The Journal of Young Investigators
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Volume 20, Issue 5
November 2010

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ABSTRACT

Anthropogenic activities may influence rates of microbial activity through the increased concentrations of pollutants. One relatively understudied pollutant is lithium. In addition to natural sources of lithium, lithium is also derived from a variety of manufactured goods such as pharmaceuticals and electronic devices. Thus, environmental concentrations of lithium are increasing. We studied the effect of lithium on microbial activity measured as respiration, nutrient uptake, and nitrification rates. Homogenized sediment samples were collected from the White River, in central Indiana for *in vitro* activity assays. Lithium treatments used in assays were representative of the range of lithium concentrations found in natural environments. We found that microbial respiration rates decreased linearly with increasing lithium concentrations. Moreover, no effect of lithium was observed on nitrate and phosphate uptake. These data indicate lithium does influence microbial activity, even at trace concentrations currently measured in natural ecosystems. Lithium contamination in freshwater ecosystems may thus threaten not only microbial communities but also higher organisms. A better understanding of lithium poisoning can lead to the informed development of ways to mitigate harmful effects of lithium in the natural environment.

INTRODUCTION

I. *Importance of Microbes*

Microbes act as the backbone of our world by recycling elements in ecosystems, degrading pollutants, and serving as a basal food resource (delGiorgio and Cole 1998). Microbes provide many beneficial functions for society. For example, microbes play a vital role in human health through symbioses. Such an example is the human digestive process through which microbial lining of the small and large intestines helps to degrade consumed food. Some microbes even produce natural antibiotics that are used as human pharmaceuticals (Willey, Sherwood and Woolverton 2008). In the environment, microbes are equally essential. Microbial communities are utilized in sewage treatment plants where they help degrade waste products. Microbes are also fundamental to elemental fluxes in biogeochemical cycles including methanogenesis and decomposition (carbon cycle); nutrient assimilation (all nutrients); nitrification (nitrogen cycle); and sulfide reduction (sulfur cycle).

II. *Microbial respiration & nitrification*

One fundamental, but understudied role of microbes is the potential degradation of pollutants. Microbial respiration uses carbon and trace elements to produce energy and as a metabolic consequence, many microbes are capable of breaking down pollutants in the process. For example, biodegradation of hydrocarbons by natural populations of microorganisms is one primary mechanism by which petroleum and other hydrocarbon pollutants are eliminated

from the environment (Leahy and Colwell 1990). Microbes have also been known to degrade phenols (Hwang and Hudson 1986). Mitigation of contaminants in ecosystems in the future will require a comprehensive understanding of microbial activity.

Although all microbes respire, only some are capable of nitrification despite the ubiquitous nature of the process. Nitrification is the oxidation of ammonia to nitrate under aerobic conditions by chemoautotrophic bacteria. The process is maximized in industrial settings such as wastewater treatment plants and wetland remediation. Rates of nitrification are highest in areas where concentrations of ammonium are high, typically in sewage plants or in freshwater affected by agricultural runoff (Willey, Sherwood and Woolverton 2008). Nitrification is fundamental to all organisms in an ecosystem as it transforms and provides nitrogen for assimilation by other organisms and can utilize carbon from macromolecules, including pollutants.

There are numerous factors that might influence rates of nitrification and respiration including pH, temperature, moisture, oxygen, and growth inhibitors (Willey, Sherwood and Woolverton 2008). Microbes are very sensitive to oxygen concentrations greater than atmospheric, which potentially inhibit their activity (Poole 2002). Temperature can affect the biodegradation of crude oil and the way the microbes are able to digest hydrocarbons (Leahy 1990). Many bacteria are also affected by the presence of toxins (Russell 1979). Toxins such as anthropogenic contaminants could lyse or disrupt normal activity causing cell death. Most bacteria are also demonstrate decreased activity when pH is < 5.7(Russell, Sharp and Baldwin 1979).

Anthropogenic activities may also influence rates of microbial activity through increasing concentrations of pollutants such as metals. These compounds can be either beneficial to activity, if they can be utilized as a nutritive source, or inhibitory if the compound is toxic. Many microbes are able to use pollutants as electron donors or acceptors in energy metabolism (i.e., respiration and nitrification) whereby enzymatic microbial detoxification of pollutants may be converted to a less toxic or non-toxic entity by enzymatic oxidation or reduction (Ehrlich 1997). In nature, microbial interaction with pollutants frequently manifests itself through chemical immobilization or mobilization (Ehrlich 1997). However, the understanding of microbe-pollutant interactions has been limited due to the complexity of both the microbiology and chemistry of natural systems (Ford 1999).

