

Efficacy of Algae Phytoremediation vs Sand

Filtration of Bisphenol-a Solution

Science Capstone

2021

PURPOSE

The purpose of this research was to determine if a culture of *Chlorella pyrenoidosa* could remove BPA from an aqueous solution, and if so, to compare the efficacy of this phytoremediation to sand filtration of the same solution.

HYPOTHESIS

It was hypothesized that a culture of *C. pyrenoidosa* would reduce BPA content of a solution by 50%, and sand filtration would do the same by 25%.

BACKGROUND

15 million Americans rely on private wells for their drinking water (1). Currently, sewage is treated in a multistep process to remove fats, oils, sugars, and excess nutrients such as nitrogen and phosphorus, especially if the sewage is to be released into a sensitive ecosystem (2). Despite these efforts at water filtration, many municipalities fall short in providing clean drinking water to their residents. In an effort to combat the deleterious health effects of toxic concentrations of nitrogen, arsenic, and phosphorous in the drinking water supply, states such as Nebraska have enacted oversight agencies to monitor the levels of nutrients in the water supplies; if concentrations exceed these standards, these small communities have to assume the financial burden of either implementing an expensive water-filtration system or laying pipe to a new water supply (3).

Toxic levels of arsenic, nitrogen, and phosphorus in the water supply have been a concern in the United States since the 1970s; therefore, their effects on the human body have been studied extensively, and multiple measures have been put in place both by the United States

Environmental Protection Agency (USEPA) and by individual state agencies to reduce the concentrations of these nutrients to acceptable levels (usually measured in parts per billion, 4).

Although the world has made great strides in eliminating the risk of contamination from these chemicals in the water supply, in recent years new concerns have arisen about the presence of hormones and their mimics, endocrine disrupting chemicals (EDCs). The presence itself of EDCs in the water supply is not the only cause for concern. New studies are beginning to demonstrate that these compounds may persist in the environment for longer than originally expected (5). Additionally, these compounds may react with each other to form new substances deleterious to our regulatory systems (5). Endocrine disruptors not only affect humans, but aquatic organisms as well (5).

Currently, the EPA monitors the presence of several EDCs in the water supply, including estrogens, progestogens, androgens, and Bisphenol A, or BPA (6). In this research, the EDC of focus is BPA.

BPA is a chemical used in the production of plastics and epoxy resins (7). Polycarbonate plastics have an incredible range of applications, from water bottles to medical devices, and epoxy resins are used to coat the inside of common objects such as bottle caps and metal pipes, making BPA virtually ubiquitous in our environment (7).

Because of its use in manufacturing, the presence of BPA in the water supply is more commonly associated with urbanized areas (8). In fact, BPA has been detected in multiple areas, giving the compound multiple pathways by which to contaminate humans: wastewater effluent, runoff, and atmospheric aerosols from industry (8). However, recent studies have found BPA contamination in the urine and water supplies of people from all over the globe and in a range of socioeconomic strata, demonstrating that the presence of BPA in the environment is a concern

worldwide (8). The primary method by which BPA can get into the human body is through the diet (7). For example, BPA in water bottles can seep into the water which we drink (7). BPA contamination is also widespread. To illustrate, “the 2003-2004 National Health and Nutrition Examination Survey (NHANES III) conducted by the Centers for Disease Control and Prevention (CDC) found detectable levels of BPA in 93% of 2517 urine samples from people six years and older.”

Multiple studies in the past two decades have indicated that BPA is an endocrine disruptor for its ability to imitate the body’s naturally occurring hormones and therefore interfere with the mechanisms of these (9). Research has shown that BPA has a variety of effects on human health. For example, a study done in 2013 by Brigham and Women’s hospital demonstrated BPA can interfere with human egg maturation (9). Additionally, a review of studies published in 2015 suggested that BPA can adversely affect the function of the pituitary and hypothalamus glands in the brain (9). This suggests that BPA exposure may have a negative effect on puberty, ovulation, and may even cause infertility and male impotence (9). Currently, research is being done to determine if the effects of BPA are long-lasting or transgenerational (9). Beside its effects on the reproductive system, BPA has been linked to a variety of other bodily disorders, including heart disease, insulin resistance, asthma, abnormal fetal brain development, and certain cancers (9).

