Deep Ultraviolet Raman Microspectroscopy - Novel Technique to Characterize Inorganic Phosphorus in Soil

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Abstract:

Backgrounds and Aims

Phosphorus is of fundamental importance for virtually all living organisms including humans, and is not substitutable. Phosphorus, among others is an essential plant nutrient that is required for crop production. A sustainable phosphorus management would mitigate the negative impacts of extensively using phosphate rock as the single source for mineral fertilizer production. Its availability for plants depends highly on the chemical state of phosphorus in soils, which is difficult to analyze due to the limits of state of the art wet chemical and instrumental approaches.

Methods

In this paper, deep ultraviolet Raman microspectroscopy was successfully investigated as a new approach to analyze the chemical state of mineral phases directly in soil.

Results

Deep ultraviolet Raman microspectroscopy has the advantage that no fluorescence interference occurs which is principally caused by organic matter in soils. Therefore, the chemical state of inorganic minerals can be analyzed. Furthermore, deep ultraviolet Raman microspectroscopy as a high-resolution imaging technique also provides the opportunity to analyze local interactions between soil compounds.

Conclusions

The experimental technique presented here shows potential to become the analytical key to improve the understanding of transformation mechanisms of inorganic mineral phases in soils.
Introduction:

Phosphorus (P) has fundamental importance for all living organisms. It is necessary for the metabolic process (ADP/ATP), and an integral part of the DNA molecule and the cell membrane. For that reason P in the form of phosphates is applied as fertilizer in the agricultural industry. Currently, organic and phosphate rock based fertilizers are used.

In order to improve the phosphorus uptake of plants by fertilization, substantial knowledge of the chemical state of soil P is necessary. Determination of the chemical state of soil mineral phases and especially soil P is a difficult field of research due to the limits of wet chemical and instrumental analytical approaches. Phosphorus in soils occurs primarily in the form of orthophosphates, typically with low total mass fractions between 0.02 and 0.5 wt %.

In the past, soil P was analyzed by many researchers (Kizemski et al. 2011, Doolette and Smernik 2011, Kruse et al. 2015) with different analytical methods (sequential extraction, X-ray absorption near edge structure (XANES), infrared, Raman and $^{31}$P nuclear magnetic resonance (NMR) spectroscopy). Each method has advantages and disadvantages and can be used within certain limits for the detection of phosphate phases.

Up to now vibrational spectroscopic techniques were not often used for soil P characterization. Infrared spectroscopy was successfully applied by Beaton et al. (1963) to determine fertilizer-soil reactions. However, this technique was only applicable for high fertilizer/soil ratios, which is not in agreement with the realistic amounts of P-fertilizers added to the soils. Furthermore, Raman spectroscopy studies of soils were done with lasers which excite in the visible (VIS) spectrum. Tomic et al. (2010) investigated Raman (VIS) microspectroscopy to analyze silicates, carbonates and sulfates in soil. Lanfranco et al. (2003) presented a Raman (VIS) spectrum of a phosphate in a soil-phosphate-mixture but had considerable difficulties with fluorescence interference.

Previously, we applied synchrotron infrared and Raman (VIS) microspectroscopy with the intention of determining P-phases in soils, in P-fertilizers and to follow respective P-
fertilizer-soil reactions (Vogel et al. 2013a and 2013b). Phosphate phases in P-fertilizers were successfully determined with both methods but it was not possible to analyze phosphate phases in the soil with both techniques for various reasons. Raman (VIS) microspectroscopy has the advantage over infrared microspectroscopy in that a high lateral resolution is applicable (approx. 300 nm with a 514 nm Raman excitation laser). However, due to fluorescence interference observed in Raman (VIS) microspectroscopy, we were not able to analyze soil mineral phases by this method. In contrast, with synchrotron infrared microspectroscopy we were able to analyze soil mineral phases such as silicates, carbonates and sulfates, but did not detect phosphate phases (Vogel et al 2013b). While synchrotron infrared microspectroscopy is diffraction limited, the use of infrared radiation places limits the lateral resolution to approx. 5-10 μm). For this reason P-phases in soils, which are often present in the form of small particles, were not detectable.

Deep ultraviolet (DUV) Raman microspectroscopy has the advantage over:

i) excitation in the VIS, in that no fluorescence interference occurs when the excitation is at wavelengths below about 250 nm (Asher and Johnson 1984, Nelson and Sperry 1991, Asher 1993, Tarcea et al. 2007) and;

ii) infrared microspectroscopy due to the high lateral resolution that can be applied.

