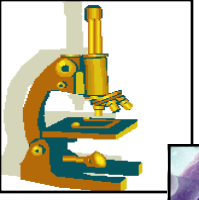
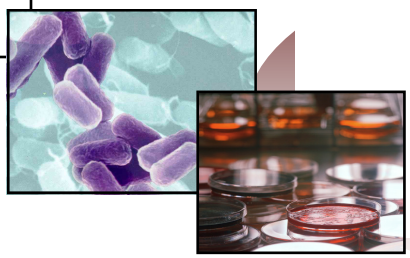
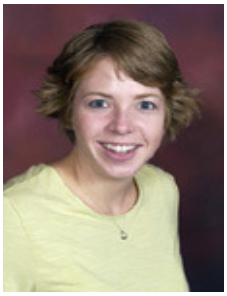




IBASM NEWSLETTER

Volume 13, Issue 1
February, 2011

Greetings from the President: Jennifer Metzler



Greetings to all!! I hope you had a wonderful holiday season and enjoyed some well-deserved time-off, which included some rest and relaxation. I also hope that your 2011 has started off with a bang and will be a wonderful and productive year for each of you.

I do think 2011 will be a great year for our Branch. First and foremost, we have our upcoming annual meeting to be looking forward to. This event will be held at Brown County State Park on April 15th and 16th. Please mark your calendars now and plan on attending and strongly encourage your students to do so as well. Also, encourage your students to give oral presentations, as our meeting is a wonderful environment to get some practice with and feedback on such a presentation. In the message from our President-Elect, Rebecca Sparks-Thissen, you will find all sorts of valuable information about this meeting. I know she has been working hard on planning an exciting and intellectually stimulating event for us. In this edition of the newsletter you can also find the needed registration materials as well as a tentative agenda. I know Brown County State Park has recently renovated their pool facilities at the lodge to include a waterslide, so it should be quite enjoyable for families to attend as well. So get those abstracts and registration materials in soon!!

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I am pleased to announce that the Executive Committee awarded a $500 grant to Lindsey Steiner, our IBASM student representative, from Ball State University in our inaugural offering of the student mini-grant opportunity. We will look forward to a presentation at an upcoming meeting on her project entitled "Effect of Carvacrol on Diminishing Infection Progression of Bacillus cereus-mediated Endophthalmitis in Mice"

I am also very excited to announce that we will be offering student grant opportunities again this spring for undergraduates as well as graduate/professional students. Two awards of $500 each will be awarded in each category to students who are members of IBASM. These awards can be quite helpful in furthering your research endeavors, but also look great on a CV or on a graduate or professional school application. Please look in this edition of the newsletter for the official announcement of this opportunity as well as guidelines. We look forward to receiving many competitive applications by the deadline of March 15, 2011!

I also want to remind everyone to renew their IBASM memberships for this year. You can easily do this

through ASM National when you renew with them, just look for Branch membership. Or, you can renew when you register for the meeting. I would also encourage you to have your students become members as well, if they have not done so already. A student membership is only $5 and students must be members to present at the meeting. Membership in our Branch is just one more way to round out a CV or application.

In closing, I wish much success to all in 2011. I hope you will take advantage of all our Branch has to offer you. I look forward to seeing you all in April at our meeting in Brown County. Please feel free to contact me with any questions, ideas, or concerns at jametzler@bsu.edu.

Message from the President– Elect: Becky Sparks-Thissen

Our spring IBASM meeting will be held April 15 &16 at Brown County State Park in Nashvillle, IN. It promises to be a fantastic meeting with student oral and poster presentations. Our Branch speaker, Kenneth Noll, will also present a talk entitled "Do Thermophiles Have Roles to Play in our Energy Future?" We also will have ASM secretary Jim Tiedje presenting a talk. His research interests are the ecology, physiology, and genetics/ genomics underlying important microbial processes in nature. The meeting promises to have lots of exciting science to discuss!

In addition to the science, Brown County State Park promises many indoor and outdoor activities including hiking and enjoying the beauty of the outdoor setting (http://www.browncountystatepark.com/). In addition to the state park, there are numerous activities available in nearby Nashville, IN (http://www.brown-county-indiana.com/).

As always, we recommend that you make reservations early. A block of rooms have been reserved at the Abe Martin Lodge at Spring Mill State Park for $104 + tax. You should contact them directly at 1**-877-563-4371** and reference **group code 0415IB** to make your reservation. Note that payment is due at the time of your reservation. **You must make your reservation by 3/15/11**. The rooms will be released on 3/15/11 and we will not be able to guarantee you a room.

A note for students: Please consider giving an oral presentation! It's great practice for upcoming seminars, thesis defenses, etc. If you are presenting, don't forget to apply for travel assistance to help defray the costs of attending the meeting.

I look forward to seeing you in April! Best wishes for a productive spring semester.

Save the Date!

Next IBASM

Spring Meeting

Brown County State Park

April 15-16, 2011

Message from the Student Representative: Lindsey Steiner

I hope everyone had a great holiday! The 2011 IBASM meeting will take place April 15th and 16th at Brown County State Park. I am very much looking forward to presenting the results of my current research, and I can't wait to see what other students have been working on in their research labs across the state. Don't forget to watch for the IBASM grant opportunity for undergraduate and graduate students (more information will be given in the spring)! I received a $500 grant to complete my research on endophthalmitis in a mouse model. My project, titled "Effect of Carvacrol on Diminishing Infection Progression of Bacillus cereus-mediated Endophthalmitis in Mice," confirms in-vitro work conducted by Pierre Nimmer (M.S. Student) in a mouse model, and will allow me to determine whether carvacrol will be a proper substitute for antibiotics in the treatment of eye infections.

This opportunity through IBASM allowed me to contribute financially to my research and will help me stand out as I apply for research internships and medical/graduate school. The ability to write grants is invaluable for students planning to pursue a career in research. I highly encourage any graduate and undergraduate student to prepare a grant for the upcoming IBASM grant competition, as a way to hone their grantsmanship abilities and stand out to prospective employers.