BPA contamination of surface water seems to be more of a problem for Europe and Asia than for the United States (10). To illustrate, a meta-analysis conducted by Arnold et al in 2012 concluded that BPA concentrations were above the detection limit in only 5% of studies conducted in the U.S., whereas BPA concentrations were above the detection limit in 52% of European studies and 59% of Asian studies (10). Additionally, these studies concluded that of

either surface water and groundwater, surface water is much more likely to be contaminated with detectable levels of BPA (10).

Current drinking water treatment technologies, such as activated granulated carbon filtration systems, are capable of reducing BPA concentrations in contaminated surface water sources to below detectable levels (detection limit=0.005 µg/l). However, the manufacturing of these filtration systems and the construction of new facilities or retrofitting of existing ones can incur significant costs, which are shouldered most heavily by rural communities (3). Additionally, activated granulated carbon, though inexpensive for consumers, necessitates a multi-step process in its production (4).

In recent years, plants have been studied for their ability to purify aqueous environments in a process known as phytoremediation. In general, plant phytoremediation of accumulated toxins has been shown to be advantageous over conventional remediation methods due to its low cost and high efficiency (13). In principle, phytoremediation is the process of plants accumulating toxins in themselves in quantities much higher than present in their normal environment (13). In order to be an effective purifying agent, the candidate plant must have a high capacity for absorption of a particular chemical compound, a high surface area to volume ratio, a high growth rate, and the potential for genetic manipulation (13). In addition, recent literature has demonstrated that algae are able to remove heavy metals and arsenic from a water supply (13). In this case, compounds within the algae interact with the toxic compounds and transport them to the algae's vacuoles, where they are stored and metabolized (13). Following studies have suggested that phytoremediation is also applicable to the cleanup of other toxic compounds (13).

Some participants within the agricultural industry have utilized a phytoremediation technique known as Algae Turf Scrubbing (ATS) in order to remove excess nitrogen and phosphorus from irrigation runoff (12). The ATS system works by pumping water over a sloped surface or “raceway” seeded with algae (12). The algae restores the nutrient balance of the water by absorbing excess nutrients and releasing dissolved oxygen during the photosynthetic process (12). Once a week during the growing season, the algae on the raceway are harvested, removing the waste nutrients and rejuvenating the community (12). In a high-light environment, algae are very productive, meaning that they are photosynthetic powerhouses (12). The more productive they are, the more nutrients they can absorb from the water (12). Currently, water can be treated by ATS at a rate of 40 to 80 million liters per day (12).

One genus of algae commonly used in research is *Chlorella*. *Chlorella* are single-celled, freshwater green algae also commonly taken as a nutritional substance (14). Two species of *Chlorella*, *Chlorella pyrenoidosa* and *Chlorella vulgaris*, are commonly used in research for their ability to bind to heavy metals and other toxins, including the hormone disrupting chemical dioxin (14). For this reason, *Chlorella* is often taken as a “detox” supplement in powdered form.

Chlorella pyrenoidosa, in scientific literature, is known as a “model” organism for several distinct traits. It is a simple organism; therefore, there is little risk for nuances of the organism disrupting research experiments (15). Additionally, *C. pyrenoidosa* is resilient and not sensitive to minute changes in the environment (15). Finally, as another benefit of its simplicity and resilience, *C. pyrenoidosa* can be genetically manipulated to fit the needs of individual research using today’s technology (15). For these reasons, *C. pyrenoidosa* is an excellent candidate for a research focus in phytoremediation, which necessitates not only a hardy organism, but one that can potentially be genetically altered to remove specific contaminants.

