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ENVIRONMENTAL SCIENCES DIVISION
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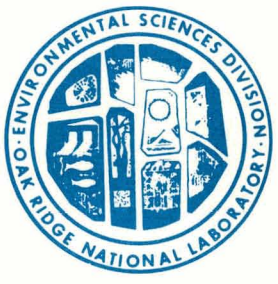

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DISTRIBUTION OF MERCURY IN THE ENVIRONMENT AT ALMADÉN, SPAIN[1]

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S. A. Janzen, J. A. Solomon, and K. D. Kumar

ENVIRONMENTAL SCIENCES DIVISION
Publication No. 1570

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ABSTRACT

HILDEBRAND, S. G., J. W. HUCKABEE, F. S. DIAZ, S. A. JANZEN, J. A. SOLOMON, and K. D. KUMAR. 1980. Distribution of mercury in the environment at Almadén, Spain. ORNL/TM-7446. Oak Ridge National Laboratory, Oak Ridge, Tennesee. 98 pp.

An ecological survey of the concentration and distribution of mercury in terrestrial and aquatic systems near the mercury mine at Almadén, Spain, was initiated in 1974. Field studies were completed in 1977, and chemical analyses were completed in 1979. This research was a joint effort of the Consejo de las Minas de Almadén, la Direccion General de Sanidad (Spain) and the U.S. Environmental Protection Agency, and Oak Ridge National Laboratory (USA). Sample collection at Almadén followed a trophic-level approach in which certain compartments were sampled at a given instant in time (fall 1974, fall 1975, spring 1976, fall 1976, spring 1977). The majority of total mercury analyses of field samples was performed by the Minas de Almadén. Methylated mercury analyses were performed by Oak Ridge National Laboratory.

Mean total mercury concentration in terrestrial plants (8 taxa combined) ranged from > 100 μg/g within 0.5 km of the mine to 1 μg/g 20 km distant from the mine. Different plant species had different affinities for mercury, but moss species usually had higher total mercury concentration than vascular plants. Woody plants were lower in mercury concentration than forbs. Total mercury concentration in muscle, brain, kidney, and liver tissue from mice was highest at a station near the stream receiving liquid effluent from the mine (mean total mercury at this station ranging from 0.18 μg/g in muscle to 4.74 μg/g in kidney). Approximately 15 to 30% of total mercury in

mouse tissue was in the methylated form. Total mercury concentration in muscle tissue from house sparrows varied inversely with distance from the mine, with highest concentrations exceeding 0.1 µg/g. Approximately 1 to 4% of total mercury in sparrow muscle was in the methylated form.

Total mercury concentration in fish muscle tissue decreased with distance downstream from the mine. Mean total mercury concentration on a given date ranged from 2.4 µg/g in barbo nearest the source to approximately 0.3 µg/g (boga) at control stations. The mean percentage methylmercury for all fish analyzed was 82%. Total mercury concentration in water and sediment was highest near the mine (> 1000 µg/g in sediment, > 300 µg/liter in water), then decreased downstream. Limited information for benthic invertebrates indicates that mercury concentration in these taxa follows a pattern similar to that observed for fish, water, and sediment. A maximum of 50% of the variance in total mercury concentration in fish muscle was explained by distance from the source and sampling date.

The results of this ecological survey confirm that both aquatic and terrestrial species in the vicinity of the mercury mine at Almadén contain elevated levels of mercury. Mercury concentration in both plant and animal tissue generally decreased with distance from the mine. The level of mercury in fish, if consumed, could be a significant source of mercury to local inhabitants. Our estimates indicate that exposure concentrations > 1.0 µg/g are possible 29 km downstream from the mine liquid effluent. The level of mercury in asparagus plants, if consumed, could also be a source of mercury to local inhabitants (exposure conditions near 1 µg/g).

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1. INTRODUCTION

The mercury deposits at Almaden are one of the most remarkable mineral occurrences on earth. Exploitation of the ore - cinnabar and quicksilver - began with the Carthaginians at least two centuries before the Christian era, was expanded by the Romans, and was continued by the Moorish caliphs and the Spaniards until the present. At least 2.8×10^8 kg of mercury have been taken from the mine, but the ore body, a vertical bed of quartzite in the flank of a great plunging syncline, still shows no indication of exhaustion.

The Almadén mining operation generates the oldest and possibly the most extensive case of mercury effluent to the land and air in the world. The flora and fauna of the region are exposed to elevated levels of environmental mercury derived both from rock weathering and from the mining/smelting processes. Effluents are dispersed to both terrestrial and aquatic ecosystems, but neither the extent nor the effect of this dispersal is known.

Although mankind has been aware of the unique and peculiar properties of mercury for millennia, concern about the metal's environmental effects is a comparatively recent phenomenon. It is only two decades since the first manifestations of the Minamata tragedy in which over fifty Japanese died of eating fish contaminated with mercury released to Minamata Bay from an acetaldehyde factory. This disastrous occurrence, along with the similar events at Niigata (Japan), the decrease in Swedish bird populations because of bioaccumulation of mercury derived from agricultural and industrial sources, and

accidental poisonings from the misuse of mercury biocides in the United States and Canada, has now become litany for environmentalists.

All these events were found to be preventable, and, with proper vigilance, it is unlikely that similar cases will occur on such scales again. Overt effects of the noxious element are still present in some instances, however, serving to warn us against the misuse of mercurials and of their environmental persistence.

Mercury is one of the rarer elements in the earth's crust, but it is so widely disseminated by natural processes, that it can be found in practically everything, including the tissues of biota. Indeed, mercury in trace quantities has been detected in virtually all organisms in which it has been sought, indicating that, in spite of the low solubility of naturally occurring HgS, mercury is mobilized and absorbed by plants and animals whether it is derived from natural or cultural sources.

There are four main reasons why a study of mercury cycling and transport in the environment at Almadén was a unique opportunity: (1) the release is continuous and of long term, meaning that cycling processes would tend to be at steady state; (2) the effluents are from a virtual point source, at least on a regional basis, meaning that transport gradients and rates are easier to measure; (3) there is apparently no other significant source of mercury within a radius of hundreds of kilometers; and (4) the region is semi-arid so that nonvaporous cycling processes are not accelerated through excessive leaching.

The most obvious, and probably the most important, mercury source at Almadén is the 30-m stack from which mercury vapor and sulphur dioxide generated in the ore-roasting ovens are released. Another source of mercury vapor is the forced ventilation of the mine. Air is drawn through the shafts and galleries by large fans and is dissipated to the atmosphere through a stack quite near the main shaft. There are other discharges of mercury vapor to the atmosphere, such as that from the flasking operation, but they are minor compared to those from ore-roasting and mine ventilation. Particulate matter (such as road dust) containing mercury is distributed by wind and vehicular activity to unknown distances from the mine.

The liquid effluent from mine and smelter is little more than a trickle, but it is nearly constant and contains very high concentrations of mercury, as evidenced by drops of metallic mercury accumulating in the discharge channels. Before 1975, the effluent from the smelter, flowed into trapezoidal sedimentation ponds that collect most of the elemental mercury and mercury-containing particulates. Overflow was continuous and was released into a small stream called Arroyo Azogado, which runs for 7 km until it joins a larger river, the Rio Valdeazogues. In 1975, a water treatment plant was installed to reduce aqueous mercury releases. The river has large fish populations, some of which are used eventually for food by the local residents.

The Arroyo Azogado also receives an unknown quantity of dissolved mercury leached out of the mine tailings by rain and groundwater. The average annual rainfall at Almaden is about 50 cm, but it falls mainly during January and February. This means that mercury contributions to

the aquatic environment from the tailings may vary widely, reaching maximum levels following the seasonal rains.

The ecological survey discussed in this report was conducted in both terrestrial and aquatic systems in the vicinity of the mine at Almadén, with the objectives of defining the range of mercury concentrations in certain ecosystem components and determining the distribution of mercury in these compartments with distance from the mining area.

This ecological study of the distribution of mercury in the environment in the vicinity of the mercury mine at Almadén, Spain, was initiated in the fall of 1974. The research was funded by the National Science Foundation Office of International Programs, in accordance with agreements for scientific collaboration between the United States and Spain. This study is a joint effort of the Consejo de las Minas de Almadén, la Direccion General de Sanidad, the United States Environmental Protection Agency (EPA) Environmental Sciences Research Laboratory, and the Environmental Sciences Division of the Oak Ridge National Laboratory (ORNL).

Francisco Sanz Diaz was the co-scientific investigator with the Minas de Almadén, responsible for assistance in all aspects of field sample collection and all analytical work done in Spain. John W. Huckabee and Stephen G. Hildebrand were co-scientific investigators on the project at Oak Ridge National Laboratory (ORNL), responsible for ORNL activities in terrestrial and aquatic systems, respectively. Deva Kumar and Jean Solomon of ORNL assisted in the statistical analysis of the data.

This report will place emphasis on the levels of mercury in the environment at Almadén and the distribution of mercury with distance from the mine.

Section 2 of this report is a discussion of the field and analytical methods employed. Section 3 discusses results of the terrestrial portion of the study, and section 4 includes results of the aquatic portion of the study. Section 5 presents a concise summary of major observations and conclusions.

2. METHODS

Sample collection at Almadén followed a trophic-level approach in which certain compartments were sampled at a given time. Major groupings sampled in the terrestrial environment included native plants, small mammals, and house sparrows. Major groups sampled in the aquatic environment were water, sediment, fish, and benthic invertebrates. The majority of chemical analyses for total mercury concentration in field samples were performed by the Laboratorio de Minas de Almadén. All determinations of methylmercury concentration were performed by ORNL. In addition, ORNL performed total mercury analyses on a portion of the samples analyzed at the mine as a cross-check on analytical techniques.

