4. Digestions

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

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4.5.2 Estimation of plant-available P in soil samples

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In soil, phosphorus is bound to various soil components and only a small proportion is dissolved in the soil solution. This means that in different soils P is differently mobilisable and bioavailable. The proportion of bioavailable or plant-available P therefore depends not only on how high the P concentration in the soil is, but also on how P is supplied from the soil particles into the soil solution and is thus available to plants at all (Abdu 2006, Zheng and Zhang 2012). In particular, the proportion of nutrients such as P available during a growing season is important for agricultural crops and their fertilization according to their requirements. Therefore, when determining the potentially bio- or plant-available P, not only the current P concentration in the soil solution, but also the P solubility or P replenishment from the soil solid phase must be taken into account (e.g. Abdu 2006, Zheng and Zhang 2012). Various extraction methods have been developed since the first half of the 20th century to estimate the fertilizer requirements for the most important nutrients such as P, K and Mg; later, so-called ion-sink methods were added. The methods for evaluating the bio- or plant-available P attempt to simulate the conditions around the plant roots (e.g. the release of low-molecular weight organic acids by the plant root). In general, a distinction is made between chemical extractions and ion-sink methods. All of these methods have their specific advantages and disadvantages. The chemical extracts in particular were often developed for special soils with special properties, on which they can be used very effectively; however, they can only be used to a limited extent on other soils (Abdu 2006). The pH value and the concentration of carbonate play a particularly important role here (e.g. Schüller 1969, Zheng and Zhang 2012). The advantage of ion-sink methods lies in their universal applicability, as they are largely

independent of soil properties and, similar to plant roots, mimic P removal. In addition, the chemical properties of the soil are not changed and the anion exchange resins in particular can be reused without losing their extraction capacity (Abdu 2006, Zheng and Zhang 2012).
Some methods for estimating plant-available phosphorus are presented below.

4.5.2.1 DL and CAL extract

Principle and suitability of the extracts

In Germany, double lactate (DL) and calcium lactate (CAL) extracts are used in agriculture to estimate the proportion of phosphorus, potassium and magnesium that is potentially available to plants. This makes it possible to estimate the amount of fertilizer to be applied. Only P is discussed in the following. In other countries, other extracts are used (e.g. with $NaHCO_3$) to estimate the phosphorus that is potentially available to plants and can be used as fertilizer. **The CAL extract according to Schüller (1969) is used in Germany for calcareous soil samples and the DL extract according to Riehm (1948) for lime-free soil samples.** The specifications of the "Landesuntersuchungs- und Forschungsanstalten (LUFen)" as to whether a DL or CAL extract is to be used in agriculture depend on the federal state. The DL extract consists of a calcium lactate solution with a pH value of 3.8. The CAL extract is a buffered solution of calcium acetate, calcium lactate and acetic acid with a pH value of 4.1 (VDLUFA 2015).
The following section was taken from Schüller (1969), van Laak et al. (2018) and VDLUFA (2012): Since apatitic phosphates do not or hardly dissolve in neutral to alkaline soils, in contrast to acidic soils, and thus have hardly any P fertilization effect, the extraction agent should not or only slightly dissolve the apatites in neutral to alkaline soils. Both DL and CAL dissolve monocalcium and dicalcium phosphates. However, due to the higher Ca activity of the CAL extract, in contrast to the DL extract, the poorly soluble Ca phosphates such as apatites are not dissolved with the CAL extract. Therefore, in contrast to the DL extract, an overestimation of P availability can be avoided with the CAL extract on neutral and alkaline soil. On acidic soils, however, the CAL extract may incompletely dissolve P in the presence of apatitic phosphates. Due to the higher buffering capacity of the CAL extract compared to the DL extract (increase in the pH value of the extraction solution for carbonate-containing soil samples), the

CAL extract can be used for $CaCO_3$ concentrations of up to 8 or up to 15 %, respectively. To make DL and CAL extracts more comparable, van Laak et al. (2018) developed pH-dependent equations to convert P concentrations between DL and CAL extracts.