Beyond phytoremediation, or biological filtration methods, lay sand filtration. This method involves either human-engineered layers of sand or the environment itself (16). For example, at the wastewater treatment plant in Ogallala, Nebraska, sewage from the town is filtered not only through manufactured filters, but also sediment as it flows toward the South Platte River.

The focus of this study will be to determine the efficacy of *Chlorella pyrenoidosa* phytoremediation compared to sand filtration of a 0.2 ppb concentration of BPA. Analyzing the low concentration of solution following experimentation necessitated the use of Liquid Chromatography - Tandem Mass Spectrometry (LC-MS-MS). LC-MS-MS is used for a variety of analyses, including soil and water quality and pharmaceutical samples. This system is highly sensitive and involves several steps. First, the analyte of interest, or the mobile phase, is forced by high pressure through a column or the stationary phase. Distinct chemical interactions between the mobile and stationary phases determine how the sample will separate. Once separation occurs, the effluent is directed to the mass spectrometer, where it becomes ionized. Through the application of electromagnetic magnetic fields, these particles are directed through a series of physical interactions that determine their identity and mass with a high degree of selectivity (17).

MATERIALS/METHODS

Materials

- 12 Algae Research Supply 50ml plastic algae culture tubes
- 2 50 ml tubes of Algae Research Supply *Chlorella Pyrenoidosa* inoculum
- Incubator
- Vernier light sensor
- BPA powder
- Algae Culture Media:
 - 2 1 Liter bottles of water, unopened
 - 2 bags of freshwater algae culture salts
- Washed sand
- Paper filters
- Colorimeter app

Procedure:

1. Prepare a solution of BPA at $0.2 \times 10^{-6}\text{g}$ / 1 liter of water by doing a serial dilution:

- a. Dissolve 0.02 g BPA in 10 ml 95% ethyl alcohol
- b. Dilute to 100 ml with water
- c. Take 1ml of this solution and add it to 99ml of water, making the concentration of the solution $2 \times 10^{-3}\text{g BPA}/100 \text{ ml}$
- d. Repeat step c until concentration reaches $2 \times 10^{-6}\text{g BPA}/100\text{ml}$
- e. Dilute to 1000 ml

2. Prepare BPA algae culture

- a. Open a 500 ml bottle of water and dump out its contents. Fill it with 500 ml of BPA solution.

- b. Add 1 bag of culture salts and 1 vial of nutrient solution to the water. Shake until dissolved.
- c. Discard 50ml of the solution and add 50 ml of algae inoculum.

3. Prepare the Control algae culture

- a. To a separate 500ml bottle of water, add 1 bag of culture salts and 1 vial of nutrient media. Shake until dissolved.
- b. Discard 50ml of the solution and add 50 ml of algae inoculum.

4. Prepare the culture tubes.

- a. Add 50 ml of the BPA algae culture to each of three separate culture tubes.
- b. Add 50 ml of the Control Algae culture to each of three separate culture tubes.
- c. Add 50 ml of BPA solution to each of three separate culture tubes.
- d. Add 50 ml of pure water to each of six culture tubes.

5. Incubation/Growth Period

- a. Set an incubator facing a light source.
- b. Set the temperature to 24° Celcius.
- c. Place culture tubes in an incubator for 14 days, shaking and rotating every 24 hours.

6. Analyze concentrations of solutions using Liquid Chromatography Tandem Mass Spectroscopy (LC-MS-MS).

DATA

The concentration of BPA in the stock solution was 236 parts per billion. The concentration of BPA in filtered pure water in which algae was grown was 0.779 ppb. The BPA concentration of a filtered algae culture grown in the stock solution was 32.3, and the BPA concentration of the stock solution filtered through sand was below the detection limit of the LC-MS-MS. Because only a single sample per group was tested, there was not enough data to perform a statistical analysis.

