Diploma project
Natural Sciences
Umeå Elitidrottsgymnasium
2023/2024

PFOA: Threat to vegetation?

How plants are affected by PFOA from the textile industry in Bangladesh

Source: Environment and Social Development Organization (2019). "PFAS: Bangladesh Situation Report"

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Abstract

A hot topic for debate is the impact per- and polyfluoroalkyl substances (PFAS) have on plants and animals. Southeast Asia being the world's largest textile producer, contributes greatly in water pollution through hazardous waste. PFAS are a group of persistent chemical compounds with destructive properties that affect our ecosystems. Studies show high concentrations of PFOA, a type of PFAS, coming from Bangladesh's textile industry of which measured values exceed Europe's limits. Yet there are few economical and sufficient purification methods today, leading to these substances accumulating in nature.

The purpose of the project was to examine the influence PFOA has on vegetation. This was achieved through cultivating populations of cress plants on agarose gels prepared with different concentrations of the chemical. The plants' developments were documented over two weeks and subsequently a timelapse video was created.

During analysis, it was concluded that cress has a delayed sprouting in high concentrations of PFOA. Furthermore, PFOA treated plants showed a slower growth rate throughout the duration of the experiment. An average height of the plants in each concentration was calculated and statistical analysis showed a significant difference between non-treated plants and plants treated with the highest concentration of PFOA in dependent cultivations. In conclusion, treating plants with PFOA leads to an impairment in its development. In independent cultures, no difference in growth could be detected.

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1. Introduction

Have you ever noticed where most of your clothes are being produced? A majority of today's clothing is produced in Southeast Asia, more particularly in Bangladesh. The situation in Bangladesh is serious with harmful emissions coming from the textile industry, contaminating the environment including nearby water sources. The polluted water does not only affect human health and living circumstances, but also plants. One class of over-represented chemicals from the textile industry, PFAS, are very difficult to break down and have very harmful effects on our ecosystem. Only a few studies have been made on analyzing the chemical composition of water pollution, but findings from these studies indicate a large number of chemicals in which PFOA dominates. When does the concentration of chemicals become devastating for the plants' existence?

1.1 Purpose and research question

The project aimed to investigate when the impact of PFAS, particularly PFOA, becomes devastating to the existence of plants. It mainly focuses on the challenges posed by harmful emissions from the textile industry in Bangladesh and how plants adapt in the presence of these chemicals. Our report discusses the issue with PFAS usage and its effects on cress growth. The study explores the implications of elevated PFOA levels in the environment for future vegetation.

- How is the growth of cress affected in the presence of PFOA?

1.2 Background

1.2.1 PFAS and the textile industry

Per- and polyfluoroalkyl substances (PFAS) is a group of numerous chemical compounds with an exceptional stability attributed to their polar covalent carbonyl fluoride bond. This stability renders them resistant to degradation in nature, leading to their gradual accumulation over time. Currently, there are a few efficient methods for purifying water contaminated with PFAS and they are complicated and expensive. PFAS are industrially produced by humans and the use of the chemicals can be found in various industries, notably in Bangladesh's textile industry for manufacturing water-resistant clothing. However, Bangladesh law lacks regulatory frameworks concerning PFAS usage, thus leading to its widespread use driven by its efficiency. Major textile brands like H&M, Zara, Target and Levi's source their products from Bangladesh. Consequently, inadequate waste management systems result in the release of PFAS into nearby rivers, thereby exposing vegetation to prolonged contamination. Notably, the textile industry constitutes a significant portion, accounting for 26% of all industries in Bangladesh utilizing PFAS (ESDO, 2019).

1.2.2 Perfluorooctanoic acid (PFOA)

Highly fluorinated substances share a common characteristic: they persist in the environment indefinitely. One of the most prevalent PFAS compounds from textile industries' emissions in Bangladesh is perfluorooctanoic acid (PFOA). PFAS are highly soluble, allowing plants to easily absorb the substance through their roots. PFOA is a man-made chemical used in textiles to impart water-repellent properties. In 2017, the Stockholm Convention's POPs Review Committee highlighted the consequences of exposure to this chemical, revealing its carcinogenic risks. PFOA has been contaminating the global environment for a long time, dating back to the 1950's. PFOA is persistent and does not degrade in nature (ESDO, 2019). This chemical belongs to the group of perfluorinated alkyl acids (PFAA) and PFOA consists of a fluorinated hydrophobic alkyl chain with a hydrophilic group (Figure 1). PFOA can withstand high temperatures and has the ability to be, among other things, water repellent, grease repellent and dirt repellent. PFAS substances have also been widely used in fire extinguishing foam and thus spread in nature (Livsmedelsverket, 2024).

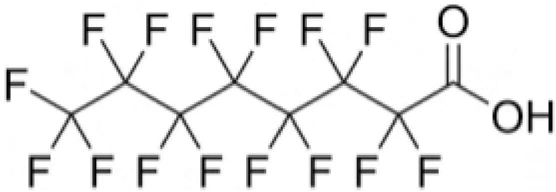

Figure 1: Shows the structural formula of PFOA. Source: Wikipedia

1.2.3 Sweden's exposure to PFAS

PFAS use and its impact can also be connected to Sweden. Sweco Environment AB's report on PFAS contamination at Umeå Airport shows that there are still high levels of PFAS that pose acute risks to the environment, despite the fact that fire extinguishing foam with PFAS stopped being used in this area in 2008. According to the report, there is a risk that the contaminated water will be pumped up onto arable land and then make its way further into the food chains. Guideline values for the occurrence of PFOS in surface water are 0.65 ng/l and in groundwater 45 ng/l. (Sweco Environment AB, 2019). In addition, at least two million Swedes drink water with PFAS levels that exceed the Danish limit value of 2 ng/l. In addition to that, there are around 200 000 Swedes who drink water with such high levels of PFAS that it exceeds EFSA's (EU Food Safety Authority) guideline for total intake of the chemicals (Naturskyddsföreningen, 2022).

