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# OCCURRENCE OF MERCURY-RESISTANT MICROORGANISMS IN MERCURY-CONTAMINATED SOILS AND SEDIMENTS IN PAVLODAR, KAZAKHSTAN

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**ABSTRACT:** There is extensive mercury contamination of soil surrounding a chloralkali plant in Pavlodar, Kazakhstan, that operated from 1970 to 1990. High-level mercury contamination exists within the confines of the plant, at nearby off-site waste storage and evaporation ponds, and in Balkyldak Lake, which adjoins the waste ponds. The Irtysh River, a major river in Kazakhstan and a drinking water source, is threatened by a plume of mercury-contaminated groundwater and may already be affected. Estimates of the extent and levels of contamination were made based on available information, the amount of mercury used at the plant over the period it operated and field monitoring. Bacteria, *Actinomyces* sp., and fungi from soil and sediment samples collected at the plant and Balkyldak Lake were cultured. A high percentage of these microorganisms were resistant to mercury chloride concentrations of up to 0.1 mM. These mercury-resistant microorganisms are being characterized for their potential to be used in bioremediation at this site and other contaminated sites in Kazakhstan and the world.

## INTRODUCTION

Mercury is a naturally occurring element in the earth's crust. Anthropogenic emissions and releases account for some of the largest inputs of mercury to the environment. Exposure of animals and humans to mercury can cause numerous ill health effects. Historically, large quantities of mercury have been used in the extraction of gold, in chloralkali plants, as pesticides and in the medical and dental fields. Significant release of mercury can occur at chloralkali plants used in the production of chlorine. Chlorine is produced by the amalgam process involving electrolytic dissociation of chloride solutions with $Hg^{\circ}$ serving as the cathode. Production of chlorine in this manner accounts for 60% of the total production in Europe.

Some areas in republics of the former Soviet Union are heavily contaminated with mercury as a result of releases from industrial plants. These include industrial zones in the following cities: (1) Sumgait in Caucasus, (2) Volgograd, Belgorod, Shvarts, Kirovo-Chepetsk, Sterlitamak in the European part of Russia, (3) Usolie-Sibirskoe and Sayansk in Siberia, and (4) Temirtau and Pavlodar in Kazakhstan. Ingress of mercury from the plants to the environment has resulted in significant pollution of soils, silts ground and surface waters. Although pollution of groundwater and surface water by other toxic metals, such as arsenic, copper, and lead is more common than mercury pollution, the high levels of mercury encountered impose a considerable risk to the populations of these regions.

The northern suburb of Pavlodar City is contaminated by mercury as a result of activity at a chlor-alkali chemical plant (Khimprom), which produced chlorine from the 1970s to the 1990s. It is estimated that 1000 tons (10 tons of which is $HgCl_2$) of mercury lay in the soil 2-4 m beneath the plant. Soil and groundwater in the surrounding vicinity of the plant are also contaminated with high concentrations of mercury. The plant is 5km from the Irtysh River, a major river in Kazakhstan. According to some reports, a plume of mercury from the plant has already reached the river. In addition, an estimated 10 tons of mercury in wastewater was discharged into Balkyldak Lake during operation of the plant.

To date, the amount of mercury released from chlor-alkali plants in Kazakhstan has not been recorded or published. Therefore, we will consider estimates of the total maximum amount of mercury possibly released by the plant in Pavlodar to provide insight into the potential magnitude of the problem. The total amount of mercury used at Pavlodar in the electrolytic process at any time was 187 tons. If this entire amount were released each year the total amount released for the entire time the plant was in operation (19 yrs) would be 3550 tons. We know however, that some amount of mercury was recycled as part of the process. Another estimation can be based on the consumption of mercury by the plant. Annual mercury consumption was estimated to be 120 tons (Novosibirk. 1995). If 75% of this is assumed to be lost every year, then about 1700 tons was released during operation of the plant. The actual total losses of mercury are maybe closer to 3000 tons and are quite likely more than 1700 tons.