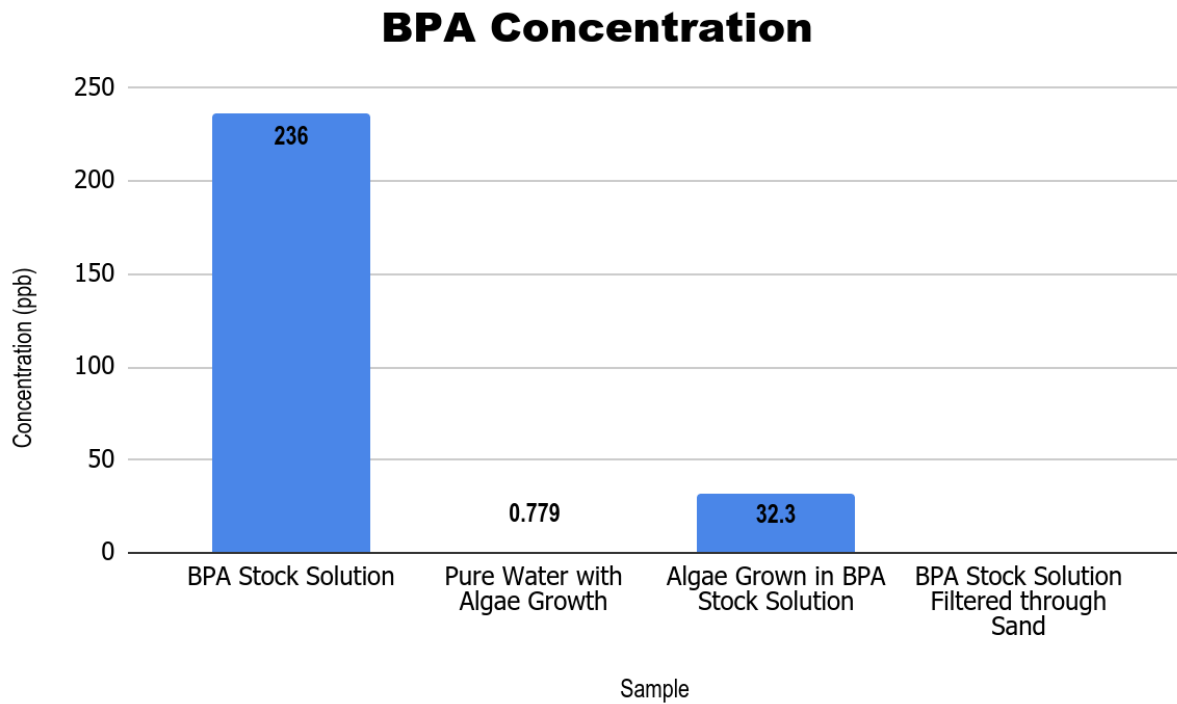


Figure 1

Additionally, light levels during the algae growth cycle were analyzed:

