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# Module 1: Theoretical background

## Urine project at Wetsus

One of the research projects at Wetsus is the recovery of nutrients and energy from urine. The urine that we carelessly flush down the toilet contains many useful and valuable nutrients such as phosphate and nitrogen compounds. Moreover, it takes a lot of energy to remove all kinds of substances at a wastewater treatment plant: 70% of the total energy of this system.

## Phosphorus

Phosphorus is an irreplaceable and indispensable element for plants and animal life. Phosphorus is a part of the DNA and it plays a role in the energy supply in organisms.

Phosphorus is mined in mines. Then, the ore is processed into fertilizer. A part of the phosphorous compounds end up directly after scattering, by rinsing, in the environment. Another part ends up via animal faeces and via consumption and excretion by the humans in the environment.

Recovering phosphorus from mines is finite. The demand for phosphorus will increase by the strong growth of the world population and the growing of energy crops for the production of biofuel. The stock of phosphorus will run out in a matter of about 100 years.

Phosphorus is difficult to recover because it is being used and spread across the planet. Since phosphorus is an essential element for life, we have to fertilize more efficiently and find a method for recovery and recycling to maintain life on earth.

Phosphate is now partly recovered in the Netherlands by wastewater treatment plants (Figure 1).

## Nitrogen

Air consists of 80% nitrogen. Nitrogen is an indispensable element in the formation of proteins. People discharge nitrogen through their urine. Nitrogen is present in urine in the form of urea.

In contrast to phosphorus, there is no infinite supply of nitrogen. The problem with nitrogen is the amount of energy that is required to recycle it into a form so that it can be re-used by organisms.

In nature nitrogen is converted in different forms in the so-called nitrogen cycle (Figure 2).

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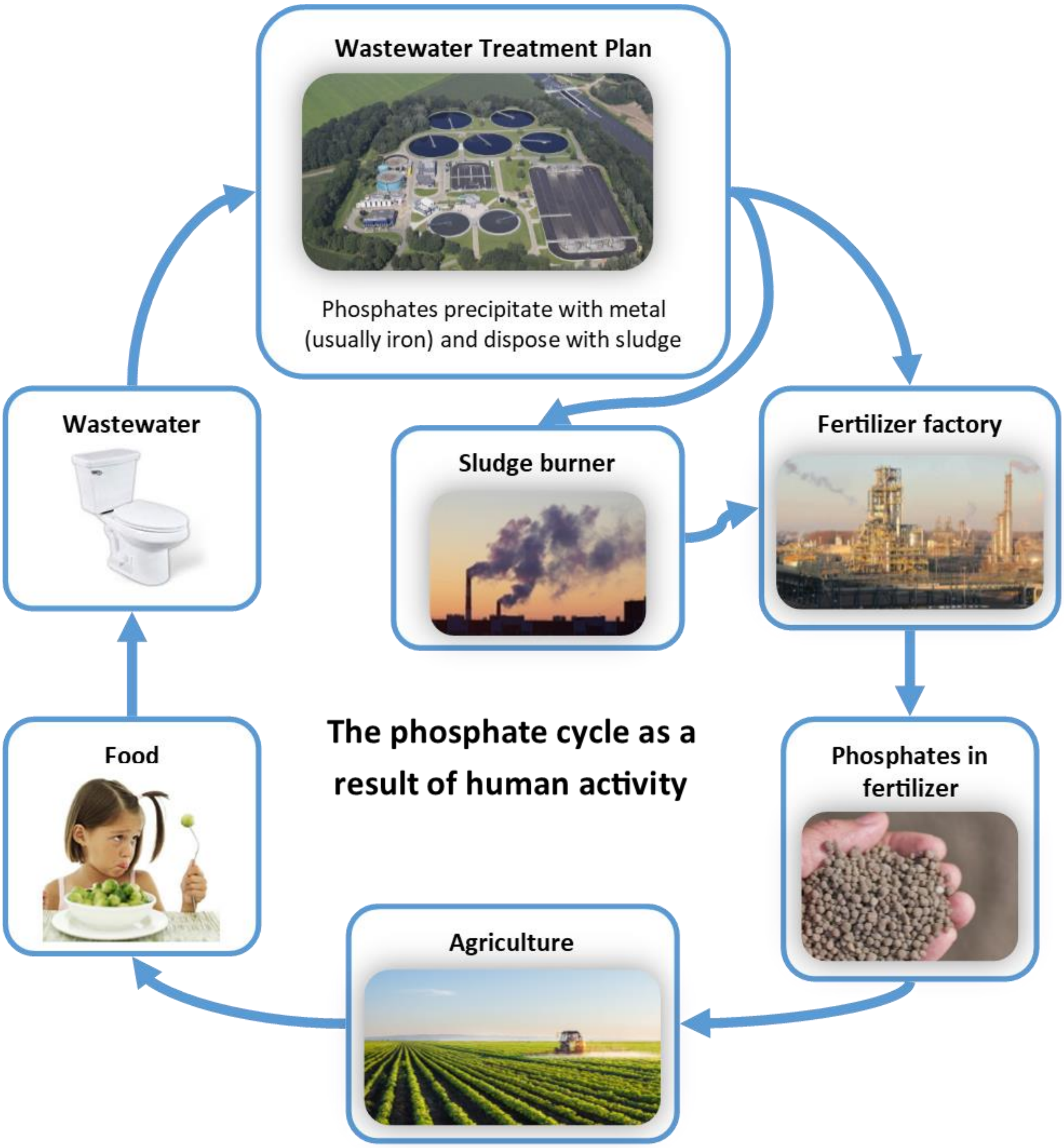


