HIGH SELENIUM CONCENTRATION AND SELENITE-HYPERRESISTANT BACTERIA IN THE LOWER STREAM OF MISSISSIPPI RIVER

***Wen-Hsun Yang, *Amin Baaree, *Jen-Rong Yang, and **Andy Yee**

***Bioremediation Education, Science and Technology Center, and Biology Department, School of Science and Technology Jackson State University**

****Earth Science Department, Lawrence Berkeley Laboratory University of California Berkeley, Berkeley, California**

INTRODUCTION

Increases in the concentration of trace-elements of natural waters and sediments have been documented in both North America and Scandinavia (Smith, Alexander, and Wolman 1987; Borg 1987). Group V and VI elements such as selenium (Se), arsenic (As), chromium (Cr), and thallium (Tl), are commonly found as pollutants in a number of locations. They are found in groundwater, agricultural and municipal waste water, power plant cooling reservoirs, oil refining waste streams, and waste disposal sites (Burton 1987; Tracy, Oster, and Beaver 1990). Se is one of the trace elements which is strongly linked to fossil-fuel consumption. In the heavily industrialized interior valleys of California, over 20% of the wells sampled had Se levels above the NAS or EPA levels of safety (Tracy, Oster, and Beaver 1990). Because of its similarity to sulfur, increases in Se can greatly affect living organisms. Selenite, for example, can cause cytolysis by precipitating in protoplasm and cell membranes (Yang et al. 1993). Metal or metalloid ions, and even organometallic compounds, can activate or inhibit enzymes by binding their catalytic regulatory sites (Foster 1983). These compounds can also interfere with electron transfer by binding to hemoglobin and other oxygen carriers (Bragg and Rainnie 1974). Arsenate can inhibit phosphate kinase and, consequently, inhibit the activation of many other enzymes. Arsenated-sugars can hydrolyze spontaneously in the absence of enzymes and result in the loss of a fuel source for glycolysis (Summers and Silver 1978). Toxicity may result from such interference with cellular activity. For example, endemic necrotic hoof disease afflicts horses that feed on plants indigenous to a region of China known to be rich in Se (Comb and Comb 1986). This was first described by Marco Polo during his travels during the thirteenth century (Polo 1958). For over a century now, consumption of As-polluted water in southern Taiwan has been known to cause necrotic damage to the foot (blackfoot disease) and skin cancer in man. This region of Taiwan was contaminated long before industrialization took place (Chen and Wu 1962; Tseng, Chu, and How 1968; Tseng 1989).

Many techniques are currently available for qualitative and quantitative analysis of trace element concentration in environmental samples. These techniques include graphite furnace and flame atomic absorption spectrometry (Borg 1987; Jeffries and Snyder 1981), neutron activation analysis (Galloway and Likens 1979), inductively coupled plasma atomic mass spectrometry (ICP-MS) (Henshaw, Heithmar, and Hinners 1989), and inductively coupled plasma atomic emission spectrometry (ICP-AES) (Johnson, Culp, and George 1986). Of these instruments, both ICP-AES and ICP-MS are suitable for rapid multi-element analysis of environmental samples.

This study utilizes the ICP-AES for multi-element analysis of surface water from the Mississippi River. This investigation was carried out as a follow up to the earlier report (Yang et al. 1993) which showed the presence of selenite hyperresistant bacteria in the soil along the Mississippi River, an early indication of early sign of selenium pollution in the river.

MATERIALS AND METHODS

Water Samples, Standards, and Solutions

Sample Collection. River surface water was collected on May 27, 1993, from the Mississippi River at Vicksburg (VB or M1), Natchez (NC or M2), Baton Rouge (BR or M3), New Orleans (NO or M4), Belle Chasse (BC or M5), and Venice (VN or M6). For comparison, samples were also collected from the Pearl River at North Bay (NB or P1), Park side (PS or P2), Jackson (JS or P3), Monticello (MC or P4), Foxworth (FW or P5), and West Pearl River (WPR or P6). The samples were collected from the surface layer of the actively moving stream at least 8 feet

from the river bank by floating a glass bottle attached to a rod.

Sample Preservation. Following the collection of the water, 100 ml of the river water was acidified with 16 drops of concentrated HCl, adjusted to a pH of 3, and passed through a sterilized filter with a pore size of 0.45 um. All samples were kept on dry ice and transferred to a -80°C freezer as soon as possible. They were usually assayed within two weeks from the time of collection. The sample was thawed overnight, and nitric acid was added to a final concentration of 0.7% before assay.

Calibration Standards for Simultaneous ICP-AES analysis. ASTM Type 1 water was used for all preparations. Ultrapure hydrochloric acid and nitric acid were used whenever possible. Certified multi-element plasma standards (purchased from Spex Industries) containing the 24 EPA required elements were used in preparing the standards. Elements not present in the Spex mixtures were added separately from single standards. Dilutions were made using 5% v/v nitric acid. Element concentration and analytical wavelength of the standards are all listed in Table 1.

Standards and Solution for Se Analysis. 1000 ppm Se reference standard was purchased from Fisher Scientific and diluted with de-ionized water to 10, 20, 50, 100, and 200 ug/L for calibration of the ICP-AES. The standard solutions were prepared fresh daily. 10 M-HCl was prepared from reagent grade HCl for acidification of samples in the hydride generator. 0.6% w/v sodium borohydride solution in 0.1N-NaOH was prepared for the reduction of selenite to the gaseous hydrogen selenide.