As a member of the Ball State chapter of the American Society for Microbiology (BSUASM) over the last year, I have participated in hands-on activities, seminars, and community service opportunities, such as our recent donation drive for the Madison County Sexual Assault Treatment Center. The recent donation drive collected an entire truck full of teddy bears, toys, clothing, and hygiene items which I helped deliver on January 5th. While at the center, Holly Renz, R.N., Director of the Madison County Sexual Assault Treatment Center, gave Dr. McKillip and me a brief tour of the facility. Having an ASM chapter at Ball State is helping to draw interest in the field of microbiology, and I am excited to leverage the Ball State chapter to bring in new members and participants for the IBASM meeting this spring. Not only will we bring our own new members, but we will also use advertising to make sure it is a well-known event to other Ball State students.

Please let me know if you have any questions or suggestions at lmsteiner@bsu.edu

Dr. McKillip (left), the IBASM Councilor, and Lindsey Steiner (right) delivered a truck full of BSUASM-donated supplies to Holly Renz (center), the director of the Madison County Sexual Assault Treatment Center.

From the Desk of Jim Mitchell-...Educational Representative

Student Poster Competition

Abstract submission form will be distributed by email separately but is also located at IBASM website: http://users.ipfw.edu/merkel/IndianaASM.html. We will be utilizing 4x4 sq.ft. tri-fold styrofoam poster boards and each student is limited to one board. Tacks will be supplied but it wouldn't hurt to bring some extras in case we run short. You may participate in both oral (limited # of slots available) and poster sessions but you will only be judged for an award in the poster session. Awards will be presented in the following divisions: Undergraduate, MS graduate and PhD graduate. Post-Doctoral Fellows are welcome to participate in either session but are not eligible for the award competition.

Students will be judged in 5 categories:

***Professional Appearance*:** Jeans and sweat pants are unacceptable; torn, dirty, or frayed clothing is unacceptable. Business casual dress is the standard dress code. (20 points)

***Scientific Thought*:** Is there a clear hypothesis? Are the goals of the study defined? Were data correctly analyzed? Were statistical analyses performed? Did a logical conclusion result? (20 points)

***Creativity*:** Was the topic original? Is there anything new in the approach to answering the question? Were new methods developed? (20 points)

***Thoroughness*:** Was the study as complete as possible? Does the student understand the background material? Were subject headings (e.g. Introduction, Materials & Methods, etc.) presented? Is the student aware of the drawbacks of the study? (20 points)

***Presentation (poster)*:** Were the results/conclusions clearly presented? Was the student's verbal expression clear and concise? Was the student able to answer questions? How well did the poster convey the information? (20 points)

2011 IBASM Spring Meeting

Tentative Agenda

Friday April 15, 2009

All meals will be served in the main dining room at the Abe Martin Lodge. All other events are in the Allison Peabody Room at the Abe Martin Lodge.

4:30-5:30 Registration

5:30-6:30 **Dinner**

6:30-7:30 **ASM Branch Lecture**- Kenneth Noll, University of Connecticut, "Do Thermophiles Have Roles to Play in our Energy Future?"

7:30-9:00 Welcome reception

Saturday April 16, 2009

All meals will be served in the main dining room at the Abe Martin Lodge. All other events in the Allison Peabody Room at the Abe Martin Lodge.

7:30-8:30 **Breakfast**

8:30-10:00 Poster Judging/Registration

10:00-11:30 Poster Viewing

10:30-11:30 Student Oral Presentations

11:30-12:30 IBASM Business Meeting

12:30-1:30 **Lunch**

1:30-2:30 Student Oral Presentations

2:30-3:30 Presentation by ASM Treasurer, Jim Tiedje

3:30-4:00 Announcement of Student Award Winners and Closing Remarks

IBASM Annual Meeting Registration and Meal Reservation Form

April 15 and 16, 2011
Brown County State Park
Nashville, IN

Please use this form to register for the IBASM meeting and reserve your room and meals. The meeting registration fee is $25 for regular members and $5 for student members. **You must be an IBASM member to participate in the meeting.** Family members are encouraged to attend, however, they do not have to pay registration fees. Upon completion, **e-mail** this form to Becky Sparks-Thissen (thissenr@wabash.edu) no later than March 11, 2011. If necessary, forms may also be mailed to Becky Sparks-Thissen at the address given at the end of the form. **Registrations received after March 17th will be subject to a $7.00 late fee (regular members) or a $4.00 fee (student members).** Please feel free to contact Becky at the email address provided above if you have any questions.

Please fill in the requested information.

Name:_____ **#Adults** _____ **#Children**_____

Address:_____

Phone:_____ **Fax:**_____ **Email:**_____

IBASM member: ___Yes ___No If you are not a member, you will need to become a member and include your dues with your payment for the meeting.

Please indicate which sessions you plan to attend:

___ Friday evening session
___ Saturday morning session
___ Saturday afternoon session

If you are a student presenter, do you request travel assistance?

___ Yes
___ No

Lodging

A block of rooms have been reserved at the Abe Martin Lodge at Brown County State Park for $104 + tax. You should contact them directly at **1-877-563-4371** and reference **group code 0415IB** to make your reservation. Note that payment is due at the time of your reservation. **You must make your reservation by 3/15/11**. The rooms will be released on 3/15/11 and we will not be able to guarantee you a room.

