Columbia University Superfund Basic Research Program
Annual Project and Core Updates – 2008

Health Effects and Geochemistry of Arsenic and Manganese

Director: Joseph H. Graziano

Deputy Director: Alexander van Geen

Highlight #1 - (Project #2): Arsenic exposure from drinking water and all-cause and chronic disease mortality in Bangladesh

Chronic arsenic exposure through drinking water has become a growing public health issue affecting millions of people worldwide, including 35 to 77 million in Bangladesh alone. Studies from the United States, Taiwan, and Chile have reported increased mortality due to chronic diseases among arsenic-exposed populations. However, previous studies were limited to group-level data and utilized a retrospective design. Methodological limitations, including individual-level inferences based on aggregate data and measurement error, leave in question the association between mortality and arsenic exposure. Additionally, no such research has been conducted among the population of Bangladesh. The Health Effects of Arsenic Longitudinal Study (HEALS) established as part of the Columbia NIEHS-funded program project provides us an invaluable opportunity to investigate the association between arsenic exposure and mortality using a prospective design and repeated individual-level assessment of arsenic exposure.

The aim of this study was to prospectively evaluate whether arsenic exposure and changes in exposure are associated with all-cause and chronic disease mortality in a Bangladeshi population. The data utilized for this analysis was from 11,746 population-based participants recruited between 2000-2002 into the HEALS cohort. These participants have been followed-up biennially, with mortality data available through August 2006. Cox proportional hazards models were used to estimate hazard ratios (HR) of all-cause mortality and chronic disease mortality at different doses of arsenic exposure measured in drinking water and urine. We found an increased risk for both all-cause mortality (HR=1.75, 95% CI: 1.20, 2.56) and chronic disease mortality (HR=1.97, 95% CI: 1.28, 3.06) among individuals in the highest quartile of water arsenic concentrations. These associations were similar using other arsenic exposure constructs. Additionally, the population attributable mortality risk was estimated as 17.8% for all-cause mortality and 22.6% for chronic disease mortality predicted from well water arsenic concentrations greater than 10 µg/L in our study sample.

Utilizing four-years of follow-up data from the HEALS cohort, Dr. Ahsan and his colleagues have shown an increased mortality risk due to chronic arsenic exposure. The association persisted across different individual-level exposure constructs, including well water arsenic and urinary total arsenic concentrations. These study findings not only corroborate the prior research, but contribute to the public health literature on arsenic by providing precise individual-level estimates for mortality risk among an arsenic-exposed population. Dose-response effects have been previously demonstrated in this population for the association between arsenic exposure and premalignant skin lesions, and evaluations of children from our study area have also shown significant dose trends of arsenic exposure on developmental effects. Future data from this cohort will provide us with the unique opportunity to assess dose-response effects of arsenic exposure on mortality with adequate study power. Additionally, prospectively collected arsenic exposure and mortality data will strengthen our understanding of the impact of changes in exposure on longer-term mortality risk.

These findings emphasize the public health challenge millions of Bangladeshis and others worldwide face due to arsenic exposure through drinking water. We see that even with short-term reductions in exposure, our study population remains at increased risk of mortality due to chronic arsenic exposure. This research highlights the need for continued remediation processes in arsenic-exposed populations and further public health research to address the residual health effects of chronic arsenic exposure.

Kalra T, Argos M, Rathouz P, Chen Y, Pierce B, Islam T, Ahmed A, Rakibuz-Zaman M, Hasan R, Hasan K, Slavkovich V, Parvez F, Graziano J, Ahsan H. Arsenic Exposure from Drinking Water and All-cause and Chronic Disease Mortality In Bangladesh; Under Review 2008.

Highlight #2 - (Project 7): Suppression of Arsenic Release to Shallow Groundwater of Bangladesh by Precipitation with Reduced Sulfur

Reductive dissolution of iron oxyhydroxides and reduction of arsenic are both widely invoked as leading causes of high arsenic levels in groundwater of Bangladesh and neighboring countries draining the Himalayas. This perspective was recently challenged by a study initiated under Project 7, with additional support provided by the Biogeochemical Core laboratory, showing that As levels in groundwater would severely impact the health of many more millions of villagers were it not for the reduction of As(V) to As(III) followed by precipitation as arsenic sulfides (e.g., orpiment or realgar). Detailed time-series covering two successive monsoons at an unusually sensitive location show that the formation of these sulfides is dependent on recharge of shallow aquifers with surface water containing sulfate and results in the sequestration of As(III) in the sediment in preference to phosphate, the structural analogue for A(V). X-ray absorption spectroscopy data obtained by our collaborator Dr. Benjamin Bostick at Dartmouth College unmistakably shows that the loss of As from groundwater is coupled to the formation of As sulfides in the sediment. These observations challenge the view that As levels increase with depth in Bangladesh groundwater primarily because of reductive dissolution of Fe oxyhydroxides. Re-analysis of groundwater data obtained by the British Geological Survey and the Department of Public Health Engineering of Bangladesh for 1509 wells <30 m deep distributed across the country confirms that the formation As sulfides probably is equally important at regulating dissolved As in shallow groundwater.

The documented response of As in the dissolved and solid phase to a seasonal perturbation combined with regional patterns at a larger scale in Bangladesh show that, given a sufficient supply of sulfur, reducing conditions promotes the sequestration of As in sediment rather than its release to groundwater. The observations help explain that As is released from the sediment only after a certain threshold of reduction of Fe oxyhydroxides has been reached if a local supply of sulfate prevents the sediment from crossing a certain threshold of reduction of Fe oxyhydroxides, as shown by previous Columbia-based SBRP work in Bangladesh. Another significant implication of the demonstrated As-S interactions is that an enhanced supply of sulfate driven by irrigation pumping could lead to reduced As concentrations in shallow groundwater, reducing previous concerns that irrigation pumping might instead enhance the release of As by promoting the reductive dissolution of Fe oxyhydroxides. A final potential implication of a practical nature relates to As removal. Competition with phosphate has long been recognized as one of the leading factors that reduce the capacity of removal systems, which are based primarily on adsorption and/or co-precipitation with Fe oxyhydroxides. Our field observations suggest that precipitation of As with sulfide might provide a way of circumventing this problem in those areas where P levels in groundwater are particularly high.

It is worth emphasizing that, by and large, As concentrations do not vary over time in Bangladesh groundwater. The large seasonal fluctuations observed at this particularly shallow well located near a stream had previously been identified as part of a large-scale sampling effort on behalf of cohort recruitment under biomedical projects supported by the Columbia SBRP. Without this inter-disciplinary activity, the observed fluctuations would probably not have been recorded and, therefore, the underlying processes not identified in a follow-up.

Aziz, Z., B.C. Bostick, Y. Zheng, M.R. Huq, M.M. Rahman, K.M. Ahmed, and A. van Geen, Suppression of arsenic release to shallow groundwater of Bangladesh by precipitation with reduced sulfur, submitted to *Nature*, December 2008.

Project 1: ***Genotoxic and Cell Signaling Pathways of Arsenic in Mammalian Cells***
P.I.: Tom Hei

1) The overall goals of this project remain unchanged.

2) During the current funding period, we assessed whether arsenic induces both heteroplasmic and homoplasmic point mutations in mitochondrial DNA. Human hamster hybrid (A_L) cells were treated with arsenic (1 µg/ml for 5 days). A direct sequencing approach was used to reduce the occurrence of experimental errors and all detected base changes were cross-checked with databases of known polymorphisms. Our results showed that, while base changes did occur, there was no difference between the number of changes in treated and untreated cells. Furthermore, in human lymphocyte samples from subjects exposed to arsenic from our cohort studies in Bangladesh, most of the base changes have previously been reported. Interestingly, there was an increase in the number of transversions (purine to pyrimidine) in smokers from the human samples, but not in the total number of base changes. These data suggest that only a change in the number of rare transversions would be indicative of an increase in point mutations in mtDNA after exposure to arsenic.

