



Development of a cobalt electrode for the determination of Phosphate in soil extracts and comparison with standard methods

Journal:	<i>Analytical Letters</i>
Manuscript ID	Draft
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Ebuele, Victor; Bangor University, School of Chemistry Congrave, Daniel; Durham University Department of Chemistry Gwenin, Christopher; Bangor University, Chemistry Thoss, Vera; Bangor University, Chemistry
Keywords:	ammonium lactate-acetic acid extraction, potentiometry, phosphate determination, cobalt electrode, soil extract

SCHOLARONE™
Manuscripts

1 **Development of a cobalt electrode for the determination of**
2 **Phosphate in soil extracts and comparison with standard methods**

3 **Victor O. Ebuele^a, Daniel G. Congrave^a, Chris D. Gwenin^a and Vera**
4 **Fitzsimmons-Thoss^{a*}**

5 ^a School of Chemistry, Bangor University, Bangor, LL 57 UW, Wales

6 * Corresponding author (vera.thoss@bangor.ac.uk)

7 **Abstract**

8 A phosphate-sensitive cobalt electrode was evaluated in detecting orthophosphate
9 ions (H_2PO_4^-) in ammonium lactate-acetic acid soil extracts. The dependence of the
10 mixed potential of a cobalt electrode on H_2PO_4^- concentration was investigated via
11 potentiometry. The mechanism of detection is based on the consumption of a
12 surface cobalt (II) oxide layer to form $(\text{Co}_3(\text{PO}_4)_2)$, which leads to a concentration-
13 dependent shift of the mixed potential. Two reference electrodes were evaluated:
14 Ag/AgCl (3 M) KCl and a platinum (Pt) wire. A linear response was observed using
15 both reference electrodes. However, application of a Pt wire quasireference
16 electrode increased the linear dynamic response range of the detector from $10-10^3$
17 mg L^{-1} or $10^{-4}-10^{-1}$ M (Ag/AgCl (3 M) KCl) to $0.1-10^5$ mg L^{-1} or $10^{-6}-10^1$ M. In
18 addition, the response time using the Pt wire was less than 5 minutes compared to a
19 minimum of 10 minutes using Ag/AgCl (3 M) KCl. There was close agreement
20 between the response of the phosphate-sensitive cobalt electrode with a standard
21 colorimetric method. As dissolved organic substances can potentially interfere with
22 electrochemical techniques, an investigation into the use of a nonpolar resin for
23 decolorization and removal of organic matter in soil extracts was carried out and
24 successfully employed. The phosphate-sensitive cobalt electrode was found to be a
25 fast method for the analysis of soil extracts with high sensitivity and selectivity. It has
26 the potential to be developed into a sensor for the *in situ* measurement of phosphate
27 in various environmental matrices.

28
29 **Key words:** ammonium lactate-acetic acid extraction, potentiometry, phosphate
30 determination, cobalt electrode, soil extract.

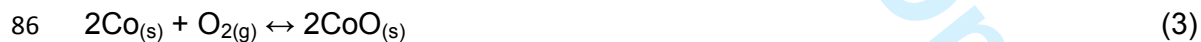
31 Introduction

32 Phosphorus (P) is one of the essential elements for plant growth and development
33 because P plays a vital role in the cell physiology and biochemistry of all living
34 organisms (Hanrahan et al. 2005; Adeloju 2013). Phosphorus naturally exists in either
35 organically or inorganically-bound forms, mostly as insoluble phosphate minerals
36 (Hanrahan et al. 2005; Adeloju 2013; Warwick et al. 2013). In soil extracts (water and
37 dilute salt solutions such as KCl and CaCl₂), P is usually found in the form of the
38 orthophosphates H₂PO₄⁻ and HPO₄²⁻ (0.01 – 3.0 mg L⁻¹) (Sims 2000; Warwick et al.
39 2013), which are the main readily available forms of P for plant growth. In rivers,
40 orthophosphate concentrations vary greatly from 0.02 – 1 mg L⁻¹. (Quintana et al.
41 2004; Warwick et al. 2013). In natural and waste waters orthophosphate
42 concentration varies from 0.2 - 10 mg L⁻¹, while in soil it varies from 0.2 - 50 mg kg⁻¹
43 (Warwick et al. 2013). In recent decades, a large increase in the use of phosphate-
44 containing fertilisers has resulted in increased concentrations of orthophosphate in
45 land runoff, which can lead to eutrophication. This process is regarded as one of the
46 major threats to the aquatic environment (Abowei et al. 2005). Thus, there is a need
47 for the development of improved orthophosphate detection techniques in order to
48 enhance our knowledge regarding the major sources of orthophosphate input to
49 water bodies and to improve our understanding of P-cycling in various environments.

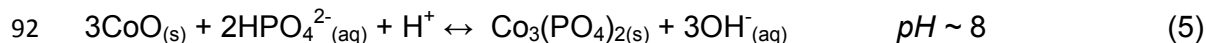
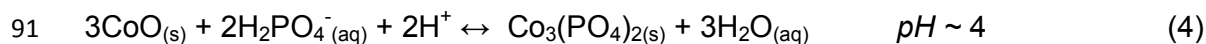
50 The methods used for the monitoring and management of environmental P, based
51 on its accurate determination in soil and water samples, were reviewed by Worsfold
52 et al (2005). Most often the Murphy and Riley (1962) colourimetric method is used to
53 quantify soluble reactive P because of its low limit of detection (≤ 0.01 mg L⁻¹)
54 (Murphy and Riley 1962; Motomizu 1983; Pierzynski 2005). For total P analysis
55 (inorganic and organic-bound P), samples can be acid digested and then analysed
56 via Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), with a
57 limit of detection of 0.03 mg L⁻¹ (Thompson and Walsh 2003; Pierzynski 2005). Ion-
58 Chromatography (IC) directly measures orthophosphate concentration with a limit of
59 detection of 0.002 mg L⁻¹ (Mckelvie et al. 1995). Orthophosphate has also been
60 determined using indirect techniques: precipitation with Ca²⁺ or Pb²⁺ in flow injection
61 analysis (FIA) (Midgley 1979; Chen et al. 1997) or using ion selective electrodes
62 (ISEs) (Marco et al., 1998; Vanstaden 1993; Xiao et al. 1995; Lee et al., 2009).
63 However, the indirect methods are often affected by matrix interferences due to the

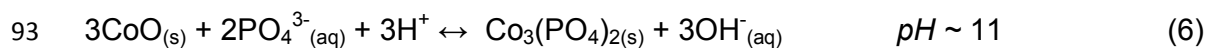
64 formation of Pb^{2+} and Ca^{2+} compounds with other anions; (Midgley 1986; Alexander
65 and Koopetngarm 1987; Xiao et al. 1995; Berchmans et al. 2012; Warwick et al.
66 2013).

67 In recent years a number of methods have been reported for the electrochemical
68 detection of orthophosphate (Xiao et al.1995; Meruva and Meyerhoff 1996; Adeloju
69 2013; Berchmans et al. 2012; Warwick et al. 2013). Many of these methods are
70 discussed in a recent review (Berchmans et al. 2012). The potentiometric detection
71 of orthophosphate using simple metal electrodes is a promising method of
72 quantification. Although the potential for selectivity issues may be increased
73 compared to biosensors, systems involving ion selective membranes and other
74 indirect techniques (Berchmans et al. 2012), metal electrodes are cheap, simple and
75 robust. For example, while salophen-based electrochemical sensors show good
76 selectivity for HPO_4^{2-} down to 10^{-5} M with short response times, they have short
77 lifetimes and require strict laboratory conditions (Berchmans et al. 2012; Kim et
78 al.2008). Xiao et al (1995) reported the first comprehensive evaluation of a
79 phosphate-sensitive electrode based on a redox reaction between cobalt and
80 dissolved orthophosphates. The dynamic range was $1\text{--}1000$ mg L^{-1} or $10^{-5}\text{--}10^{-2}$ M
81 using 0.025 M potassium hydrogen phthalate as a buffer solution. The hypothesised
82 mechanism for the phosphate-sensitive Cobalt electrode is dependent on the
83 formation of an oxide layer (Eqs (1)- (3)) (Mervu and Meyerhoff (1996)



87 The cobalt oxide layer reacts with orthophosphates (Eqs (4) – (6)), the exact nature
88 of which are dependent on pH. The potentiometric response given by these
89 reactions can then be related to phosphate concentration because it affects the
90 concentration of available CoO (Eq 1).





