USEPA LEAF methods for characterizing phosphorus and potentially toxic elements in raw and thermally treated sewage sludge

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Abstract

Biologically available phosphorus supports plant growth but can also cause environmental contamination. Sequential extraction methods, such as Hedley fractionation, are the most widely used to assess available phosphorus from solids. However, such methods exhibit numerous deficiencies. The USEPA Leaching Environmental Assessment Framework (LEAF) is a tiered system developed to evaluate releases of Potentially Toxic Elements (PTEs) from solids. This study compared the Hedley fractionation method to the LEAF pH-dependent, parallel batch tests (Method 1313) and dynamic leaching column test (Method 1314) to assess the bioavailability of phosphorus. The three methods were applied to wastewater treatment plant sludge before and after thermal treatment. Both methods revealed similar qualitative trends, namely that thermal treatment transformed phosphorus into less immediately available forms. However, the Hedley and LEAF methods were inconsistent in the forms and amounts of available phosphorus recovered from the solids. The Hedley method left 40% of phosphorus unextracted from sludge and 20% from ash, suggesting that it may be less appropriate for organic materials. Moreover, only 2 of the 6 Hedley phosphorus pools were within environmentally relevant pH conditions. Furthermore,

25   the Hedley method overpredicted the readily available phosphorus. In contrast, the LEAF methods

26   allowed for a more detailed analysis of phosphorus availability - while simultaneously assessing PTEs -

27   across a controlled pH range. Moreover, LEAF used simpler procedures and provided more easily

28   interpreted results. Thus, LEAF facilitates more robust and valuable assessment of organic and inorganic

29   solids being considered for land application.

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1. Introduction

Land application of solids, such as manure, inorganic fertilizers, and wastewater treatment plant (WWTPs) sludge, are valuable sources of phosphorus and other nutrients. However, accumulation of phosphorus within soils can increase leaching (1) as previously studied with applied manures (2,3) and inorganic fertilizers (1,4). Released phosphorus is transported through the subsurface or via runoff to surface waters (5); contributing to eventual eutrophication (6). Determining the biologically available (i.e., bioavailable) phosphorus in land applied solids is important for developing application guidelines, maximizing beneficial use, and establishing regulatory compliance.

Numerous methods have been developed to quantify phosphorus, especially bioavailable phosphorus, in a wide range of solids. Most literature and analytical methods focus on soils and the Hedley sequential fractionation method (7) dominates the field (8–10). Hedley uses progressively stronger chemical extractants to recover increasingly recalcitrant forms of phosphorus (11), inferring potential sources and sinks of phosphorus based on phosphorus quantity within various fractions. Bioavailable phosphorus is typically divided into several "pools" ranging from highly available to unavailable (12).

Hedley forms the basis for many related sequential fractionation methods (13–16). Common modifications to the Hedley method include (i) an initial deionized water step prior to anion exchange resin (15), (ii) excluding quantification of microbial phosphorus (16), (iii) eliminating sonication during the extraction of moderately-bound phosphorus (13), and (iv) using heated digestion to quantify residual (unavailable) phosphorus (14). Sequential phosphorus fractionation methods vary significantly in the type of chemical extractant and molarity used to quantify each pool (a summary of published methods can be found in Appendix A of the Supplementary Materials). Since phosphorus dissolution is highly pH dependent (17), extraction methods are likely to yield different results for available phosphorus depending on chosen extractants and procedure order (18,19). Furthermore, strong extractants can

56 change the chemical structure of phosphorus species (20–23). As a consequence, conclusions on

57 phosphorus availability from studies using fractionation methods are inconsistent (18,24) and sometimes

58 contradictory (25).

59 Studies assessing phosphorus availability in other solids including animal manure and sewage sludge have

60 used modified versions of Hedley and soil phosphorus test procedures (15,26–29). Only a few studies have

61 attempted to fractionate phosphorus in sewage sludge (15,27,30,31). Such evaluations are anticipated to

62 suffer from the same problems identified for sequential fractionation of phosphorus in soils.

63 Following decades of development, the USEPA released the Leaching Environmental Assessment

64 Framework (LEAF) in 2010. LEAF is a characterization-based leaching framework combining experimental

65 data on relevant intrinsic leaching behaviour with scenario-specific information for environmental

66 assessments (32). USEPA Method 1313 from LEAF consists of a series of parallel batch experiments to

67 produce a liquid-solid partitioning curve of the material of interest as a function of pH (33). The

68 complementary USEPA Method 1314 involves a column percolation experiment to obtain eluate

69 concentrations and/or cumulative release as a function of the liquid-to-solids ratio (34). LEAF has been

70 used to assess leaching behaviour of potentially toxic elements (PTEs) from coal fly ash (e.g., 33);

71 municipal solid waste (MSW) incinerator ash (e.g., 34); concrete waste (37); and sewage sludge compost

72 (38). Using LEAF to assess phosphorus is less common and has been used for inorganic phosphorus from

73 mining waste (39,40). The methodology seems promising to evaluate bioavailable phosphorus for a wide

74 range of organic/inorganic matrices.

75 The aim of this research was to evaluate analytical procedures for assessing bioavailable phosphorus from

76 sewage sludge before and after thermal treatment. The two procedures chosen for comparison were the

77 widely used Hedley fractionation method and USEPA LEAF. This study demonstrates that the LEAF

78 provides a more consistent method for analyzing phosphorus bioavailability in sludges and suggests that

79   it may be more widely applicable to soils and other solids under consideration for land application. This

80   study also illustrates how the LEAF methods provide valuable quantification of PTEs that may be present

81   in these materials with no further analytical steps required.

82   **2. Materials and Methods**

83   The sludge utilized in this study was collected from Greenway Wastewater Treatment Plant (Greenway),

84   London, Ontario, Canada. At Greenway, sludge is produced as a by-product of primary and secondary

85   treatment. Primary clarification removes settleable solids (i.e., primary sludge). Following aerobic

86   digestion and thickening, secondary clarification settles out waste activated sludge via dissolved air

87   flotation units and rotating drums. Centrifugation with polymer addition is used to dewater a combined

88   slurry of primary and waste activated sludge to produce a cake sludge. All sludge was collected as cake

89   sludge in a single batch on July 26th, 2018, to minimize compositional variability.