III. *Abundance of Lithium in the environment*

Lithium is one contaminant that could pose a threat to microbial activity. Lithium is primarily found in the earth's core and is the 27th most abundant element present in our system. Lithium is typically bonded to another element due to its one valence electron and is naturally coupled with another ion thus forming salts, phosphates, silicates, and micas; all of which are various forms in which lithium naturally occurs in the environment (Aral and Vecchio-Sadus 2008; Schrauzer 2002). Lithium is most concentrated in the earth's core, with lithium minerals mined predominantly in Zaire, Zimbabwe, and Australia (Aral and Vecchio-Sadus 2008). In addition to natural sources, lithium is also derived from a variety of manufactured goods such as pharmaceuticals and electronic devices (Aral and Vecchio-Sadus 2008). Importantly, it has not been determined whether microbes can naturally degrade lithium. Moreover, with the rise in lithium manufacturing for human use, lithium concentrations in the environment are expected to increase. The United States is the largest consumer of lithium-based items such as: ceramics, glass, aluminum, pharmaceuticals, and batteries (Kszos and Stewart 2003). The United States also uses

lithium as a central component in nuclear related studies with the Department of Energy (Kszos and Stewart 2003). This high lithium usage increases the likelihood of environmental contamination, particularly in the United States.

Lithium is typically found at low concentrations in freshwaters throughout the United States. In central Indiana, lithium concentrations average approximately 0.002 mg/L **(**Bunch 2009) in freshwater systems**.** The United State Geological Survey (USGS) reported lithium concentrations in the Missouri Creek to be < 10 mg/L. Lithium is able to enter the groundwater and stream systems through waste-disposal areas, chemical manufacturing, spills, or recycling centers (Kszos, Beauchamp and Stewart 2003; Kszos and Stewart 2003).

Geochemically, lithium is a highly mobile element; therefore, the environmental and occupational health and safety risks are high (Aral and Vecchio-Sadus 2008). Lithium can be toxic to citrus plants although nightshade species are lithium tolerant and may reach lithium contents of up to 1000 μg/g. All plants assimilate lithium, although it appears not to be required for their growth and development. It has been noted that high concentrations of lithium in the soil can become toxic to plants causing a chlorosis-like condition (Schrauzer 2002). Contaminated lakes associated with alkaline mining have produced high concentrations of lithium (3 μmols Li/L) allowing for some study. Higher concentrations of lithium in these ecosystems resulted in altered lipid composition of fish. Additionally, an increase in ATPase, a degradation enzyme that stops ATP, occurred when higher concentrations of lithium were experimentally exposed to fish (Tkatcheva et al. 2007).

In humans, lithium chloride can be fatal. At 10 mg/L of lithium in blood, a person is mildly lithium poisoned; at 15 mg/L they experience confusion and speech impairment; and, at 20 mg/L lithium concentrations in blood there is a risk of death (Aral and Vecchio-Sadus 2008). Lithium has also been found to have some biological impacts on human emotional states (Schrauzer 2002)**.** Humans consume small concentrations of lithium (~ 650 μg) daily from food sources. The lithium is completely absorbed through the small intestine via sodium-channels (Schrauzer 2002)**.**

To further study potential effects of lithium on organisms, we quantified microbial response to lithium in experimental microcosms. Several specific research questions were developed for a more comprehensive understanding of the influence of lithium on microbial communities. Specifically, the effects of lithium on microbial activity were quantified as changes in freshwater sediment microbial respiration, nitrification, and nutrient uptake. We hypothesized that lithium would decrease microbial respiration rates by inhibiting both aerobic and anaerobic metabolism.

METHODS

Sediment and stream water were collected from the White River, in Muncie, Indiana, USA. The sediment and water provided natural microbial inoculum used to assess microbial activity. Sediment was collected approximately 11/2 ft from the shoreline at the ~12 in depth. All sediment and water used in experiments were collected from the same sampling site. Experimental mesocosms were prepared by sieving collected sediment through a No.14 USGS sieve to remove invertebrates and debris and homogenize the sediment for placement into individual replicate mesocosms. Lithium treatments, dissolved in stream water, added to mesocosms were: 0.00, 0.05, 0.1, 0.15, 0.3, 1.0, 2.0, 5.0, and 10 mg Li/L initially as a range-finding assessment. A second experiment tested the effects of lower lithium concentrations including 0.00, 0.375, 0.5, 0.75 and 0.15 mg Li/L treatment concentrations. Negative controls were incorporated as no-lithium

addition treatments; no positive controls were tested. All experiments were conducted at room temperature and sediment was allowed to equilibrate for 24h prior to experiment start.

