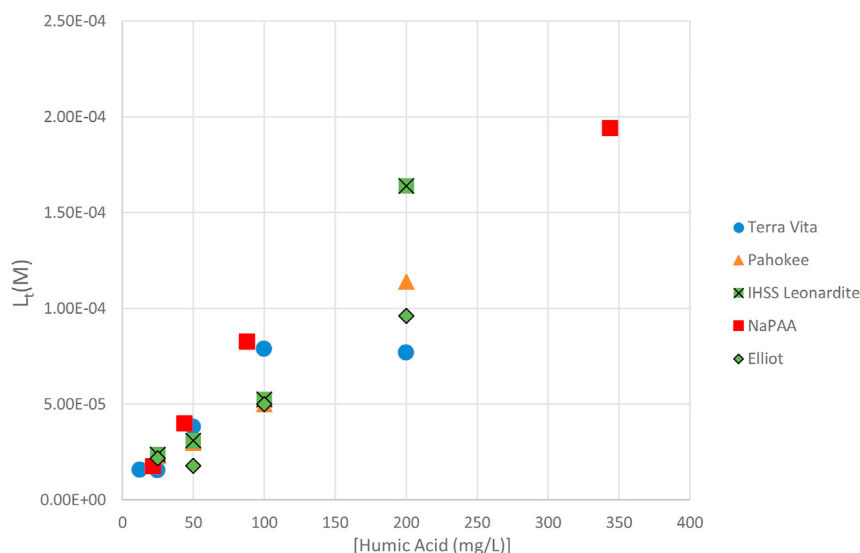


Figure 10. Pahokee peat humic acid isotherms measured in the presence of 0.24 mM PAR. [PbHA] and [Pb<sup>2+</sup>] are reported as in mol/L (M).





**Figure 11.** The best fit value of  $L_t$  (M) from the Langmuir equation is correlated with the concentration of the polyelectrolyte in the solution in mg/L.

mg/L. The solution was prepared in 0.1 M HEPES buffer (pH 8). The solution color darkened considerable over the course of a few days. The best fit results for the fresh solution was  $K = 10^{8.78}$  and  $L_t = 3.18 \times 10^{-4}$  ( $r^2 = 0.993$ ). After four days the  $K$  was  $10^{8.87}$ , which was fairly close to the original solution value, however  $L_t$  was reduced to  $2.55 \times 10^{-4}$  M ( $r^2 = 0.997$ ). From our observations, humic acid solutions also show this aging effect. For the results presented here, all experiments were conducted within 5 h of placing the humic acid into solution.

The interaction of  $Pb^{2+}$  with Suwanee River NOM (an aquatic humic substance) was investigated at different concentrations. Lead binding increased with the concentration of the NOM. However, the lowest Suwanee river NOM concentrations did not yield viable fits to the Langmuir model.

The Langmuir isotherm can also be fit to mass normalized absorption of  $Pb^{2+}$  to the humic materials or the polyelectrolytes. These fitting parameters are presented in Table 2.

When the humic acid bound lead concentrations are normalized to the mass of humic acid (Table 2) the sorption isotherm can still be described by the Langmuir equation, however the isotherms for the various concentrations of humic acid clearly do not merge into a single curve. The calculated  $L_t$ , on a moles per gram basis, varied over the range of concentrations we examined by more than an order of magnitude, for each material. The  $\log K_{HA}$  values shows similar variations. While  $L_t$  values calculated on a mol/L basis appears to be related to the concentration of the humic materials, binding site concentrations on a mol/g basis vary considerably.

Several experiments were conducted to determine if the concentration of the competing ligand (PAR) would have a large impact on the analytical results and the values of  $L_t$  and  $K_{HA}$ . Results for several of the materials studied are presented in Table 3. In general, higher PAR concentrations resulted in Langmuir fits with higher  $K_{HA}$  values. This implies that the higher PAR concentration prevented  $Pb^{2+}$  from interacting with lower energy complexation sites. It is clear that a change in the completing ligand concentration has shifted the analytical window (van den Berg et al., 1990; Wells et al., 2013). This results are consistent with other competitive ligand studies. In addition, the  $L_t$  values generally tend to increase with lower PAR concentration.

