

Field Laboratory Workshop

Hands-On Biogeochemical Field and Laboratory Experiences for Underrepresented Minority Undergraduates



Figure 1. After collecting samples underground, the workshop participants gathered at the shaft 3 mine entrance.

PURPOSE

The purpose of this field laboratory workshop (FLW) was to provide a field laboratory experience for minority undergraduate students within the United States and South Africa in the fields of Earth and Biological Sciences and Environmental Sciences and Engineering. This was done in conjunction with our LExEn research funded by the National Science Foundation (NSF), which provided an excellent venue from which to complement training, technology transfer and retention of necessary skills in South Africa. Anticipated products included: (1) a safe and successful workshop that could be expanded into the Research Experiences for Undergraduates (REU) Site program, (2) positive impacts on underrepresented students, and (3) lead in for future educational opportunities.

The FLW was funded as an auxiliary educational outreach function of the NSF-funded Collaborative Research: South African Ultradeep Mines-Long Term Sites for Interdisciplinary Studies (LSLIS) into Extreme Environments Project (EAR-9978267) that is examining biochemical approaches for life in hot deep subsurface environment. The research is finding that diverse thermophilic microbial communities are present in the deep gold mines of South Africa. These communities are capable of growing under extreme living conditions and surviving by oxidizing or reducing metals, likely relying on hydrogen as a major electron donor. These processes are of interest in basic research and biotechnology, as well as for research and development. In addition, the project is helping the South African mining community determine and monitor gases (hydrogen and methane) that may be harmful to miners and the mining operations. Importantly, the NSF-supported project serves as a focus for training the next generation of scientist involving three S.A. institutions, University of the Free State, University of the North, and University of Witwatersrand.

WORKSHOP GOALS

- To recruit and engage undergraduate students in unique and exciting research not normally available to them by offering state-of-the-art experimental opportunities on specific scientific topics through interaction with faculty.
- To foster a science and technology collaboration between the South African and U.S. communities through the development of an undergraduate research experience workshop. This workshop will provide the basis for the development of an undergraduate exchanges program to facilitate the transfer of innovative technologies and increase the retention of minorities in fields of Earth and Biological Sciences and Environmental Sciences and Engineering.

The long-term goal of this educational workshop and future opportunities is to encourage the retention of black and underrepresented minority undergraduates within the United States and South Africa in the fields of Earth and Biological Sciences, Environmental Sciences and Engineering. LExEn has provided an opportunity to build linkages with South African and North American educational institutions, U.S. Department of Energy laboratories, the U.S. Geological Survey and industry.

HOSTING INSTITUTIONS

Dr. Susan M. Pfiffner and Ms. Kimberly Davis of the Center for Environmental Biotechnology, University of Tennessee (UT), Knoxville, Tennessee, USA, organized and conducted the workshop in collaboration with Profs. D. Litthauer and E. van Heerden of the Department of Microbiology and Biochemistry, University of the Free State (UOVS), Bloemfontein, South Africa. The workshop was held the week of December 17-21, 2001 and was based at the UOVS campus. UOVS provided a conference room, laboratory space, and a computer work area, in addition to arranging daily-catered lunches and evening meals at the Bloemfontein zoo, potjiekos at the UOVS, and Touch of Africa (the Friday night river ride and closing ceremony).

LIST OF ATTENDEES

A total of 26 undergraduate students, graduate students, technicians and mentors participated in the Field Laboratory Workshop. These participants included five U.S. and eight S.A. undergraduates representing seven institutions of higher learning: Florida A&M, the New Mexico Institute of Technology, Princeton University, the University of the North, the University of Witwatersrand, UT and UOVS. Two technicians and one graduate



Figure 2. Group photograph of workshop participants.

student from the UOVS and Dr. E. Abotsi of the University of the North also attended the workshop, participated in field and laboratory exercises, and provided assistance with workshop logistics. The mentors included Dr. T. Kieft of the New Mexico Institute of Mining and Technology (New Mexico Tech.), Dr. T. Phelps of Oak Ridge National Laboratory, Mr. M. Davidson of the University of Witwatersrand, Dr. S. Pfiffner and Ms. K. Davis of the University of Tennessee, Profs. D. Litthauer and E. van Heerden, and Ms. E. Botse of the University of the Free State, and Mr. C. Ralston of the Harmony Evander mine. Mr. Davidson was a graduate of the Department of Microbiology at the University of Witwatersrand who had just completed his honors project on the NSF-LExEn project. Mr. Ralston is a mining geologist at Evander mine who has been collaborating with investigators on the NSF-LExEn project.

