Evaluation on the determination of available phosphorus using blue molybdate in Organosols

Raihan Garin, Fransisca Simanjuntak, Darmawan and Basuki Sumawinata
Department of Soil Science and Land Resources, IPB University, Bogor, Indonesia
Email: raihangarinn@gmail.com

Abstract Bray and Olsen methods have widely been used for measuring available phosphorous (P) in soil. Those methods have been known resulting good correlation between available P in soil and crops, especially for corn. Correlation between P uptake by plant and P concentration in Bray extractant ranges from 0.74 to 0.94; the method is, therefore, recommended for neutral and acid soils (pH ≤ 7.0). Meanwhile for Olsen method, correlation could span from 0.73 to 0.96 for alkaline soils. Both, however, have been observed to potentially result in false-high available P in Organosols. This might be due to dark color of Bray and Olsen extracts that reacting with blue molybdate and resulting in high values of absorbance during spectrophotometric measurement. In the case for Organosols, dissolved organic substance originates dark color of the extract. Therefore, organic substance should be eliminated prior to the measurement. This research explored an improvement for available P measurement in Organosols by treating the extract with H₂O₂ and an activated charcoal. This treatment the Olsen extracts has resulted in a notable available P gap, i.e. 102.7 ppm of treated extracts compared to 1207.7 ppm of untreated one.

1. Introduction
Availability of P in soils plays an important role for plant growth [1]. Either Bray or Olsen approach is employed to measure available P in soil. A good correlation and calibration between P content in soil and in maize was demonstrated [2]. However, these methods are inappropriate when used on Organosols, in which having high organic matters. As found by Nagul et al. [3], organic matters interfere measurements because molybdate blue is reduced by organic matters or humus compounds, which cause dark colors (black or deep blue). In turn, when measured with a spectrophotometer, they interfere incoming light, resulting the absorbance becomes high and so measured P.

A research by Astiana and Rachim [4] discovered that total P in Delta Upang Organosols reached 1209 ppm, with available P using Bray of 91 ppm. P levels in these Organosols were very high, however, field observation suggested P deficiency, indicated by purple leaves on corn. According to Jackson [5], the extract before being reacted with molybdate blue must be acidified with H₂SO₄, HClO₄, or Na₂CO₃ until pH 3.0 so that organic matters can be precipitated. However, treatment by acidifying remains a perplexing problem because there are many acid solutions involved. If acid solution is reactive to organic matters, it would expose to new problems. Treatment by oxidizing organic matters using H₂O₂ can be a solution. This research investigated the retrievals of available P measured on Organosols by removing organic matters using H₂O₂ and activated charcoal.
2. Methods
This research was carried out from June to September 2020 at the Laboratory for the Development of Land Physical Resources, Bogor Agricultural University. Soil materials consisted of Organosols from Riau, South Sumatra, and Central Kalimantan.

2.1. Determining P using Bray 1, Bray 2, and Olsen
Fifteen 15 ml Bray solution was added into 1.5 grams of soil, and was shaken for 1 minute. The extract was then filtered to produce a clear-colored solution. Around 5 ml extract was reacted with 5 ml molybdate blue yielding a dark blue-colored solution. This color blocks the lights entering spectrophotometer and therefore resulting in a high P level.

2.2. Proposed treatment
In this research, additional treatment was carried out by adding 5 ml of 30% H$_2$O$_2$ PA in a porcelain. Next, oxidation process was conducted by heating the extract in a water bath until it became ashes. They were then dissolved into 2 ml of 0.1 N HCl to clear extracts. Extract solution of Olsen method was given the same treatment as Bray’s, except that after removals of organic matter using H$_2$O$_2$, the extract remained yellow. In this case, Olsen extracts must be added by 0.1 grams of activated charcoal and then filtered. Clear extract was subsequently reacted with 5 ml of molybdate blue, resulting in a light color solution. This treatment is summarized in Table 1.

Table 1. Treatment of P determination in soil using Bray 1, Bray 2, and Olsen

| Soil   | Method            | Extract           | Treatment                        | Color Extract | Reactor         | Color Result    |
|--------|-------------------|-------------------|----------------------------------|---------------|-----------------|-----------------|
| Bray 1 | NH$_4$F 0.03 N + HCl 0.025 N | Oxidation with H$_2$O$_2$ 30% | Clear | Blue Molybdate | Light Blue |
| Bray 2 | NH$_4$F 0.03 N + HCl 0.1 N | Oxidation with H$_2$O$_2$ 30% | Clear | Blue Molybdate | Light Blue |
| Olsen  | NaHCO$_3$ 0.5 N   | Oxidation with H$_2$O$_2$ 30% & Activated Charcoal | Clear | Blue Molybdate | Blue |