Theoretically, the lateral resolution of Raman (DUV) microspectroscopy is even higher than for excitation in the VIS because the lateral resolution is proportional to the excitation wavelength (Zoubir 2012). Equation (1) shows the relationship between lateral resolution (d), the wavelength (λ) and the numeric aperture (NA) of the objective lens:

$$d = \frac{0.61\lambda}{NA}$$

(Equation 1)
Unfortunately, objective lenses with a high NA~1 and a simultaneous transparence for the VIS to focus on the sample are currently not available for the DUV. Thus, it is technically limited to the resolution of Raman (VIS) microspectroscopy. However, the resolution is sufficient to analyze soil P and could even be improved by the development of appropriate objective lenses.

**Materials and Methods**

Three types of soil samples were used for the investigation.

1) An alluvial soil (pH(H$_2$O) = 7.4; pH(CaCl$_2$) = 7.2; total P = 3648 mg/kg) from an area periodically exposed to river flooding was chosen as it is a well characterised reference material provided by Bundesanstalt für Materialforschung und –prüfung (BAM-U110). The alluvial soil was collected from a depth between 0.2 and 0.8 m. The raw material was dried in a convection oven at 30 °C to constant mass and then passed over a vibrating 2 mm screen discarding the fraction > 2 mm. The material passing the screen was ground in a ball mill completely to particle sizes below 63 μm.

2) The Nowra soil (pH(H$_2$O) = 8.1; total P = 15111 mg/kg; organic P = 2552 mg/kg; texture: clay loam (Doolette et al. 2011)) is from New South Wales, Australia.

3) The Togari control soil (pH(H$_2$O) = 5.5; total P = 465 mg/kg; organic P = 220 mg/kg; texture: loamy sand (Doolette et al. 2011)) is from South Australia, Australia. Soils 2) and 3) were sampled, after removal of the litter layer, to a depth of 0.1 m and air-dried.

The soil samples were pressed in form of a pellet to get a smooth surface for the spectroscopic investigations. Raman images and spectra were collected with a Horiba LabRam HR Evolution micro-Raman system (80× objective, NA = 0.55, Mitutoyo) with an Ar ion laser (244 nm, approx. 120 mW). The system was operated by LabSpec 6 software. The samples were mapped with a 1-3 μm lateral step size. The spectral resolution obtained
was 8 cm$^{-1}$. The duration time for Raman mapping of the alluvial soil (Fig. 4) was approx. 45 min. (XX $\times$ YY measurement points) Raman chemical images were constructed by color coding the areas of major bands in the spectra (blue: low concentration; green: medium concentration; red: high concentration). From the point with the highest intensity (red dot or area) of the Raman images, a single spectrum was extracted and displayed below the chemical image (middle).

The following reference compounds were used for the analysis: CaCO$_3$, MgCO$_3$ (both Fluka, Steinheim, Germany), CaSO$_4$·2H$_2$O (Acros, Geel, Belgium), SiO$_2$ (quartz), (NH$_4$)$_2$HPO$_4$ (both Merck, Darmstadt, Germany), NH$_4$H$_2$PO$_4$ (J.T.Baker, Deventer, the Netherlands), Na$_2$HPO$_4$·2H$_2$O (AppliChem GmbH, Darmstadt, Germany), K$_2$HPO$_4$, KH$_2$PO$_4$, Al$_2$O$_3$ (all Carl Roth GmbH, Karlsruhe, Germany), Na$_4$P$_2$O$_7$·10H$_2$O (AnalR, England), Ca$_5$(PO$_4$)$_3$OH, β-Ca$_3$(PO$_4$)$_2$, CaHPO$_4$·2H$_2$O, AlPO$_4$ (berlinite), FePO$_4$·H$_2$O, Mg$_3$(PO$_4$)$_2$·8H$_2$O, (all Sigma-Aldrich, Steinheim, Germany), NH$_4$MgPO$_4$·2H$_2$O, MgHPO$_4$·2H$_2$O, Ca(H$_2$PO$_4$)$_2$·2H$_2$O (all Alfa Aesar, Karlsruhe, Germany). AlPO$_4$ (christobalite) was prepared from AlPO$_4$ (Sigma-Aldrich, Steinheim, Germany) in platinum crucibles by thermal treatment (24 h) at 1300°C in a muffle furnace (Nabertherm LH 15/14, Lillenthal, Germany). Chlorapatite (Ca$_5$(PO$_4$)$_3$Cl) and CaNaPO$_4$ were thermally prepared from CaHPO$_4$·2H$_2$O, CaCl$_2$·6H$_2$O, CaNO$_3$·4H$_2$O and Na$_2$CO$_3$, respectively, in a platinum crucible at 1000°C (all chemicals: Sigma-Aldrich, Steinheim, Germany). Calcium pyrophosphates were precipitated from aqueous solutions of Na$_4$P$_2$O$_7$·10H$_2$O (AnalR, England) with CaCl$_2$·6H$_2$O, (Sigma-Aldrich, Australia). After precipitation the solid material was filtered and dried at 200°C in a drying oven.