*Figure 1, The phosphate cycle as a result of human activity.*

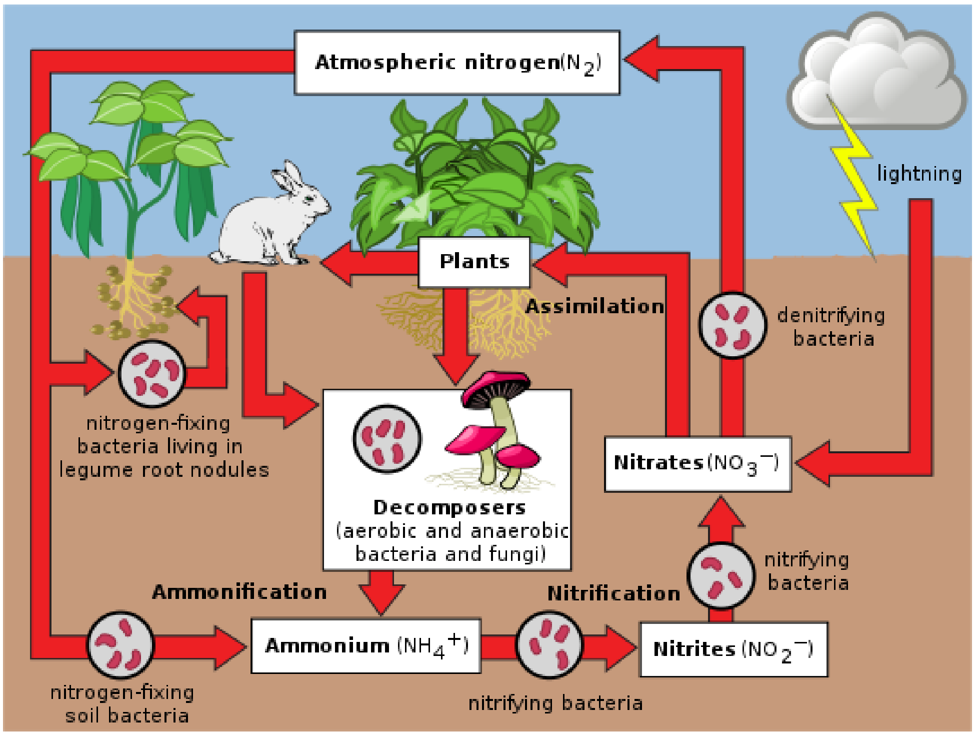


*Figure 2, The nitrogen cycle.*

In the nitrogen cycle three processes can be distinguished:

1. Ammonification: the conversion of organic nitrogen to ammonium
2. Nitrification: the conversion of nitrogen to nitrogen in nitrate form
3. Denitrification: the conversion of nitrogen in nitrate form of nitrogen

In the Netherlands nitrogen in the form of urea ends up in the form of human urine in a wastewater treatment plant. In the wastewater treatment process urea is converted into nitrogen in a number of steps (Figure 3). The produced nitrogen can be discharged without problems in the environment, where it is again part of the nitrogen cycle.