Standards and Solutions for As analysis. 1000 ppm As reference standard was purchased from Fisher Scientific and diluted with de-ionized water to 10, 20, 30, and 50 ug/L. 8 M urea solution was prepared by dissolving 120 gm of urea in 250 ml of water. It is used to mask the nitrite interference generated from the nitrates. 40% w/v KI solution by dissolving 40 gm KI in 100 ml water. It is used as a reductant to convert the AsVI to AsIII.

Standards and Solutions for Hg Analysis. 100 ug/ml mercury standard was purchased from Spex and diluted to 5, 10, 20 ug/L with de-ionized water for cold-vapor ICP-AES analysis. Other reagents for cold-vapor analysis of Hg are listed in the EPA method 7470. Sulfuric acid, 0.5N; concentrated nitric acid; stanneous sulfate solution, 25 gm dissolved in 250 ml of 0.5N sulfuric acid; sodium chloride-hydroxylamine sulfate solution, 12 gm each of sodium chloride and hydroxylamine sulfate in 100 ml of water; 5% w/v potassium permanganate solution. A 0.6% sodium borohydride solution can be substituted for the stanneous chloride solution.

Simultaneous ICP-AES Analysis. For elements other than Se, As, and Hg, analyses were performed on a Fision ARL 3560 instrument fitted with a Meinhard C-2 nebulizer. Instrument parameters are as forward power, 1200W; nebulizer flow, 860 ml/min; carrier flow, 1000 ml/min; coolant flow, 14 liters/min; sample uptake rate, 2.2 ml/min; integration time 10 seconds x 3.

Hydride Generator-ICP-AES Analysis for Total Se (196.09 nm). A hydride-generation apparatus assembled from a 3-channel peristalic pump and gas-liquid separator purchased from Spectra Company was employed together with the ARL 3510 sequential ICP-AES. The pump tubings were connected in series with plastic "T"s with the first channel sample uptake, the second channel in 10M-HCl for acidifying the sample, and the third channel in sodium borohydride for the reduction of the selenite to the gaseous hydride. A two foot long chromatographic teflon tubing at the end served as a reaction coil before the mixture entered the gas-liquid separator. From the outlet of the gas-liquid separator, a 1/4" o.d. teflon tubing was inserted directly into the bottom of the injector tube of the plasma torch. Flow rates of sample:acid:borohydride were adjusted to 7:1:1 ml/min, respectively. The argon flow rate through the gas-liquid separator was adjusted to yield the most stable plasma and the highest sensitivity, and this was found to be 800-1000 ml/min. Detection limit of this hydride system was found to be 0.1 ug/L with a background equivalent concentration (BEC) of 4 ug/L. Linearly was found to exceed 4000 ug/L, many times above our normal analytical range. Sample pre-treatment involved pipeting out 5 ml of the sample into a culture test tube, 5 ml concentrated hydrochloric acid, and 0.2 ml of a 2% potassium persulfate solution. The mixture was heated in a boiling water bath for 10 minutes. After cooling, it was ready for analysis.

HG-ICP-AES Analysis for As (193.76 nm). The same hydride-generation apparatus for selenium analysis is used for arsenic analysis. In As analysis, sample pre-treatment involved pipeting 5 ml sample blanks and standards into culture test tubes to which equal volume of concentrated HCl, 0.25 ml KI, and 0.25 ml 8M urea were added. The mixtures are then heated in a 70°C water bath for 30 minutes. Upon cooling samples are ready for analysis.

Cold Vapor-ICP-AES Analysis for Hg (194.76 nm). The hydride-generation system described above can be used for Hg analysis. Sample pre-treatment consists of pipeting 10 ml of sample or Hg standard solution into a culture test tube to which 0.5 ml concentrated sulfuric acid, 0.25 ml nitric acid, and 1.5 ml 5% potassium permanganate were

added. The mixture was heated in a boiling water bath for 30 minutes. After cooling to room temperature, the excess permanganate in the solution was removed by the addition of 0.6 ml NaCl-hydroxylamine solution and then analyzed.

Hyperresistant Bacteria from the Riverside Soil

Soil Sample Collection and Treatment. Soil samples along the riverbank slightly above water level were also collected in a sterile bottle at the time of water collection. An equal weight of sterile distilled water was added, and the tube was heated to 100°C for 5 minutes with frequent inversion of the tube. After cooling to room temperature, various volumes of supernatants were spread-cultured on plates containing TBAB and 1 mM sodium selenite.

Assessment of Bacterial Growth. After incubation for 48 hours at 38°C on spread-culture plates, the nine fastest growing bacteria colonies from each soil sample were selected from about 200 colonies. They were transferred to 9 evenly-distributed positions on each of 4 culture plates: TBAB with inclusion of 0 mM, 0.1 mM, 1.0 mM, or 10 mM sodium selenite in disposable Petri dishes (88 x 15 mm). During culture for 6 days at 38°C, colonies in each set of 4 plates were photographed at intervals of every 24 hours by transillumination of light from a regular 15 watt electric lamp set at 50 cm above a sheet of 8 x 10 Kodabrome RC print paper in contact with the bottoms of the culture plates. A transparent section paper printed with individual squares at 0.0169 square centimeter per unit was used to calculate the area formed by negative image of each colony in the photograph. A planimeter was also used to measure the area formed by larger colonies. After consecutive measurement of colony sizes for 5 days, the growth curve for each bacterium was plotted for comparison. Then each colony was transferred from the TBAB plate into 2 ml 2SG broth in a shaker incubator at 38°C to allow for spore formation. Spores were preserved by the addition of 0.1 ml chloroform to destroy the vegetative forms of bacteria. For final assessment of the growth of a rapidly growing colony in each plate, the spore stock was germinated for 24 hours on a TBAB plate. A single colony of the bacterium was isolated and patched on the center of each plate in a set of 4 plates containing various concentrations of selenite. After daily measurement of colony size for 5 days, the growth curve for each bacterium at each different selenite concentration was plotted. Those bacteria which grew to more than 400 square mm in colony size after 5 days of incubation on 10 mM selenite plate were designated as selenite hyperresistant bacteria (Figure 2).