6 94 The formation of cobalt (II) phosphate in the reactions outlined (Eqs (4) – (6))
7
8 95 consumes cobalt (II) oxide, perturbing the equilibrium (Eq (3)). This equilibrium must
9
10 96 be restored by further oxidation of cobalt from the electrode surface to form cobalt
11
12 97 (II) oxide, which shifts the mixed potential. The measured mixed potential for this
13
14 98 system is not a true equilibrium potential, but a steady state potential. It occurs when
15
16 99 the anodic current (i_a) formed by the dissolution (oxidation) of the metal at the anode
17
18 100 (Eq (1)), is equal in magnitude to the cathodic current (i_c) formed by the reduction of
19
20 101 oxygen (Eq (2)) at the cathode ($|i_a| = |i_c|$). This results in the reaction having a net
21
22 102 current of zero, but the reactions involved are still taking place at a measurable rate.
23
24 103 The shift in potential is directly proportional to the logarithm of the phosphate
25
26 104 concentration according to the Nernst equation. The electrode response also
27
28 105 depends on ionic strength, buffer salts and sample stirring rate (Meruva and
29
30 106 Meyerhoff 1996; Engblom 1999). Engblom (1999) applied the cobalt electrode to soil
31
32 107 extracts, using ammonium lactate-acetic acid as extractant for available P, achieving
33
34 108 a linear range 10–100 mg L⁻¹ or 10⁻⁴–10⁻³ M. However, this is not sufficiently
35
36 109 sensitive for environmental applications because the concentration of
37
38 110 orthophosphate in fertile arable soil extracts is usually between 0.01–3.0 mg L⁻¹
39
40 111 while for freshwater analysis concentrations of less than 0.1 mg L⁻¹ (UK
41
42 112 environmental agency guidelines) need to be reached. A common matrix
43
44 113 interference encountered in the analysis of soil extracts by electrochemical methods
45
46 114 is the deposition of dissolved organic matter on the electrode surface. Dissolved
47
48 115 organic matter can be reduced by washing extracts with carbon black; however,
49
50 116 slight increases in P concentration have been noted due to the leaching of P from
51
52 117 carbon black (Engblom 1999). For the electrochemical quantification of
53
54
55
56
57
58
59
60

1
2
3 118 orthophosphate, an easy method for the removal of organic matter which does not
4
5 119 influence the available P concentration is highly appealing and vital for accurate and
6
7 120 reliable measurements.
8

9
10 121 In this study, the response of the phosphate-sensitive cobalt electrode in ammonium
11
12 122 lactate-acetic acid soil extracts is investigated. The removal of organic matter using
13
14 123 Amberlite XAD-4 resin is also introduced as an advantageous alternative to the
15
16 124 aforementioned carbon black. The electrochemical sensor was also examined to
17
18 125 improve its detection range. The electrochemical performance of the cobalt-based
19
20 126 sensor was analysed in the presence of two difference reference electrode systems:
21
22 127 a Pt wire (a single phase reference and so a 'quasireference' electrode) and an
23
24 128 Ag/AgCl (3 M) KCl reference electrode (an electrode of the 2nd kind, three phases in
25
26 129 equilibrium) and the detection range, response time and reproducibility of both
27
28 130 methods were compared.
29
30
31

32 **Materials and Methods**

33 **Cobalt electrode**

34
35 132 The electrodes used in this work were fabricated from 99.99% pure cobalt rods (50
36
37 133 mm length, 2 mm diameter, 3.14 mm² cross sectional area, Goodfellow, Cambridge,
38
39 134 UK). The rods were cut in half and each half was encased in a
40
41 135 Polytetrafluoroethylene (PTFE) housing, leaving one third exposed as a point of
42
43 136 contact for the multimeter (Figure 1). The surfaces of the electrodes were polished
44
45 137 mechanically using a polishing wheel (Buehler Metaserv 2000 grinder/polisher,
46
47 138 USA), fitted with abrasive polishing pads (Buehler, USA), alongside different grades
48
49 139 of alumina powder (15 μ m, 3 μ m and 1 μ m, Buehler micropolish, USA), prepared as
50
51 140 aqueous suspensions. The electrodes were then washed with water and sonicated
52
53 141 (Branson 1510 sonicator, USA). Each freshly polished electrode was then
54
55 142
56
57
58
59
60

1
2
3 143 conditioned for 2 hours by immersion in deionised water. Figure S1 (in supporting
4
5 144 information) shows the experimental setup. Two reference electrodes were used, an
6
7 145 Ag/AgCl (3 M) KCl reference electrode (MI-401, Microelectrodes Inc. USA), and a
8
9 146 platinum wire (Pt) of 99.9% purity (Goodfellow, Cambridge, UK).

11
12 147 For measurements involving the Ag/AgCl (3 M) KCl reference, the potential
13
14 148 difference between the two electrodes (cobalt working and Ag/AgCl (3 M) KCl
15
16 149 reference) was monitored using a digital multimeter (Metex M-3000, USA, input
17
18 150 impedance < 10 M ohms) during conditioning in deionized water until a stable value,
19
20 151 usually below -280 mV, was attained. The electrodes were then placed in buffer
21
22 152 solution (0.1 M ammonium lactate and 0.4 M acetic acid solution) until a new steady
23
24 153 state was established (~45 minutes). At this point, the electrode was ready for use.
25
26 154 During measurements, the electrode was immersed for an equilibration period of 10
27
28 155 minutes in the sample/ orthophosphate standard solution before the potential was
29
30 156 recorded. This requirement was determined based on the observed response times
31
32 157 of the sensor when analyzing orthophosphate standard solutions of concentrations
33
34 158 below 1 mg L⁻¹ (10⁻⁵ M). The stability of the electrode in the ammonium lactate-acetic
35
36 159 acid solution was also investigated for a period of 7 days.

37
38
39 160 The platinum wire quasireference electrode was first calibrated to determine its
40
41 161 potential against a true reference electrode. This was determined against an
42
43 162 Ag/AgCl (3 M) KCl reference electrode in ammonium lactate-acetic acid solution
44
45 163 using the multimeter and the data is given in the supporting information (Table S1).
46
47 164 The experimental setup (Figure S1) was slightly modified when using the Pt wire.
48
49 165 The potential difference between the two electrodes (cobalt and Pt wire), was
50
51 166 monitored during the deionized water conditioning until a stable potentiometric
52
53 167 response (below -400 mV) was reached. The electrode was placed in buffer solution
54
55
56
57
58
59
60

1
2
3 168 (0.1 M ammonium lactate and 0.4 M acetic acid solution) until a new steady state
4
5 169 was established (~45 minutes). Before being used, the Pt wire quasireference was
6
7 170 sterilised on a Bunsen burner and left to cool and equilibrate in the solution for 5
8
9 171 minutes.

10
11
12 172 For measurements involving both reference electrodes (Ag/AgCl (3 M) KCl and Pt
13
14 173 wire), the solutions were stirred constantly using a magnetic stirrer (HANNA
15
16 174 instruments Hi-190M, UK). The cobalt electrode was always rinsed with deionized
17
18 175 water after each reading to extend the lifetime of the sensor by reducing
19
20 176 interferences that could occur due to accumulation of phosphate on the surface of
21
22 177 the electrode. The electrodes were freshly polished and conditioned after each
23
24 178 batch of samples had been analysed. Prolonged use without polishing, particularly in
25
26 179 the analysis of soil extracts, lead to a lower sensitivity and longer response time. All
27
28 180 calibration measurements were carried out in triplicate and the averages of these
29
30 181 measurements were used to plot the calibration curve.

31
32
33
34
35 182 The cyclic voltammograms of the phosphate layer formation were recorded using an
36
37 183 auto lab electrochemical analyser (Autolab potentiostat PGSTAT30). A three
38
39 184 electrode system was used: an Ag/AgCl (3 M) KCl reference, a Pt wire counter and
40
41 185 the cobalt working electrode. The electrolyte consisted of a 0.1 M solution of
42
43 186 potassium dihydrogen phosphate (mono-basic) in 0.1 M ammonium lactate and 0.4
44
45 187 M acetic acid buffer (pH 3.75). The experiment was performed at a scan rate of 100
46
47 188 mV s^{-1} over a potential range of $-800 \text{ mV} - 800 \text{ mV}$ for 50 scans. The electrolyte
48
49 189 was degassed by sparging it with Nitrogen (N_2) for 10 minutes prior to the
50
51 190 experiment to remove dissolved oxygen (O_2).

52
53
54
55
56 191 **Sample Collection and Preparation.**
57
58
59
60

1
2
3 192 Soil samples (n=5) were collected in June 2012 from the Henfaes Research Centre,
4
5 193 (Abergwyngregyn, United Kingdom) from the following coordinates; (53°14'28.08N,
6
7 194 4°01'17.97" W). The site has been a permanent pasture over the last 11 years and
8
9
10 195 has constantly been grazed by sheep. Samples were collected using an auger
11
12 196 (Eijkelkamp, Holland) up to a soil depth of 30 cm. The soil cores (inner diameter, 8
13
14 197 cm), were split into two sections: Surface sA (0 cm - 15 cm) and subsurface sB (15
15
16 198 cm - 30 cm). Each sample was air-dried, passed through a 2 mm sieve and stored
17
18 199 for analysis.

