**Determination of phosphate in soil extracts**

**by malachite green (MG) colorimetric procedure**

(d'Angelo et al., 2001, slightly modified)

The color reaction based on complexation of malachite green with phosphomolybdate under acidic conditions is provided by mixing Reagents 1 and 2.

**Reagent 1** is 14.2 mmol L\(^{-1}\) ammonium molybdate tetrahydrate in 3.1 M H\(_2\)SO\(_4\).

**Reagent 2** is 3.5 g L\(^{-1}\) aqueous polyvinyl alcohol (PVA) reagent (molecular weight between 89 000 and 98 000) (Sigma Aldrich Co), prepared by stirring with deionized distilled water at 80°C. After cooling to room temperature, MG carbinol hydrochloride (Sigma Aldrich Co) was added at 0.35 g L\(^{-1}\). Both reagents are stable at room temperature.

150 µl of acid or neutralized NaHCO\(_3\) extract is mixed with 30 µl **Reagent 1** in disposable 96-well polystyrene microplates for 10 min on an orbital shaker at low rate (< 90 rev min\(^{-1}\)). Then, 30 µl of **Reagent 2** is added and the plate is shaken more rapidly for an additional 20 min. After the shaking, microplates are exposed to 40°C for 30 – 40 min in a dryer (thermostat). In 1-1.5 h the microplates can be read on the plate reader **Victor** at 630 nm using “Peroxidase” protocol. For better results, the microplates can be left for 10-12 hours (e.g. overnight) before reading. It would decrease variability of replicates.

Standards should be prepared in triplicate and with the same extractant as used for phosphate extraction (e.g. H\(_2\)SO\(_4\), HCl, water etc). The standards set can vary
depending on the P concentrations range, typically – 0, (0.005); 0.1; 0.2; 0.4; 0.6; 0.8; 1.0; (1.25); 1.5; 2.0; (3.0) μg P ml⁻¹. P concentrations range with linearity of calibration depends on extractant; typically, the concentrations > 2 μg P ml⁻¹ should be omitted. P concentrations are calculated according to the linear equation y = Bx+A, where y is P concentration, μg P ml⁻¹, x is optical density at 630 nm; B is usually varied from 1.6 to 2.0.

**Note:** malachite-reactive P (MRP) does not represent all the P pools in P extract. However, it comprises more than 95% of extractable P (Cheesman et al., 2010), and therefore can be used as an index of “total” phosphorus in soil extracts.

**References**