If both  $K_{HA}$  and  $L_t$  for the mass normalized sorption isotherms are examined on a logarithmic scale, there is a consistency in the order of magnitude of these parameters. This is illustrated in Figure 12 which shows about an order of magnitude variation in the average value of both parameters for the mass normalized  $Pb^{2+}$  absorption by the humic materials examined in this study.

The measurements were generally reproducible with the caveat that humic acid solutions do show signs of aging and need to be measured within a day. If the estimated carboxylic and phenolic groups reported by IHSS can serve as an upper limit for binding sites, it is clear, that the binding sites concentrations determined by the fit to the Langmuir isotherms are significantly lower than the total carboxylic acid and phenolic concentrations estimated by the IHSS (less than 10%) (<https://humic-substances.org/acidic-functional-groups-of-ihss-samples/>).

Linearized Langmuir isotherm plots were constructed and also indicate heterogeneity in the lead binding sites. The linearized Langmuir equation used in this study is presented in Eq. (5).

**Table 2.** Summary of Langmuir isotherm fitting parameters with  $Pb_{HA}$  normalized to the mass of humic acid.

Specimen	mg/L	$\log K_{HA}$	$L_t$ (mol/g)	r (correlation coefficient)
TV	200	7.617	3.84E-04	0.998
	100	7.223	9.00E-04	0.993
	50	7.049	1.38E-03	0.984
	25	7.614	6.11E-04	0.994
	12.5	6.635	5.46E-03	0.986
Pahokee	200	7.686	5.72E-04	0.999
	100	7.679	4.84E-04	0.997
	50	7.831	5.97E-04	0.991
	25	7.681	9.14E-04	0.996
IHSS Leo	200	7.230	8.19E-04	0.996
	100	7.565	5.26E-04	0.999
	50	7.669	6.17E-04	0.992
	25	7.593	9.46E-04	0.991
Elliot	200	7.994	4.80E-04	0.996
	100	7.917	5.00E-04	0.998
	50	8.111	3.56E-04	0.989
	25	7.526	8.74E-04	0.963
Suwanee	400	7.655	1.14E-04	0.999
	200	7.644	9.66E-05	0.979
	100	7.223	2.59E-04	0.997
NaPAA	344	8.760	5.63E-04	0.988
	88	8.294	9.60E-04	0.990
	44	8.270	9.02E-04	0.993
	22	8.467	7.84E-04	0.996

**Table 3.** Summary of Langmuir isotherm parameters for variable PAR experiments.

	PARt (M)	Log $K_{HA}$	$L_t$	r (correlation coefficient)
Tannic	2.40E-04	9.204	1.05E-04	0.984
	8.00E-05	9.033	1.14E-04	0.967
NaPAA	2.40E-04	8.469	8.10E-05	0.989
	8.00E-05	8.025	1.18E-04	0.983
IHSS Leo	2.40E-04	8.070	2.25E-05	0.992
	1.20E-04	7.510	3.78E-05	0.994
Pahokey	8.00E-05	7.450	3.53E-05	0.998
	2.40E-04	7.971	3.59E-05	0.997
	1.20E-04	7.816	4.45E-05	0.996
	8.00E-05	7.572	4.66E-05	0.999