Payment

Registration

Member ($25)	$_____
Student ($5)	$_____

Meals

Dinner 4/15 ($19)	# _____	$_____
Breakfast 4/16 (12.00)	# _____	$_____
Lunch 4/16 ($15)	# _____	$_____

Dues (if applicable)

Non-student ($15)	$_____
Student ($5)	$_____

Late fees (if applicable) $_____

Total Enclosed $_____

A check payable to "Indiana Branch ASM" for the total costs of registration, dues (if applicable), and meals must be sent by mail by **March 18, 2010** to: Becky Sparks-Thissen, Department of Biology, Wabash College, Crawfordsville, IN 47933

2011 Membership Application/Renewal

If you have not done it already, it is time to pay your IBASM dues for 2011. You can do it either online when you pay your dues to the ASM National Organization (www.asm.org) or by using this form. Dues are $15.00 for non-students and $5.00 for students (per year). Please return the completed form with check, payable to IBASM, to

Dr. Christian Chauret
Science, Mathematics, and Informatics Department
Indiana University Kokomo
2300 South Washington Street
Kokomo, IN 46904-9002
Phone: (765) 455-9371; email: cchauret@iuk.edu

Please check:
_____ New Member Application
_____ Renewal for 2011

and
_____ Student Member in 2011 ($ 5)
_____ Full Member in 2011 ($15)

Name:

Current Position & Title:

Institution:

Mailing Address (new address Yes / No ?) :

Phone:
Email:
Fax:

National ASM Member #::

Background

Highest Degree:
Institution:
Professional Interests:

An Exciting Opportunity

I. SUMMARY & ANNOUNCEMENT

The Indiana Branch of ASM announces an opportunity for undergraduate or graduate/professional students in current good standing with IBASM to apply for research grants of up to $500. Two awards will be made for undergraduates and two for the graduate/professional student category. The awards are intended to offset costs to purchase laboratory/field supplies, to support travel, and provide other items required to conduct novel scientific research. The deadline for the electronic submission of the proposal to the IBASM President is March 15, 2011 (see below for additional details). Awardees are expected to deliver an oral presentation of their findings at the Annual Spring IBASM meeting within two (2) calendar years of the award being made.

III. ELIGIBILITY

Student applicants who meet any of the following criteria are eligible to apply for research grants:

- Current student members of IBASM in good standing during the preceding year and at the time of the application who are undergraduates, graduate students (masters or doctoral level), or professional students with a G.P.A. of 3.25 or higher (on a 4.0 scale).

Awards will be made to the academic institution through the relevant grants/sponsored programs office, not to an individual. Therefore, each application must be signed by the organization's official with the authority to approve the request (e.g., President, Chief Academic Officer, College or University Research Officer, etc.) and commit the institution to the conditions of the award. This information is to be included on the mini-grant cover page and, if awarded, will necessitate an account be set up for the student use.

IV. APPLICATION PROCEDURE

Submit the grant application to the President of IBASM (Dr. Jennifer Metzler, Ball State University, jametzler@bsu.edu). Each research proposal should be brief but complete and must include the following information arranged in the following order, with each section starting on a new page:

1. A COVER PAGE FORM (see attached) giving the name, mailing and e-mail addresses, and affiliation of the student investigator and of the faculty mentor, together with a descriptive title for the proposed project. The cover page should also include the date of submission; the level of funding requested; the name of the person authorized to make grant-related commitments on behalf of the applicant organization; and, the name and address of the institutional official to whom the check should be sent (again, note that award checks are made out to the institution,

not to any individual). Student applicants are also asked to include a statement as to whether the requested funding is for a 'new' project, or an 'ongoing' one with current funding. Also include status of compliance.

2. An ABSTRACT of no more than 300 words.
3. A NARRATIVE of *no more than three pages* (single or double spaced with double spaces between paragraphs, 10-12 point font, 1" margins). This section must

   - describe the problem to be investigated and justify the proposed study,
   - provide a review of the published relevant literature,
   - state the research objectives, hypothesis, scope of the problem, and methodology to be followed,
   - describe previous research, if applicable,
   - include 1-2 sentences on the timeline of the proposed activities, and
   - a brief statement on how the research results will be disseminated.
   - If applicable, include approx. ½ page of preliminary data

   This is an important part of the proposal and will be scrutinized by the reviewers. Applications with a narrative exceeding three pages may be returned without review.
4. A REFERENCES CITED page listing all literature cited in the application (ASM style, not included in 3 page limit).
5. A *detailed* BUDGET. The budget may include

   - supplies (defined as items that by their nature will be consumed during the course of the research),
   - vehicular travel to field sites or related; travel outside of Indiana is not supported

   The budget *may not* include funds to

   - attend meetings,
   - pay publication costs,
   - support institutional administrative or overhead/indirect costs,
   - pay salaries to applicants or sponsors,
   - sponsor pedagogical research, or
   - purchase computers or computer time.

   The budget must also describe the expected or actual source of funding for resources essential for the project not included in the request to IBASM.

   It is essential to understand that enough detail must be provided to allow members of the IBASM Executive Committee to evaluate the appropriateness of each element of the budget. The Committee may delete items from the request that it feels are not justified as long as it believes the project would not be unduly impaired; such a conclusion usually results in the application's rejection. Consequently, it is essential that each item be explained and justified for the request to receive full funding.

6. For work involving Human Subjects, Animal Care & Use, infectious agents, and/or recombinant DNA, a statement needs to be included indicating how and when compliance approval will be obtained from the sponsor institution. Formal compliance approval is not needed for grant awards to be made, but is required prior to the commencement of any research work.
7. Current transcript(s)
8. Support letter from faculty mentor

V. REVIEW PROCESS

The IBASM Executive Committee will review every application. The Executive Committee members represent a wide range of subdisciplines within the field of microbiology, and the results of their discussions determine which requests will be awarded and for what elements of the request. The IBASM President will inform applicants by email of the Committee's final decision and will contact the Branch Secretary/Treasurer who will then mail award checks to the official identified in the application. The review process is normally completed within six weeks of the application deadline. Names of awardees will be published in the IBASM newsletter. The decision of the Committee is final and all questions concerning awards and the decision process should be directed to the IBASM President.

In reviewing proposals for possible support, the IBASM Executive Committee will consider the following criteria:

- Significance of project
- Soundness of scientific idea
- Appropriateness of methodological approach
- Feasibility of study within given timeframe and environment
- Quality of preliminary data (if applicable)
- Appropriateness of budget
- Completeness and clarity of proposal

No grant proposal, however worthy, may request more than $500; proposals requesting more than this amount will not be reviewed.