3. Results and discussion

3.1. Chemical analysis on Organosols
Table 2 shows that all soil samples had a high C-Organic content (43.0% - 48.8%). They had acidic reaction and relatively low nutrient contents; Ca content of Kalimantan (O3) Organosols was the highest. Meanwhile, the levels of K, Cu, Zn, and Mn in Organosols from Riau (O5) and South Sumatra (O6) were higher due to ash content. Andrejko et al. [6] argued that ash content contains a number of inorganic materials that can increase nutrients in the soil. Nutrient content in Organosols does not affect P levels in the soil, because Organosols do not contain metals, such as Al, Fe, or Ca, that bind P. According to Karjalainen et al. [7] P available in Organosols is in dissolved or suspended form in water. Dissolved P can be absorbed into Organosols surface or in the form of humus structure. Hence, soil P should be low, despite measurements made by Bray 1, Bray 2, and Olsen methods produce very high amount. In general, measured P with Bray method recommends neutral and acid soils (pH ≤ 7.0). Bray method suggests ammonium fluoride as extraction element to unbound Al-P and Fe-P in acid soil, whereas Olsen method uses sodium bicarbonate in alkaline condition or for calcareous soils (pH ≥7.0), because the extract unbounds Ca-P in soils. With pH spanned about pH 3 – pH 4, Bray was a suggested method for P measurement.
Table 2. Chemical analysis on Organosols

| Samples | pH 1:5 | C-Org (%)* | Bray 1 (ppm) | Bray 2 (ppm) | Olsen (cmol(+)/kg) | Nutrient Content (ppm) |
|---------|--------|------------|--------------|--------------|-------------------|----------------------|
| O1      | 4.0    | 48.1       | 82           | 88           | 237               | 4                    | 2                    | 0.1                  | 0.1                  | 720                  | 11                   | 3                    | 34                  |
| O2      | 3.9    | 44.6       | 320          | 268          | 593               | 5                    | 2                    | 0.1                  | 0.1                  | 1387                 | 21                   | 5                    | 46                  |
| O3      | 4.5    | 43.0       | 438          | 198          | 287               | 15                   | 4                    | 0.1                  | 0.2                  | 2024                 | 12                   | 3                    | 89                  |
| O4      | 3.9    | 48.8       | 31           | 29           | 210               | 4                    | 2                    | 0.1                  | 0.1                  | 614                  | 7                    | 2                    | 35                  |
| O5      | 3.5    | 43.4       | 54           | 46           | 1208              | 1                    | 0.7                  | 19                   | 4                    | 27                   | 15                   | 22                   | 1367                |
| O6      | 3.4    | 46.9       | 35           | 42           | 407               | 2                    | 1                    | 24                   | 2                    | 29                   | 25                   | 33                   | 303                 |

Measurement using Olsen method Organosols from Riau (O5) exceeded the one of Bray 1 and Bray 2, which was 1207.7 ppm. This was caused by black extract produced by Olsen that dissolved lignin and humus from organic compounds at pH 8. Black extract reacted with molybdate blue and remained black. This extract blocks incoming light during analysis using spectrophotometer, resulting in a high absorbance value or measured P. Source of phosphorous in Organosols includes leaves that contain only around 0.2% P; P contained in woods is even lower than in leaves. Wowor et al. [8] found that averaged P nutrients of nantu and matoa fresh leaf litter was 0.18% and 0.1%, respectively. Those levels were similar to the ones available in soil type studied in this research; hence, very high amount of phosphorous in Organosols was unrealistic.

Organic compounds also interfered the determination of P through Bray 1 and Bray 2. Recorded P levels obtained using Bray 1 was very high in Central Kalimantan (O3) Organosols, i.e. 438.2 ppm; similar to the one from Bray 2 (267.8 ppm). The extracts from both methods 2 were visually clear; however, reaction between organic compounds and molybdate blue yielded dark blue solution. It blocks the light entering spectrophotometer; hence, absorbed light becomes high.

3.2. Proposed treatment

Specifically for Central Kalimantan (O3) Organosols, P quantification measured using Bray 1 was very high, i.e. 438.2 ppm (Table 3). When the extract was added H₂O₂, measured P was about 27.6 ppm. This discrepancy was considerably significant, around 15 times. Similar outcome was observed using Bray 2; eight times over Central Kalimantan (O2) Organosols. Measured P levels before treatment were generally very high, exceeding maximum critical limits for correlation and calibration on plants. According to Locke and Hanson [9], P measurement exploiting Bray has a maximum critical limit on plants of 50 ppm. Meanwhile, Watson and Mullen [10] found that 15 ppm P was a good correlation and calibration that required by plants.