20
21 200 Compost samples (T1 to T4) were collected from the Treborth botanical gardens,
22
23 201 (Bangor, United Kingdom), and were used to compare the capability of absorbing
24
25 202 organic matter using Amberlite XAD-4 (Sigma Aldrich, UK) and carbon black (Fisher
26
27 203 scientific, UK).

28
29
30 204

31 205 **Sample treatment and P extraction**

32
33
34 206 Soil pH values were measured with an Orion (Boston, USA) 420A pH meter.
35
36 207 Samples were prepared in a 1:2.5 w/v soil to deionised water ratio and were stirred
37
38 208 (HANNA instruments Hi-190M, UK) for 15 minutes prior to the measurement.
39
40 209 Organic matter was determined by the loss of ignition method, heating 10 g of
41
42 210 sample to 450 °C in a Muffle furnace (Carbolite, UK) for 4 hours.

43
44
45
46 211 The nitric acid (HNO₃) digestion used for total P determination was based on a
47
48 212 modified procedure recommended by the United States Environmental Protection
49
50 213 Agency (USEPA, Method 3050, 1989). One gram of soil was placed in a 100 mL
51
52 214 pyrex digestion tube and a pre-digestion step was run at room temperature for 16
53
54 215 hours with 10 mL nitric acid (Sigma Aldrich reagent grade, 70%). Then, the
55
56 216 suspension was digested at 120 °C for 4 h, in a Stuart SBH 200D13 digester (Bibby

1
2
3 217 scientific UK). The obtained suspension was then filtered and diluted to 100 mL with
4
5 218 deionised water, before being stored in polyethylene bottles at 4 °C for analysis by
6
7 219 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).
8
9

10 220 Ammonium lactate-acetic acid extraction for available and total dissolved P was
11
12 221 based on the protocols of Engblom (1999). Five grams of the soil was weighed into a
13
14 222 250 mL Erlenmeyer flask and mixed with 100 mL of 0.1 M ammonium lactate and 0.4
15
16 223 M acetic acid solution (pH 3.75). This was shaken using a Thermo Scientific Max Q
17
18 224 Shaker, UK for 90 minutes, filtered and stored at room temperature for analysis by
19
20 225 Murphy Riley (1962) colourimetry, ICP-OES and electrochemical methods.
21
22
23

24 226 The four compost samples (T1 to T4) were analysed for extractable P (Olsen P in
25
26 227 mg kg⁻¹). For the carbon black method, the removal of organic matter from the
27
28 228 compost extracts was carried out by adding 0.5 g of carbon black to 50 mL of an
29
30 229 extract (T1 to T4) and shaking it for 15 minutes. For the resin method, 10 g of wet
31
32 230 resin (corresponding to ca. 4 g dry resin) was added to 50 mL of an extract (T1 to
33
34 231 T4) and shaken for 20 hours. After filtration through Whatman no.42 filter paper, the
35
36 232 orthophosphate concentrations of the extracts were determined using Murphy and
37
38 233 Riley (1962) colourimetry.
39
40
41
42

43 234 **Reagent and solutions**

44
45
46 235 Deionised water (Elga option 4 water purifier, USA) was used for the preparation of
47
48 236 samples, extracts and standard solutions. All other chemicals were of analytical-
49
50 237 reagent grade such as HNO₃ (Sigma Aldrich reagent grade, 70%). All phosphate
51
52 238 standards used for the electrochemical analysis were prepared in ammonium
53
54 239 lactate-acetic acid solution daily from a monobasic potassium phosphate stock
55
56 240 solution (Sigma Aldrich, UK purity of >98%) across a concentration range of 0.001 –
57
58
59
60

1
2
3 241 10^5 mg L⁻¹ or 10^{-8} – 10^1 M. Method interferences caused by contaminants in
4
5 242 deionised water, reagents, glassware, and other sample processing was reduced by
6
7 243 washing all glassware with 2% HCl, rinsing with deionised water and allowing it to
8
9 244 dry before use.

10 11 12 245 **Instruments**

13
14
15 246 A Varian Agilent 710-ES model ICP-OES with a Varian SPS-3 auto sampler was
16
17 247 used for P determination of the soil extracts. The operating parameters of the
18
19 248 instrument were: Wavelength: 213.618 nm, RF Generator power: 1.2 kW, plasma
20
21 249 argon flow: 15.0 L min⁻¹, auxiliary argon flow: 15.0 L min⁻¹, nebulizer pressure;
22
23 250 200Kpa, nebulizer argon flow; 0.75 L min⁻¹, replicate time; 5s, sample uptake: 5 mL,
24
25 251 rinse time 30s, pump rate: 12 rpm.

26
27
28
29 252 A Jasco V550 UltraViolet Visible Spectrophotometer (Maryland court, USA) was
30
31 253 used for the colorimetric analysis using the Murphy Riley method. Measurements
32
33 254 were carried out at a wavelength of 880 nm and path length of 5 cm.

34 35 36 37 255 **Data Analyses and Quality Control**

38
39 256 All soil results were reported based on their air-dried weight at room temperature
40
41 257 (25°C). Statistical differences among means were evaluated using student t-test
42
43 258 (paired two sample for means), ANOVA-single factor and F- Test. All analytical
44
45 259 results are means of three replicate analyses with standard errors less than $\pm 5\%$ of
46
47 260 the mean value.

48 49 50 51 261 **Results**

52 53 54 262 **Phosphate-Sensitive Cobalt Electrode: Calibration and Stability**

1
2
3 263 Cyclic voltammetry was applied to the cobalt electrode in the presence of
4
5 264 orthophosphate. The cyclic voltammograms (Figure 1) show the oxidation of cobalt to
6
7 265 cobalt (II) oxide (Co^0 to Co^{2+}), which leads to the formation of cobalt (II) phosphate in
8
9
10 266 the presence of orthophosphate (Meruva and Meyerhoff 1996). This is recognisable
11
12 267 by the anodic processes commencing between -200 to -100 mV vs. Ag/AgCl (3M)
13
14 268 KCl on the forward scans. On the reverse scans, no significant cathodic processes
15
16 269 are observed, as expected (Meruva and Meyerhoff 1996). This indicates that the
17
18 270 formation of Co^{2+} is irreversible within the potential range. The anodic peak therefore
19
20
21 271 decreases in magnitude with successive scans as the electrode surface becomes
22
23 272 increasingly coated with cobalt (II) phosphate, effectively reducing the electrode
24
25 273 surface area (Figure 1). This also has the effect of shifting the anodic processes to
26
27 274 more positive potentials with successive scans.

28
29
30 275 The potentiometric response of the cobalt electrode coupled to an Ag/AgCl (3 M) KCl
31
32 276 reference electrode to different concentrations of phosphate standards was also
33
34 277 examined (Figures S2 and S3 in supporting information). To gain insight into the
35
36 278 lifetime of the sensor, the detection range of the electrode calibrated in the same
37
38 279 beaker, several times over 7 days (stored under air without polishing between runs)
39
40
41 280 was carried out. After three days, a change in the response of the electrode at low
42
43 281 concentrations was observed (Figure S2).

44
45
46 282 The original linear characteristics were retained after polishing and sonicating of the
47
48 283 electrode. Since the electrode had been exposed to air, the cobalt surface may have
49
50 284 been oxidized and covered by particles and precipitates. These particles were
51
52 285 removed by the polishing and sonication of the surface of the electrode. Coupled to a
53
54
55 286 Pt wire quasireference electrode, the cobalt electrode showed a linear response to
56
57 287 varying orthophosphate concentration. The modified system exhibits improved
58
59
60

1
2
3 288 sensitivity towards phosphates ions, resulting in an increase in the linear range of
4
5 289 detection to $0.1 - 10^5 \text{ mg L}^{-1}$ or $10^{-6} - 10^1 \text{ M}$ (Figure S2). Repeatability was also
6
7 290 observed for the low concentration orthophosphate standards.
8
9

10 291 **Phosphorus Quantification in Soil Extracts: Comparison between the cobalt**
11
12 292 **electrode coupled to an Ag/AgCl (3 M) KCl Reference Electrode and**
13
14 293 **Colourimetry**
15

16
17 294 Ammonium lactate-acetic acid soil extracts were chosen to test the phosphate-
18
19 295 sensitive cobalt electrode as they provide higher concentrations of orthophosphate
20
21 296 compared to freshwater. Table 1 shows the total P concentration of all ten soil
22
23 297 samples, which ranged from $384 - 1189 \text{ mg kg}^{-1}$ (Measured using ICP-OES on nitric
24
25 298 acid extracts). The organic matter content ranged from $2.9 - 7.6 \%$ and pH in water
26
27 299 ranged from $5.4 - 8.0$. The Murphy and Riley colourimetric technique is the most
28
29 300 accepted standard method for the measurement of orthophosphate in soil extracts
30
31 301 (Worsfold et al. 2005). The cobalt electrode coupled to a Ag/AgCl (3 M) KCl
32
33 302 reference electrode showed no significant differences compared to the colourimetric
34
35 303 P determination for eight of the ten soil extracts while samples s3A and s3B showed
36
37 304 a significantly lower concentration ($p = 0.02$). This deviation does not appear to be
38
39 305 linked to total P concentration, organic matter content, or pH (Table 1), indicating
40
41 306 that the sensor is potentially susceptible to other matrix interferences.
42
43
44
45

