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ASSESSMENT OF IN SITU BIOREMEDIATION OF CYANIDE AND NITRATE AT A HEAP LEACH MINING OPERATION IN NEW MEXICO

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INTRODUCTION

The Ortiz Mountains in southern Santa Fe County are the location of one of the oldest gold-producing areas in the United States. Mining began there with the discovery of gold in 1828 and has continued sporadically ever since. Gold Fields Mining Corporation took a long-term lease on property in the district in 1973 and began milling the ore in 1980.

From 1980 to 1987, the Ortiz Mine utilized the heap leach process (Hickson 1982) for gold recovery. The gold bearing ore was crushed and placed on an impervious leach pad. A cyanide solution was sprayed on and percolated through the heap for 90 days. Nitric acid was used to partially neutralize the solution after the gold was removed from the heap pile and to remove scaling from activated carbon later in the mining process. The pile was then rinsed with fresh water. The rinsed residue was hauled from the impervious pad to an unlined arroyo for disposal.

Despite washing and natural removal mechanisms, the residue pile at the Ortiz Mine has been

identified as the source of cyanides and nitrates found in groundwater more than 1100 feet down-gradient from the residue pile. Some of the nitrate nitrogen in the residue pile may be a result of nitric acid used in the gold recovery process. However, nitrate contamination may also be a consequence of microbially mediated cyanide degradation. In some groundwater sampling wells, total cyanide and nitrate values exceed Environmental Protection Agency (EPA) and New Mexico standards for groundwater. Groundwater contamination is caused by cyanides and nitrates leaching from the residue pile.

These contaminants are regulated at the state and federal level because of the potential health threats associated with their ingestion from drinking water. Cyanide is a well known toxin which inhibits aerobic respiration. Nitrate itself is relatively innocuous until it is converted to nitrite in the intestinal tract. The nitrite is then absorbed into the bloodstream and interferes with hemoglobin's ability to carry oxygen. A comparison of the EPA groundwater and state drinking water regulations to contaminant levels found at the Ortiz site is shown in Table 1.

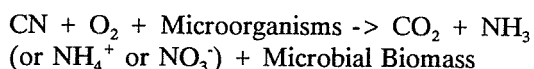
Table 1. Regulatory Levels of Cyanide and Nitrate

EPA Groundwater/State Drinking Water Regulations		Contamination Levels Found at the Ortiz Site	
		<u>Groundwater</u>	<u>Residue Pile</u>
Cyanide Guideline	0.2 ppm	<0.01-0.76	<0.3-12.4
Nitrate Standard	10 ppm	<0.1-41.3	0.3-72.0

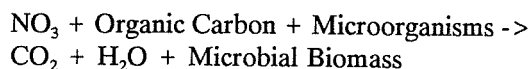
A potential method for reducing cyanide and nitrate concentrations at the Ortiz site involves the microbial remediation of these contaminants. In 1991, Pegasus Gold Corporation contracted Dr. Carleton White, James Markwiese and Lisa Valle from the University of New Mexico's Department of Biology to explore this possibility. Their objective was to assess the potential for bioremediation of cyanide and nitrate contamination at the Ortiz site. Meeting the objective required a literature survey related to microbial cyanide degradation; enumerating total indigenous heterotrophic microorganisms in the pile; enumerating cyanide degraders in the residue pile/groundwater-soil system downslope of the residue pile; and evaluating amendments that promote cyanide degradation and nitrate uptake by the indigenous microbial communities.

LITERATURE SEARCH

In an extensive literature search, over 80 articles on microbial degradation of cyanide were identified. In these articles, more than 20 species (and numerous unidentified microorganisms) are reported to degrade cyanide compounds. Microorganisms including fungi, bacteria, and actinomycetes are able to obtain the energy and raw materials for growth by metabolizing cyanide. Researchers (Allen and Strobel 1966; Bunch and Knowles 1980; Castric and Strobel 1969; Raef et al. 1977; Rodgers 1982; Skowronski and Strobel 1969; Strobel 1967) using ^{14}C N and/or C^{15}N , have shown that the general pathway (without intermediates) for microbial cyanide decomposition is:



Nitrate is not degraded by microorganisms; it is taken up as a nutrient unless anaerobic conditions prevail in which case denitrification may occur. There exists extensive literature on microbial nitrate uptake and therefore less emphasis was placed on this aspect of the literature search. The general reaction for the aerobic uptake of nitrate is:



The literature also reports that microbes in natural and contaminated settings are likely to be limited in growth by the availability of organic carbon. To build proteins for the synthesis of new cells, all organisms use amino acids as basic building blocks. The ratio of carbon to nitrogen is roughly 10:1 in an amino acid. The carbon and nitrogen ratio obtained from the metabolism of cyanide is 1:1, 9 carbons less than the typical amino acid. By adding carbon to this type of system, the growth of microorganisms should be greatly enhanced. A proliferation of microbes capable of degrading a contaminant should result in an accelerated rate of contaminant removal.

ENUMERATION OF HETEROTROPHIC BACTERIA

Literature search results showed that many microorganisms are capable of degrading cyanide. The next step was to identify whether microbes were present at the Ortiz site. This involved sampling the residue pile in various locations.

The sampling involved drilling with a hollow-stem auger to a depth of 100 feet or until native soil was reached. Since all of the residue pile had contact with the atmosphere during the mining, milling, and extraction process, potential contamination by exposure to the atmosphere was not a concern. Care was taken to minimize cross-sample contamination. Samples were transported in a cooler on ice to the Biology Annex at the University of New Mexico. All analyses were carried out using standard methods employed at the Department of Biology, UNM.

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Microbiological procedures generally followed those in *Methods of Soil Analyses, Part 2, Chemical and Microbiological Properties*, in the section by Wollum (1982). The entire sample was weighed and oven dried to determine total sample dry weight. The remainder of each soil sample was separated into greater than and less than 2 mm fractions. Approximately one tenth of the <2 mm fraction was weighed and dried to determine moisture content. Only the organisms associated with the <2 mm fraction were used in plating experiments. Plating results were extrapolated to the whole sample for number of organisms per gram of residue material.

Enumerating heterotrophic bacteria was done on three types of media (see Appendix for formulas). Preliminary plating was performed with minimal dilution of the sample because few organisms were expected. Nearly all plates had too many colonies; thus, later platings used standard conditions (soil methods). Ten grams of moist residue pile material were added to 95 ml of sterilized water, yielding a solution with 0.1 gram residue material/ml. Triplicate plates were made by spreading 0.1 ml of this solution on each agar type (0.1 ml of this plus the original solution gives 0.01 gram of residue material spread on each plate or a hundred-fold dilution). Additional 10- and 100-fold dilutions of the residue material were plated in triplicate for each agar type. These procedures gave 100-, 1000- and 10,000-fold dilutions of the original residue pile solution. Viable colonies were counted after incubating for 72 hours at room temperature on plates that contained between 30 and 300 colonies.

There were no clear preferences for any of the three agar types used in this study. The results from the plating indicate that the residue pile has unusually high numbers of viable heterotrophic organisms, given that the pile was mined rock. After incubating at room temperature for 72 hours, microorganism numbers ranged from 10^3 to 10^6 per gram of residue material.

Given the high winds and large amount of dust (soil) in the air typical for this region, inoculation of the pile probably occurred by air-borne microorganisms during leaching and before burial under other residue material. Some microorganisms have adapted to the pile conditions and are able to utilize cyanide, which is the major source of available carbon in the pile.

ENUMERATION OF CYANIDE DEGRADING MICROORGANISMS

Residue Pile

Growth of microorganisms provided with cyanide as their sole source of carbon and nitrogen for new biomass would confirm that they are capable of degrading cyanide. Plates were made with a mineral-salts media that had no other source of nitrogen or carbon (formula given in Appendix). Thus, any microorganisms growing in the agar will derive their energy (carbon) and nitrogen from the cyanide. Sample preparation was as described earlier, with subsamples of the <2 mm fraction prepared and spread to yield 100-, 1000- and 10,000-fold dilutions. Plates were incubated at room temperature and viable colonies counted starting after 6 days of incubation.