Organosols from Riau (O5), employing Olsen method prior treatment, yielded 1207.7 ppm, which also exceeded maximum critical limit. According to Higgs et al. [11], Olsen has a critical limit value of 25 ppm so that plants can grow well with a maximum limit of 60 ppm. Measuring P through Olsen needed pre-treatment with H₂O₂ and activated charcoal as the reading was beyond the critical limit. After treatment, measured P was lower than the one before treatment, i.e. 102.7 ppm. This research found that measured P by Olsen method was higher than of Brays because Olsen method dissolved organic matters on the extract and reacted with molybdate blue producing a denser color than Brays. Olsen method is, therefore, not recommended for Organosols because sodium bicarbonate with a high alkaline content, pH 8, reacts with an acidic soil with a pH 3 – pH 4 and dissolves organic matters on the extract. Bray methods can be utilized to measure P in Organosols because the extract suits soil conditions. Comparison of P measurements before and after H₂O₂ treatment is presented in Table 3.
References

1. Malhotra H, Sharma S and Pandey R 2018 Phosphorus nutrition: plant growth in response to deficiency and excess Plant Nutrients and Abiotic Stress Tolerance: Springer pp 171-90
2. Geisseler D and Miyao G 2016 Soil testing for P and K has value in nutrient management for annual crops Calif. Agric. 70 152-9
3. Nagul E A, McKelvie I D, Worsfold P and Kolev S D 2015 The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box Anal. Chim. Acta 890 60-82
4. Astiana and Rachim A 1978 Pengaruh pemupukan fosfor pada tanah organik terhadap padi varietas pelita I/1. In: Prosiding Simposium Nasional III Pengembangan Daerah Pasang Surut di Indonesia, (Palembang: Direktorat Jendral Pengairan Departemen PU - IPB)
5. Jackson M L 1958 Soil chemical analysis (Englewood Cliffs: Prentice Hall)
6. Andrejko M J, Fiene F and Cohen A D 1983 Comparison of ashing techniques for determination of the inorganic content of peats Testing of Peats and Organic Soils: ASTM International)
7. Karjalainen S M, Ronkanen A-K, Heikkinen K and Kløve B 2016 Long-term accumulation and retention of Al, Fe and P in peat soils of northern treatment wetlands Ecol. Eng. 93 91-103
8. Wowor A E, Thomas A and Rombang J A 2020 Kandungan unsur hara pada serasah daun segar pohon (mahoni, nantu dan matoa) Eugenia 25
9. Locke M A and Hanson R G 1991 Calibration of corn response to Bray I, Bray II, and Mehlich II extractable soil phosphorus Commun. Soil Sci. Plant Anal. 22 1101-21
10. Watson M and Mullen R 2007 Understanding soil tests for plant-available phosphorus. In: Unpublished, (Columbus, Ohio: School of Environment and Natural Resources. The Ohio State University)

Table 3. Proposed treatment for determination of P on Organosols

| Samples | pH  | C-Org (%) | Before Treatment | After Treatment |
|---------|-----|-----------|----------------|----------------|
|         |     |           | Bray 1 | Bray 2 | Olsen | Bray 1 | Bray 2 | Olsen | Treatment (1) | Treatment (2) |
| O1      | 3.95| 47.0      | 82     | 88    | 237   | 25     | 39    | 164   | 125   |
| O2      | 3.88| 50.0      | 320    | 268   | 593   | 24     | 36    | 170   | 108   |
| O3      | 4.54| 48.4      | 438    | 198   | 287   | 28     | 38    | 149   | 97    |
| O4      | 3.86| 51.4      | 31     | 29    | 210   | 29     | 37    | 153   | 93    |
| O5      | 3.46| 43.4      | 54     | 46    | 1208  | 31     | 32    | 136   | 103   |
| O6      | 3.44| 46.9      | 35     | 42    | 407   | 20     | 28    | 133   | 106   |

Note: Treatment (1) : Bray 1 + H2O2, Bray 2 + H2O2, Olsen + H2O2; Treatment (2) : Olsen + H2O2 + Activated Charcoal

4. Conclusion
This research found that Bray 1 and Bray 2 methods suit peat soil conditions. Bray extract releases P in Organosols bound with free ions or with Fe-P. Olsen method in Organosols was not recommended because its extract has a high pH capable to dissolve organic materials with a low pH and insignificant Ca-P contents. Dissolved organic matter can interfere with P measurement because the extract becomes denser. Adding H2O2 into soil extracts was important especially on Bray and Olsen extracts to remove organic matter that disturbed the measurement. Besides H2O2, applying activated charcoal is needed for Olsen method to obtain a clear extract. The extract was free of organic matters and staining with molybdate blue did not produce a dense color that blocked the light during analysis with a spectrophotometer. This process prevented of getting impaired results so that the outcome shall realistically show the availability of P in the field.

The test carried out in this study should be amplified by using P levels using an HPLC device, capable of precisely measuring P, even with unclear extracts.
[11] Higgs B, Johnston A E, Salter J L and Dawson C J 2000 Some aspects of achieving sustainable phosphorus use in agriculture *J. Environ. Qual.* 29 80-7