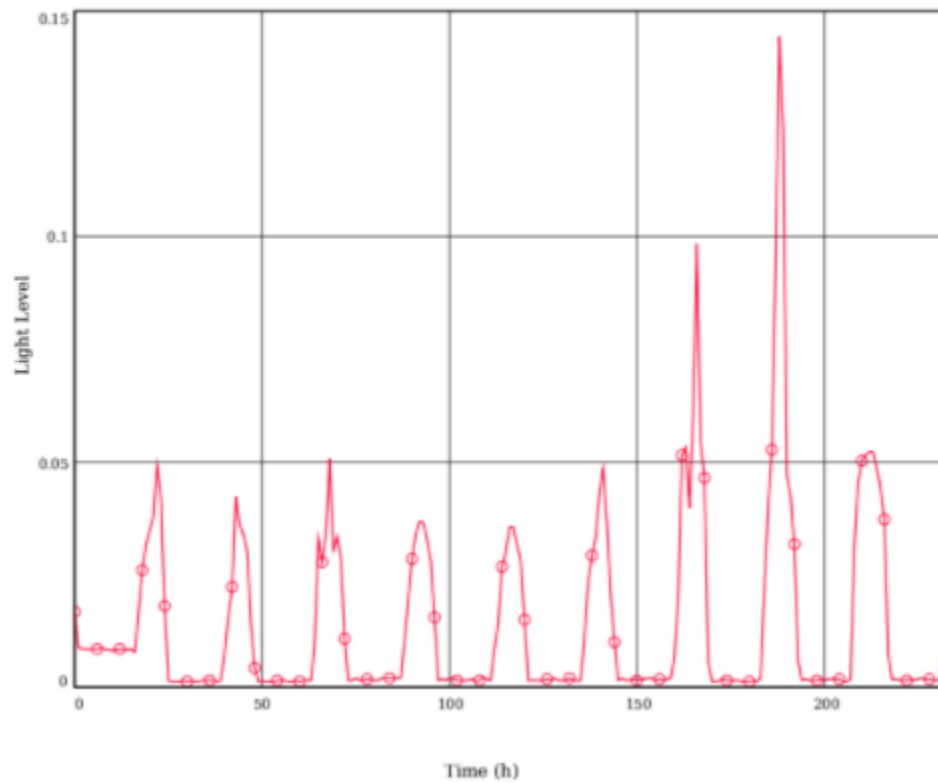


Figure 2

Finally, no changes in algae growth were observed between the algae grown in pure water and those grown in the BPA stock solution.