VI. GENERAL POLICIES

Grants will normally be made for investigative periods of one year beginning on the date of the award letter unless otherwise requested in the grant application, approved by the Committee, and specifically mentioned in the award letter.

Unexpended funds are to be returned to the IBASM Secreatry/Treasurer.

Oral presentation of research results at the Annual Spring IBASM meeting expected.

Publications or presentations resulting from projects supported by IBASM mini-grant funds must acknowledge Branch support.

VII. REQUESTS FOR CHANGES IN FUNDED REQUESTS

Requests for extensions, without additional funds, or requests for redirection of funds, will be made on a case-by-case basis through individual requests to the IBASM President.

VIII. APPLICATION DEADLINES AND SUBMISSION DETAILS

Applications for IBASM Student Research Mini-Grants **must be postmarked or received by Tuesday, March 15, 2011** and must arrive within three days of this deadline. Receipt of application will be acknowledged by electronic mail within a week of the final deadline date. Grant announcements will normally be made by the IBASM President within six weeks of the deadline date. Applicants whose requests are denied will be notified on or soon after the corresponding award announcement dates.

CHECKLIST

- ✓ Cover sheet with all requested information (included with this announcement)
- ✓ Abstract of 300 words or less
- ✓ Project description that follows general guidelines provided in this announcement
- ✓ Statement of compliance approval
- ✓ Preliminary data, if applicable
- ✓ Budget of $500.00 or less with justification(s)
- ✓ Transcripts (e-mail electronic copies or post hard copies)
- ✓ Faculty mentor support letter (e-mail electronic copies or post hard copies)

Applications should be mailed or e-mailed to:

Dr. Jennifer Metzler
IBASM President
Ball State University
Department of Biology
2000 W. University Ave.
Muncie, IN 47306
jametzler@bsu.edu

IBASM Student Research Grant Cover Page

Complete the following information below and save/send this cover page with the other proposal components to the contact provided in guidelines. ***Deadline:*** March 15, 2011

Name: Institution:

Address:

Phone: Fax: email:

Please indicate your student status:
__ Undergraduate
__ Graduate (M.S/M.A. or Ph.D/Ed.D.)
__ Professional

Research advisor: Institution:

Address:

Phone: Fax: email:

What is the title of your proposed project?

Date: Total funding amount requested:

Indicate whether this project is:
__ New project/no current funding
__ Ongoing project/current funding

Compliance status:

Name & address of the institutional official to whom the check should be sent, if funded:

Second Place Graduate (M.S. Division) Winner

Pharmaceutical Abundance and Degradation in Indiana Streams

Allison Veach and Melody J. Bernot
Department of Biology, Ball State University, Muncie, IN

Introduction

Pharmaceuticals have been recognized as an environmental threat since the 1970's (Tabak and Bunch 1970; Kummerer 2004) when compounds were first detected in freshwater. Both prescription and non-prescription pharmaceuticals can enter fresh-water ecosystems via multiple sources including disposal of surplus drugs, human excretion into sewage, and runoff associated with therapeutic treatment of livestock (Jorgenson 2000) (Figure 1). Environmental transport and fate of these compounds has not been quantified to assess regulatory need.

Pharmaceutical contaminants most frequently detected in water include caffeine, acetaminophen, cotinine (nicotine metabolite), ibuprofen, carbamazepine, and sulfamethoxazole (Kolpin et al. 2002; Ashton et al. 2004; Kolpin et al. 2004; Glassmeyer et al. 2005; Barnes et al. 2008; Focazio et al. 2008; Walraven and Laane 2009). Once pharmaceuticals enter into freshwater, they may be transformed, retained, or exported to downstream systems (Figure 1). Pharmaceutical compounds can be transformed via biodegradation or photo degradation; they can be retained via assimilation or sediment sorption, or if the compound is recalcitrant they will be exported downstream unaltered. All of these pathways may influence freshwater integrity.

Microbial degradation is an integral component to pharmaceutical persistence in freshwater. Although surface water communities of bacteria are less diverse and lower in number than in sewage treatment (Kummerer 2009), organic pollutants can serve as a carbon source for microbial assimilation in natural ecosystems (Winkler et al. 2001; Lawrence et al. 2005). However, multiple physiochemical factors will influence the rate and likelihood of degradation. To quantify the abundance and frequency of pharmaceutical pollutants in central Indiana streams and potential for microbial degradation, we addressed three primary research questions:

How do pharmaceutical concentrations vary temporally in urban and agriculturally-influenced streams of central Indiana? We hypothesized that pharmaceutical concentrations would be higher in urban-influenced streams due to combined sewer overflow (CSO) input.

What physiochemical factors are related to pharmaceutical concentrations? We hypothesized that stream flow would influence pharmaceutical concentrations.

Can microbes degrade pharmaceuticals at trace environmental concentrations? We hypothesized that microbes can degrade trace concentrations of pharmaceuticals under limiting conditions.

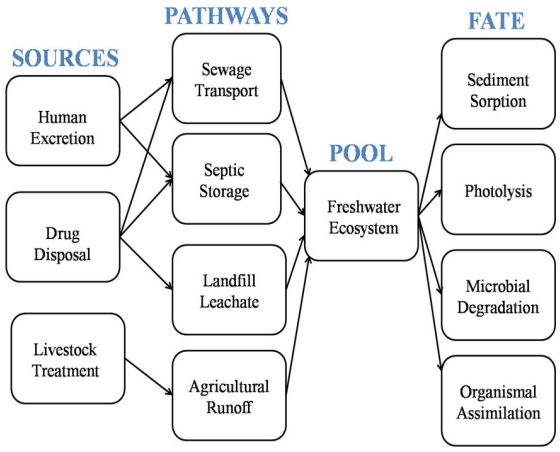

Figure 1. Conceptual model describing sources and pathways for pharmaceutical introduction to freshwater ecosystems. Once pharmaceuticals are present, their fate will vary depending on specific conditions.