2.1 COLLECTION METHODS FOR TERRESTRIAL SPECIES

The terrestrial sampling stations at Almadén are within a radius of 25 km from the mine/smelter complex (Fig. 1 and Table 1). They were located by field examination, as nearly equidistant from the mine as possible along north-south and east-west transects. The distances and directions vary with the local differences in topography, geology, cultural activities, and ecology. However, the stations were generally about 2, 5 and 20 km from the mine. There was no station 2 km east, nor at 2 km south because of range fires two years in a row. Thus, there were ten terrestrial stations at which the following samples were collected for mercury analysis: plants that include Quercus sp., Asparagus acutifolius, Centaurea calcitrapa, Centaurea sp., Avena fatua, and Retama sphaerocarpa. Composite samples of moss species were

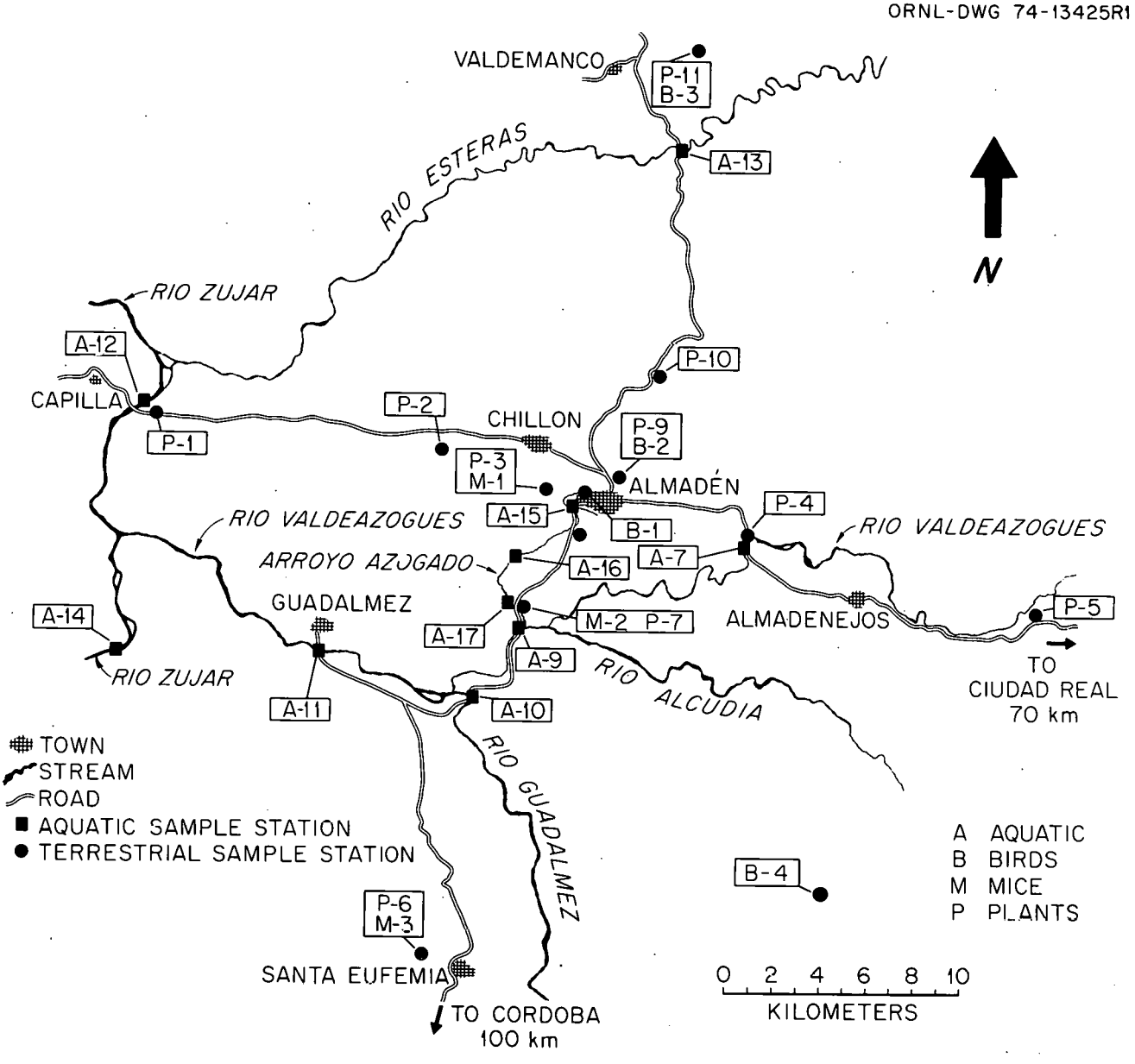

Fig. 1. Map of the Almadén area showing approximate locations of aquatic and terrestrial sampling stations.

Table 1. Description of sampling stations where terrestrial plants were collected. See Fig. 1 for approximate location of plant stations (designated by P-X) 1-7, 9-11.

Station number	Distance (km) and direction from mine
1[a]	20.0 W
2	5.0 W
3	1.0 W
4	5.0 E
5	20.0 E
6	20.0 S
7	5.0 S
9	2.0 N
10	5.0 N
11	20.0 N
12[b]	3.0 W
13[b]	1.0 N
14[b]	0.5 N
15[b]	8.0 SE
16[b]	1.5 NE
17[b]	2.0 ENE
18[b]	2.0 NE
19[b]	2.0 W

[a]Moss species were not collected.

[b]Only moss species were collected.

collected at 9 of 10 plant stations. All moss species were mat-forming types. Because mosses have been shown to be reliable indicators of airborne mercury contamination, composite samples of several species were collected at eight additional sites, making a total of 17 moss stations.

House sparrows (Passer domesticus), abundant in the Almadén area, were collected independently of the established stations because of unique characteristics of the species. House sparrows were collected at the mine, the chalets 1.6 km from the mine, and a control site 25 km from the mine.

Rodents were collected in snap traps at three locations: 1, 5, and 25 km from the mine. The most frequently caught species was Apodemus sylvaticus (wood mouse), with a few Mus musculus (house mouse) and shrews occasionally appearing.

At each of the terrestrial plant sampling stations, a 200-m-diameter circular plot was established by locating a permanent feature (easily identified rock or tree) as the center. An imaginary circle describing the plot was drawn and divided into square subplots 20 m on a side. These subplots (only those falling entirely within the circle were used) were assigned consecutive numbers. When plants were sampled, a random-numbers' list was used to select the subplots in which the samples were collected. Three samples of each species were taken until 12 samples of each species - four subplots - were obtained. If less than three samples could be found in each subplot, the random number selection process continued until the collection was complete. A surveyor's chain and compass were used for locating the

subplots. The plants were placed in plastic bags and transported to the laboratory. Green (fresh) samples were frozen, and dried samples were stored on the shelves.

The three stations at which small mammals were collected coincided with a plant sampling station. In each station, Victor snap traps were arranged in transects, with a trap about every 5 m. The traps were baited with peanut butter in the afternoon and checked the following morning. Twenty samples of each species were sought at each station. The animals caught were transported to the laboratory, weighed, sexed, and frozen.

The sparrows had to be netted where they occurred, which in no case coincided with the other terrestrial stations. Birds were caught in mist nets or were shot. The nets were erected at favorable locations and birds were removed at intervals each day until 10 had been taken at each of the two stations where netting was feasible. At the third station they were shot-gunned with fine pellets. If pellets penetrated the tissues to be analyzed for Hg, the sample was discarded.

Preliminary sampling of the terrestrial environment was conducted in the fall of 1974. The results of this sampling, and mercury analyses of these samples performed at ORNL, were used to design the terrestrial study. The terrestrial system components were sampled again in fall 1975, spring 1976, fall 1976, and spring 1977.

2.2 COLLECTION METHODS FOR AQUATIC COMPARTMENTS

The liquid effluent from the mine enters the Arroyo Azogado (Fig. 1) and flows approximately 7 km before joining the Rio

Valdeazogues, the main study stream. Three temporary stations (A-15, A-16, A-17) were established on the Arroyo Azogado for limited sampling of water and sediment. Three stations (A-7, A-9, A-11) were established on the Rio Valdeazogues, with A-7 being 12 km above the confluence with the Arroyo Azogado, and A-9 and A-11 being 0.3 km and 11 km below the confluence with the Arroyo Azogado, respectively. Station A-12 was established on the Rio Zugar downstream from the confluence of the Rio Valdeazogues, a total distance of 29 km below the Arroyo Azogado. Station A-14 was a temporary station established on the Rio Zujar above the confluence with the Rio Valdeazogues for limited water and sediment sampling. Station A-10 was established on the Rio Guadalmez, a tributary to the Rio Valdeazogues not directly influenced by the liquid effluent from the mine. Station A-13 was established on the Rio Esteras, a tributary to the Rio Zujar not directly influenced by the liquid effluent from the mine.

We attempted to collect three fish species at each station sampled; the barbo (Barbus spp.), the cacho (Leuciscus cephalus), and the boga (Chondrostoma polylepus). Limited numbers of largemouth bass (Micropterus salmoides) were collected where present. The majority of fish were collected by electrofishing with a battery-operated back-pack device (Dirigo Model 500). Beach seines were used to supplement electrofishing where necessary. Fish species were collected at stations A-7, A-9, A-10, A-11, A-12 (fall 1974, fall 1975, spring 1976, fall 1976, spring 1977). Fish were collected at station A-13 in spring 1976, fall 1976, and spring 1977. All fish samples were frozen for later chemical analysis.

Benthic invertebrates were collected at stations A-9, A-12, and A-13 in spring 1977. Qualitative samples of benthic invertebrates were obtained by physically disrupting the substrate upstream from a collecting screen ("kick sampling"). The invertebrate samples were frozen upon collection and returned to ORNL for both total and methylmercury analysis.

Water samples were collected at stations A-7, A-9, A-10, A-11, A-12, A-13, A-15, A-16, A-17 in fall 1975, fall 1976, and spring 1977. Water was sampled at station A-14 in spring 1977. Water samples were collected in the field by filling a previously prepared 100-ml volumetric flask (see Section 2.3). Water samples were not filtered prior to analysis, so water concentrations represent total mercury (dissolved plus particulate) in the water column.

Sediment samples were collected at stations A-7, A-9, A-10, A-11, and A-13 in spring 1976, fall 1976, and spring 1977. Sediment samples were collected at station A-12 in spring 1977 only. Sediment samples were collected by hand, placed directly into plastic bags, and frozen within 2 h of collection. We attempted to collect sediment from depositing areas at all stations. The sediment samples were thawed prior to analysis, dried at 35°C, and sieved through 104-μm mesh. The fraction smaller than 104-μm was analyzed for total mercury (see Section 2.3).

