# Determination of inorganic and organic P dissolved in water and Olsen extracts by inductively coupled plasma optical emission spectroscopy (ICP-OES) and colorimetry

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## Abstract

A lab experiment was carried out to investigate the feasibility of calculating soluble ("plant available") organic soil P in water and Olsen extracts from P measurements by ICP-OES and colorimetry. To this aim, mixed solutions of water soluble organic P (sodium phytate) or polyphosphates common in organic soil compounds (sodium polyphosphate and sodium triphosphate pentabasic) with orthophosphate ($KH_2PO_4$) were produced at different mixing ratios and P was measured by both methods. While ICP-OES was able to determine total dissolved P (with an error of up to about 13 %), as expected only orthophosphate was measured by colorimetry (with an error of up to around 9 %). Thus, it is possible to calculate "non orthophosphate" (organic P and polyphosphates) dissolved in the respective soil extracts with reasonable accuracy. However, some error may occur due to hydrolysis of non-orthophosphate as well as due to the fact that, in real soil extracts, suspended colloidal P and soluble complexes of P with Fe, Al and/or Ca, which are not plant available, may increase total P measured by ICP-OES.

**Keywords:** *inorganic P, organic P, soil extracts, plant availability, colorimetry, ICP-OES*

## Zusammenfassung

### Bestimmung von anorganischem und organischem P im Wasser- und Olsenextrakt mittels optischer Emissionsspektroskopie (ICP-OES) und Kolorimetrie

Ein Laborexperiment wurde durchgeführt, um herauszufinden, ob es möglich ist, aus der Messung von P in Wasser und im Olsenextrakt mittels ICP-OES und Kolorimetrie das lösliche organische (pflanzenverfügbare) P zu berechnen. Zu diesem Zweck wurden Mischlösungen aus organischem P (N-Phytat) bzw. Polyphosphaten, die in organischen Bodenverbindungen auftreten (Na-Polyphosphat, pentabasisches Na-Triphosphat), mit Orthophosphat ($KH_2PO_4$) hergestellt und mit beiden Methoden P darin gemessen. Während mittels ICP-OES das gesamte gelöste P bestimmt werden konnte (mit einem Fehler bis zu maximal 13 %), erfasste die Kolorimetrie erwartungsgemäß nur das Orthophosphat (mit einem Fehler bis zu rund 9 %). Demnach ist es möglich, das in den beiden Bodenextrakten gelöste "Nicht-Orthophosphat" (d. h. organisches P oder Poly-P) mit ausreichender Genauigkeit zu berechnen. Allerdings können Fehler zum einen aufgrund von Hydrolyse der Nicht-Orthophosphate sowie in realen Bodenextrakten auch dadurch entstehen, dass suspendiertes kolloidales P sowie lösliche P-Komplexe mit Fe, Al und /oder Ca das mit ICP-OES gemessene Gesamt-P erhöhen.

**Stichwörter:** *anorganisches P, organisches P, Bodenextrakte, Pflanzenverfügbarkeit, Colorimetrie, ICP-OES*

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## Background and aim of this study

Various extractants are in use to estimate the amount of plant available P in soils. Traditionally, the molybdenum blue method was used to determine the amount of extracted P. This coloring agent only reacts with the inorganic orthophosphate ($PO_4$) compound. Therefore, it is assumed that organic P dissolved by the applied extractant will not be accounted for by this method of determination. In contrast to this, ICP-OES is thought to measure all P forms present in the soil extract, including organic as well as other inorganic compounds (Mallarino, 2003; Ziadi et al., 2009; Matula, 2010, 2011; Paz-Ferreiro et al., 2012). Therefore, it can be hypothesized that the "organic" P (including non-orthophosphate P-forms common in organic compounds, such as polyphosphates) dissolved by a given extractant may be estimated by subtracting P measured by colorimetry („dissolved inorganic P") from P measured by ICP („total dissolved P"). While this combined methodology is already in use in some research labs, it has not yet been validated as such. The aim of the reported experiment was to validate the underlying assumptions using pure lab grade chemicals at different mixing ratios.

## Material and methods

A water soluble organic phosphate (sodium phytate) as well as two water soluble polyphosphates (sodium polyphosphate and sodium triphosphate pentabasic) were each mixed with $KH_2PO_4$ at different inorganic/"organic" P ratios and dissolved in water and Olsen (bicarbonate) extractant (Olsen et al., 1954) (Table 1).