Interpretation of the results

For both the DL extract and the CAL extract, so-called content classes ("Gehaltsklassen") A (very low content) to E (very high content) are defined for the P concentrations (as well as Mg and K) (VDLUFA 2015, Table 4.5.2-1). The guideline values for the P concentrations for the content classes can also be found in Table 4.5.2-1 (VDLUFA 2015). The fertilizer recommendations are derived from these content classes. The specific fertilizer recommendation depends on the type of crop and the possible and targeted yield on the respective soil. In the VDLUFA regulation, the P concentration is traditionally determined using molybdenum blue on a photometer. However, according to VDLUFA (2015), it is also possible to determine P using ICP-OES (in parallel with Mg and K), as carried out in the working group Soil Science. It generally provides comparable results. It is assumed that the determination using MB and ICP-OES provides approximately the same results because DL and CAL are mainly used to extract inorganic phosphate and not organically bound phosphate. However, this should be adjusted specifically for the soil to be extracted.
<u>Note:</u> In organic farming, content class B is often aimed for, so that the fertilizer recommendations are correspondingly lower.

Tab. 4.5.2-1 Definition of content classes, P concentrations and fertilizer recommendation according to VDLUFA (2015)

Content class	Short definition for P in soil	mg P per kg soil	Fertilizer recommendation
A	very low content	up to 20	Strongly increased fertilization compared to the recommendation in content class C
B	low content	21 to 44	Increased fertilization compared to the recommendation in content class C
C	target content	45 to 90	Maintenance fertilization usually after removal
D	high content	91 to 150	Reduced fertilization compared to the recommendation in content class C
E	very high content	>150	no fertilization

Note from VDLUA (2015): Vertically rotating shaking machines have become widely established in routine operation. The optimum rotational speed depends on the diameter of the rotor. Soil and extraction solution must be mixed reliably, but centrifugal effects caused by excessive rotational speeds must be avoided.

Sample preparation for DL and CAL extract:

- Drying soil samples (see chapters 2.4 and 3.1)
- Sieve soil samples <2 mm and use this <2 mm fraction (fine soil)
- Determine total element concentrations in a subsample (e.g. using aqua regia extract, see chapter 4.1.2)

Protocol for the DL extraction

Material and chemicals required for the DL extract

DL storage solution

- Place 120 g of calcium lactate ($C_6H_{10}CaO_6$ $5H_2O$) in a 1-liter graduated flask and pour approx. 0.8 L of boiling ultrapure water (UW) over it and stir until everything is dissolved.
- Add 40 ml of 10 M HCl to the still warm solution and fill up to 1 L with UW after cooling down.

DL solution for use (prepare fresh daily)

- Fill 500 ml of the DL stock solution with UW to 10 L or fill 50 ml to 1 liter
- Important: pH control 3.6

Procedure DL extraction:

- Prepare at least 3 replicates per soil sample and at least 1 blank value per 10 extraction samples.
- Normally, 4 g of soil is weighed into 250 ml shake flasks and mixed with 200 ml DL working solution; however, 0.6 g of soil can also be weighed into 50 ml centrifuge tubes and mixed with 30 ml working solution; in this case, pay more attention to the homogeneity of the sample
- Shake overhead for 90 minutes at approx. 20 rotations per minute
- Filter DL extract through P-free filters in Erlmeyer flasks
- If no P-free filters are used, discard the first 10 to 30 ml of the filtrate (rinse the filters), replace the Erlmeyer flasks that have collected the filtrate or place the funnels with the filters on new Erlmeyer flasks after the first ml of filtrate and filter the rest
- Transfer the aliquot to ICP tubes and store in the refrigerator or freeze until measurement (if the extracts must be stored for >1 day)
- Determination of the elements K (766.490 nm), Mg (285.213 nm) and P (213.617 and/or 214.914 nm) by ICP-OES and/or P photometrically with molybdenum blue, see below

Protocol for the CAL extraction

Material and chemicals required for the CAL extract

CAL stock solution

- Dissolve 77 g Ca-lactate pentahydrate ($C_6H_{10}CaO_6 * 5H_2O$) in approx. 300 ml boiling RW and 39.5 g Ca-acetate ($Ca(CH_3COO)_2 * H_2O$) (or 35.5 g Ca(CH3COO)2 anhydrous) in approx. 300 ml RW (heat if necessary)
- Combine both solutions in a 1-liter volumetric flask and add 89.5 ml 100 % acetic acid p.a.
- Fill the mixture with UW in the graduated flask to 1 L.