Estimates can also be based on the averaged concentrations of mercury measured in the plant's buildings, structures, soils, wastes and adjoining sediments of Balkyldak Lake. Other factors and any longer distance dispersion of mercury are not included in the estimation. Figure 1 is a map of the chloralkali plant and its vicinity showing the extent of mercury contamination. Studies carried out within the confines of the chemical plant showed it is intensively contaminated with mercury (Sintez, 1991; KHIF GOSNIIHLORPROEKT, 1989a; NTS Tehnolog, 1990; HIF GOSNIIHLORPROEKT, 1989b; KNII Sinteko, 1992.). The total aerial extent of contaminated soil at the plant is 521150 $м^2$. Soils in the area east of the electrolysis workshop are the most contaminated. There, the average concentration of mercury in topsoil (0-25см) is 14 mg/kg. High contaminations of mercury were found more than 4 m below the surface and mercury concentrations exceeded the maximum permissible concentration (MPC) to depths of approximately of 1.5 m. From these data, release of mercury from the plant can be considered to have been about 1400 tons minimally with a maximum amount of approximately 3500 tons.

Spills of process saline solutions and water around the electrolysis workshop building resulted in centers of highly saline groundwater containing chlorides and sodium up to 62-72 g/l and extremely high concentrations of mercury (12.5-103.0 mg/l; 25000-206000 times higher of MPC which is 0.5 μg/l for water). Mercury has spread in the southwest, western, northwest and northern directions up to 800 m away from the workshop. The aerial extent of the contaminated first horizons of groundwater and stationery groundwater is 0.55 $km^2$; the total content of mercury dissolved in them is about of 10 tons.

Balkyldak Lake had once been used to receive considerable volumes of mercury-containing wastewater from the Khimprom plant. This has resulted in the accumulation of high concentrations of mercury in bottom sediments (up to 1200 mg/kg). The concentration of mercury in the lake water reached 25 mg/l (50000 times higher than the MPC) during some periods. Subsequently, evaporation storage ponds were constructed to receive waste from the plant. Mercury in one of the evaporation ponds receiving wastes from the plant reached 2.3-51.5 mg/l (4600-10300 times higher of MPC) and the accumulated solid material enriched with mercury is estimated at 270,000 tons. After the wastewater storage ponds had been put into operation, the mercury levels in the water of Balkyldak Lake decreased and varied in the range of 0.001-0.01 mg/l (2-20 times higher of MPC). However, the ponds were constructed adjacent to the lake and, because of periodic or emergency discharges of wastewater from the ponds, mercury levels in the lake could sharply rise. For example, in 1992 mercury concentrations in the lake water increased to 10 mg/l.

High concentrations of mercury at the waste ponds resulted in a gradual extension of contaminated groundwater (more then MPC, i.e. 0.5 μg/l) in the area. In 1985, mercury above the MPC was discovered only in wells located 10-150 m to the south and southwest of the bank line, and the area of the contaminated ground water was 2-3 $km^2$. In 1987, the contaminated area increased up to 12-15 $km^2$ in the same direction. In addition, centers of groundwater contamination (up to 0.4-0.5 $km^2$) were noted in northern and eastern directions.

Mercury concentrations found in snow exceeded the MPC of 0.5 μg/l over an area of about 255 $km^2$. Mechanisms of mercury migration were probably wind transport of dust, snow and ice from the surface of Balkyldak Lake and the wastewater storage ponds, and dispersion of ventilation releases from the electrolysis workshop at Khimprom plant. In 1992, a considerable decrease in the area of contaminated snow cover was observed when there was a reduction in the industrial activity of the chemical plant. This further supports the likelihood of dust and gas emissions from the plant as a major source of the mercury transported to the atmosphere and deposited on the ground.

Integrated studies of mercury distributions in (1) the water and sediments of Balkyldak Lake, (2) in groundwater in the vicinity of the plant, (3) in snow cover, (4) in the waters and sediments of the waste and storage ponds, including an ash lagoon, and (5) nearby reservoirs in the northern industrial section of Pavlodar City has confirmed a high level of industrial contamination in this region (Zhetekshi 1988,1991,1993)

At present various projects have been proposed to mitigate the contamination under and around the electrolysis workshop since these are the areas with the highest mercury levels. These areas are a source for mercury input by groundwater ingress into the waters of Balkyldak Lake and Irtysh River. However, even after the implementation of any demercurization project for the Khimprom plant, a substantial amount of mercury will still remain in the topsoil and beneath the surface at the plant and waste ponds. This mercury will continue to be a source of pollution entering the Balkyldak Lake and Irtysh River via groundwater inflow.

Research into further determining the extent of contaminated soil and sediments around the plant, and a study into the microbial communities at contaminated areas is underway by an international team of scientists from Kazakhstan and the U.S.A. Soil and sediment samples were obtained from contaminated sites around the electrolysis plant. Sample sites and sampling methodology are described in a section below. The samples were analysed by researchers in Khazakhstan and in the U.S.A. These research efforts are aimed at isolating mercury resistant bacteria from contaminated soils for possible future bioremediation applications.