**Table 1**
Salt mixtures and mixing ratios used in the experiment

| Mix | Inorganic (ortho-P) salt | Organic/poly-P salt | Inorg./org. P (%) |
|---|---|---|---|
| 1 | $KH_2PO_4$ [mono potassium phosphate] | $C_6H_{18}O_{24}P_6$ * x Na+ * y $H_2O$ [sodium phytate] | 100/0 |
| | | | 75/25 |
| | | | 50/50 |
| | | | 25/75 |
| | | | 0/100 |
| 2 | $KH_2PO_4$ | $(NaPO_3)_n$ [sodium polyphosphate, 96 %] | 100/0 |
| | | | 75/25 |
| | | | 50/50 |
| | | | 25/75 |
| | | | 0/100 |
| 3 | $KH_2PO_4$ | $Na_5P_3O_{10}$ [sodium triphosphate pentabasic, 98 %] | 100/0 |
| | | | 75/25 |
| | | | 50/50 |
| | | | 25/75 |
| | | | 0/100 |

All compounds used were in the form of lab grade salts. Since Na phytate came in hydrate form and thus no defined P content was declared, the exact P concentration in this salt was determined after digestion in aqua regia and the salt mix prepared based on the analyzed P concentration. Each treatment was done in four repetitions. The resulting solutions were measured by ICP-OES as well as by colorimetry (using the ascorbic acid – ammonium molybdate method based on Murphy & Riley, 1962) and it was calculated how much of the added inorganic and organic/poly-P was detected by the different methods of determination.

## Results and Discussion

Tables 2 to 4 show the results for the three different salt mixtures. Using ICP-OES for detection, between 95 to 113 % of the total P amount added to the extract were detected in the three salt mixtures. Apparently, a measuring error of up to 13 % may occur, including errors in sample preparation (determination of P content in salts, dosage/weighing of salt mixtures, dilution of samples) as well as instrumental inaccuracy (which was calculated for ICP-OES to be no larger than 3.6 % based on repeated measurements of matrix adapted standards).

**Table 2**
Comparison of P in water and Olsen extractant measured by ICP-OES and colorimetry using salt mix 1 ($KH_2PO_4$ / sodium phytate), expressed as relative share of total added P (% $P_{tot}$ = $P_{measured}$ *100/total P added) and of added organic P (% $P_{org}$ = ($P_{measured}$ – added inorganic P)*100/added org P), mean values of 4 repetitions. Significant differences (ANOVA, $p < 0.05$; post hoc test: LSD5) between recovery rate of $P_{tot}$ ($^{a}$, $^{b}$, …) and $P_{org}$ ($^{A}$, $^{B}$, …) and measuring "error" by colorimetry ($^{A}$, $^{B}$, …), respectively, for different mixing ratios are indicated by different letters (tested separately for water and Olsen, respectively).

| Ratio inorg/org P | | ICP-OES | | Colorimetry | |
|---|---|---|---|---|---|
| | | Water | Olsen | Water | Olsen |
| 100/0 | % $P_{tot}$ | 113.3 $^{a}$ | 104.5 $^{a}$ | 96.4 $^{a}$ | 99.0 $^{a}$ |
| | % $P_{org}$ | - | - | - | - |
| 75/25 | % $P_{tot}$ | 103.6 $^{b}$ | 100.8 $^{b}$ | 77.5 $^{b}$ | 81.4 $^{b}$ |
| | % $P_{org}$ | 114.5 $^{A}$ | 103.6 $^{A}$ | 0.0 $^{A}$ | 12.8 $^{A}$ |
| 50/50 | % $P_{tot}$ | 104.7 $^{b}$ | 102.0 $^{b}$ | 56.0 $^{c}$ | 59.1 $^{c}$ |
| | % $P_{org}$ | 109.4 $^{B}$ | 104.6 $^{A}$ | 1.8 $^{B}$ | 8.7 $^{B}$ |
| 25/75 | % $P_{tot}$ | 105.6 $^{b}$ | 102.3 $^{bc}$ | 32.0 $^{d}$ | 33.7 $^{d}$ |
| | % $P_{org}$ | 107.5 $^{BC}$ | 103.2 $^{A}$ | 4.1 $^{C}$ | 6.6 $^{C}$ |
| 0/100 | % $P_{tot}$ | 105.8 $^{b}$ | 103.8 $^{ac}$ | 5.7 $^{e}$ | 5.5 $^{e}$ |
| | % $P_{org}$ | 105.8 $^{C}$ | 103.8 $^{A}$ | 5.7 $^{D}$ | 5.5 $^{C}$ |
| $LSD_{\alpha = 5\,\%}$ | $P_{tot}$ | 6.6 | 1.7 | 0.4 | 0.7 |
| $LSD_{\alpha = 5\,\%}$ | $P_{org}$ | 2.0 | 4.3 | 0.2 | 1.1 |