"Miljösamverkan Sverige" has together with the Swedish County Administrative Boards, Swedish Environmental Protection Agency, Swedish Board of Agriculture and Swedish Agency for Marine and Water Management produced a report, "PFAS vid deponier", which deals with the high levels of PFAS found in leachate from landfills. The report highlights the risk that PFAS can spread from the landfills to the surrounding soil, air, groundwater and surface water, where they cause damage to animals and nature. This is because the existing purification methods are not designed in an efficient way to separate PFAS from the leachate. In addition, it is mentioned in the report that leachate from landfills is one of the largest spread routes for PFAS in Sweden. Furthermore, PFAS spreads easily in the ground, which means that surface- and groundwater will be contaminated for a long time ahead. Different purification techniques are more or less effective for PFAS and thus several techniques may be needed to achieve cleaner water. The results from the report showed that PFOA had among the highest median levels (Miljösamverkan Sverige, 2022).

1.2.4 Values and concentrations of PFOA

According to studies carried out by the organization in Bangladesh, ESDO, surface water can contain up to 50 ng/l PFAS (ESDO, 2019), which is 11.5 times greater than the maximum PFAS limit that is allowed to exist in Europe, which is 4 ng/l (Livsmedelsverket, 2023). The more of the chemical that is released, the more of the non-degradable content of PFAS in nature increases (Naturskyddsföreningen, 2022).

In Sweden, studies have also been carried out on what levels of PFAS could be found in leachate from landfills. The maximum measured content of PFAS 11 (the sum of the 11 most common PFASs, including PFOA) in the report "PFAS vid deponier" was 194 367 ng/l in a single leachate sample. The maximum measured PFOA content was approximately 9 500 ng/l, where the frequency of detection in the leachate was 98%. This makes PFOA the second most common type of PFAS (Miljösamverkan Sverige, 2022).

In 2019, Sweco Environment AB presented a report on PFAS contamination at Umeå Airport. Within two source areas where samples were taken from the groundwater, a maximum value PFAS 11 of 300 000 ng/l was found. For PFOS, which is another very common type of PFAS, the maximum value was 24 000 ng/l (Sweco Environment AB, 2019). PFAS contamination is a global problem and not an isolated problem in Bangladesh.

1.2.5 Cress

In the studie cress was used as a model plant for investigating the effect of PFOA on plant development. Cress is a plant that begins to germinate just a few days after sowing seeds. The growing process is fast and cress grows well with roots that can absorb a lot of nutrients. Cress that is allowed to grow in agarose gel becomes stable and then it is possible to to only affect the plant with PFOA. In addition, it is also difficult to fail in growing cress, making cress a good model for the study.

1.2.6 Dependent and independent cultivations

In this report, dependent and independent cultivations are two different experimental methods. Method 1, dependent, has several seeds in the same beaker (same conditions) and thus the seeds can influence each other just like in nature. Method 2, independent, has only one seed in each cultivation, which means that there are fewer sources of errors.

1.2.7 Raspberry Pi

In order to observe the growth and development of cress a Raspberry Pi-system was used. The company Raspberry Pi Foundation developed a high-performance single-board computer, the Raspberry Pi, which is cheap, small in size and has a wide range of uses. The choice of operating system is determined by the purpose of use and thus programming of the computer can be carried out as needed. These computers can be programmed to take pictures in a specific time interval during a selected period of time (Raspberry Pi, 2024).

1.2.8 T-test for mean difference in length

The results obtained were scrutinized through statistical analysis where a T-test was used. T-test is a statistical analysis that can provide statistical evidence for an alternative hypothesis if there is a difference between two mean values. The test thus shows whether the results are significant. This is done by using mean value, sample standard deviation and number (n) in the samples to obtain a probability value (p-value). In statistical tests, a null hypothesis is tested against an alternative hypothesis for which statistical evidence can be found. If the p-value falls below a chosen significance level, the null hypothesis can be rejected in favor of the alternative hypothesis. In the study, the significance level is chosen to be 5%. A p-value of 0.05 means that there is a 5% chance that the result of the experiment or worse (speaks even more for H1) is due to chance given that H0 is true. Expected values and hypotheses for Experiments 1 and 2 can be seen in Figure 2.

Experiment 1	Experiment 2
μ_1:expected value for mean height in population 1 μ_2:expected value for mean height in population 2 μ_3:expected value for mean height in population 3	μ_1:expected value for mean height in population 1 μ_2:expected value for mean height in population 2
$\begin{cases} H_0: \mu_1 = \mu_2 \\ H_1: \mu_2 < \mu_1 \end{cases}$ $\begin{cases} H_0: \mu_1 = \mu_3 \\ H_1: \mu_3 < \mu_1 \end{cases}$	$\begin{cases} H_0: \mu_1 = \mu_2 \\ H_1: \mu_2 < \mu_1 \end{cases}$

Figure 2: Shows expected values for average height for each population, the null hypotheses and our hypotheses. These were used for the respective T-tests for mean difference in Experiment 1 (left) and Experiment 2 (right).