CAL usage solution:

- Fill up 1 L of the stock solution with UW in the graduated flask to 5 L (= 0.1 M Ca-lactate; 0.1 M Ca-acetate; 0.3 M acetic acid; pH 4.1)

Procedure CAL extraction:

- Prepare at least 3 replicates per sample and at least 1 blank value per 10 samples.
- Normally, 5 g of soil is weighed into 300 ml shake flasks and mixed with 100 ml CAL working solution; however, 1.5 g of soil can also be weighed into 50 ml centrifuge tubes and mixed with 30 ml working solution; in doing so, pay more attention to the homogeneity of the sample
- Shake overhead for 90 minutes at approx. 20 rotations per minute
- Filter CAL extract through P-free filters in Erlmeyer flasks
- If no P-free filters are used, discard the first 10 to 30 ml of the filtrate (rinse the filters), replace the Erlmeyer flasks that have collected the filtrate or place the funnels with the filters on new Erlmeyer flasks after the first ml of filtrate and filter the rest
- Transfer the aliquot to ICP tubes or similar and store in the refrigerator or freeze until measurement (if storage is required for >1 day)
- Determination of the elements K (766.490 nm), Mg (285.213 nm) and P (213.617 and/or 214.914 nm) by ICP-OES and/or P photometrically with molybdenum blue, see below

P determination in DL and CAL extract with molybdenum blue (according to Murphy and Riley, 1962)

Photometric P determination is only possible on colorless, non-turbid extracts!

Reagents:

For **1 liter** of detection reagent A

- Pour approx. 500 ml of UW into a 1-liter flask
- Dissolve 6 g of ammonium heptamolybdate $(NH_4)_6Mo_7O_{24} \times 4H_2O$ in it
- Add 74 ml conc. H_2SO_4, allow to cool slightly
- Dissolve 0.149 g potassium antimony oxide tartrate $K(SbO)C_4H_4O_6 \times 0{,}5\ H_2O$ (KAT) in it
- As the KAT dissolves poorly, add the KAT when the solution is still warm and, if necessary, place in an ultrasonic bath and swirl repeatedly until completely dissolved.
- Add more UW to slightly below the mark, allow to cool to room temperature and fill to 1 liter with UW.

For **2 liters** of detection reagent A:

12 g ammonium heptamolybdate, 148 ml conc. H_2SO_4 and 0.298 g KAT are required.

For **250 ml** detection reagent B:

- Add 1.32 g ascorbic acid to a 200 ml volumetric flask
- Add reagent A and swirl until the ascorbic acid has dissolved
- Fill up to 200 ml with reagent A

For **500 ml** detection reagent B:

- Add 2.64 g ascorbic acid to a 500 ml volumetric flask
- Add reagent A and swirl until the ascorbic acid has dissolved
- Fill up to 500 ml with reagent A

Note:

Reagent B must be **prepared fresh daily**, as it **cannot be kept for longer than 24 hours**. For a 25 ml volumetric flask, 4 ml of detection reagent B is required per sample/standard. Depending on the amount of detection reagent B required, the weight of ascorbic acid and the volume must be adjusted.

Standard series approach for photometric P determination:

Target concentration for the P stock solution: prepare 10 mg P per liter in a 100 ml flask

- To prepare the calibration standards (Table 4.5.2-2), fill the required volume of stock solution into a 25 ml or 50 ml flask and fill up to half full with UW. (Different pipette sizes are required!)
- Add 4 ml (in a 25 ml flask) or 8 ml (50 ml flask) of the detection reagent
- Fill up to 25 or 50 ml with UW
- Wait 30 min and measure absorbances at 882 nm
- Set the standard S0 in the photometer to “zero”, observe the manufacturer's instructions for the photometer
- Calculate calibration line

Tab. 4.5.2-2 Target concentrations for the P standards for the calibration line

Standard	Target concentration P in mg l^{-1} for the calibration line
S0	0
S1	0,025
S2	0,05
S3	0,1
S4	0,2
S5	0,3
S6	0,4
S7	0,5
S8	0,6
S9	0,7
S10	0,8

Note:

If the absorbances of most samples are below the standard S2, additional standards must be inserted between S0 and S2 (Tab. 4.5.2-3). Appropriate µl pipettes are required for this.