Urea is decomposed by bacteria which produces ammonia and water. → Under aerobic conditions, bacteria convert ammonium into successively, nitrite and nitrate. → Under anaerobic conditions, bacteria convert nitrate into nitrogen.

*Figure 3, The nitrogen at a wastewater treatment process.*

Nitrogen is an essential element in the production of fertilizers. Nitrogen from the air is converted to nitrate, a component of fertilizer. In studying the schedule of the path that nitrogen in human life in the Netherlands, it is noticeable that in the wastewater treatment urea is converted through nitrate into nitrogen and that the fertilizer industry converts it back to nitrogen in the nitrate form (Figure 4.).

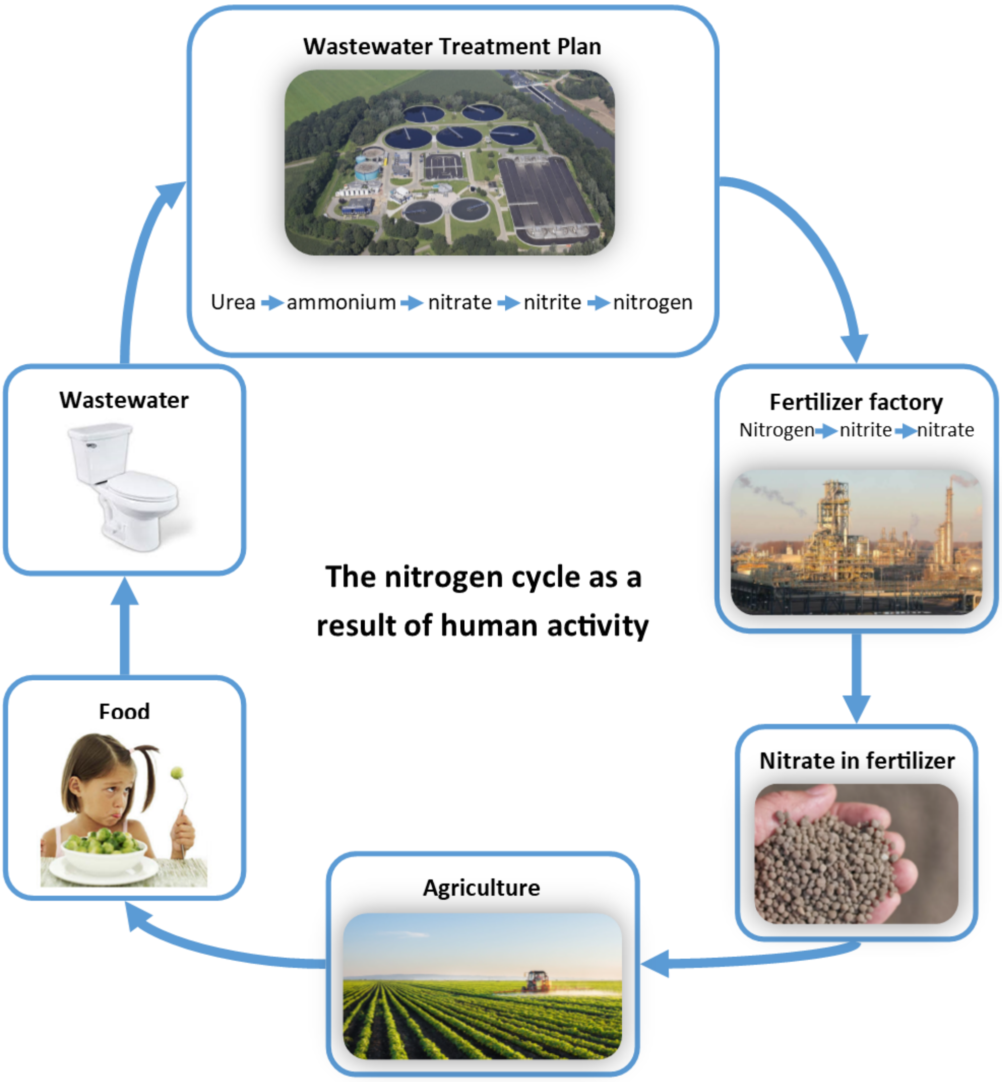


*Figure 4, The Nitrogen cycle as a result of human activity.*

## Struvite

Struvite ($(NH_4) Mg PO_4 . 6H_2O$) is a mineral composed from ammonium, phosphate and magnesium, and excellent for use as a fertilizer. Struvite can be made from urine in a simple manner. The advantages of making a fertilizer directly from urine are:

- Save energy, while phosphate and nitrogen don't need to be removed from urine
- Keep phosphorus in the cycle
- To keep a valuable product (fertilizer)

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In this module, students can produce struvite from morning urine. An alternative for morning urine is a synthetic form of urine (see Module 2 – Struvite Synthesis).

At first the enzyme urease is added to the urine. Urease causes the conversion of urea into ammonia.

$$(NH_2)_2CO\ (aq) + H_2O\ (l) \rightarrow CO_2\ (g) + 2NH_3\ (g)$$

The ammonia reacts with water to form ammonium:

$$NH_3(g) + H_2O(l) \rightarrow NH_4^+\ (aq) + OH^-\ (aq)$$

An increase of pH is the result.

Subsequently, magnesium chloride is added, and in this step struvite occurs:

$$NH_4^+\ (aq) + Mg^{2+}\ (aq) + PO_4^{3-}\ (aq) + 6\ H_2O\ (l) \rightarrow NH_4MgPO_4 \,.\, 6\ H_2O\ (s)$$

