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EFFECTS OF COPPER AND NITRATE POLLUTION ON SURVIVAL OF THE FRESHWATER AMPHIPOD, *GAMMARUS MINUS*

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ABSTRACT

The amphipod *Gammarus minus* inhabits freshwater springs of central Pennsylvania. These amphipods are extremely sensitive to water chemistry, (i.e. ion content, pH, dissolved oxygen, etc.). This sensitivity allows *G. minus* to be a good bio-indicator. We experimented to determine whether or not *G. minus* would be sensitive to copper and nitrate concentrations. Our experiments revealed that *G. minus* is, in fact, sensitive to both copper and nitrate.

INTRODUCTION

Copper and nitrates have many ill-effects on humans. Copper, though an essential nutrient, can at high doses cause stomach and intestinal distress, liver and kidney damage, and anemia. It is widely distributed in nature in the form of sulfides, arsenites, chlorides, and carbonates. Copper can get into the environment artificially through several human activities including smelting operations. In the vicinity of copper mines or smelting operations, water and soil have been found to be contaminated with copper. According to the Toxics Release Inventory, copper compound releases to land and water totaled nearly 450 million pounds, primarily from copper smelting industries (http://www.epa.gov/ogwdw000/dwh/t-ioc/copper.html).

Nitrates also have effects on human health. In excess, nitrate in drinking water can interfere with the oxygen-carrying capacity of blood. This can lead to serious illness or death. Infants and young children are especially vulnerable to high levels of nitrates. Chronic exposure may lead to hemorrhaging of the spleen. Primary sources of organic nitrate pollution include human sewage and livestock manure. The largest inorganic source of nitrates is fertilizers. According to the Toxics Release Inventory, over 112 million pounds of nitrates were released into land and water between 1991 and 1993 (http://www.epa.gov/ogwdw000/dwh/t-ioc/nitrates.html).

Because of the potential health risks to humans, the Environmental Protection Agency (EPA) has set guidelines for copper and nitrate levels in water. The acceptable level for copper is 1.3 ppm and for nitrates it is 10 ppm. In this experiment we set out to discover whether the freshwater amphipod *Gammarus minus* is a good indicator of copper and nitrate pollution. If they are good indicators of these compounds, they can be used as preliminary indicators of water pollution in the field. Also, they could be used in laboratory situations testing for copper and nitrate levels. If they are good bio-indicators, we believe the nitrates and copper will cause increased mortality to exposed specimens over that seen in a control group.

FIELD SITE

The amphipods in our study were collected from the lower pool of Petersburg Spring near Petersburg, PA. The spring is located in a wooded area between the railroad tracks and the Juniata River, near Petersburg Pike. The great majority of the pond surface is covered with watercress, making it an ideal habitat for many invertebrate species including amphipods, which are abundant there. One can easily collect a large number of specimens in only a few minutes. Petersburg Spring has an approximately neutral pH, and low levels of copper and nitrate. It was important to our study that the background levels of copper and nitrate were low, because we assumed that they were zero when preparing our copper and nitrate treatment levels using Petersburg springwater as a starting medium. Petersburg spring is used as a source of drinking water, and therefore meets the EPA regulations for drinking water

METHODS AND MATERIALS

To begin this experiment, we collected 70 amphipods from Petersburg Spring. They were randomly placed into specimen cups (without regard to sex) each filled with 10 mL of spring water. Solutions of 0.76 ppm cupric sulfate and 21.88 ppm sodium nitrate were made in distilled water. An amount of the original spring water was drawn out of each cup and replaced with an amount of the stock solution according to the equation C1V1=C2V2 such that the final solutions for copper were 0.165 ppm, 0.327 ppm, and 0.66 ppm. The original volume of 10 mL was maintained. Each of the 3 treatments contained 10 specimens. The nitrate treatment was carried out in the same way. The final concentrations for nitrate were 3.3 ppm, 6.6 ppm, and 15.3 ppm. A control group of 10 specimens was set up in only spring water.