Experimental Methods

Two sites, Buck and Killbuck Creek, in the Upper White River watershed of central Indiana (Delaware County) were selected for bi-monthly sampling from June 2009-May 2010. Sites were selected to maximize differences in surrounding land use. Buck Creek is an urban/suburban stream with combined sewer overflow (CSO) input points upstream of sampling. Killbuck Creek is an agriculturally influenced stream with potential pharmaceutical input via septic or animal sources. Physiochemical parameters (discharge, pH, turbidity, conductivity, temperature, chlorophyll, oxygen) were measured every two weeks using a Hydrolab minisonde and Marsh McBirney flow meter. Filtered water samples (GF/F, 0.7 µm pore size) were also collected every two weeks for analysis of anions (Cl^-, Fl^-, Br^-, NO_2^-, NO_3^-, PO_4^{3-}) and cations

collected into an amber-baked glass bottle for pharmaceutical analysis via mass spectrometry at the IA Hygienic Laboratory. Bonferroni corrected Pearson correlations were calculated to assess relationships between stream physiochemical parameters and pharmaceuticals.

Microbial degradation of pharmaceutical compounds was assessed using a selective basal salt media. Basal salt media amended with pharmaceuticals was prepared and poured into 5 replicate glass test tubes for each pharmaceutical tested including a nicotine, acetaminophen, ibuprofen, and cotinine treatment (100 µg/L each) and controls with no pharmaceutical amendment. Media was subsequently inoculated with stream sediment. Absorbance was measured at incubation times of 66, 168, and 216 hours as a proxy for microbial growth (as turbidity) via a Schimadzu dual-beam spectrophotometer (660 nm wavelength).

Results

Acetaminophen, caffeine, cotinine, and DEET were detected most frequently at our two sampling sites (Figure 2). Other compounds detected included carbamazepine, gemfibrozil, ibuprofen, sulfadimethoxine, sulfamethazine, sulfamethoxazole, triclosan, and trimethoprim. Across sites, DEET, trimethoprim, and sulfamethazine were correlated with stream depth whereas acetaminophen, caffeine, and sulfamethazine were correlated with dissolved oxygen concentrations (Figure 3). Stream flow, as discharge or velocity, was not correlated ($p>0.1$) with individual pharmaceutical compound concentrations.

Microbial degradation experiments using pharmaceutical-amended basal salt media inoculated with stream sediment indicated potential microbial degradation of pharmaceutical compounds (Figure 4). An initial increase in media turbidity was observed after 168 h incubation, potentially due to organic matter introduced during inoculation. After this initial increase, turbidity declined for all treatments. However, pharmaceutically-amended media treatments had higher turbidity indicating more microbial growth relative to control (no pharmaceutical) treatments. Given higher turbidity of pharmaceutical treatments, microbes were likely using pharmaceuticals as a nutritive source. Cotinine exhibited highest turbidity at the end of the incubation period indicating greatest potential for microbial degradation; whereas, nicotine and acetaminophen exhibited the lowest turbidity. Control broths did not include a carbon source therefore little microbial growth would be expected.

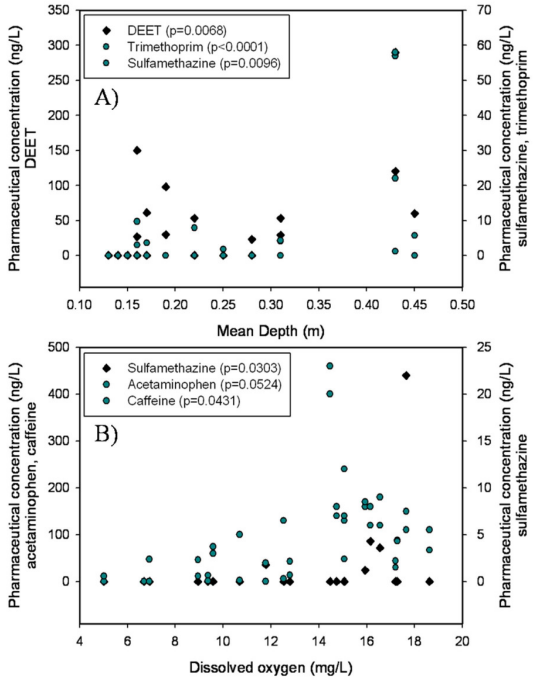

Figure 3. Correlations of A) mean and B) dissolved oxygen and pharmaceutical compounds in Buck and Killbuck Creek. Signifcance noted by p-values for each pharmaceutical.

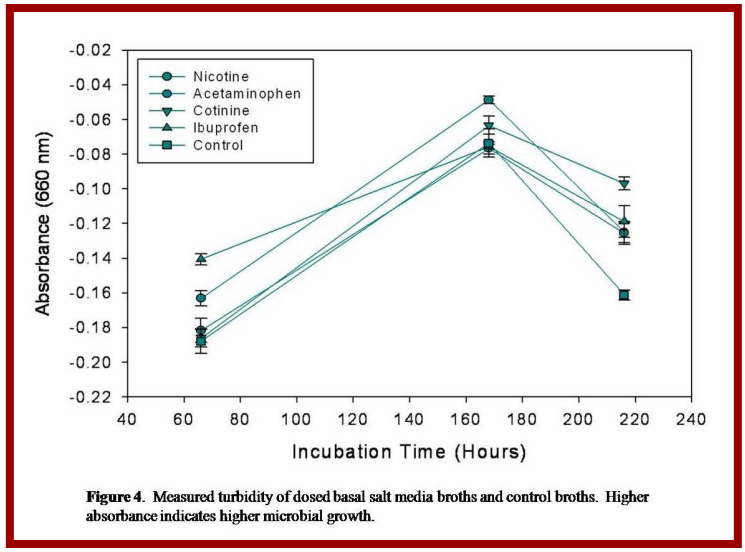

Figure 4. Measured turbidity of dosed basal salt media broths and control broths. Higher absorbance indicates higher microbial growth.