   Our previous studies have shown arsenic to be an efficient inducer of apoptosis in human melanomas. In the past year, we extended the studies to normal human epithelial cells. Human small airway epithelial cells were continuously exposed to 0.5 µg/ml arsenic for 28 weeks, and apoptosis was examined after 24 h treatment with either Fas L or hydrogen peroxide (H_2O_2). Among arsenite-treated SAEC cells, there was a significant decrease in cell viability and an increase in apoptosis after treatment with Fas L and H_2O_2, compared to non-arsenic treated control cells. Furthermore, treatment of arsenic-exposed SAEC with Fas L or H_2O_2 induced cleavage of the DNA damage recognition protein, poly (ADP-ribose) polymerase (PARP), and the 'effector' caspase, Caspase-3, both canonical indicators of apoptosis. We further observed an increase in phosphorylation of p38, a member of the MAP kinase family, following treatment with Fas L or H_2O_2. To further confirm the involvement of p38 in the regulation of apoptosis, we pretreated cells with the p38 kinase inhibitor, SB 203580 and observed a significant decrease in apoptosis. These data indicate that in long term arsenic-treated human epithelial cells, p38 mitogen activated protein kinase signaling pathway plays an essential role in mediating apoptosis upon treatment with both intrinsic and extrinsic stimuli.

3) Arsenic is an important environmental carcinogen that affects millions of people worldwide through contaminated water supplies. A better understanding of the mutagenic/carcinogenic mechanism of arsenic should provide a basis for better interventional approach in both treatment and prevention of arsenic induced cancer.

4) In the coming year, we will continue using multiplex PCR and DNA sequencing to identify the incidence and types of mitochondrial DNA mutations in arsenic-induced skin lesions with or without concurrent UV exposure based on the Bangladeshi cohort. Furthermore, we will determine the effects of both mitochondrial mediated and non-mitochondrial associated signaling pathways in regulating cell survival and apoptosis in normal and cancer cells.

Publications:

Ivanov, V., Partridge, M.A., Johnson, J.E., Huang, S.X.L., Zhou, H., and Hei, T.K., Resveratrol sensitizes human melanomas to TRAIL through modulation of anti-apoptotic gene expression in conditions of partial suppression of mitochondrial function. ***Exp. Cell Research*** 314: 1163-1176, 2008.

Wen, G.Y., Calaf, G.M., Partridge, M.A., Chau-Echiburu, C., Zhao, Y., Huang., S.X.L., Chai, Y.L., Li, B.Y., Hu, B. and Hei, T.K., Neoplastic transformation of human small airway epithelial cells induced by arsenic***. Mol. Medicine*** 14: 2-10, 2008.

Wen, G.Y., Partridge, M.A., Calaf, M., Meador, J., Hu, B., Chau-Echiburu, C., Hong, M., and Hei, T.K., Increased susceptibility of human small airway epithelial cells to apoptosis after long term arsenate treatment. **Sc. Total Environment**: 2008 Oct 30. [Epub ahead of print]

Partridge, M.A., Huang, S. X.L., Kibriya, M.G., Ahsan, H., Davidson, M.and Hei, T.K., Environmental mutagens induced transversions but not translations in regulatory region of mitochondrial DNA. ***J. Toxicology & Environmental Health*** (In press, 2008).

Project 2: *A Cohort Study of Health Effects of Arsenic Exposure in Bangladesh*
P.I.: Habibul Ahsan

This prospective cohort study recruited 11,746 men and women in Araihazar, Bangladesh, during 2000-2002 to investigate the health effects of arsenic exposure, with an initial focus on skin lesions and skin cancers. Between 2006 and 2008, the cohort was expanded to 20,033 individuals to increase our statistical power to detect arsenic-induced cancers (see below). Expansion participants were enrolled into the cohort through in-person interviews and had blood and urine samples collected in the same manner as the baseline cohort. The design of this multidisciplinary project and cohort description has been published (Ahsan et al., 2005). We have numerous publications utilizing the baseline cross-sectional data on arsenic exposure and adverse health effects, including prevalent skin lesion cases (Ahsan, Chen et al. 2007; Argos, Parvez et al. 2007; Chen, Hall et al. 2007; Heck, Chen et al. 2008; Parvez, Chen et al. 2008) and are currently conducting longitudinal analyses with the prospective data.

In-person interviews and clinical examinations are conducted every 2 years. In our previous progress report, we summarized the two- and four-year follow-up visits. The six-year follow-up visit of the original cohort is nearly complete and, we initiated the 2-year follow-up visit of the expansion cohort in the beginning of November 2008. In under a month we have administered more than 100 questionnaires and have collected surveillance cardiovascular disease data on more than 500.

Based on the data collected from the first two follow-up visits (2-year and 4-year visits) of the original cohort, we see that the incidence of cancer within the study population is lower than what we expected based on extrapolation from other studies of arsenic health effects in other countries and also WHO cancer incidence data for Bangladesh (based on incidence in India). Nevertheless, our prospective analyses based on individual level data clearly suggest an increased total mortality in relation to increases in arsenic exposure based on water and urinary arsenic, as described in Highlight #1. Since the prior progress report, we have been working to enhance our detection of the cancer cases that occur within our study cohort. In order to enhance our chronic disease surveillance, we are in the process of opening a clinic facility specifically geared towards chronic disease diagnosis and treatment. We have trained field workers to administer a simplified chronic disease questionnaire in which they are able to encourage participants visit our chronic disease unit if symptoms point towards a chronic disease ailment. There, trained physicians administer more detailed questionnaires and clinically diagnose disease.

The objectives of our current analyses and the follow-up interviews are to specifically examine the aims stated in the renewal of this project related to cardiovascular disease; these have not been modified since the last progress report. Several other manuscripts are being drafted for publication

reporting results of our prospective cohort analyses of modification of the effects of arsenic on mortality and incidence of premalignant skin lesions.

In addition to the above-mentioned components, resources from this prospective cohort study have also yielded many other ancillary studies and publications including three additional R01 grants from NIH to investigate the genetic and nutritional aspects as well as chemoprevention of arsenic health effects.

Project 2-supported 2008 publications:

Chen, Y., R. M. Santella, et al. (2007). "Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules." Environ Health Perspect **115**(10): 1415-20.

Heck, J. E., Y. Chen, et al. (2008). "Arsenic exposure and anemia in Bangladesh: a population-based study." J Occup Environ Med **50**(1): 80-7.

Parvez F, Chen Y, Brandt-Rauf PW, et al. Nonmalignant respiratory effects of chronic arsenic exposure from drinking water among never-smokers in Bangladesh. Environ Health Perspect. Feb 2008;116(2):190-195.

Zablotska, L. B., Y. Chen, et al. (2008). "Protective effects of B vitamins and antioxidants on the risk of arsenic-related skin lesions in Bangladesh." Environ Health Perspect **116**(8): 1056-62.

Project 2-supported 2008 submissions:

Kalra, T.K. Ahsan, H. (Submitted 2008) “Arsenic Exposure from Drinking Water and All-Cause and Chronic Disease Mortality in Bangladesh” JAMA.

Project 3: *Consequences of Arsenic and Manganese Exposure on Childhood Intelligence*
P.I.: Joseph Graziano

This project builds upon our discovery that arsenic and manganese exposure from drinking water have adverse effects on intelligence in children. Two ongoing studies, in Bangladesh and New England, are in progress. Two years ago we launched a study of elementary school children in New Hampshire to determine whether exposure to arsenic impairs intellectual functioning in a U.S. population. Because recruitment in New Hampshire was not proceeding at a sufficient rate, we expanded our study into Maine during the past year. While progress has improved, it is still lagging far behind our aims and expectations. We were not able to obtain the cooperation of any additional schools in New Hampshire. However, we have been well received in several schools in Maine.

In the period from Jan-June, 2008 we recruited as many children for study in Maine as we had in the prior 18 months in New Hampshire. However, our late start in Maine, in conjunction with an extremely severe winter there, hampered progress. By the end of June, 2008 (the school year) we had sent out a total of 1725 recruitment packages. Of the 203 positive responses 75 families were scheduled and tested, 37 from New Hampshire and 38 from Maine. Sample analysis and data entry and analysis for these subjects are ongoing.

The second portion of this project takes place in Bangladesh, where our research seeks to determine whether exposure to arsenic and manganese has an adverse effect on motor function and on intelligence. This portion of our work has gone well in the past year. This study involves a 2 x 2 design, i.e., high/low drinking water As and high/low water Mn, with 75 children in each cell. Last month, we successfully completed the recruitment of all 300 children, 7-9 years of age. Data entry is

ongoing, as is the analysis of blood and urine samples for the measurement of biomarkers of exposure.