2.3 ANALYTICAL METHODS FOR TOTAL MERCURY ANALYSIS, MINAS de ALMADÉN

All analyses completed in Spain were performed in the Laboratório, Minas de Almadén, which is responsible for production control,

geochemistry, clinical analyses, and pollution control activities at the mine.

2.3.1 Apparatus

The equipment used for total mercury analyses in Spain is depicted schematically in Fig. 2. The major analytical system components are the following:

(1) Cold Vapor Flameless Atomic Absorption Spectrophotometer, 254-nm, 300-mm double cell, LDC mercury monitor (Laboratory Data Control).

(2) LDC Recorder, 1-100 mV sensitivity, with speeds of 23.54 cm/h to 20.3 cm/min (Laboratory Data Control).

(3) Digital Voltmeter, 0 to 199.9 mV sensitivity (Digitec Model 261C, United Systems Corporation).

(4) Flowmeter (Fisher and Porter, Lab Crest Model 448-225), 100 to 1800 cm^3/min.

(5) 100-ml heart-shaped aeration vessels.

(6) 1500-liter/h aspiration pump (Alver).

(7) Thermolyne Model 9425 hot plates.

(8) Surface thermometers, 10 to 400°C (PIC Instruments).

(9) 250-ml volumetric flasks, borosilicate glass, flat bottom (Kimax).

(10) Special condensers ("Feldman Chimneys") as described in Feldman (1974).

(11) Furnace (Thermolyne Model F-6020).

(12) Imperial II Radiont Heat Oven (Lab Line Instruments Inc.).

(13) Mettler Model PL 200 balance.

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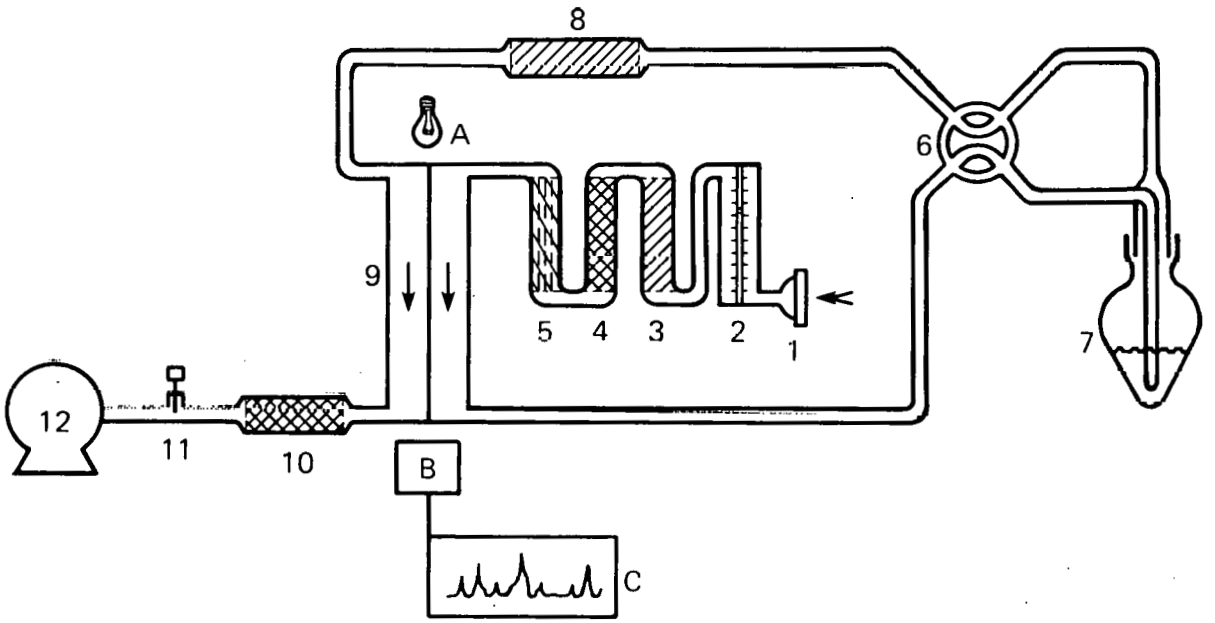

1. DUST FILTER
2. FLOWMETER
3. & 8. $Mg(ClO_4)_2 \cdot H_2O$ DESICCANT
4. & 10. HOPCALITE
5. SILVER WOOL
6. FOUR-WAY VALVE
7. HEART-SHAPED AERATION VESSEL
9. 300-mm DOUBLE CELL
11. FLOW-LIMITER VALVE
12. PUMP
A. ULTRAVIOLET MERCURY LAMP
B. 254 - nm PHOTODETECTOR
C. RECORDER

Fig. 2. Schematic representation of equipment used for total mercury analyses at the Minas de Almadén.

All chemical analyses were performed in a "clean room" which included a forced-air stream, previously filtered through Hopcalyte, and a double entry door to avoid contamination.

Reagents utilized in total mercury analyses included: mercury free 65% HNO_3 (less than 5 μg/liter, Merck Catalogue No. 452), mercury free 65% $HClO_4$ (less than 5 μg/liter, Merck Catalogue No. 514), $K_2Cr_2O_7$ (Mallinchrodt Catalogue No. 6770), $Mg(ClO_4)_2 \cdot XH_2O$ (Merck Catalogue No. 5874), Hopcalyte (MSA Catalogue No. 26599), micro silver wool (Fisher Catalogue No. 737148), $HgCl_2$ (Analar Catalogue No. 5552), and double-distilled water.

2.3.2 Sample Preparation

All field samples of fish, birds, and rodents were placed in a freezer within a few hours after collection, and all sample preparation was conducted in the "clean room" to avoid external contamination. Samples were then thawed for preparation for wet-ashing. Five grams or less of fish axial muscle from above the lateral line and below the dorsal fin was removed for analysis (all skin removed). Pectoral muscle, brain, and kidney tissues were obtained from all bird samples. Skeletal muscle from one foreleg and one hindleg, brain, and liver tissue were removed from rodent samples for analysis.

Green plant samples were also frozen soon after collection. After thawing, individual plants were separated from the sample. Where appropriate, each individual plant was segregated into samples of leaves, stems, and fruits. The different parts of each plant were placed in individual beakers and rinsed several times with distilled

water to remove external contamination (shake beaker covered with watch glass). After rinsing, the individual beakers and contents were dried overnight in an oven at 35°C prior to wet-ashing.

Water samples were collected in the field with 100-ml volumetric flasks containing 15 ml of HNO_3 and 20 mg $K_2Cr_2O_7$. Water was collected to fill the flask to the 100-ml mark. In the laboratory, each water sample was transferred to a 250-ml flask and $HClO_4$ added for processing.

Samples of river sediment were dried at 35°C, then sieved to separate the <105-μm size fraction.

2.3.3 Chemical Analysis

All samples of animal and plant tissue were wet-ashed using the procedure of Feldman (1974). Sample weight was obtained by determining the difference between the empty flask and the flask containing the sample. Digestion products were diluted to 50 ml, and aliquots were analyzed in the atomic absorption (AA) system (Fig. 2). Readings were compared with the straight-line calibration obtained with different amounts of 20 ng Hg^{2+}/ml standard solution prepared in the same manner as the unknown sample.

All the glassware used in mercury analysis was cleaned utilizing the following procedure:

(1) rinse with tap water and detergent,

(2) several rinses with tap water,

(3) several rinses with distilled water,

(4) rinse with 10% HNO_3,

(5) several rinses with distilled water,

(6) bake in oven at 450°C overnight, and

(7) cover with parafilm until next use.

2.3.4 Quality Control

Quality control of total mercury analyses performed at the Laboratory Minas de Almadén was achieved in two ways. National Bureau of Standards (NBS) bovine liver (NBS Standard Reference Material 1577), orchard leaves (NBS Standard Reference Material 1571), and water (NBS Standard Reference Material 1642a) were used to check the procedure and standardization. In addition, approximately 10% of all samples were separated into two parts, one part to be analyzed by Minas de Almadén and one part by ORNL. These "check" samples were then analyzed for comparability.

2.4 ANALYTICAL METHODS FOR TOTAL MERCURY AND METHYLMERCURY CONDUCTED AT ORNL

Approximately 10% of all animal and plant samples collected were returned to ORNL at the conclusion of each sampling sequence (shipped frozen on dry ice). These samples were primarily "check" samples described in Section 2.3, but also included samples to be analyzed for methylated mercury. The samples analyzed for total mercury at ORNL were processed and analyzed in a manner similar to that described in Section 2.3 and in Smith (1957) and Feldman (1974).

Samples analyzed for methylated mercury followed the procedure of Talmi (1975). The analytical detection system for organomercurials consisted of a gas chromatograph (g.c.) equipped with a

microwave-emission spectrometric detector. The microwave-emission detector sensitivity is generally independent of the molecular structure of the mercurial analyzed, in contrast to the widely used electron capture detector. Thus, the detectability of the system for either CH_3HgCl or $(CH_3)2Hg$ is at the 3- to 8-pg range.

The procedure for analysis of methylated mercury in plant and animal tissue consisted of the following general steps:

(1) Homogenize the tissue and weigh 0.5 to 1.0 g into a centrifuge tube.

(2) Add 1 ml of concentrated hydrochloric acid and 2 ml of water to the sample.

(3) Mix for 3 min.

(4) Add 3 to 5 ml of benzene to the centrifuge tube, centrifuge to separate phases.

(5) Inject 1 to 20 ml of the dried benzene extract (over anhydrous sodium sulfate) into the gas chromatograph column.

Methylated mercury concentrations were obtained by monitoring the emission intensity at the 253.7-nm Hg spectral line, and comparing it to a standard curve resulting from chromatography of a pure standard. Because it was found that the extraction efficiency is in the 75 to 90% range, the extraction procedure was repeated.

The excellent selectivity provided by the g.c. microwave-emission system eliminates the need for the tedious and time-consuming cleanup procedures of the organic extract. Also, the extracts can be injected at the rate of 30 to 50/h compared to 1 to 2/h with conventional systems. Typical accuracy values at 10 to 40 μg/liter CH_3HgCl in

fish were 4 to 12%; reproducibility, expressed as relative standard deviation, was 3 to 10%. A detailed description of analytical techniques used for aquatic samples is given in Hildebrand et al. (1980).