**Table 3**
Comparison of P in water and Olsen extractant measured by ICP-OES and colorimetry using salt mix 2 ($KH_2PO_4$ / $(NaPO_3)_n$), expressed as relative share of total added P (% $P_{tot}$ = $P_{measured}$* 100/total P added) and of added poly-P (% $P_{poly}$ = ($P_{measured}$ – added inorganic P)*100/added poly-P), mean values of 4 repetitions. Significant differences (ANOVA, $p < 0.05$; post hoc test: LSD5) between recovery rate of $P_{tot}$ ($^{a}$, $^{b}$, …) and $P_{poly}$ ($^{A}$, $^{B}$, …) and measuring "error" by colorimetry ($^{A}$, $^{B}$, …), respectively, for different mixing ratios are indicated by different letters (tested separately for water and Olsen, respectively).

| Ratio inorg/org P | | ICP-OES | | Colorimetry | |
|---|---|---|---|---|---|
| | | Water | Olsen | Water | Olsen |
| 100/0 | % $P_{tot}$ | 97.3 $^{a}$ | 108.7 $^{a}$ | 96.5 $^{a}$ | 100.2 $^{a}$ |
| | % $P_{poly}$ | - | - | - | - |
| 75/25 | % $P_{tot}$ | 95.9 $^{a}$ | 99.6 $^{b}$ | 73.8 $^{b}$ | 75.6 $^{b}$ |
| | % $P_{poly}$ | 83.6 $^{A}$ | 98.4 $^{A}$ | 0.0 $^{A}$ | 2.9 $^{A}$ |
| 50/50 | % $P_{tot}$ | 95.7 $^{a}$ | 98.2 $^{b}$ | 50.5 $^{c}$ | 53.3 $^{c}$ |
| | % $P_{poly}$ | 91.3 $^{B}$ | 96.5 $^{A}$ | 1.0 $^{B}$ | 6.6 $^{A}$ |
| 25/75 | % $P_{tot}$ | 96.2 $^{a}$ | 98.2 $^{b}$ | 27.2 $^{d}$ | 29.6 $^{d}$ |
| | % $P_{poly}$ | 95.0 $^{C}$ | 97.6 $^{A}$ | 2.9 $^{C}$ | 6.1 $^{A}$ |
| 0/100 | % $P_{tot}$ | 94.9 $^{a}$ | 95.9 $^{c}$ | 3.6 $^{e}$ | 5.6 $^{e}$ |
| | % $P_{poly}$ | 94.9 $^{C}$ | 95.9 $^{A}$ | 3.6 $^{D}$ | 5.6 $^{A}$ |
| $LSD_{\alpha = 5\,\%}$ | $P_{tot}$ | 2.3 | 2.1 | 0.3 | 2.3 |
| $LSD_{\alpha = 5\,\%}$ | $P_{org}$ | 1.8 | 4.7 | 0.3 | 2.7 |

**Table 4**
Comparison of P in water and Olsen extractant measured by ICP-OES and colorimetry using salt mix 3 ($KH_2PO_4$ / $Na_5P_3O_{10}$), expressed as relative share of total added P (% $P_{tot}$ = $P_{measured}$* 100/total P added) and of added poly-P (% $P_{poly}$ = ($P_{measured}$ – added inorganic P)*100/added poly-P), mean values of 4 repetitions. Significant differences (ANOVA, $p < 0.05$; post hoc test: LSD5) between recovery rate of $P_{tot}$ ($^{a}$, $^{b}$, …) and $P_{poly}$ ($^{A}$, $^{B}$, …) and measuring "error" by colorimetry ($^{A}$, $^{B}$, …), respectively, for different mixing ratios are indicated by different letters or numbers (tested separately for water and Olsen, respectively).

