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A new mechanism of macrophyte mitigation: How submerged plants reduce malathion's acute toxicity to aquatic animals

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HIGHLIGHTS

- We examined the mechanisms driving malathion's mitigation by submerged plants.
- Sorption by plants played virtually no role in mitigating malathion's toxicity.
- Plants mitigated malathion's toxicity by increasing water pH during photosynthesis.
- These results can improve insecticide effects models and mitigation strategies.

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ABSTRACT

A growing body of evidence suggests that aquatic plants can mitigate the toxicity of insecticides to sensitive aquatic animals. The current paradigm is that this ability is driven primarily by insecticide sorption to plant tissues, especially for hydrophobic compounds. However, recent work shows that submerged plants can strongly mitigate the toxicity of the relatively hydrophilic insecticide malathion, despite the fact that this compound exhibits a slow sorption rate to plants. To examine this disparity, we tested the hypothesis that the mitigating effect of submerged plants on malathion's toxicity is driven primarily by the increased water pH from plant photosynthesis causing the hydrolysis of malathion, rather than by sorption. To do this, we compared zooplankton (*Daphnia magna*) survival across five environmentally relevant malathion concentrations (0, 1, 4, 6, or 36 $\mu g\ L^{-1}$) in test containers where we chemically manipulated water pH in the absence of plants or added the submerged plant (*Elodea canadensis*) but manipulated plant photosynthetic activity via shading or no shading. We discovered that malathion was equally lethal to *Daphnia* at all concentrations tested when photosynthetically inactive (i.e. shaded) plants were present (pH at time of dosing = 7.8) or when pH was chemically decreased (pH = 7.7). In contrast, when photosynthetically active (i.e. unshaded) plants were present (pH = 9.8) or when pH was chemically increased (pH = 9.5), the effects of 4 and 6 $\mu g\ L^{-1}$ of malathion on *Daphnia* were mitigated strongly and to an equal degree. These results demonstrate that the mitigating effect of submerged plants on malathion's toxicity can be explained entirely by a mechanism of photosynthesizing plants causing an increase in water pH, resulting in rapid malathion hydrolysis. Our findings suggest that current ecotoxicological models and phytoremediation strategies may be overlooking a critical mechanism for mitigating pesticides.

1. Introduction

A major contemporary challenge in ecotoxicology is to identify and understand the factors that can mitigate the effects of contaminants in aquatic communities. In the past 15 years, aquatic plants have emerged as one factor that can have a strong influence on the transport, fate, and ecological effects of many contaminants (Cooper et al., 2004; Reichenberger et al., 2007; Moore et al., 2011). However, the degree to which plants mitigate contaminant effects in aquatic ecosystems is highly variable and there is a critical need for research that examines the mechanisms driving contaminant mitigation.

The current approach used in models designed to predict the influence of aquatic plants on the fate and effects of insecticides in surface waters (e.g., AQUATOX, CATS, etc.; Park et al., 2008) is

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based primarily on the octanol–water partition coefficient (K_{ow}) of an insecticide. For highly hydrophobic insecticides (i.e. $\log K_{ow} > 5$) such as DDT and pyrethroids, plants can remove nearly all of a compound from the water column within a few hours (Gao et al., 2000a; Hand et al., 2001; Leistra et al., 2003). For less hydrophobic insecticides ($\log K_{ow} < 3$), however, plants remove the insecticides from the water column much more slowly. As a result, these chemicals can be detected in the water for several days after application (Crum et al., 1999; Gao et al., 2000a). Under the current sorption paradigm, one is left to conclude that plants should have weak mitigating effects on insecticides with low $\log K_{ow}$ values (e.g., Gao et al., 2000b). While this is a logical extrapolation, new evidence suggests that this is not always the case.

Recent studies demonstrate that aquatic plants can strongly mitigate the toxicity of the widely used organophosphate insecticide malathion to the sensitive aquatic zooplankter *Daphnia magna*, despite malathion's relatively low octanol–water partition coefficient ($\log K_{ow} = 2.75$). In one study, Brogan and Relyea (2013a) tested the lethality of malathion to *Daphnia* across a range of densities of the submerged plant *Elodea canadensis* and demonstrated that with each increase in plant density, malathion's toxicity to *Daphnia* decreased. Further, by removing samples of water at several time points following malathion applications and then exposing *Daphnia* to them, the researchers discovered that in the absence of *Elodea*, water treated with as little as $5\ \mu g\ L^{-1}$ of malathion was still toxic to *Daphnia* after 48 h, whereas *Elodea* detoxified water treated with up to $30\ \mu g\ L^{-1}$ of malathion within 2–6 h. In a subsequent study, Brogan and Relyea (2013b) found that four species of submerged plants all strongly and equally mitigated malathion's toxicity to *Daphnia* relative to systems containing no plants or plastic-plant controls. These studies demonstrated clear mitigating effects of submerged plants on malathion's toxicity, but they were not designed to elucidate the mechanism by which the plants were able to mitigate an insecticide that current toxicological models suggest should be weakly mitigated.