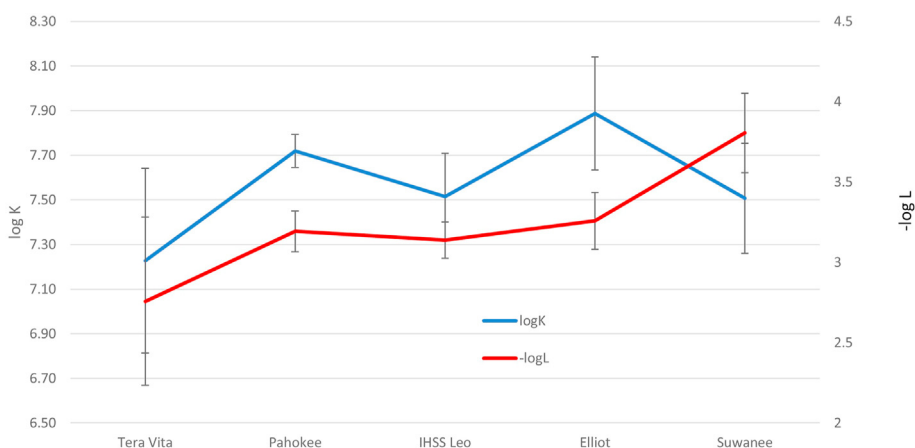
$$\frac{[Pb^{2+}]}{[PbHA]} = \frac{1}{K_{HA}L_t} + \frac{[Pb^{2+}]}{L_t} \tag{5}$$

Some examples of linearized Langmuir plots are shown in Figure 13 for humic acid concentrations of 100 mg/L. The Suwanee River NOM, Tera Vita and Pahokey humic acids show pronounced curvature, which can be interpreted as adsorption site heterogeneity. Considering the complexity of humic materials, site heterogeneity is a reasonable conclusion (Logan et al., 1997).

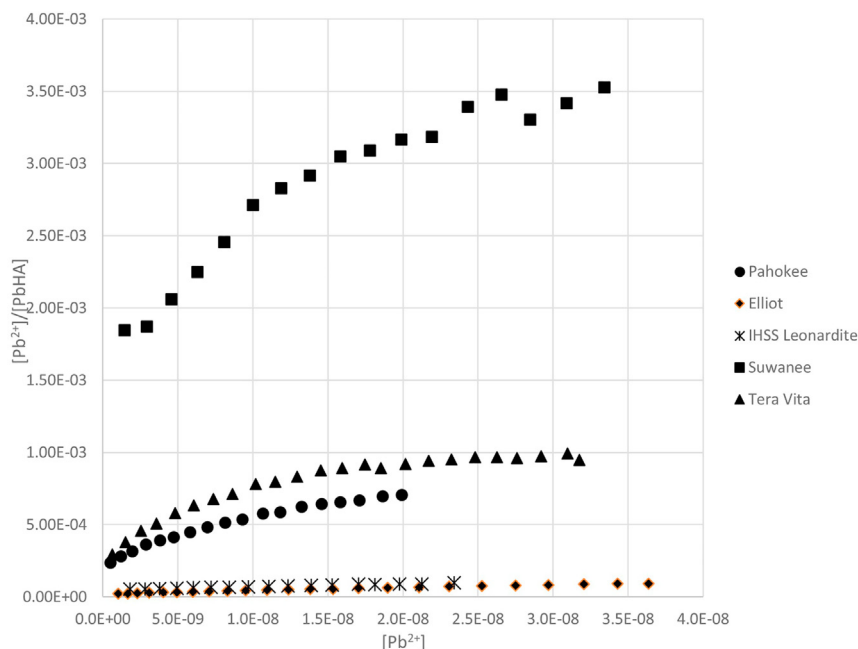
The results from this study can be compared with observations from our previous measurements that were performed using DPP (Steinberg and Hodge, 2020). We previous noted that according to the Lingane equation (Eq. (6)), the shift in the DPP reduction potential should be a function of  $\log(K_{HA} * L_t)$ .