Unexpectedly high numbers of cyanide degrading microorganisms were obtained from the residue pile, ranging from 10^3 to 10^5 microbes per gram of residue material. The incubation period for these plates was extended to allow maximum development of the organisms into colonies. Some plates reached near maximum numbers after 6 days, while other plates took as long as 13 days.

Well Water Samples

Triplicate spreads of 0.1 ml of each water sample were made on KCN plates as described above. Dilutions of 10- and 100-fold were prepared and triplicate spreads with 0.1 ml of each dilution were made. Plates were incubated at room temperature and viable colonies were counted after 13 days of incubation.

After 13 days, all water samples had viable cyanide degrading organisms ranging from 10 to 430 microbes per ml of water. These results are particularly encouraging since most wells show declining cyanide and nitrate levels. The presence of cyanide degrading organisms lends support to the hypotheses that the decline is at least in part due to in situ biological activity.

The fact that the residue pile and well-water samples all have microorganisms capable of degrading cyanide may be explained by several factors. First, the pH of the residue pile and groundwater systems (about 7.5-8.1) are near optimum for the majority of cyanide degrading microorganisms

reported in the literature. Second, the only carbon source readily available in the residue pile should be cyanide, which would select for organisms capable of growth on that substrate.

AMENDMENTS TO PROMOTE CYANIDE DEGRADATION AND NITRATE UPTAKE

Based on earlier studies and the current status of the residue pile and groundwater system, aerobic pathways appear the most feasible for bioremediation. In reviewing microbial and cyanide metabolism, Knowles and Bunch (1986) referred to a number of fungi (mainly snow moulds) and bacteria (*Pseudomonas* species) capable of growth with the apparent sole source of carbon and nitrogen being cyanide. As noted previously, these microorganisms may often be carbon limited. An experiment using cyanide degrading bacteria (*Pseudomonas fluorescens*) and glucose as a source of carbon and energy with KCN or NH₄Cl as a nitrogen source, (Harris and Knowles 1983) showed that microbial growth was terminated due to glucose depletion from the medium. For the following experiments then, a base medium was prepared with all nutrients necessary for growth other than carbon and nitrogen. To this medium, cyanide and/or glucose was added as a source of carbon and energy for the microorganisms. Nitrogen was added as cyanide and nitrate because nitrate contamination is also a significant problem in this system. Nitrate served as a potential source of nitrogen (to be immobilized into amino acids) for the microorganisms.

Sample sets were prepared with three different levels of cyanide (2.6, 13, and 26 mg/l CN) and three levels of nitrate (6, 30, and 60 mg/l NO₃-N) (complete matrix shown in Appendix). These concentrations bracket those found in well water at the site (Newcomer 1991). The sets of the 3×3 matrix solutions were treated with glucose to produce a set with a 5:1 and a set with a 10:1 total carbon:nitrogen ratio in the solutions. Two other sets of solutions had no carbon amendments. One-gram subsamples of the <2 mm fraction sample with the highest number of cyanide degraders were added to each bottle of the glucose amended sets and to one of the unamended sets. Another portion of this sample was sterilized for 15 minutes prior to adding 1 gram to each bottle of the remaining unamended set of solutions.

All samples were placed on a shaker stand during incubation at room temperature. A 2 ml

portion was taken from each bottle of all sets 0, 4, 10, 17 and 38 days after introducing the sample. This portion was diluted to within working range for the nitrate analysis and for the cyanide analysis. Nitrate was determined with a Technicon Auto-Analyzer using a cadmium reduction method. Cyanide was determined by automated analysis with a cytochrome T method (ASTM Designation: D 2036-75). As of September 6, 1991, a total of 180 analyses on the batch culture had been performed for cyanide and nitrate. After 38 days of incubation, the highest carbon amendments resulted in significantly reduced concentrations of nitrate and cyanide in the culture.

All statistical analyses were performed on StatView SE using analysis of variance and significance set at the 95% confidence interval.