3. RESULTS AND DISCUSSION OF THE TERRESTRIAL SURVEY

3.1 CONCENTRATION OF MERCURY IN PLANTS AND DISTRIBUTION IN THE ENVIRONMENT AT ALMADEN

The vegetation of the Almadén area strongly reflects the semiarid climate: in spring, the forbs (nonwoody plants) are green and growing; in the fall, they are completely dry and dead. Although Asparagus is woody and evergreen, this species also shows succulent new growth in April and May, but only stiff and nonpliant needles and stems by September. Of the plant species collected, only oak (Quercus sp.) and Retama showed little or no obvious external differences in leaves (oak) or stems (Retama has no leaves) between spring and fall. Moss responds to rain, which was more prevalent in spring, but remains green at all seasons.

Vascular plants may accumulate mercury by two routes of uptake: through the roots from the soil (ionic) or through the stomates from the atmosphere (Hg°) (Lindberg et al. 1979). Moss, on the other hand, accumulates most of its mercury content from the atmosphere, retaining particulate mercury ("dry fall") and ionic mercury (in rain) but not Hg° (Huckabee 1973, Huckabee and Janzen 1975).

It was expected that all plants nearer the mine/smelter would have higher ΣHg concentrations than plants more distant from the mine/smelter, and that moss would usually have higher ΣHg concentrations than the vascular plants in the same area. A further distinction must be made in comparing the plant ΣHg data. The forbs that senesce or die will not accumulate, and may not retain, mercury at the same rates all year. The plants that continue to metabolize all

year would tend to accumulate and retain ΣHg at the same rates all year. The moss responds to rainfall whenever it occurs, and greatest uptake would follow a rain event preceded by an extended dry period.

The plant ΣHg data were examined by station, by season, and by species to determine if plant mercury content was a function of distance from the mine/smelter, and if there were differences in mercury concentration between species. Table 1 shows the distance from the mine/smelter of each plant station.

3.1.1 Statistical Analysis

Individual analytical determinations (2483) of total mercury concentration were obtained from the various tissues of seven plant species over the study period (Table 2). For all statistical analyses that follow, estimates of mercury concentration were transformed (natural log) to stabilize the variance. Consequently, all means reported for plants are geometric means.

Station comparison of mercury concentration in plants (all species included) was accomplished (Table 3) using the Duncan's multiple range test, with the mean square error and degrees of freedom from a nested analysis of variance (with types nested within dates, dates nested within species, and species nested within stations).

The comparison of mercury concentration between species (all stations included) was done in a similar manner (Table 4). A nested analysis of variance was performed (types within dates within stations) to determine the degrees of freedom and the mean square error. The Duncan's multiple range test was used to rank the means.

Table 2. Number of samples of plant species analyzed for total mercury concentration over the study period (individual tissue samples lumped when analyzed). One sample of moss was analyzed from each station and on each date where available (total number analyzed = 75).

Station	Fall 1974	Fall 1975	Spring 1976	Fall 1976	Spring 1977
			Avena fatua		
1	1	1	12		12
2			12		12
3	1	2	12		12
4		2	12		12
5		2	12		12
6	1	2	12		12
7		2	12		12
9	1		12		12
10	1	2	12		12
11			12		12
			Centaurea calcitrapa		
1		2	12		12
2	3		13		13
3	3		12		12
4	3		8		12
5			12		11
6			12		12
7	3		12		12
9		4	12		12
10			9		12
11					
			Asparagus acutifolius		
1	1	9	4	8	2
2	1	11	13	12	12
3	1	14	12	12	12
4	1	12	17	12	12
5			13	12	12
6		10	12	12	12
7					13
9	1	12	17	12	12
10	1	10	8	12	
11					

Table 2. (continued)

Station	Fall 1974	Fall 1975	Spring 1976	Fall 1976	Spring 1977
			Centaurea sp.		
1			12		13
2	3		12		12
3			12		12
4			12		12
5					
6		4	12		12
7			12		
9		2	12		12
10		4	12		12
11			12		12
			Quercus sp.		
1	1	40	29	28	24
2	1	34	19	24	24
3	1	30	24	28	24
4	1	32	24	26	24
5		30	24	28	24
6	1		24	24	22
7	1		24	24	24
9	1	21	24	28	24
10	1	30	24	24	24
11		27	24	34	24
			Retama sp.		
1		9	12	13	19
2		13	12	15	14
3				12	12
4		11	12	13	15
5		11	12	12	15
6		20	10	14	15
7		13	12	13	20
9					
10					
11					

Table 3. Station ranking in order of total mercury (ΣHg) concentration in plants, using all plant data (all species, all seasons)

Grouping[a]	N	Station	Geometric mean (μg/g)	Distance (km) from source
I	4	14[b]	108.85	0.5
I	2	18[b]	35.16	2.0
I	3	16[b]	32.79	1.5
I	4	13[b]	21.98	1.0
I	2	19[b]	19.11	2.0
I	4	17[b]	16.44	2.0
I	4	12[b]	15.49	3.0
I	4	15[b]	5.87	8.0
I	253	3[c]	2.77	1.0
I	227	9[c]	1.16	2.0
I I	194	7[c]	0.98	5.0
I I	217	10[c]	0.96	5.0
I	282	4[c]	0.90	5.0
I	281	2[c]	0.89	5.0
I I	250	6[c]	0.79	20.0
I I	244	5[c]	0.73	20.0
I	271	1[d]	0.69	20.0
I	161	11[c]	0.53	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

[b]Only moss collected at station.

[c]All plant species collected at station.

[d]All plant species except moss collected at station.

Table 4. Geometric mean of total mercury (ΣHg) concentration in each plant species from all stations at all seasons and from spring only

All seasons			Spring only		
Grouping[a]	Species	Mean (μg/g)	Grouping[a]	Species	Mean (μg/g)
I	Moss	10.73	I	Moss	6.05
I	*Centaurea* sp.	2.80	I	*Centaurea* sp.	2.79
II	*Avena fatua*	1.82	II	*Avena fatua*	1.82
II	*Centaurea calcitrapa*	1.69	II	*Centaurea calcitrapa*	1.69
II	*Asparagus acutifolius*	0.82	II	*Quercus* sp.	0.71
II	*Quercus* sp.	0.78	II	*Asparagus acutifolius*	0.69
II	*Retama sphaerocarpa*	0.38	II	*Retama sphaerocarpa*	0.32

[a]Species connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Individual analyses of variance to detect station differences were performed for each plant species (Tables 5-12). Due to the unbalanced nature of the sampling design, date was used as a blocking factor. Using the information from these analyses, the Duncan's multiple range test was used to rank the station means within each species. The following constraints were placed on this analysis of individual species:

(1) For Avena fatua, only observations for spring 1976 and spring 1977 were included.

(2) For Centaurea calcitrapa, only observations for spring 1976 and spring 1977 were used. No data were available for station 11.

(3) For Asparagus, stations 5 and 7 were eliminated from the analysis; all dates were included; and only whole plant tissue data were used.

(4) For Centaurea sp., stations 5 and 7 were eliminated from the analysis; only dates spring 1976 and spring 1977 were used. Only whole plant data were used.

(5) For moss, station 1 was excluded, all dates were used.

(6) For Quercus, separate analyses were performed for each tissue type. All dates were included for analysis of leaves and stems, but only dates fall 1974 and fall 1976 were used for the acorn and involucre analysis. Acorns and involucres are only available in the fall.

(7) For Retama sp., fall 1974 data and station 3 data were excluded.

To compare plant species within each station, one-way analyses of variance were performed separately for each station.

Table 5. Geometric mean values of total mercury (ΣHg) concentration in *Avena fatua* collected between 30 April and 8 May 1976, and between 4 and 16 May 1977. See text for explanation.

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
	10	2.673	5.0
	1	2.479	20.0
	6	2.330	20.0
	4	2.214	5.0
	3	2.063	1.0
	2	1.734	5.0
	5	1.592	20.0
	9	1.560	2.0
	7	1.463	5.0
	11	0.937	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 6. Geometric mean values of total mercury (ΣHg) concentration in *Centaurea calcitrapa* collected between 30 April and 8 May 1976 and between 4 and 6 May 1977. See text for explanation.

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
	6	2.137	20.0
	5	2.121	20.0
	10	2.121	5.0
	3	2.001	1.0
	9	1.781	2.0
	2	1.730	5.0
	4	1.411	5.0
	7	1.329	5.0
	1	1.142	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 7. Geometric mean values of total mercury (ΣHg) concentration in *Asparagus acutifolius* collected between 30 April and 8 May 1976 and 4 and 16 May 1977 near Almadén, Spain. See text for explanation.

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
I	3	1.88	1.0
II	9	0.88	2.0
II	10	0.76	5.0
II	4	0.62	5.0
II	1	0.62	20.0
II	2	0.52	5.0
III	6	0.49	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 8. Geometric mean values of total mercury (ΣHg) concentration in *Quercus* sp. leaves collected between 30 April and 8 May 1976 and 4 and 16 May 1977 near Almadén, Spain. See text for explanation.

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
I	3	4.20	1.0
II	9	1.43	2.0
III	2	0.69	5.0
III	10	0.62	5.0
III	5	0.61	20.0
III	7	0.59	5.0
III	1	0.56	20.0
III	4	0.55	5.0
III	11	0.39	20.0
III	6	0.30	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 9. Geometric mean values of total mercury (ΣHg) concentration in *Retama sphaerocarpa* collected between 30 April and 8 May 1976 and 4 and 16 May 1977 near Almadén, Spain

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
	4	0.36	5.0
	7	0.35	5.0
	2	0.31	5.0
	5	0.26	20.0
	1	0.25	20.0
	6	0.20	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 10. Geometric mean values of total mercury (ΣHg) concentration in *Centaurea* sp. collected between 30 April and 8 May 1976 and between 4 and 16 May 1977 near Almadén, Spain

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
	10	5.64	5.0
	4	5.41	5.0
	3	3.80	1.0
	1	2.65	20.0
	6	2.16	20.0
	11	2.05	20.0
	7	2.03	5.0
	9	1.45	2.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 11. Geometric mean values of total mercury (ΣHg) concentration in composite moss species collected near Almadén, Spain, during spring 1976-1977 and fall 1974, 1975, and 1976

Grouping[a]	Station	Mean (μg/g)	Distance from source (km)
	14	107.770	0.5
	18	35.165	2.0
	16	32.813	1.5
	3[b]	22.789	1.0
	13	22.031	1.0
	19	19.133	2.0
	17	16.399	2.0
	12	15.482	3.0
	9[b]	13.405	2.0
	7[b]	9.269	5.0
	4[b]	6.984	5.0
	15	5.864	8.0
	2[b]	5.100	5.0
	11[b]	4.019	20.0
	10[b]	3.717	5.0
	5[b]	3.290	20.0
	6[b]	2.544	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

[b]Stations at which other plants were collected.

