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Occurrence of Bacterial Resistance to Arsenite, Copper, and Selenite in Adverse Habitats

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The effects of metal pollution on biotic communities has been extensively studied, particularly in the areas of ecotoxicology and species composition effects. Impact studies on bacterial communities have focused on adaptation via resistance mechanisms or biogeochemical cycling alterations (Barkay and Olson 1986, Rother et al 1982). It has been shown that microbial communities in polluted environments are frequently resistant to higher levels of organics and metals than those in unimpacted areas (Barkay and Olson 1986, Olson and Thornton 1982, Mills and Colwell 1977). Increases in bacterial resistance to metals and metalloids has been attributed to selection and molecular mechanisms, such as gene transfer via plasmids (Olson and Thornton 1982).

Relatively few natural environments have been surveyed for metal resistant bacterial populations, with most studies measuring mercury, lead or zinc resistance (Burton et al 1987, Barkay and Olson 1986, Olson and Thornton 1982, Timoney et al 1978). Thus, the incidence of naturally-occurring and pollutant-related microbial resistance is poorly defined, as are the environmental factors which influence resistance (Barkay and Olson 1986, Babich and Stotzky, 1980). Elevated levels of arsenic, copper, and selenium have caused environmental impacts which are linked to agricultural, industrial, and municipal activities. The impacts of these metalloid/metals on natural microbial communities and the levels of resistance are poorly defined. The present study reports the incidence of aerobic heterotrophic bacterial resistance to arsenite, selenite and copper in a variety of habitats in the United States. These included soil, water, and sediments with known copper, arsenic, or selenium pollution, as well as control sites.

MATERIALS AND METHODS

Samples were collected from 33 sites within 13 ecosystems. The survey consisted of one soil, nine water, and twenty-seven sediment samples. Samples were collected from the following locations. Kesterson Wildlife Refuge, San Joaquin Valley, California; Volta Reservoir, San Joaquin Valley, California; Clark Fork River, Western Montana; Whitewood Creek, Black Hills of South Dakota; Lake Lavon, Dallas, Texas; Varsity Pond, University of Colorado at Boulder; Emerald and Bear Lakes, Rocky Mountain National Park, Colorado; Agricultural Research Station, Bennett, Colorado; and Holbrook Creek, Como, Blue No. 2, and Crater Lakes, Mount Blanca, Colorado.

Replicate sediments were collected by using a ponar dredge or aseptically scraping the upper 2 cm of surface into sterile, acid-washed, polyethylene bottles. The composite soil sample was collected similarly by scraping. Waters were collected, aseptically, in sterile, acid-washed, polyethylene bottles. Samples were immediately placed on ice and returned to the laboratory within 24 h for immediate processing. Most analyses, however, were begun within 2 h of sample collection.

Samples were homogenized and split for metal analyses. Sediment dry weights were determined, in triplicate, by drying overnight at 110 C. Total recoverable arsenic, copper, and selenium concentrations were determine in sediments using an acid digestion procedure (U.S. EPA 1979) followed by analysis on an atomic absorption spectrophotometer. Arsenic was measured using the hydride generation method with nickel nitrate addition to reduce background interference. Copper was measured by the flame method and selenium by graphite furnace (U.S. EPA 1979).

Aliquots of well mixed samples were removed and serially diluted in cold phosphate buffer (0.06 M, pH 7.5). Aliquots (0.1 ml) of each dilution were spread on casein-peptone-starch (CPS) agar plates (Collins and Willoughby 1962) for total aerobic heterotrophic bacterial counts. Enumeration of metal resistant populations was performed on CPS agar plates amended with either sodium arsenite, cupric sulfate, or sodium selenite (Sigma Chemical Company, St. Louis, Mo.) Metals were filter sterilized prior to adding to autoclaved CPS media. Final concentrations of metals were as follows: 10 mM arsenite, 1 mM copper, and 10 mM selenite. These concentrations were chosen after preliminary testing to determine detectable resistance levels. Inoculated plates were incubated in plastic

sleeves and incubated at room temperature for 7-10 days before counting. Duplicates of four dilutions were counted and averaged. The percentages of heterotrophs exhibiting resistance and total recoverable metal concentrations were compared for relationships using Pearson's standard correlation coefficient with the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

A wide range of total recoverable metal concentrations were found among the test site sediments (Table 1). Selenium levels ranged from 14.4 ug/g dry wt on the Clark Fork River (Station 2) to less than 0.005 ug/g at Volta Reservoir. Arsenic sediment concentrations ranged from 218.8 ug/g at Station 4 on the Clark Fork River to 0.782 ug/g at Station 10. Finally, total copper results revealed extreme contamination at 1,078.1 ug/g at Station 4 on the Clark Fork River and a low of 0.38 ug/g in Bear Lake in Rocky Mountain National Park. Metal contamination on the upper Clark Fork River and Whitewood Creek was expected due to historical copper and gold mining activities upstream from the impact sites (Montana 1986). Selenium concentrations observed in samples from Kesterson National Wildlife Refuge were not high when compared with the other test samples (Table 1). Selenium contamination, from agricultural drainage has been well documented in the Refuge, resulting in severe impacts to waterfowl populations. Studies of metal contamination in Kesterson sediments have shown extensive variability ranging from below detection limits to 100 mg/kg dry weight (U.S. Bureau of Reclamation 1986).

