For Teachers **Struvite from Urine**

Module 1: Theoretical background

Wetsus

Wetsus, the name, is a composition of the Frisian word for water "wetter" and the English word "sustainable". Wetsus is a research institute that researches various phenomena and techniques in water. This creates sustainable solutions for clean drinking water, energy and food. Wetsus cooperates with many companies, universities and laboratories.

The idea is that we spread the sustainable solutions as an export product around the world. We also want to make sure that no one has to die through the lack of clean drinking water and that we no longer destroy our planet by the use of dirty and wasteful techniques.

Main applications are the purification of drinking water and removing compounds from wastewater. This allows the health of all peoples and the living conditions can be improved. This is necessary because contaminated water is currently number one cause of death of children.

It is only real sustainable water if the water is filtered with smart technologies, that can purify salt or polluted water and at the same time produce energy. Also, all kinds of organisms from nature, such as worms, bacteria and algae are here to help us in many processes

We are not finished with inventing smart solutions in water technology and it will take a long time before everyone has access to clean drinking water on earth and that the earth will be saved forever from pollution and waste. We are working hard and are in need of young researchers with fresh ideas and big plans.

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).

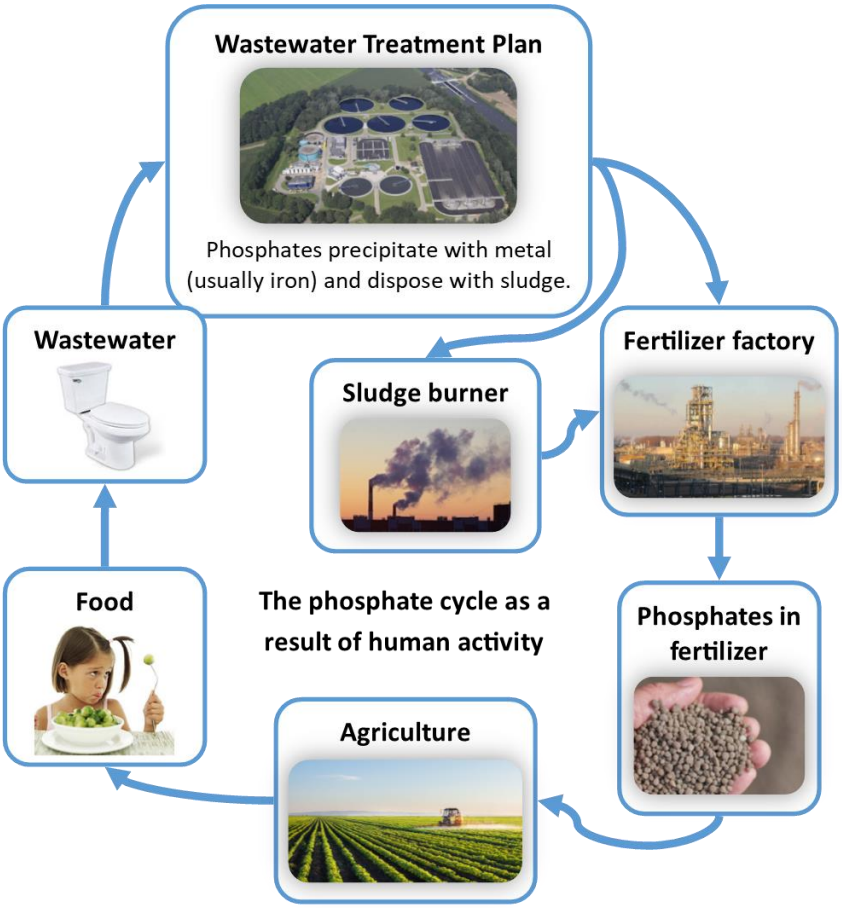

Figure 1, The phosphate cycle as a result of human activity.

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).