Table 12. Total mercury (ΣHg) concentrations (μg/g) in *Quercus* sp. leaves, stems, involucres, and acorns collected in 1975, 1976, and 1977 near Almadén, Spain

Grouping[a]	N	Station	Geometric mean [Hg] (μg/g)	Distance from source (km)
			Leaves	
	47	3	4.99	1.0
	45	9	1.49	2.0
	35	7	1.40	5.0
	45	2	0.93	5.0
	46	4	0.72	5.0
	45	5	0.65	20.0
	46	10	0.61	5.0
	47	1	0.59	20.0
	35	6	0.52	20.0
	47	11	0.38	20.0
			Stems	
	47	3	4.71	1.0
	36	7	1.29	5.0
	44	9	1.25	2.0
	47	1	0.98	20.0
	45	5	0.92	20.0
	41	2	0.91	5.0
	35	6	0.90	20.0
	46	4	0.75	5.0
	46	10	0.55	5.0
	47	11	0.47	20.0
			Involucres	
	2	3	4.88	1.0
	4	4	2.33	5.0
	7	2	1.03	5.0
	11	1	0.80	20.0
	3	10	0.76	5.0
	6	5	0.47	20.0
	1	9	0.40	2.0
	4	11	0.27	5.0
			Acorns	
	10	3	0.37	1.0
	7	9	0.11	2.0
	7	10	0.09	5.0
	11	1	0.07	20.0
	10	4	0.07	5.0
	7	2	0.06	5.0
	10	5	0.05	20.0
	11	11	0.04	20.0

[a]Stations connected by the same vertical line are not significantly different from one another ($\alpha = 0.05$).

Table 3 shows the ranking of the stations when all plant data (all seven species and all four seasons plus the fall 1974 moss collection) are included. Stations 12-19 are only moss stations, while stations 1-11 are moss plus vascular plant stations. The groupings showing statistical difference indicate that proximity to the mine/smelter is related to ΣHg concentrations in plants, but distance is not a perfect indicator. A more accurate picture would be obtained when wind directions are considered. Unfortunately, no wind patterns (windrose) have been determined for the Almadén area.

Further confounding the issue is the mercury distribution downstream from the mine/smelter via the Arroyo Azogado. Soil and gravels along this stream, including those underlying station 7, have high concentrations of cinnabar.

Table 4 lists the ΣHg concentration in each plant species from all stations at all seasons and in spring only. Clearly, different plant species have different affinities for ΣHg. As indicated by the literature, moss accumulates ΣHg to a much greater extent than do vascular plants. It can be concluded from these data (Table 4) that herbaceous plants (grass and forbs) accumulate more ΣHg than do woody plants, even evergreens. Tables 5 through 12 show the results of the analyses for significant differences in ΣHg concentrations of each species at all stations collected in the two springs. These analyses show that any one plant species does not predict the level of mercury in other species at the same station. One would expect this to be the case if plants take up various forms of mercury at different rates and if different forms of mercury are prevalent at different stations.

Virtually no data exist on the differences in uptake coefficients of different forms of mercury in a given plant species.

Quercus sp. was sampled to test for ΣHg distribution in leaves, stems, acorns, and involucres. Table 12 shows the derived means for each tissue at each station. Clearly, there is no difference in ΣHg concentration in leaves, stems, and involucres. The acorns, which are used for pig food and are occasionally eaten by people, contain much less Hg than the other tissues. The involucres, which may be consumed by the pigs (but not by people), apparently trap Hg particles by virtue of their rugosity and surface roughness.

The young sprouts of Aspargus acutifolius are often consumed by humans. In April 1976, some of these sprouts were collected along with the adult plants and analyzed for ΣHg. Table 13 shows that the concentrations of ΣHg in the sprouts and in adults overlap, but at station 4, the six sprouts averaged over 1 μg/g.

Retama sphaerocarpa was also sampled for ΣHg distribution in different tissues. The whole plant, flowers, and seed pods were analyzed. The data are insufficient for conclusions, but the flowers seemed to have higher concentrations of ΣHg than the whole plant.

3.1.2 Discussion

The data reported here show that ΣHg concentration does, in general, vary directly with distance from a strong mercury source (Tables 3 and 11). Because wind rose data are not available for the Almadén site, this trend cannot be quantified in a directional sense. Our results also indicate that moss almost always accumulates ΣHg three to five times more than any vascular plant tested, that the R.

Table 13. Total mercury (ΣHg) (μg/g ± 1SU) in young and mature *Asparagus acutifolius* plants collected in April 1976 near Almaden, Spain

Station	Distance from source (km)	Young ΣHg		Mature ΣHg	
1	5.0	1.47	0.44	0.52	0.31
9	2.0	0.36	0.23	1.74	1.5

sphaerocarpa always had the least ΣHg concentration of all plants tested, and that woody plants averaged less mercury than forbs.

All ΣHg concentrations in plants measured during this study greatly exceed most other reported values. Normal or background levels of ΣHg in plants are in the range of 80 to 100 ng/g (Wallace et al. 1971). Lindberg et al. (1979) grew alfalfa on Almadén soils (from station 3 and near station 5) and found 2.3 ± 0.8 g/g and 1.4 ± 0.2 μg/g, respectively, in leaves and stems at these stations. These values compare very well with those for the forbs collected at those stations (Table 14). This may indicate that the forbs quickly reach equilibrium with the soil mercury burden. Shacklette (1970) reported ΣHg at 3.5 μg/g in a shrub near a cinnabar vein, but the analysis unfortunately was on a dry-weight basis and thus is not comparable to that in the recent literature. Byrne and Kosta (1970) reported ΣHg in herbaceous vegetation (elderberry, crocus, and coltsfoot) ranging between 1.1 and 0.04 μg/g (wet weight) and 0.51 to 0.08 μg/g (wet weight) in cherry wood from the Idrija, Yugoslavia, mercury mine/smelter area. The unwashed bark of the same cherry trees was up to 59 μg/g Hg (wet weight). The background ΣHg values they reported were 0.63 μg/g for bark and 0.002 μg/g for wood. These values correspond well with our data.

We apparently did not sample a background area near Almadén, because the lowest mean ΣHg concentrations we found are at least 10 times greater than the reported background values (vide supra). We therefore conclude that the circular area within 25 km radius from Almadén has elevated ΣHg, even though economic deposits are limited to

Table 14. Geometric mean total mercury (ΣHg) concentration (all seasons) for each plant species collected at each station near Almadén, Spain, 1974-1977. Solid lines above the concentration values connect values not significantly different from each other at the 5% level. All *Retama* values are distinct from all other species.

	Total mercury (ΣHg) concentration, geometric mean (μg/g)					
Station	Moss	*Centaurea* sp.	*Avena fatua*	*Centaurea calcitropa*	*Asparagus acutifolius*	*Quercus* sp.	*Retama sphaerocarpa*
1	a	2.65	2.48	1.14	0.62	0.56	0.25
2	5.10	2.03	1.73	1.73	0.52	0.69	0.31
3	22.79	3.80	2.06	2.00	1.88	4.20	a
4	6.98	5.41	2.21	1.41	0.62	0.55	0.36
5	3.29	a	1.59	2.12	a	0.61	0.26
6	2.54	2.16	2.33	2.14	0.49	0.30	0.20
7	9.27	a	1.46	1.33	a	0.59	0.35
9	13.41	1.45	1.56	1.78	0.88	1.43	a
10	3.72	5.64	2.67	2.12	0.76	0.62	a
11	4.02	2.05	0.94	a	a	0.39	a

[a]Not present.

the immediate environs of Almadén. Of course, undiscovered or undisclosed emplacements of Hg ore could be present throughout this region, or Hg° and Hg-containing particulates may be distributed from the ore body mine/smelter operation sufficiently to produce the elevated ΣHg levels in the biota we tested.

3.2 MERCURY CONCENTRATION IN MICE

The number of individual mouse (Apodemus sylvaticus) tissue samples analyzed for total mercury is given in Table 15. The statistical analysis described below was utilized to test for differences in mercury concentration in mice among the three stations sampled, and between the fall and the spring sampling periods. A natural logarithm transformation was employed to stabilize variance.

To compare stations, each tissue type was analyzed separately. For muscle tissue, a three-way analysis of variance was performed, with blocking on sex and date (all four dates included). Date was significant at the 0.0001 level, but sex was not significant ($\alpha = 0.05$).

Only data from the fall 1975 and spring 1977 collections were used in analyzing mercury concentration in the other three tissue types at the three stations. Again, a three-way analysis of variance was performed, blocking on sex and date. Sex never accounted for a significant ($\alpha = 0.05$) portion of the variance in mercury concentration in other tissues, and date was an important factor only in brain tissue.

A t-test of differences between two means was used to detect seasonal differences in muscle tissue.

Table 15. Number of *Apodemus sylvaticus* tissue samples analyzed for total mercury (ΣHg) (see Fig. 1 for station location)

Station	Tissue	Fall 1975	Spring 1976	Fall 1976	Spring 1977
1	Muscle	5	22	4	15
1	Liver	5	-	1	10
1	Brain	5	-	1	10
1	Kidney	5	-	1	10
2	Muscle	7	20	6	9
2	Liver	7	-	2	9
2	Brain	6	-	2	8
2	Kidney	7	-	2	9
3	Muscle	14	21	-	14
3	Liver	14	-	-	14
3	Brain	13	-	-	12
3	Kidney	14	-	-	14

Table 16. Geometric mean total mercury (ΣHg) concentration (μg/g) in *Apodemus sylvaticus* muscle, liver, kidney, and brain tissue in animals caught near Almadén, Spain, in 1975-1977

Grouping[a]	Station	Muscle	Liver	Kidney	Brain
I	1	0.066	0.200	1.62	0.089
I	2	0.181	0.558	4.74	0.217
I	3	0.017	0.019	0.086	0.033

[a]Stations connected by the same vertical line are not significantly different from each other ($\alpha = 0.05$).