The next phase of the Bangladesh work involves remediation of the ongoing As and Mn exposures via the installation of deep tube wells in each child's village by our earth science investigators (Project #7). Our objective is to reassess motor function and intelligence in one and two years to determine whether the consequences of exposure are reversible. The installation of deep wells, which will be achieved not with NIEHS funding but with funds provided by an anonymous donor to the Columbia Earth Institute, has been delayed due to Columbia University's insistence that a third party, preferably the Bangladesh government, be responsible for well installation. The position taken by our university trustees reflects their recent desire to minimize any theoretical risks to the university that exist in its relatively extensive global health research portfolio, which spans more than 100 different countries. We have therefore worked to achieve their goals through extensive negotiations with the in-country office of the World Health Organization (WHO) and the Bangladesh Department of Public Health Engineering (DPHE). This issue is discussed more extensively below in Project #7. We remain optimistic that we will soon be able to proceed with deep well installation.

Student Involvement:

Christine George, Dr. Graziano's PhD student, is deriving her dissertation from the Bangladesh portion of this project.

Lauden Behrouz is deriving her MPH dissertation in Epidemiology from this project.

Khalid Khan, is a Bangladeshi DrPH student in EHS who is supported by our Fogarty Training Grant. Under the PI's direction, he is conducting an assessment of the impact of a school-based As education program in Araihazar, Bangladesh, in reducing As exposure. He has worked extensively with school teachers, administrators and elementary school students during the past year.

Publications:

There have been no publications from project #3 during the past year, though the PI has been a co-author of 6 other publications derived from projects #2 and #4. This is due to the fact that we have been conducting field work throughout the year. We anticipate, however, that the fruits of that fieldwork, and the ongoing statistical analyses, will very soon yield a series of novel findings and publications concerning the effects of As and Mn exposure in children.

Project 4. *One-Carbon Metabolism, Oxidative Stress and Arsenic Toxicity in Bangladesh*
P.I.: Mary V. Gamble

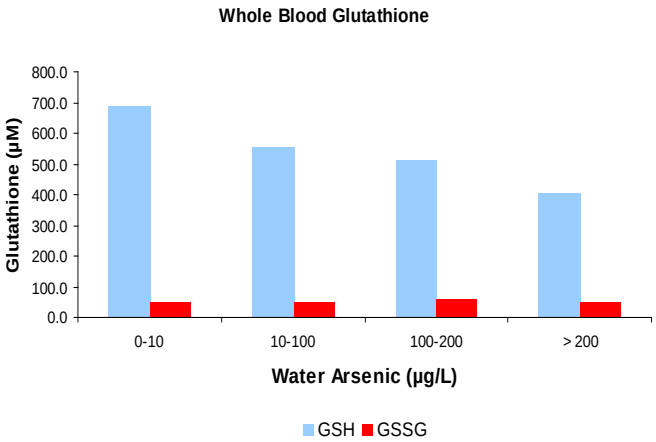
Nutritional status may account for some of the considerable variability in progression from arsenic (As) exposure to manifestations of disease. Methylation of ingested inorganic As (InAs) to methylarsonic- (MMA) and dimethylarsinic acids (DMA) relies on folate-dependent one carbon metabolism and facilitates urinary As elimination. Our SBRP project builds upon our Nutritional Influences on As Toxicity (NIAT) studies which demonstrated that folic acid supplementation increases As methylation[1,2] and lowers blood As and blood MMA concentrations[3].

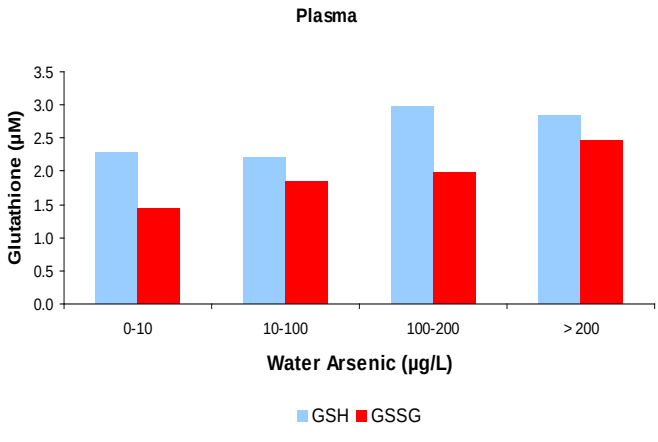
The first aim utilizes the repository of biological samples (from Project #2) to conduct a nested case-control study. We tested the hypothesis that at the time of enrollment, participants who subsequently developed As-induced skin lesions (SLs) had lower folate and/or higher homocysteine as compared to non-skin lesion controls; we also analyzed genomic methylation of leukocyte DNA. The odds ratios (95% C.I.s) for subsequent development of SLs for participants who had low folate, high Hcys, or hypomethylated PBL-DNA were 1.6 (1.05 – 2.5; p = 0.005), 1.7 (1.1 - 2.6; p = 0.01), and 1.7 (1.1 – 2.7; p = 0.008), respectively, indicating that folate deficiency, hyperhomocysteinemia, and hypomethylation of genomic DNA are risk factors for As-induced skin lesions[4].

In this same study, we also proposed to analyze biomarkers of oxidative stress. Analyses of urinary 8-hydroxy-2'-deoxyguanosine suggest that this biomarker is not associated with risk for skin lesions. An inverse association was observed between plasma folate and 8OHdG, suggesting that the role of folate in maintaining the intracellular nucleotide pool may be important for the repair of oxidative DNA lesions. Additional biomarkers will include malondialdehyde and protein carbonyls.

For the second aim, we proposed to examine the extent to which urinary As metabolites reflect As metabolites in blood in 130 Bangladeshi adults. The Spearman correlations between As in blood and urine ranged from 0.68 to 0.81 ($p < 0.0001$ for all metabolites). However, when the As metabolites were expressed as a percentage of total As, the correlations were less strong (0.32 – 0.44; $p < 0.001$). Most striking were the differences in %MMA (13% in urine vs. 40% in blood) and %DMA (72% in urine vs. 34% in blood)[3], consistent with a short circulating half-life of DMA which is rapidly excreted in urine. These findings suggest that studies analyzing As metabolites in urine have more limitations than previously believed.

Fieldwork for aims 3 and 4 began in January 2008. All 375 participants have been enrolled, and baseline biological samples are currently being analyzed for reduced and oxidized glutathione. Preliminary data for the first 102 samples suggest that As exposure is associated with decreased concentrations of reduced glutathione (GSH) in whole blood and increased concentrations of oxidized glutathione (GSSG) in plasma in a dose-dependent fashion (see figure). These data are consistent with the hypothesis that As exposure is associated with depletion of glutathione. Since GSH is the body's primary antioxidant, this would be expected to be associated with increased oxidative stress. Other measures of oxidative stress that we propose to evaluate for this aim include malondialdehyde, protein carbonyls and urinary 8-hydroxy-2'-deoxyguanosine.





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1. Gamble MV, Liu X, Ahsan H et al. Folate, homocysteine and arsenic metabolism in Bangladesh. Environ Health Perspect 2005;113:1683-8.

2. Gamble MV, Liu X, Ahsan H et al. Folate and Arsenic Metabolism: A double-blind placebo controlled folic acid supplementation trial in Bangladesh. Am J Clin Nutr 2006;84:1093-101.

3. Gamble MV, Liu X, Slavkovich V et al. Folic Acid Supplementation Lowers Blood Arsenic. Am J Clin Nutr 2007;86:1202-9.

4. Pilsner JR, Liu X, Ahsan H et al. Folate deficiency, hyperhomocysteinemia, low urinary creatinine and hypomethylation of leukocyte DNA are risk factors for arsenic-induced skin lesions. Environ Health Perspect 2008;(in press).

5. Chen Y, Parvez F, Gamble M, Islam T, Graziano JH, and Ahsan H. Health Effects of Arsenic Exposure from Drinking Water in Bangladesh: Key Findings from the Health Effects of Arsenic Longitudinal Study (HEALS) (in review, Toxicology and Applied Pharmacology).

6. Hall MN, Liu X, Slavkovich V, Ilievski V, Alam S, Factor-Litvak P, Graziano JH, and Gamble MV. Influence of Cobalamin on Arsenic Metabolism (manuscript in preparation).

7. Pilsner JR, Liu X, Hall M, Rahman M, Factor-Litvak P, Graziano JH, and Gamble MV. Influence of maternal arsenic exposure on genomic DNA methylation in maternal-newborn pairs. (manuscript in preparation).

8. Pilsner JR, Liu X, Ahsan H, Ilievski V, Slavkovich V, Levy D, Factor-Litvak P, Graziano JH, Gamble MV. Influence of folic acid supplementation on DNA methylation in Bangladeshi adults. (manuscript in preparation).