The five U.S. students were female: three African Americans, one African American-Caucasian, and one Puerto Rican. The S.A. students consisted of seven female and one male, three of whom were Caucasian.

U.S. STUDENT APPLICATION AND SELECTION

Due to the short time between notification of funding and the beginning of the workshop (3 months), recruitment of undergraduate students was mainly word of mouth to investigators within the NSF-LExEn project. The investigators sent email messages to departments or specific individuals. We received requests for information from students from five universities (Princeton University, University of Tennessee, New Mexico Institute of Mining and Technology, Florida A&M, and Tennessee State University). Students were asked to submit a copy of their transcript, a resume and a letter of interest. The two Princeton University candidate participants were selected on the basis of personal interviews from a pool of six undergraduates interested in participating. Based on this information and on phone conversations with the students, the final student participants were chosen. Some of the students not selected had incomplete packages at the time the selections were made.

Students who were selected received the UT student handbook on rules, standards and instructions for student behavior and were given the URL (<http://web.utk.edu/~kdavis/>) for additional information about the workshop. The students fulfilled additional requirements under the UT guidelines for study abroad programs. These requirements included acquiring an international student identification card and travel insurance, filling out health, emergency information, and records access authorization forms, and signing a statement of responsibility and assumption of risk form. All parents were sent a letter that provided information on the field laboratory workshop, the URL, the agenda, the travel and accommodations, the mentor contact numbers, and the emergency numbers. Prior to traveling, the students or their parents (if the student was under 21 years old) signed a mine access form that was required by the mines from every person intending to travel underground. In addition to the information posted on the web site, students were provided with three review papers on subsurface microbiology and microbiological field techniques (references 1-3 below) prior to travel.

S.A. STUDENT APPLICATION AND SELECTION

The recruitment of the undergraduate students was hampered by the short notice and summer holidays starting early in South Africa, therefore the participating SA investigators involved in the LExEn project were contacted and students identified. The students applied to attend the workshop with a short motivational report and the selections were based on the criteria discussed above.

The students were informed of the program and asked to view the information on the mentors websites as well as reading through the URL (<http://web.utk.edu/~kdavis/>) for additional information. The parents were contacted and informed about scope of the workshop as well as the underground sampling procedure. Each student signed a mine access form which was required by the mines for access to sample underground. In addition to information of the web site, students were provided with three references (listed below) prior to attending the workshop.

MENTORS

Mentors were recruited beginning with the initial conception of the workshop; as soon as we were notified that this workshop was funded, potential mentors volunteered their time for the workshop.

PREWORKSHOP

Preparation for the workshop included arranging travel and accommodations, deciding on an agenda, organizing caterers and outside activities, ordering supplies, making growth media, collecting and organizing field gear, and performing a preworkshop underground sampling trip. Dr. Pfiffner organized travel for the U.S. participants, while Dr. van Heerden organized travel for the South African participants. A draft agenda was distributed to all mentors by email and the U.S. mentors discussed the agenda over a



Figure 3. Dr. Kieft used a packer system to collect fissure water.

conference call. The final agenda was hammered out at the UOVS during the week prior to the workshop when some of the U.S. and S.A. mentors were present. Dr. van Heerden organized the caterers, outside activities, including the workshop mine sampling trip, and the workshop T-shirt. Most supplies were ordered through UT and UOVS. Carlien Pohl and Christelle Möller made media for the laboratory exercises. Field equipment, tools, supplies, sampling containers and backpacks were gathered from the LExEn research field house by Mark Davidson, Drs. Pfiffner and Phelps, and taken to the UOVS. A preworkshop sampling trip had two purposes, to familiarize the mentors with the sampling gear and working underground, and to collect backup

samples for the workshop. Colin Ralston and Walter Seymour of Harmony Mines arranged the pre-workshop sampling trip. Mentors and assisting graduate students that participated in this sampling event were Mark Davidson and Drs. Pfiffner, Kieft, Phelps, and Litthauer. During the mine sampling trip, the packers and sampling tools for collecting fissure water, the sampling tools for air and biofilms collection and the on-site chemical test kits and pH, Eh, and temperature probes were tested to ensure they were functioning properly. Samples and sampling gear were taken to UOVS for use during the workshop.