Table 16 shows the mean Hg in A. sylvaticus muscle, brain, kidney, and liver tissue. The three stations were distinctly different (5% level) with mice from station 2 having the highest concentration of ΣHg in all tissue. Station 2 is adjacent to the Arroyo Azogado, the small stream that receives the liquid effluent from the mine. There is too great a range of concentrations to distinguish any seasonal differences in liver, kidney, and brain. No differences in Hg concentration attributable to sex were detected.

Seasonal differences in ΣHg and MeHg concentration were discernible only in muscle tissue (all stations combined). Table 17 shows that ΣHg was higher in muscle tissue in the fall, and that the MeHg concentration averaged up to 29% of ΣHg.

The food habits of A. sylvaticus in the Almadén area are insufficiently known to make conclusions about the relationship of Hg in plants and A. sylvaticus taken at the same locations.

Bull et al. (1977) reported ΣHg in fescue grass (F. rubra) and ΣHg and MeHg in two species of rodents (Cleithroenomys glareolus and Apodemus sylvaticus) from an uncontaminated area in Great Britain. They measured 103 ± 8 ng/g (1 SE) ΣHg in fescue, reported as dry weight. This value expressed as wet weight is approximately 26 ng/g ΣHg. The lowest average ΣHg concentration we measured in grass (Avena) was 940 ng/g (Table 5).

In mouse skeletal muscle, Bull et al. (1977) found a ΣHg concentration of 60 ng/g ± 10 (1 SE) and 70 ng/g (1 SE not significant) in C. glareolus and A. sylvaticus, respectively. In liver, ΣHg was 60 ng/g ± 20 (1 SE) and 40 ng/g ± 10 1 (SE) in C. glareolus

Table 17. Geometric mean total mercury (ΣHg) and methylmercury (MeHg) concentration (μg/g) in muscle tissue of *Apodemus sylvaticus* (combined data for all stations) from near Almadén, Spain, in 1975-1977

Grouping[a]	Date	ΣHg	MeHg	% MeHg
I	Fall	0.161	0.046	29
I	Spring	0.078	0.011	14

[a]Seasons connected by the same vertical line are not significantly different from each other ($\alpha = 0.05$).

and A. sylvaticus, respectively. At station one in our study (Table 16), A. sylvaticus had muscle and liver ΣHg concentrations of 66 and 200 ng/g, respectively; at Station 3, the comparable values were 17 and 19 ng/g. Liver concentrations of ΣHg increase faster than muscle concentrations in rodents following high experimental doses and then decrease faster following peak levels (Norseth and Clarkson 1970). This implies that at low ingestion rates of ΣHg, muscle concentrations would exceed liver concentrations. This was indeed documented in the present study (Table 16) and by Bull et al. (1977).

> These results [(compared with those of Bull et al. (1977)] indicate that plants are better indicators of the presence of ΣHg than are rodents, inasmuch as the plants, but not the A. sylvaticus, showed Hg concentrations above putative background levels.

Little information on the natural occurrence of MeHg in the terrestrial environment is available. It has been shown that inorganic mercury is methylated in soil and plants (Rogers 1976, Fortmann et al. 1977). Bull et al. (1977) showed that MeHg occurs in feral rodents as well; they reported MeHg values for both A. sylvaticus and C. glareolus as 2.9 ng/g in muscle and 6.9 ng/g in liver.

Our data are insufficient for comparisons beyond those shown in Table 17. Clearly, MeHg is present in significant quantities in these mice, apparently at higher percentages than found by Bull et al. (1977) in Great Britain.

3.3 MERCURY CONCENTRATION IN BIRDS

The number of house sparrow (Passer domesticus) tissue samples analyzed for total mercury are given in Table 18. The purpose of the statistical analysis that follows was to detect differences in mercury concentration among three stations sampled (1, 2, 3) and also between the fall and spring sampling periods. As was the case for plants and mice, all mercury concentrations were log-transformed to stabilize variance. Since station 3 was not sampled in the fall of 1975, mercury concentrations in birds for station 4 in fall 1975 were used for station 3 at this date (the stations are about the same distance from the mine).

A three-way analysis of variance, blocking on date and sex was performed for each tissue type separately. If differences among stations were found to account for a significant portion of the variance ($\alpha = 0.05$), then the Duncan's multiple range test was used to rank the means.

Table 19 shows the mean ΣHg concentration in Passer domesticus muscle, liver, and brain tissue. For muscle and liver, the three stations are significantly different, but brain tissue of birds from stations 2 and 3 were not distinguishable at the 5% level. No differences attributable to sex were detected at the 5% level. Table 20 shows seasonal differences in muscle and liver (all stations pooled); no significant differences in brain were detected. Table 20 also shows percentage MeHg in P. domesticus muscle tissue. Although ΣHg concentration was different in muscle at the 5% level, MeHg was not. Clearly, Hg concentration in P. domesticus varies inversely with

Table 18. Number of *Passer domesticus* tissue samples analyzed for total mercury concentration during the study period (see Fig. 1 for station location)

Station	Tissue	Fall 1975	Spring 1976	Fall 1976	Spring 1977
1	Muscle	11	18	4	12
1	Liver	11	18	4	12
1	Brain	10	17	4	12
2	Muscle	15	19	-	18
2	Liver	15	19	-	18
2	Brain	15	19	-	17
3	Muscle	-	14	13	10
3	Liver	-	9	8	10
3	Brain	-	9	8	10
4	Muscle	19	-	-	-
4	Liver	19	-	-	-
4	Brain	19	-	-	-

Table 19. Geometric mean total mercury (ΣHg) (μg/g) in muscle, liver, and brain tissue of *Passer domesticus* near Almadén, Spain, in 1974-1977.

Station	Muscle	Liver	Brain
1	0.115	0.233	0.086
2	0.028	0.046	0.029
3	0.018	0.023	0.019

Table 20. Geometric mean total mercury (ΣHg) and MeHg in muscle (μg/g) and geometric mean ΣHg (μg/g) in liver and brain tissue of *Passer domesticus* in spring and fall, 1974-1977, near Almadén, Spain

	Muscle				
Date	ΣHg	MeHg	% MeHg	Liver ΣHg	Brain ΣHg
Fall	0.046	0.002	4	0.097	0.086
Spring	0.070	0.001	1.4	0.172	0.080

distance from the mine/smelter complex, but the reasons for the seasonal differences are unknown.

3.4 CHECK-SAMPLE COMPARISON

Sixty-six samples collected during the study were divided in half for ΣHg quality control analysis (see Sect. 2.3). Each laboratory analyzed one-half of each of the 66 samples (9 P. domesticus, 23 A. sylvaticus, and 34 plants). Correlation coeffients (r) were calculated for each sample type. The r-value for P. domesticus was 0.94, for A. sylvaticus 0.79, and for plants 0.57.

The reason for the much better agreement between the two laboratories for the bird tissue compared with the mouse tissue is not apparent. It is possible that the poor agreement between the two laboratories on the plant analyses may be caused by very unequal distribution of Hg, especially particles, in the plant tissue. Indeed, when the Quercus and moss samples - those most likely to retain particles - are ignored, the r-value for the rest of the plants becomes 0.72.

One other possible explanation for differences between mercury concentration in check samples analyzed by the Minas de Almadén and Oak Ridge National Laboratory stems from the fact that check samples were handled differently from the other samples. For mice and birds, muscle samples were removed and placed in screw cap bottles and stored in freezers at Almadén. Plant check samples were cut into many small segments prior to freezer storage. Regular samples were stored in freezers intact. It is possible that dehydration of the samples

occurred during long periods of freezer storage at Almadén. During dehydration, samples would lose weight but not mercury content. If dehydration occurred to a greater extent for check samples stored at Almadén than for those stored at Oak Ridge National Laboratory, estimates of mercury concentration in individual check samples by the Minas de Almadén could be higher than those obtained by Oak Ridge National Laboratory. Dehydration could occur more rapidly in small check samples (mouse tissue, plant tissue) than in larger check samples (birds). Dehydration would occur at a slower rate for the intact regular samples than for the smaller-sized check samples. Partial support for this explanation comes from our general observation that for several stations and different tissue types, estimates of mercury concentration for regular samples obtained by the Minas de Almadén were similar to estimates of mercury concentration in ORNL check samples. For similar comparisons, however, estimates of mercury concentration for Almadén check samples were higher than ORNL check samples.

4. RESULTS AND DISCUSSION OF AQUATIC SURVEY

The major sampling effort in the aquatic portion of this study was devoted to collection of fish species, because it was felt that fish would be a general indicator of mercury distribution in the aquatic environment. In addition, mercury that may be accumulated by fish in the environment of the mine potentially represent a direct dietary source of mercury to local inhabitants. Sampling effort devoted to determining mercury concentration in sediment, water, and benthic invertebrates was less extensive, but we hoped information on mercury concentration in these compartments of the aquatic system would assist in the interpretation of the fish data.

4.1 COMPARISON OF ESTIMATES OF TOTAL MERCURY CONCENTRATION IN FISH BETWEEN THE MINAS DE ALMADÉN AND OAK RIDGE NATIONAL LABORATORY

The majority of analyses for total mercury concentration in fish species were performed by the Minas de Almadén (Section 2.3). ORNL analyzed muscle samples for total mercury concentration from 126 individual fish for which comparison analyses (check samples) were performed in Spain. Figure 3 shows the results of the check-sample analyses. The simple correlation coefficient (r) for these data is 0.93. We believe this "check-sample" comparison for total mercury concentration in fish muscle indicates that possible mercury contamination of fish samples analyzed in Spain is not a serious problem.