1.2.9 Phytoremediation

A very interesting and appealing idea is to use plants for decontamination of contaminated water. The use of plants to purify water is called phytoremediation. In the degree project "Fytoremediering - Ett hållbart sätt att tillgängliggöra förorenad mark?" made by Jennie Kind at the Swedish University of Agricultural Sciences (SLU) describes what phytoremediation is and how it can be used to clean contaminated land and water. Phytoremediation is a collective name for purification methods that utilize the ability of plants to purify or reduce pollution from, among other things, water. Cleaning with vegetation is a technique that is used directly on the contaminated site and utilizes nature's own processes, photosynthesis as the foundation of plants autotrophy. The process is thus powered by solar energy and is more sustainable compared to other purification processes. In addition to these positive aspects, the method also has a great potential to become cost-effective. To be able to use phytoremediation, plants that have a high tolerance to harmful substances are required. (Kind, 2012).

Researchers Maria Greger and Tommy Landberg at Stockholm University have conducted a study on the purification of PFAS using wetland plants in the report "Removal of PFAS from water by aquatic plants". The levels of PFAS in fresh water from the lake Sänksjön decreased drastically by 40% in just one day, where the size of the plant biomass proved to be more important for effective purification than a specific plant species. One of the plants that the report puts forward as most effective in its purification is sedge plants (a type of carex). These plants collect PFAS in the shoots which can then be harvested. Hopefully, the method can be developed and also used on one's own property where PFAS contamination is present (Greger, Landberg. 2024).

1.2.10 Umeå University - collaboration

In collaboration with Umeå University (UMU), the project was made possible. Christine Gallampois from UMU provided us with a total of 2 ml of PFOA with a concentration of 6.15 ng/ul diluted in methanol. In order to document the growing process, Sara Raggi, also from UMU, helped us with equipment for timed photography. The laboratory process was thus documented by Raspberry Pi camera module using Raspberry Pi 3 (Model B).

1.3 Risk analysis

PFAS is a group of chemicals that, in high concentrations, can lead to possible cancer, just like all other industry-causing highly fluorinated substances in our vicinity. Handling of PFAS always takes place with safety glasses and gloves. Actions that risk contact with chemicals are only handled by teachers. The experiment is carried out in a fume hood to which only teachers have access. After the project, the plants must be sealed in plastic bags and the contaminated water in bottles. This waste must be given to the authorized person at Umeå University for destruction.

2. Material and method

2.1 Dependent growth experiment - Method 1

Plant nutrition was diluted with 300 ml of distilled water according to the dosing instructions on the package to a concentration of 3 ml/l. Then 3 grams of agarose was added to the solution which was boiled under low heat. The solution was distributed evenly between three sterilized beakers (100 ml in each) named 0 ng/l, 500 ng/l and 5000 ng/l according to their final PFOA-concentrations. The added PFOA had a concentration of 6.15 ng/ul. In the control (0), 81.3 ul of methanol was added using a pipette, in beaker 500 8.13 ul of PFOA and 73.17 ul of methanol were added and finally 81.3 ul of PFOA was added in beaker 5000. Methanol was added as PFOA was diluted in methanol. The agarose solutions were allowed to stand and solidify completely during two days. Subsequently, 20 cress seeds were distributed over the agarose surface using a sterilized tweezer (Figure 3). A watch glass was then placed on top of each beaker and plant lighting was set up according to Figure 4.

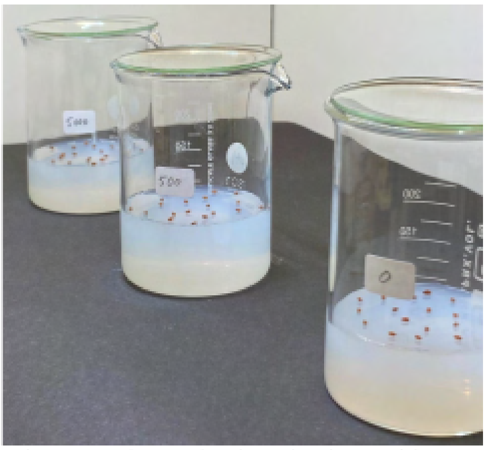

Figure 3: Shows the three beakers with concentrations of 0, 500 and 5000 ng/l PFOA respectively where cress seeds were grown on agarose.

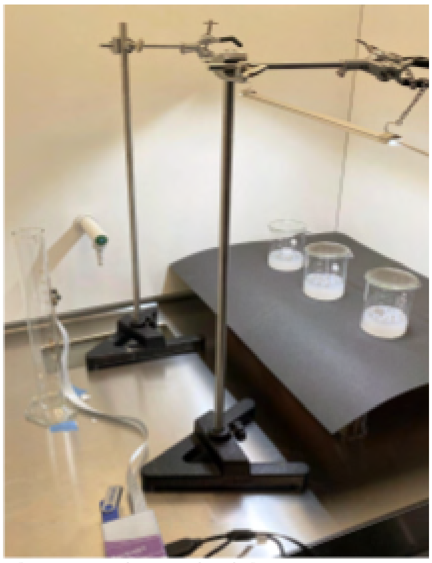

Figure 4: Shows the laboratory setup of method 1.

2.2 Independent growth experiment - Method 2

60 test tubes were placed in aluminum foil and sterilized in the oven at 150°C for 5 hours. Plant nutrition was added in 400 ml of distilled water to a concentration of 3 ml/l. Then 4 grams of agarose was added to the solution, which was allowed to boil under low heat. After that, the solution was distributed between three beakers with 130 ml in each. PFOA and methanol were added to the beakers and named according to their prospective concentrations; 0 ng/l, 500 ng/l and 5000 ng/l. In the control (0) 106 ul of methanol was added and in the 500-beaker 10.6 ul of PFOA was added together with 95.4 ul of methanol. Finally, 106 µl of PFOA was added to the 5000-beaker. Using an electronic pipette, 6 ml of each solution was taken and transferred to each test tube. A total of 60 test tubes were used (20 for each concentration). All test tubes were placed in test tube racks and covered with ParaFilm. The agarose solutions were allowed to solidify completely for two days. After that, a single seed was

Figure 5: Shows the test tubes with one seed in each. The control on the left, 500 ng/l in the middle and 5000 ng/l on the right.

placed in each test tube and ParaFilm was again used to cover the tubes (Figure 5). Figure 6 shows the setup.