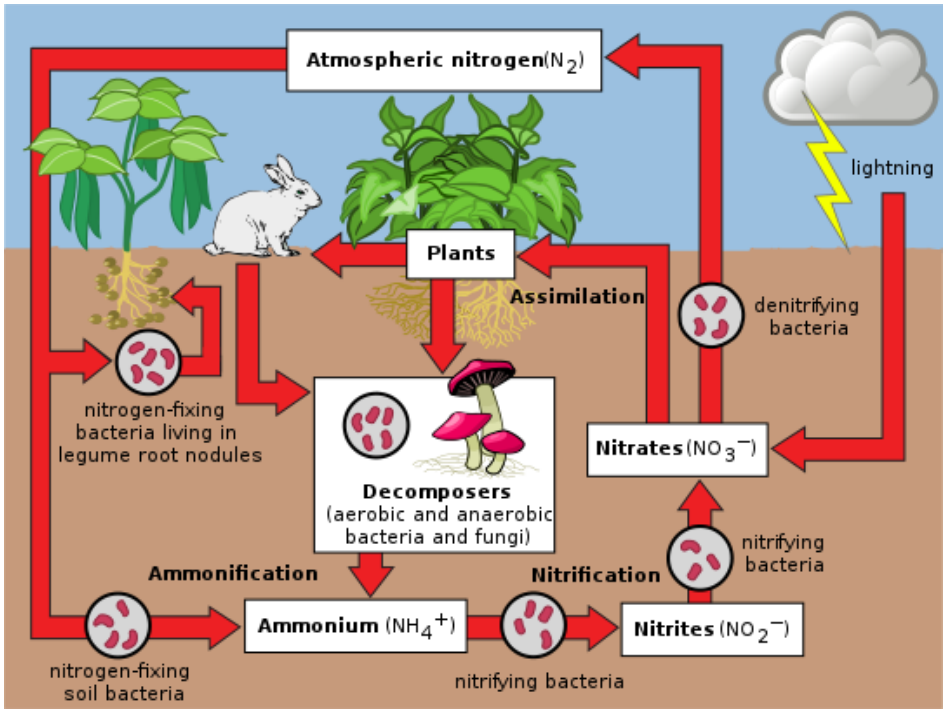

Figure 2, ***Cycle of nitrogen.***

In the Netherlands nitrogen in the form of urea ends up 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 is discharged in the environment, where it is again part of the nitrogen cycle.

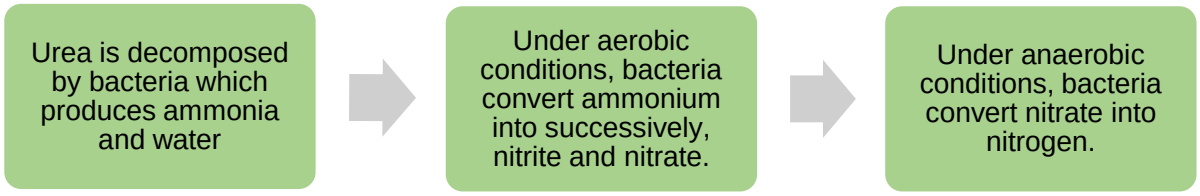

Figure 3, ***Nitrogen process at a wastewater treatment plant.***

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).