Classification of Selenite-Hyperresistant Bacteria Isolated. The selenite-hyperresistant, gram-positive, spore-forming bacteria isolated in the above experiments were further analyzed for their genus according to the procedures of Microstation system using differences in carbon source utilizations for identification (Biolog Inc.) (Miller and Rhoden 1991; Klingler et al. 1992).

RESULTS

AES-assay of Elements. The results of ICP-AES assay of 27 elements in water samples collected from 6 locations in the Mississippi River are found on Table 1. The data for water samples collected from 6 locations along the Pearl River are listed in Table 2. Comparing the results, it is clear that the Mississippi River water had significantly higher concentrations of Ca (t=17.80), K (t=6.84), Si (t=3.73), Sr (t=15.88), As (t=5.05), and Se (t=7.08) as compared to Pearl River water. In contrast, the Pearl River water had significantly higher concentration of Ba (t= 4.80), B (est t=4.55), Fe (t= 3.21), and Mn (t=2.95) as compared to Mississippi River water.

Hyperresistant Bacteria. The spore-forming gram-positive selenite-hyperresistant bacteria isolated from Mississippi River soil were able to grow rapidly at selenite concentrations from 1 to 10 mM (Figures 1 and 2). (The rate of growth from these bacteria were generally as good or faster than Bacillus subtilis which are known to thrive in selenium.) Figure 3 demonstrated rapid growth of 3 isolated hyperresistant bacteria in the 10 mM selenite plates with marked reddish discoloration of the colonies. In contrast, spore-forming gram-positive selenite-hyperresistant bacteria isolated from the Pearl River soil did not grow as rapidly (Figures 1 and 2).

Classification of Selenite-Hyperresistant Bacteria. When 10 of the isolated hyperresistant bacteria were subjected to identification of genus according to the procedures of Biolog Microstation System, 2 of them have been identified as Bacillus mycoides (MR#2, MR#4), 1 of them as Bacillus coagulans (MR#1), 1 of them as Kurthia Gibsonii (MR#6), 1 of them as Staphylococcus carnosus (PR2), 1 of them as Erysipelothrix rhusiopathiae (MR#8), and 5 of them were still not identified.

DISCUSSION

The increase in As and Se in the Mississippi River are of great concern because of the potential health hazards. Consequently, it is important to determine the possible sources of pollution. Leakage of Se from power plants ranks high on the list of possibilities, and leakage of Se from power plant coal ash disposal sites has been observed in other locations (Woock, Gsarret, Partin, and Bryson 1987; Cumbiee and Van Horn 1987, Gillespie and Bauman 1986). Indeed, there are a number of power generation plants along the Mississippi River (6 between Vicksburg

and Baton Rouge over a distance of about 100 miles). In contrast, there are no power plants along the Pearl River. The higher concentrations of Se found in the section of river between M2 (Natchez) and M4 (New Orleans), as compared to the section between M5 (Belle Chasse) and M6 (Vincent), also correlates with the density of power plants in those areas. In contrast, the presence of higher concentrations of Ca, K, Si, and Sr in the Mississippi River and higher concentrations of Ba, B, Fe, and Mn in the Pearl River are probably attributable to geochemical differences because no compounds containing such elements are generated by industrial activity along these rivers.

Fossil-fuel combustion, primary metals manufacturing, pesticides, herbicides, and phosphate-containing fertilizers and detergents are also known to cause As pollution as in the northern basins of the midwest (Scow et al. 1982; Delos 1985). However, these sources of pollution usually cause an increase in both As and Cd. Consequently, the absence of Cd contamination suggested that these may not be the cause of As pollution. Furthermore, pollution by fossil-fuel combustion should also result in atmospheric deposition of As in the Pearl River which runs in parallel with the nearby Mississippi River. If these were the scenario, the concentrations of As and Se should be similar in both rivers.

While industrial activity appeared to be the primary difference between the lower Mississippi River and the Pearl River, other differences do exist. For example, the Mississippi River passes through other industrial states of the north; the Pearl River does not. The pollution of the Mississippi River may be partly explained by the accumulation of industrial wastes from northern states. However, the increase in Se from M1 (Vicksburg) to M2 (Natchez) suggested significant pollution from sources in Mississippi and Louisiana. The Mississippi River also passes through an area of Arkansas and Tennessee where hot spring activity occurs. Furthermore, the Mississippi River passes through two areas rich in fossil fuels along the I-10 and I-20 highways in Mississippi and Louisiana and through the strategic national reservoir of crude oil in Louisiana. Leakage of Se from petrochemical fossils has been known to occur. For example, in California, agricultural drain water percolated through seleniferous soils and was later discharged into the Kesterson National Wildlife Refuge. This water contained selenium levels that were 100 times higher than normal (Izbecki 1984; Presser and Barnes 1984). Deaths and deformities in the wildlife eventually compelled the closure of this area in 1986 (Ohlendorf et al. 1986).