Discussion

Overall, pharmaceutical concentrations detected in central Indiana streams were within the range previously documented in freshwaters across the United States (Kolpin et al. 2002). Because individual compounds had different temporal patterns and were influenced by different physiochemical parameters, fate of pharmaceuticals is likely variable among compounds. Significant positive correlations between pharmaceuticals and dissolved oxygen may indicate *in situ* microbial degradation is more effective under low-oxygen conditions. Further, positive correlations with water depth may be a result of less contact between dissolved pharmaceuticals and the benthos with deeper water, decreasing potential microbial degradation.

Contrary to our hypotheses, urban and agriculturally influenced streams had comparable pharmaceutical concentrations not associated with stream flow metrics. This indicates pharmaceutical persistence in the environment may be less related to input and more dependent on microbial activity, photolysis, sorption, or assimilation. Turbidity measurements of pharmaceutical amended media indicate *in vitro* microbial degradation of pharmaceuticals likely occurs with cotinine exhibiting the highest potential for degradation.

These data show pharmaceutical concentrations in freshwaters of central Indiana are spatially and temporally variable with the highest concentrations in winter. To assess regulatory need for pharmaceuticals in freshwaters, a more comprehensive assessment of factors controlling these contaminants is needed. Further, future management will demand a better understanding of degradation potential for these compounds.

Literature Cited

Ashton, D., Hilton, M., and Thomas, K. (2004). Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Science of the Total Environment. 333: 167-184.

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Second Place Undergraduate Winner

The Effect of Nicotine on the Growth and Biofilm Formation of *Streptococcus mutans*

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Abstract

Streptococcus mutans plays a major role in the formation of dental caries. Oral bacteria are exposed to a variety of factors that affect growth and biofilm formation. However, there are different environmental conditions under which the growth and biofilm formation of the bacteria are affected, including exposure to nicotine. There are several proteins that play a role in the biofilm formation of the *S. mutans*. Previous work had indicated that increased concentrations of nicotine upregulated expression of *S. mutans* antigen I/II, a surface adhesin responsible for binding to salivary agglutinin. The objective of this project was to determine the effect of nicotine on the growth and biofilm formation of *S. mutans* on salivary-coated microtiter plates. In order to examine whether the growth and biofilm formation of *S. mutans* UA159 on saliva-coated microtiter plates are affected by nicotine, microtiter plates were coated with diluted saliva. The plates were incubated with *S. mutans* cells that had grown in varying concentrations of nicotine, ranging from 0.125 to 4.0 mg/ml for 16 h. The planktonic cells were transferred to a second microtiter plate, and their growth was measured at 600 nm. The first microtiter plate containing biofilm cells was incubated with a formaldehyde solution overnight to fix the biofilm cells to the plate. The formaldehyde was removed by washing with saline, and the plate was stained with crystal violet and read at 600 nm to measure the amount of biofilm formation. The experiments indicated a significant decrease ($p<0.05$) in the amount of planktonic cells in all concentrations of nicotine, which correlated to an increase in the amount of adhered cells. The results also established a significant increase in the amount of biofilm formation of the cells grown in 2.0 mg/ml of nicotine when compared to the 0 mg/ml control. This data establishes that nicotine has an effect on the growth and selective biofilm formation of *S. mutans* to saliva-coated microtiter plates, possibly related to the increased expression of antigen I/II previously reported by our laboratory. The increased biofilm formation by *S. mutans* after exposure to nicotine provides one explanation for increased dental caries in smokers.

Introduction

According to the CDC, roughly 1 in 5 adults is a smoker and approximately 3% of Americans are smokeless tobacco users[1]. The smoking of cigarettes is known to be a carcinogen, as well as the major causative agent of several other deadly diseases. In addition, smokers have increased dental caries. The main site of interaction between tobacco, and specifically nicotine, is the oral cavity. Nicotine makes up 0.6-3% of tobacco, with each smoker receiving approximately 1-2 mg of nicotine from each cigarette[2]. This helps to explain why the use of cigarettes and smokeless tobacco is a major factor in the development of dental caries in those who use them. Our laboratory has been systematically assessing the effect of nicotine and tobacco on *Streptococcus mutans*, the major causative agent of caries. Nicotine has many effects on *S. mutans*, such as on growth and biofilm formation. The initial attachment of the bacteria to saliva-coated surfaces is aided by antigen I/II, which is a 185 KDa surface adhesin[3]. We have previously reported that antigen I/II is up-regulated after exposure to various concentrations of nicotine[4]. This up-regulation of antigen I/II is hypothesized to lead to an increased amount of adhesion of nicotine-treated bacteria to saliva-coated microtiter plates.

Materials and Methods

Preparation of bacteria: *S. mutans* UA159 was cultured in TSB (tryptic soy broth) overnight in a 5% CO_2 incubator at 37°C. 100 µl of this culture was added to 7 ml of TSB containing various concentrations of nicotine, ranging from 0.125 to 4.0 mg/ml, including a control tube containing no nicotine, and incubated overnight.

Adhesion procedure: 5 mg of hydroxyapatite beads were weighed. The beads were washed three times with phosphate buffer. Pooled de-identified filter-sterilized saliva was diluted 1:5 with saline. The beads were incubated with 125 µl of the diluted saliva for 30 min on a rocker. The beads were washed with buffer to remove nonabsorbed salivary material. 500 µl of 1% BSA was added to each vial to block unbound sites, and incubated for 30 min at room temperature. The BSA was removed and the beads were washed with buffer. The beads were incubated with 125 µl of bacteria at room temperature for 30 min and washed with buffer to remove unbound cells. The beads were resuspended in 1 ml saline, vortexed for 10 sec and sonicated at max setting for 10 sec to remove bound cells. 0.3 ml of the saline was removed and added to 2.7 ml saline to make a 1:10 dilution. The dilutions were spiral-plated onto blood agar plates and incubated in 5% CO_2 for 48 h and counted using an automated colony counter.