Figure 6: Shows the laboratory setup of method 2.

2.3 Growth documentation and analysis

The plants were allowed to grow for two weeks. During these weeks, the Raspberry Pi with the associated camera module was programmed to take a picture every 4 hours. The computer and the camera can also be seen in figures 4 and 6.

Then all plants were cut off at the root of each population and placed in Petri dishes (Figure 7). Furthermore, each individual plant of the populations was measured according to Figure 8. Measured values were entered into Google Sheets and compiled to T-test for mean difference in length in GeoGebra.

Figure 7: Shows the cut plants from the populations from Experiment 1 which are in Petri dishes.

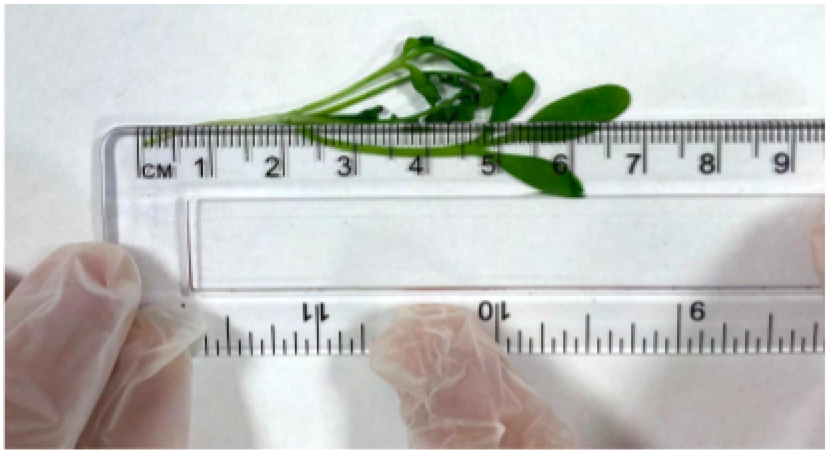

Figure 8: Shows how each plant was measured.

3. Results

3.1 Visual results: Experiment 1 (Dependent growth)

The result of the experiment showed that higher levels of PFOA have a negative impact on cress seeds. When treated with 5000 ng/l, a greatly delayed germination was observed and a greater proportion of the seeds did not germinate at all; 19/20 seeds germinated from the control, 15/20 from the 500 beaker, and 10/20 from the 5000 beaker. Furthermore, the treated plants had impaired growth throughout the course of the project. At the end of the experiment, after fourteen days, a clear difference in growth was observed between untreated and treated plants (Figure 9). Figure 10 shows how the plants changed in germination and growth with four days between each image photographed by the Raspberry Pi.

Figure 9: Shows cress that has grown for 2 weeks in the different concentrations. Descending concentration from the left.

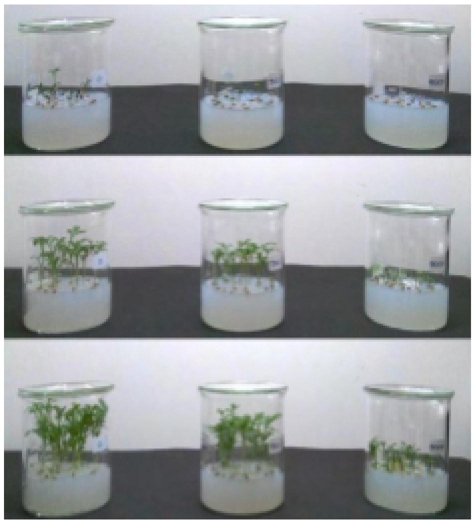
Figure 10: Shows the difference in germination and growth. There are four days between each picture. Increasing concentration from the left.

3.2 Statistical analysis: T-test for mean difference in length Experiment 1

The observations were confirmed by measuring the plants (Table 1 and Figure 11). The average length of the control plants was 5.121 cm and the average length in PFOA treatment decreased with increasing concentration where treatment with 5000 ng/l almost halved the average length (2.27 cm). The mean length of the middle concentration was 4,533 cm. The standard deviations for all populations were calculated (blue marked in Table 1). The values were used in T-tests for mean difference which showed reliable responses in the population with 5000 ng/l of PFOA (Figure 11). In the plants with 500 ng/l there was a possible statistical basis for significant results.

Table 1: Shows the length of the plants in cm from each population and the calculated average values at the bottom. The blue-marked area shows the standard deviations.

0	500	5000
6,9	5,3	3
4,2	4,2	2,8
4,5	5	2
4,7	3,8	2,5
5,3	5,2	2
6,2	3,7	2,8
2,3	2,9	2,1
2,5	5	2,5
5,8	4,1	1,9
6,5	3,8	1,1
6,6	4,7	
6,2	4,8	
5,4	5,8	
4,6	4,7	
4,5	5	
4,4		
4,7		
6,9		
5,1		
5,121052632	4,533333333	2,27
1,310461728	0,7631388815	0,5657836257