46
47 307 The actual solution orthophosphate concentrations in the soil extracts ranged from
48
49 308 $0.3 - 1.9 \text{ mg L}^{-1}$. As the actual orthophosphate concentrations were below the linear
50
51 309 detection range for the Ag/AgCl (3 M) KCl reference electrode (Figure S2), the
52
53 310 results reported (Table 1) were calculated based on the lower and upper limiting
54
55 311 standards bracketing these concentrations (Engblom 1999).
56
57
58
59
60

1
2
3 312 In order to evaluate the sensitivity of the cobalt electrode coupled to a Pt wire
4
5 313 quasireference electrode as a system for detecting orthophosphate concentrations in
6
7 314 ammonium lactate-acetic acid soil extracts, five soil samples were tested and the
8
9 315 data were compared to those obtained using the Ag/AgCl (3 M) KCl reference
10
11 316 electrode method and colourimetry. Results (Figure 2) showed no significant
12
13 317 differences compared to the colourimetric P determination for two out of the five
14
15 318 samples but significantly lower concentrations for the other three samples ($p = 0.04$
16
17 319 s2A, $p = 0.03$ s2B and $p = 0.02$ s4B). Although the platinum quasireference method
18
19 320 provides a wider linear range of detection compared to the Ag/AgCl (3 M) KCl
20
21 321 reference electrode method for standard solutions, it appears that it may be more
22
23 322 susceptible to matrix interferences in natural media, potentially due to the adsorption
24
25 323 of organic compounds onto the surface of the platinum electrode.
26
27
28
29

30 324 **Organic matter influence on P determination**

31
32 325 Four compost extracts (T1-T4) high in organic matter content were used to compare
33
34 326 carbon black and amberlite XAD-4 for the removal of dissolved organic matter. From
35
36 327 loss on ignition analysis the organic matter content was determined as T1 = 96 %,
37
38 328 T2 = 95 %, T3 = 93 % and T4 = 96 %.

39
40
41 329 Results from the ANOVA-single factor analysis revealed that there was a significant
42
43 330 difference between the treatments ($p < 0.05$) within each sample. Treatment of the
44
45 331 compost extracts with non-polar resin resulted in a noticeable change in colour.
46
47 332 Further analysis to differentiate between each treatment, using the Fisher test (F-test
48
49 333 two sample for equal variance, ($\alpha = 0.05$)) revealed that for sample T3 there was no
50
51 334 significant difference between no-treatment, carbon black and resin treatment ($p >$
52
53 335 0.05). For samples T1, T2, there was no significant difference between no-treatment
54
55 336 and resin treatment, but a significant increase in P concentration after carbon black
56
57
58
59
60

1
2
3 337 treatment was observed ($p < 0.05$). This could be due to the fact that phosphoric acid
4
5 338 is often used in the preparation of carbon black/ activated charcoal. T4 showed a
6
7 339 significant difference between the three treatments, with lowest values found in resin
8
9 340 treatment and highest in the carbon black treatment (Figure 4).

11 341 Overall, the results showed that the non-polar resin adsorbed organic matter from
12
13 342 extracts without increasing the P concentration, while carbon black treatment for all
14
15 343 samples added P to the compost extracts. However, this effect seems to be less
16
17 344 relevant in samples with lower organic matter content (i.e. T3) but leads to significant
18
19 345 differences in P results in samples with higher organic matter content (i.e. T4).

22 346 The non-polar resin was applied to the ten surface (s1A–s5A) and sub-surface (s1A–
23
24 347 s5B) soil extracts and P was determined using colourimetry (C) and the phosphate-
25
26 348 sensitive cobalt electrode coupled to the Ag/AgCl (3 M) KCl reference electrode
27
28 349 method (P). The results obtained with the two different methods before and after
29
30 350 resin treatment are shown in Table 2.

33 351 Results from the electrode measurements showed that there was a 21–55%
34
35 352 decrease in orthophosphate concentration across the surface soil while a 16–56 %
36
37 353 significant decrease was observed across the sub-surface soils ($p = 0.03$). It was
38
39 354 also noticed that the electrode attained a steady potential earlier (< 5 min) after resin
40
41 355 treatment. The results from the colourimetric analysis also showed a 8–26 %
42
43 356 reduction in orthophosphate concentration across the surface soils, while the sub-
44
45 357 surface soils showed a 24–42% significant reduction ($p = 0.004$) (Table 2).

51 358 Discussion

52 359 The formation of a cobalt (II) phosphate layer in the presence of orthophosphate was
53
54 360 confirmed by cyclic voltammetry. The anodic peak decreased with an increasing
55
56 361 number of scans because of the accumulation of cobalt phosphate on the surface of
57
58
59
60

1
2
3 362 the electrode, which forms a layer preventing the oxidation of Co^0 to Co^{2+} in
4
5 363 successive scans (Meruva and Meyerhoff 1996; Engblom. 1999).

6
7
8 364 Using the cobalt electrode coupled to an Ag/AgCl (3 M) KCl reference electrode in
9
10 365 standards prepared in ammonium lactate-acetic acid solution, the linear detection
11
12 366 range attained was consistent with that reported by Engblom (1999). However, this is
13
14 367 still higher than most orthophosphate concentrations routinely measured in
15
16 368 freshwater (Sims 2000; Warwick et al. 2013). The maximum permissible
17
18 369 orthophosphate concentration in river water according to Environment Agency
19
20 370 guidelines in the UK is 0.04–0.12 mg L⁻¹ which implies that the limit of quantification
21
22 371 ought to be one tenth of this: 0.01mg L⁻¹. Our results also showed that stirring the
23
24 372 solution during measurement did not adversely affect the calibration curve, which
25
26 373 maintained its linear relationship with orthophosphate concentrations. This may
27
28 374 make this system advantageous for applications in flowing conditions for *in situ*
29
30 375 measurements. This result also confirmed previous observations by Engblom (1999)
31
32 376 and Lee et al (2009) who demonstrated that the stirring rate did not affect the
33
34 377 sensitivity and stability of the electrode response. Using the Pt wire in place of the
35
36 378 Ag/AgCl (3 M) KCl reference electrode led to an increase in the detection range of
37
38 379 the electrode by four orders of magnitude compared to that reported by Engblom et
39
40 380 al. of between ~10–10³ mg L⁻¹ (10⁻⁴–10⁻¹ M) (Engblom 1999). A simple Pt wire could
41
42 381 be considered a good replacement for the Ag/AgCl (3 M) KCl and calomel reference
43
44 382 electrodes that often have a limited range of applications due to the liquid junctions
45
46 383 in these cells, which are not stable and often prone to degradation. Platinum wire
47
48 384 has also been used as a solid state reference electrode because of its rapid
49
50 385 response and physical form, which yields reproducible results as a reference
51
52 386 electrode (Chaston 1974; Kasem and Jones 2008). The proposed method using a Pt
53
54
55
56
57
58
59
60

1
2
3 387 wire exhibits a lower dynamic linear potential response in the concentration range of
4
5 388 $0.1\text{--}10^5 \text{ mg L}^{-1}$ ($10^{-6} - 10^1 \text{ M}$). This range is wider and extends to lower
6
7 389 concentrations compared to most other cobalt based phosphate sensors previously
8
9 390 described: (Berchmans et al. 2012): $1\text{--}1000 \text{ mg L}^{-1}$ ($10^{-5} - 10^{-2} \text{ M}$) reported by Xiao
10
11 391 et al. (1995), $10\text{--}1000 \text{ mg L}^{-1}$ ($10^{-4} - 10^{-2} \text{ M}$) by Meruva and Meyerhoff (1996), 1--
12
13 392 10000 mg L^{-1} ($10^{-5} - 10^{-1} \text{ M}$) by Lee et al. (2009) and $1 - 500 \text{ mg L}^{-1}$ ($5 \times 10^{-3} - 10^{-5}$
14
15 393 M) by Chen et al. (1997).