I. Microbial Respiration

A dehydrogenase enzyme activity assay (DHA) was performed to quantify microbial respiration (Severin and Seidler 1992; Smith and McFeters 1997; Hill and Kaufmann 2002). A mixture of collected homogenized sediment (2.5 mL) and water (2.0 mL) was placed into acid-washed 8 mL falcon tubes. Each treatment concentration of lithium had five replicates prepared. After adding sediment and water, the appropriate lithium treatment was added to each tube along with 1 mL of 0.75% Iodonitroetrazolium (INT)-Chloride. The INT-Chloride acted as the electron acceptor for microbial respiration. All samples were incubated for 3 h at room temperature. Subsequently, 8 mL of methanol was added to all 45-falcon tubes to stop the reaction. All samples were then centrifuged on a tabletop centrifuge for 5 min. Supernatant of each tube was then placed into cuvettes followed by measurement of absorbance (428 nm) on an Schimadzu dual-beam UV spectrophotometer. Standards were also prepared using INT-formazan. Absorbance data was converted from absorbance to oxygen consumed per hour.

II. Nutrient Assimilation

The effects of lithium on nutrient assimilation were measured using the lower treatment concentrations of lithium (0-0.15 mg Lithium/L). Each treatment (N = 30) had five replicates and samples were prepared with sediment and water as before then incubated for 3 d. After incubation, supernatant was collected for measurement of nitrate and phosphate concentrations on a Dionex Ion Chromatograph and compared to initial measurements of nitrate and phosphate concentrations. Net nutrient assimilation was calculated as the change in dissolved nutrient concentration per unit dry mass and time.

III. Microbial Nitrification

Nitrification was measured as the difference in ammonium concentrations between paired microcosms treated with nitrapyrin and no nitrapyrin control microcosms (APHA 1995; Aminot, Kirkwood and Kerouel 1997; Koroleff 1997). Nitrification assays were conducted on samples treated with lithium concentrations of 0.0-0.15 mg/Li/L. Each treatment had 5-paired replicates with a nitrapyrin and a control. Sediment and water was collected from the White River and homogenized as before with 150 mL of sediment and water added to 250 mL Ehrlenmeyer flasks (N = 48). Flasks were incubated on a shaker at 100 rpm for 3d. After incubation, 10 mL of 1N KCl solution was added to each flask and allowed to incubate for 10 min for extraction of ammonium from sediment. Filtered supernatant was then collected for analysis of ammonium concentrations via the phenol-hypochlorite technique (APHA 1995; Aminot, Kirkwood and Kerouel 1997; Koroleff 1997).

IV. Statistical Analyses

All statistical analyses were conducted using MiniTab Software (v15.1.3, 2008). Microbial respiration and nitrification response to lithium was assessed using linear and non-linear regression with the lithium concentration as the independent variable. The influence of lithium on microbial nutrient assimilation was analyzed using Pearson correlation coefficients.

RESULTS

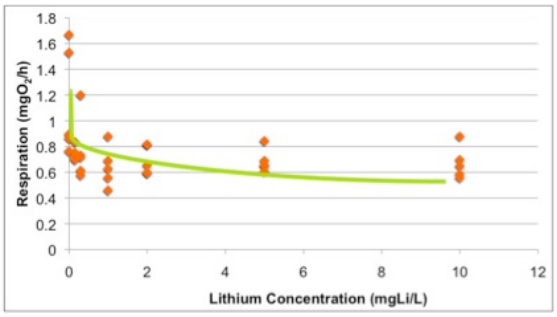

Figure 1: *Influence of lithium on freshwater sediment respiration.* There was a signficant non-linear decline in respiration. Beginning at the top left corner you see where the first treatment was administered which was no added lithium as our negative control. As the lithium treatment increased you saw a non-linear delined in respiration. Respirations rates ranged from 0.765 to 1.670 mgO_2/h. (P = 0.0024) ;

$$respiration = 0.68 + 0.34(-1740296.21 * lithium) + 0.12 * \exp(-2.71 * lihtium)$$