Tab. 4.5.2-3 Additional standards A, B and C for the calibration line if the absorbances of most samples are below the standard S2

Standard	Target concentration P in mg l^{-1} for the calibration line
S0	0
A	0,01
B	0,02
S1	0,025
C	0,04
S2	0,05

Photometric determination of the P concentration in the samples with molybdenum blue

- Pipette 1 to max. 10 ml (or 0.5 to max. 5 ml) of the filtrate into each 25 ml (or 50 ml) volumetric flask and then fill up to max. half full with UW
- Add 4 ml (or 8 ml) of detection reagent and make up to 25 (or 50 ml) with UW
- After a reaction time of 30 min, measure at 882 nm, leaving standard S0 at “zero”. Observe the photometer manufacturer's specifications
- The blank values are measured in the same way as the samples and the mean value of the absorbance values of the blank values is subtracted from those of the samples
- Calculate the P concentration in the samples using a calibration line

Note:

The sample quantity required for the molybdate reagent depends on the DL or CAL extractable P concentration in the soil. For soil samples from Ap horizons, try 2.5 ml of sample filled up to 25 ml!

The DL and CAL extracts can be carried out in the Soil Science and Agronomy working groups (both Faculty of Agricultural and Environmental Sciences, University of Rostock).

4.5.2.2 $NaHCO_3$ extract

Principle and suitability of the extract

In general, there is a balance between the following species in water:

$$CO_2\ (g) \leftrightarrow CO_2\ (aq) + H_2O \leftrightarrow H_2CO_3\ (aq) \leftrightarrow H^+(aq) + HCO_3^-(aq) \leftrightarrow\ H^+(aq) + CO_3^{2-}(aq)$$

If $NaHCO_3$ is added to the soil solution, it decomposes in the water to Na^+ and HCO_3^-. In alkaline soil, HCO_3^- reacts with the Ca^{2+} in the soil solution and precipitates as $CaCO_3$ (Olsen et al. 1954, Soinne 2009). The equilibrium is shifted to the right-hand side of the equation. This allows the P previously bound to Ca to be extracted. In acidic soils, in which P is mainly bound to Al- and Fe-(hydroxides), HCO_3^- reacts with the H^+ to form H_2CO_3 and further and further to the left to form CO_2, which can also bubble out ($CO_2(g)$) and is thus removed from the equilibrium. All ions from the above equilibrium reaction compete with P for the binding sites on the pedogenic oxides (Hartikainen, 1981), so that P also dissolve from these. Due to the CO_2 bubbling out, no constant equilibrium can be established between the species. Since no equilibrium is established during extraction and the extracted P concentrations can therefore fluctuate, exact compliance with shaking time, temperature, etc. must be ensured for comparability (Miller et al. 2002). The extract according to Olsen et al. (1954) is used in some states in the USA, for example, to estimate the plant-available P content (Wuenscher et al. 2015). It is preferably used for calcareous soils, but can also be used for non-calcareous soils (Wuenscher et al. 2015).

Protocol

Sample preparation:

- Drying soil samples (see chapters 2.4 and 3.1)
- Sieve soil samples <2 mm and use this <2 mm fraction (fine soil)
- Determine total element concentrations in a subsample (e.g. using aqua regia extract, see chapter 4.1.2)

Preparation of chemicals

1 liter 0,5 M $NaHCO_3$ (pH 8,5)

- Add 42 g NaHCO3 to 1 L flask and fill up to approx. 800 ml with UW
- Adjust the pH value with 1 M NaOH (approx. 10 to 20 ml required)
- Fill up to 1 liter with UW

1 liter 1 M NaOH for pH adjustment

- Fill 1-liter flask with approx. 700 ml RW, add 40 g NaOH pellets, fill incompletely with UW
- After the pellets have completely dissolved and cooled down to room temperature, fill up to 1 liter with UW

Procedure:

- Weigh 1.00 g of air-dry soil into a 50 ml centrifuge tube
- Add 20 ml of 0.5 M $NaHCO_3$ solution
- Shake for 30 min
- Centrifuge for 10 min at 3500 x g
- Filter into Erlenmeyer flasks
- Possibly fill 20 ml for photometric molybdenum blue measurement (see chapter on sequential P fractionation F2, no destruction of HCO^- with HCl)
- Acidify 10 ml sample for ICP measurement with HCl (destruction HCO^-):
  - Add 10 ml sample + 1 ml conc. HCl to Erlenmeyer flask, leave to stand overnight (outgassing CO_2)
  - Add 9 ml UW the next day (dilution factor: 2)
- Filling for P determination on ICP-OES, wavelength: 213.617 nm and/or 214.914 nm

This extraction can be carried out in the Soil Science and Agronomy working groups (both Faculty of Agricultural and Environmental Sciences, University of Rostock).