90   *2.1. Sample Preparation and Storage*

91   Sludge was oven-dried to prevent decomposition and moulding. Prior to subsequent analyses, the oven-

92   dried sludge was pulverized into a homogenous powder using an immersion blender and mortar and

93   pestle. Batches of ~100 g of dried sludge were placed in large crucibles and heated in a muffle furnace at

94   950°C for 2 hours to produce incinerated sewage sludge ash (herein referred to as ash). The ash appeared

95   to be a relatively homogenous powder; no further grinding was done. All materials were stored in sealed

96   20 L pails at 5°C prior to use.

97   *2.2. Preliminary Analysis*

98   Moisture content, volatile matter, ash content, and fixed carbon of the sludge were determined following

99   EPA Method 1684 (41), with three replicates analyzed for each. The sludge had an average moisture

100   content of 73 ± 0.2%, volatile matter content of 18 ± 0.3%, ash content of 7 ± 0.1%, and fixed carbon

101   content of 2 ± 0.4%, all on a wet-mass basis. Laboratory incineration at 950°C resulted in mass loss of

102   ~93%.

103   Pseudo-total elemental concentrations (herein referred to as total concentrations) were determined for

104   aluminum, cadmium, cobalt, chromium, copper, iron, manganese, molybdenum, nickel, phosphorus, lead,

105   and zinc within sludge and ash. Elements were extracted from the solid phase through microwave assisted

106   acid digestion following USEPA Method 3051A (42). Acid digestions were performed in triplicate.

107   *2.3. Hedley Method*

108   In the Hedley method, inorganic and organic phosphorus fractions are extracted using progressively

109   stronger chemical reagents. Figure 1a illustrates the series of 6 extracts (H1 – H6). In each extraction, 1 g

110   dried sample was added to a 250 mL polyethylene bottle. An anion exchange resin bag (Dowex™ resin,

111   Sigma-Aldrich Canada Co., within 41 μm nylon mesh, Fisher Scientific Co Ltd.) and 30 mL deionized water

112   were added and shaken at 170 RPM on a rotary shaker for 16 hours. Phosphorus was removed from the

113   resin with 20 mL 0.5 M hydrochloric acid (HCl) (H1). The supernatant was decanted and disposed of

114   followed by the addition of 30 mL 0.5 M sodium bicarbonate ($NaHCO_3$). The bottle was shaken for 16

115   hours, then the supernatant was collected (H2). This process was repeated, adding 30 mL 0.1 M sodium

116   hydroxide (NaOH) (H3), 20 mL 0.1 M NaOH with 2 min sonication (H4), and 30 mL 1 M HCl (H5). Following

117   the resin extraction procedure, a repeat sample was used to determine the microbial biomass phosphorus

118   by spiking the solid material with 1 mL concentrated chloroform ($CHCl_3$), leaving the sample to fumigate

119   for 16 hours, then extracting the phosphorus with 30 mL 0.5 M $NaHCO_3$. The microbial biomass

120   phosphorus is calculated as the difference between the total labile phosphorus extracted with only

121    $NaHCO_3$ and pretreated with $CHCl_3$ (43). The residual phosphorus is determined by digesting (H5) in 10 M

122   sulfuric acid (H_2SO_4) (H6). Importantly, pH is not dictated in this method. Instead, following each

123   procedure step, eluate samples were analyzed for pH and conductivity using a Fisher Scientific accumet™

124   AB200 pH/mV/Conductivity meter (Waltham, MA, USA), then preserved for further analysis of inorganic

125   and total phosphorus. Concentrations of organic phosphorus were calculated as the difference between

126   total and inorganic phosphorus in each extract. All extractions were performed in triplicate with results

127   presented as averages including standard error.

128   Each extract is associated with a phosphorus pool and mechanism of phosphorus binding to solid surfaces,

129   as illustrated in Figure 1a. Whether each of the chemical extractants can accurately dissociate the target

130   phosphorus compounds from the solids is not fully understood, and it is likely that each pool contains a

131   combination of compounds (44).

132   *2.4. USEPA LEAF Methods 1313 and 1314*

133   USEPA LEAF Method 1313 consists of 9 parallel batch extractions (Figure 1b) to produce a liquid-solid

134   partitioning curve of the material of interest over eluate pH range $2 \leq pH \leq 13$. For each extraction, 10 g of

135   material was combined with 100 mL of extraction solution consisting of deionized water with either 2 N

136   nitric acid (HNO_3) or 1 N potassium hydroxide (KOH) to achieve 9 target pH values ± 0.5 for each (Figure

137   1b). The liquid-to-solids ratio was 10 mL/g-dry. Quantities of HNO_3 or KOH were determined after

138   measuring the native pH of the material using deionized water. Mixtures were prepared in 125 mL HDPE

139   bottles, sealed, and shaken end-over-end at 170 RPM for 24 hours. Following shaking, bottles were

140   centrifuged. pH was measured to confirm the final solution remained within target ranges; electrical

141   conductivity was also measured. Eluate samples were filtered, preserved, and analyzed for total and

142   inorganic phosphorus. Additionally, cadmium, cobalt, chromium, copper, molybdenum, nickel, lead, and

143   zinc were analyzed because they are typically monitored and/or regulated at WWTPs.

144   USEPA LEAF Method 1314 uses a percolation column experiment to evaluate constituent release from the

145   material of interest as a function of the liquid-to-solid ratio (L/S). The L/S is computed as the quantity of

146   solution (mL) passed through the fixed quantity of solid material (g) within the column. A glass column

147   (DWK Life Sciences, KIMBLE®) of 5 cm outer diameter and 30 cm height was used for each test. A 5 cm

148   layer of acid-washed, air-dried sand (Number 12, Bell & Mackenzie) was packed into the base of the

149   column. Dried material (sludge or ash) was added to the column in successive layers (~60 g), tamping each

150   using a glass rod, until 300 g of sample was added. A second 5 cm layer of acid-washed sand was added

151   to the top of the material pack to minimize material loss from the column. Deionized water (neutral pH)

152   was pumped using a Masterflex® L/S® digital peristaltic pump (Cole-Parmer Instrument Company, IL, USA)

153   upwards at a L/S of 1.0 mL/g/day until breakthrough occurred. The pump was stopped, letting the

154   saturated column rest for 24 hours. After resting, water flow was reintroduced and maintained at rate of

155   0.75 ± 0.5 mL/g/day to collect the nine eluate samples (T01-T09) at specified L/S of 0.2, 0.5, 1.0 ± 0.1, and

156   1.5, 2.0, 4.5, 5.0, 9.5, and 10.0 ± 0.2 mL/g-dry matter. The pH and electrical conductivity of each eluate

157   sample were measured within an hour of collection. Eluate samples were filtered through 0.45 μm filters

158   (Whatman 0.45 μm celluacetate filters, VWR International) via a vacuum pump. Filtered samples were

159   preserved with concentrated HNO_3 and analyzed for total and inorganic phosphorus as well as cadmium,

160   cobalt, chromium, copper, molybdenum, nickel, lead, and zinc.