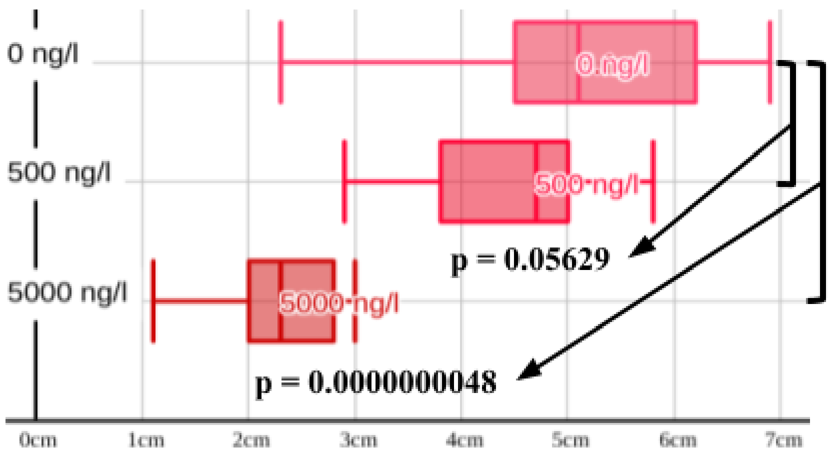

Figure 11: Shows min, max, mean and median length for each population. The p-values indicate the significance.

3.3 Timelapse video: Experiment 1

The timelapse video for Experiment 1 showing how the cress crops grew over two weeks can be viewed by scanning the QR code on the right (Figure 12).

Figure 12: Shows the QR code that can be scanned to view the timelapse video for Experiment 1.

3.4 Visual results: Experiment 2 (Dependent growth)

The result of Experiment 2 showed a difference in germination and growth when treated with PFOA. Experiment 2 treated only concentrations 0 and 5000 (two replicates of each concentration). In the far left beaker (control 1 (0 ng/l)), 20/20 seeds grew. In control 2 (left center), 18/20 seeds grew. In the beaker right middle (5000 ng/l) 15/20 seeds grew and in the treated beaker far right (5000 ng/l) 18/20 seeds grew. Figure 13 shows the change in growth for the cress cultivations with 3 days between each picture.

Figure 13: Shows the difference in germination and growth in Experiment 2. There are three days between each image. The two beakers on the left are controls (0 ng/l) and the two beakers on the right are treated (5000 ng/l).

3.5 Statistical analysis: T-test for mean difference in length Experiment 2

The average length of the two controls on the left was 5,147 cm and the average length of the two right treated populations was 3,951 cm which can also be read in the white area at the bottom of Table 2. In the blue marked area (Table 2) there are two calculated standard deviations for the populations. The values were used in T-tests for mean difference which showed reliable results in the populations treated with 5000 ng/l of PFOA (Figure 14).

Table 2: Shows the length of the plants in cm from each population and the calculated average values at the bottom. The blue-marked area shows the standard deviations.

Left (0)	Middle (0)	Middle (5000)	Right (5000)
5,9	1,6	2,3	6,5
7,9	1,9	2	5,7
5	2,6	2,7	6,5
6	5,1	2,2	5,5
7,8	4,5	2	4,2
4,3	5,3	4,5	4,5
6,8	3,8	3,6	5,8
6,2	4,5	3	3,8
6,2	6,2	4,7	3,2
5,9	4,8	3,6	4,5
3,5	5	5,6	2,3
6,5	5,6	5,3	5,1
4,1	5,5	4,8	5,2
5,2	6,4	3,8	3,2
4	5,7	1,4	3,7
6,1	6,3		4
4,9	1,6		3,6
6	5,3		1,6
5,8			
1,482827256	5,147368421	1,411807621	3,951515152

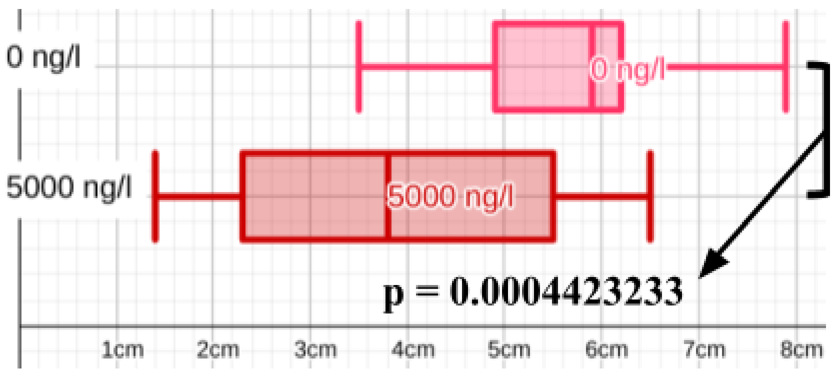

Figure 14: Shows min, max, mean and median length for each population. The P-value indicates the significance.

3.6 Timelapse video: Experiment 2

The timelapse video for Experiment 2 showing how the cress grew over two weeks can be viewed by scanning the QR code on the right (Figure 15).

Figure 15: Shows the QR code that can be scanned to view the timelapse video for Experiment 2.

3.7 Visual results: Experiment 3 (Independent growth)

Experiment 3 of method 2 gave no visible differences in the length of the populations. Thus, no measurements were made of this experiment. In Figure 16 and Figure 17 the result from Experiment 3 can be seen.

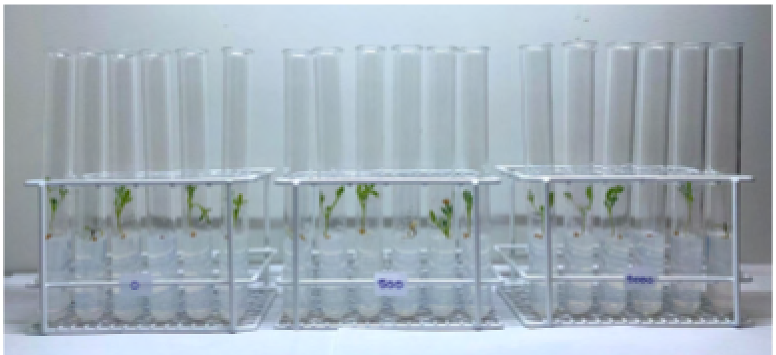

Figure 16: Shows Experiment 3 where cress seeds were grown in test tubes with three different concentrations (0 ng/l, 500 ng/l and 5000 ng/l) of PFOA. Increasing concentration from the left.