Growth and biofilm formation procedure: Pooled de-identified filter-sterilized whole saliva was diluted 1:5 with a carbonate-bicarbonate buffer. Sterile microtiter plates were coated with 100 µL of saliva and incubated at 37°C for 60 min. The saliva was removed and 100 µL of bacteria was added. The plate was incubated in a 5% CO_2 incubator at 37°C for 16 h. The planktonic cells were transferred to a second microtiter plate and their absorbance was read at 600 nm. The first microtiter plate containing biofilm cells was incubated with a formaldehyde solution overnight to fix the biofilm cells to the plate. The formaldehyde was removed by washing with saline, and the plate was stained with 0.5% crystal violet for 30 min. 70% isopropanol was added to release the dye and the absorbance at 600 nm measured the amount of biofilm formation.

Results

Adhesion: Nicotine had a trend toward increased adhesion of the bacteria to the saliva-coated hydroxyapatite (Fig. 1). The experiment exhibited an increase in the amount of bacteria that adhered to the beads. There was an increase in adhesion seen in bacteria grown in 0.5, 2.0, and 4.0 mg/ml nicotine.

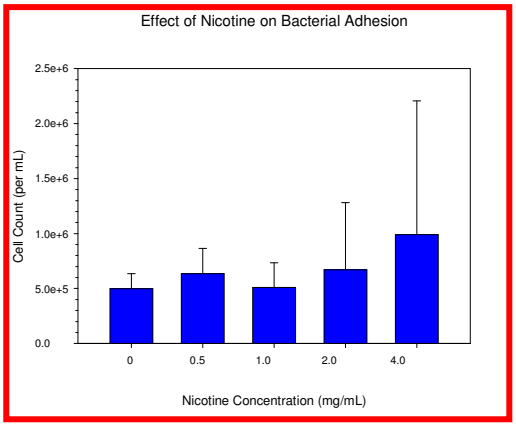

Fig. 1: Effect of nicotine on the adhesion of bacteria to saliva-coated hydroxyapatite.

Growth and biofilm formation: These experiments indicated a significant decrease ($p<0.05$) in the amount of planktonic cells grown in 0.25 and 2.0 mg/ml of nicotine (Fig. 2). The results also established a significant increase in the amount of biofilm formation of the cells grown in 0.125, 2.0, and 4.0 mg/ml of nicotine when compared to the 0 mg/ml control (Fig. 3). This data establishes that nicotine has an effect on the growth and selective biofilm formation of *S. mutans* to saliva-coated microtiter plates, possibly related to the increased expression of antigen I/II previously reported by our laboratory. The increased biofilm formation by *S. mutans* after exposure to nicotine provides one explanation for increased dental caries in smokers. The results indicated that only the 0.25 and 2.0 mg/ml of nicotine exhibited significance in the decrease of planktonic cells and 0.125, 2.0, and 4.0 mg/ml of nicotine demonstrated significance in the amount of biofilm formation.

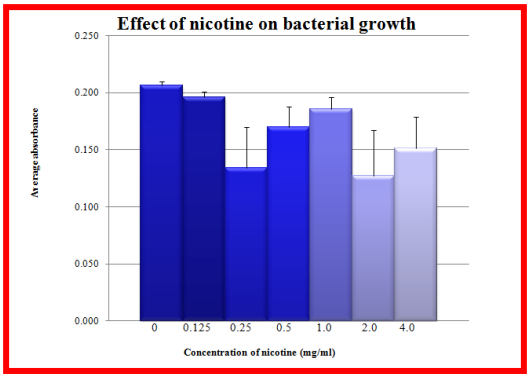

Fig. 2: Effect of nicotine on the decrease in the amount of planktonic cells (absorbance at 600 nm) when compared to the 0 mg/ml control.

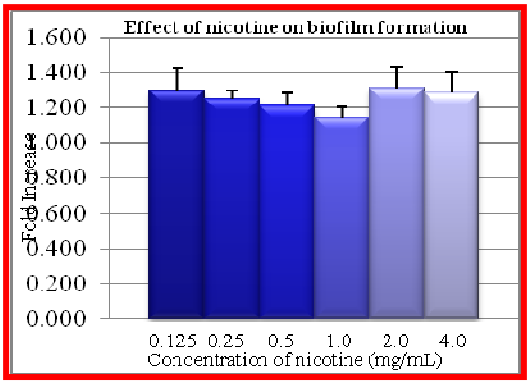

Fig. 3: Effect of nicotine on the fold increase in biofilm formation (absorbance at 600 nm) when compared to the 0 mg/ml control set at 1.0.

Summary

Nicotine was shown to induce a significant decrease in the amount of planktonic cells of *S. mutans*, as well as an enhanced effect on biofilm formation. Concentrations as low as 0.25 mg/ml of nicotine causes the bacteria to have a decrease in the amount of planktonic cells. Conversely, as little as 0.125 mg/ml of nicotine is enough to cause the bacteria to have a significant increase in biofilm formation on saliva-coated microtiter plates. The results established an overall decrease in the growth of planktonic bacteria and an increase in the amount of biofilm formation. This is likely due to the increased expression of antigen I/II. And with most cigarettes sold in the U.S. today delivering approximately 1 mg or more of absorbed nicotine, it does not take many cigarettes to affect the oral bacteria[5]. This increase in the adhesion and biofilm formation of the bacteria to the saliva-coated hydroxyapatite and microtiter plates helps to explain the increased likelihood for smokers to have a greater amount of plaque buildup, which leads to an increased amount of dental caries.

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Mallory Wilson receiving her award from former IBASM President **Dr. John McKillip**

More Photos from the 2010 Annual Meeting

Allison Veach receiving her award from former IBASM President **Dr. John McKillip**

POSTER JUDGING

Highlights from the Journals of the ASM, January 2011 (from ASM Tipsheet)

Nanoparticle Vaccine Protects Against Stomach Flu

A new vaccine strategy using nanoparticles as carriers may be the key to developing a vaccine against norovirus, one of the most common causes of foodborne disease in the United States. Researchers from the Cincinnati Children's Hospital Medical Center report promising findings in the January 2011 isse of the *Journal of Virology*.