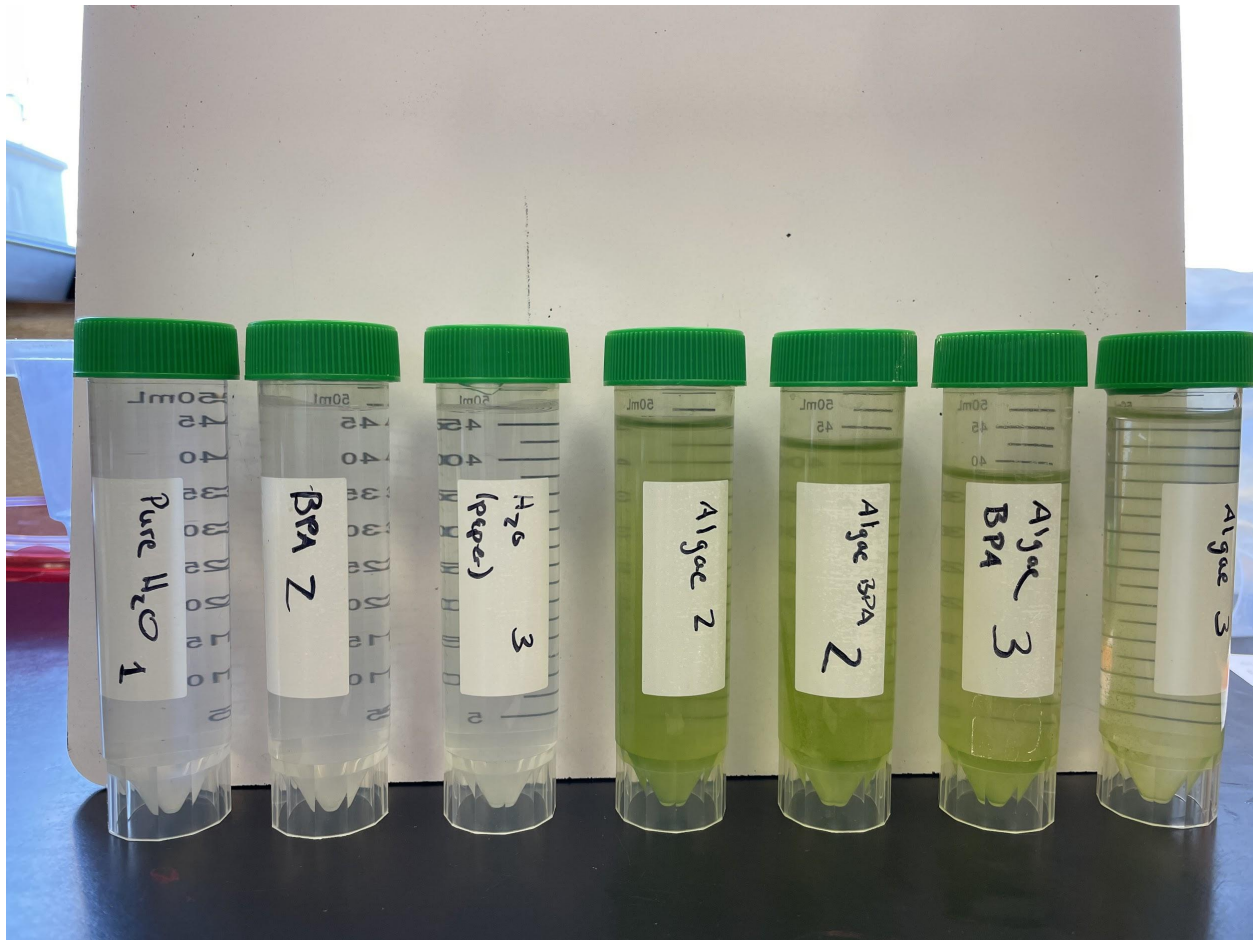


Figure 3

ANALYSIS

236 ppb was the concentration of the BPA stock solution used in this experiment. The 0.779 ppb concentration of BPA measured in the pure water algae culture indicates the BPA levels that leached into the water from the plastic tubes used in this experiment. The sum of these values is the total BPA concentration capable of being filtered from the water by an algae growth or sand. The results of the experiment indicate that an algae culture removed 86.36% of the BPA from the solution it grew in, and sand filtration of the same concentration of solution was almost 100% effective.