From this resulting struvite the phosphate concentration can then be determined with colorimetry. In addition, it is possible to test whether the created struvite has a positive effect on the growth of plants.

## Questions

1. **Why is nitrogen and phosphate recovery from wastewater important?**

2. **What step in the wastewater treatment process is not logical when you look at the nitrogen and phosphorus cycle in Figure 1 and 4?**

3. **Can you think of several advantages when making struvite directly from urine?**

4. **Where would you make struvite from urine? Think big: how could this way of resource recovery be implemented in the Netherlands and abroad?**


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# Module 2: Struvite Synthesis

Struvite is a mineral composed of ammonium, phosphate and magnesium. It turns out to be a good fertilizer. Struvite can be easily made from urine. In this module, you are going to synthesize your own struvite.

## Necessities

- 200 ml morning urine or synthetic urine (see table 1.)
- cup with a lid (for urine)
- 2 cups (250 mL)
- stirrer
- spoon
- pH paper or pH meter
- soybeans
- bean grinder
- parafilm
- magnesium chloride
- vacuum flask with funnel
- filter (round)
- water pump
- refrigerator

| compound | formula | molmass g/mol | concentration g/L |
|---|---|---|---|
| Urea | $CH_4N_2O$ | 60,062 | **20** |
| Sodium hydrogen carbonate | $NaHCO_3$ | 84,008 | **2,1** |
| Sodium sulphate | $Na_2SO_4.10\ H_2O$ | 322,16 | **3,2** |
| Ammonium chloride | $NH_4Cl$ | 53,49 | **1,3** |
| Sodium chloride | $NaCl$ | 58,44 | **5,2** |
| Potassium dihydrogen phosphate | $KH_2PO_4$ | 136,086 | **0,95** |
| Potassium hydrogen phosphate | $K_2HPO_4$ | 174,78 | **1,2** |
| Calcium chloride | $CaCl_2.\ 2\ H_2O$ | 147,032 | **0,37** |
| Magnesium sulphate | $MgSO_4$ | 120,37 | **0,499** |

*Table 1, Recipe for 1 litre of synthetic urine*

## Lab Procedure

*First decide if you want to use morning urine or if you want to make synthetic urine.*