Aerobic heterotrophic bacterial densities were at levels previously reported in waters and sediments (Wetzel 1975). Water densities ranged from 4.90×10^2 CFU/ml in oligotrophic Emerald Lake (Rocky Mountain National Park) to 4.03×10^5 CFU/ml in eutrophic Volta Reservoir (San Joaquin Valley, California). Sediment populations showed similar ranges from 2.00×10^5 CFU/g dry wt in oligotrophic Crater Lake (elevation 3,871 m) to 1.17×10^9 CFU/g dry wt in the Clark Fork River.

The portion of these bacterial populations which were resistant to selenite (10 mM) and copper (1 mM) showed marked differences between sites. The greatest range in resistance levels occurred with the metalloid, selenite (Table 1). The lowest level of resistance (as determined by % population recovery) occurred at Bear Lake (0.20%) in Rocky Mountain National Park, where low background levels of selenium existed (0.263 ug/g dry wt). The highest level of selenite resistance was

Table 1. Metal resistance[a]

Site[b]	Media	Total bacterial counts[c]	Resistant population[a](%) Se	As	Cu	Metal concn[d] Se	As	Cu
Kesterson	sediment	1.04×10^6	58.88	0.00	0.10	1.36	12.20	1.04
	water	2.35×10^3	53.83	0.21	0.21	0.15	ND	ND
Volta	sediment	1.88×10^8	1.25	0.01	0.06	<0.01	12.06	3.40
	water	4.03×10^5	2.21	0.00	0.00	<0.01	ND	ND
Clark Fork								
Station 1	sediment	3.62×10^8	4.20	0.04	0.86	4.18	2.74	452.06
2	sediment	1.17×10^9	3.22	0.17	0.42	14.36	62.90	618.28
3	sediment	3.72×10^8	1.28	0.08	1.18	4.98	27.49	229.44
4	sediment	6.12×10^8	2.48	0.05	0.15	2.19	218.75	1078.13
5	sediment	1.58×10^8	2.98	0.08	0.18	2.57	25.67	82.33
6	sediment	3.40×10^8	2.05	0.04	0.06	6.64	108.60	177.87
7	sediment	1.60×10^8	2.06	0.04	0.08	1.93	11.55	57.75
8	sediment	1.30×10^8	4.99	0.08	0.08	2.06	15.78	65.29
9	SEDIMENT	1.74×10^8	2.45	0.02	0.16	1.87	9.28	40.03
10	sediment	3.65×10^8	6.36	0.18	0.50	7.73	0.68	72.96
11	sediment	1.63×10^8	6.19	0.17	1.68	3.57	12.50	106.79
12	sediment	2.48×10^8	12.22	0.16	0.28	2.14	3.51	13.33
13	sediment	1.42×10^8	10.70	0.10	0.76	1.71	1.14	31.36
Reference	sediment	2.09×10^8	3.02	0.01	0.03	0.03	5.41	4.90

a. Percentage of bacterial population resistant to the test metals: selenite (10 mM), arsenite (10 mM), copper (1 mM).
b. Site locations given in text.
c. Total bacterial colony forming units on CPS agar as CFU/ml or g dry wt.
d. Metal concentration of test sample as ug/ml (waters) or ug/g dry wt. (soil or sediments).
e. Not determined.

(Table 1 cont'd)

