4. Digestions

4.5 Methods for the determination of P fractions or binding forms in soil samples

Dana Zimmer, Karen Baumann

As explained in more detail in chapter "1.1.1 P binding forms in soils", phosphorus is generally present in the soil as phosphate, but in very different **organic** and **inorganic compounds**. Inorganic phosphates can be divided into orthophosphates, pyrophosphates and polyphosphates, for example, and organic phosphates into orthophosphate monoesters, orthophosphate diesters and phosphonates (Cade-Menun and Liu 2013, Turner et al. 2005). These different P-compounds can be **bound to soil minerals** such as Fe and Al(hydr)oxides or clay minerals as well as organo-mineral complexes. The type of P-compound and its binding to the soil matrix influences the **turnover** and **bioavailability** of the P-compound for soil organisms and plants. Various wet-chemical methods such as sequential P fractionation or DL extract are used to estimate the P binding forms and their bioavailability, e.g. with regard to plant nutrition or P leaching into water bodies. In general, it is assumed that the extraction agents used attack certain target compounds and thus allow an estimation of the binding form and bioavailability. However, it should be noted that, in contrast to spectroscopic methods such as ^{31}P-NMR, all **wet chemical extractions** are only **operationally defined**, i.e. they extract other binding forms in addition to the target compounds or transfer the target compounds only incompletely into the extract and the extraction agent itself can lead to changes in the binding forms (e.g. Bacon and Davidson 2008). This is particularly important when naming and interpreting the extracts.
In a number of (sequential) extractions, the P concentration in the extract is/can be determined by ICP-OES (or MS) and/or photometrically, e.g. using molybdenum blue (MB). If P in an extract is determined using both methods, the P concentration using ICP-OES (Chapter 5.1) is interpreted as total P ($\mathbf{P_t}$) and that using MB as inorganic P ($\mathbf{P_i}$) and the difference between the two is interpreted as organic P ($\mathbf{P_o}$) in the extract. However, it is not advisable to interpret this determination on a one-to-one basis, as the acidic environment of the MB reagent causes an unknown proportion of the labile organic P to be converted to phosphate, thus overestimating the proportion

of P_i and underestimating the proportion of P_o. Alternatively, there are unknown proportions of non-reactive inorganic P, which leads to an underestimation of P_i and an overestimation of P_o (e.g. Cade-Menun and Liu 2013, Condron and Newman 2011). For this reason, the term **molybdate-reactive P** and **non-reactive P** is the better term (Haygarth and Sharpley 2000, Felgentreu et al. 2018).

4.5.1 Sequential P fractionation of soil samples

Dana Zimmer, Karen Baumann

Principle and suitability of sequential P fractionation

In the soil, phosphorus is bound to different soil components and can therefore be mobilized and bioavailable in different ways. There are various inorganic and organic P fractions in the soil P pool, which can be regarded as **labile**, **moderately labile**, **relatively insoluble** and **stable (long-term availability) P pools** from the point of view of **P plant availability**. Various fractionation methods have been developed to differentiate between these P-forms. Most sequential P fractionations first extract a **"weakly bound"** fraction with a **salt solution** (e.g. NH_4Cl), followed by an extraction of **Fe- and Al-bound P** with an **alkaline extractant** (e.g. NaOH) and finally an **acidic extraction** (e.g. HCl) to extract **Ca-bound P** (Condron and Newman, 2011). In addition, a distinction is made in some cases between organic and inorganic P in the individual fractions by means of P determination using molybdenum blue (MB) and ICP-OES/-MS. If P in the extracts is determined using both methods, the P concentration using ICP-OES or -MS is interpreted as total P ($\mathbf{P_t}$) and that using MB as inorganic P ($\mathbf{P_i}$) and the difference between the two as organic P ($\mathbf{P_o}$). Since the acidic environment of the MB reagent converts an unknown proportion of the labile organic P to phosphate and thus overestimates the proportion of P_i or, alternatively, unknown proportions of non-reactive inorganic P are present, which leads to an underestimation of P_i (e.g. Cade-Menun and Liu 2013, Condron and Newman 2011), the term **molybdate-reactive P** and **non-reactive P** is the better term (Haygarth and Sharpley 2000, Felgentreu et al. 2018).