Project 5: *Mobilization of Natural Arsenic in Groundwater*
P.I.: Yan Zheng
Co-Investigators: Martin Stute, and Alexander van Geen

We continued intensive field campaigns in Bangladesh (Oct. 07, Jan. 08, Mar. 08, Jul. 08) and New England (Oct. 08) in the past year. Especially noteworthy was the completion of two large group experiments jointly with Project 7 and the Hydrogeology Core that assessed As mobility *in situ* in shallow and deep aquifers in Bangladesh.

Kinetics and Equilibrium of As mobilization: *in situ* and laboratory experiments

The partitioning of As between groundwater and sediment is hypothesized to be controlled locally by sorption equilibria. This applies to the shallow aquifer where groundwater As is high because sediment As can be high and the affinity of As to reducing sediment is low, i.e., because of a low partitioning coefficient (Kd). This also applies to the deep aquifer where groundwater As is low because sediment As is low and the affinity of As to oxidized sediment can be high (high Kd).

In the deep aquifer, both batch and *in situ* experiments demonstrate that As(III) and As(V) sorption occur with half-times of hours. The Kd of the orange colored sand is high, with sorption

capacity between 35-40 mg/kg. The results support the potential of using this aquifer as a sustainable drinking water source.

In the shallow aquifer, both batch and *in situ* experiments demonstrate that sorption and desorption of As(III) to sediment are also fast. The Kd of the mostly gray colored sand with variable grain size vary widely, with sorption capacities between 6-8 mg/kg. The results suggest that groundwater As concentrations are determined by the "upstream" groundwater concentrations and the local adsorption partitioning (Kd) of the sediment.

The implication is that clean up of As contaminated reducing aquifer is challenging due to quick re-establishment of local sorptive equilibrium.

Arsenic during Groundwater Discharge: Field, Laboratory and Modeling Study

In groundwater discharge areas along the Meghna River in Bangladesh and in Waquoit Bay, MA, the partitioning of As between groundwater and sediment is also controlled by sorptive equilibrium. Data from high spatial density piezometers show systematic Fe and As attenuation from well water to riverbank or bayside porewater, indicating trapping of As in a reactive barrier consisted of amorphous Fe-oxides determined by XAS. A reactive-transport model has successfully simulated the concentrations of the As plume and the precipitated Fe-oxides at the redox interfaces by incorporating a semi-mechanistic surface-complexation model based on experimentally determined sorption isotherms (JUNG et al., 2008).

Bedrock Geology Control As, U, Rn Spatial Distribution in Greater Augusta, Maine

Two sets of private well samples were collected in 2006 (n=787) in 13 towns encompassing 1000 km^2 area of Greater Augusta, Maine, and in 2007 (n=343) in 4 cluster areas that are chosen to represent low, intermediate and high As occurrence. A geostatistical analysis demonstrates for the first time that bedrock geology controls the spatial pattern of As distribution at intermediate scale (10^0-10^1 km) relevant to community planning applicable to New England (YANG et al., 2008). Two high As wells were surveyed with down-hole geophysical methods in October 2008 to link individual fracture characteristics with elevated As concentrations.

Students and Post-docs whose research is supported at least in part by Project 5:

Bethany O'Shea, Post-doc fellow, Columbia University Science Fellow
Hun Bok Jung, 5th yr PhD candidate, Earth and Environmental Sciences, City University of New York (CUNY)
Kathleen A Radloff, 5th yr PhD candidate, Environmental Engineering, Columbia
Qiang Yang, 3rd yr PhD candidate, Earth and Environmental Sciences, CUNY
Ivan Mihajlov, 2nd yr PhD student, Earth and Environmental Sciences, Columbia
Mohammed Wahid Rahman, 1st yr PhD student, Earth and Environmental Sciences, CUNY
Margaret Bounds, Undergraduate, Environmental Science, Barnard College
Hosea Siu, Intel-Westinghouse Contestant, Bronx High School of Sciences

Publications in 2008 (7 published, 4 submitted):

Aziz Z, van Geen A, Stute M, Versteeg R, Horneman A, Zheng Y, Goodbred S, Steckler M, Weinman B, Gavrieli I, Hoque MA, Shamsudduha M, Ahmed KM. (2008) Impact of local recharge on arsenic concentrations in shallow aquifers inferred from the electro-magnetic conductivity of soils in Araihazar, Bangladesh. *Water Resources Res* **44**(7).

Dhar RK, Zheng Y, Stute M, van Geen A, Cheng Z, Shanewaz M, Shamsudduha M, Hoque MA, Rahman MW, Ahmed KM. (2008) Temporal variability of groundwater chemistry in shallow and deep aquifers of Araihazar, Bangladesh. *J Contaminant Hydrology* **99**(1-4), 97-111.

He Y. and Zheng Y. (2008) Comparison of the Urinary Excretion of Arsenic Metabolites Before and After Rice Diet. *Toxicol. Appl. Pharmacol.*

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Jung HB, Charette MA, Zheng Y. (2008) A field, laboratory and modeling study of reactive transport of groundwater arsenic in a coastal aquifer. *Env. Sci. Tech.* **submitted**.

Loose B, Stute M, Alexander P, Smethie WM. (2008) Design and deployment of a portable membrane equilibrator for sampling aqueous dissolved gases. Water Res. Res., submitted.

Radloff KA, Manning AR, Mailloux B, Zheng Y, Moshiur Rahman M, Rezaul Huq M, Ahmed KM, and Geen Av. (2008) Considerations for conducting incubations to study the mechanisms of As release in reducing groundwater aquifers. *Applied Geochemistry* **23**(11), 3224.

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Weinman B., Goodbred S. L., Zheng Y., Aziz Z., Steckler M., van Geen A., Singhvi A. K., and Nagar Y. C. (2008) Contributions of floodplain stratigraphy and evolution to the spatial patterns of groundwater arsenic in Araihazar, Bangladesh. *Geological Society of America Bulletin* **120**(11-12), 1567-1580.

Yang Q., Jung H. B., Culbertson C. W., Marvinney R. G., Loiselle M. C., Locke D. B., Cheek H., Thibodeau H., and Zheng Y. (2008) Spatial Pattern of Groundwater Arsenic Occurrence and Association with Bedrock Geology in Greater Augusta, Maine, USA. *Env. Sci. Tech.* **submitted**.

Project 6: *Mobilization of anthropogenic arsenic in groundwater*
P.I.s: Steven Chillrud, Martin Stute, H. James Simpson, Brian Mailloux, John Stolz

Project 6 examines arsenic behavior associated with the Vineland Superfund site in southern New Jersey, both at the former biocide manufacturing facility as well as downstream at a recreational reservoir impacted by arsenic transport from the site. Despite remediation of unsaturated zone soils (where pre-treatment soil arsenic was >500 ppm) and eight years of pump-and-treat remediation, groundwater arsenic can still exceed 1000 ppb on site.

During the past year we have utilized field experiments, hydrological modeling, and laboratory work to evaluate the potential for subsurface chemical additions to accelerate arsenic remediation. Extrapolations from laboratory column experiments of aquifer solids have suggested that the current aquifer clean-up strategy relying on pump-and-treat to flush ambient groundwater through the aquifer, could require hundreds of years before remediation targets are reached, whereas this timeframe could be decreased substantially (to <5 years) by introducing oxalic acid and/or phosphate to the

subsurface. This year's lab experiments focused on refining the chemical cocktail to be used in field trials as well as improving approaches to scale up field experiments.

Five injection well nests and one sampling well were installed in the most contaminated zone and within the depression cone of an existing pump-and-treat recovery well on site. A tracer experiment was performed showing a peak in concentration at the sampling well 4.8-4.9 days after injection, equivalent to average groundwater flow of ~1.3 m/d. From this tracer experiment, a modified hydrological model is being constructed to help plan chemical injection rates, volumes, and concentrations as well as to optimize sampling frequency for the pilot field study.

Additionally, we have performed molecular studies to elucidate effects of elevated arsenic concentrations on the microbial ecology of the subsurface. We have found that the arsenic reductase gene, *arrA*, is present at only low copy numbers and is non-detectable at many locations. This is in contrast to environments such as Mono Lake where *arrA* is thought to drive arsenic cycling. Instead, we observe a diverse population of microbes with the arsenical compound resistance *acr3* gene that is responsible for encoding enzymes which transport arsenite out of cells. Further analysis of *acr3(2)* from 11 diverse worldwide locations indicates this gene is ubiquitous and may play an important role in arsenic cycling in a wide range of environments.