$$\Delta E \approx \frac{-0.0592}{n} \text{Log}(K_{HA} * L_t) \tag{6}$$

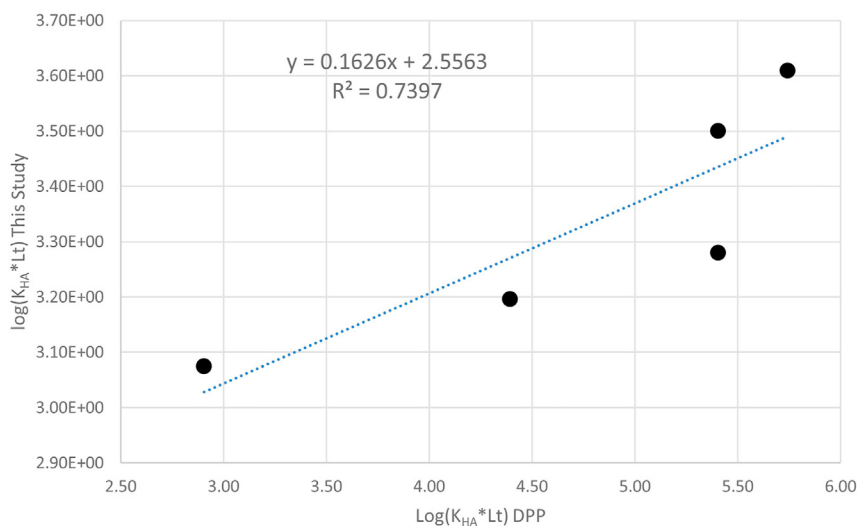
At low total  $Pb^{2+}$  concentrations  $K_{HA} * L_t$  is the ratio of  $[PbHum]/[Pb^{2+}]$ . In our previous study utilizing the DPP method and we did not have any way to experimentally measure the  $L_t$  values. Therefore, we used the published total ionizable functional group concentration, from IHSS, as an estimate of  $L_t$ .



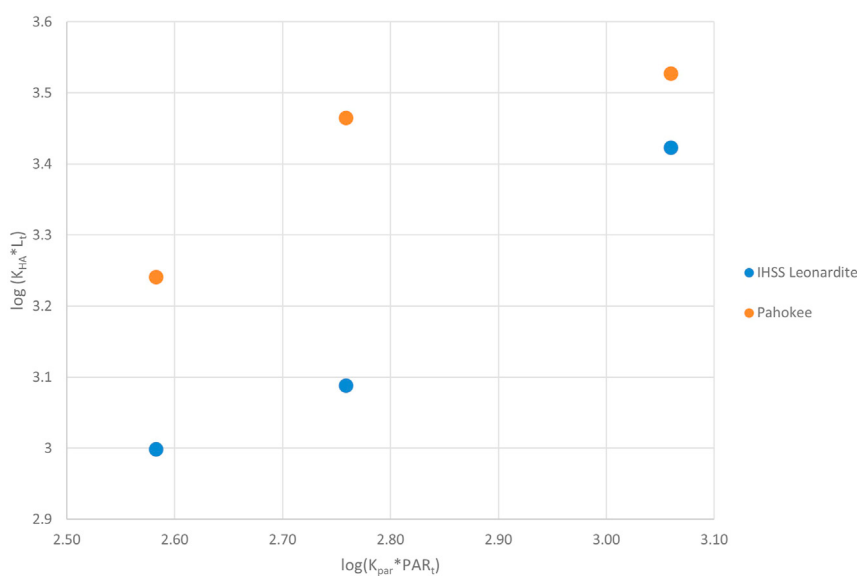
**Figure 12.** The average and the standard deviation for the mass normalized  $\log K_{HA}$  and  $\text{Log } L_t$  for the various humic materials investigated. These results were obtained in the presence of 0.24 mM PAR.



**Figure 13.** Linearized Langmuir plots for the various humic materials investigated. The curvature in these plots can be interpreted as indicating a heterogenous distribution of complexation sites in the humic material.



**Figure 14.** Our previous results for Log ( $K_{HA}L_t$ ) obtained by DPP for these humic materials in consistent with observations made using the competitive ligand approach discussed in this work.



**Figure 15.** Correlation of Log ( $K_{par} \cdot PAR_t$ ) with the Log ( $K_{HA} \cdot L_t$ ) demonstrates how the varying the concentration of the competing ligand can change nature of the humic acid complexation that is observed.