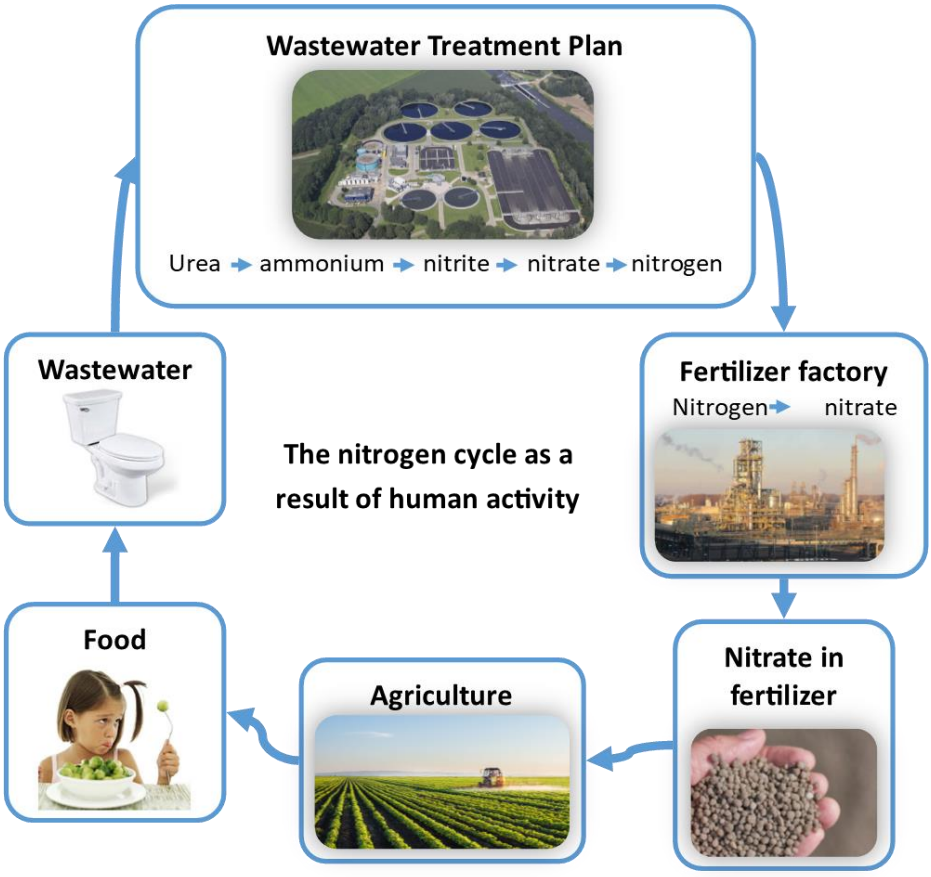

Figure 4, The Nitrogen cycle as a result of human activity.

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)

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.

Procedure

- *Let the students read or present the background information*
- *Optional: discuss the content or the questions in the group*

Questions and answers

1. *Why is nitrogen and phosphate recovery from wastewater important?* Answer should include the fact that mining resources is finite. Other answers are the advantages of a circular system: better for the environment, less energy needed, less CO2 emission, less transport, a more self-sustainable way of having resources / not dependent on the countries that own the mines.
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?* The wastewater treatment plant discharges phosphate compounds (mainly from urine) with sludge to the incinerator. In the wastewater treatment plant urea is converted via nitrate into nitrogen. Nitrogen is a raw material for the fertilizer industry. Here they transfer nitrogen back into a nitrate compound.
3. *Can you think of several advantages when making struvite directly from urine?*
   • Save energy, while phosphate and nitrogen don't need to be removed from urine and urine is not diluted in a waste water plant.
   • Keep phosphorus in the cycle
   • to keep a valuable product (fertilizer)
4. *Where would you make struvite from urine? Think big: how could this way of resource recovery be implemented in the Netherlands and abroad?* Urine from animals or people can be collected easily. The possibility of making a cheap and easily-made fertilizer has applications in different countries and setups. Think of places like a farm, a hospital urine collection system, a toilet system that separates urine, etc. Mention the possibilities abroad: in poor regions in Africa people use the same toilet and there is no wastewater plant. Here urine can be collected and used for fertilizer production.

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Module 2: Struvite Synthesis

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

Procedure

First decide if you want to let the students use morning urine or if you want to make synthetic urine with them or for them.

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.

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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 answers

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.

1. *Write the formulas of the ions which struvite exists.* Mg^{2+}, PO_4^{3-}, NH_4^+
2. *Give the formula of struvite.* $NH_4MgPO_4.6\ H_2O$

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.

3. *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?* Answer of the pupil should contain a motivation which describes the advantages and disadvantages of the methods.

