

## Determination of Orthophosphate in Water and Soil Extracts-Malachite Green Method

### **Reagents:**

**Reagent I:** Add 106 ml concentrated H<sub>2</sub>SO<sub>4</sub> to 500 ml DI water in a 1 liter volumetric flask. Dissolve 17.55 g ammonium heptamolybdate [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>]-4H<sub>2</sub>O] in the acid and bring up to volume.

**Reagent II:** Heat approximately 800 ml DI water to 80° C and add 3.5 g polyvinyl alcohol; stir until completely dissolved. Add 0.35 g malachite green and stir until dissolved. Cool to room temperature and dilute to 1 liter with DI water. The reagent is stable for 1 yr.

**Standards:** known standards in fridge.

### **Procedure:**

1. Pipette replicate 200 ul aliquots of your standards into the appropriate wells of a microplate. The Tech Info sheet of the attached excel file, Data for P Microplate Determination, outlines the protocol for standard curves.
2. Pipette replicate 200 ul aliquots of your samples into the appropriate wells of a microplate.
3. Pour about 10 ml of Reagent I and II into separate reagent basins.
4. Add 40 ul of Reagent I to all the wells using the manual 8-channel pipettor. Allow to react for 10 min on the microplate shaker\* at setting ~ 300.
5. Add 40 ul of Reagent II to all the wells using the manual 8-channel pipettor. Mix thoroughly by shaking more rapidly (setting ~ 600) for 19 min.
6. Determine absorbance in the microplate reader at 630 nm. The protocol for this method includes a 1 min shaking step just before the plates are read.

\*Shaker must be balanced; use an empty plate opposite your plate.

### **References:**

D'Angelo E, Crutchfield J, Vandiviere M. 2001. J. Environ. Quality 30:2206-2209

Linge KL, Oldam CE. 2001. Anal.Chim. Acta 450:247-252