161   *2.5 Analytical Methods*

162   Total elemental concentrations in the microwave extracts, Hedley method extracts, and LEAF Method

163   1313 and 1314 samples, were analyzed using an Agilent 720 Inductive Coupled Plasma Optical Emission

164   Spectrometer (ICP-OES) following USEPA Method 6010D (45).

165   Total phosphorus in the Hedley method extracts and USEPA method 1313 and 1314 samples was

166   measured using ICP-OES to avoid interferences that affect colorimetric analysis (46). Inorganic phosphorus

167   was measured as dissolved orthophosphate (PO_4^{3-}) by High-Performance Liquid Chromatography (HPLC)

168   using direct injection by a Water® 515 pump following USEPA Method 300 (47).

169   All extracts were run at dilution factors of 1:1 – 1:100 (1:10 – 1:100 for digested samples) on both ICP-OES

170   and HPLC to ensure all elements were within detection ranges for every sample. Triplicates, method

171   blanks, and spiked extracts were also run on both ICP-OES and HPLC to ensure quality assurance and

172   quality control.

173   Due to differences in the sludge versus post-treatment ash, all results were normalized in terms of the

174   starting material: mass of element per mass of initial dry sludge (mg/kg–DS). Results in terms of dry matter

175   (i.e., mg/kg–dm) can be found in Supplementary Materials (Appendix C).
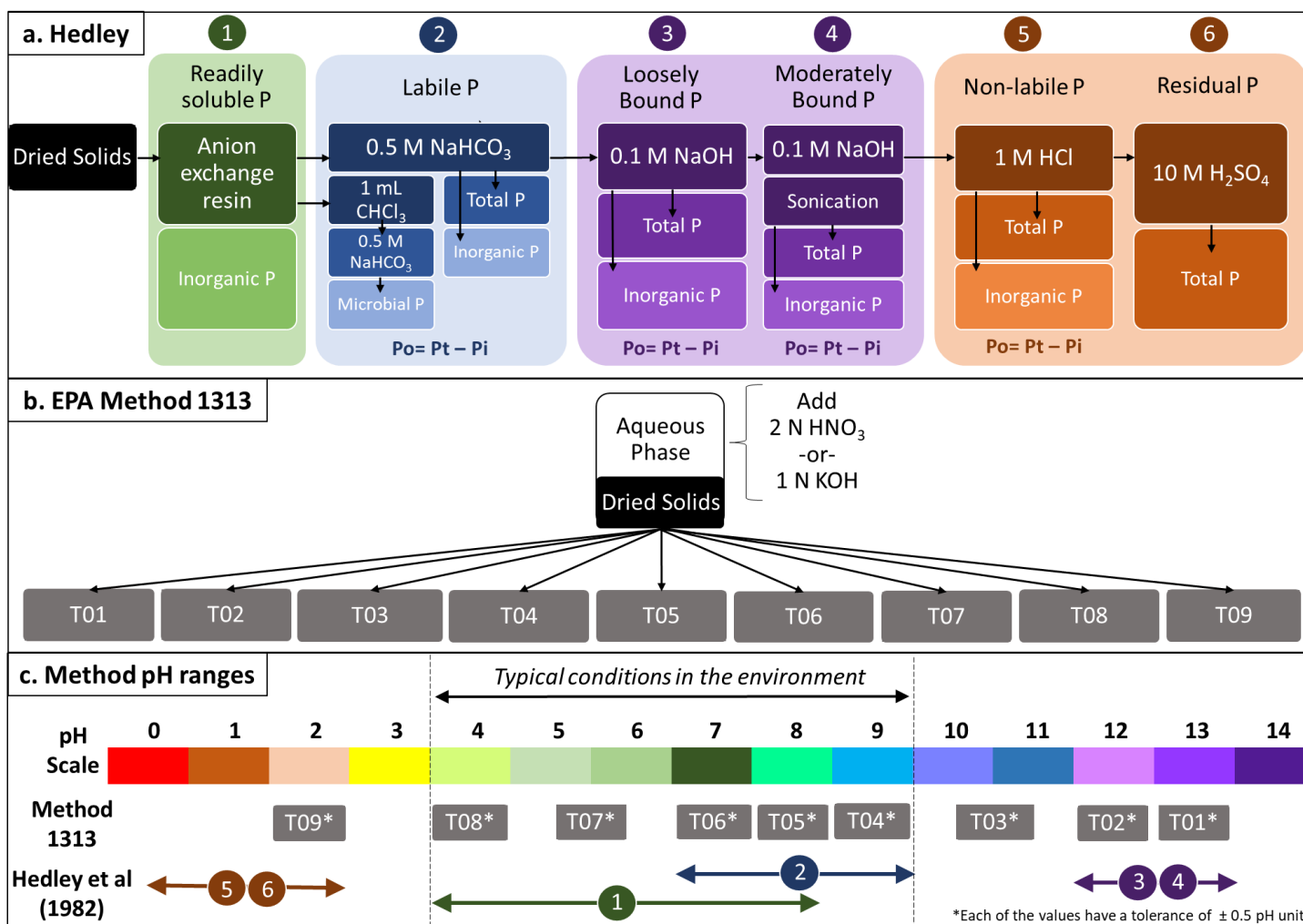
Figure 1: procedural schematics for a. the Hedley et al. (1982) fractionation procedure and b. USEPA Method 1313 parallel batch extraction. The 6 steps of the Hedley procedure (H1-H6) are outlined in a., including the chemical extractant and molarity used for to quantify each phosphorus pool. The phosphorus pools are assumed to decrease in plant availability from step (H1) being immediately available to step (H6) being unavailable. H1 is associated with readily soluble inorganic phosphorus (14). H2 is correlated to labile inorganic phosphorus from P-esters bound to surfaces of aluminum and iron (9). H3 and H4 are moderately-labile phosphorus pools assumed to contain phosphorus chemisorbed to amorphous and some crystalline aluminum and iron oxides/hydroxides (13). H5 is assumed to be non-labile phosphorus bound to calcium-species (9). The pH ranges corresponding to Hedley method pools and USEPA Method 1313 samples are provided in c. and compared to typical environmental pH conditions.