The application of nanoparticles as carriers to present small peptide antigens is a growing field within vaccine development. Researchers led by Xi Jason Jiang of Cincinnati Children's Hospital Medical Center, have described a new nanocarrier, called a P particle, which holds promise as a scaffold for a variety of vaccines. In the current study they inserted rotavirus antigen into the P particle, which boosted immune response to rotavirus, as well as norovirus, in mice.

Both rotavirus and norovirus are important causes of acute gastroenteritis. The former causes severe diarrhea in children, and kills an estimated 527,000 worldwide, annually. Norovirus is a notably highly transmissible, and particularly unpleasant flu, which can result in one to three days of vomiting and diarrhea in otherwise healthy adults, and which kills 200,000 children annually.

"The dual vaccine holds promise for controlling gastroenteritis in children," says Jiang.

The P particle's unique feature is the scaffold. The P particle consists of 24 copies of an outer coat protein from norovirus. The beauty of the P particle is that it contains three types of surface loops, which are ideal for presenting a wide variety of antigens. Additionally, it is highly immunogenic and extremely stable, the latter an important quality for use in developing nations. The antigens can easily be inserted during the manufacturing process. Production is a simple matter of expressing the cloned P particle in *E. coli*.

In addition to the rotavirus antigen, the team has succeeded in inserting a number of antigens into the P particle, varying in size up to more than 200 amino acids. The resulting vaccines have induced significantly stronger immune responses in mice than have free antigens.

Jiang is principal investigator for a five year, $4.1 million grant from the National Institute of Allergy & Infectious Disease (NIAID) that Cincinnati Children's received last May to develop the P particle vaccine against norovirus. "With the unique features of high efficiency, easy production, and low cost, this new platform will find a broad application in the biomedical sciences," says Jiang.

(M. Tan, P. Huang, M. Xia, P.A. Fang, W. Zhong, M. McNeal, C. Wei, W. Jiang, and X. Jiang, 2011. Norovirus P Particle, a Novel Platform for Vaccine Development and Antibody Production. J. Virol. 85:753-764.)

Staph Vaccine Shows Promise in Phase I

A new experimental vaccine against *Staphylococcus aureus* has been shown to be well-tolerated, and to boost antibodies, according to a paper in the December, 2010 issue of the journal *Clinical and Vaccine Immunology*. The vaccine was developed by Merck.

In the study, investigators led by Clayton Harro of the Johns Hopkins Bloomberg School of Public Health, Baltimore, gave a single vaccination in one of three different doses, or placebo, to four groups of 31 healthy volunteers, each, who ranged in age from 18-55. All three doses stimulated a rise in antibodies, the two higher doses significantly more so than the lowest dose. Antibody levels reached high levels after about 14 days, and they remained at those levels after three months.

"Based on this and other studies, the vaccine is now being tested in people who are at high risk of getting infected by *S. aureus* to see if the resulting antibodies can protect them from disease," says Harro. The need for such a vaccine is critical. *S. aureus* is the leading cause of hospital-acquired infections. "Invasive *S. aureus* infections (blood stream, deep wound, prosthetic device) have high associated morbidity and mortality," says Harro. In the US and Europe, 6 million people become infected annually, and 140,000 die. Multidrug-resistant *S. aureus* is an increasing problem.

Vaccine design has been a big challenge, says Harro. *S. aureus* has a complicated structure, a vast array of strains, and an uncanny capacity to evade immune surveillance systems in our bodies. But these complexities may have been rendered largely moot when the researchers discovered a single protein on the bacterial surface that is common to most *S. aureus* strains. Modern antigen discovery techniques, not available until recently, enabled the protein's discovery.

(C. Harro, R. Betts, W. Orenstein, E.-J. Kwak, H.E. Greenberg, M.T. Onorato, J. Hartzel, J. Lipka, M.J. DiNubile, and N. Kartsonis, 2010. Safety and Immunogenicity of a Novel *Staphylococcus aureus* Vaccine: Results from the First Study of the Vaccine Dose Range in Humans. *Clinical and Vaccine Immunology*, 17:1868-1874.)

MICROBIOLOGY IN THE NEWS

Nanoparticle Vaccine Protects Against Stomach Flu

ScienceDaily
January 20, 2011
A new vaccine strategy using nanoparticles as carriers may be the key to developing a vaccine against norovirus, one of the most common causes of foodborne disease in the United States.
http://www.sciencedaily.com/releases/2011/01/110119191230.htm

More than a game: Researchers design video games that feature real microorganisms

Scientific American
January 20, 2011
Do video games change behavior? This question may be the subject of debate for years, but researchers have now shown the answer to be yes—for microorganism behavior, at least.
http://www.scientificamerican.com/blog/post.cfm?id=more-than-a-game-researchers-design-2011-01-20

Need a new metabolic pathway? Steal a few genes

New Scientist
January 20, 2011
Haloarcula marismortui, a micro-organism that lives in the salty waters of the Dead Sea, is the proud owner of a unique metabolic pathway. So far it is only the third pathway of its kind uncovered in organisms that use oxygen, seemingly cobbled together using genes stolen from bacteria.
http://www.newscientist.com/article/dn20009-need-a-new-metabolic-pathway-steal-a-few-genes.html

Bacteria Work as Hard Drives

Discovery
January 10, 2011
A group of students at Hong Kong's Chinese University are making strides towards storing such vast amounts of information in an unexpected home: the E. coli bacterium better known as a potential source of serious food poisoning.
http://news.discovery.com/tech/bacteria-work-as-hard-drives-110110.html

Information on other research developments can be found at these sites:
Science News:
http://www.microbeworld.org/
http://www.scicentral.com/
http://sciencedaily.com

Important Dates

March 15, 2011:	Grant applications due
March 18, 2011:	Abstract deadline for Annual IBASM meeting
April 15-16, 2011:	Annual IBASM meeting at Brown County State Park
May 21-24, 2011:	111th Annual Meeting of the ASM, New Orleans, LA

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