## MATERIALS AND METHODS

**Sample Collection.** Samples of soil were collected from areas near the electrolysis workshop at the Khimprom plant in Pavlodar in September 2001. Soils and sediments were also obtained near waste storage ponds used by the Khimprom plant, and from the edge of Balkyldak Lake. Soil samples, about 1 cm beneath the surface, were collected using spatulas. Sediment samples were collected using a 170 mm x 60 mm diameter coring device. Samples were placed in acid-washed and sterilized plastic bottles or whirlpak bags.

Locations of the sampling sites (1-13) are shown in Figure 1. Samples labeled P were refrigerated within 2 days and processed in the Almaty, Kazakhstan laboratories within one month. Samples labeled U were shipped to the EPA laboratory in Cincinnati and processed within one month. The holding temperature for the U samples during the 25day transit is not available, but is likely to be variable room temperatures. The U samples were stored at 4°C upon receipt at the laboratory.

**Analyses of Samples in the Kazakhstan Laboratory.** Soil and silt samples were inoculated onto plates containing the following nutritious media: fish peptone agar (FPA), peptone iron agar (PIA), and R2A agar. To identify resistance of microorganisms to mercury, 0.01 and 0.1 mM of $HgCl_2$ were added to the media. Controls consisted of media without added mercury. Colonies of bacteria, fungi and *Actinomyces* sp. were identified by colony morphology.

**Analyses of Samples in the U.S Laboratory.** An initial screening for microorganisms was done using the 'U' soil samples. One gram each of the soil samples was extracted using 10 mL of sterile deionized (DI) water. The soil samples were added to flasks containing 10 mL of the sterile DI water and shaken on a shaker for 2 hrs at 150 rpm. Undiluted extracts were plated on YM agar (Glucose-10.0g; Peptone-5.0g; Yeast Extract-0.3g; Malt Extract-0.3g) supplemented with mercuric chloride. Three different concentrations of mercury, 0.005mM (1.36mg/l), 0.02mM (5.43mg/L) and 0.05mM (13.6mg/L) in plates were tested.

Based on the results from the initial screening, second plating was done using YM agar supplemented with 0.05mM $HgCl_2$ in order to enumerate mercury resistant bacteria and unsupplemented-YM and R2A agars for total counts of bacteria. Dilutions of the soil extract were prepared in Butterfield's phosphate buffered solution (U.S. FDA). A stock solution of $KH_2PO_4$ was prepared in DI water. 1.25 mL of the stock was diluted to 1L and the pH was adjusted close to 7.2 and the buffer was sterilized. This buffer solution was used in the preparation of dilutions. Fungi and Actinomyces were identified by colony morphology. Pure cultures of selected microbes were isolated on YM plates containing 0.05mM $HgCl_2$. The cultures were stored at –80°C.