183 **3. Results and Discussion**

184 *3.1. Phosphorus Analysis*

185 *3.1.1. Hedley Method*

186 Figure 2 presents Hedley method results for sludge and ash. In the immediately soluble phosphorus pool

187 (H1), 1100 mg/kg – dry sludge (i.e., mg/kg–DS) was extracted from both sludge and ash, comprising 2 and

188 5% of total phosphorus, respectively. Majority was from unavailable pools (H3 – H6): 57% and 79% for

189 sludge and ash, respectively. The fraction of total phosphorus released from non-labile (H5) and residual

190 (H6) pools increases from 32% in sludge to 62% in ash. Thermal treatment seems to have transformed a

191 fraction of phosphorus to less available forms.

192 Hedley assumes organic phosphorus is the difference between total and inorganic phosphorus. The sludge

193 seems to have larger proportions of organic phosphorus compared to the ash. Phosphorus in the sludge

194 extracted by Hedley consists of 32% organic phosphorus distributed as 54% labile (H2), 16% loosely bound

195 (H3), 10% moderately bound (H4), and 85% non-labile (H5). In contrast, only 10% of phosphorus in the

196 ash is present as organic, distributed as 14% labile (H2), <1% loosely bound (H3), 10% moderately bound

197 (H4), and <1% non-labile (H5). This reduction in organic phosphorus during thermal treatment is also

198 evident when comparing microbial phosphorus: ~310 mg/kg–DS of microbial phosphorus for sludge

199 versus negligible for ash. This is due to the destruction of any microbial biomass present in the sludge

200 which would cause subsequent release of any microbially-bound phosphorus.

201 Phosphorus recovered from all steps of Hedley accounted for only 60% of total phosphorus extracted by

202 microwave digestion from sludge and 80% from ash (Figure 2). This is consistent with unextracted

203 phosphorus of 20-70% in other studies (13,48). Hedley was developed for use in soils that have

204 significantly less organic matter than sludges. In sludges, the optimal application of Hedley may be to

205 compare the relative changes to functional phosphorus pools between samples.
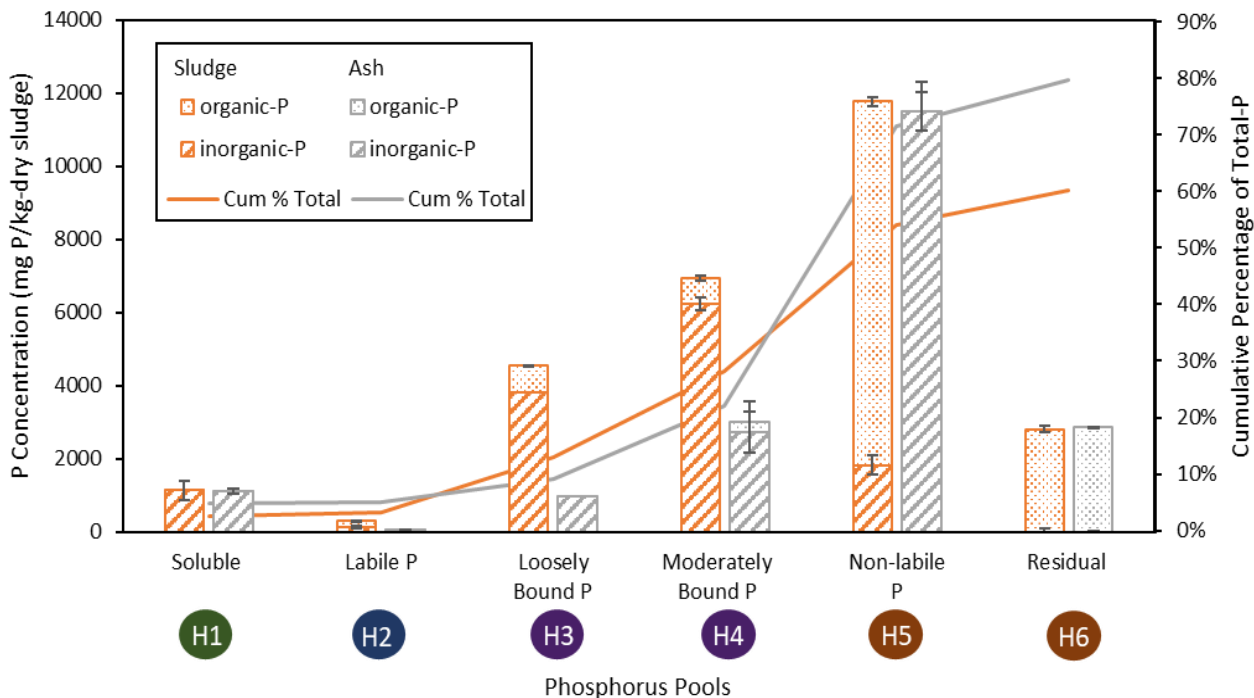
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Figure 2: Results from the Hedley method on sludge and ash presented in orange and gray, respectively. The different P-pools are shown on the x-axis and labelled with numbers corresponding to the respective extraction steps shown in the Hedley procedural schematic (Figure 1a.). The full bar represents the total-P in that fraction. The bars are subdivided into inorganic- and organic-P which are shown with diagonal stripes and dots, respectively. The P concentration is given in mg of P per kg of dry sludge. The cumulative percentage of P extracted by the Hedley method compared to the total-P for the sludge and ash are plotted as lines on the secondary axis.

207 *3.1.2. USEPA Method 1313*

208 USEPA Method 1313 was used to quantify pH-dependent phosphorus availability (Figure 3). Sludge is

209 slightly acidic while ash is slightly alkaline with native pH of 5.8 and 8.0, respectively. At native pHs,

210 phosphorus availabilities were at a minimum from both sludge and ash; increasing significantly under

211 strongly acidic and alkaline conditions.