**Method:**

1. Add 200 ml of urine to the cup. Make a note of the colour and smell.
2. Determine the PH of the urine.
3. Grind the beans in a coffee grinder.
4. Add two spoons of soy flour to the urine and stir well.
5. Cover the beaker with parafilm and place in the refrigerator for 1.5 hours.
6. Note the smell of the mixture.
7. Determine the pH of the mixture.
8. The pH-value should be 9 or higher. If this is not the case, the mixture should again rest for some time in the refrigerator. Only when the pH-value reaches 9, it can be used.
9. At pH> 9: Filter the mixture over a filter with the use of a water jet pump. Making sure that there is as little as possible soybean flour in the funnel.

10. Transfer the filtrate into a cup and then add two teaspoons of magnesium chloride to the filtrate and stir well. After several minutes, a white precipitate appears. This precipitation is struvite. It takes several hours till all struvite is formed.
11. Filter the suspension obtained over a suction filter with the use of water.
12. Allow the filtrate to air dry. Don`t heat to accelerate the drying process.

## Questions and Calculations

Struvite is a double salt: it consists of two types of positive ions and one type of negative ion. The struvite molecule also includes six water molecules.

**5. Write the formulas of the ions which struvite exists.**

**6. Give the formula of struvite.**

Because urine, among other things consists of ammonium and phosphate, struvite can, in a simple manner, be produced. Struvite could be made by collecting urine through decentralized sanitation (separate collection of urine in every household) or by having struvite precipitation at wastewater treatment plants.

**7. If you were involved in this project, which of these two ways of Struvite production, through separate collection of urine or by precipitating struvite at a wastewater treatment plant, would you choose and why?**

The lab procedure describes how struvite from urine can be made. In the first step urease is added to the urine. Hydrolysis of urea takes place in accordance with the following equation:

$(NH_2)_2CO\ (aq) + H_2O\ (l) \rightarrow CO_2\ (g) + 2NH_3\ (g)$

**8. What is the role of urease in this reaction and what do we call this type of material?**

The pH of the solution increases in the hydrolysis step.

**9. Write the reaction that causes the pH value.**

You can calculate how much magnesium chloride you should add to the urine to precipitate all the available phosphate as struvite. For this you have the following information listed in Binas (Table 85B) need:

- You can fill 1.0 g.ml-1 for the density of urine;

- After the hydrolysis ammonium is present in excess.

**10. Calculate the amount of magnesium chloride that should be added.**

If magnesium chloride is added to the urine, the mixture can go effervescence.

**11. Give a possible explanation for this phenomena.**

**12. Calculate how many moles of nitrogen 1 gram of struvite contains if it is 100% pure.**

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# Module 3: Colorimetric Phosphate Determination

Colorimetric is a determination method in which uses the light-absorbing capacity of a coloured solution. The higher the concentration of a coloured substance in solution, the more light this solution absorbs. In other words, the degree of light absorption is a measure of the amount of substance.

As struvite is dissolved in water, there is no coloured solution. Phosphate, however, can be made visible by allowing it to form a complex which turns blue using a phosphate measuring kit.

The colorimeter measures the degree of light absorption in Absorbance (E). To translate the absorbance to a specific concentration, a calibration curve is used as a reference. If you don't have a colorimeter at school, you can us e the spectophotometre in this toolkit. With this you will measure the amount of light in lux that will pass through the cuvet.

A calibration curve, also called a standard curve or reference curve, is a general method to determine a concentration of a substance in analytical chemistry. An unknown sample is compared to a set of standard samples with a known concentration. Study figure 5 to understand the basic principles of a calibration curve.