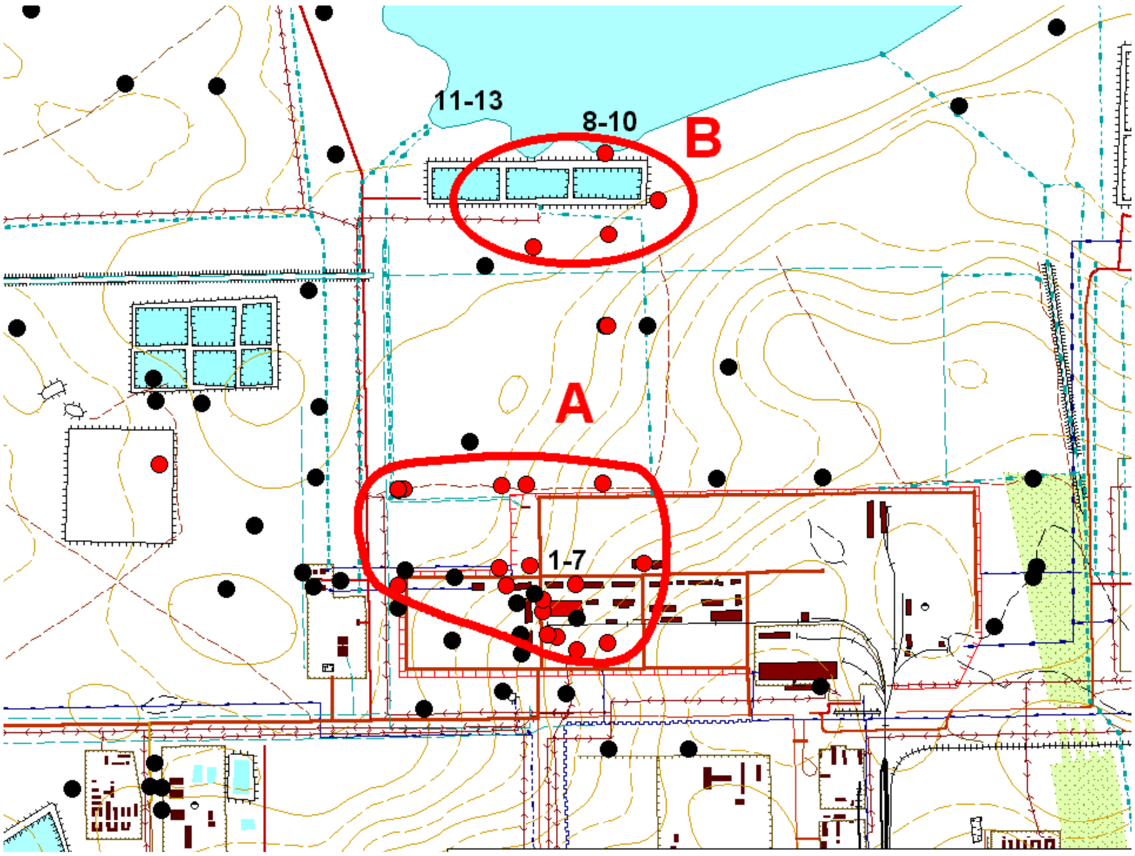


**FIGURE 1. Focus of mercury pollution in the North Industrial Site of Pavlodar City, KZ. Light boreholes (circles) in A and B contained mercury concentrations greater than the MPC in groundwater samples. Black boreholes did not contain mercury in the ground water samples. The three sludge lagoons circled in B are together about 1 KM in length.**

## RESULTS AND DISCUSSION

Table 1 shows the results of analyses done at the Kazakhstan laboratory. There was variation in the number of aerobes and saprophytic microorganisms recovered on different media. The results also showed that the isolated microorganisms were tolerant to 0.001 mM $HgCl_2$ in the media. Increasing the $HgCl_2$ concentration in the media to 0.1 mM however, resulted in a reduction in the recovery of microorganisms. Fungi and *Actinomyces* were found only in the soil (not in silt samples) and found to grow in media with $HgCl_2$ contents of 0.1 mM. On PIA and some PFA agar plates however, the numbers from soil samples increased with an increase in the $HgCl_2$ concentration in the media.

A total of 125 bacteria strains, 1 fungus and 4 *Actinomyces* were isolated from the plates containing $HgCl_2$. Cultivation of pure cultures of bacteria in liquid complex media with $HgCl_2$ has shown that only 50 strains of bacteria were resistant to 0.01 mM $HgCl_2$ and 41 strains of bacteria to 0.1 mM $HgCl_2$. Fourteen isolated strains of bacteria were gram-negative and 27 were gram-positive. These data show that mercury contaminated soils and silts from the chlor-alkali plant Pavlodar City contain a wide variety of microorganisms resistant to high levels of mercury.

Results from the analysis conducted in the EPA lab are shown in Table 2. Undiluted extracts from the initial screening (U samples) plated on mercury-supplemented YM plates resulted in colonies too numerous to count. Fewer colonies grew on plates with increasing concentrations of mercury. Colonies from plates containing 0.05mM mercury were picked to isolate mercury resistant bacteria. Unique colony types on the 0.005mM plates not seen on 0.05mM plates were also selected. Plate counts of total soil bacteria recovered on YM agar, R2A agar and YM agar supplemented with $HgCl_2$ at a concentration of 0.05mM are shown in Table 2.

The results are represented as counts per gram of soil. R2A agar (without added mercury) yielded a higher total count of bacteria from all the samples. Comparisons of counts on YM agars with and without mercury (Figure 2) show that for five of the six samples, 16-54 percent of the total soil bacteria are resistant to a mercury concentration of 0.05mM.