One alternative mechanism that could mitigate malathion's toxicity is the increase in pH caused by plants that can in turn cause the breakdown of malathion. During photosynthesis, submerged plants take up dissolved carbon dioxide from the water; this initiates the conversion of carbonic acid into CO_2 and increases water pH by shifting the bicarbonate buffer system towards the more alkaline molecules bicarbonate and carbonate (Wetzel, 2001). This increase in pH may be important because malathion is rapidly hydrolyzed under alkaline aquatic conditions. For example, malathion's half-life in water is approximately 3 d at a pH of 7, but it is 19 h at a pH of 8 and 2.4 h at a pH of 10 (no temperature data provided; Seaman and Riedl, 1986). Wolfe et al. (1977) reported similar half-lives at 30 °C, although at pH 10 malathion's half-life was substantially less than 1 h. While these studies suggest that alkaline hydrolysis could potentially play an important role in the detoxification of malathion in water, no studies to date have examined whether alteration of pH is a mechanism whereby plants can mitigate the lethal effects of insecticides on animals. This is an important step as some of malathion's breakdown products resulting from alkaline hydrolysis (e.g., malaoxon, diethyl fumurate) can be more toxic to aquatic animals than malathion itself (Bender, 1969; Aker et al., 2008).

We addressed this important gap in our knowledge by exploring whether plant-mediated and chemical-mediated changes in water pH can alter malathion's toxicity to *Daphnia*. Our approach allows us to tease apart the independent influence of pH on malathion's toxicity from the effects of other potential interactions between plants and malathion, such as sorption. Based on our hypothesis that aquatic plants mitigate the effects of insecticides on sensitive animal taxa by a mechanism of increasing water pH, we made the following predictions: (1) adding aquatic plants in full sunlight to allow photosynthesis will mitigate malathion's effect on *Daphnia*, (2) chemically increasing water pH by the same amount as a photosynthesizing plant will mitigate malathion's effect on *Daphnia* to a similar degree, and (3) adding aquatic plants in complete shade to prevent photosynthesis but to allow other plant-insecticide interactions such as sorption will not mitigate malathion's effect on *Daphnia*.

2. Materials and methods

2.1. Experimental design

We tested these predictions using an experiment conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology in July 2012. We exposed *D. magna* to five nominal malathion concentrations (0, 1, 5, 10, 50 $\mu g\ L^{-1}$) crossed in a factorial manner with four aquatic environments (plants in full sunlight, plants in complete shade, chemical additions to maintain low pH without plants, and chemical additions to maintain high pH without plants). The 20 treatments were replicated four times for a total of 80 experimental units.

2.2. Insecticide selection

While a major reason that we performed the present study using malathion was to test hypotheses arising from discoveries in previous studies (Brogan and Relyea, 2013a,b), we also selected this insecticide because it is widespread usage and potential for contamination of surface waters. With approximately 10–14 million kg applied annually (Kiely et al., 2004, National Pesticide Use Database, www.ncfap.org/database/national.php), malathion is one of the most common active ingredients applied in the U.S. (Grube et al., 2011). The nominal concentrations in the present study span a range of estimated environmental concentrations for malathion exposure in aquatic environments (0–36 $\mu g\ L^{-1}$), accounting for application frequencies and estimated drift (Odenkirchen and Wente, 2007).

2.3. Species collection and husbandry

For our malathion toxicity assays, we used a mixture of four genetically distinct *D. magna* (hereafter *Daphnia*) clones originating from Katholieke Universiteit Leuven, Belgium that we had reared in the lab since winter 2010 using methods described in Brogan and Relyea (2013a,b). Approximately 1 month prior to the start of our experiment, we stopped culling the populations and pooled individuals from all *Daphnia* families together in a 15-L container so that we could generate enough gravid females to use <24-h old neonates for our 48-h survival test. No ephippia were observed in our *Daphnia* cultures at any point within 2 months prior to our experiment.

We collected and cultured *E. canadensis* (hereafter *Elodea*) from three artificial ponds located in northwestern Pennsylvania, USA (41°35′19.12″N, 80°14′40.61″W) on 15 June. More detailed descriptions of the ponds and culturing methods for *Elodea* are described in Brogan and Relyea (2013a,b). We cultured the plants for 25 d before adding them to the experiment.

2.4. Experimental setup

The experimental units were 0.95-L glass jars containing 700 mL of UV-filtered well water. On 9 July, we set up all 80 jars and added *Elodea* shoots to the appropriate treatments. To set up the treatments containing plants, we harvested the top 12 cm of *Elodea* shoots in our culturing tanks and rubbed each shoot under

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