16
17
18
19 394 Total extractable P in ammonium lactate-acetic acid soil extracts was quantified
20
21 395 using ICP-OES (both organically and inorganically-bound P) (Mckelvie et al. 1995),
22
23 396 as was reviewed by Pierzynski et al. (2005). Bioavailable inorganic P was quantified
24
25 397 colourimetrically using the blue reduced phosphor-molybdenum complex method
26
27 398 established by Murphy and Reily (1962) - the standard method for the determination
28
29 399 of orthophosphates in soil extracts. The differences between the two methods were
30
31 400 proportionally larger when the extractable P concentration in the soil was below 20
32
33 401 mg kg^{-1} . The largest difference between the two methods was observed for sample
34
35 402 s4a, which features the highest organic matter content and so the largest content of
36
37 403 P not present as bioavailable orthophosphate. Potentiometry on the other hand,
38
39 404 gave lower values. The largest difference was observed in samples s3A and s3B,
40
41 405 where the electrode measurements were lower compared to colourimetry and ICP-
42
43 406 OES values (Table 1). Thus, resulting in lower values than the actual
44
45 407 orthophosphate concentration in the extracts. This occurred likely as a result of the
46
47 408 presence of organic substances and iron in the extracts not removed during the
48
49 409 carbon black treatment (Table 2), which could have led to slight drifts towards more
50
51 410 positive potentials during measurements. This is supported by the very low organic
52
53 411 matter removal efficacy shown by s3A and s3B (Table 2). This hypothesis is also
54
55
56
57
58
59
60

1
2
3 412 supported by Engblom (1998), who investigated the effect of organic matter and iron
4
5 413 on the electrode measurements. He reported that the presence of other competing
6
7 414 oxidizing substances (i.e. iron) in the soil extracts could compete with the reduction
8
9 415 of oxygen, if present, causing drifts towards higher potential values. Thereby
10
11 416 resulting in the underestimation of the actual orthophosphate concentration in the
12
13 417 soil extracts.

14
15
16
17 418 The standard method for the determination of orthophosphates in soil extracts using
18
19 419 the reduced phosphor-molybdenum blue complex and colourimetry has been
20
21 420 established by Murphy and Reily (1962). It is highly sensitive in detecting inorganic P
22
23 421 and orthophosphate ions (Limit of detection = 0.01 mg L⁻¹) and a stable complex is
24
25 422 formed in the presence of antimony. However, the antimony-phospho-molybdate
26
27 423 complex can be reduced to an intensely blue-colored complex (required to measure
28
29 424 absorbance) by ascorbic acid within 30 minutes. Barium, lead, silver and silica might
30
31 425 interfere with the reaction by forming precipitates overestimating orthophosphate
32
33 426 concentrations (Murphy and Reily 1962). Coloured humic material in soil and water
34
35 427 extract can influence the absorption and is often removed prior to measurement. In
36
37 428 addition, the acidic nature of the reagent might lead to the overestimation of
38
39 429 orthophosphates in soil extracts (Pierzynski et al. 2005; Worsfold et al. 2005.), due to
40
41 430 hydrolysis of weakly bonded inorganic or organic-condensed P species (Mckelvie et
42
43 431 al. 1995).

44
45
46
47
48 432 This is not the case with the phosphate sensitive cobalt electrode, and its simple
49
50 433 instrumentation and low fabrication cost, making it an attractive analytical tool
51
52 434 suitable for a wide variety of applications. Apart from its numerous advantages such
53
54 435 as increased opportunity for miniaturisation, and greater durability, it is not
55
56 436 dependent on colouring reagent, less prone to interferences which affect
57
58
59
60

1
2
3 437 colourimetry and can analyse lower P concentrations. This was confirmed by the
4
5 438 small difference in P concentration between the techniques shown by samples s1A
6
7 439 and s1B, which also contained the lowest concentration of dissolved organic matter
8
9
10 440 as shown in Table 1.

11
12 441 The difference in solution orthophosphate values between the Pt wire quasireference
13
14 442 and the Ag/AgCl (3 M) KCl electrode could also have been due to the presence of
15
16 443 dissolved organic compounds in the soil extracts (Table 1), which have been
17
18 444 reported to cause drifts in potential readings in soil extracts (Engblom 1999). In order
19
20 445 to try and reduce this effect on the electrode a resin amberlite XAD-4 was used and
21
22 446 shown to be able to adsorb organic matter. Unlike carbon black, the resin amberlite
23
24 447 XAD-4 did not increase the P concentrations in the soil extracts.

25
26
27
28
29 448 The phosphate sensitive cobalt electrode has already been applied in real
30
31 449 applications

32
33 450 (Engblom, 1998; Chen et al., 1997) and has proven successful for the measurement
34
35 451 of orthophosphate in ammonium lactate-acetic acid extracts of soils (Engblom 1998).

36
37 452 The suitability of the proposed method using the Pt wire quasireference electrode,
38
39 453 makes it an attractive tool suitable for a wide variety of applications. The potential of
40
41 454 the cobalt electrode coupled to a Pt wire for field-based measurements provides a
42
43 455 versatile and indeed potentially invaluable screening option for measuring
44
45 456 orthophosphate ions. Interest in the use of *in-situ* measurements originate from a
46
47 457 need to provide a quick on-site assessment. Thus, alleviating much of the costs, time
48
49 458 delays and loss of sample integrity associated with standard laboratory-based
50
51 459 analysis which can be prone to interferences. The proposed method provides a quick
52
53 460 and quantitative method for field measurements and can also be carried out by non-
54
55 461 experts. However, going by the established history of potentiometric sensors, they
56
57
58
59
60

1
2
3 462 have been reported to be prone to errors, resulting in significant drifts in potentials
4
5 463 when measurements of very low concentrations are involved. However, despite the
6
7 464 Pt wire set up having a lower resolution, it provides a viable platform for *in-situ*
8
9
10 465 monitoring of phosphate and for trace analysis. For this to be feasible issues of
11
12 466 matrix interferences still need further investigation.

13 14 15 467 **Conclusions**

16
17
18 468 The two reference electrode (Ag/AgCl (3 M) KCl and Pt wire) performed linearly and
19
20 469 showed high sensitivity towards orthophosphate standards. However, the Pt wire
21
22 470 quasireference electrode method gave a faster response time (less than 5 minutes)
23
24 471 and a much wider dynamic detection range ($0.1 - 10^5 \text{ mgL}^{-1}$). It also showed good
25
26 472 reproducibility with orthophosphate standards. Further investigation of the cobalt
27
28 473 electrode coupled to an Ag/AgCl (3 M) KCl reference showed that its simplicity
29
30 474 makes it an alternative to spectroscopic methods such as Ultraviolet Visible
31
32 475 Spectroscopy and ICP-OES for the determination of P in soil extracts. Because of
33
34 476 the good selectivity shown by the electrode, the results suggest that it gives a more
35
36 477 reliable interpretation of the actual concentration of phosphate in the soil extracts,
37
38 478 making it an attractive analytical tool for the direct determination of orthophosphate.
39
40 479 Although the Pt wire method gave a better range when analysing standards, when
41
42 480 tested on soil extracts, due to the complex matrices involved and interferences
43
44 481 caused by the presence of organic matter, slight drifts in electrode response were
45
46 482 noticed. Interferences due to the presence of organic substances in the extracts
47
48 483 were reduced to a large extent by the use of a hydrophobic resin (Amberlite XAD-4),
49
50 484 which was more reliable in both the removal of organic matter, and also in the
51
52 485 indirect removal of organically bound P from extracts, compared to carbon black.
53
54
55
56
57
58
59
60

1
2
3 486 These preliminary results indicate that the use of the Pt wire as a reference electrode
4
5 487 in the experimental set up is promising. A wide dynamic linear range of detection can
6
7 488 be obtained: $0.1 - 10^5 \text{ mg L}^{-1}$. This is a significant improvement over some more
8
9 489 complex potentiometric cobalt based phosphate sensors reported in the literature,
10
11 490 which display linear responses within $1 - 10^4 \text{ mg L}^{-1}$ (Xiao et al. 1995; Meruva and
12
13 491 Meyerhoff 1996; Kim et al. 2008; Lee et al. 2009; Chen et al. 1997; Berchmans et al.
14
15 492 2012). The technique has considerable potential for use in fresh water analysis,
16
17 493 where organic matter influence would likely be minimal. Although this improvement is
18
19 494 promising, further investigation is still needed to lower the limit of quantification by a
20
21 495 further order of magnitude (to ca. 0.01 ppm) for accurate monitoring of
22
23 496 orthophosphate levels in natural waters that are not yet subject to eutrophication
24
25 497 (Quintana et al. 2004; Warwick et al. 2013, UK Environment Agency, 2012).

29 30 498 **Acknowledgements**

31
32
33
34 499 Victor Oghogho Ebuele would like to thank the Delta State Government of Nigeria,
35
36 500 for funding. Anna Santoro and Simon Curling are acknowledged for ICP-OES
37
38 501 measurements. The authors acknowledge ERDF funding through the WISE Network.
39
40 502 The authors acknowledge the Analytical Chemistry Trust Fund for financial support.

41 42 43 503 **References**

- 44
45
46 504 Abowei, J.N. and F.D. Sikoki. 2005. Water Pollution Management and Control,
47
48 505 Double Trust Publications Company, Port Harcourt.
- 49
50
51 506 Adeloju, S. B. 2013. Progress and recent advances in phosphate sensors: A review.
52
53 507 *Talanta* 114: 191-203.