212 At pH < 7, phosphorus availability was greater in ash than sludge, whereas at pH > 7, the opposite was

213 true. Under acidic conditions (pH < 6), the concentration of total available phosphorus in ash increased

214 from 370 to 3800 mg/kg–DS and from 150 to 1300 mg/kg–DS in sludge under the same conditions. Organic

215 phosphorus accounts for an important fraction of available phosphorus in both materials in these

216 conditions: 15 – 80% in sludge and 17 – 83% in ash. Most of the available organic phosphorus in ash

217 becomes available at pH = 4, whereas, in sludge, available organic phosphorus varies more with pH. This

218 difference likely reflects the absence of organic matter in ash.

219 Above pH 7, the concentration of available phosphorus in sludge increases almost linearly with increasing

220 pH. In slightly alkaline conditions ($7.5 \leq pH \leq 9.5$), available phosphorus from ash was 0.4% of available

221 phosphorus from sludge. As alkalinity increased (pH > 9.5), available phosphorus increased linearly in both

222 sludge and ash. Available phosphorus in ash remained 20 – 40% of that available in sludge (3100 – 17,000

223 mg/kg–DS and 650 – 6400 mg/kg–DS in sludge and ash, respectively). Organic phosphorus accounts for a

224 significantly larger fraction of available phosphorus in sludge than ash in alkaline conditions: 29 – 68%

225 versus 0 – 61%.

226 In summary, sludge shows generally higher concentrations of immediately available phosphorus across

227 environmentally relevant pH conditions (3.5 < pH < 8.5) while ash contains higher available phosphorus

228 under increasingly acidic conditions ($pH \leq 4$). Thermal treatment of sludge is likely transforming a portion

229 of immediately available phosphorus at native pH into more recalcitrant forms (27). This transformation

230 may have important benefits since, after land application, immediately available phosphorus may be

231 flushed rapidly from the system causing eutrophication of nearby surface waters. Conducting dynamic

232 leaching tests (Method 1314) allows this potential impact to be directly studied.
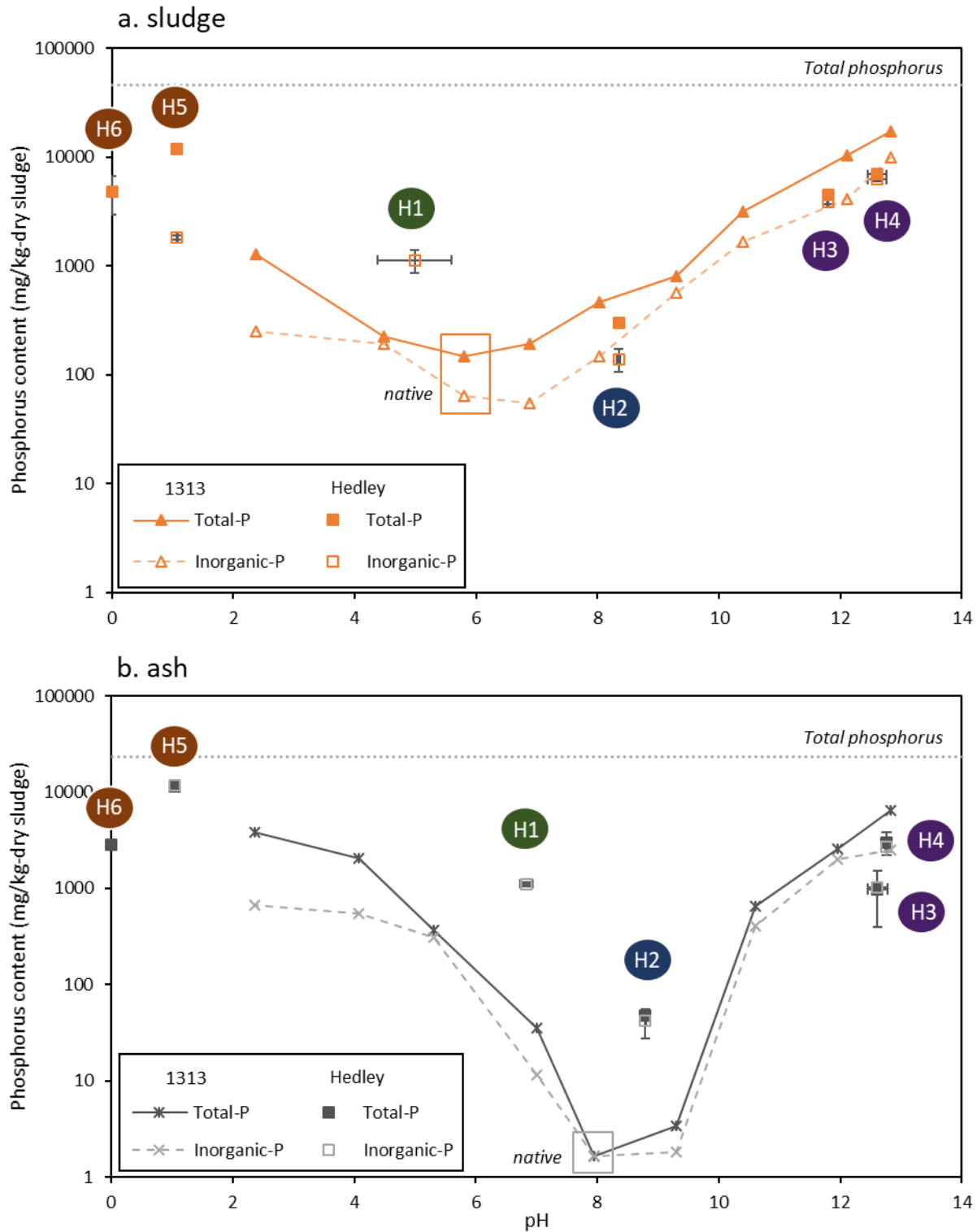
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a. sludge