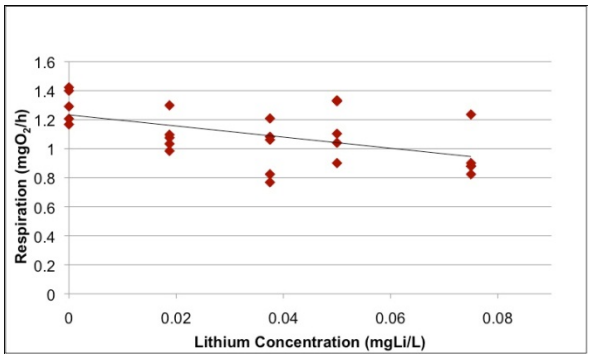

Figure 2: *Influence of trace-concentrations of lithium on freshwater sediment respiration*. Linear decline is shown as lithium concentration increases. Beginning at the top left corner you see where the first treatment was administered which was no added lithium as our negative control. As the lithium treatment increased you saw a linear delined in respiration.(P = 0.007). The following equation was commuted;

$$respiration = -3.842(lithium) + 1.233.$$

In the first experiment, respiration rates ranged from 0.88 to 1.48 mg O_2/h (Figure 1). As lithium concentrations increased, microbial respiration declined (Figure 1, Table 1, p = 0.0024). An exponential decline in respiration occurred between 0.0 and 0.15 mg Li/L lithium exposure (p < 0.0001). The second experiment assessing a lower range of lithium concentrations also identified linear decline in respiration in response to lithium at trace concentrations (Figure 2, p = 0.007).

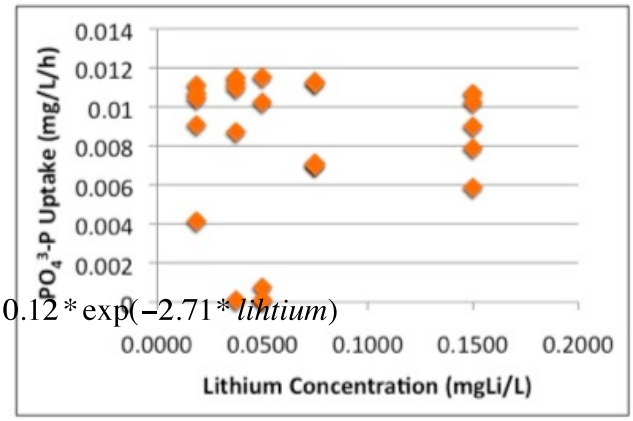

Figure 3: *Influence of trace-concentrations of lithium on freshwater sediment nitrate uptake.* Lithium treatments were added at the same concentrations as respiration. Lithium didn't response to the uptake of nitrates, thus showing no significant effects observed (P > 0.05).

Nitrate uptake ranged from 0.001 to 0.012 mg NO_3-N/h. Phosphate uptake ranged from 0.0045 to 0.0091 mg PO_4^{3-}-P/h (Table 1). No significant effects of lithium on nutrient uptake was found with either nitrate or phosphate assimilation (Figures 3,4). Further, no significant differences in carbon to nitrogen assimilation ratios were observed with lithium treatment indicating consistent use of elements across increasing lithium exposures.

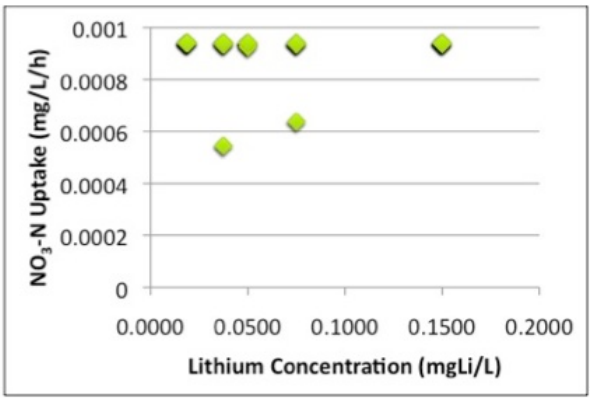

Figure 4: *Influence of trace-concentrations of lithium on freshwater sediment phosphate.* Lithium treatments were added at the same concentrations as respiration. Lithium didn't response to the uptake of phosphates, thus showing no significant effects observed (P > 0.05).

Nitrification rates ranged from 0.00088 to 0.00094 mg NH_4-N/h (Table 1, Figure 5). Nitrification rates increased linearly with lithium concentration (Figure 5, p = <0.0001).