4.5.2.3 Water extracts

Principle and suitability of the extracts

Water extracts simulate the P pool in the soil that can potentially be mobilized by rain events. This P pool can also be regarded as directly available to plants. However, water extracts largely disregard the subsequent supply of the labilely bound P, which goes into solution as soon as the dissolved P has been absorbed by plants. A distinction can be made between cold and hot water extracts. In addition to the mineral elements of interest, the hot water extract can also be used to extract the easily mobilizable or convertible organic soil substance (OBS), i.e. also C and N (Leinweber et al. 1995).

Sample preparation for the water extracts:

- Drying soil samples (see chapters 2.4 and 3.1) and
- Sieve soil samples <2 mm and use this <2 mm fraction (fine soil)
- If naturally moist soil (e.g. rhizosphere soil) is to be used, at least <5 mm should be sieved and stones, roots etc. removed
- Determine total element concentrations in a subsample (e.g. using aqua regia extract, see chapter 4.1.2)

Protocol cold water extraction (soil + water ratio: 1 + 10)

Procedure:

- Weigh 2.00 g of air-dry fine soil (<2 mm) into 50 ml centrifuge tubes
- Add 20 ml ultrapure water
- Shake over head for 60 min
- Centrifuge at 3000 x g for 10 min
- Filter the supernatant through a P-free filter into a 100 ml Erlenmeyer flask
- Acidify with 20 µl conc. HCl to pH 2-3 to prevent element precipitation and possible microbial changes (“slime”)
- When acidified, the samples should be able to be stored in the refrigerator for several days. However, as the elements have always been determined promptly up to now, there is no experience of storage life. For longer storage, the extracts should be frozen.
- Element determination on ICP-OES (select e.g. Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, Pb, Zn)

Protocol hot water extract for the simultaneous determination of the easily convertible OBS

Provedure:

- Weigh (10) or 25 to 30 g of air-dry fine soil (<2 mm sieved) into a 250 ml glass flask (the heating block offers 12 places, prepare at least one of them as a blank)
- Add a few boiling stones and add 50 to 60 ml of water
- Note the exact weight and volume of water
- Place the glass bulb in the heating block
- Place the reflux condenser on top, heat the suspension in the heating block and boil vigorously for 60 minutes so that soil and water are intensively swirled through
- Cool the sample quickly (place containers in cold water)
- Add 3 drops of 2.5 M CaCl2 solution as a filtration aid and filter through P-free pleated filters into 100 ml Erlenmeyer flasks
- Determination of C and N concentrations in liquid extract as for C_{mic}
- Determination of P (and possibly other elements) on ICP-OES
- If a solid is required for the determination of organic C-compounds using mass spectrometry (MS), the extract should be freeze-dried.

Both water extracts can be carried out in the Soil Science working group (Faculty of Agricultural and Environmental Sciences, University of Rostock).

4.5.2.4 Extraction with low-molecular weight organic acids

Principle and suitability of the extracts

In the soil, only small amounts of P are present as dissolved phosphate in the soil solution; the largest amounts are bound more or less strongly to the soil particles. Extraction with low molecular weight organic acids (LMWOA) simulates the exudation of root exudates from plants and thus the dissolution of potentially available nutrients in the rhizosphere. Feng et al. (2005) describe the extraction with LMWOA for plant-available heavy metals, while Wei et al. (2010) used citric acid solution of similar molarity for the extraction of plant-available P.