One of the most common sequential P fractionations is the fractionation according to Hedley et al. (1982) or Thiessen and Moir (1993) (Alamgir and Marschner 2013 a, b). The **modified Hedley fractionation**, as carried out

in the Agronomy and Soil Science working groups of the Faculty of Agricultural and Environmental Sciences (University of Rostock), comprises the following extraction steps in sequence (F1) water-anion resin, (F2) $NaHCO_3$, (F3) NaOH and (F4) HCl or H_2SO_4.

Note:

- This P fractionation is normally used for agricultural soils but is generally suitable for terrestrial mineral soils. If it is applied to semi-terrestrial (e.g. gleys), semi-sub-hydric (e.g. mudflats) and sub-hydric (e.g. gyttja) soils, bogs, marine sediments or substrates such as manure, the results must be interpreted with even greater caution, as these substrates may have pH and Eh values (see Chapter 2.3) and binding partners for P (e.g. concentration of organic matter) that differ greatly from terrestrial soils.

Interpretation of the results

In this sequential P-fractionation, the fractions can generally be interpreted as follows, although it should be noted that, as with all sequential fractionations, the fractions are operationally defined and do not correspond 100% to the interpretations (Bacon and Davidson 2008).

- **F1**: Resin-P (labile P): exchangeable P, superficially sorbed, readily available to plants, reflects the removal of phosphate from the extraction water by the anion exchange resin, the removal by the plant roots (compared to a cold-water extract, where a solubility equilibrium between the soil sample and the extraction water is established more quickly).
- **F2**: $NaHCO_3$-P (labile P), easily mineralizable, plant-available P (simulates root respiration: formation of HCO_3^- from CO_2 release)
- **F3**: NaOH-P is moderately labile P and therefore available in the medium or long term, NaOH-P is considered to be P bound to Al-Fe or humic substances.
- **F4**: H_2SO_4 P: P bound in Ca or carbonate
- **F5:** residual P = total P (TP from aqua regia extract of the soil sample and ICP-OES measurement) minus the sum of fractions F1...F4 (P from ICP-OES measurements); or determine TP in the extraction residue, only long-term available P

The ICP-OES measures total P in the respective extract, while the MB method can be used to measure the reactive phosphate P and thus approximately the inorganic P content (P_i) in the extract. The difference

between the two measurements gives approximately the organic P content (P_o). It should be noted that the use of HCl and H_2SO_4 in the course of extraction can already convert parts of organic P compounds into free phosphate-P. Therefore, the terms molybdate-reactive P and non-reactive P should generally be used instead of P_i and P_o (Cade-Menun and Liu 2013, Haygarth and Sharpley, 2000).

The photometric P determination with the molybdenum blue method is only possible on colorless, undimmed extracts! Particularly in soil samples with high concentrations of organic matter (e.g. peat), the extracts are often dark-colored (especially the NaOH extract); this means that P determination with MB is not useful. An attempt can be made to dilute the extracts accordingly so that the extracts lighten in color.

Protocol for sequential P fractionation

Material and chemicals required for 24 samples + 2 blank values + solutions for ICP-OES standards

- Sufficient solutions must be prepared for the extractions themselves and for preparing the standards for ICP and, if necessary, MB measurement.
- If several runs of sequential P fractionation or a higher number of samples are planned, correspondingly larger quantities of chemicals should be prepared in order to use the same solutions for all extracts and for the standards for the calibration lines.