Whitewood								
Station 3	sediment	9.42×10^6	3.70	0.14	ND[e]	11.40	100.88	16.49
5	sediment	1.05×10^8	3.09	0.01	ND	5.20	63.75	37.38
7	sediment	8.27×10^7	3.87	0.03	ND	1.32	49.89	5.75
9	sediment	2.74×10^7	3.64	0.00	ND	9.10	156.02	14.76
Lake								
Lavon	sediment	1.95×10^6	0.95	0.05	ND	2.04	0.99	3.03
Varsity								
Pond	sediment	1.04×10^9	7.40	0.05	0.10	4.08	1.18	4.01
	water	3.20×10^5	1.06	0.02	0.02	ND	ND	ND
Emerald								
Lake	sediment	1.43×10^8	1.01	0.01	0.48	2.63	3.42	3.68
	water	4.90×10^2	6.12	0.00	1.02	ND	ND	ND
Bear Lake	sediment	1.45×10^8	0.38	0.01	0.05	0.26	5.53	0.38
	water	7.00×10^3	0.43	0.00	0.00	ND	ND	ND
Bennett	soil	1.82×10^7	0.57	0.01	ND	3.17	1.48	1.81
Crater Lake	sediment	9.30×10^5	1.32	0.05	2.40	4.26	1.31	3.59
	water	1.10×10^4	1.14	0.00	0.00	ND	ND	ND
Blue 2 Lake	sediment	4.13×10^5	1.74	0.00	0.09	4.27	1.07	2.03
	water	2.04×10^5	0.20	0.00	0.10	ND	ND	ND
Como Lake	sediment	2.00×10^5	6.90	0.00	1.21	1.78	1.44	1.09
	water	4.00×10^4	2.12	0.01	0.63	ND	ND	ND
Holbrook								
Creek	water	2.38×10^3	14.32	0.21	0.63	ND	ND	N

a. Percentage of bacterial population resistant to the test metals: selenite (10 mM), arsenite (10 mM), copper (1 mM).
b. Site locations given in text.
c. Total bacterial colony forming units on CPS agar as CFU/ml or g dry wt.
d. Metal concentration of test sample as ug/ml (waters) or ug/g dry wt. (soil or sediments).
e. Not determined.

observed in Kesterson Refuge (58.9%) where selenium contamination exists. Correlations between selenium concentrations in water, algae and sediment at Kesterson and nearby Volta Reservoir showed a significant ($P \leq .05$) nonparametric correlation ($r = 0.95$) between selenium concentration and bacteria resistant to selenite, as previously reported (Burton et al 1987).

Arsenite proved to be more toxic than selenite as evidenced by lower recoverable populations of resistant heterotrophs, ranging from 0-0.21%. Highest resistance occurred at Kesterson (water), Holbrook Creek, and on the Clark Fork River (Stations 10, 11, and 12). A portion of the section of the Clark Fork River has been placed on the National Priority List for Superfund activities due to severe arsenic contamination of groundwater supplies (Montana 1986). High arsenic concentrations were observed in the Clark Fork River sediments (Table 1). Resistance to arsenite was significantly correlated ($P < 0.008$, $r = .52$) with selenite resistance at all test sites.

Copper (1 mM was the most toxic of the 3 test metals (Table 1). Its high degree of bacterial toxicity has been reported by others (Albright et al 1972). Resistant populations ranged from 0 to 2.40% of the total recoverable heterotrophic bacteria. Highest resistance occurred at Crater Lake, however, sediment copper concentrations were much higher at several Clark Fork River sites. Elevated copper resistance did occur at contaminated sites along the Clark Fork River, but statistically significant correlations did not exist. Arsenite and copper resistance were significantly ($P < 0.005$) correlated ($r = 0.60$) as were concentrations in the sediment ($r = 0.84$).

In those sites where both sediment and water resistant populations were enumerated, greater proportions of resistant microorganisms were recovered in the sediment 62.5% of the time. This is likely a result of sediment populations being exposed to higher concentrations of metals in sediments. The lack of significant correlations between total recoverable metals in sediments and levels of phenotypic resistance with data from all test sites is not surprising, even though such correlations have been reported (Barkay and Olson 1986). Numerous environmental variables, e.g., pH, cation-exchange capacity, redox potential, affect metal toxicity to microorganisms (Babich and Stotzky 1980). Physiochemical factors will influence bioavailability, metal speciation, and various metabolic activities which in turn will determine both the degree and mechanisms of resistance. Correlations have been

observed between bacterial resistance and both metal speciation and bioavailability (Hornor and Hilt 1985, Hallas et al 1982). Metal effects on microbial populations, e.g., metabolic adaptation via resistance pathways, will be affected most by the bioavailable fraction of metals (Hines and Jones 1982). Therefore, measuring total recoverable metals by rigorous acid digestions measures both available and unavailable fractions, the ratio of which likely varies between sites due to a multitude of physicochemical factors. In addition, resistance was only determined using single chemical species, e.g., As^{+3}, Se^{+4}, Cu^{+2} and not for all possible species as measured in total metal analyses. Two other critical factors affecting resistance determinations are metal concentration and the type of enumeration media. Media effects on metal speciation, complexation, and bioavailability have been established (Gadd and Griffiths 1978). The media used in this present study, CPS, has lower concentrations of nutrients than more commonly used isolation media, e.g., plate count agar, thus a less likelihood of complexation with the test metals (Gadd and Griffiths 1978). Unfortunately, there is not a standardized method for measuring metal resistance, therefore comparisons between sites by different methods are difficult (Trevors et al 1985).

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