Coincident with the high concentrations of Se in the Mississippi River is the appearance of spore-forming, gram-positive, Selenite-hyperresistant soil bacteria. Soil along the Mississippi River contained many more bacteria which were hyperresistant to Se than the Pearl River. Among the Se hyperresistant bacteria, those from along the Mississippi River had even higher growth rates as compared to those from the Pearl River. The presence of hyperresistant bacteria in the Mississippi River may be due to a selection process caused by the presence of high concentrations of Se in the river. However, the presence of two nuclear power generation plants in the region may result in the formation of active radicals in the water that cause mutagenic changes in bacteria. This mutagenesis may be responsible for the increased number of bacteria hyperresistant to selenite as well as the high degree of hyperresistance.

SUMMARY AND CONCLUSIONS

Much evidence indicates that the quality of water from the Mississippi River is deteriorating with the intense industrialization of the area along the river. The purpose of this survey is to investigate the source of pollution in the river. An inductively coupled-plasma atomic emission spectrometer (ICP-AES) was used to assay 24 elements from samples collected in May of 1993. Se and As were analyzed by hydride generation ICP-AES. Hg was determined using the cold vapor technique with the ICP-AES. The range of concentrations in microgram per milliliter for each element in the Mississippi River water samples were: Al, 0.01-0.29; As, 0.68-2.20; Ba, 0-0.06; Ca, 28.86-37.25, Fe, 0-0.11, Mn, 0-0.03, Mg, 8.53-12.03; K, 1.76-3.41; Si, 1.32-3.47; Na, 10.43-17.47; Sr, 0.12-0.18; V, 0.02-0.05; Zn, 0-0.01. The range of concentration for Hg is 1.82-5.63 ug/L. The range of concentration for As is 0.68-2.19 ug/L. Not detectable were Ag, Be, Cd, Cr, Co, Cu, Pb, Mo, Ni, Sb, and Tl. Samples from the Pearl River, which runs parallel to the Mississippi River and does not have any industrial discharges, were also collected and analyzed. Data from the latter is used to serve as a control blank since the geochemical composition of the two rivers are similar. Selenium levels were found to be elevated from samples collected at Vicksburg, 176 ug/L; Natchez, 351 ug/L; Baton Rouge, 368 ug/L; New Orleans, 489 ug/L; Belle Chasse, 265 ug/L; and Venice, 258 ug/L. For comparison, Se concentration in the Pearl River at North Bay Reservoir, Parkside Reservoir, Jackson, Monticello, Foxworth, and West Pearl River were 12.97, 3.27, 0.54, <0.25, <0.25, and <0.25 ug/L, respectively. It was also found that the soil bacteria isolated from the Mississippi sediment showed an increase in resistance to selenite in culture as compared to bacteria isolated from the Pearl River. These results showed that the concentration of Se is higher in the lower stream of the Mississippi River (Natchez to New Orleans), and this increase may be attributed to power generation plants and petrochemical

factories along this part of the river. Such industrial activities were scarcely noticed along the Pearl River.

ACKNOWLEDGEMENTS

This paper is dedicated to the 44th anniversary of the heroic death of my upperclassman friend, Dr. Sheng-Chi Yeh. This work was supported by the Department of Energy Grants DE-FG05-86ER75274 and also by a special grant from the Science Consortium formed by JSU, LBL, and AGMUS.

REFERENCES

Borg, H. 1987. Trace metals and water chemistry of forest lakes in northern Sweden. Water Res. 21(1): 65-72.

Bragg, P.D. and D.J. Rainnie. 1974. The effects of silver ions on the respiratory chain of *Eschericia coli*. Can. J. Microbiol. 20: 883-889.

Burton, G.A. 1987. Occurence of bacterial resistance to arsenite, copper, and selenite in adverse habitats. Bull Environ. Contam. Toxicol. 39: 990.

Burton Jr., G.A., T.H. Giddings, P. DeBrine, and R. Fall. 1987. High incidence of selenite-resistance bacteria from a site polluted with selenium. Applied and Environmental Microbiology. 53: 185-188.

Holak, W. 1980. Analysis of foods for lead, cadmium, copper, zinc, arsenic, and selenium, using closed system sample digestion: colaborative study. J. Assoc. of Anal. Chem. 63: 485.

Chen, K. P., and H.Y. Wu. 1962. Epidemiologic studies on blackfoot disease. 2. A study of source of drinking water in relation to the disease. J. Formosan Med. Assoc. 61: 611-618.

Clark, Jr., D.R., P.M. Ogasawara, G.J. Smith, and H.M. Ohlendorf. 1989. Selenium accumulation by racoons exposed to irrigation drainwater at Kesterson National Wildlife Refuge, California. Arch. Environ. Contam. Toxicol. 18: 787-794.

Combs G.F., Jr. and S.D.G. Combs. 1986. The role of selenium in nutrition. Academic Press, New York.

Cumbie, P.M. and S.L. Van Horn. 1978. Proc. Ann. Conf. S.E. Assoc. Fish Wildl. Agencies 32: 612.

U.S. Environmental Protection Agency. 1982. Methods for chemical analysis of water and wastes. EPA-600/4-79-020.

Delos, C.G. 1985. Cadmium contamination of the environment: an assessment of nationwide risks. EPA-440/4-85-023. Environmental Protection Agency. Washington, DC.

Foster, T.J. 1983. Plasmid-determined resistance to antimicrobial drug and toxic metal ions in bacteria. Microbiol. Rev. 47: 361-409.

Galloway, J.N. and G.E. Likens. 1979. Atmospheric enhancement of metal deposition in Adirondack lake sediments. Limnol. Oceanogr. 24(3): 427-433.

Gillespie, R.B. and P.C. Baumann. 1986. Effects of high tissue concentration of selenium in reproduction by bluegills. Trans. Amer. Fish. Soc. 115: 208.

Gianessi, L.P. and H.M. Peskin. 1984. An overview of the RFF Environmental Data Inventory: Methods, Sources, and Preliminary Results. Resources for the future. Washington, DC.