Comparison of the results in Table 1 and 2, show the counts of mercury resistant bacteria obtained in both screenings are within the same order of magnitude. The concentrations used by researchers in Kazakhstan were 0.01mM and 0.1mM while 0.05mM were used in the U.S. laboratory. The concentration of mercury also does not seem to have a large effect on the recovery of bacteria for many of the sites. Low numbers at 0.1mM concentration were seen on R2A supplemented with mercury (Table 2). These results suggest that the very high concentrations of mercury at the sample sites naturally selected for survival of mercury resistant microorganisms.

Pigment-producing bacteria were found in U samples from all the sites. Many different pigment colors were observed. Approximately 132 cultures of bacteria and Actinomycetes were isolated from the soil samples in Table 2.

The focus of the research was to isolate microorganisms capable of growth in the presence of high concentrations of mercury that might be useful in the mitigation of soils and sediments and ground water. Numbers of total soil bacteria and mercury resistant bacteria are difficult to extrapolate to the sampling sites because of errors associated with extended holding times. In addition, it is widely recognized that not all the microorganisms inhabiting soils can be extracted or recovered on plates. The numbers however, show a relative abundance of the resistant microorganisms obtained in culture from site to site.

## CONCLUSIONS

Operation of the chlor-alkali facility at the Khimprom chemical plant in Pavlodar City, Kazakhstan, resulted in high-level mercury contamination of soil within the plant and ground water flowing beneath it. It is likely that Irtysh River, one of Kazakhstan's main rivers, will become contaminated, if not already, by a plume of mercury in

**TABLE 1. Quantity of aerobes in mercury contaminated soil and silts of Pavlodar City (counts per 1g of soil or silt) *F= fungi, A*=Actinomyces**

| Sample Number | Sampling location | PFA | | | R2A | | | PIA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Without $HgCl_2$ | 0.01 mM $HgCl_2$ | 0.1 mM $HgCl_2$ | Without $HgCl_2$ | 0.01 mM $HgCl_2$ | 0.1 mM $HgCl_2$ | Without $HgCl_2$ | 0.01 mM $HgCl_2$ | 0.1 mM $HgCl_2$ |
| P1 | Soil near the workshop south-west | $3.8x10^6$ | $5.2x10^6$ | $4.6x10^6$ | $9x10^6$ | $9x10^6$ | $7x10^4$ (F: $10^4$) | $8x10^5$ | $3x10^6$ | $4x10^6$ (A: $5x10^4$) |
| P2 | Soil near the workshop south-east | $5.6x10^6$ | $3.6x10^6$ | $7x10^6$ (F: $2x10^4$) | $6.7x10^6$ | $10^7$ | $1.6x10^5$ (A: $10^4$) | $1.8x10^6$ | $5x10^6$ | $9x10^6$ (A: $3x10^4$) |
| P3 | Soil near tanks | $3.3x10^6$ | $3.5x10^6$ | $2.3x10^6$ | $4.2x10^6$ | $3.1x10^6$ | $10^4$ (A: $10^4$) | $1.7x10^5$ | $10^6$ | $3.6x10^6$ (A: $10^4$) |
| P8 | Lake sediment N 3, surface layer | $1.7x10^6$ | $1.1x10^6$ | $1.9x10^5$ | $2x10^6$ | $2.4x10^6$ | 0 | $4.6x10^5$ | $3.8x10^5$ | $5.5x10^5$ |
| P9 | Lake sediment N 3, the depth is of 5 cm | $1.8x10^7$ | $1.2x10^7$ | $2.6x10^6$ | $1.5x10^7$ | $3.7x10^6$ | $4x10^4$ | $1.6x10^7$ | $1.2x10^7$ | $3x10^6$ |
| P10 | Lake sediment N 3, the depth is of 10 cm | $2.2x10^6$ | $9x10^5$ | $1.6x10^5$ | $4.5x10^6$ | $2.8x10^6$ | $10^4$ | $7x10^5$ | $7x10^5$ | $3x10^5$ |
| P11 | Lake sediment N 1, surface layer | $3.2x10^6$ | $2.6x10^6$ | $10^6$ | $2.4x10^6$ | $2.8x10^6$ | $7x10^4$ | $1.4x10^6$ | $1.8x10^6$ | $9x10^4$ |
| P12 | Lake sediment N 1, the depth is of 5 cm | $4.8x10^6$ | $3.2x10^6$ | $2x10^6$ | $7x10^6$ | $5x10^6$ | 0 | $3.4x10^6$ | $4.2x10^6$ | $2.6x10^6$ |

