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Effects of Antidepressant Metabolites on the Fresh Water Algae, Pseudokirchneriella subcapitata

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SENIOR THESIS APPROVAL

This Honors thesis entitled

Effects of Antidepressant Metabolites on the Fresh Water Algae,

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meets the criteria for acceptance
and has been approved by the undersigned readers.

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Abstract

Quantities of antidepressant metabolites are known to be released through our wastewater facilities into the environment. The effects of many of these metabolites on the environment are unknown. To understand more fully the effect of these metabolites, an assay was conducted using the green alga, *Pseudokirchneriella subcapitata.* Antidepressants are the number one prescribed pharmaceutical in the United States. For this work, Zoloft was utilized as a representative antidepressant in the aquatic environment. It was found that Zoloft significantly impacted the growth of the algae in controlled laboratory experiments.

Introduction

The residues of pharmaceuticals and other chemical household products have been found in significant quantities in the discharge released from water treatment plants (Brennan, Brougham, Roche, & Fogarty, 2006). There are growing concerns among scientists on not only how these residues may affect human health but also how they might adversely affect the environment (Caccia, 1998). Waste water treatment plants (WWTP) receive their water from any drainage of water, such as rain and household sewage. Waste water treatment facilities have been primarily designed to rid water of traditional pollutants and biological waste. Sewage is first treated by holding the water in a temporary stasis to allow all of the heavy and light elements that are not dissolved in water to separate out. The heavy and light elements are removed and continues on to secondary treatment. In this step the sewage is usually chlorinated to rid the water of pathogenic microorganisms (US Department of Interior, 2013). The resulting treated water is subjected to various chemical and biological tests as required by the Environmental Protection Agency to insure that traditional pollutants have been adequately removed. Typically, the treated waste water is also de-chlorinated since chlorine is considered a significant environmental contaminant. Wastewater treatment facilities constructed over the last century were not designed to remove smaller molecules and compounds. These materials would require more expensive and stringent procedures to effectively eliminate. Currently no such procedure exist in the WWTP.

Unfortunately, more than just solid waste gets put down the drain. Household products are routinely disposed of in this manner. These household products include many unused or out-of-date medications. Antidepressants have become more prescribed as depression has become more recognizable and diagnosed (Celiz, Tso, & Aga, 2009). While different antidepressants may have different methods of action in the human body, they all have essentially the same goal, to increase the amount of neurotransmitters within the brain (Geisler & Murphey, 2006). After the drug is metabolized by the body to produce its desired effect, the metabolic products of the drug are flushed from the body through the circulatory system and the urinary tract. These metabolites end up in our wastewater and are not being effectively removed prior to its release into the environment. They are too small in concentration and size for the WWTP or wastewater treatment plants to undergo the expense and difficulty it would take to remove them successfully from the water.

Antidepressant metabolites often have significantly different biological and chemical properties from the original drug due to the antidepressant being extensively metabolized by the body. Sertraline, more commonly known under its commercial name Zoloft, is metabolized to form the metabolite desmethylsertraline, which has a high affinity for P-glycoproteins (Wang, Zhu, Gibson, Markowitz, Donovan, & Devane, 2008). P-glycoproteins are not only found in animals but also plants in which they are important during the development and growth of the cell. Zoloft was the antidepressant selected for this study due to its well-known brand name and metabolite (Geisler & Murphey, 2006).

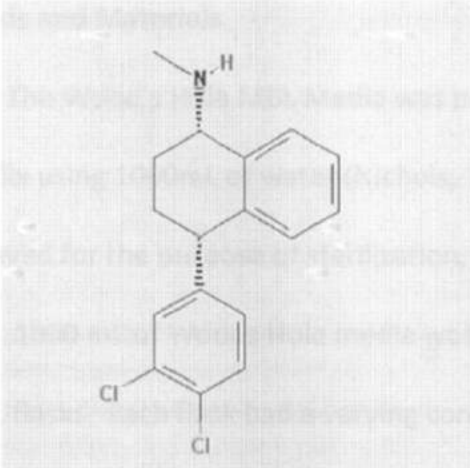

Figure1: The structure of Zoloft. Its metabolite, desmethylsertraline, is the same compound with a second Hydrogen on the Nitrogen replacing the R group (National Center for Biotechnology Information, 2005)

Pseudokirchneriella subcapitata is a species of crescent-shaped freshwater green alga which has a single chloroplast found within the algal cell and can form non-mucilaginous colonies of 4 to 16 cells. These colonies are rarely intertwined, instead forming a matrix by attaching their dorsal sides to other cells. *P. subcapitata* is important in bioassays of water quality, algaecides and environmental assessment. It is an extremely sensitive species and can detect toxins and nutrient levels in extremely limited amounts. *P. subcapitata* also grows at an exponential rate allowing for a large number of algal cells to be grown in limited amount of time (Guiry, 2011). All of these factors make the algae, *P. subcapitata,* an excellent model species to test for the effects of metabolites on living organisms.