We have also undertaken an investigation of remobilization of arsenic from sediments into the bottom waters of Union Lake as a result of seasonal anoxia. Preliminary data indicate that even during anoxia, arsenic remobilization is relatively minor, with maximum arsenic concentrations in lake waters reaching only 28 ppb for several weeks during the summer months. Manganese mobilization is more persistent indicating that the system may be redox buffered by reduction of Mn(IV) to Mn(II). *AroA,*and *ArrA,* which encode arsenite oxidizing and reducing enzymes, are present in the lake throughout the summer redox changes indicating that in the past, [As] may have been high enough to be a meaningful source of energy to the microbial community.

Students and Postdocs involved in studies in 2008:

Alison Keimowitz, PhD 2005, Earth and Environmental Science, currently postdoctoral fellow at Columbia University
Karen Wovkulich, 4th year graduate student, Earth and Environmental Science, Columbia University
Ekaterina Alexandrova, undergraduate student, Barnard College
Alison Powell, undergraduate student, Barnard College
Peter Wagner, undergraduate student, Columbia University
Sasha Harbajan, George Washington Carver High School Harlem Children Society. www.harlemchildrensociety.org
Lauren Thompson, undergraduate student, Barnard College
Jared Fox, Frederick Douglass Academy III in the south Bronx. This past summer as a part of Columbia University's Science Teacher Research Program

2008 abstracts and meetings:

Keimowitz, A. R., S. N. Chillrud, J. N. Ross, B. J. Mailloux, M. Stute, and R. Waldholz. 2008. Arsenic Cycling in Union Lake, NJ. Geological Society of America Abstracts with Programs, Vol. 40, No. 6, p. 459.
Keimowitz, A. R., B. J. Mailloux, S. N. Chillrud, J. M. Ross, K. Wovkulich, P. McNamara, E. Alexandrova, and L. Thompson. 2008. Arsenic Movement From Sediment to Water: Microbes and Mobilization in a Contaminated Lake. Eos Trans. AGU, 89:Fall Meet. Suppl., Abstract H41E-0916.

Wagner, P. J., B. J. Mailloux, R. Foster, J. F. Stolz, M. Scholz, K. Wovkulich, A. R. Keimowitz, B. Lanoil, A. Van Geen, and G. Freyer. 2008. Worldwide Diversity and Occurrence of Arsenite Transporter acr3(2) Suggests An Important and Overlooked Pathway. Geological Society of America Abstracts with Programs, Vol. 40, No. 6, p. 523.

Wovkulich, K., M. Stute, H.J. Simpson, B. Mailloux, A. Lacko, A.R. Keimowitz, Z. Cheng, S. Chillrud, Increasing Arsenic Mobilization from Contaminated Sediments to Improve Remediation Efficiency, Poster at the 2nd International Congress on Arsenic, Valencia, Spain, May 2008.

Wovkulich, K., M. Stute, B. Mailloux, H.J. Simspon, A.R. Keimowitz, A. Powell, S.N. Chillrud, Accelerating Pump-and-Treat Remediation of Arsenic: Preparations for In Situ Arsenic Mobilization, Poster at the annual Superfund Basic Research Program Meeting, Pacific Grove, CA, Dec 2008.

Wovkulich K., B. Mailloux, M. Stute, H.J. Simpson, A.R. Keimowitz, A. Powell, A. Lacko, S.N. Chillrud, Arsenic Remediation Enhancement through Chemical Additions to Pump-and Treat Operations, Oral presentation at the fall annual American Geophysical Union Meeting, San Francisco, CA, Dec 2008.

Project 7: *Mitigation of Arsenic Mobilization in Groundwater*
P.I.: Alexander van Geen
Co-Investigators: Yan Zheng, Martin Stute, Zhongqi Cheng, Peter Schlosser, Andrew Gelman, and Alex Pfaff

Work carried out under this project has focused on 2 themes: (1) broadening our understanding of adsorption/desorption and transport for regulating temporal and spatial fluctuations in groundwater As levels and (2) generating data in support of clearance from various authorities for installing low-As community wells to support health interventions under projects 3 and 4. The activities have resulted in 9 published papers and 3 submissions under review.

(1) Our extensive groundwater monitoring network in Araihazar has shown that As levels in groundwater are surprisingly constant given the large changes in groundwater levels and flow in response to the monsoon, whereas concentrations of other constituents of groundwater are much more variable (Dhar et al., 2008). This suggests that As concentrations in groundwater might be buffered by adsorptive equilibrium with a larger pool in the particulate phase. Evidence for such exchange has been obtained by sampling groundwater and aquifer sands simultaneously across a wide range of settings in Bangladesh and subjecting the sands to a chemical extraction to isolate the exchangeable fraction (van Geen et al., 2008). Monitoring has recently unveiled another process that dampens fluctuations in groundwater As linked to the precipitation of As-sulfides (Aziz et al., submitted – see Highlight).

(2) The decision of Columbia University's Office of the General Council to delay our team's plan to install about 100 community wells in Araihazar (with support from a private donor), discussed above in Project #3, has forced us to look for alternative approaches. The avenue we are exploring is to provide the private funds to the local World Health Organization (WHO) office in Dhaka, which would then pass these funds on to the Department of Public Health Engineering (DPHE) to carry out the installations on the basis of our suggestions. Our team would provide the analytical support to monitor these wells, ensure that the WHO guideline for As in drinking water of 10 µg/L is met, and that the exposure of the population to Mn is, on average, greatly reduced, even if not not every single well achieves the WHO guideline of 0.4 mg/L. This plan is acceptable to Columbia's Office of the General Council, WHO and DPHE. However, WHO now requires a letter of approval from the Bangladesh Medical Research Council (BMRC), i.e., the Bangladesh IRB. Despite the fact that BMRC has

approved our protocols for projects #3 and #4 for deep well installation by DPHE, and that DPHE is specifically mentioned in the informed consents, they have expressed the opinion that they will not provide WHO and Columbia University such a letter because it is simply "not required." Needless to say, these developments have been a source of frustration and delay to our remediation efforts in Bangladesh. Nevertheless, we are continuing to work with WHO, DPHE and BMRC to resolve this matter and are also considering other alternatives for deep well installation.

Peer-reviewed publications in 2008

Auffan, M., J. Rose, O. Proux, D. Borschneck, A. Masion, P. Chaurand, J.L. Hazemann, C. Chaneac, J.P. Jolivet, M. Wiesner, A. van Geen, J.Y. Bottero (2008) Enhanced adsorption of arsenic onto maghemites nanoparticles: As(III) as a probe of the surface structure and heterogeneity, *Langmuir* 24, 3215-3222.

Aziz Z., van Geen A., Stute M., Versteeg R., Horneman A., Zheng Y., Goodbred S., Steckler M., Weinman B., Gavrieli I., Hoque M. A., Shamsudduha M., and Ahmed K. M. (2008) Impact of local recharge on arsenic concentrations in shallow aquifers inferred from the electromagnetic conductivity of soils in Araihazar, Bangladesh. *Water Resources Research* **44**(7).

Dhar R. K., Zheng Y., Stute M., van Geen A., Cheng Z., Shanewaz M., Shamsudduha M., Hoque M. A., Rahman M. W., and Ahmed K. M. (2008) Temporal variability of groundwater chemistry in shallow and deep aquifers of Araihazar, Bangladesh. *Journal Of Contaminant Hydrology* **99**(1-4), 97-111.

Horneman A., Stute M., Schlosser P., Smethie W., Santella N., Ho D. T., Mailloux B., Gorman E., Zheng Y., and van Geen A. (2008) Degradation rates of CFC-11, CFC-12 and CFC-113 in anoxic shallow aquifers of Araihazar, Bangladesh. *Journal Of Contaminant Hydrology* **97**(1-2), 27-41.

Métral, J., L. Charlet, S. Bureau, S. B. Mallik, S. Chakraborty, K.M. Ahmed, M.W. Rahman, Z. Cheng, and A.van Geen (2008). Comparison of dissolved and particulate arsenic distributions in shallow aquifers of Chakdaha, India, and Araihazar, Bangladesh, *Geochemical Transactions* 9:1 doi:10.1186/1467-4866-9-1.