**TABLE 2. Average counts of microbes on media with and without added mercuric chloride. Standard deviations are shown in parentheses.**

| Sample #, location | R2A (no added Hg) (CFU/g of soil) | YM (no added Hg) (CFU/g of soil) | YM and 0.05mM $HgCl_2$ (CFU/g of soil) |
|---|---|---|---|
| U2, near electrolysis workshop | $2.07(\pm0.17)x10^7$ | $1.30(\pm0.22)x10^7$ | $3.70 (\pm1.59)x10^6$ |
| U8, lake shore | $1.22(\pm0.14)x10^7$ | $5.23(\pm2.87)x10^6$ | $2.83(\pm0.35)x10^6$ |
| U12, lake shore | $1.56(\pm0.43)x10^7$ | $7.53(\pm0.45)x10^6$ | $2.60(\pm0.35)x10^6$ |
| U16, lake shore | $2.95(\pm0.1)x10^7$ | $2.68(\pm0.25)x10^7$ | $4.30(\pm0.17)x10^6$ |
| U17, lake shore | $8.20(\pm0.7)x10^6$ | $7.20(\pm0.46)x10^6$ | $1.23(\pm0.35)x10^6$ |
| U18, lake shore | $7.77(\pm1.42)x10^6$ | $4.73(\pm1.27)x10^6$ | $2.67(\pm0.58)x10^5$ |

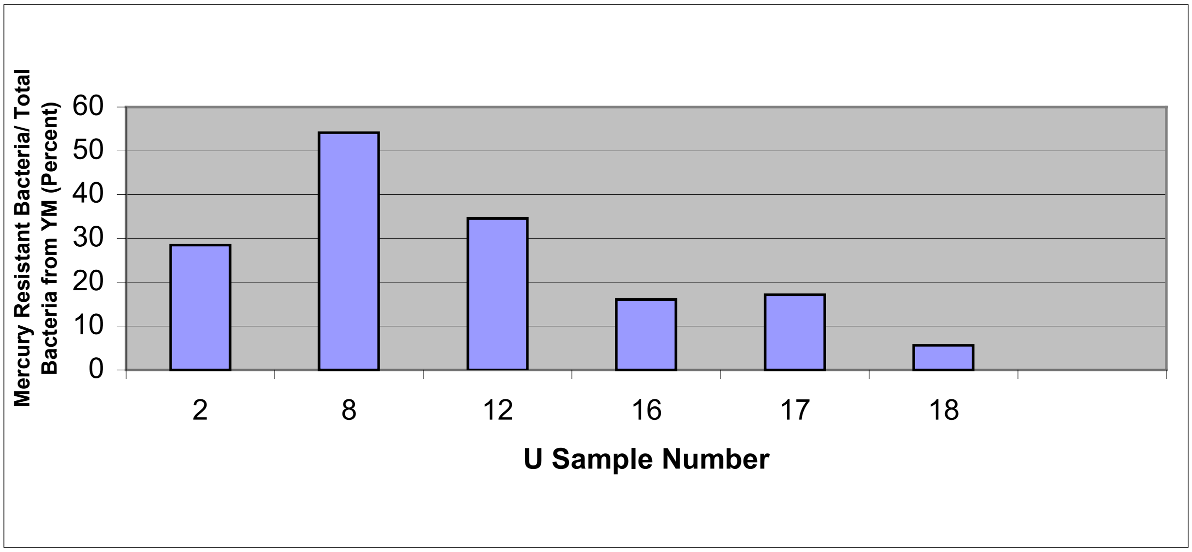


**FIGURE 2: Mercury resistant bacteria represented as percentage of Total bacteria recovered on YM not supplemented with mercury**

groundwater coming from the plant. Early waste disposal practices, and high concentrations of mercury in waste treatment ponds, resulted in contamination of Balkyldak Lake. Deposition of atmospherically transported mercury has caused mercury contamination of the surrounding countryside. Microbiological treatments are being considered as cost effective approaches to help mitigate the mercury contamination. Cultivation of soil and sediment bacteria from mercury contaminated sites in and around the chemical plant showed that high numbers of microorganisms can be recovered and these organisms from the site will work most efficiently at the site. Most of the cultures were resistant to high concentrations of mercury. Pure cultures of many microbes resistant to mercury have been isolated and preserved. Research will continue with the cultures isolated to determine whether they will be useful for remediation of the mercury contaminated soils and sediments.

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