b. ash

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Figure 3: Comparison of the available-P as a function of pH for a. sludge and b. ash using the results of the EPA leaching method 1313 and the Hedley fractionation procedure. Method 1313 results are plotted along the curves while the Hedley results are plotted as discrete points using square markers. The extraction steps corresponding to each of the points are labelled as 1-6 (see Figure 1 for the full procedure). Total phosphorus is presented as the dotted line. The native pH of each material is outlined in a box.
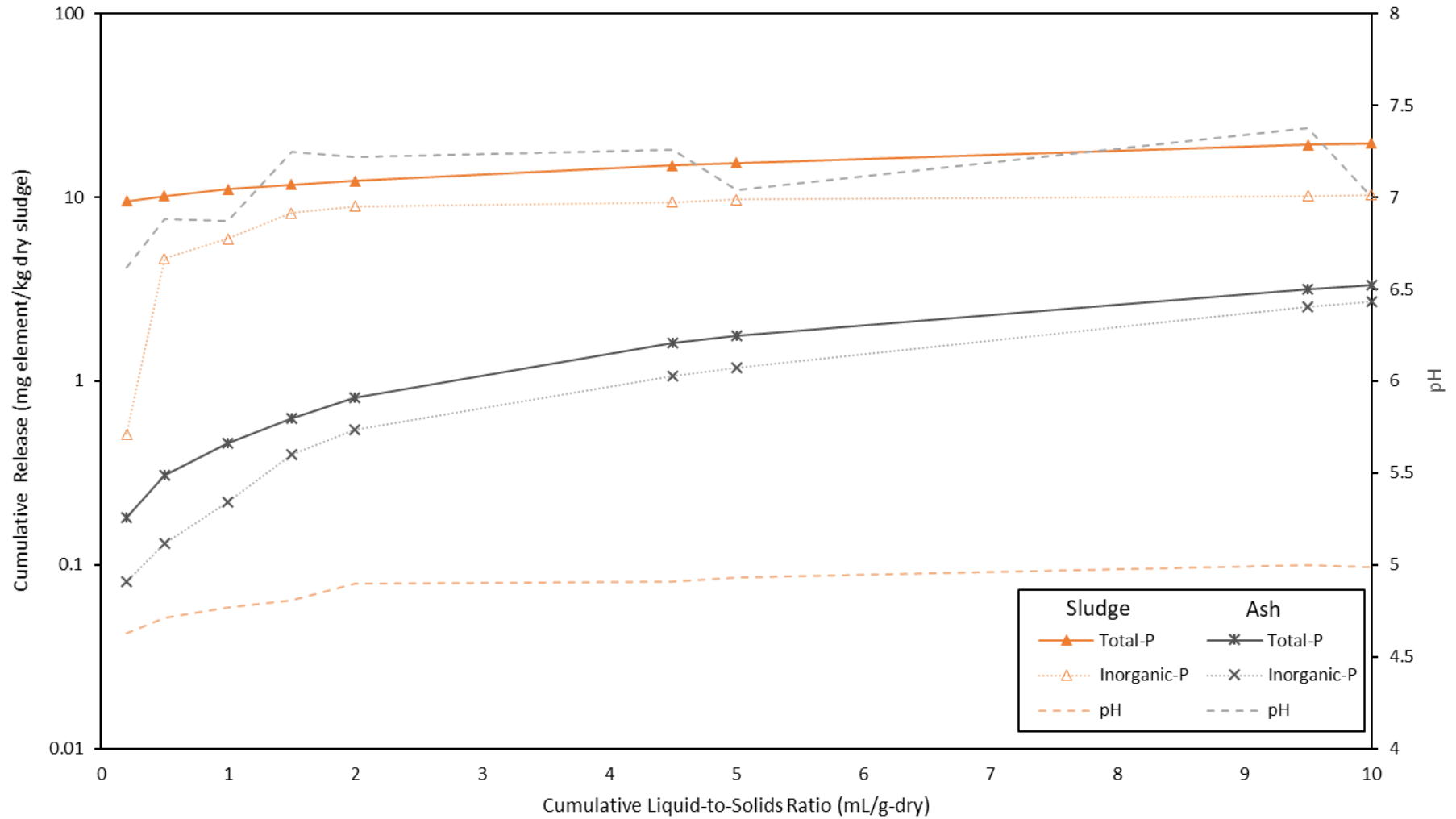
3.1.3. USEPA Method 1314

USEPA Method 1314 dynamic leaching tests evaluate constituent release from materials as a function of increasing L/S ratio. The highest concentration of phosphorus is released immediately from both sludge and ash (Figure 4); however, important differences were observed between their release profiles.

In sludge, an initial slug of 9.5 mg/kg–DS of phosphorus was released (L/S = 0.2) followed by diminishing rates. Phosphorus rapidly becomes availability – limited as minimal release occurs with additional percolation (0.5 ≤ L/S ≤ 10), leading to a cumulative release of 19.7 mg/kg–DS. Of the total eluted phosphorus, 48% is released immediately. This behaviour is consistent with applications of sludge to soil where a rapid initial release of phosphorus, which can be linked to eutrophication (5).

In ash, the initial slug of phosphorus leached was smaller - 92% less than in sludge - and released more slowly (0.8 mg/kg–DS over 0.2 ≤ L/S ≤ 2.0). Organic phosphorus accounted for 37% of this initial slug as compared to 95% of the initial slug in sludge. Total and inorganic phosphorus then exhibited a continued slow release for the remainder of the ash experiment (2.0 ≤ L/S ≤ 10), exhibiting solubility-limited behaviour. Importantly, approximately 81% of total eluted phosphorus from ash was in the inorganic form, compared to 52% from sludge.

Early washout of soluble ions did not have a substantial impact on eluate pH in either material, although thermal treatment affected the initial pH (Figure 4). The elevated temperatures of incineration are associated with processes such as denaturation of organic acids and combustion of organic materials that have been observed to cause similar increases in soil pH (49). Combustion of organic matter within sludge resulted in about 50% of phosphorus being released through volatilization (see Supplementary Materials Appendix A), illustrating an important mechanism for recovery during thermal treatment. Most of what remained within the ash was likely transformed into more crystalline forms (50). The net result was a decrease in immediate phosphorus leaching, agreeing with the Method 1313 results at native pH.

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Figure 4: The USEPA method 1314 column percolation experiments for sludge (orange) and ash (grey). The concentrations of released phosphorus are shown in mg of phosphorus
per kg dry sludge. The darker solid lines and lighter broken lines show total- and inorganic-P release, respectively. The pH changes over the column leaching experiment are plotted
as dotted lines on the secondary y-axis.

