Selective dissolution of soil iron for mineralogical and microbiological evaluation

These reagents extract different fractions of iron as indicated, but no extractant is perfect, and depending on the soil there is some overlap, i.e. a small amount of another iron fraction may be extracted at the same time.

1. Pyrophosphate extraction – removes iron present in organic-metal complexes (complexed with soil organic matter.
2. Dithionite extraction – reduces and extracts Fe(III) in secondary minerals such as goethite, hematite, and ferrihydrite; also removes aluminum and silicon from some short-range order aluminosilicates
3. Oxalate extraction – extracts iron from ferrihydrite and other short-range order oxyhydroxides. Includes all amorphous Fe(III) potentially available for microbial reduction, but is not necessarily a good indicator of the true amount of Fe(III) available for microbial reduction, since poorly reducible forms of Fe(III) are also extracted. Also dissolves short-range order aluminosilicates and extracts some iron and aluminum from organic complexes.
4. Dilute acid – a measure of the Fe(II) produced during reduction of Fe(III)
5. Hydroxylamine – selective extractant for microbially reducible Fe(III)

Procedure:

1. Pyrophosphate: Extract 1 g soil for 16 hours with 100 ml 0.1 M tetrasodium pyrophosphate. Centrifuge at 15600 x G for 30 minutes or until fine colloids are removed.
2. Dithionite: to 0.5 g soil add 50 ml 0.3 M sodium citrate and 0.5 g sodium dithionite (sodium hydrosulfite). Shake overnight.
3. Oxalate: to 0.5 g soil add 50 ml ammonium oxalate solution buffered at pH 3.0 (per liter: 16.2 g ammonium oxalate monohydrate and 10.8 g oxalic acid monohydrate). Shake for 4 hours in the dark (light affects dissolution of iron in the presence of oxalate).
4. Dilute acid: usually 1 g soil plus 50 ml 0.5 M HCl. Lovley and Phillips 1986. Appl Env Microb 51:683
5. Hydroxylamine: to 1 g soil add 50 ml 0.25 M hydroxylamine hydrochloride in 0.25 M HCl (per liter: 17.4 g hydroxylamine hydrochloride and 20.8 ml concentrated HCl). Shake for one hour. Lovley and Phillips 1987. Appl Env Microb 53:1536.

Determination of iron in oxalate extracts

Prepare the reagent (from Dominik and Kaupenjohann 2000. Talanta 51:701): dissolve 3 g ammonium acetate and 0.1 g ferrozine in 100 ml water. Just before use, add 0.2 g ascorbic acid. Into cuvets, pipet 0.2 ml sample or standard, then add 0.8 ml reagent. Cap, mix, and wait until color is fully developed.

This is usually within an hour, but it is good to confirm. The color is stable until the next day. Read absorbance at 565 nm. The working range is about 0.2 to 10 mg/L.

Determination of iron in pyrophosphate and dithionite extracts

Pipet 0.2 ml sample or standard into cuvettes. For 0.1 M pyrophosphate solutions, add 0.04 ml 6 M HCl to neutralize. If the samples have been diluted with water, add proportionally less HCl. Add 0.04 ml water to the standards if they are not made in a pyrophosphate matrix; if they are made in pyrophosphate, add 0.04 ml HCl. Then add 0.8 ml reagent (same as oxalate analysis), mix, and read absorbance at 565 nm once color is fully developed. Do not wait until the next day – some iron may begin to precipitate. If the samples are heavily colored, prepare a corresponding correction sample for each sample by using a reagent without ferrozine; subtract the absorbance from this sample (the background absorbance) from the absorbance of the samples with iron color developed.