**Results and Discussion:**
Figures 1 to 3 show Raman (DUV) spectra of different inorganic phosphates, pyrophosphates, sulfates, carbonates and oxides. Most of the inorganic phosphates have the strongest Raman band between 900-1000 cm\(^{-1}\). Only iron and aluminum phosphates have them in the range 1000-1100 cm\(^{-1}\). In the latter range, Raman bands of pyrophosphates are also detectable. In addition to this strong Raman band all phosphates show also weaker Raman bands which are useful to explicitly identify the species and distinguish between those species with similar strongest bands.

Figure 4 (top) shows the Raman (DUV) chemical images of the same alluvial soil that we already studied using synchrotron infrared and Raman (VIS) microspectroscopy (Vogel et al. 2013b). Using Raman (DUV) mapping of the soil, calcium carbonate (CaCO\(_3\)), calcium sulfate (CaSO\(_4\)) and quartz (SiO\(_2\)) were detected in accordance with previous synchrotron infrared investigations. In addition, also hydroxylapatite was detected by Raman (DUV) microspectroscopy which was not detectable by infrared and Raman (VIS). The extracted Raman spectra from the red areas of the chemical images (middle) of each compound fit very well with the Raman (DUV) spectra of pure reference compounds (bottom). Hydroxylapatite (Ca\(_5\)(PO\(_4\))\(_3\)OH) was detected as the single inorganic mineral phosphate phase present in the soil. The soil spectrum and the reference spectrum of hydroxylapatite are shown in Fig. 4. The soil Raman bands are slightly shifted towards the direction of the main band (971 cm\(^{-1}\)) of \(\beta\)-tricalcium phosphate (\(\beta\)-Ca\(_3\)(PO\(_4\))\(_2\)). However, the second main band (951 cm\(^{-1}\), see also Fig. 1) was not present in the soil spectrum. One probable explanation for the shift could be a tricalcium phosphate with an apatite structure or a calcium deficient apatite.

Furthermore, Raman bands of decomposed organic material were detected (1586 and 1380 cm\(^{-1}\); Figure 4 right). Raman bands of quartz at 468 cm\(^{-1}\) and CaCO\(_3\) at 1088, 1435 and 1750 cm\(^{-1}\) are also visible in the extracted spectrum. Due to the use of the UV-laser most of the organic material decomposed during the Raman measurements. The strong Raman band at around 1600 cm\(^{-1}\) belongs to carbon black (Nakamizo et al. 1974).
Figure 5 shows the Raman (DUV) chemical images (top) of the nowra soil. In addition to quartz and calcium carbonate also apatite could be detected as P phase. The apatite is not a pure hydroxylapatite. Also here the Raman band is slightly shifted which most likely comes from defects in the apatite structure. Doolette et al. (2011) found by $^{31}$P NMR spectroscopy, that approx. 90% of the total P in this soil is orthophosphate. Because this soil is from dairy pasture the detection of hydroxylapatite as orthophosphate is comprehensible. Furthermore, strong Raman signals of carbon black were detected (at approx. 1600 cm$^{-1}$). Obviously, this soil also contains a large amount of organic material.

Figure 6 shows the Raman (DUV) chemical images (top) of the Togari control soil. In this soil only the minerals quartz and calcium carbonate were detected. Phosphate phases were not detected in this soil. Probably the P mass fraction of this soil is too low for detection (465 mg/kg total P (245 mg/kg inorganic P) instead of 3648 mg/kg total P for the alluvial soil and 15111 mg/kg total P for the nowra soil). Thus, 245 mg/kg inorganic P is below the limit for the determination of the chemical state of P in soils by Raman (DUV) microspectroscopy. In addition, some small Raman signals of decomposed organic material (band at approx. 1600 cm$^{-1}$) were detected whereby this soil contains only small amounts of organic material.