The hypothesis of this research was that the antidepressant Zoloft would negatively affect the growth and survival of *P. subcapitata* as the concentration of Zoloft increased. It was hypothesized that relatively high levels of metabolites in the medium would prove toxic to the algae and prevent the formation of more algal cells.

Methods and Materials

The Wood's Hole MBL Media was prepared as outlined in Table 1 of the appendix using 1000mL of water (Nichols, 1973). After the solution had been autoclaved for the purpose of sterilization, one mL of solution 5 was added to the media. 1000 mL of Woods Hole media was made and 250 mL was distributed to four 500 mL flasks. Each flask had a varying concentration of Zoloft: 0 ng/L, 1 ng/L, 2 ng/L and 3 ng/L. The Zoloft was obtained via prescription from Dr. Kluck. After inoculation of 1 mL of $6x10^6$ cells/mL one week old alga was allowed to grow under artificial light in a 16 hour light and 8 hour dark cycle.

After a week of growth a sample was taken of each flask, diluted if necessary, and the cells/ml counted using a hemacytometer. Algal solutions were usually diluted until the hemacytometer had approximately 200 to 300 cells. Algal cells were counted using a hemacytometer, a device originally designed to count blood cells. The hemacytometer determines the number of cells in a predetermined amount of solution using a series of grid lines. The cell concentration in the overall solution is then calculated using the equation:

$$\text{Number of cells counted in } 1\ \mu\text{L} * \text{Dilution factor} * 1000$$
$$= \text{Concentration of cells in orginal solution/milliliter}$$

Results and Discussion

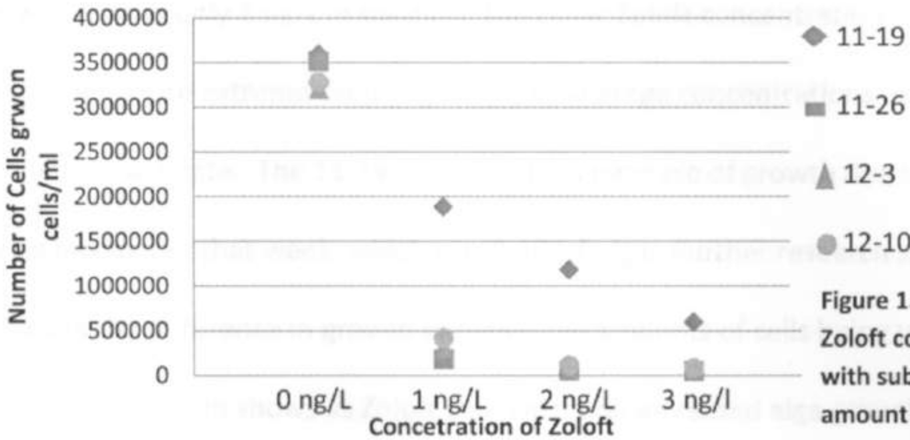

Figure 1. High levels of Zoloft corresponded with subsequent low amount of algal cells.

The cell count in the control was around 3.6 million cells/mL which was expected from growth in the Wood's Hole media from previous research in this lab. The week of 11-19's deviation from the other weeks in the Zoloft-added media differed from the others due to the use of 5 mL of alga while the other weeks' media were only inoculated with 1 mL of alga. In all cases, the cells grown in the presence of Zoloft did show a huge drop in number to the control. Zoloft in as small a dose as 1ng/L inhibited the growth of alga substantially. See table 2 in the appendix for a list of the concentrations.