Figure 17: Shows the remaining 42 test tubes from Experiment 3 where cress seeds were grown in test tubes.

3.8 Timelapse video: Experiment 3

The timelapse video for Experiment 3 showing how the cress grew over two weeks can be viewed by scanning the QR code on the right (Figure 18).

Figure 18: Shows the QR code that can be scanned to view the timelapse video for Experiment 3.

4. Discussion

4.1 Dependent cultivations (Method 1)

4.1.1 Conclusion

Based on our observations, it was concluded that high concentrations of PFOA over a short period of time have a negative impact on plant germination and growth. With increasing concentrations of PFOA, the length of the cress plants decreased. In addition, fewer seeds actually became plants with increasing PFOA concentration. As in Figures 9, 10 and 13, a clear visual difference in growth can be read between the cups and thus PFOA had a negative effect on the development of the plants. The measured values that were made on each plant also show a clear difference in growth and the average values (Table 1 and Table 2) also indicate this. In Figure 11 and Figure 14 there are boxplots where we see clear differences in measurement values for the length of the different populations. Both Experiments 1 and 2 had statistical evidence of significant results at the 5000 concentration.

Before the start of the project, the Raspberry Pi was programmed to take pictures at a specific time interval (14 400 seconds). This gave us six images per day four hours apart. Through the images taken by the Raspberry Pi, timelapse videos were created (Figure 12 and Figure 15) where clear results can be seen. This gave us the opportunity to see what happened to the plants even when we were not on site at the project for review. In the videos, it can be seen that the higher the concentration of PFOA that is found, the fewer seeds started to grow, which resulted in poorer germination and impaired growth in the cress farms.

The reason Experiment 2 only treated the concentrations 0 ng/l and 5000 ng/l was our limitation of PFOA. We didn't have enough chemical in batch 1 and thus needed to prioritize the concentration that already had significant results and see if these matched. Experiments 1 and 2 were done before Experiment 3, so a different batch was used for Experiment 3.

4.1.2 Analysis of T-test for mean difference in length

For the T-tests, normally distributed populations were assumed. When we examine the height of a population of plants, normal distribution is a reasonable assumption. There is a null hypothesis and a chosen alternative hypothesis for different T-tests. μ is the expected value for the mean length of the different populations (Figure 2). T-test for mean difference gave significant results for the 5000 concentration in both Experiments 1 and 2 and the null hypotheses could thus be rejected. The probability values, the p-values, were far below the significance level of 0.05 (5%), which indicates that it is very likely that the concentration of PFOA is the explanatory factor in the growth difference. With the 5000 concentration in both Experiments 1 and 2, there is therefore statistical evidence for significant results as the p-values were below 0.05.

The T-test for the 500 concentration of Experiment 1 was just above the significance level ($p = 0.05629$) which is not a statistical basis for a significant result. However, the value is very close to the significance level and with more experiments a smaller p-value will likely emerge. There is thus a very possible prerequisite for a statistical basis for significant results even at this concentration.

4.2 Independent cultivation (Method 2)

4.2.1 Conclusion

From Experiment 3, done on Method 2 which is independent cultivations, no conclusions could be drawn. No visible differences in the length of the plants in the different populations could be read and thus no statistical analysis was performed. The reason why no result appeared can be due to several reasons. A new batch of PFOA was used in Experiment 3 and the results may therefore vary. Since the cress still grew, there is probably nothing wrong with the cress seeds. However, the control did not initially grow as it should, which makes the whole experiment less reliable. Otherwise, we cannot know more about why the results were like that in comparison with both Experiments 1 and 2.

4.3 General discussion

4.3.1 Choice of concentrations

Reported levels of PFAS in Bangladesh were around 50 ng/l, which is approximately 11.5 times greater than Europe's limit value of 4 ng/l (ESDO, 2019). During the project, clearly higher levels of PFOA were used; 500 ng/l and 5000 ng/l. The plants in Bangladesh are exposed to a lower concentration than we are used to, but its exposure takes place over the entire lifetime. We had limited time for our experiments and increasing concentrations is a necessity to demonstrate results over that period. With our diploma work, we want to show the negative effect PFOA has on plant development in germination and growth, which means that the concentrations must be increased to achieve clear results. These values give a good picture of the effects of PFOA on plants. In contrast, the guideline values for PFOS around Umeå Airport in the surface water are 0.65 ng/l and in the groundwater 45 ng/l (Sweco Environment AB, 2019). In Bangladesh there is surface water with PFAS of 50 ng/l, which is an incredibly much higher level. If there is the same percentage difference between surface water and groundwater PFOS in Umeå as PFAS in Bangladesh, the groundwater should be approximately 68 times greater than the surface water. This gives a value of just over 3 400 ng/l. These values, even much higher, can possibly be found in the groundwater of Bangladesh and thus our values in the experiment are reasonable.

In addition to the values presented in the report from Bangladesh, we learn that a finding frequency of PFOA of 98% gave a maximum value of 9500 ng/l from samples in Sweden. These values are found in leachate and have a high risk of being spread into the environment (Miljösamverkan Sverige, 2022). Our choices of concentrations are therefore absolutely reasonable. In addition, the report states that PFAS 11 was at 194 367 ng/l in a leachate sample. We only investigate a single chemical, but in the environment there are several chemicals in the same place that affect the plants together. There is thus a risk that as much as 194 367 ng/l of PFAS will enter the environment and affect the plants.