FLW AGENDA

The format of the workshop consisted of a sampling trip underground, lectures by the mentors, laboratory sessions, and computer time. Emphasis was placed on hands-on laboratory and field exercises, with lectures providing background information. Coffee/tea breaks, lunch, and some of the dinner activities were catered. Some evening activities allowed the workshop participants to tour Bloemfontein. Students worked in pairs and where possible each pair was comprised of one U.S. and one S.A. student. Otherwise, the students were paired from different institutions. Most workshop participants arrived on Sunday, December 16 and departed on Saturday, December 22. The Shawu Lodge, where non-local participants stayed, was in walking distance to the UOVS Department of Microbiology and Biochemistry.

Monday, December 17. The students and mentors convened at 08:30 in the UOVS Department of Microbiology and Biochemistry conference room for introductions and orientations on the layout of the building, safety, and conduct which was led by Drs. Litthauer and Phelps. After a short break, Dr. Pfiffner gave an introduction to the LExEn funded research project and the workshop's overall program. Dr. Phelps covered the theory of sampling and logistics of field operations. The group then moved into the laboratory to discuss sampling and analytical equipment to be used that week, which was led by Mr. Davidson. After a catered lunch buffet in the departmental courtyard, Mr. Ralston gave a talk on the geology of mines in South Africa and provided the workshop and the LExEn field house with a display box (6cm by 200cm) of the different geological formations (and depth ranges) found in the Evander mine. Dr. Litthauer discussed extreme environments and bacterial protein structure. Next, the group moved to the lab to finish packing supplies and sampling equipment into backpacks for the Dec. 18 mine trip. The evening closed with a tour of the Bloemfontein zoo and catered BBQ dinner.



Figure 4. In the laboratory, Mark Davidson described and demonstrated how to use the field equipment.

Tuesday, December 18. The students and mentors left with sack breakfasts at 05:30 from Shawu Lodge for the trip to Beatrix. Arriving at 07:30, the group was greeted by mine representatives Ian Clare, Christian Rampa-Luhembwe and Evert Kleynhans. After a short

presentation and hors d'oeuvres, two groups were formed for visiting 1 Shaft (866 meter depth with a 2 kilometer hike to the sampling destination) and 3 Shaft (1400 meter depth with a 300 meter hike to the sampling destination). The mine provided protective clothing, safety equipment and instructions for their use. Each group was escorted with 6-8 mine personnel. Samples consisted of fissure water, biofilm and sediments (from the mine floor around the sampling sites). Students assisted the mentors in collection of the samples, as well as in gathering and recording temperature, pH, conductivity, and CHEMmet (dissolved oxygen, sulfide, iron, ammonia, chloride) data. At noon, the groups surfaced, changed back into street clothes, and enjoyed a catered lunch at the "Lapa," a picnic area on the Beatrix game reserve. Gifts were exchanged between the mine personnel and the workshop participants. The workshop participants arrived back at UOVS at 16:00, where students helped to process the mine samples for use in the workshop throughout the week. The evening was open for participants to tour and have dinner in Bloemfontein.



Figure 5. Field sampling trip at the Beatrix mine.

Wednesday, December 19. The workshop participants met at 08:00 for lectures on water geochemistry and microbial processes (Dr. Phelps), microbial characterization and diversity (Dr. Kieft) and molecular techniques and applications (Ms. Botse). During breaks the workshop participants wrote thank you notes to be added to a large poster that was sent to the Beatrix mine. After a short break, the group reconvened in the laboratory where they spent the rest of the day conducting molecular and microbial practical work (DNA extraction and microbial enumeration and enrichment inoculations, inoculation of *Thermus* strain for a study of iron-reducing activity, visualization of biofilm samples by light and fluorescent microscopy). At the end of the day, a special Potjiekos supper was catered in the departmental courtyard (in addition to hosting a catered lunch at 01:00).