Gonzales, S.R., E. Otero, A. Resto, A. Yee, W.H. Yang, S. Ekunwe, and B. Chapman. Bioremediation of selenium and chromium using the bacteria, *Bacillus subtilis*. International Waste Management Conference, San Juan, Puetro Rico, April 29, 1993.

Hailton, S.J., and K.J. Buhl. 1990. Acute toxicity of boron, molybdenum to fry of Chinook Salmon and Coho Salmon. Acute Environ. Contam. Toxicol. 19: 366-373.

Heinz, Z.H., G.H. Pendleton, A.J. Krynitsky, and L.G. Gold. 1990. Selenium accumulation and elimination in mallards. Arch Environ. Contam. Toxicol. 19: 374-379.

Heit, M., Y. Tan, C. Klusek, and C. Burke. 1981. Anthropogenic trace elements and polycyclic aromatic hydrocarbon levels in sediment cores from 2 lakes in the Adirondack. Water Air Soil Pollut. 15(4): 441.

Henshaw J.M., E.M. Heithmar, and T.A. Hinners. 1989. Inductively coupled plasma mass spectrometric determination of trace elements in surface waters subject to acidic deposition. Anal Chem 61(4): 335-342.

Jeffries, D.S. and W.R. Snyder. 1981. Atmospheric deposition of heavy metals in central Ontario. Water Air Soil Pollut. 15: 127-152.

Izbeckin, J.A. 1984. Open File Report 84-582. U.S. Geological Survey.

Johnson, M.G., R.L. Culp, and S.E. George. 1986. Temporal and spatial trends in metal loadings to sediments of the Turkey Lakes, Ontario. Can. J. Fish. Aquat. Sci. 43: 754-762.

Klingler, J.M., R.P. Stowe, D.C. Obenhuber, T.O. Groves, S.K. Nishra, and D.L. Pierson. 1992. Evaluation of the Biolog automated microbial identification system. Applied and Environmental Microbiology. 58: 2089-2092.

Miller, J.M. and D.L. Rhoden. 1991. Preliminary evaluation of Biolog, a carbon source utilization methods for bacterial identification. J. of Clinical Microbiology. 29: 1143-1147.

Milton, J., Alexy and Thressa, C., Stsadtman. 1989. Metabolism and selenium-dependent enzymes in microorganisms. Ann. Rev. Nutri. 9: 127-137.

Nriagu, J.O. 1983. Geochim. Cosmochim. Acta. 47: 1523.

Ohlendorf, H.M., D.J. Hoffman, M.K. Saiki, and T. Aldrich. 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. Sci. Total Environ. 52(1/2): 49-64.

Polo, M. 1958. In The travels of Marco Polo, translated by R. Latham. Penguin Books, New York.

Presser, T.S. and I. Barnes. 1984. Report 84-41222. pp. 1-25. U.S. Geological Survey.

Scow K. 1982. An exposure and risk assessment for arsenic. Environmental Protection Agency. Washington, DC.

Slavin, W., G.R. Carnrick, D.C. Maning, E. Pruszkowska. 1983. Recent experience with stabilized temperature platform furnace and Zeeman background correction. Atomic Spectroscopy. 4: 69-86.

Smith, R.A., R.B. Alexander, and M.G. Wolman. 1987. Water-Quality Trends in the Nation's Rivers. Science 235: 1607-1615.

Summers, A.O., and S. Silver. 1978. Microbial transformation of metals. Ann. Rev. Microbiol. 32: 637-672.

Tracy, J.E., J.D. Oster, and R.J. Beaver. 1990. J. Environ. Qual. 19: 46.

Tseng, W.P., H.M. Chu, and S.W. How. 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J. Nat. Cancer Inst. 40: 453-463.

Tseng, W.P. 1989. Blackfoot disease in Taiwan. In: Special Report on Inorganic Arsenic and Skin Cancer. Env. Prot. Agency. 625/3-87/013.

Welz B., M. Melcher, and G. Schlemmer. 1983. Determination of selenium in human blood serum. Comparison of two atomic-abdsorption spectrometric procedures. Fresenius Z. Anal. Chem. 316: 271-276.

Woock, S.E., W.R. Gsarrett, W.E. Partin, and J.F. Moore. 1987. Bull Environ. Contam. Toxicology. 39: 998.

Yang, W.H., J.R. Yang, A. Yee, B.B. Buchanan, and T. Leighton. 1993. Microbial removal of sodium selenite from the culture media by *Bacillus subtilis*. Mississippi Water Resources Conference 23: 161-171.

Table 1. Concentration and Wavelength of 25 elements used in the Simultaneous Inductively Coupled Plasma Atomic Absorption Spectrometry.

Element	Wavelength (nm)	Std #1 (ppm)	Std #2 (ppm)	Std #3 (nm)
Aluminum (Al)	237.34	0	5	10
Antimony (Sb)	217.59	0	5	10
Arsenic (As)	189.04	0	5	10
Barium (Ba)	493.41	0	5	10
Beryllium (Be)	234.86	0	5	10
Boron (B)	249.68	0	5	10
Cadmium (Cd)	226.50	0	5	10
Calcium (Ca)	317.93	0	5	10
Chromium (Cr)	267.72	0	5	10
Cobalt (Co)	228.62	0	5	10
Copper (Cu)	324.75	0	5	10
Iron (Fe)	259.94	0	5	10
Lead (Pb)	220.35	0	5	10
Magnesium (Mg)	279.08	0	5	10
Manganese (Mn)	257.61	0	5	10
Molybdenum (Mo)	202.03	0	5	10
Nickel (Ni)	231.60	0	5	10
Potassium (K)	766.49	0	100	200
Silver (Ag)	328.07	0	5	10
Silicon (Si)	288.16	0	5	10
Sodium (Na)	589.59	0	5	10
Strontium (Sr)	421.55	0	5	10
Thallium (Tl)	351.92	0	5	10
Vanadium (V)	309.31	0	5	10
Zinc (Zn)	213.86	0	5	10

Table 2. Inductively Coupled Plasma Atomic Emission Spectrometry of 27 Major Metal or Non-Metal Elements in the Surface Water of the Mississippi River.