Radloff K. A., Manning A. R., Mailloux B., Zheng Y., Moshiur Rahman M., Rezaul Huq M., Ahmed K. M., and Geen A. v. (2008) Considerations for conducting incubations to study the mechanisms of As release in reducing groundwater aquifers. *Applied Geochemistry* **23**(11), 3224.

van Geen A., Zheng Y., Goodbred S., Horneman A., Aziz Z., Cheng Z., Stute M., Mailloux B., Weinman B., Hoque M. A., Seddique A. A., Hossain M. S., Chowdhury S. H., and Ahmed K. M. (2008a) Flushing history as a hydrogeological control on the regional distribution of arsenic in shallow groundwater of the Bengal Basin. *Environmental Science & Technology* **42**(7), 2283-2288.

van Geen A., Radloff K., Aziz Z., Cheng Z., Huq M. R., Ahmed K. M., Weinman B., Goodbred S., Jung H. B., Zheng Y., Berg M., Trang P. T. K., Charlet L., Metral J., Tisserand D., Guillot S., Chakraborty S., Gajurel A. P., and Upreti B. N. (2008b) Comparison of arsenic concentrations in simultaneously-collected groundwater and aquifer particles from Bangladesh, India, Vietnam, and Nepal. *Applied Geochemistry* **23**(11), 3244.

Weinman B., Goodbred S. L., Zheng Y., Aziz Z., Steckler M., van Geen A., Singhvi A. K., and Nagar Y. C. (2008) Contributions of floodplain stratigraphy and evolution to the spatial patterns of groundwater arsenic in Araihazar, Bangladesh. *Geological Society of America Bulletin* **120**(11-12), 1567-1580.

Manuscripts submitted in 2008

Aziz, Z., B. Bostick, Y. Zheng, M.R Huq, M.M. Rahman, K.M. Ahmed, and A. van Geen, Suppression of arsenic release to shallow groundwater of Bangladesh by reduced sulfur, submitted to *Nature*, December 2008.

Garnier, J.-M., F. Travassac, V. Lenoble, J. Rose, Y. Zheng, M. Hossain, S. Chowdhury, A. Biswas, K.M. Ahmed; Z. Cheng, A. van Geen, Temporal variations in arsenic uptake by rice plants: The role of iron plaque in paddy fields of Bangladesh irrigated with groundwater, submitted to *New Phytologist*, December 2008.

Leber, J., M.M. Rahman, M.T. Chowdury, K.M. Ahmed, B. Mailloux, A. van Geen, Seasonal variation of *E. coli* concentrations in the shallow aquifer of two geologically contrasting villages in Bangladesh, submitted to *Ground Water*, September 2008

Students supported at least in part under Project 7 in 2008

Kathleen Radloff, Earth & Environmental Engineering, Columbia, PhD candidate.
Zahid Aziz, Earth & Environmental Sciences, Columbia, PhD candidate.
Ivan Mihajlov, Earth & Environmental Sciences, Columbia, PhD candidate.
Jessica Leber, Earth & Environ. Sci. and Journalism, Columbia MS 2007.
Christine George, Mailman School of Public Health, Columbia, PhD candidate.

Administrative Core.

Director: **Joseph Graziano**
Deputy Director: **Alexander van Geen**

General Activities: The Administrative Core continues to function smoothly. Dr. Graziano, the Program Director, and Dr. van Geen, the Associate Director, communicate continuously with regard to the integration of our biomedical and non-biomedical research programs. This communication is evidenced by the number of truly multi-disciplinary publications that have come from our program, involving close collaboration between biomedical, earth, and social scientists. Our monthly two-hour seminars (one hour biomedical and one hour non-biomedical) have been very well attended. The seminars held in the past year are listed below:

December 17, 2007 — **"Arsenic-stimulated angiogenesis and vascular remodeling: Receptor-mediated events?" Aaron Barchowsky**
Dept of Environmental & Occupational Health Graduate School of Public Health University of Pittsburgh

"A new mechanism for arsenic removal from shallow aquifers of Bangladesh linked to local hydrology" Zahid Aziz
Dept of Earth & Environmental Sciences Lamont-Doherty Earth Observatory Columbia University

February 18, 2008 — **"Exposure from arsenic in rice: from Bangladesh to babies"**
Andrew Meharg
Chair of Plant and Soil Science, School of Biological Sciences - University of Aberdeen

March 24, 2008	**“Arsenic and Rice: From Yield Reductions to Human Safety”** **John M. Duxbury** Department of Crop and Soil Sciences – Cornell University
	“Bangladesh Vitamin E and Selenium Trial (BEST): Current Progress and Challenges" **Maria Argos** Department of Epidemiology, Mailman School of Public Health - Columbia University Department of Health Studies - University of Chicago
April 21, 2008	**“Nutritional Influences on Arsenic Metabolism Among Children in Bangladesh”** **Megan Hall** Department of Epidemiology - Mailman School of Public Health – Columbia University
	“Effectiveness of Household-Level Arsenic Removal in Shahrasti, Bangladesh” **Christine Marie George** Environmental Health Sciences - Mailman School of Public Health - Columbia University
May 16, 2008	**"Coupled Iron and Sulfate Reduction Rates in Sediments, and Their Effect on Groundwater Arsenic Concentrations in Cambodia"** **Benjamin Bostick** Department of Earth Sciences - Dartmouth College
September 15, 2008	**"Evaluation of an Elementary School-based Educational Intervention on Arsenic in Araihazar, Bangladesh: A Progress Report"** **Khalid Khan** Department of Environmental Health Sciences - Mailman School of Public Health - Columbia University
	"Arsenic in Bangladesh: A Geological and Human Perspective" **Yan Zheng** Department of Geochemistry - Lamont-Doherty Earth Observatory – Columbia University
November 17, 2008	**"Genomic Methylation of Peripheral Blood Leukocytes among Arsenic Exposed Maternal-Newborn Pairs"** **Richard Pilsner** Robert Wood Johnston Fellow - University of Michigan
	"Coupled Hydrologic and Biogeochemical Processes Controlling Arsenic in Groundwater of Cambodia" **Matthew Polizzotto** School of Earth Sciences - Stanford University
December 15, 2008	**"Groundwater Arsenic Contamination: An Overview on Magnitude of Calamity and Some Analytical Approaches for its Trace Level Detection in Environmental Samples"** **Mrinal Kumar Sengupta**

Department of Chemistry & Biochemistry - University of Texas at Arlington

"Genomic Predictors of Exposures and of Responses to Environmental Agents"
Leona Samson
Center for Environmental Health Sciences - Massachusetts Institute of Technology

Our External Advisory Committee continues to provide valuable input to our program. The next meeting of the Committee is scheduled for March 2-3, 2009. The composition of the committee includes: a) Chien-Jen Chen, Committee Chair, and Chairman of the Graduate Institute of Taiwan; b) Andrew Gelman, Professor of Statistics at Columbia University; c) Zoltan Szabo, Research Hydrologist, USGS; d) Margaret Karagas, Chair, Section of Biostatistics and Epidemiology, Dartmouth University; e) Allan Smith, Professor of Epidemiology, University of California, Berkeley; f) X. Chris Le, Professor of Public Health Sciences, University of Alberta; g) Peggy O'Day, Associate Professor of Natural Sciences, University of California, Merced; and h) James Davis, USGS. Each of our seven research project PIs, as well as the PIs of the Research Translation Core, will make presentations to the Committee at the March meeting.

The Administrative Core was also instrumental in assuring an excellent representation of investigators, post-doctoral fellows and students at the recent December, 2008, annual meeting of SBRP programs held at Asilomar, California. Seven doctoral students and one post-doctoral fellow presented a total of six posters and one platform session.

Core A: *Data Management Core*
PI: Diane Levy

During the past year the data management staff provided services to each of the three projects with which it works. The following summarizes the tasks that were performed.

1. Project 2:
    a. We supervised the design and programming of a customized system by an outside consultant; the system allows data entry to occur in Bangladesh, eliminating the need to send completed questionnaires to New York. Data are collected on the local area network in Bangladesh. When a stable Internet connection is available, data are sent to our Columbia University SQL database located in New York. This system is also being used to enter the first follow up questionnaire for the baseline expansion participants. Currently 6,995 questionnaires have been entered in Bangladesh and transmitted to New York.
    b. We also supervised the data entry of 3,177 baseline expansion questionnaires that were added to our database since December, 2007; the total number of baseline questionnaires in our Arsenic study database is currently 18,068. Data entry is continuing for this effort.
    c. We also provided data sets to Chicago based staff as requested.
2. Project 3:
    a. New Hampshire/Maine
        i. We completed programming, testing and implementation of web based questionnaires and tests for the US based children's study.
        ii. We also supervised data entry for this study. Currently the data for 58 participants have been entered. Data entry includes a demographic/well

questionnaire, WISC and WASI tests, and a home assessment questionnaire for each participant and family.

b. Bangladesh
    i. Based on a successful test period during 2007, home questionnaires and WASI, Bruininks and WISC exams have been administered in Bangladesh; data entry has been supervised in New York. Currently data for 136 participants have been entered.