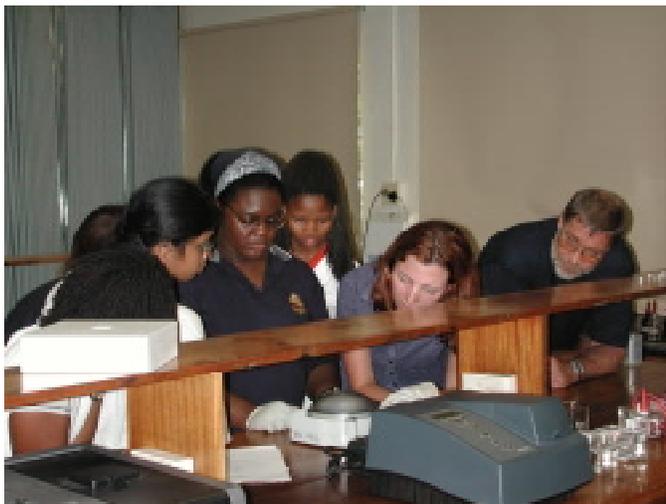


Figure 6. Students concentrated their DNA samples.

Thursday, December 20. The workshop participants met at 08:00 to continue the laboratory molecular analyses, which included amplifying the DNA by PCR and DNA purification, and

performing lipase assays. The morning session ended with lectures on extremozymes and biotechnological applications by Dr. van Heerden, on membrane lipid analysis for determining microbial community structures by Dr. Pfiffner and on environmental sustainability by Ms. K. Davis. The afternoon laboratory work involved the observation of the previous days microbial enumerations and enrichments and making clones using the TA cloning kit. The final afternoon session involved a computer exercise where the students performed a BLAST search using DNA sequences from previous mine samples provided by Dr. Kieft. These sequences were provided because there was not enough time to perform the sequence analysis on the students' clones before the end of the workshop. The evening was open for participants to tour and have dinner in Bloemfontein.



Figure 7. Students performed BLAST search.

Friday, December 21. The morning session was spent reading and recording laboratory results, discussing and interpreting the results and writing a final report on their workshop results. Lunch was catered at the UOVS courtyard. The students provided each mentor with a card and small gift of appreciation. Following lunch the workshop participants traveled to the National Women's Memorial and War Museum. There was free time for participants to shop prior to the closing ceremonies. The final workshop session included a riverboat ride and a catered dinner provided by Touch of Africa. During the dinner, a slide presentation of a photo diary of the week's events was shown. Certificates of completion and parting gifts were then exchanged. During the gift exchange for the mentors, one of the students, Liandre van der Merwe, gave an eloquent synopsis of her impressions of each mentor. The closing ceremony was laced with sense of emotional reward for all participants (few dry eyes were seen). The students had clearly bonded during the short time they spent together.



Figure 8. Drs. Pfiffner and van Heerden presented a certificate of completion to Khosi Diamini.

CONFERENCE FINDINGS AND OUTCOMES

Students expanded their knowledge base and laboratory skills in field and laboratory biology. They also gained an appreciation for interdisciplinary research through this one-week immersion in biogeochemistry of the subsurface.

The students began to develop international networks among themselves and with established scientists. In fact, Dr. Pfiffner has already submitted a letter of recommendation for one of the US students, who has applied to participate in a summer undergraduate research program at New York University School of Medicine. We are still communicating with several of the students by email.

The bonding that developed between the US and SA students resulted not only from time spent in the classroom and laboratory, but also from the evening activities and especially the times the students would gather in each other rooms for late night conversations. The extent of the bonding was evident during the closing ceremonies and was emphasized in the flurry of emails sent after the New Year.