	M1(VB)	M2(NC)	M3(BR)	M4(NO)	M5(BC)	M6(VN)	Mean	SE*
Al	+ 0.289*	+ 0.080	+ 0.139	+ 0.128	+ 0.104	+ 0.014	+ 0.127	0.037
Sb	- 0.039	- 0.290	- 0.275	- 0.210	- 0.242	- 0.428	- 0.247	0.052
Ba	- 0.057	+ 0.057	+ 0.062	+ 0.052	+ 0.054	+ 0.047	- 0.247	0.052
Be	- 0.017	- 0.051	- 0.041	- 0.024	- 0.032	- 0.024	- 0.032	0.005
B	+ 0.518	+ 0.706	+ 0.690	+ 1.522	+ 1.357	+ 0.839	+ 0.939	0.165
Cd	- 0.008	- 0.018	- 0.012	- 0.006	- 0.009	- 0.018	- 0.012	0.002
Ca	+28.855	+36.006	+38.240	+37.294	+36.320	+30.244	+34.489	1.599
Cr	- 0.006	- 0.022	- 0.013	- 0.008	- 0.009	- 0.020	- 0.013	0.016
Co	- 0.008	- 0.017	- 0.013	- 0.010	- 0.013	- 0.020	- 0.014	0.002
Cu	- 0.006	- 0.027	- 0.023	- 0.012	- 0.013	- 0.033	- 0.019	0.004
Fe	+ 0.113	- 0.018	- 0.010	- 0.004	- 0.016	- 0.011	+ 0.013	0.021
Pb	- 0.068	- 0.136	- 0.120	- 0.072	- 0.114	- 0.154	- 0.111	0.014
Mg	+ 8.994	+10.258	+11.182	+11.549	+12.026	+ 8.532	+10.432	0.586
Mn	+ 0.006	+ 0.005	+ 0.027	+ 0.012	+ 0.002	+ 0.008	+ 0.010	0.004
Mo	- 0.107	- 0.278	- 0.230	- 0.146	- 0.185	- 0.317	- 0.211	0.033
Ni	- 0.197	- 0.725	- 0.549	- 0.320	- 0.413	- 0.795	- 0.499	0.095
K	+ 3.409	+ 2.228	+ 2.418	+ 3.033	+ 3.051	+ 1.760	+ 2.650	0.252
Ag	- 0.018	- 0.061	- 0.046	- 0.030	- 0.037	- 0.069	- 0.044	0.008
Si	+ 3.472	+ 1.499	+ 1.518	+ 1.828	+ 2.156	+ 1.325	+ 1.966	0.324
Na	+10.433	+17.468	+17.118	+14.453	+16.804	+12.602	+14.813	2.847
Sr	+ 0.124	+ 0.180	+ 0.176	+ 0.157	+ 0.160	+ 0.146	+ 0.157	0.008
Tl	- 0.422	- 1.233	- 0.977	- 0.584	- 0.755	- 1.369	- 0.890	0.151
V	+ 0.032	+ 0.027	+ 0.034	+ 0.044	+ 0.045	+ 0.023	+ 0.034	0.004
Zn	- 0.011	- 0.035	- 0.032	- 0.037	- 0.008	- 0.039	- 0.024	0.008
As	1.465**	1.858	1.694	0.677	1.498	2.187	1.563	0.208
Se	176.554#	351.450	368.232	489.802	265.184	258.660	318.314	44.525
Hg	5.626@	3.733	2.696	2.929	2.929	1.815	3.288	0.531

*: Results of simultaneous ICP-AES assays. Data are expressed in microgram/milliliter.
**: Results of ICP-AES assay with hydride generation apparatus. Data for As are expressed in microgram/liter.
#: Results of ICP-AES assay with hydride generation apparatus. Data for Se are expressed in microgram/liter.
@: Results of ICP-AES assay with mercury cold vapor generator. Data for Hg are expressed in microgram/liter.

Table 3. Inductively Coupled Plasma Atomic Emission Spectrometry of 27 Major Metal or Non-Metal Elements in the Surface Water from the Pearl River.