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:

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

4. *What is the role of urease in this reaction and what do we call this type of material?* Urease is an enzyme. Another word for this is biocatalyst. These are substances that ensure that a reaction takes place at a low temperature such as body temperature. A catalyst is not consumed during the reaction and is therefore not in the reaction equation.

The pH of the solution increases in the hydrolysis step.

5. *Write the reaction that causes the pH value.* $NH_3(g) + H_2O(l) \rightarrow NH_4^+\ (aq) + OH^-\ (aq)$

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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.

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

   Urine contains 0.4% phosphate (M = 94.97 g / mol) and 0.02% magnesium (M = 24.31 g / mol)

   $M_{\text{magnesium chloride}}$ = 95,22g/mol

   200ml of urine ≡ 200g urine

   200g of urine is 0.8g phosphate ≡ 0.8/94.97 mol phosphate ≡ 0.8/94.97 mol of magnesium

   It is therefore necessary that 0.8 / 94.97 mol of magnesium is added. The urine already contains 0.02% magnesium (= 0.04 g ≡ 0.04/24.31 mol magnesium).

   Still 0.8/94.97 - 0.04/24.31 mol of magnesium needs to be added.

   0.8/94.97 to 0.04/24.31 mol magnesium ≡ (0.8/94.97 to 0.04/24.31) x 95.22 = 0.64 g of magnesium chloride.

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

7. *Give a possible explanation for this phenomena.* The solution is hot and residual gases, CO_2 and NH_3, can escape.
8. *Calculate how many moles of nitrogen 1 gram of struvite contains if it is 100% pure.*

   1g struvite corresponds to 1/245.28 mol struvite.

   1/245.28 mol struvite corresponds to 1/245.28 mol nitrogen = 4.08 mmol N_2

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.

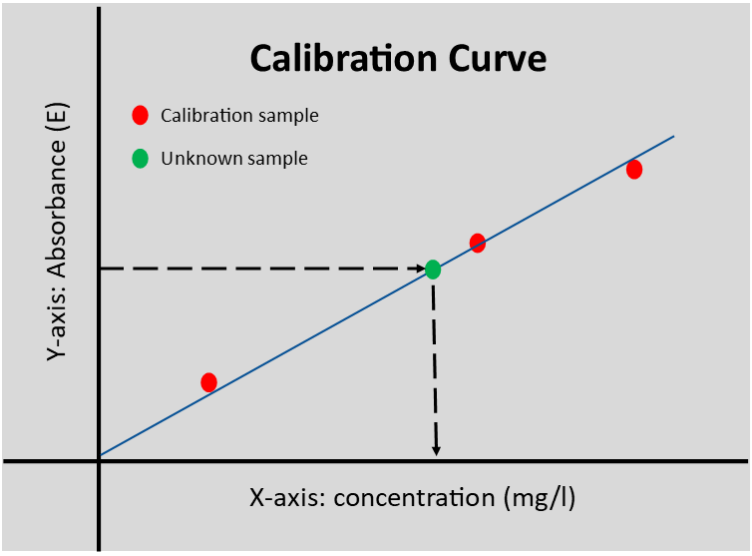


Figure 5, principle of a calibration curve.

For Teachers **Struvite from Urine**

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

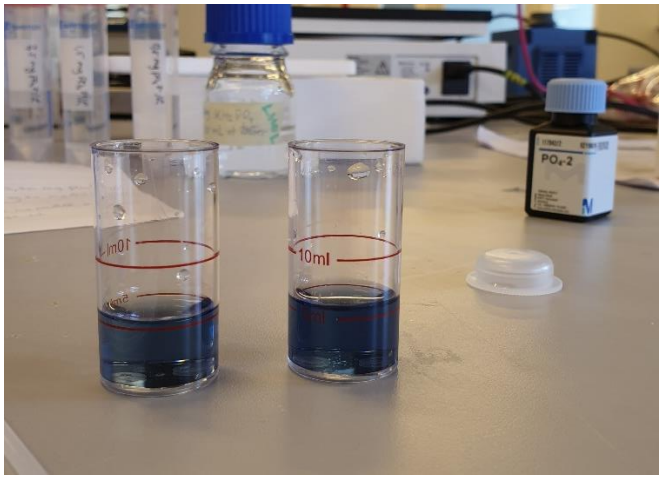
Wear a labcoat and safety glasses. When working with the phosphate measuring kit, wear gloves.