The mentors expanded their networks to include workshop students and other mentors. Some of these connections are already leading to new collaborations: Kieft (New Mexico Tech) and van Heerden (Free State) are collaborating to isolate thermophiles with lipases that may be useful to industry. Van Heerden and Kieft are working with Dr. Abotsi (University of the North) to isolate potentially useful metal reducing and metal oxidizing bacteria from a contaminated site. Kieft plans to visit Abotsi at the University of the North in June or July 2002.



Figure 9. Namisha (Univ. Wits) and Sabriya (Florida A&M) collected souvenirs.

The students selected the trip down into the mine as the most exciting part of the workshop, even though they were a bit apprehensive prior to going underground.

Beatrix mine was very cooperative and generous with their resources while hosting the undergraduate student visit. The mines in South Africa in general are very interested in participating in educational outreach, particularly to the previously disadvantaged South Africans. Mr. Ralston is every excited about hosting students at the Evander mine.

Mr. Colin Ralston, one of the mentors and a geologist, was motivated by the workshop to enrolled in a Masters level graduate program at the Department of Microbiology and Biochemistry at the UOVS. Under the direction of Prof. Litthauer, Mr. Ralston will pursue on biotechnological applications of mine microorganisms. Colin's decision to learn more biology for the purpose of a second career in science was made in large part as a result of his participation in the Field Laboratory Workshop.

The students made extra efforts to let each mentor know that their participation was essential to the success of the workshop and to their growth as young scientists. The importance of mentors involved in educational opportunities will rely in part on support for mentors. In a every increasing population of scientists and academicians whose livelihood is based on total cost recovery, considerations for support will be an issue in future education programs.

SAMPLE COMMENTS ABOUT THE WORKSHOP

In their final report, Patience Modise and Ana Inés Gracia Zalisñak wrote "... we studied different geomicrobiological and biochemical procedures during the 5-day workshop in South Africa, and learned the techniques used for the above-mentioned processes. Thank you for giving us this great, unforgettable opportunity, not only to learn science, but also to experience an amazing cultural exchange of ideas, language, customs, and country."

Hasina Outtz wrote to us when she got back to Princeton: "... I had an absolutely AMAZING time! The week was definitely well spent, and I was able to gain a lot of experience in lab areas as well as have a lot of fun."

Namisha Muthraparsad wrote to us as her next term started: "... I am back at Wits to do my Honours. I think we will still be sampling from the Beatrix and Oryx mines...a lot of my friends want to see it (the web site). They think its pretty cool!! Could you please give me the address... had the greatest time down in Bloemfontein."

Patience Modise, one of the SA students, said that the workshop gave her a new outlook on an area of research to investigate. She is now considering an advanced degree in food safety.

The following excerpt is from a thank you email sent by Mr. Ralston sent to T.C. Onstott at Princeton. "... It was for me an incredible experience in many aspects including the science, the social interaction of all the participants who gelled instantly (beautiful to see) and the general atmosphere of learning a lot of science, but having great fun at the same time, the social issues raised, discussed and resolved. All the students were great ambassadors for their respective countries. The final highly emotionally charged awards presentation (there was hardly a dry eye there) bears testimony to what was a great week. I certainly learnt a lot, had great fun, was inspired and energized. I have been reading on the Internet for several months now to get some more basic knowledge. The week inspired me, gave me confidence in my own abilities to the extent that I have decided to enroll for an M.Sc. Biotechnology with Derek Litthauer as my mentor... For the next workshop, I will do my best to vie for the privilege of hosting the underground sampling trip on one of Harmony's mines in the Free State..."

When asked if the students would participate in an extended version (summer) program, all students said "yes!" Some students asked if they could participate in it.

Profs. D. Litthauer and E. van Heerden wrote: "To all the workshop friends: Thanks for a wonderful week of getting to know new people and sharing some great experiences. All our best wishes for the new year may all your wildest dreams come true!!!"

SUMMARY OF SURVEYS

The results of the workshop survey were that the students definitely enjoyed the workshop and found it enriching. They felt there was very good interaction with both students and mentors, and the interactions was a valuable learning experience. Although the students came from different

disciplines of study, they each felt they had gained knowledge that would help them in their career path. The high point of the workshop was the sampling underground in the mine and the low point was the computers going down when they were working on their reports. When some of the laboratory experiments did not work well, whether it was using the wrong dilution or contaminated plates, there was always someone's experiment that worked correctly that was available for group examination. The students would recommend the workshop to someone else and they would like to be involved in a similar program if it was extended to six weeks.