3. Project 4:
    a. Periodic uploads of laboratory data to Microsoft SQL server database were completed. The following data have been added: plasma folate, B12, homocysteine, cysteine, Cystatin C; leukocyte DNA methylation; urinary 8-OHdG (8-hydroxydeoxyguanosine); serum retinol and carotenoids (including leutein/zeaxanthin (one variable); beta-cryptoxanthin, lycopene, alpha-carotene, and beta-carotene) serum tocopherol.
    b. Targeted data sets have been distributed to PI and biostatistician (as requested) for analyses for studies explained in detail in the project related sections.
4. Additional efforts:
    a. Health Education of School Children in Bangladesh:
        i. Consultations have been provided to graduate student Khalid Khan regarding the design of his questionnaire for interviews of children in arsenic education project; this project is supported by our Fogarty Training grant, "Building capacity to reduce arsenicosis in Bangladesh."
        ii. Programmed Access database tables and data entry screens.
    b. Maintenance of secure database and web servers has continued. All projects detailed above are the direct beneficiaries of these services.
    c. Management of data flow continues using the Issue Tracking System and the Query Tracking Systems.
    d. The P.I. attended weekly project team meetings to provide data management consultation as projects move forward.

Core B: *Trace Metals Core Laboratory*
PI: Joseph Graziano
Laboratory Director: Vesna Slavkovich

The primary purpose of the Trace Metals Core Laboratory is to provide Center investigators with the capability to obtain analyses of biological samples for a broad array of metals. In addition, the facility provides method development for these analyses, standardization, and quality control. The Trace Metals Core provides analytical support to projects #2, #3 and #4.

During the past year, this Core Lab conducted more than 17,000 "routine" analyses of biological samples from projects 2, 3 and 4. Notably, the Trace Core has supported the expansion of the HEALS Cohort Study (Project #2) from a sample size of roughly 12,000 to 20,000 participants; urinary As and creatinine were measured for each of the 8,000 new study participants. In support of various studies, urinary arsenic metabolites were analyzed for 880 study participants. Two years ago, the Trace Metals Core developed a new method for the analysis of arsenic in blood, using ICP-MS-DRC. That method allowed us to demonstrate that blood As is an extremely useful biomarker of exposure. Moreover, the method allows for the simultaneous measurement of other metals of interest that are covariates in many analyses, namely Pb, Mn and Se. During the past year the lab has continued to provide these blood analyses to several projects, notably Project #4.

Given the potential utility of blood arsenic measurements in epidemiologic research, we are now devoting time to the development of a new blood arsenic method that could use tiny blood samples obtained by fingerstick. Epidemiologic studies employing repeated blood arsenic measurements over time have the potential to answer many issues concerning the bioavailability and toxicokinetics of arsenic. Yet people in the developing world are generally reluctant to give blood samples for research purposes. For this reason, we have embarked on a new study to assess the reliability of these analyses in small capillary blood samples obtained via fingerstick. This protocol, approved by the Columbia University IRB and by the Bangladesh Medical Research Council, will compare venous and fingerstick blood As concentrations in 50 individuals with varying concentrations of As in their drinking water.

Students involved with this work:

Marni Hall, a former Ph.D. student in Environnmental Health Sciences, Mailman School, and now employed at the FDA at Rockville Pike, Maryland.

J. Richard Pilsner, a former Ph.D. student in Environnmental Health Sciences, Mailman School, now a Robert Wood Johnson Fellow in the EHS Department at the University of Michigan.

Christine George, Dr. Graziano's minority Ph.D. student, who has a degree in Environmental Engineering from Stanford University.

Christopher Capelli, an undergraduate from Wagner College, was given a job in the lab during the summer of 2008. Mr. Capelli has now applied for entry into the MPH degree program in the Department of Environmental Health Sciences (EHS).

Mr. Zhongyuan Mi, an MPH student in EHS, carried out his Master's thesis research in the lab during the past year. His experiments demonstrated that the methyl group of methyl cobalamine (vitamin B12) can be non-enzymatically transferred to arsenite to form monomethylarsonic acid (MMA). He has also demonstrated that that methyl group cannot be transferred to MMA to form DMA. This work was driven by the observation in Project #4 that blood B12 levels are positively associated with urinary MMA (but not DMA). Mr. Mi is now applying to the PhD program in EHS.

Publications: Virtually every publication listed for Projects 2, 3 and 4 has relied on the Trace Metal Core.

Core C: *Biogeochemistry Core*
PI: Alexander van Geen
Co-investigators: Yan Zheng, Steve Chillrud, Brian Mailloux,
Outside collaborators: Benjamin Bostick (Dartmouth U.), John Stolz (Duquesne U.)

The Biogeochemical analytical core laboratory is housed at Lamont-Doherty Earth Observatory (LDEO). It provides sample preparation and analyses to six projects of the Columbia Superfund program. Analyses have been carried out by high-resolution inductively coupled plasma mass spectrometry for up to 33 elements for water, soil, sediment, leachate, and plant material (HR ICP-MS). In addition, subcontracts with two outside collaborators have boosted the core's capabilities in two new areas to include extended X-ray absorption spectroscopy (XAS) of aquifer particles for Fe

and As and detailed characterization of bacterial species interacting with As using 16S rRNA techniques.

On behalf of biomedical projects 2, 3, or 4 a total of 1300 new groundwater samples collected from Araihazar, Bangladesh, and 100 wells samples from Maine were analyzed by HR ICP-MS in 2008. In support of project 5, over 800 groundwater or sediment leachates collected in Maine and Bangladesh were analyzed by HR ICP-MS for As, Mn, U and additional constituents of potential geochemical interest or health concern. A total of 600 groundwater and leachate samples for material collected from the Vineland Superfund sites were analyzed by HR ICP-MS for project 6. Almost 400 groundwater and sediment leachate samples from Bangladesh were analyzed on behalf of project 7. Another 400 raw groundwater and water treated by As-removal systems that are widely used in Bangladesh were also analyzed for As, Mn, Fe, P, S, Si and major cations under project 7. Finally, 290 samples were analyzed n support of the Research Translation Core.

This past year, the core laboratory generated a significant number of findings on the basis of XAS of preserved aquifer material. In support of Project 7, a total of 40 intervals within a shallow 3-m section of aquifers sands from Bangladesh corresponding to a wide range of As concentrations in groundwater was analyzed for As speciation by X-ray absorption near-edge spectroscopy (XANES) at the Stanford synchrotron facility. The new results document a gradual transition with depth from adsorbed As(V) to adsorbed As(III) in the solid phase that is consistent with other measured redox parameters. Less expected was a clear signature As-sulfide mid-way through this transition, within a region where groundwater time series indicate that sulfate is supplied by recharge from above, reduced to sulfide, to then combines with dissolved As(III). An additional 11 samples were analyzed by XANES for projects 5 and 6.

In terms of microbiology, we have performed diversity studies and analysis of arsenic specific genes in the last year. Microarrays and terminal restriction fragment length polymorphism (TRFLP) of the 16s rRNA gene were performed on Bangladesh and Union Lake samples. Sequencing of the arsenic reductase gene *arrA* was performed on samples form Union Lake and Vineland. Analysis of the detoxification gene *Acr3(2)* that is responsible for encoding enzymes responsible for transport of arsenite out of cells was analyzed from diverse worldwide locations. In summary, a wide range of sequencing projects were undertaken to better constrain the role microbes play in mobilizing arsenic at both pristine and contaminated sites.

Manuscripts involving collaborators supported by Core C:

Mailloux, B. J., Alexandrova, E., Keimowitz, A., Wovkulich, K., Freyer, G., Herron, M. M., Stolz, J. F., Kenna, T., Pichler, T., P. Knappett, 2007. Microbial mineral weathering for nutrient acquisition releases arsenic. Resubmitted to *Appl. Environ. Microbiol*., October 2008.

Aziz, Z., B. Bostick, Y. Zheng, M.R Huq, M.M. Rahman, K.M. Ahmed, and A. van Geen, Suppression of arsenic release to shallow groundwater of Bangladesh by reduced sulfur, Submitted to *Nature*, December 2008.