In the report on PFAS around Umeå Airport there was an area with a maximum value of PFAS 11 which was 300 000 ng/l in groundwater samples. In the same report, PFOS, a very common PFAS, was investigated, which in the same area had a maximum value of 24 000 ng/l. These high levels are thus also found in our immediate environment in Sweden (Sweco Environment AB, 2019). If these high levels are found in Sweden, which nevertheless has limit values and guideline values these days, higher levels than what the report "PFAS: Bangladesh Situation Report" puts forward should be found in Bangladesh. Bangladesh has no rules regarding how the chemicals may be used, and large amounts are thus found in all types of industries. Again, our choices of concentrations are not unreasonable.

4.3.2 Analysis of error sources and limitations

The 2 ml of PFOA we received from Umeå University was diluted in methanol. Therefore, methanol was added to achieve the same concentration in all beakers to exclude the possible but very minimal source of error in plant development. Methanol in such low concentrations should not affect the plant's development. Had this been the case, however, our results would still be reliable as all plants were exposed to the same concentration. We therefore believe that this possible but minimal source of error can be excluded from our results.

The project was limited to a few experiments because only 2 ml of PFOA was available for the entire work. This obviously leads to a possible source of error as only 3 experiments could be performed. Carrying out the experiment several times is a matter of course in the scientific world. It is clear that our report would have become more reliable if more experiments could have been carried out as more data could have been analyzed and given more fair values, but since this is a high school project we have to make do with the equipment we had access to.

The diploma project has limited time and the time aspect has thus been a major limitation for our continued work. This meant that we could not perform as many experiments as desired. The optimal thing would have been to test each method several times so that more reliable answers could have been discussed. Moreover, Experiment 3 showing no difference in growth may be due to some source of error. What exactly happened during the experiment and why the results turned out the way they did is difficult to determine. What we can discuss is that dependent growth, just like our nature works, is affected by PFOA. The reason why plants living together grew worse in the presence of PFOA and that individual seeds (independent cultures) were not affected by PFOA is difficult for us to say. In contrast, the control plants grew differently at first and of the test tubes that were filmed during Experiment 3 there were also seeds that had not even begun to germinate. This makes the results difficult to use and discuss.

4.3.3 Possible effects on future vegetation in Bangladesh

With the help of our report, we can put forward a possible idea of what the future vegetation will look like. From our data on dependent growth, we know that the plants are negatively affected in their development when exposed to high levels of PFOA. This means that the nature around the textile industries is very likely also affected by the large chemical emissions. Assumptions of impaired growth in length and affected vegetation in Bangladesh vegetation can be assumed. These persistent chemicals accumulate over time and affect the plants even more negatively as long as the industries continue to pollute the water as there is currently no law on emission management. The plants around the industries will be affected with continued high exposure to PFOA. This means that the seeds will either not germinate, the plants will not reach their normal length, or the plants will die prematurely to a large extent. These possible situations are of course devastating for future plant life. The companies, including Zara and H&M, that use these industries need to take this information into account and review their textile manufacturing decisions. The companies contribute to the success of the industries and thereby also contribute to the release of PFOA and other chemicals.

Our project only deals with a single chemical, PFOA. In nature, however, there are numerous chemicals, including several PFASs, which together have a negative effect. The levels of chemicals found around the textile industries as a whole are much higher than the values we have used. If we get results from experiments with only PFOA, we can only imagine how the plants are affected in conditions with the majority of chemicals.

4.3.4 The project's connection to Sweden

If we then link our project to Sweden's exposure to these chemicals, high levels of PFOS and PFAS 11 at Umeå Airport have been found. Although PFOA was not the main chemical the report studied, PFASs work in much the same way. This gives us a picture of how the high levels in Umeå affect the vegetation. Fire extinguishing foam with PFAS stopped being used already in 2008, which is 16 years ago. After 16 years, there are still excessively high levels of PFAS 11 (maximum value of 300 000 ng/l) around the airport (Sweco Environment AB, 2019). This proves the chemical's persistent ability and how it stays in our circuits. We therefore believe that a purification method is possibly the only way to remove the high levels. The chemicals around Umeå's airport that have polluted the water will reach farmland and make their way further into the food chains. From there, the chemicals will not only affect the crops but also us humans who ingest the substances. Not only have high levels been found around Umeå, drinking water in southern Sweden has also been discovered with high levels of PFAS. At least two million Swedes therefore drink contaminated water that exceeds the Danish limit value of 2 ng/l. In addition, the drinking water for 200 000 Swedes exceeds EFSA's guideline value for the total intake of PFAS (Naturskyddsföreningen, 2022). Sweden's exposure to the chemicals PFAS is therefore incredibly serious. In addition to these areas, there are also incredibly high levels of PFAS 11 in leachate from landfills. 194 367 ng/l is the maximum value found in a single leachate sample. The maximum value of PFOA was approximately 9500 ng/l which is still much higher than the highest level we used. A finding frequency of 98% thus makes PFOA the second most abundant chemical of PFAS (Miljösamverkan Sverige, 2022). The contaminated areas around Umeå Airport are connected to the groundwater system and thus have direct contact with both the Umeå Älvdal and other land.

4.3.5 Hypothesis

In our report there is no hypothesis because we have not found scientific data behind how plants are affected by PFOA although more studies have been done on PFAS substances in recent times. According to our research, there are few scientific designs linked to the chemical PFOA, and thus a hypothesis is not possible as it must have a scientific basis. This means that our hypothesis would consist of pure guesswork, which is not scientifically correct, and thus the hypothesis is not included in the report.