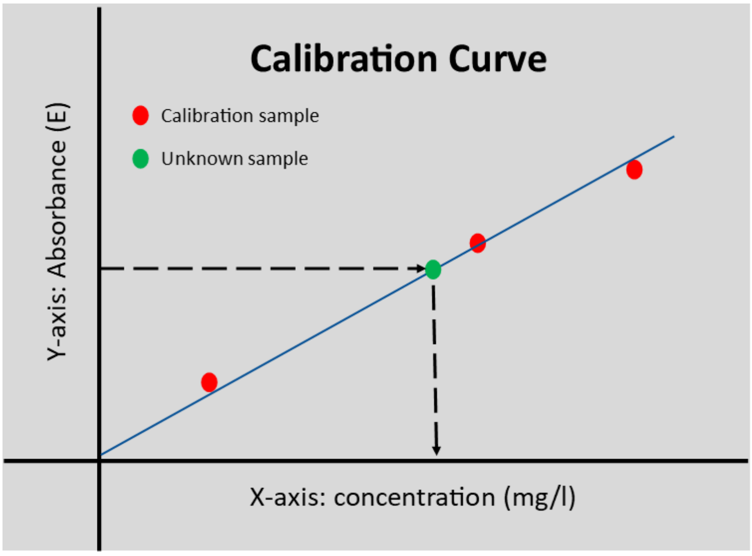


*Figure 5, principle of a calibration curve.*

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## Necessities

**List of materials/tools**

- Glassware (erlenmeyers/beakers 20 mL – 500 mL)
- Magnetic stir bars
- Stirrers
- Balance (0.01 g significant)
- Measuring cylinder (10-100 mL)
- Spoons
- Phosphate measuring kit
- Spectrophotometre
- Plastic cuvets
- Samples from Module 2 and 3

## Additional Safety Notes

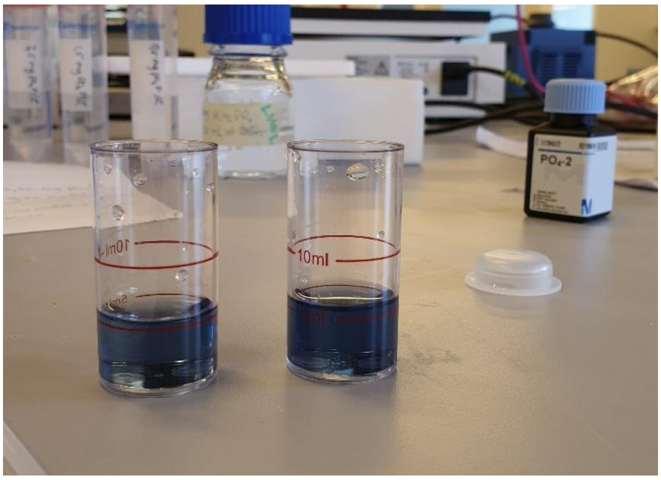
Wear a labcoat and safety glasses. When working with the phosphate measuring kit, wear gloves.

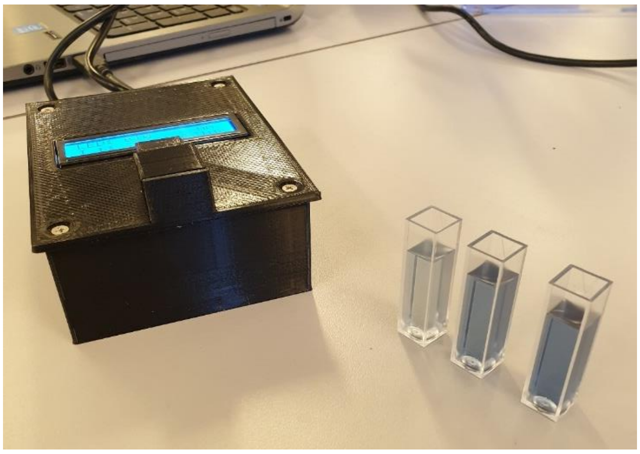
Waste procedure: measure the pH of the solutions before disposing them. Dispose them accordingly. The spoons etc. can be cleaned in the sink.