	P1(NB)	P2(RP)	P3(JS)	P4(MC)	P5(FW)	P6(WPR)	Mean	SE*
Al	-0.009*	+0.030	-0.239	-0.112	-0.135	-0.182	-0.108	0.042
Sb	-0.111	-0.133	-2.976	-2.619	-1.178	-1.882	-1.483	0.499
Ba	-0.000	+0.011	-0.010	-0.003	+0.007	+0.010	+0.003	0.003
Be	-0.015	-0.016	-0.148	-0.137	-0.144	-0.132	-0.048	0.028
B	+1.357	+1.951	+2.041	+2.009	+2.023	+1.684	+1.844	0.111
Cd	-0.002	-0.008	-0.045	-0.032	+0.001	+0.005	-0.014	0.008
Ca	+3.909	+3.909	+5.765	+5.669	+6.117	+4.092	+4.910	0.426
Cr	-0.006	-0.007	-0.083	-0.054	-0.061	-0.065	-0.046	0.013
Co	-0.007	-0.013	-0.035	-0.023	-0.012	-0.027	-0.020	0.004
Cu	+0.009	-0.009	-0.010	-0.014	-0.034	-0.040	-0.013	0.009
Fe	+0.050	+0.056	+0.977	+0.581	+0.689	+0.850	+0.534	0.162
Pb	-0.016	-0.084	-0.666	-0.626	-0.798	-0.433	-0.437	0.162
Mg	+1.248	+1.253	+0.990	+0.819	+1.036	+0.636	+0.997	0.099
Mn	-0.001	+0.021	+0.105	+0.087	+0.110	+0.135	+0.076	0.022
Mo	-0.104	-0.100	-1.086	-0.969	-0.969	-0.757	-0.664	0.183
Ni	-0.191	-0.181	-2.631	-2.462	-2.584	-2.462	-1.752	0.496
K	+1.716	+1.737	-0.055	-1.298	-1.081	-0.884	+0.023	0.566
Ag	-0.017	-0.016	-0.106	-0.079	-0.109	-0.109	-0.073	0.018
Si	+0.827	+0.498	-0.321	-0.107	+1.450	-0.212	+0.356	0.285
Na	+4.890	+4.841	+9.965	+9.907	+8.668	+9.192	+7.911	0.983
Sr	+0.021	+0.029	+0.025	+0.027	+0.032	+0.019	+0.026	0.002
Tl	-0.386	-0.388	-3.579	-3.333	-3.441	-3.210	-2.390	0.635
V	-0.000	-0.001	-0.046	-0.041	-0.043	-3.210	-0.557	0.531
Zn	-0.008	-0.015	+0.056	+0.025	+0.096	-0.017	+0.023	0.019
As	0.513**	0.414	0.645	0.410	0.402	0.413	0.466	0.040
Se	12.970#	3.274	0.538	0.250	0.250	0.250	2.922	2.067
Hg	5.029@	4.148	3.422	2.384	2.262	1.923	3.195	0.499

*: Results of simultaneous ICP-AES assays. Data are expressed in microgram/ mililiter.
**: Results of ICP-AES assay with hydride generation apparatus. Data for As are expressed in microgram/liter.
#: Results of ICP-AES assay with hydride generation apparatus. Data for Se are expressed in microgram/liter.
@: Results of ICP-AES assay with mercury cold vapor generator. Data for Hg are expressed in microgram/liter.

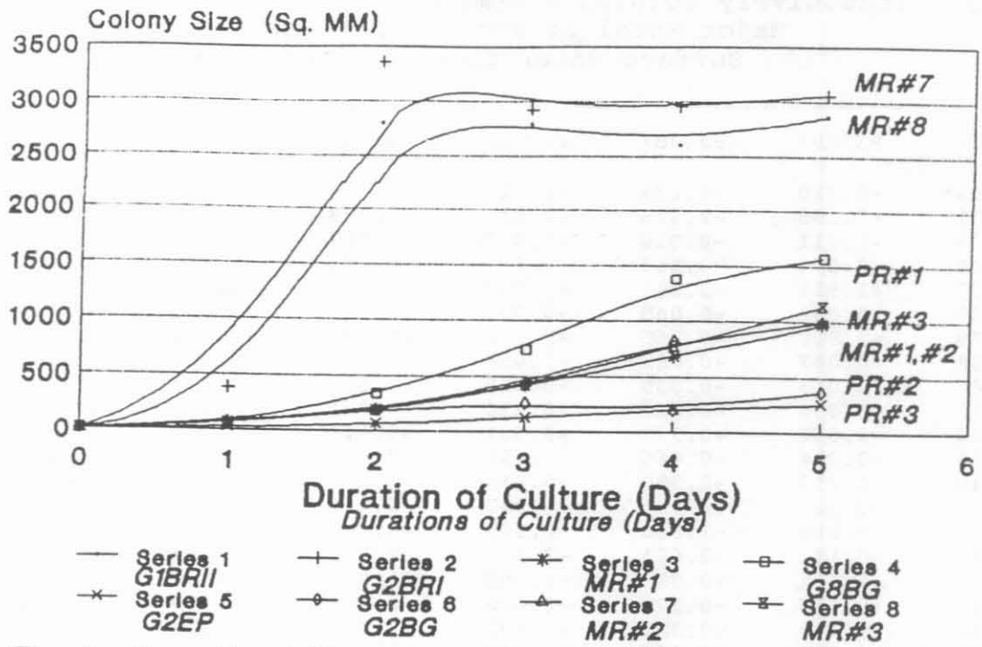

Fig. 1. Growth of Hyperresistant Bacteria in 1 mM Selenite.