- 1
2
3 508 Alexander, P.W. and J. Koopetngarm. 1987. Flow-Injection Determination of
4 509 Phosphate Species in Detergents with a Calcium Ion-Selective Electrode", Anal.
5 510 Chim. Acta 197: 353-359.
6
7
8
9 511 Berchmans, S, T., B. Issa, and P. Singh. 2012. Determination of inorganic phosphate
10 512 by electroanalytical methods: A review. *Analytica Chimica Acta* 729: 7-20.
11
12
13 513 Chaston, J. C.1965. Reactions of oxygen with the platinum metals. *Platinum Met.*
14 514 *Rev*, 9:51-56.
15
16
17
18 515 Chen, Z.L., R. DeMarco, and P. W. Alexander, 1997. Flow-injection potentiometric
19 516 detection of phosphates using a metallic cobalt wire ion-selective electrode.
20 517 *Anal. Chim. Acta* 34: 93-95.
21
22
23
24 518 Engblom, S. 1999. Determination of inorganic phosphate in a soil extract using a
25 519 cobalt electrode. *Plant and Soil* 206:173-179.
26
27
28
29 520 Environmental, a. 01/10/2012, Water Quaiity [Homepage of Environmental agency
30 521 uk], [Online].
31
32
33 522 Hanrahan, G., T. M. Salmassi, C. S. Khachikian, and K.L Foster. 2005. Reduced
34 523 inorganic phosphorus in the natural environment: significance, speciation and
35 524 determination *Talanta* 66: 435-444.
36
37
38
39 525 Kasem, K. K., and S. Jones. 2008. Platinum as a reference electrode in
40 526 electrochemical measurements. *Platinum Metals Review*, 52 :100-106.
41
42
43
44 527 Kim, J., D. M. Kang, S. C. Shin, M. Y. Choi, J. Kim, S. S. Lee, and J. S. Kim. 2008.
45 528 Functional polyterthiophene-appended uranyl-salophen complex:
46 529 electropolymerization and ion-selective response for monohydrogen phosphate.
47 530 *Analytica chimica acta* 614: 85-92.
48
49
50
51 531 Lee, W.H., Y. Seo, and P. L Bishop. 2009. Characteristics of a cobalt-based
52 532 phosphate microelectrode for in situ monitoring of phosphate and its biological
53 533 application. *Sensors and Actuators B-Chemical* 137:121-128.
54
55
56
57
58
59
60

- 1
2
3 534 Marco, 1998. Flow injection potentiometric determination of phosphate in waste
4 535 waters and fertilisers using a cobalt wire ion-selective electrode. *Analyst* 123 (7):
5 536 1635-40.
6
7
8
9 537 Mckelvie, I.D., D.M.W. Peat, and P. J. Worsfold. 1995. Techniques for the
10 538 Quantification and Speciation of Phosphorus in Natural-Waters. *Anal. Proc.* 32:
11 539 437-445.
12
13
14
15 540 Meruva.R,K, M.E. Meyerhoff, 1996. Mixed potential response mechanism of cobalt
16 541 electrode towards inorganic phosphate. *Anal. Chem* 68: 2022-2026.
17
18
19
20 542 Midgley, D. 1986. Sulfate and Phosphate Ion-Selective Electrodes", *Ion-Selective*
21 543 *Electrode Reviews.* 8: 3-54.
22
23
24 544 Midgley, D. 1979. Solid-State Ion-Selective Electrodes for the Potentiometric
25 545 Determination of Phosphate. *Talanta* 26: 261-266.
26
27
28
29 546 Motomizu, S., T. Wakimoto, and K. Toei. 1983. Determination of Trace Amounts of
30 547 Phosphate in River Water by Flow-Injection Analysis", *Talanta*, 30: 333-338.
31
32
33 548 Murphy, J. and H. P. Riley. 1962. A modified single method for the determination of
34 549 phosphate in natural waters. *Anal.Chim. Acta*, 27: 31-36.
35
36
37
38 550 Okamoto, Y., Kanda, K., Kishiwada, S. and T. Fujiwara. 2004. Determination of
39 551 phosphorous and sulfur in environmental samples by electrothermal vaporization
40 552 inductively coupled plasma atomic emission spectrometry", *Applied*
41 553 *Spectroscopy*, 58: 105-110.
42
43
44
45 554 Pierzynski, G., H. Zhang, A. Wolf, P. Kleinman, A, Mallarino, D, Sullivan. 2005.
46 555 Phosphorus Determination in Waters and Extracts of Soils and By-Products:
47 556 Inductively Coupled Plasma Spectrometry versus Colorimetric Procedures.
48 557 SERA-17 Policy Workgroup Paper [Online]. SERA-17. Available at
49 558 http://www.sera17.ext.vt.edu/Documents/P_Analysis_Comparisons.pdf (verified
50 559 20 Mar 2012).
51
52
53
54
55
56
57
58
59
60

- 1
2
3 560 Quintana, J.C., L. Idrissi, G. Palleschi, P. Albertano, A. Amine, M. El Rhazi, and D.
4 561 Moscone. 2004. Investigation of amperometric detection of phosphate
5 562 Application in seawater and cyanobacterial biofilm samples. *Talanta* 63: 567-
6 563 574.
- 7
8
9
10
11 564 USEPA, Method 3050. 1989. Acid digestion of sediments, sludge and soils. Test
12 565 methods for evaluating solid waste.Vol.1A. 3rd ed. Rep EPA/SW-846.
13 566 Natl.Techical Info. Serv., Springfield, VA.
- 14
15
16
17 567 Sims, T. 2000. "Soil fertility evaluation" in Hand Book of Soil Science., ed. M.
18 568 Sumner.E., CRC Press, Boca Raton, London, New York, Washington DC., pp.
19 569 25-65.
- 20
21
22
23 570 Van Staden., J F. 1993. Behaviour of silver orthophosphate as the electroactive
24 571 sensor of a coated tubular solid-state phosphate-selective electrode in flow
25 572 injection analysis (FIA). *S. Afr. J. Chem.* 46:14 - 19.
- 26
27
28
29 573 Thompson, N. and N. Walsh. 2003. inductively coupled plasma atomic emission
30 574 spectroscopy, Viridian publishing.
- 31
32
33
34 575 Warwick, C., A. Guerreiro, and A. Soares. 2013. Sensing and analysis of soluble
35 576 phosphates in environmental samples: A review. *Biosensors and Bioelectronics*
36 577 41: 1-11.
- 37
38
39
40 578 Worsfold, P.J., L. J. Gimbert, U. Mankasingh, O.N. Omaka, G. Hanrahan, P. C. F. C,
41 579 Gardolinski, P.M. Haygarth, B. L. Turner, M. J. Keith-Roach, and I. D. McKelvie,
42 580 2005. Sampling, sample treatment and quality assurance issues for the
43 581 determination of phosphorus species in natural waters and soils. *Talanta* 66:
44 582 273-293.
- 45
46
47
48
49 583 Xiao, D., H.Y. Yuan, J. Li, and R.Q. Yu. 1995. Surface-Modified Cobalt-Based
50 584 Sensor as a Phosphate-Sensitive Electrode. *Anal. Chem.* 67: 288-291.
- 51
52
53
54 585
55 586

587 Table 1. Properties of the soils ((A) soil surface 0-15 cm depth, (B) subsurface 15- 30 cm depth) and the P concentration as mg kg⁻¹
 588 of nitric acid digested extracts for total P and ammonium lactate-acetic acid extracts determined by three different analytical
 589 techniques. Electrochemical quantification was done with the Ag/AgCl (3M) KCl reference electrode as the
 590 average of triplicate measurements \pm the standard deviation.

Soil	pH (H ₂ O)	Organic matter (%)	Total P (mgkg ⁻¹)	Ammonium lactate-Acetic Acid soil extract (mg kg ⁻¹)		
				Inductive Coupled Plasma Optical Emission Spectroscopy	Colourimetry	Cobalt Electrode
s1A	8.0	2.9	417.5 \pm 0.5	21.4 \pm 0.10	19.8 \pm 0.03	18.4 \pm 4
s1B	7.9	2.6	383.5 \pm 6.5	13.7 \pm 0.03	11.0 \pm 0.01	10.5 \pm 2
s2A	5.5	5.1	546.6 \pm 5.2	9.8 \pm 0.03	7.0 \pm 0.20	6.4 \pm 4
s2B	5.6	3.9	439.3 \pm 7.4	5.1 \pm 0.03	5.0 \pm 0.06	4.8 \pm 2
s3A	6.0	6.0	988.4 \pm 9.1	49.1 \pm 0.20	47.2 \pm 0.04	19.4 \pm 4
s3B	6.3	5.4	878.6 \pm 5.7	38.8 \pm 0.01	34.2 \pm 0.08	12.2 \pm 5
s4A	6.0	7.6	1188.5 \pm 4.1	87.9 \pm 0.01	74.4 \pm 0.30	60.4 \pm 14
s4B	5.4	6.5	860.7 \pm 2.7	40.9 \pm 0.02	38.2 \pm 0.24	37.2 \pm 10
s5A	5.6	6.1	1042.5 \pm 5.5	13.5 \pm 0.01	10.9 \pm 0.40	9.9 \pm 2
s5B	5.8	5.6	790.5 \pm 3.1	9.4 \pm 0.03	5.5 \pm 0.02	4.8 \pm 3

592 Table 2: Determination of orthophosphate (mg kg^{-1}) in the ammonium lactate-acetic
 593 acid soil extracts by (C) colourimetry and (P) potentiometry with the phosphate-
 594 sensitive cobalt electrode coupled to a Ag / AgCl/3M KCl reference electrode as the
 595 average of triplicate measurements \pm the standard deviation.