If small-scale hot spots in the soil, such as rhizosphere soil, are examined with these extracts, the use of naturally moist samples is recommended in order to prevent microbial conversion and oxidative changes during drying (see chapter 2.4). This also prevents drying-induced lysis of microbial cells in the microorganism-rich hotspots such as the rhizosphere, which would cause an unwanted release of microbial P (Sparling et al. 1985, Srivastava 1998, Turner and Haygarth 2003). To prevent microbial activity and potential biodegradation of the organic acids, a biocide (e.g. chloroform) can be added (Wei et al. 2010). In addition, the prepared solution must be consumed on the same day to prevent degradation of the organic acids.

The LMWOA extracts have not yet been carried out in the Soil Science laboratories (Faculty of Agricultural and Environmental Sciences, University of Rostock), but could potentially be tested there.

Protocols

LMWOA extract modified according to Feng et al. (2005)

<u>LMWOA solution approach according to Feng et al. 2005</u>

- Total concentration of organic acids: 10 mM consisting of: acetic, lactic, citric, malic and formic acid in the ratio: 4:2:1:1:1
- 10 mM = 9/9

Tab. 4.5.2-4 Concentrations of organic acids required for the extract

Part	Acid	Concentration	Molar mass in g mol^{-1}	Available as
4/9	acetic acid	4,44 mM acetic acid	60,05	liquid
2/9	lactic acid	2,22 mM lactic acid	90,08	liquid
1/9	citric acid	1,11 mM citric acid	192,124	powder
1/9	malic acid	1,11 mM malic acid	134,09	powder
1/9	formic acid	1,11 mM formic acid	46,025	liquid

Preparation for 1-liter LMWO solution:

acetic acid	4,44 mM	266,62 mg
lactic acid	2,22 mM	199,98 mg
citric acid	1,11 mM	213,25 mg
malic acid	1,11 mM	148,84 mg
formic acid	1,11 mM	51,09 mg

<u>Procedure for extraction</u>

- Weigh 2.00 g of moist rhizosphere soil or lutro soil <2 mm into 50 ml centrifuge tubes

- Add 20 ml LMWOA solution plus 2 drops of chloroform (Wei et al. 2010) as a biocide (work under the fume cupboard)
- Shake overhead for 16 hours at approx. 20 rotations per minute
- Centrifuge at 3000 x g for 30 min
- Pipette 5 ml of the supernatant into 10 ml graduated test tubes, fill them up with 2 %HNO_3

Citric acid, oxalic acid or maleic acid extract according to Wei et al. (2010)

According to Wei et al. 2010, extraction with citric acid was most effective for tropical and subtropical soils.

Approach of the solutions

1 mM Citric acid solution

- molar mass: 192,124 g mol^{-1}
- Weigh-in weight for 1 liter of 1mM citric acid: 192 mg

1 mM Oxalic acid solution

- molar mass: 90,04 g·mol^{-1} (anhydrous) 126,07 g·mol^{-1} (dihydrate)
- Weigh-in weight for 1 liter 1 mM oxalic acid: 90 mg (anhydrous) or 126 mg (dihydrate)

1 mM Maleic acid solution

- molar mass: 116,072 g mol^{-1}
- Weigh-in weight for 1 liter of 1 mM maleic acid: 116 mg

Procedure for extraction with LMWOAs

- Weigh 4.00 g lutro soil <2mm into 50 ml centrifuge tubes
- Add 40 ml citric acid or oxalic acid or maleic acid solution plus 2 drops of chloroform as a biocide (work under the fume cupboard)
- Shake over head for 24 hours
- Centrifuge at 3000 x g for 30 min
- Filter through P-free filters

Determination of element concentrations

- Measurement of the elements of interest using ICP-OES
- If necessary, measure P_i using molybdenum blue

4.5.2.5 Ion-sink methods - anion exchange resin

Ion sink methods include extracts with anion exchange resin and Fe-coated strips or filter papers (Myers et al. 2005). These methods simulate the conditions in the rhizosphere, i. e. the plant roots (or in this case the exchangers) absorb the P present in the soil solution. Following the solubility equilibrium, P is then desorbed from the soil particles and the dissolved P is taken up again by the roots or the exchanger. These methods have the advantage that, in contrast to chemical extraction agents, they can be used independently of the soil properties, do not chemically alter the soil and, in the case of anion exchange resin, can be reused several times (50 to 500 times) (Saggar et al. 1990, Schoenau and Huang 1991).