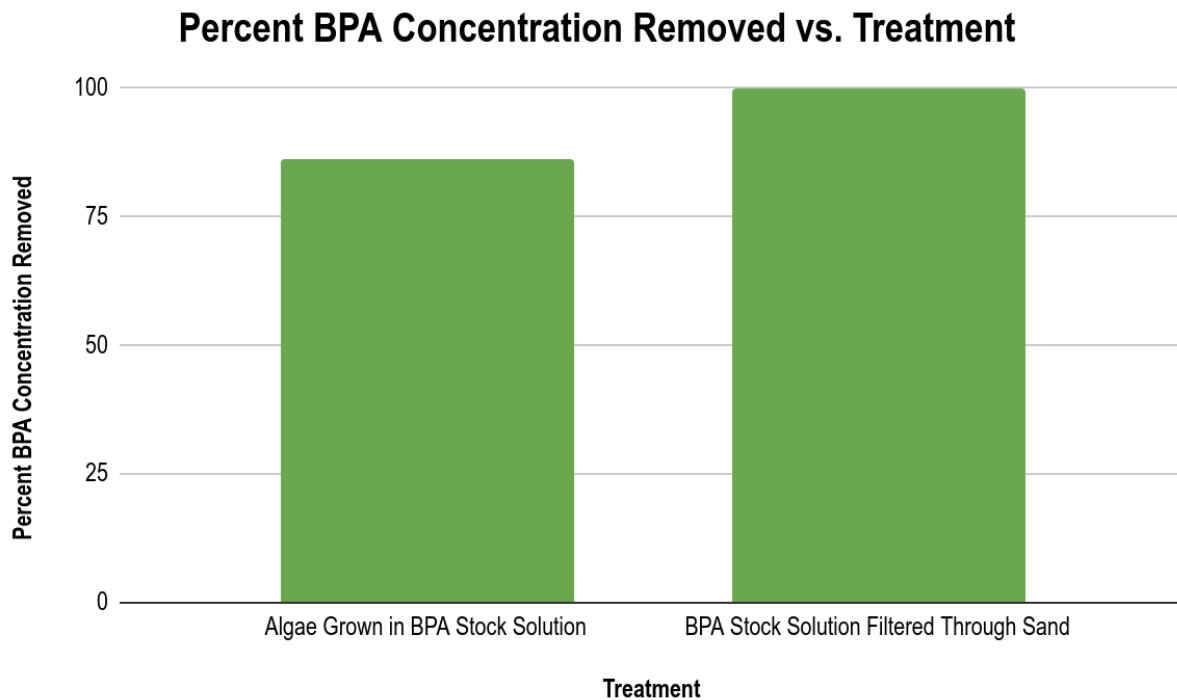


Figure 4

DISCUSSION

Typically, algae growth is measured using a secchi stick. However, the small sample size and short growth period for the experiment resulted in low-density algae cultures that could not be measured in this way. To prove algae growth, besides observational data, a colorimeter app was used to determine the absorbance of red light from the algae cultures and from the BPA solution and pure water samples. An unpaired t-test between these groups produced a two-tailed p-value of 0.0062, indicating statistically significant differences in red light absorbance and proving that the algae cultures were thriving.

The colorimeter app was also used to determine a difference between the algae grown in pure water and the algae grown in the BPA solution. A paired t-test between the two groups produced a non-statistically significant p-value of 0.5000, indicating that the presence of BPA had no effect on the health of the algae cultures.

Ultimately, this experiment produced enough data to yield conclusive results: algae phytoremediation of the BPA solution was over 80% effective, but sand filtration was almost 100% effective at removing BPA from the stock solution. However, the reliability of this data is in question due to the limited sample size that was tested. The initial experimental design proposed three samples for each group, effectively simulating three trials of each treatment. This was to improve the reliability of the data by providing enough data to perform a statistical analysis and determine if the results were statistically significant. However, data collection proved to be a difficult problem: the initial spectrophotometric method offered at no cost for the use of the experiment failed to sense BPA in the samples. The back-up analytical method, Liquid Chromatography Tandem Mass Spectroscopy, was provided by Applied Industrial Technologies. Unfortunately, due to a limited timeline and limited resources, the company was unable to process every sample; only a maximum of four could be sent. These problems illustrate the challenges of conducting a complex scientific experiment in a rural high school setting with limited resources.

Further investigation is necessary to determine what happened to BPA during both the sand-filtration and algae-phytoremediation processes. Moreover, it will be necessary to determine if sand can remove BPA from the environment, and if algae is capable of metabolizing BPA or simply stores it.

REFERENCES

1. Lant, Karla. "Thirsty Moss Can Remove Arsenic From Drinking Water". Environmental Monitor, 2018,
<https://www.fondriest.com/news/thirsty-moss-can-remove-arsenic-from-drinking-water.htm>. Accessed 3 Sept 2020.
2. "17.3B: Wastewater And Sewage Treatment". Biology Libretexts, 2018,
[https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_\(Boundless\)/17%3A_Industrial_Microbiology/17.3%3A_Wastewater_Treatment_and_Water_Purification/17.3B%3A_Wastewater_and_Sewage_Treatment#:~:text=In%20primary%20treatment%2C%20sewage%20is,separate%20process%20called%20sludge%20digestion.](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/17%3A_Industrial_Microbiology/17.3%3A_Wastewater_Treatment_and_Water_Purification/17.3B%3A_Wastewater_and_Sewage_Treatment#:~:text=In%20primary%20treatment%2C%20sewage%20is,separate%20process%20called%20sludge%20digestion.)
Accessed 26 Oct 2020.
3. "Small Communities Pay High Costs For Clean Water | UNL Water". Water.Unl.Edu, 2020,
<https://water.unl.edu/article/groundwater/small-communities-pay-high-costs-clean-water>.
Accessed 7 Nov 2020.
4. Wilsonweb.Physics.Harvard.Edu, 2020,
http://wilsonweb.physics.harvard.edu/arsenic/Historical_Survey.doc. Accessed 7 Nov 2020.
5. Hinnefeld-Indiana, Steve. "Hormones For Cattle Stick Around In Streams - Futurity". Futurity, 2015, <https://www.futurity.org/hormones-beef-cattle-921542/>. Accessed 28 Oct 2020.
6. Epa.Gov, 2020,
https://www.epa.gov/sites/production/files/2016-12/documents/lenehan-hormones_in_water_using_spe_and_lc-ms.pdf. Accessed 14 Oct 2020.