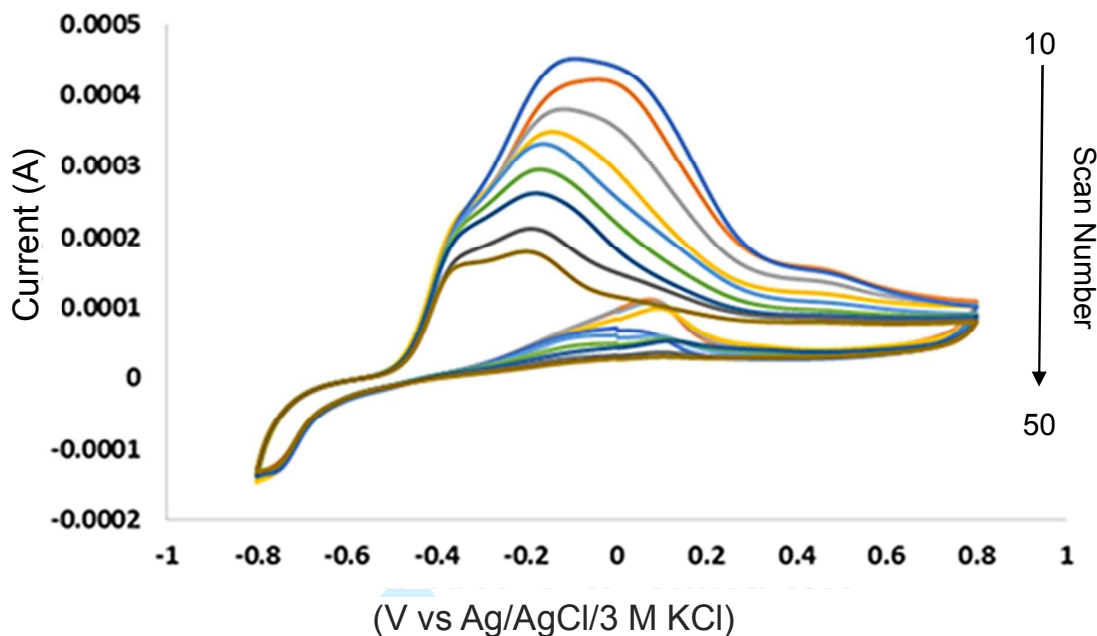
Surface Soil	Treatment			
	Colorimetry (C)		Potentiometry (P)	
	No Resin	Resin	No Resin	Resin
	mg kg^{-1}			
s1A	19.8 ± 0.03	15.4 ± 0.04	18.4 ± 4	10.9 ± 2
s2A	6.5 ± 0.3	5.1 ± 0.01	6.4 ± 4	4.34 ± 2
s3A	46.6 ± 0.7	40.9 ± 0.06	19.4 ± 4	14.7 ± 5
s4A	73.5 ± 0.4	68.8 ± 0.01	60.4 ± 14	35.8 ± 10
s5A	9.7 ± 0.5	8.96 ± 0.03	8.9 ± 2	4.3 ± 1

596

Sub-Surface Soil	Colorimetry (C)		Potentiometry (P)	
	No Resin	Resin	No Resin	Resin
	mg kg ⁻¹			
s1B	10.6 ± 0.02	7.9 ± 0.3	10.5 ± 2	6.5 ± 5
s2B	5.1 ± 0.1	3.7 ± 0.02	4.8 ± 2	3.0 ± 1
s3B	34.3 ± 0.08	19.7 ± 0.02	12.2 ± 5	10.9 ± 4
s4B	38.2 ± 4	29 ± 0.02	37.2 ± 10	15.9 ± 3
s5B	5.5 ± 0.4	3.7 ± 0.02	4.8 ± 3	2.1 ± 2

597

598



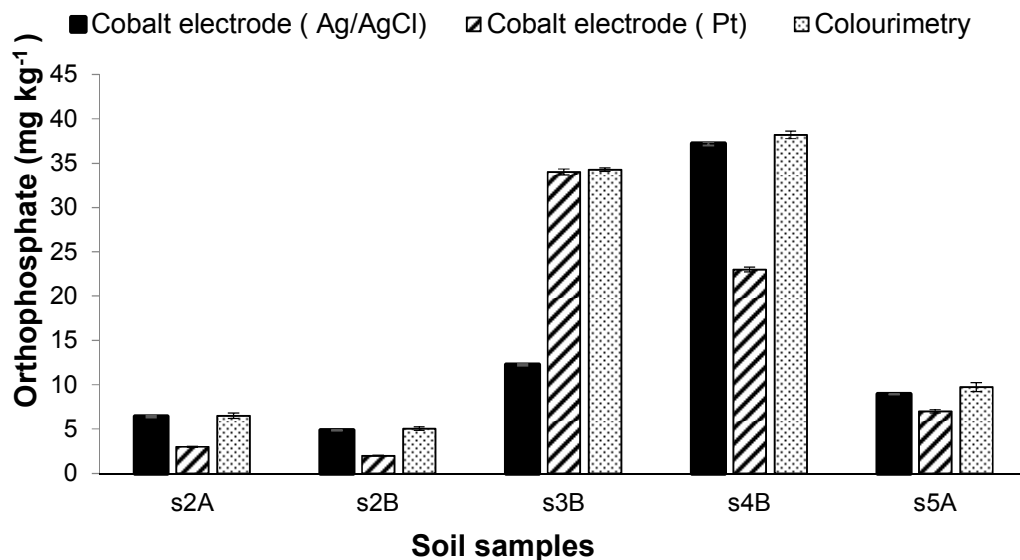
599

600 Figure 1. Cyclic voltammogram of the cobalt electrode with Ag/AgCl (3M) KCl
601 reference and Pt wire as counter electrode in 0.1M KH_2PO_4 recorded with
602 scan rate of 0.1 mV s^{-1} in the potential range of -800 mV to 800 mV showing
603 10 to 50 scans.

604

605

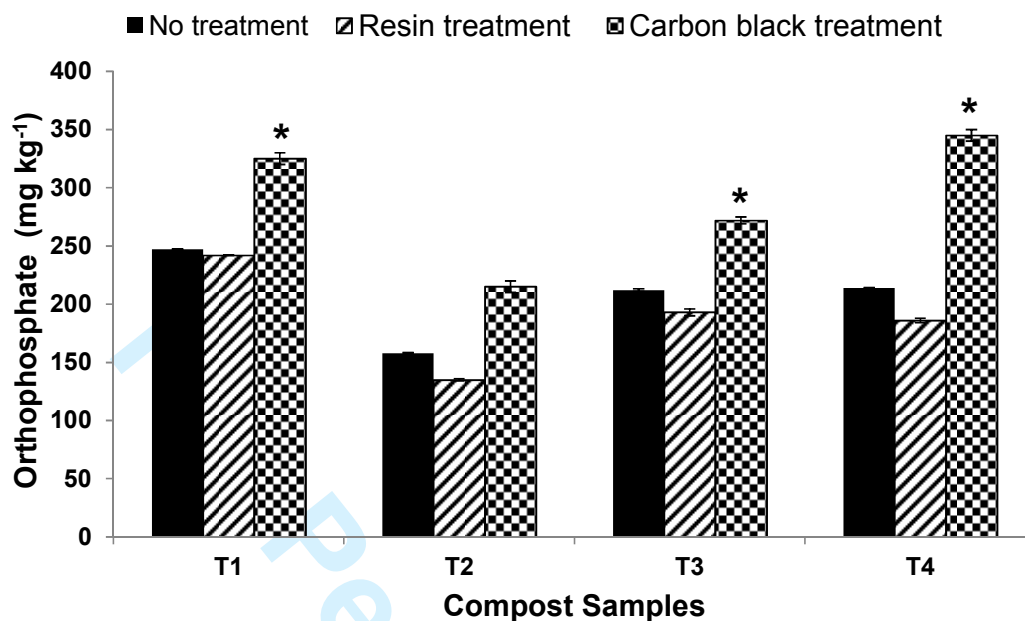
606



607

608 Figure 2. Comparison between the orthophosphate concentrations (mg kg⁻¹) in
609 ammonium lactate-acetic acid soil extracts (pH=3.75) determined by cobalt electrode
610 coupled to an Ag/AgCl (3M) KCl reference electrode (black bars), cobalt electrode
611 coupled to a Pt wire quasireference electrode (shaded horizontal bars) and
612 colourimetry (shaded spotted bars). (n=3, error bar = standard deviation)