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267   *3.2. Potentially Toxic Element Availability and Leaching*

268   One of the barriers to land application of sludge, with or without further treatment, is potential release

269   of PTEs. LEAF Methods 1313 and 1314 provide additional data to evaluate their potential release. Figure

270   5 shows Method 1313 plots of 8 elements of concern identified in Ontario Regulation 338/09 (O. Reg. 338

271   CM1 NASM) from the Nutrient Management Act (2002) (see Supplementary Materials for PTE results from

272   Method 1314).

273   All elements generally had higher availabilities from sludge compared to ash. The exceptions typically

274   occurred in limited circumstances not relevant to conditions for land application (pH = 4). Cadmium,

275   molybdenum, and lead all had similar availabilities from sludge and ash under neutral to acidic conditions

276   and higher availabilities from sludge than ash under alkaline conditions. To understand how availability

277   translates to potential release behaviour upon potential land application, results from Methods 1313 and

278   1314 must be viewed together.

279   Cumulative releases of all elements apart from molybdenum were higher from sludge than ash, often

280   significantly higher (see Figure C2, Supplementary Materials). Importantly, although lead had higher

281   available concentration at neutral pH (Figure 5), lead release from ash was small and 5% of lead released

282   from sludge (Figure C2, Supplementary Materials). Among the other 6 PTEs, similar release trends were

283   observed; cumulative releases from ash were <1-10% of the cumulative releases from sludge, usually

284   because of a relatively large initial slug released from the sludge. The thermal treatment process appears

285   to affect the availability and leaching of PTEs in the resulting material, similar to phosphorus.

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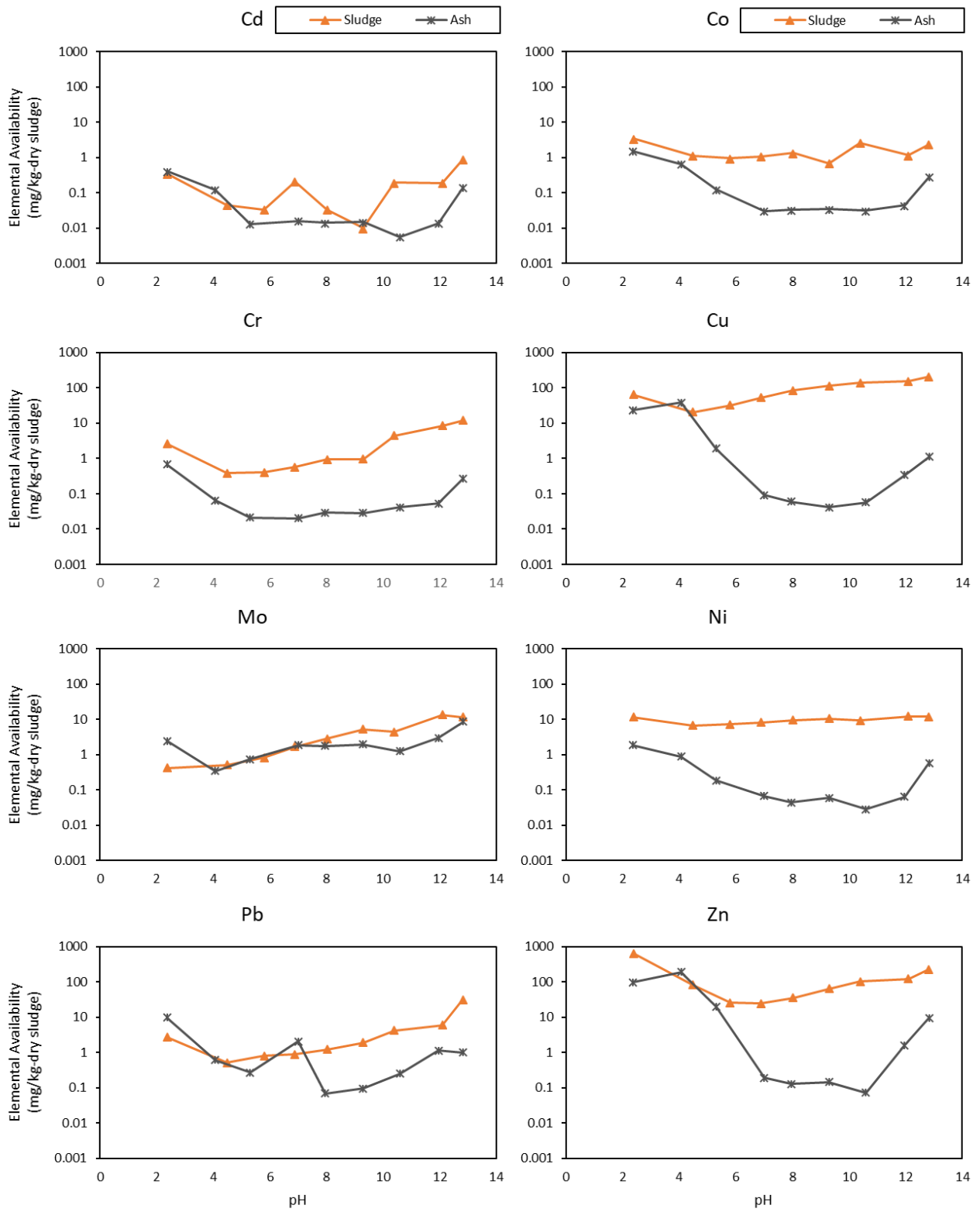
Figure 5: pH-dependent leaching curves for 8 PTEs of concern from O. Reg. 338 CM1 NASM for both sludge and ash, following
USEPA Method 1313. Values have been normalized per kg dry sludge.

3.3. Discussion: Comparing Hedley and LEAF Methods

LEAF methods are easier to execute than sequential fractionation procedures. Hedley uses 6 different chemical extractants of varying molarities while Method 1313 requires only 2 and Method 1314 only 1 (deionized water). Method 1313 consists of 9 parallel batch extractions that are performed simultaneously, yielding independent results. Although Method 1314 is technically also a series extraction from the percolation column, compared to sequential fractionation the sample collection is simplistic. Sample quantity may also influence results. Hedley indicates 1 g dried sample which may not fully represent material characteristics. Method 1313 utilizes 10 g (or more) dried sample for each extraction and Method 1314 uses at least 300 g dried sample in the column. Although likely more characteristic, the larger sample sizes used in LEAF may also be a drawback compared to Hedley if sample amount is limited.