Preparation of the resin strips

- Anion exchanger membrane BDH #55164 2S, cut into 12 strips of 6 x 2 cm each
- Storage in ultrapure water (UW) in the refrigerator
- Prepare 2 L 0.5 M NaHCO3 and fill into two 1-liter beakers
- Place resin strip in first beaker for 1 h, transfer to second beaker with tweezers for 1 h
- Wash resin strips 3 times by placing them in beakers with UW (move with tweezers, place tweezers in UW before use)
- Storage in UW in the refrigerator (24 h before use, after preparation with HCO_3^-)

Preparation of chemicals

2 liters 1 M HCl (washing of the resin strips) for F1

- Fill 2-liter flask to approx. 1.7 L with UW, add 166 ml 37 % HCl
- After cooling, fill up to 2 liters with UW

5 liters 0,5 M $NaHCO_3$ (pH 8,5) for F2

- Add 210 g $NaHCO_3$ to a 5 L flask and fill up to approx. 4 liters with UW
- Adjust the pH value with 1 M NaOH (approx. 50 to 100 ml required)
- Fill up to 5 liters with UW

1 liter 1 M NaOH for pH adjustment

- Fill 1-liter flask with approx. 700 ml UW, add 40 g NaOH pellets, fill incompletely with UW
- Allow to cool, fill up to 1 liter with UW

3 liters 0,1 M NaOH for F3: Prepare 1 liter + 2 liters (if no 3-liter flask is available)

- Fill 1-liter flask to approx. 700 ml with UW, add 4 g NaOH pellets, after cooling fill to 1 liter with UW
- Fill 2-liter flask to approx. 1.5 L with UW, add 8 g NaOH pellets, after cooling fill to 2 liters with UW

3 liters 1 M H_2SO_4 for F4: Prepare 1 liter + 2 liters (if no 3-liter flask is available)

- Fill a 1-liter flask with approx. 700 ml UW and add 55 ml H_2SO_4 (95-97 %)
- Fill up to 1 L with UW the next day after cooling down
- Fill a 2-liter flask with approx. 1.5 liters of RW and add 110 ml of H_2SO_4 (95-97 %),
- Fill up to 2 L with UW the next day after cooling down

Sample preparation:

- Drying soil samples (see chapters 2.4 and 3.1)
- Sieve soil samples <2 mm and use the <2 mm fraction (fine soil)
- Determine total element concentrations in a subsample (e.g. using aqua regia extract, see chapter 4.1.2)
- Sequential extraction must be started on Monday so that the fourth fraction is ready on Friday
- The sample can also be weighed in the previous week.
- Prepare the anion exchange resin (see above: Preparation of the resin strips)

Procedure:

- Prepare at least 3 replicates per soil sample and at least 1 blank value per 10 extraction samples.
- Weigh 0.5 g of fine soil into 50 ml centrifuge tubes
- **For F1**: Add 30 ml ultrapure water (UW) and a strip of anion exchange resin, shake in an overhead shaker for 18 hours (start at approx. 2 pm)
- Remove resin strips with tweezers, rinse adhering soil particles with UW (spray bottle) back into the centrifuge tube
- Wash P from resin strips with max. 45 ml 1 M HCl via funnel with filter (P-free) in 50 ml volumetric flask
- Place the resin strips in beakers with UW, later place in the refrigerator
- Fill the graduated flask with 1 M HCl to 50 ml (**F1**)
- Fill aliquots into ICP tubes ((1.) Determine P_t of F1 on the ICP and if necessary (2.) P_i photometrically, difference = P_o)
- **For F2**: Add 30 ml 0.5 M NaHCO3 to the soil sample, mix briefly and shake in an overhead shaker for 18 h (start approx. 2 pm)
- Centrifuge at 2500 x g for 20 min
- Filter the supernatant into a 100 ml volumetric flask (funnel + filter)
- For washing, add another 30 ml of 0.5 M $NaHCO_3$ to the soil sample, mix by hand and centrifuge at 2500 x g for 20 min
- Add the supernatant to the volumetric flask (combine the filtrates) and make up to 100 ml with 0.5 M $NaHCO_3$, shake well
- Fill 10 ml of the extract into an Erlmeyer flask and slowly (!) add 1 ml of conc. HCl to destroy HCO_3^- for the ICP measurement!
- Leave the Erlmeyer flask under the fume cupboard overnight for outgassing and add 9 ml UW to the sample (for ICP measurement) in the Erlmeyer flask the next day (fraction F2 labile P_t at the ICP)
- if necessary, determine labile P_i photometrically (second tube) with MB, difference to P_t = P_o); do not destroy this sample with HCl
- **For F3**: Add 30 ml 0.1 M NaOH to the soil sample in the centrifuge tube, shake in an overhead shaker for 18 h (start approx. 2 pm)
- Centrifuge at 2500 x g for 20 min
- Filter the supernatant into a 100 ml volumetric flask
- For washing, add another 30 ml of 0.1 M NaOH to the soil sample, mix by hand and centrifuge again at 2500 x g for 20 min
- Also filter the supernatant into the volumetric flask and make up to 100 ml with 0.1 M NaOH (**F3**)