| Ratio inorg/org P | | ICP-OES | | Colorimetry | |
|---|---|---|---|---|---|
| | | Water | Olsen | Water | Olsen |
| 100/0 | % $P_{tot}$ | 98.5 $^{a}$ | 110.3 $^{a}$ | 100.3 $^{a}$ | 99.3 $^{a}$ |
| | % $P_{poly}$ | - | - | - | - |
| 75/25 | % $P_{tot}$ | 100.5 $^{a}$ | 100.2 $^{b}$ | 77.0 $^{b}$ | 77.1 $^{b}$ |
| | % $P_{poly}$ | 102.1 $^{A}$ | 100.8 $^{A}$ | 8.1 $^{A}$ | 8.4 $^{A}$ |
| 50/50 | % $P_{tot}$ | 98.8 $^{a}$ | 100.8 $^{b}$ | 52.0 $^{c}$ | 49.7 $^{c}$ |
| | % $P_{poly}$ | 97.5 $^{A}$ | 101.5 $^{A}$ | 3.9 $^{B}$ | 0.1 $^{B}$ |
| 25/75 | % $P_{tot}$ | 98.6 $^{a}$ | 99.6 $^{b}$ | 27.2 $^{d}$ | 26.9 $^{d}$ |
| | % $P_{poly}$ | 98.2 $^{A}$ | 99.5 $^{A}$ | 3.0 $^{C}$ | 2.5 $^{C}$ |
| 0/100 | % $P_{tot}$ | 98.8 $^{a}$ | 98.9 $^{b}$ | 1.8 $^{e}$ | 1.5 $^{e}$ |
| | % $P_{poly}$ | 98.8 $^{A}$ | 98.9 $^{A}$ | 1.8 $^{D}$ | 1.5 $^{C}$ |
| $LSD_{\alpha = 5\,\%}$ | $P_{tot}$ | 1.8 | 3.4 | 0.5 | 1.0 |
| $LSD_{\alpha = 5\,\%}$ | $P_{org}$ | 8.0 | 5.1 | 0.6 | 1.1 |

As expected, the amount of P measured by colorimetry came very close to the amount of added inorganic P, while the organic/poly-P remained largely undetected. However, depending on the salt mixture and the extractant used, colorimetry resulted in up to nearly 9 % more P being measured than was added as ortho-P compound (see Table 2: 33.7 % of $P_{tot}$ measured by colorimetry in Olsen solution while only 25 % of the total were added as ortho-P). This error is a little higher than observed by Torres-Dorante et al. (2004), who measured different polyphosphate solutions by colorimetry and found orthophosphate-concentrations in the range of 5 to 6 % of total P. While this might at least partly be attributed to the detection method's inherent inaccuracy, it could also be interpreted as an indication that some of the organic (and poly-) P was transformed into orthophosphate by the acid coloring reagent (which brought the pH of the measured samples down to <1) and therefore detected by colorimetry. This would concur with Matula (2010) and Baldwin (1998) who argued that at pH values below 2, hydrolysis of less stable esters of phosphorus may take place, increasing the amount of assumed inorganic P measured by colorimetry. It is also known from the literature that spontaneous hydrolysis of polyphosphates back into the ortho-P form may occur (Torres-Dorante et al., 2005), with the rate of hydrolysis increasing at decreasing pH (Robertson, 2001). It should be noted, however, that in the study by Baldwin (1998), less than 2 % of the tested tripolyphosphate and inositol hexaphosphate were hydrolysed. The assumption that the degree of hydrolysis by the acid coloring agent is rather limited is also supported in our experiment by some control measurements with ion chromatography (IC), which agreed well with or even exceeded the colorimetric measurements (data not shown here). However, similar to the observations reported by Torres-Dorante et al. (2004), some of the "excess" ortho-P determined in our experiment might also stem from the declared minor impurity of the lab chemicals (see Table 1), which contain small traces of ortho-P.

## Conclusion and outlook

Based on the presented results from the lab experiment, the combination of ICP-OES and colorimetry promises to be a valid method for estimating the amount of dissolved organic (and polyphosphate-) P in standard soil P extracts. However, due to hydrolysis of organic P compounds and polyphosphates back into ortho-P, a range of error in the order of up to 13 % must be accounted for. In addition, ICP-OES does not differentiate between dissolved organic P and non-orthophosphate inorganic P compounds dissolved in the solution,

so it will not give a precise estimate of organic P. In order to further specify the composition of P compounds in solution, speciation by weak-ion exchange chromatography may be performed (Baldwin, 1998). Nevertheless, measuring total dissolved soil P by ICP-OES may still allow an assessment of potentially plant available soil P, keeping in mind that according to Torres-Dorante et al. (2005), hydrolysis – and thus transformation into a plant available form – of polyphosphates may take as long as 30 days up to a couple of months, depending on the type of compound. A possible limitation of the method's accuracy when using it for real soils, however, is indicated by Pierzynski et al. (2012) who pointed out that soil extracts may contain suspended colloidal (i.e. particle bound) P not removed by filtering, as well as soluble complexes of P with Fe, Al and/or Ca, which are not plant available, but may increase the total P measured by ICP-OES.

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