Students supported in part by the Biogeochemistry Core over the past year

Lauren Thompson, undergraduate student, Barnard College
Talia Arbit, undergraduate student, Barnard College
Jennifer Cheung, undergraduate student, Barnard College

Core D: Hydrogeology Support Laboratory – Progress report

Martin Stute, Peter Schlosser, Juerg Matter, Steve Chillrud

The Hydrogeology Support Core provides information on the groundwater and surface water flow and transport regime at our field sites in the US and Bangladesh, and supports projects number 5, 6, 7, and the RTC.

Notable advances for the last period:

Field efforts: In the late spring of 2008, we installed 15 injection and 1 observation well in the pumping induced depression cone of an extraction well at the Vineland Superfund site (Project 6). The purpose of the array is to conduct field scale experiments on the chemically enhanced mobilization of arsenic that we have tested in laboratory experiments. A tracer experiment was performed to establish communication between wells and the groundwater residence time of the system. If successful and scalable to the whole site this technology could potentially reduce the treatment of the site by at least an order of magnitude.

Also in the winter/spring of 2008, we conducted two field trips to Bangladesh (P5 & 7). We performed so called 'push/pull' experiments during which we injected (and recovered) low or high arsenic water into shallow and deep aquifers to study the release and the adsorption of arsenic. We also built a monitoring well surrounded with small additional well screens through which we injected a concentrated salt solution on the outside of the well casing. The movement of the salt can be monitored with geophysical tools from the inside of the monitoring well and will allow us to constrain the potential pumping induced vertical motion of groundwater through clay layers. This is important for the evaluation of the sustainable use of low arsenic water from deep aquifers.

Finally, in the fall of 2008 we studied high arsenic drinking water wells near Augusta, Maine (P5). Several wells were logged with temperature, conductivity, and gamma probes, a heat pulse flow meter, as well as optical and acoustic televiewers to identify the factures conducting flow into the well. Vertical dissolved As profiles were collected to identify which fractures contributed to the high dissolved As levels in the wells.

Sample Measurements We performed about 50 ^{3}H and 20 $^{3}H/^{3}He$ as well as 35 ^{18}O and ^{2}H analyses on samples from Bangladesh community wells and shallow observation wells (P5 & 7) and wells in Rockland County (RTC). 50 measurements were performed on soil gases from 3 sites in Bangladesh (P5), as well 100 SF_6 measurements and 200 Br measurements for tracer experiments in Bangladesh and NJ (P5 & 6).

Modeling Core personnel continued to support groundwater flow modeling activities at site 'X' in Bangladesh (P5&7) with the goal to quantify the groundwater/surface water interactions. An MT3D model was refined to design the enhanced As mobilization pilot experiment at the Vineland Superfund site (P6).

Instrument development We finished testing a prototype of a novel sampler for gases dissolved in groundwater. This sampler separates gases from the water in the field with a membrane and potentially considerably simplifies the transport of the samples to the lab and the measurement by gas chromatographs and mass spectrometers (projects 5-7).

All publications listed in Projects 5, 6, and 7 have been supported by the Hydrogeology Core.

Research Translation Core: *Collaborating with Government & the Public: As & Mn Exposure via Groundwater*

Co-PIs: Steve Chillrud, Meredith L. Golden
Co-investigators: Joseph Graziano, Mark Becker, Martin Stute, H. James Simpson, Yan Zheng, Alexander van Geen

The mission of Columbia's Research Translation Core (RTC) is to facilitate communication within the project, between Columbia scientists and other SBRP-funded groups, and with members of the public. In addition a central theme of RTC activities is direct involvement with ongoing priorities of governmental agencies responsible for minimizing human exposure to arsenic and manganese. The intra-project communication has been facilitated by ongoing seminars, regular email communications, and maintenance of the project website. The goal of inter-SBRP communications is being met by the coordination of the upcoming Research Translation workshop for SBRP RTC scientists around the country.

The government agencies with which we are involved are located in NJ, NY, NH and ME and include county, state, and federal personnel. In Maine, public outreach has included the dissemination of new domestic well water chemistry results, including As, U, Mn and Rn concentrations to >1000 homeowners in 13 towns in greater Augusta, Maine.

In NY, following detection of arsenic in public-supply wells in Rockland County in 2007, RTC scientists entered into a memorandum of agreement and began collaboration with County Department of Health officials and the private water supply company on a sampling program of both public supply and private wells. This program should help determine the spatial and temporal extent of groundwater arsenic contamination in the region and test reductive dissolution as the primary mobilization mechanism. Columbia scientists have contributed scientific expertise to the design of the sampling program and will continue to collaborate with the county in analyzing splits of all the samples and interpreting results. Initial data from two public-supply wells show pre-treatment arsenic concentrations of 28 and 23 ppb, in excellent agreement with the county's data. Sampling of other wells is currently underway. Furthermore, RTC scientists have initiated development of a public information website regarding water quality and supply in Rockland County, NY. This county is typical of many suburban areas in that water consumption is very near the capacity of the supply system and it faces immediate major decisions on how to best preserve and increase its water resources, including the proposed installation of a desalination plant by United Water New York. Our new website should help provide access to current and historical scientific information to allow interested citizens of Rockland County to more fully participate in decision making processes. The web site will be ready for beta testing in December 2008, after which we will publicize it to interested citizen groups and K-12 educators.

Columbia SBRP scientists have continued collaboration with scientists from the NYS Department of Environmental Conservation on the issue of naturally-occurring arsenic mobilization at old landfills. This collaboration has resulted in further understanding of iron-rich flocs that are often associated with groundwater seeps down gradient from landfills. We have demonstrated that strong correlation exists between floc composition and groundwater arsenic concentrations approximately two years prior to floc collection. This information may allow flocs to be used as triage tools for arsenic mobilization at old landfills, many of which do not have monitoring wells.

Students and Postdocs involved in RTC activities in 2008:

Alison Keimowitz, PhD 2005, Earth and Environmental Science, currently postdoctoral fellow at Columbia University

Karen Wovkulich, 4th year graduate student, Earth and Environmental Science, Columbia University.
Marco Balletta, visiting student from University of Naples, Italy

Publications:

Balletta, M. S. Chillrud, A. Keimowitz, K. Wovkulich, H.J.. Simpson, J. Ross, M. Stute, R. Hon, B. Brandon, S. Parisio and J. Giordano. 2008. Developing Triage Assessment Tools for Old Landfills and Arsenic. 2008 Joint Meeting , The Geological Society of America. 5-9 October. Houston, Tx. Geological Society of America *Abstracts with Programs*, Vol. 40, No. 6, p. 355).

Chillrud, S., A. Keimowitz, M Balleto, K. Wovkulich, H.J. Simpson, J. Ross, M. Stute, R. Hon, B. Brandon, and S. Parisio. Developing triage assessment tools for old landfills and arsenic. Presented at the 2nd International Congress on Arsenic. Valencia, Spain, 2008.

Training Core:
P.I.: Pam Factor-Litvak

Our Director of the Training Core, Dr. Paul Brandt-Rauf, recently left Columbia University to become the Dean of the School of Public Health at the University of Illinois, Chicago. He has been replaced by Dr. Pam Factor-Litvak, Associate Professor of Epidemiology at the Mailman School of Public Health. Dr. Factor-Litvak has an extensive resume in the field of environmental epidemiology, and has published extensively with many investigators in this SBRP program. She also has a strong history of student mentorship as the director of training in the Department of Epidemiology. She is currently working on the development of a new agenda for our SBRP trainees.

The Training Core continued its annual Workshop Program with a week-long, all-day workshop on "EPA Lead Paint Inspection and Risk Assessment" given by Dr. Marco Pedone, an expert on hazardous waste management. In addition to covering the didactic requirements for EPA training in this area, the workshop included hands-on exercises on the evaluation of lead paint hazards. Besides the monthly Superfund Seminar Series, the Training Core also participated in the Annual Granville H. Sewell Distinguished Lecture in Environmental Health Sciences which this year featured: Dr. William E. Rees, Professor of UBC School of Community and Regional Planning speaking on "*Our Ecological Footprint: Reducing Human Impact on the Earth"*.

In addition, the Training Core continued to offer trainees participation in a web-based course on "Hazardous Waste and Public Health" during the summer semester which can be done by anyone with a computer and internet access from any location and which has proven highly successful in past years. The course includes practical case studies in managing hazardous waste issues as problem-solving exercises for the participants.

Patent Updates: None.

Superfund Site Updates: None.

Contact Information Updates: Updated in the attached file.

Student Information Updates: Embedded within text and also updated in attached excel file