- Fill aliquots into ICP tubes ((1.) Determine P_t in NaOH (F3) on the ICP and if necessary (2.) P_i photometrically, difference = P_o)
- **For F4**: Add 30 ml 1 M H_2SO_4 to the soil sample in the centrifuge tube under the fume cupboard, shake in an overhead shaker for 18 h (start approx. 2 pm)
- Filter the extract into a 100 ml flask. Since H_2SO_4 vapors corrode the centrifuge, do not centrifuge!
- Fill the flask with 1 M H_2SO_4 to 100 ml (**F4**)
- Fill aliquots into ICP vessels ((1.) Determine P_t in H_2SO_4 on the ICP and if necessary (2.) P_i photometrically, difference = P_o)
- **For F5**: either dry the extraction residue and determine the total element concentrations in it using aqua regia and ICP-OES or subtract the sum of fractions F1 to F4 from the total element concentration (e.g. in the aqua regia extract) of the untreated soil samples.

Notes:

- If F5 is determined in the extraction residue, the sum of the P concentrations of F1 to F5 should theoretically correspond to the total element concentration in the untreated soil sample after aqua regia extract. However, it is possible that the sum of the 5 fractions is greater than the total element concentration. This is caused by the fact that aqua regia extracts only provide so-called pseudo-total concentrations (silicates are not broken down) and sequential P fractionation may release higher proportions of P. Therefore, F5 is usually calculated as the difference between the total P concentration and the sum of F1 to F4 as residual P.
- Only aliquots of the fractions are required for P determination. The retained samples of the extracts should be frozen until the end of all analyses if repeat measurements are necessary.
- The alkalis and acids must be added under the fume cupboard!
- The appropriate protective clothing must be worn, especially when working with H_2SO_4.

Sequential P fractionation is carried out in the working groups Soil Science and Agronomy (both at the Faculty of Agricultural and Environmental Sciences at the University of Rostock).

References

Bacon JR, Davidson CM (2008) Is there a future for sequential chemical extraction? Analyst, 133, 25–46, DOI: 10.1039/b711896a

Cade-Menun B and Liu CW (2013) Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy of soils from 2005 to 2013: A review of sample preparation and experimental parameters. Soil Sci. Soc. Am. J. 78, 19–37, DOI: 10.2136/sssaj2013.05.0187dgs

Condron LM, Newman S (2011) Revisiting the fundamentals of phosphorus fractionation of sediments and soils. J Soils Sediments 11, 830–840, DOI: 10.1007/s11368-011-0363-2

Felgentreu L, Nausch G, Bitschowsky F, Nausch M, Schulz-Bull D (2018) Colorimetric chemical differentiation and detection of phosphorus in eutrophic and high particulate waters: advantages of a new monitoring approach. Frontiers in Marine Science 5, article 212, DOI: 10.3389/fmars.2018.00212

Haygarth PM, Sharpley AN (2000) Terminology for phosphorus transfer. Environ. Qual. 29, 10–1, DOI: 10.2134/jeq2000.00472425002900010002x

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. Talanta 66: 294–306, DOI: 10.1016/j.talanta.2004.11.012

For citation: Zimmer D, Baumann K (*year of download*) Chapter 4.5 Methods for the determination of P fractions or binding forms in soil samples (Version 1.0) in Zimmer D, Baumann K, Berthold M, Schumann R: Handbook on the Selection of Methods for Digestion and Determination of Total Phosphorus in Environmental Samples. DOI: 10.12754/misc-2020-0001