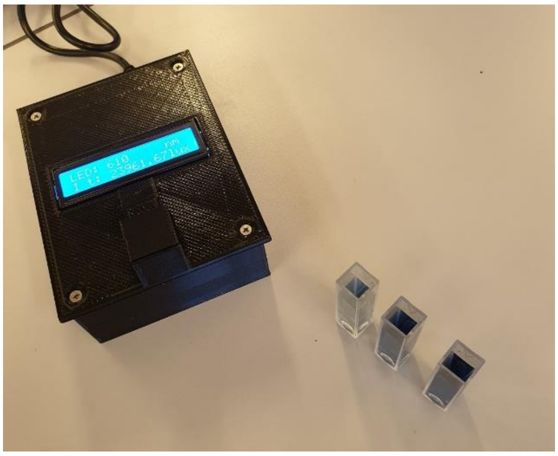
## Procedure:

1. *First of all prepare a calibration line: Make 4/5 solutions with different $PO_4^{3+}$ concentrations ranging 0-5 mg $PO_4^{3+}$/L (this is the concentration range the phosphate measuring kit can measure).*
2. *Use the phosphate measuring kit to colour the different concentrations accordingly. Read the instructions of the kit and follow them: Add 10 mL of the sample to the included plastic vial. Add 5 drops of jar 1 (wearing gloves!), next add 1 spoon (included in the cap) of jar 2 and stir until it is dissolved. You will see the solution turn blue. The higher the concentration of phosphate, the bluer the solution will become.*
3. *Measure the amount of light being absorbed by the different sampels using a spectrophotometre and cuvets: Connect the spectrophotometre to a computer. Add the coloured (or in the case of the blank the transparent) sample into a plastic cuvet and place the cuvet into the spectrophotometre. Read out the amount of lux. After measuring all the samples with the different concentrations phosphate, you can make a calibration line.*

4. *Now measure the amount of lux passing through the sample for your struvite sample. Determine with the aid of the calibration curve the unknown concentration.*





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## Module 4: Use of Struvite in an experimental design

In this module you are going to design an experiment to test your struvite as a fertilizer on pea plants. The pea plant, Lathyrus Odoratus, is a fast growing plant so you can properly observe any differences in growth rate. When using the seeds, first the plants need to germinate. Only when leaves appear, you can start fertilizing. What kind of experiment you want to design is up to you. What do you want to investigate and why?

*Figure 7, Pea plants in an experiment*

Experiments are done to study if there is a causal relationship between a variable and an effect. In an experimental design you create a certain lab procedure to test a hypothesis by changing different variables and measure the effect.

There are several steps in designing an experiment:

1. What variables are there and what do they affect? In this module you could think of:
   - Different types of urine
   - Different methods of struvite synthesis
   - Different ways of using the struvite
   - Different growth conditions of the pea plants
     affecting:
   - The yield and composition of the struvite
   - The growth of the pea plants
2. Design a hypothesis. The hypothesis should be specific and testable. A hypothesis could be:
   - Struvite from cow urine is a more potent fertilizer than struvite from human urine.
3. Design the experimental lab procedures with the variables you want to test. Make sure there are no other variables influencing your results. Therefore you have to think of the preconditions of your design. Preconditions could be:
   - All plants get the same amount of water and light
   - The used soil for the pea plants should not contain any nutrients
4. Design your samples and controls. How many samples do you have to test to get a representative outcome? Experiments also need a positive and a negative control; a positive is a control from which you know the result has to be positive, and a negative control is a control from which you know the result has to be negative. These controls give you a reference to interpret your own data to. Here controls could be:
   - A commercial fertilizer as a positive control
   - No fertilizer as a negative control
5. Make a plan to measure your results. How are you going to test the outcome of the experiment to test your hypothesis? ''Measure the growth'' as a result for how potent a fertilizer is, for instance, is not very specific. How are you going to measure the growth, and for how long? A specific measurement would be:
   - Measure the hight (cm) of the pea plant every 24 hours for a week, starting 3 days after germination.

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## Necessities

It's up to you to write this chapter now

## Lab Procedure

It's up to you to write this chapter now.

## Questions

13. **What would you do if your controls have unexpected results?**
    **For instance if your negative control plant grows the best or what if the positive control plant dies?**

14. **Which pre-conditions are important in your experimental setup? Could they fail and what would be the result of that?**

15. **How are you going to measure the outcome of your experiment? What would you do with unexpected observations that are not part of your measurement? For instance you measure the hight of a plant to measure the growth, but you also observe white leaves or no flowering while the smaller plants are green and flowering.**

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