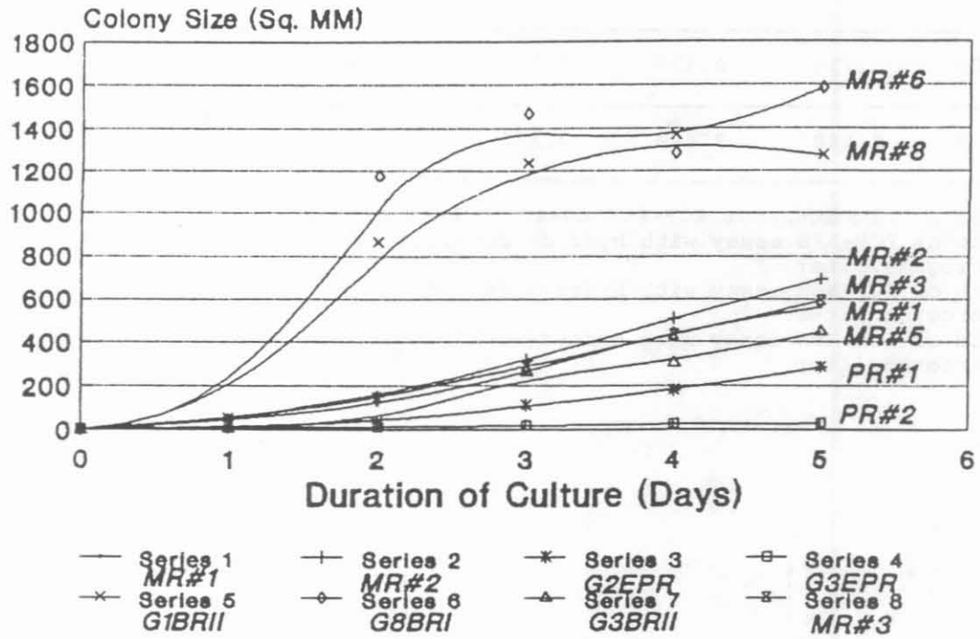

Fig. 2. Growth of Hyperresistant Bacteria in 10 mM Selenite.

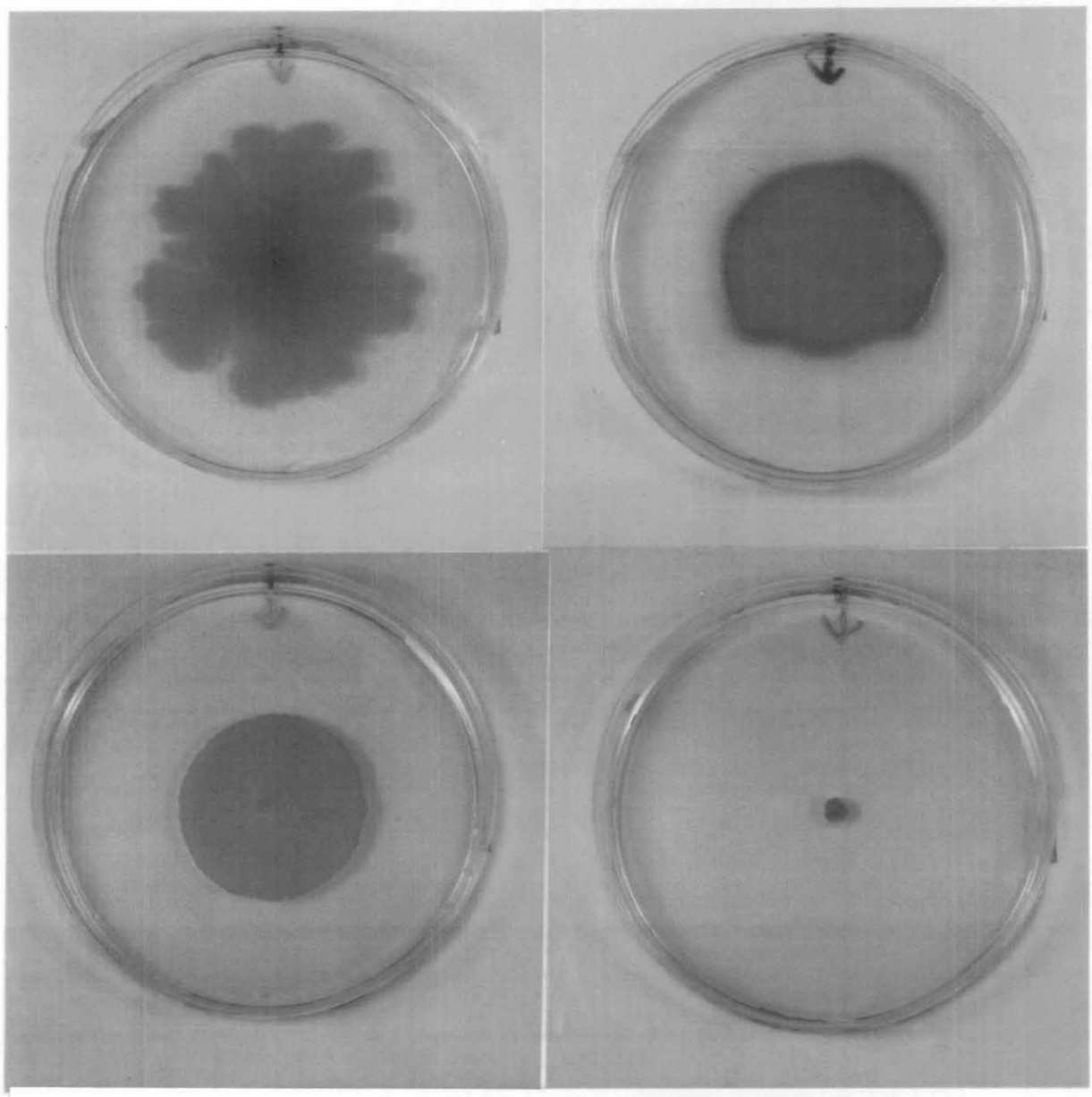

Figure 3. Large Red Colonies formed by Hyperresistant Bacteria Patched-cultured for 8 days on 10 mM Selenite.

Top Lt.: MR#2 (G2VBII) identified as Bacillus mycoides.
Top Rt.: MR#6 (G8BRI) identified as Kurthia gibsonii.
Bot. Lt.: MR#8(G1BRII) identified as Erisipelothrix rhusiopathiae.
Bot. Rt.: Control, colony appearance of Bacillus subtilis, 168 met+ lys+.