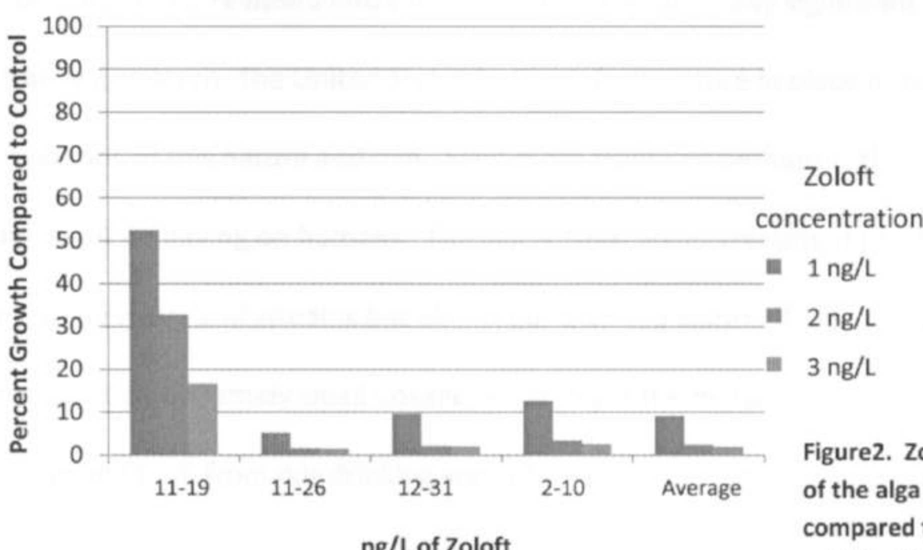

Figure2. Zoloft slowed growth of the alga tremendously when compared to the control of 0 ng/mL of Zoloft

The graph above shows the growth of algae in Zoloft media declined by, on average, 91% in only 1 ng/L in solution. The other Zoloft concentrations saw a similar but an even more extreme drop in growth; all average concentrations passing 90% in declined growth rate. The 11-19 showed a high increase of growth due to the amount of alga inoculated that week, which was 5 mL of alga. Further research should be done to explain the difference in growth between the amounts of cells being used to inoculate. The graph shows as Zoloft concentration increased alga growth decreased. Since 11-19 showed such a massive change of growth due to the difference of amount of alga inoculated, it was not taken into account for the sake of calculating an average with the 3 inoculations of 1 mL of alga. The average with 11-19 taken into account, and all other percentage data can be found in Table 3 of the Appendix.

Zoloft showing such a stark impact on algae is troubling. The depressant is very obviously toxic to the algae and possibly toxic to a number of other plants and animals. With the amount of antidepressants that are being prescribed, and the potential for metabolites to be released into the environment, ecologically significant damage is certainly a concern. The United States has no infrastructure in place to readily filter out compounds of this nature and size. Even more troubling perhaps is the effect that the drug might be having on humans. The water from water treatment plants ends up not only in the rivers and streams but also in our own tap water. If alga is this dramatically affected at an extremely small dosage, what might the metabolites be doing to us as we are exposed to it from our drinking water?

Further research needs to be done on the effect of amount of inoculated alga in the survival of the alga in stressful conditions. This research would explain the large amount of growth still seen when the media was inoculated with 5 mL of alga instead of the preceding 3. Research moving into the effect of Zoloft on animals' survivability and growth would also be informative. The alga grown in Zoloft for this project have been preserved and will be used in feeding experiments with *Daphnia magna*. This work will be conducted in summer of 2013 by another student. In this way, potential movement of drug/metabolite through the food chain may be simulated. Acute toxicity tests using Zoloft and Daphnia will be conducted to determine the adverse effects of Zoloft on animal specimens. Steps need to be taken to devise the potential mechanisms for filtering compounds such as these from wastewater. These mechanisms once found will be expensive to put in place in the water treatment plants, but it appears that as the population grows and an increasing amount of people are using this type of medication, antidepressants, the threat to ourselves and the environment will increase as well.

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Appendix

Table 1	Wood's Hole MBL Media	g/L
Solution 1	$NaNO_3$	85
	$CaC1_2 . 2H_2O$	36.76
Solution 2	K_2HPO_4	48.71
Solution 3	$NaHCO_3$	12.60
Solution 4	$MgSO_4 \bullet 7H_2O$	36.97
Solution 5	Na_2EDTA	4.36
	$FeCl_3 \bullet 6H_2O$	3.15
	$CuSO_4 \bullet 5H_2O$	.01
	$CoSO_4 \bullet 6H_2O$	.01
	$ZnSO_4 \bullet 7H_2O$	.022
	$MnCl_2 \bullet 4H_2O$	.18
	$Na_2MoO_4 \bullet 2H_2O$	.006
	$H3BO_3$	1.0

Table 2	1 ng/ml	2 ng/ml	3 ng/ml	0 ng/ml
11-19	1890000 cells/mL	1185000 cells/mL	600000 cells/mL	3600000 cells/mL
11-26	184000 cells/mL	56000 cells/mL	51000 cells/mL	3510000 cells/mL
12-3	310000 cells/mL	69000 cells/mL	68000 cells/mL	3200000 cells/mL
12-10	416000 cells/mL	112000 cells/mL	84000 cells/mL	3280000 cells/mL

Table 3	1 ng/mL	2ng/mL	3 ng/mL
11-19	52.5	32.77	16.66
11-26	5.24	1.59	1.45
12-3	9.68	2.16	2.12
12-10	12.68	3.41	2.56
Average	20.02	9.99	5.69
Average of 1 mL inoculated	9.2	2.39	2.04