For the second trial, we made 3 solutions of each of the compounds. We made solutions of cupric sulfate and filtered spring water such that the concentrations were 0.325 ppm, 0.65 ppm, and 1.3 ppm. Each solution was divided into ten cups containing 50 mL of the solution. To each cup was then added one amphipod randomly picked, regardless of sex. We made solutions of sodium nitrate in the same way such that the final concentrations were 5 ppm, 10 ppm, and 20 ppm. The solutions were then divided into 10 cups containing 50 mL and one amphipod was added to each container. We again included a control group of 10 individuals in only filtered spring water.

During both trials all of the 70 specimens were kept in the climate controlled refrigerator for 5 days. They were checked every 24 hours and the total number of dead specimens in each group was recorded. At the end of each trial, chi-square tests (using Minitab software) were performed to determine if the various nitrate and copper treatmentur affected amphipod survival (number living vs dead after five days).

RESULTS

Table 1 displays the results of both trials. Increased copper concentrations significantly increased amphipod mortality in both trials, whereas nitrate had this effect only in the second trial (Tables 2 and 3). P-values close to 1.000 are not significant, while P-values close to 0.000 are significant.

Table 1. Five-day survival of Gammarus minus in response to various concentrations of nitrate and copper in trials 1 and 2

Trial 1		alive	dead	Trial 2		alive	dead
nitrate	3.3 ppm	10	0	nitrate	5 ppm	0	10
	6.6 ppm	10	0		10 ppm	8	2
	15.3 ppm	10	0		20 ppm	1	9
copper	.165 ppm	0	10	copper	.375 ppm	0	10
	.327ppm	0	10		.65 ppm	0	10
	.66 ppm	0	10		1.3 ppm	0	10
control		10	0	control		9	1

Table 2. Chi-square and P-values for effects of nitrate and copper on Gammarus minus survival in trial 1.

Trial #1		
Nitrate	chi square value = 0.000	P-value = 1.00
Copper	chi-square value = 40.000	P-value = 0.000

Table 3. Chi-square and P-values for effects of nitrate and copper on Gammarus minus survival in trial 2.

Trial #2		
Nitrate	chi square value = 26.263	P-value = 0.000
Copper	chi square value = 34.839	P-value = 0.000

DISCUSSION

Statistical analysis shows our results to be highly significant. The only results that did not show a significant effect were the results for the first nitrate test. Both copper tests showed a very high significance, indicating that the presence of copper has a strong effect on amphipod mortality. Our experiment shows that the presence of copper, even in amounts well below the regulated levels, significantly increases the death of amphipods. We conclude that amphipods are extremely sensitive to even low levels of copper. The results of the second nitrate test were also highly significant, indicating that nitrate present in the range of EPA regulation has an effect on amphipod mortality. We conclude that amphipods are sensitive to nitrate levels in the vicinity of the regulated levels.

The results from the nitrate portion of our experiment were very unexpected. While the amphipods died at 5 ppm in the filtered spring water, they lived at similar concentrations in trial one for which a large amount of deionized water was used. One of the reasons this is so unexpected is that deionized water is often fatal to amphipods. One hypothesis for these unusual results is that when the amphipods were put into the deionized water they went into shock; their systems shut down and they stopped absorbing water from their surroundings. In this state they were resistant not only to the deionized water, but also to the nitrate levels. This hypothesis is supported by the observation that in all of the deionized tests the amphipods showed very little movement and seemed to be in poor health. In the second nitrate test, done in filtered spring water, the amphipods showed significant death at the 5 ppm and 20 ppm concentrations. This is consistent with the concept of optimum range put forth in Shelford's Law. At 5ppm the amphipods had too little nitrate and at 20ppm they had too much nitrate. While the amphipods were able to survive with little nitrate in the wild, we believe this is because decaying material, which was filtered out of our water, provides a constant source of low levels of nitrate.

We believe our results to be inconclusive. Further study is needed to verify our results and would lead to a better understanding of amphipod sensitivity to copper and nitrate. Especially useful would be studies that used a different source for copper. We used copper sulfate as our copper source. While the levels of sulfur the amphipods were exposed to were well below those regulated by the EPA, we cannot rule out the possibility that the presence of sulfur had some effect on the amphipod mortality in the copper tests. Further study of the effects of deionized water on amphipod physiology would also be useful in understanding our nitrate results.

ACKNOWLEDGMENTS

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LITERATURE CITED

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