CONCLUSIONS

The presence of microorganisms capable of growth on cyanide in the residue pile and groundwater, coupled with the significant reduction of cyanide and nitrate using carbon amendments, indicates that in situ bioremediation would be feasible at the Ortiz site. We recommend supplying additional energy to the indigenous microbial community by injecting glucose dissolved in contaminated groundwater into the pile. Pumping the groundwater and injecting glucose into the pile will keep the contaminants in a semi-closed system allowing bioremediation to occur with subsequent reduction in evaporative water loss.

At this time, it is unknown whether the nitrate problem at the Ortiz site resulted from nitric acid used during the mining process or was a consequence of microbial cyanide degradation. Determining microbial activity rates, the byproducts and end products of degradation, and the effects of additional energy supplies (organic carbon compounds) on these processes and pathways are goals for future research.

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APPENDIX

Microbiological Media

- Total Heterotrophes Agars:
 - Nutrient Agar (Difco #DF 0001-01-8)
 - 23 g agar in 1 liter H₂O
 - TSA Agar (Difco #DF 0369-01-4)
 - 40 g agar in 1 liter H₂O
 - Dilute NA Agar 2.3 g NA agar + 13.5 g granulated agar
 - *Bring agar to a boil, autoclave for 15 minutes at 18-20 pressure

- Sample Plating (1 g residue material = 0.5 ml volume)
 - 10 g residue material + 95 ml H₂O = 0.1 g residue material/ml (STOCK)
 - Plate 0.1 ml = 0.01 dilution
 - 1 ml STOCK in 9 ml H₂O = 0.01 g rm/ml
 - Plate 0.1 ml = 0.001 dilution
 - 0.1 ml STOCK in 9.9 ml H₂O = 0.001g rm/ml
 - Plate 0.1 ml = 0.0001 dilution

- Cyanide Agar:
 - Agar consisted of primary mineral salts without nitrogen.
 - Formula contained the following amounts per 800 ml of agar solution.

Ingredient (common hydrated form)	Amount (in grams)
KH ₂ PO ₄	0.4
Na ₂ HPO ₄	0.6
MgSO ₄	0.2
CaCl ₂	0.01
MnSO ₄	0.02
FeSO ₄	0.015
Agar	15.0

The above mixture was mixed and autoclaved. A separate solution of 23 g KOH with 3.256 g KCN per liter was mixed and autoclaved. Then 200 ml of the cyanide solution was added with 800 ml of the mineral salts agar immediately before pouring. This gave a 1.0 mM KCN solution in the agar. The KOH was used to insure that the pH was above 10.

- Cyanide/Nitrate Utilization: Amendments with glucose

This study examined the influence of carbon amendments on microbial degradation of CN and uptake of NO₃. The following four treatments were used: No amendment, Low amendment (carbon:nitrogen ratio 5:1), High amendment (carbon:nitrogen ratio 10:1), and a sterile control (same as no amendment but with autoclaved residue material). Each treatment consisted of nine separate containers having variable amounts of CN, NO₃ and glucose (the latter in amendment

studies only). The containers each had a total volume of 101 ml and were placed on a shaker stand for the duration of the study. Transfer of gasses in containers was facilitated by screw caps left open one quarter turn.

Each treatment was prepared in a 3×3 matrix with low, medium and high concentrations of cyanide and nitrate.

Experimental Matrix: Nitrate Level (mg/l)	Bottle # for each set CN Level (mg/l)		
	Low 2.6	Medium 13	High 26
6.0	1	2	3
30	4	5	6
60	7	8	9

Imposed on this 3×3 matrix were the appropriate amounts of glucose to give a 5:1 or 10:1 total C:N ratio, including the carbon and nitrogen in cyanide and the nitrogen in nitrate. The final concentrations of glucose in each bottle are given below:

Treatment A (C:N 5:1)		Treatment B (C:N 10:1)	
Bottle	ppm	Bottle	ppm
1	89.5	1	182.0
2	147.5	2	310.0
3	220.0	3	470.0
4	389.5	4	782.0
5	447.5	5	910.0
6	520.0	6	1070.0
7	764.5	7	1532.0
8	822.5	8	1660.0
9	895.0	9	1820.0

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