7. "Bisphenol A (BPA)". National Institute Of Environmental Health Sciences, 2020,
<https://www.niehs.nih.gov/health/topics/agents/sya-bpa/index.cfm>. Accessed 3 Nov 2020.
8. Karalius, Vytas P. et al. "Bisphenol A (BPA) Found In Humans And Water In Three Geographic Regions With Distinctly Different Levels Of Economic Development".
Environmental Health Insights, vol 8, 2014, p. EHI.S13130. SAGE Publications,
doi:10.4137/ehi.s13130. Accessed 9 Nov 2020.
9. "Bisphenol A: Hazards And Sources". Medicalnewstoday.Com, 2020,
<https://www.medicalnewstoday.com/articles/221205#hazards>. Accessed 3 Nov 2020.
10. Arnold, Scott M et al. "Relevance Of Drinking Water As A Source Of Human Exposure To Bisphenol A". Journal Of Exposure Science & Environmental Epidemiology, vol 23,
no. 2, 2012, pp. 137-144. Springer Science And Business Media LLC,
doi:10.1038/jes.2012.66. Accessed 9 Nov 2020.
11. Wqa.Org, 2020,
https://www.wqa.org/Portals/0/Technical/Technical%20Fact%20Sheets/2016_GAC.pdf.
Accessed 10 Nov 2020.
12. " Algal Turf Scrubbing: Cleaning Surface Waters With Solar Energy While Produ...:
Ebscohost ". Web.B.Ebscohost.Com, 2020,
<http://web.b.ebscohost.com/ehost/detail/detail?vid=3&sid=b476cad3-3be3-40c8-a420-458f149c50f9%40pdc-v-sessmgr01&bdata=JnNpdGU9ZWwhvc3QtbGl2ZQ%3d%3d>.
Accessed 10 Sept 2020.
13. Jmaterenvirosci.Com, 2020,
[https://www.jmaterenvirosci.com/Document/vol4/vol4_N6/118-JMES-449-2013-BenCh
ekroun.pdf](https://www.jmaterenvirosci.com/Document/vol4/vol4_N6/118-JMES-449-2013-BenCh ekroun.pdf). Accessed 2 Nov 2020.

14. "9 Impressive Health Benefits Of Chlorella". Healthline, 2020,
https://www.healthline.com/nutrition/benefits-of-chlorella#TOC_TITLE_HDR_15.
Accessed 4 Nov 2020.
15. " Model Organisms: Explora Public Libraries ". Web.A.Ebscohost.Com, 2020,
<http://web.a.ebscohost.com/ehpl/detail/detail?vid=4&sid=7a827fc4-893f-49e2-bb3b-6f743a3b7490%40sdc-v-sessmgr01&bdata=#AN=89551759&db=tol>. Accessed 4 Nov 2020.
16. Gill, Laurence W. et al. "Comparison Of Stratified Sand Filters And Percolation Trenches For On-Site Wastewater Treatment". Journal Of Environmental Engineering, vol 135, no. 1, 2009, pp. 8-16. American Society Of Civil Engineers (ASCE),
doi:10.1061/(asce)0733-9372(2009)135:1(8). Accessed 4 Feb 2021.
17. "LC-MS-MS | Liquid Chromatography | EAG Laboratories". EAG Laboratories, 2021,
<https://www.eag.com/techniques/mass-spec/lc-ms-ms/>. Accessed 4 Feb 2021.