Notes on the extracts with anion exchange resin

Extractions with anion exchange resins can be carried out either as so-called (1) batch extracts (with resin membranes (anion exchange membranes = AEM) or resin beads), with the (2) miscible displacement technique (Abdu 2006) or (3) with the placement of the exchange resins directly into the undisturbed soil (e.g. Quian and Schoenau 2002). For batch extracts, soil and ultrapure water are mixed in a wide ratio and the exchanger resin is added for shaking. The exchanger resins can be pretreated with either HCl or bicarbonate (Abdu 2006, Sibbesen 1978, Quian and Schoenau 2002). Phosphates are then exchanged for anions of the membrane. Sibbesen (1978) proved that HCO_3^- resins are more suitable than Cl^- resins, as the plants accumulate HCO_3^- in the rhizosphere, which leads to an increase in the pH value in the rhizosphere in acidic to neutral soils and to a reduction in pH in alkaline soils. If Cl^- resin is used, the Cl^- can accumulate in the solution and inhibit the exchange reaction (Myers et al. 2005). This method can therefore not be used in saline soils.

Based on sequential P fractionation, the procedure for P extraction with anion exchange resin as a membrane in the HCO_3^- form is described below. As there is no other experience with these extraction methods in the working groups, reference is made to the literature.

Protocol for extraction with strips of anion exchange resin

<u>Preparation of the resin strips</u> (see also Myers et al. 2005, Saggar et al. 1990)

- Anion exchange membrane BDH #55164 2S (see also sequential P fractionation), cut into 12 strips of 6 x 2 cm each
- Prepare 2 L 0.5 M NaHCO3 and fill into two 1-liter beakers
- Place the resin strips in the first beaker for 1 h, transfer to the second beaker with tweezers and incubate again 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 hours before use, after preparation with HCO_3^-)

<u>Sample preparation:</u>

- Drying soil samples (see chapters 2.4 and 3.1)
- Sieve soil samples <2 mm and use this <2 mm fraction (fine soil)
- Determine total element concentrations in a subsample (e.g. using aqua regia extract, see chapter 4.1.2)

<u>Procedure:</u>

- Add 0.5 g soil, 30 ml ultrapure water (UW) and a strip of anion exchange resin, shake upside down for 18 hours
- Remove resin strips with tweezers, rinse adhering soil particles with UW (spray bottle) back into the centrifuge tube
- Wash resin strips with max. 45 ml 1 M HCl via funnel with P-free filter in 50 ml volumetric flask
- Place the resin strips back into beakers with UW, later carry out another pre-treatment for bicarbonate form before storing in the refrigerator again
- Fill the graduated flask with 1 M HCl to 50 ml
- Fill aliquots (10 to 20 ml) for P determination, determine resin P_t on the ICP, if necessary, P_i using MB (see chapter 4.5.1 Sequential P fractionation)

<u>Prepare 0.5 M $NaHCO_3$</u>

Molar mass $NaHCO_3$: 84.007g mol^{-1}
Add 42 g $NaHCO_3$ to a 1-liter volumetric flask and fill up with UW

As this is a fraction of sequential P fractionation, extraction with anion exchange resin can be carried out in the Soil Science and Agronomy working groups at the Faculty of Agricultural and Environmental Sciences at the University of Rostock.

Myers et al. (1999) and (2005) deviate from the above approach as follows:
After soil extraction and rinsing of the soil particles with ultrapure water:

- Place the resin membranes in a 125 ml wide-neck bottle and add 50 ml of 0.5 M HCl to remove P from the resin strips
- The sealed bottles are shaken on a horizontal shaker (horizontal and end-to-end on a reciprocating shaker) at 125 to 130 rpm for 90 min
- Remove the resin strips and determine the P concentration in the HCl solution

Schoenau and Huang (1991) deviate as follows:
Once the soil has been washed off the resin membranes, they are placed in 50 ml centrifuge tubes and 30 ml 0.5 M HCl is added.
The resin membranes are shaken for 1 h on the horizontal shaker.

Pretreatment Anion exchange resin, if the Cl^- form is to be used, according to Myers et al. (2005):

- Store resin membranes in UW for 24 h before use
- Prepare 250 ml of 1.0 M KCl, pour into a beaker and soak the resin membranes for 30 min
- Repeat the process in a second beaker,
- then rinse the resin strips with UW before storing them in UW

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