As previously reported, it was possible to detect silicates, sulfates and carbonates in soils by Raman (VIS) and synchrotron infrared microspectroscopy (Tomic et al. 2010, Vogel et al. 2013b). Raman (DUV) microspectroscopy even makes it possible to also detect inorganic phosphate phases in soils. Due to degradation of organic compounds by the UV laser the chemical state of organic phosphates, which can be also a large part of soil P (Turner et al. 2003), cannot be detected by this method.

Raman (DUV) microspectroscopy as an imaging technique also provides the opportunity to analyze local interactions between soil compounds and could be used to explain transformation mechanisms in soils that are not understood to date. However, the localization of the small P compounds, especially in soils with low P content, can be a
problem. A combination with micro-XRF (commercial table-top instruments with a lateral resolution of 10 µm² are available) for a pre-localization of P spots in the soil might solve the problem. Thus, the phosphate phases could be localized in the soil sample with µ-XRF first and on the localized P spots Raman (DUV) microspectroscopy measurements can be applied afterwards.

Quantification of the different phosphates as it is done with P³¹ NMR spectroscopy is very challenging with the current state of DUV Raman spectroscopy. Even if large areas of the soil will be mapped, which is very time consuming, quantification would be very difficult. Advances in the optics and sampling configuration may help overcome, if not reduce these limitations, for example by improving the NA or the sensitivity of the detectors at these wavelengths.

**Conclusions:**

Raman (DUV) microspectroscopy is an easy method (almost no sample preparation required) to analyze mineral phases in soils. This technique has two advantages over other vibrational microspectroscopic techniques. Firstly, a high lateral resolution of < 1 µm is applicable, which enables to detect small particles. Secondly, fluorescence interference due to organic matter does not occur at excitation in the deep UV region. Furthermore, Raman (DUV) microspectroscopy as a high-resolution imaging technique also provides the opportunity to analyze local interactions between soil compounds. However, this fact makes the quantification of mineral phases in soils a challenge that is yet to be overcome.

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Figures Caption:

**Fig. 1**: Raman (DUV) spectra of ammonium and calcium phosphates.

**Fig. 2**: Raman (DUV) spectra of aluminum, iron, magnesium, sodium and potassium phosphates.

**Fig. 3**: Raman (DUV) spectra of pyrophosphates, sulfates, carbonates and oxides.

**Fig. 4**: Raman (DUV) chemical images (top; 15 µm × 15 µm; blue: low concentration; green: medium concentration; red: high concentration) and extracted Raman (DUV) spectra from the red areas of different compounds of an alluvial soil (middle) and related Raman (DUV) spectra of reference compounds (bottom).

**Fig. 5**: Raman (DUV) chemical images (top; 21 µm × 30 µm; blue: low concentration; green: medium concentration; red: high concentration) and extracted Raman (DUV) spectra from the red areas of different compounds of Nowra soil (middle) and related Raman (DUV) spectra of reference compounds (bottom).

**Fig. 6**: Raman (DUV) chemical images (top; 21 µm × 30 µm; blue: low concentration; green: medium concentration; red: high concentration) and extracted Raman (DUV) spectra from the red areas of different compounds of Togari control soil (middle) and related Raman (DUV) spectra of reference compounds (bottom).
Figure 1:
Figure 2:

![Raman Spectra of Different Phosphates](image)

- **AIPO₄ (Berlineite)**
- **AIPO₄ (Christobalite)**
- **FePO₄·H₂O**
- **Mg₃(PO₄)₂·8H₂O**
- **MgHPO₄·3H₂O**
- **Na₂HPO₄·2H₂O**
- **K₂HPO₄**
- **KH₂PO₄**

Raman Intensity vs. Raman Shift (cm⁻¹)
Figure 4:

![Image of chemical distributions and Raman spectra for CaCO$_3$, CaSO$_4$, SiO$_2$, Apatite, and Carbon.]

Figure 5:

![Image of chemical distributions and Raman spectra for Quartz, CaCO$_3$, Apatite, and Carbon.]
Figure 6:

- **Quartz** (515-397 cm⁻¹)
- **CaCO₃** (1108-1070 cm⁻¹)
- **Carbon** (1672-1522 cm⁻¹)