FUTURE PLANS

The University of Orange Free State has agreed to sequence one clone for each working group and send the results to the students by email.

Research will continue at UOVS, where graduate students will analyze samples for microbial growth and future isolations to build a collection of microorganisms from the mines. Additional DNA analyses will be performed.

The University of Tennessee has established the infrastructure for organizing and managing an international, multidisciplinary workshop with multi-institutional participation. The University of Free State has established the infrastructure for hosting this workshop and future educational and scientific events.

The University of Free State will approach the National Research Foundation to explore the potential for funding future workshops.

The University of Free State will archive workshop data and maintain microbial cultures garnered through the workshop.

SUMMARY

Purpose was successful. Goal was demonstrated as feasible, desired, and of major impact. Of these three anticipated products, the workshop was:

1. Highly successful,
2. Shown by survey, comments, attributes and program commitment to have a positive impact on underrepresented minority students
3. A niche found and a path demonstrated for fostering science and technology collaborations. Though the 2001 REU site proposal was not funded, many representatives of the NRF, NSF, and workshop sponsors and mentors are actively seeking mechanisms to continue and expand the educational program.

LIST OF REFERENCE MATERIALS

(provided to students before and during the workshop)

1. Fredrickson, J.K. and T.C. Onstott. 2001. Biogeochemical and geological significance of subsurface microbiology. *In: Subsurface Microbiology and Biogeochemistry*, J.K. Fredrickson and M. Fletcher (Eds). Wiley-Liss, Inc. New York, NY. pp.3-37.
2. Paul, E.A. and F.E. Clark. 1996. Methods for studying soil microorganisms. *In: Soil Microbiology and Biochemistry*. Academic Press, Inc., San Diego, CA. pp.36-68.
3. Fredrickson, J.K. and D.L. Balkwill. 1998. Sampling and Enumeration Techniques. *In: Techniques in Microbial Ecology*, R. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler (Eds.). Oxford University Press, New York, NY.
4. Kieft, T.L., J.K. Fredrickson, T.C. Onstott, Y.A. Gorby, H.M. Kostandarithes, T.J. Bailey, D.W. Kennedy, S.W. Li, A. Plymale, C.M. Spadoni, and M.S. Gray. 1999. Dissimilatory reduction of Fe(III) and other electron acceptors by a *Thermus* isolate. *Appl. Environ. Microbiol.* 65:1214-1221.
5. Takai, K., D.P. Moser, T.C. Onstott, N. Spoelstra, S.M. Pfiffner, A. Dohnalkova and J.K. Fredrickson. 2000. *Alcaliphilus auruminator* gen. nov., sp. nov., an Extremely Alkaliphilic Bacterium Isolated from a Deep South African Gold Mine. *Int. J. Syst. Evol. Microbiol.* 51: 1245-1256.
6. Onstott, T.C., K. Tobina, H. Donga, M.F. DeFlaun, J.K. Fredrickson, T. Bailey, F. Brockman, T. Kieft, A. Peacock, D.C. White, D. Balkwill, T.J. Phelps, and D.R. Boone. The Deep Gold mines of South Africa: Windows into the subsurface biosphere.
7. A window into the extreme environment of deep subsurface microbial community: The Witwatersrand deep microbiology project. 1997 NSF final report. Award # 9417921.
8. Introduction to Witwatersrand Gold Fields: location, exploration history, geology and references. Witwatersrand Gold Fields.htm.
9. Listing of all workshop participants including email and address.
10. Listing of health and safety guidelines and emergency numbers.
11. Copies of all slide or overheads from the lectures.
12. Handout on Extreme life-extreme protein by Derek Litthauer.
13. Witwatersrand microbiology project field manual and water sampling procedures table.
14. Laboratory procedures for microbial enumeration and enrichment; DNA extraction, purification, PCR, cloning and transformation, and BLAST exercise.