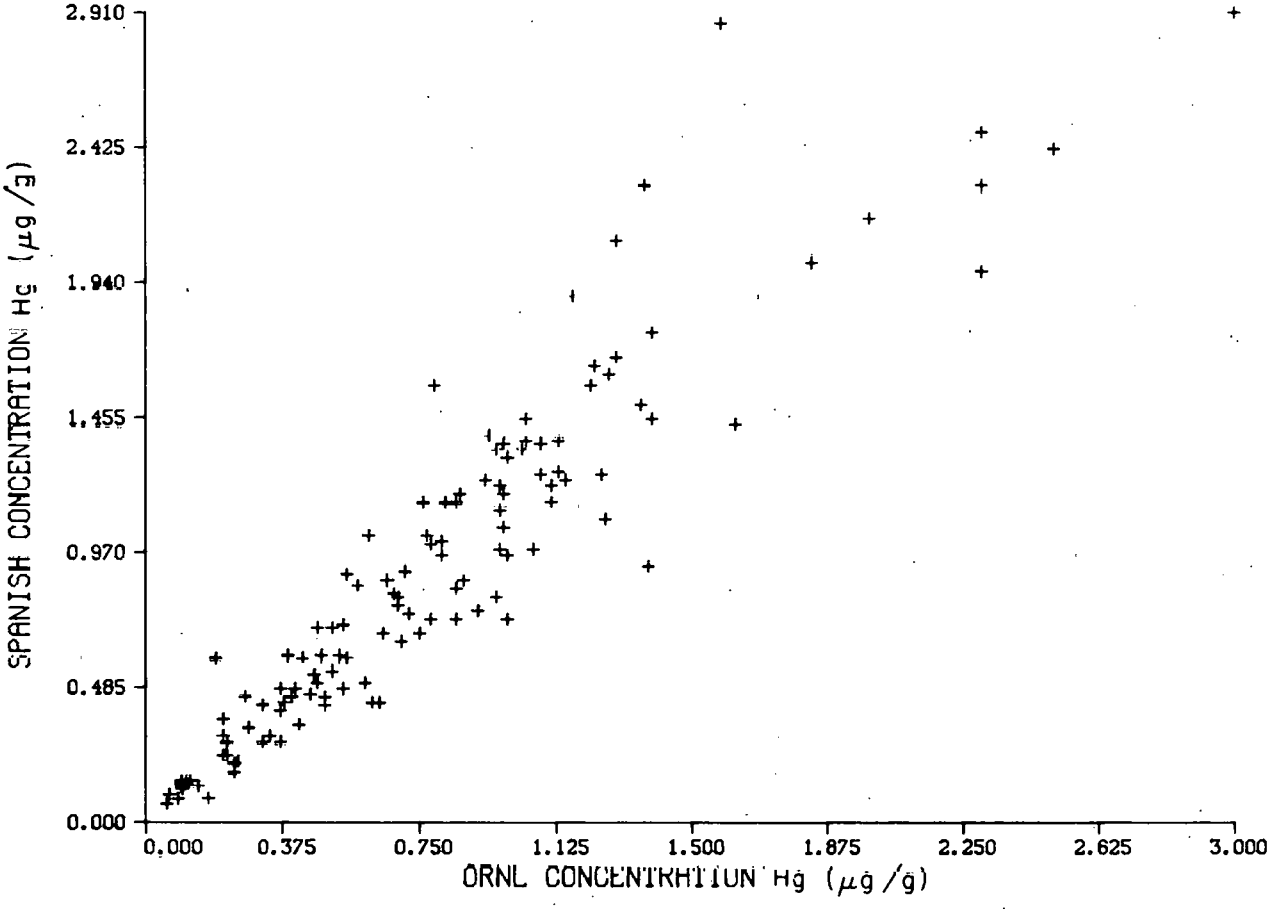

Fig. 3. Comparison of estimates of total mercury concentration for individual fish performed in Spain and at Oak Ridge National Laboratory.

4.2 MERCURY CONCENTRATION IN FISH SPECIES

A total of 1365 fish were analyzed for total mercury concentration in axial muscle. Mean total mercury concentration for the three major fish species examined (barbo, cacho, boga) for each station and date are presented in Table 21. Mean total mercury concentrations for largemouth bass collected during this study are given in Table 22. We are designating aquatic stations 10 and 13 in this discussion (Fig. 1) as control stations, because they are not directly influenced by the liquid effluent from the mine (see Section 2.2). Initially, aquatic station 7 was set up as a control station also, but subsequent mining activity in the vicinity of this station precluded the use of this station as a control area.

Mean total mercury concentration in barbo over the study period ranged from a high of 2.43 ± 0.21 μg/g ($\bar{x}$ ± 1 SE) in the fall 1976 at station 9 (0.3 km below the mine effluent), to a low of 0.33 ± 0.05 μg/g in fall 1976 at station 10 (control station). With one exception (station 13, spring 1976), mean total mercury concentrations in barbo at control stations (10, 13) not directly influenced by the mine liquid effluent were below 1.0 μg/g.

Mean total mercury concentration in cacho ranged from a high of 1.67 ± 0.27 μg/g at station 9 in fall 1976, to a low of 0.30 ± 0.02 μg/g at station 13 in spring 1977 (control station). Mean total mercury concentrations in cacho at control stations (10, 13) for all dates were below 1.0 μg/g.

Mean total mercury concentration in boga over the study period ranged from a high of 1.10 ± 0.12 μg/g at station 9 in fall 1974,

Table 21. Mean total mercury (ΣHg) concentration in axial muscle (μg/g) of three fish species collected at six stations in the vicinity of the mercury mine at Almadén, Spain, during the study period[a]

	Fall 1974						Fall 1975						Spring 1976						Fall 1976						Spring 1977					
	Station						Station						Station						Station						Station					
Statistic[b]	7	9	10	11	12	13	7	9	10	11	12	13	7	9	10	11	12	13	7	9	10	11	12	13	7	9	10	11	12	13
Barbo																														
n	8	c	6	6	12	c	21	21	12	21	21	c	18	20	19	10	17	2	20	21	20	12	8	c	21	21	21	20	21	21
$\bar{X}$	0.75	c	0.93	2.12	1.52	c	1.02	1.19	0.37	1.27	1.03	c	0.89	1.99	0.66	1.04	1.47	1.26	1.21	2.43	0.33	1.84	1.58	c	0.75	1.23	0.41	1.02	0.84	0.36
SE	0.06	c	0.17	0.21	0.15	c	0.09	0.08	0.04	0.12	0.08	c	0.08	0.21	0.16	0.15	0.21	1.03	0.12	0.21	0.05	0.23	0.20	c	0.04	0.07	0.07	0.07	0.07	0.03
Cacho																														
n	11	18	19	10	20	c	21	20	25	20	19	c	18	15	14	20	5	21	14	9	20	17	12	25	20	21	21	21	14	21
$\bar{X}$	0.74	1.46	0.47	1.38	1.26	c	0.92	1.62	0.49	1.24	1.00	c	0.54	1.18	0.62	1.34	1.12	0.46	0.92	1.67	0.33	1.08	1.12	0.44	0.82	0.92	0.30	0.77	0.72	0.30
SE	0.04	0.12	0.04	0.09	0.06	c	0.05	0.10	0.06	0.07	0.04	c	0.04	0.10	0.10	0.05	0.08	0.03	0.08	0.27	0.03	0.06	0.08	0.05	0.10	0.07	0.03	0.03	0.03	0.02
Roga																														
n	2	8	3	20	2	c	21	13	24	21	21	c	5	22	20	b	8	6	20	21	20	3	19	21	7	18	21	19	21	21
$\bar{X}$	0.30	1.10	0.14	0.59	0.54	c	0.31	1.08	0.11	0.76	0.49	c	0.28	0.64	0.07	b	0.39	0.14	0.29	0.62	0.14	0.43	0.50	0.14	0.25	0.38	0.22	0.31	0.32	0.13
SE	0.04	0.12	0.02	0.03	0.09	c	0.03	0.10	0.01	0.05	0.03	c	0.03	0.03	0.01	b	0.02	0.03	0.02	0.04	0.03	0.04	0.08	0.01	0.01	0.02	0.02	0.02	0.02	0.01

[a]See Fig. 1 for station identification.

[b]n = number in sample, x = arithmatic mean, SE = standard error or mean.

[c]Indicates species not analyzed for methylmercury.

Table 22. Mean total mercury (ΣHg) concentration (μg/g) in axial muscle of largemouth bass collected in the vicinity of the mercury mine at Almadén, Spain[a]

Station number	Date	Number n	Mean Hg concentration $\bar{X}$	Standard error of mean SE
10	Fall 1976	2	0.25	0.06
11	Fall 1975	5	2.50	0.19
11	Fall 1976	16	1.93	0.12
12	Fall 1975	5	1.34	0.30
12	Fall 1976	15	1.13	0.15

[a]See Fig. 1 for station identification.

to a low of 0.07 ± 0.01 µg/g at control station 10 in the spring of 1976. Mean total mercury concentrations in boga were below 1.0 µg/g at all other stations and dates sampled with the exception of station 9 in fall 1975.

A two-way analysis of variance was performed to contrast mercury concentration in fish species at the control stations (10, 13) against mercury concentration in fish species at stations (9, 11, 12) potentially affected by the mine liquid effluent. Sampling date was used as the blocking factor and the contrast was examined through partitioning treatment sums of squares between control and affected stations. The results of this analysis indicate the total mercury concentration in each species is significantly lower ($\alpha = 0.0001$) at the control stations than at the stations influenced by the mine effluent.

Largemouth bass were only collected at stations 10, 11, and 12 (Table 22). Mean total mercury concentration in bass at the control station (10) in fall 1976 was 0.25 ± 0.06 µg/g. Mean total mercury concentration in bass at stations 11 and 12 (influenced by mine effluent) ranged from 2.50 ± 0.19 µg/g to 1.13 ± 0.15 µg/g.

Three initial trends are evident from this gross-level analysis. First, the highest mean total mercury concentrations observed in fish species occur consistently at station 9 on the Rio Valdeazogues, 0.3 km below the entrance of the Arroyo Azogado which receives the liquid effluent from the mine. Second, total mercury concentrations in fish at the control stations are lower than at the stations potentially affected by the mine effluent. Third, the highest mean total mercury

concentrations were observed in barbo, followed by cacho and boga in decreasing order (Table 21 and Fig. 4).