Based on this reasoning we have compared the values of  $\log(K_{HA} \cdot L_t)$  estimated from the  $\Delta E$  values obtained in solutions with humic acid concentrations at approximately 100 mg/L and at the lowest lead concentrations examined during our electrochemical study ( $\sim 0.2 \mu M$ ), with the  $\log(K_{HA} \cdot L_t)$  from the best fit of the Langmuir equation for humic acid concentrations of  $\sim 100$  mg/L. When the  $\log(K_{HA} \cdot L_t)$  values calculated from DPP study are graphed against the  $\log(K_{HA} \cdot L_t)$  from the competitive ligand exchange, we do observe a correlation. This is demonstrated in Figure 14.

Within each data set,  $K_{HA} \cdot L_t$  is well correlated with the concentration of humic acid in solution, with a correlation coefficient between 0.987 and 0.998. The  $\log(K_{HA} \cdot L_t)$  values calculated by both the DPP procedure and the PAR competitive ligand method agree within an order of magnitude. This is evidence that both methods are a window into the same complexation phenomenon.

As noted above the values of  $K_{HA}$  and  $L_t$  obtained from competitive ligand experiments were dependent on the amount of competing ligand (PAR) introduced. This becomes clearer if the values of  $\log(K_{HA} \cdot L_t)$  are plotted against  $\log(K_{par} \cdot PAR_t)$ . This is shown in Figure 15 and

illustrates that the value of  $\log(K_{HA} \cdot L_t)$  obtained increases as the  $\log(K_{par} \cdot PAR_t)$  increases. This demonstrates that a spectrum of potential complexing site stabilities exists in humic materials and the concentration and stability constant associated with the competing ligand can change which sites are experimentally detected.

#### 4. Conclusions

The use of competing ligands has been demonstrated to be a useful approach for determining the stability of metal complexes with natural organic matter. We have demonstrated in this work that PAR can be used a spectroscopic probe for  $Pb^{2+}$  humate interaction. We have also demonstrated that at pH 8,  $Pb^{2+}$  forms a 1:1 complex with PAR. The competitive ligand measurements using PAR produced reproducible measurements for the interaction  $Pb^{2+}$  with various humic acids, polyacrylate and tannic acid. Under the conditions used in this study, the rate of equilibration for the adsorption of lead by humic acids was rapid. Furthermore, the adsorption of  $Pb^{2+}$  by humic materials was easily fit to a reproducible Langmuir isotherm.

For the concentration ranges measured in this study the best fit values of  $K_{HA}$  and  $L_t$  obtained for the various humic materials was surprisingly consistent. We also found that values of  $K_{HA}$  and  $L_t$  were partially determined by the concentration of the competing ligand (PAR). This observation is consistent with other studies and is consistent with the inherent heterogeneous nature of humic materials. The values of the product of  $K_{HA} \cdot L_t$  derived from this study was also consistent with the DPP measurements from our previous work.

These results are further support for the notion that humic substances can be important in determining speciation and bioavailability of metals in the soil and water environment. Furthermore, it is clear that the competitive ligand approach implemented using UV/VIS spectroscopy is a viable means of measuring interaction of metals with natural organic matter. Using UV/Vis spectroscopy the concentration ranges examined are limited to the micromolar range which is significantly higher than what would be observed in most samples. However, these results are likely to be relevant to highly polluted wastewater and soils. Lower  $Pb^{2+}$  concentrations could be examined with a longer path length cuvette. However, it is clear from the present set of measurements that a spectrum of binding site energies exists in humic material. It is possible and perhaps likely that stability constants can be order of magnitudes higher at lower Pb (nM) concentrations. Because the competitive ligand method, as implemented in this study, is relatively straight forward, the approach should be useful to generate  $Pb^{2+}$  binding data useful in the further development and fitting of sophisticated and predictive models of humic acid metal interactions. This  $Pb^{2+}$  binding data should be a valuable for refining speciation models for  $Pb^{2+}$  in soil and natural waters.

## Declarations

### Author contribution statement

Spencer M Steinberg: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted data; Wrote the paper.

Vernon F. Hodge: Analyzed and interpreted data; Contributed reagents, materials, analysis tools or data.

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### Data availability statement

Data included in article/supp. material/referenced in article.

### Declaration of interest's statement

The authors declare no competing interests.

### Additional information

No additional information is available for this paper.

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