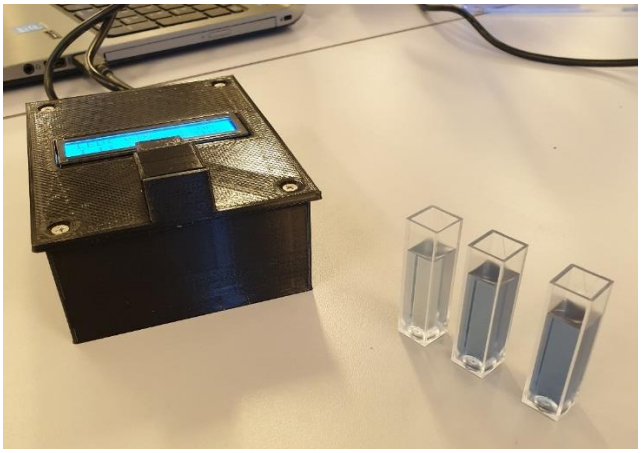
Waste procedure: measure the pH of the solutions before disposing them. Dispose them accordingly. The spoons etc. can be cleaned in the sink.

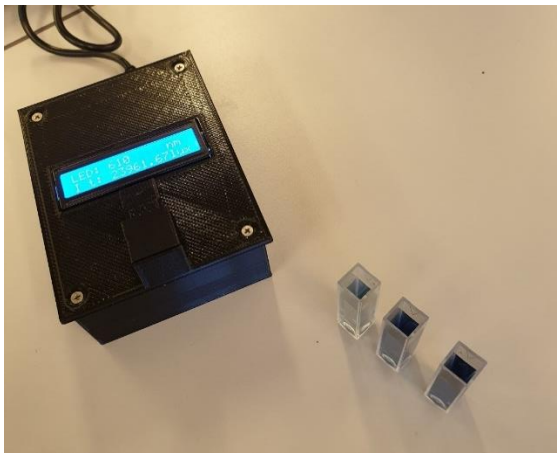
Procedure:

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.*







Calculations

The students can calculate the concentration of phosphate in their sample. Check and discuss the results.

For Teachers **Struvite from Urine**

Module 4: Use of Struvite in an experimental design

In this module the students are going to design an experiment to test their 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 is designed is up to you and the students. What do they want to investigate and why? What is possible for the project?

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

This is up to the students, but needed are at least:

- Pea seads (Lathyrus Odoratus)
- Cultivation rockwool
- A custom fertilizer as a positive control
- Water and light supply

Procedure

- *Make sure the limits of the module are clear by all students (what can they use, how much time is there for the experiment etc).*
- *The students can write their own experimental design.*
- *Check the setup: by checking written report or let them present their setup and discuss this with the group.*
- *Let the students perform their experiments and generate data, results and a conclusion.*
- *Discuss the results and conclusions with the students.*

Questions and answers

1. *What would you do if your controls have unexpected results? For instance if your negative control grows the best or what if the positive control plant dies?* It is important to measure a value that should give you a positive result and a negative result for sure, to compare your own results with. If the controls have a wrong outcome, something might have gone wrong with the experiment and the results cannot be interpret. You could start again, because now how do you know your sample results are representative if your controls are not?

2. *Which pre-conditions are important in your experimental setup? Could they fail and what would be the result of that?* What if not all plants get the same amount of light, and this influences the growth rate. Or an inequal distribution of water. Preconditions are as well a measurable outcome: what if a plant is not growing in hight, but in width.

3. *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.* In science experiments have a lot of times unpredicted outcomes. A good scientist is patient and willing to perform experiments over and over again. Sometimes you have to change the experimental setup and the way you measure results to get a more reliable outcome.

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