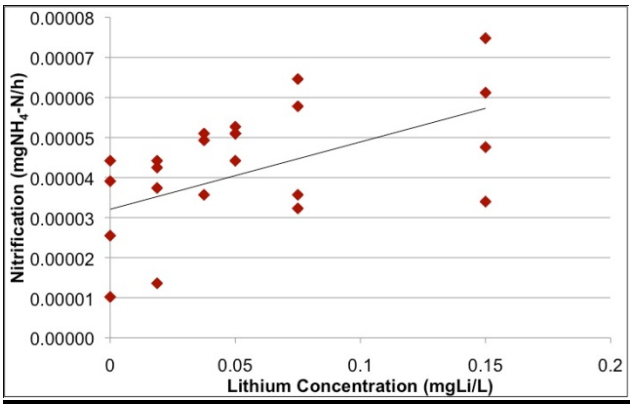

Figure 5: *Influence of trace-concentrations of lithium on freshwater sediment nitrification.* Lithium concentrations were added to river samples that corresponded to previous experiments. As lithium treatments increased you saw a steady increase in nitrification. A linear increase of nitrification was observed in response to increasing lithium concentrations (P < 0.0001);

$nitrif\ ication = 0.0002(lithium) + 3x10^{-05}$. This is common effect due to elimination of some species of bacteria.

DISCUSSION

The effects of lithium were variable depending on the specific measurement of microbial activity and lithium concentration. Increasing lithium concentrations decreased rates of microbial activity by altering or inhibiting the microbes in some capacity. Respiration is vital to microbial function. Previous studies have noted distinct changes in ecosystem function with changes in microbial respiration (Bernot 2010). Decreased microbial activity could limit bacterial production causing potential threats to the environment through alterations in biogeochemical cycles. Further, changes in microbial respiration in response to lithium could cause sewage treatment to be less effective. Thus, changes in environmental lithium concentrations may profoundly affect freshwater ecosystem functioning.

When respiration (carbon uptake) was compared to nitrogen and phosphorus uptake, a linear relationship between lithium concentrations and carbon:nitrogen (C:N) uptake was identified. However, no relationship between lithium concentration and carbon:phosphorus (C:P) uptake rates was observed (P > 0.05). This indicates that lithium may specifically affect microbial use of nitrogen, consistent with nitrification assays (Figure 3,4,5). As lithium concentrations increased, less nitrogen was utilized relative to carbon. If microbes are less able to utilize nitrogen in the presence of lithium, lithium effects may be more pronounced in nitrogen-limited ecosystems. Nitrifying bacteria are resilient bacteria; lithium could pose a threat to other species of bacteria that allows nitrifying bacteria to have a competitive advantage (delGiorgio 1998).

Treatment	Respiration ($mg0_2/h$)	Nitrification ($mgNH_4$-N/h)	NO-Uptake ($mgNO_3$-N/h)	PO-Uptake ($mgPO^{3-}_4$-P/h	C:N	C:P
0.0	1.30 (0.11)	0.00003 (1.52E-05)	*	*	*	*
0.01875	0.94 (0.17)	0.00003 (1.42E-05)	0.00088 (0.0001)	0.0087 (0.0023)	1129	112.1
0.0375	1.19 (0.01)	0.00003 (2.69E-05)	0.00094 (0.0001)	0.0045 (0.0045)	1217	5648
0.05	0.99 (0.00)	0.00005 (3.77E-06)	0.00086 (0.0002)	0.0085 (0.0085)	1238	2486
0.075	1.10 (0.00)	0.00005 (1.60E-05)	0.00094 (0.0)	0.0091 (0.0091)	1165	143.9
0.15	1.32 (0.00)	0.00005 (1.76E-05)	0.00094 (0.0)	0.0087 (0.0087)	1408	157.5

Table 1: *Mean (standard deviation) respiration, nitrogen uptake, phosphate uptake, and nitrification with lithium treatment in freshwater sediment. *Data Not Available.*

Variation among samples was minimal and variation among replicates within a treatment was significantly lower than variation across treatments (Table 1). Thus, statistically significant effects of lithium on microbial activity were identified. Potential sources of error that may have influenced results include laboratory artifacts that may yield differential effects than *in situ* conditions. Further, sediment type, microbial community within the freshwater ecosystem, oxygen concentrations, and other chemical factors could hinder microbial activity. Because sediment was homogenized and water was collected from the same site, these matrix effects likely yielded minimal influence on observed results.

Lithium concentrations used in this study are comparable to lithium concentrations measured in the environment (Bunch 2009). With increased production of pharmaceuticals and industrial products (i.e., batteries), lithium abundance in the environment is predicted to increase, potentially influencing freshwater microbial activity. Previous studies in this ecosystem noted other pharmaceuticals (e.g., ibuprofen, nicotine) also negatively influenced microbial activity (Bunch 2009). More research is needed to assess the persistence of lithium in the environment and to determine the potential for negative effects on freshwater integrity and potential entry into drinking water systems

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