613



614
615 Figure 3. Orthophosphate concentrations in compost extracts (mg/ kg) determined
616 by colorimetry with no treatment (black bars), Amberlite XAD-4 resin treatment
617 (striped bars), and carbon black treatment (checkered bars) (n=3). *Indicates a
618 significant difference ($p < 0.05$) between carbon black treatment and no treatment

619

1
2
3 **Development of a cobalt electrode for the determination of**
4 **Phosphate in soil extracts and comparison with standard**
5 **methods**
6
7
8

9 **Victor O. Ebuele^a, Daniel G. Congrave^a, Chris D. Gwenin^a, Vera**
10 **Fitzsimmons-Thoss^{a*}**
11

12
13 ^a School of Chemistry, Bangor University, Bangor, LL57 2UW, Wales

14
15
16 * Corresponding author (vera.thoss@bangor.ac.uk)
17
18
19

20 **Supplementary Information**
21
22
23
24
25
26

- 27 1) Figure S1. Schematic of the Cobalt electrode
28
29 2) Table S1. Calibration data for the Pt quasireference electrode.
30
31 3) Figure S2. Calibration curve of a cobalt electrode response
32 to the \log_{10} of orthophosphate standards dissolved in
33 ammonium lactate-acetic acid solution (pH 3.75) using an
34 Ag/AgCl / (3M) KCl reference electrode taken for a period of
35 7days.
36
37 4) Figure S3. Calibration curve of a cobalt electrode response
38 to \log_{10} of orthophosphate standards dissolved in
39 ammonium lactate-acetic acid solution (pH 3.75) using an
40 Ag/AgCl (3M) KCl reference electrode and a Pt wire
41 quasireference electrode.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

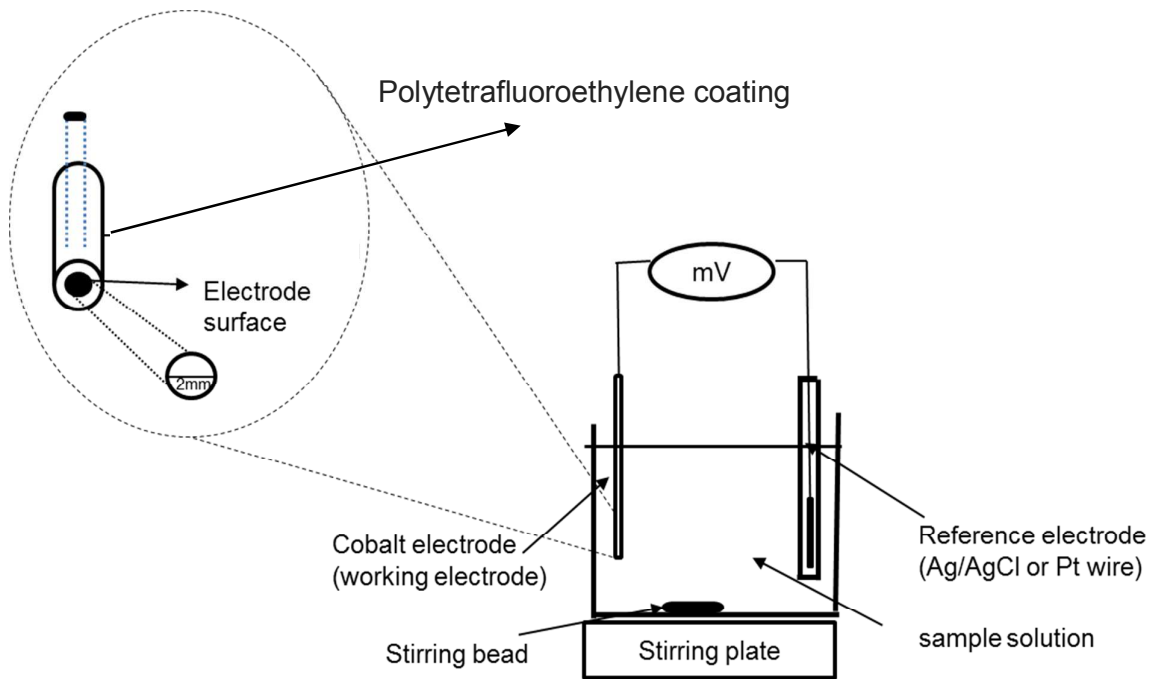


Figure S1. Schematic of the Cobalt electrode.

Table S1. Calibration data for the Pt quasireference

	Pt wire	SCE	SHE
Potential vs Pt wire	0.00 V	-0.38 V	-0.59 V
Potential vs SCE	0.38 V	0.00 V	-0.21 V
Potential vs SHE	0.59 V	0.21 V	0.00 V

For Peer Review Only

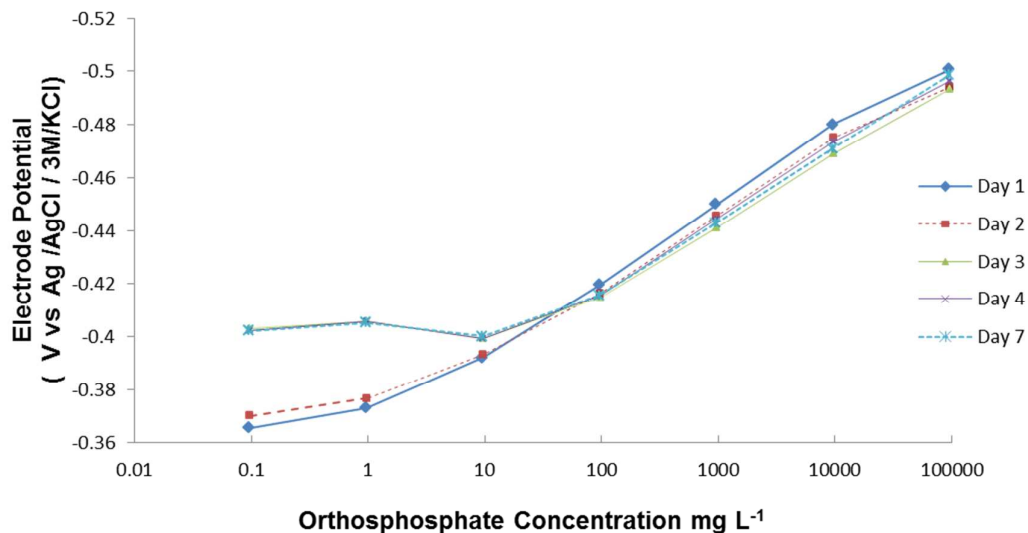


Figure S2. Calibration curve of a cobalt electrode response to the \log_{10} of orthophosphate standards ($0.1 - 10^5 \text{ mg L}^{-1}$) dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag / AgCl / (3M) KCl reference electrode taken for a period of 7 days.

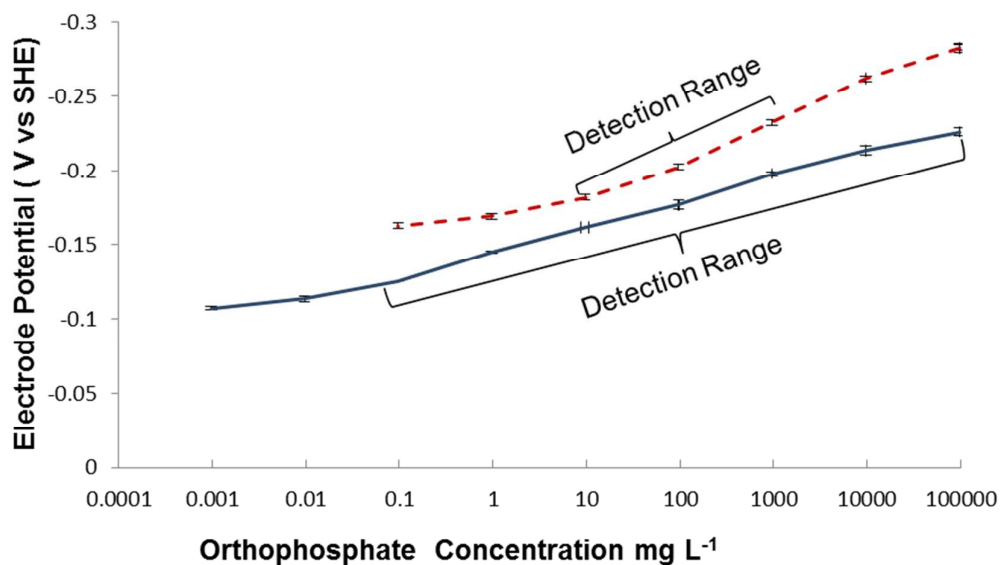


Figure S3. Calibration curve of a cobalt electrode response to \log_{10} of orthophosphate standards ($0.001 - 10^5$ mg L⁻¹) dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag / AgCl (3M) KCl reference electrode (dashed lines) and a Pt wire quasireference electrode (solid line).