ESSAI

Volume 12 Article 13

Spring 2014

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(Honors Biology 1152)

ABSTRACT

Studies are showing that waste water is contaminated with various types of chemicals, such as pesticides, flame retardants, and pharmaceuticals. The contamination of water with pharmaceuticals is of growing concern, as water treatment plants are not able to fully remove these chemicals from drinking water sources, and their effects on aquatic organisms and humans are unknown. This study looks at how water contaminated with tetracycline and ibuprofen affect the growth of common duckweed (*Lemna minor L*). Plants were placed in test tubes, treated with solutions of the medications, and growth was noted by the number of plants in each test tube after a five week period. Tetracycline was found to have no effect on plant growth, while ibuprofen had negative effects. Further testing may need to be conducted to find why this occurred.

INTRODUCTION

An increasing number of anthropogenically generated chemicals are being found in waste water and sludge (Herberer 2002). Studies have tested the quality of treated waste water released into streams, and have found that they still contain small amounts of chemicals. These chemicals cannot be fully removed during a normal filtration process, as most water treatment plants are not designed to remove persistent chemicals (Vergili 2013).

There is concern over the growing amount of pharmaceuticals found in treated waste water (Jones et al. 2005). Lipid regulators, anti-epileptic, animal growth hormone, and psychotic drugs have all tested positive in samples of treated waste water. Usually the treated waste water is either released into nearby surface waters or used for human consumption (Houtman et al. 2013). However, the effects that pharmaceuticals in drinking water have on humans and aquatic organisms are unknown (Stackelberg et al. 2004).

This experiment used common duckweed (*Lemna minor L.*, Lemnaceae), an aquatic plant that is distributed worldwide, as an indicator species to explore how tetracycline and ibuprofen affect aquatic species. The objective of this experiment was to examine how levels of tetracycline and ibuprofen similar to those found in drinking water affect duckweed growth.

METHODS

Tetracycline (Mars Fishcare North America, Inc., USA) and ibuprofen (Pfizer Inc., Kings Mountain, NC0) were the pharmaceuticals used in the treatments for this experiment, and the experiment was conducted over a period of five weeks. On the first day, 48 test tubes were filled with 55ml of water from a fish tank and three randomly selected duckweed plants were placed into each test tube. Water from a fish tank was chosen to replicate pond water. Test tubes were divided evenly into a control, tetracycline, and ibuprofen group. Concentrations of 50µg/ml of tetracycline and ibuprofen were created to simulate amounts found in drinking water in their respective category. The plants were then placed on a window sill for the duration of the experiment. Plants were treated twice with the chemical solutions and were recounted per replicate after five weeks.

Mann-Whitney rank tests were used to compare counts of the chemical treatments to the control as assumptions of parametric testing could not be met (Zar 1984).

RESULTS AND DISCUSSION

Counts of duckweed did not vary significantly between the control and tetracycline treatment, but did between the control and ibuprofen treatment (Table 1). Significance was determined at $P<0.05$. Overall, plants treated with tetracycline had growth similar to the control, with no decay in plant numbers. Ibuprofen treated plants exhibited either a decay or showed no growth in all but two test tubes. Therefore, neutral effects could not be safely rejected.

There are several reasons why these results could have occurred. First, ibuprofen is composed of various chemicals, including cyclooxygenase inhibitors (Rainsford 2012). Cyclooxygenase is an enzyme that speeds up the production of chemical messengers called prostaglandins. Thus, an inhibitor of cyclooxygenase should slow the production of prostaglandins (Groenewald et al. 1997). Studies have found prostaglandins to have roles in flowering, photosynthesis, and regulating of cell membrane permeability. The cyclooxygenase inhibitors present in ibuprofen could have affected the duckweed's ability for sunlight absorption or nutrient absorption, resulting in the overall decline in plant numbers. The negative effect of ibuprofen on duckweed does imply that if waters that supply to irrigation fields get contaminated, it may decrease crop yields. Also, ibuprofen is an enantiomer, which means that its isomers differ in shape as a result of an asymmetric carbon. This causes only one of the two isomers to be biologically active because only molecules of that form can bind to molecules of that organism. Ibuprofen is normally sold as a mixture of its two enantiomers, both the effective and less effective (Reece et al. 2011). This could have also influenced the duckweed growth, as some test tubes may have had more ineffective enantiomer than effective, which could account for replicate test tubes that showed neither growth nor decay. However, the exact nature of ibuprofen's negative effect on duckweed remained unknown and needs to be further explored through continued studies.

Finally, ibuprofen has been shown to cause gastritis in children (Young 1995), impairment of color vision and visual acuity in adults (Grant 1986), and premature closure of ductus arteriosus if taken after 34-35 weeks of pregnancy (Young 1995). Because this study indicates that ibuprofen in drinking water has negative effects on organisms, further studies investigating its effects on humans is necessary.

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Table 1. Summary (mean ± standard error; all n=16) of average plant counts per treatment. Also provided are Mann-Whitney statistics and probability values from comparisons of the control to the chemical treatment. Significance was determined at $P<0.05$.

Treatment	Mean ± standard error	U	P
Control	3.875 ± 0.256		
Tetracycline	4.063 ± 0.266	119	0.734
Ibuprofen	2.500 ± 0.398	46	<0.002