4.3.6 Further studies

Despite the project's success, there is an opportunity to develop it. This project was carried out at a high school level of work with limitations in access to data, resources and other equipment. Through our execution, it was possible to observe a visual difference in the dependent cultures, but if you take the research further to a microscopic level, it becomes possible to understand what it is that actually causes the phenotypes we see to express themselves. We can only speculate about possible mutations, destruction in cell structure but with further research, such observations can be carried out with the right equipment. With our work, we hope to inspire researchers to continue working towards effective purification methods that are both less advanced and, above all, cost-effective so that all countries with industries invest in the purification of PFAS. In addition to this, we would like to encourage the individual to buy more locally produced clothes in Europe or second hand. This is in order not to contribute to an equal spread of these dangerous chemicals, which have been shown to not only harm human health but also the existence of plants at high concentrations. In addition, more research on phytoremediation and which plants can be used in Bangladesh, among others, would be necessary for a sustainable purification method.

4.3.7 Phytoremediation

Unfortunately, we did not have the opportunity to perform tests of how much PFOA the cress plants actually took up in our experiments because the chemists at Umeå University did not have time to help us. However, we can say with a fair degree of certainty that the plants take up PFOA through their roots and into the plant mass, both based on our results but also because PFOA is water-soluble. The plants in all our experiments were still alive at high levels of PFOA. Although they grew worse, they were still resistant to a high level of hazardous substances, especially in Experiment 3. Our research can be used to discuss phytoremediation; purification methods that utilize the ability of plants to purify or reduce pollution from soil and water. The size of the plant biomass plays a more important role in effective purification than a specific plant species (Greger, Landberg. 2024). With our project, we have shown that cress grows worse under conditions with high levels of PFOA in dependent farms, but the plant still manages to be alive. This can be used to reverse our purpose of the report and instead focus on planting plants at contaminated areas for the purpose of cleaning. The plants are then harvested and PFAS is separated from the area. Sedge plants were the most effective plants in wetland areas as they reduced the level of PFAS by as much as 40% in just one day (Greger, Landberg. 2024). In areas of larger waterways, mangroves could possibly be used to clean the water from pollution as they have wide root systems completely below the surface. Using phytoremediation in particular does not have to be an expensive method to clean and can therefore also be used in Bangladesh where the focus on expensive cleaning methods is not in focus. Although the report "Removal of PFAS from water by aquatic plants" states that the size of the plant biomass is the most important for effective purification, we cannot with our project draw any conclusions about which plant species is the most optimal to use. Mangrove may be good, but it is only a suggestion we have, which has no scientific basis around phytoremediation. However, all plants work in the same way and the larger the plant, the easier it should be to absorb pollutants into the plant mass as it requires more water. The same applies to a wider root system. The wider the root system, the more water the plant species must have and the more pollutants are absorbed.

References

Environment and Social Development Organization (2019). *PFAS: Bangladesh Situation Report*. https://esdo.org/2019/03/pfas-bangladesh-situation-report/

Sweco Environment AB. (2019). *PFAS Umeå Airport*. Swedavia AB. https://www.umea.se/download/18.250f9659174ae4b9794251e/1601906473634/Kompletterande%20milj%C3%B6teknisk%20markunders%C3%B6kning.pdf (retrieved 2024-03-10)

Naturskyddsföreningen (2022). *Minst 2 miljoner svenskar har för mycket PFAS i dricksvattnet*. Retrieved 2024-03-11 from, https://www.naturskyddsforeningen.se/artiklar/minst-2-miljoner-svenskar-har-for-mycket-pfas-i-dricksvattnet/

Miljösamverkan Sverige (2022). *PFAS vid deponier*. Retrieved 2024-05-27 from, https://www.miljosamverkansverige.se/wp-content/uploads/2022-01-27-Rapport-PFAS-vid-deponier.pdf

Livsmedelsverket (2023). *PFAS i dricksvatten och livsmedel - kontroll*. Retrieved 2023-10-27, from https://www.livsmedelsverket.se/foretagande-regler-kontroll/dricksvattenproduktion/riskhantering-pfas-i-dricksvatten-egenfangad-fisk

Naturskyddsföreningen (2022). Updated 2023-09-12. *Frågor och svar om PFAS*. Retrieved 2023-10-27, from https://www.naturskyddsforeningen.se/artiklar/vad-ar-pfas/

Raspberry Pi. (2024). *Raspberry Pi 3 Model B*. Retrieved 2024-03-06, from https://www.raspberrypi.com/products/raspberry-pi-3-model-b/

Livsmedelsverket (2024). *PFAS - Per- och polyfluorerade alkylsubstanser*. Retrieved 2024-03-11, from https://www.livsmedelsverket.se/livsmedel-och-innehall/oonskade-amnen/miljogifter/pfas-poly-och-perfluorerade-alkylsubstanser

Kind, J. (2012). *Fytoremediering - Ett hållbart sätt att tillgängliggöra förorenad mark?* [Kandidatexamensarbete, Sveriges lantbruksuniversitet]. Retrieved 2024-05-29, from https://stud.epsilon.slu.se/4584/1/kind_j_120802.pdf

Greger, M. Landberg, T. (2024). *Removal of PFAS from water by aquatic plants*. [Department of Ecology, Environment, and Plant Sciences, Stockholm University]. Retrieved 2024-05-29, from Removal of PFAS from water by aquatic plants

Critical Review of Sources

The sources used are reliable as they are reports and articles from authorities in a democratic country, from universities that have reviewed the publications and from organizations. The source "Raspberry Pi 3 Model B" can be found on the website Raspberry Pi which is a company. The page exists to inform about how this computer works and how it can be used for consumers.