Quantifying release of phosphorus from each material is important to evaluate what plants may receive. Inconsistencies between Hedley and LEAF methods have important implications. Hedley soluble (H1) and labile (H2) pools typically correspond to points between T04 and T08 in Method 1313 (Figure 1c). In this work, soluble (H1) and labile (H2) pools correspond to T07 (pH = 5.5) and T05 (pH = 8), respectively, in the sludge and to T06 (pH = 7) and T04 (pH = 9), respectively, in the ash (Figure 1c). Phosphorus released at native pH in Method 1313 is inconsistent with soluble (H1) pool from Hedley, releasing ~6% and <1% from the sludge and ash, respectively (Figure 3). Furthermore, in sludge, soluble (H1) pool exceeds available phosphorus determined by Method 1313 at 3.5 < pH < 9.5 (i.e., samples T04 – T08) making it only comparable at pH extremes, which is not relevant to land application. Similarly, in ash, soluble (H1) pool only matches the magnitude of available phosphorus from Method 1313 determined in more extreme conditions (pH <5 or pH >11). Additional discrepancies arise considering Method 1314 results. Cumulative inorganic phosphorus released from Method 1314 was <1% of the quantity released in soluble (H1) pool for both sludge and ash, even when differences in L/S ratios between methods were considered.

314   This lack of alignment is problematic. Readily soluble phosphorus is the primary concern for receiving

315   waters (5). Overestimation of this pool could result in insufficient phosphorus being applied to crops

316   whereas underestimation risks contributing to eutrophication. The discrepancy between the LEAF and

317   Hedley results for readily soluble phosphorus is probably caused by the Hedley's use of anion exchange

318   resin, which likely extracts low-leachability phosphorus bound to solids (51,52).

319   Hedley is also inconsistent with Method 1313 for the labile (H2), loosely bound (H3), and moderately

320   bound (H4) phosphorus pools (Figure 3), whereas non-labile (H5) and residual (H6) pools were in line with

321   Method 1313 results; however, pH values in Hedley are outside the range of Method 1313 (Figure 1c).

322   For sludge, the quantities of labile (H2) and loosely bound (H3) phosphorus are consistently lower than

323   available phosphorus from corresponding Method 1313 points, opposite to the case for readily soluble

324   (H1) phosphorus (Figure 3). The resin used in soluble (H1) pool may have extracted some phosphorus that

325   would otherwise appear in these subsequent pools, as was observed by (19). For ash, an order of

326   magnitude more phosphorus is released in Hedley labile (H2) fraction than by Method 1313 at the same

327   pH. The phosphorus concentrated in ash following thermal treatment of sludge may have exceeded the

328   capacity of the resin used in soluble (H1), enabling release into subsequent pools. Consistent with sludge,

329   phosphorus concentrations in Hedley loosely bound (H3) and moderately bound (H4) pools in ash are

330   lower than corresponding points in Method 1313. The large portion of sorbed phosphorus removed within

331   labile (H2) pool may have caused only more strongly bound phosphorus to remain, reducing the

332   proportion of phosphorus extracted within the loosely bound (H3) pool. These discrepancies demonstrate

333   an important drawback to Hedley and other sequential fractionation procedures: pools quantified in each

334   extraction step influence pools quantified in subsequent steps (19,44). Incorporating mineralogy into

335   phosphorus analyses has the potential to improve our interpretation and understanding of these results

336   (27,31). Phosphorus mineralogy of virgin sewage sludge is challenging (53) and research in this area is

337   limited. Minerals such as hydroxyapatite, brushite, monetite, and others have been identified in sludges

338 (53,54). Mineral phases seem to depend on source materials; wastewater treatment processes and

339 operating conditions; and subsequent sludge handling processes. For examples, phosphorus mineral

340 transformations have been observed after low temperature drying processes (53). High temperature

341 treatment likely causes further transformations (27,31). This work provides important groundwork for

342 future research exploring phosphorus mineralogy of sewage sludge and transformations brought about

343 by thermal treatment.

344 **4. Conclusions**

345 Hedley and LEAF methods provide trends in available phosphorus that are qualitatively consistent. In this

346 study, both suggest that thermal treatment of the sludge changes phosphorus minerals into forms that

347 are more strongly bound to the solid surfaces. Therefore, phosphorus is less likely to leach from the ash

348 in the short term, providing a more regulated source of gradual inorganic phosphorus with less potential

349 harm to downstream water bodies.

350 However, Hedley and LEAF methods provide quantitative differences and LEAF is concluded to be superior

351 for the following reasons. First, more incomplete phosphorus extraction from sludge than ash using

352 Hedley suggests that it may be less appropriate for organic materials; this is consistent with previous

353 studies and limits its applicability. Second, Hedley phosphorus pools were mostly at extreme pH

354 conditions while LEAF (Method 1313) provided results across a range of controlled pH conditions relevant

355 to land application. Third, Hedley overpredicted readily available phosphorus and underpredicted less

356 soluble forms. Moreover, Hedley overpredicted the amount that would rapidly leach as inferred by LEAF

357 (Method 1313) and directly quantified in LEAF (Method 1314). LEAF avoids the problem of sequential

358 fractionation procedures where pools quantified in each extraction step influence pools quantified in

359 subsequent steps. Fourth, LEAF was found to be practically simpler to execute and, while requiring more

360 sample, the results may be more representative. Fifth, LEAF additionally provides analysis of PTEs, which

361 are valuable for decision-making. In this study, smaller initial releases, lower availability in

362 environmentally relevant conditions, and lower total contents in ash are promising indicators that land

363 application of ash would likely result in less PTE release to soil compared to land application of sludge.

364 Although these elements could be analyzed in the Hedley extracts, that method was not designed for such

365 purposes.

366 This analysis shows the value of the USEPA LEAF Methods in understanding phosphorus availability from

367 materials such as sewage sludge before and after treatment. Land application of a material will change

368 soil pH, which influences phosphorus availability and leaching of PTEs. LEAF was shown to provide valuable

369 and superior insights into the effects of fluctuations in pH, dynamic leaching, and availability of PTEs. This

370 information is essential for assessing material reuse and land application options. It is expected that LEAF

371 will be similarly beneficial, relative to sequential fractionation methods (e.g., Hedley), when applied to

372 soils and other relevant matrices.

373 **Acknowledgements**

380 **Supplementary Materials**

381 Supplementary data associated with this article can be found, in the online version, at:

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