A total of 230 fish were analyzed for methylated mercury. The mean percentage methylmercury of total mercury in axial muscle of three fish species (barbo, cacho, boga) at stations and dates for which methylmercury analyses were performed is given in Table 23. Mean percentage methylmercury ranged from a high of 108.8 ± 6.1% (1 SE) in boga at station 10 in fall 1976, to a low of 40.6 ± 4.2% in boga at station 9 in fall 1974. It is clear that nearly 100% of the mercury in fish muscle in the vicinity of the mine at Almaden is methylated mercury (Table 23). The mean percentage methylmercury of all 230 fish analyzed was 82.5 ± 1.2%. The mean percentage methylmercury for all barbo, cacho, and boga analyzed was 87.6 ± 1.9%, 87.5 ± 2.6%, and 81.0 ± 1.9% respectively. Thus, there do not appear to be any substantial differences in percentage methylmercury in these three species (see also Table 23).

One trend regarding percentage methylmercury in fish species deserves note (Table 23). It appears that percentage methylmercury at station 9 is consistently lower than that at other stations. A one-way analysis of variance was performed to detect station differences in percentage methylmercury, with all species and all dates pooled. The station effect was significant at the < 0.01 level. A Duncan's multiple range test indicates that the means of percentage methylmercury in fish at stations 7, 10, 11, and 12 are not significantly different from each other ($\alpha = 0.05$), but are significantly different (higher) than means at stations 9 and 13

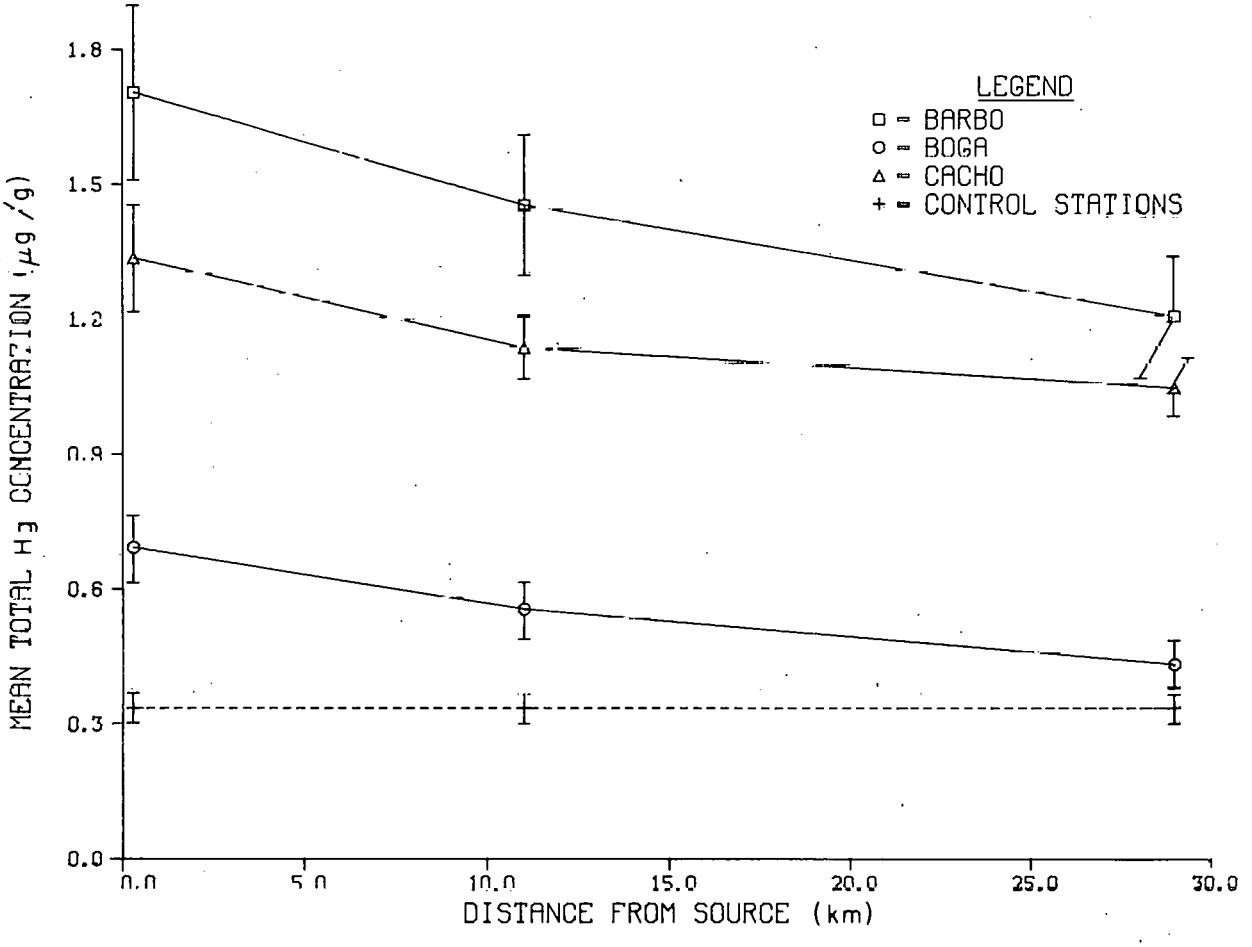

Fig. 4. Mean total mercury concentration of all fish of each species collected on all dates at stations downstream from the mine effluent.

Table 23. Means of percentage methylmercury (MeHg) of total mercury (ΣHg) concentration in axial muscle of three fish species at six stations in the vicinity of the mercury mine at Almaden, Spain, for the study period[a,b]

	Fall 1974					Fall 1975					Spring 1976		Fall 1976	
	Station					Station					Station		Station	
Statistic[c]	7	9	10	11	12	7	9	10	11	12	9	13	7	10
Barbo														
n	8	d	6	6	9	5	5	5	5	5	d	1	5	5
$\bar{X}$	95.4		87.8	93.5	92.7	91.6	54.9	91.8	94.5	99.3		66.1	67.6	89.3
SE	2.5		2.5	4.5	3.6	3.9	4.2	4.5	2.0	2.3		-	1.5	8.7
Cachc														
n	10	10	10	10	9	5	5	5	5	5	5	5	5	5
$\bar{X}$	91.0	68.1	83.9	108.4	79.4	93.1	72.1	83.7	96.9	93.4	68.2	75.0	76.0	81.3
SE	2.2	3.6	3.1	7.8	9.5	1.6	5.4	4.9	1.8	3.2	2.7	6.9	7.8	6.1
Boga														
n	2	8	4	10	2	5	5	5	5	5	5	1	5	5
$\bar{X}$	75.7	40.6	89.2	85.0	90.5	87.7	56.3	85.1	91.3	92.1	71.1	51.2	77.0	108.8
SE	13.2	4.2	4.4	1.9	1.6	3.7	3.2	4.3	2.3	2.3	6.2	-	5.3	6.1

[a]See Fig. 1 for station identification.

[b]Percentage methylmercury for two largemouth bass collected at station 11 in fall 1975 was 102.3 ± 2.3% (1 SE). Percentage methylmercury for two largemouth bass collected at station 12 in fall 1975 was 97.9 ± 6.1% (1 SE).

[c]n = number in sample, x = arithmatic mean, SE = standard error of mean.

[d]Indicates species not analyzed for methylmercury.

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(Table 24). Means of percentage methylmercury in fish at stations 9 and 13 are not significantly different from each other, but are significantly lower than those at the other stations. It appears that percentage methylmercury in fish is lower at the station where total mercury concentration in fish is higher (9). The comparably small sample size in this analysis at station 13 (Table 24) precludes evaluation of why percentage methylmercury is lower at this station.

4.3 FACTORS AFFECTING TOTAL MERCURY CONCENTRATION IN FISH SPECIES IN THE VICINITY OF THE MERCURY MINE AT ALMADÉN

Estimates of total mercury concentration and percentage methylmercury concentrations in fish species in the vicinity of the mercury mine at Almadén are presented in Section 4.1. In this section, we include results of our analysis of trends in mercury concentration in fish species with distance from the mine, taking into account size of fish and date of fish collection. This analysis is predicated on the following assumptions/observations:

(1) The main source of mercury to the Rio Valdeazogues and Rio Zujar, is the Arroyo Azogado which receives the liquid effluent from the mine (see Fig. 1).

(2) Stations 10 (Rio Guadalmez) and 13 (Rio Esteras) represent control stations not directly influenced by the mine.

(3) Station 7 on the Rio Valdeazogues upstream from the mercury source (Fig. 1) was not included in this analysis, due to the unknown influence of mining activities near this station initiated during the study period.

Table 24. Duncan's multiple range test to detect station differences in percentage methylmercury (MeHg) in fish muscle

Grouping[a]	Mean % MeHg	No.	Station
A	90.5	37	12
A	88.5	50	10
A	88.3	43	11
A	85.8	50	7
B	70.4	7	13
B	60.9	43	9

[a]Stations with the same letter are not significantly different from each other ($\alpha = 0.05$).

Under the above assumptions/observations, three stations (9, 11, 12) were selected for analysis of the distribution of mercury in fish species downstream from the source (Arroyo Azogado). Estimates of mean total mercury concentration in axial muscle of barbo, cacho, and boga at each sampling date are plotted against distance (km) from the source in Figs. 5-7 (see Table 21 for estimates of variability). It appears that there are two general trends in total mercury concentration in these fish species with distance from the mine. First, total mercury concentration in each species appears to decrease with distance from the mine; and second, there appear to be differences in mean total mercury concentration at some of the stations on the different sampling dates. We examined these trends through analysis of a model proposed to explain observed mercury concentration in fish species at these stations discussed below.

4.3.1 Linear Model of Mercury Concentration in Fish at Stations Downstream from the Mercury Source

We hypothesize that mercury concentration in fish downstream of the mercury source is a function of date collected, size of fish (length), and distance from the source. Sampling date can influence mercury concentration if the source of mercury is episodic and if hydrologic conditions vary with date. Size of fish has been shown to influence mercury concentration (see discussion in Huckabee et al. 1979). Distance from the mine could influence mercury concentration in fish through a change in exposure conditions via dilution or attenuation downstream from the source.

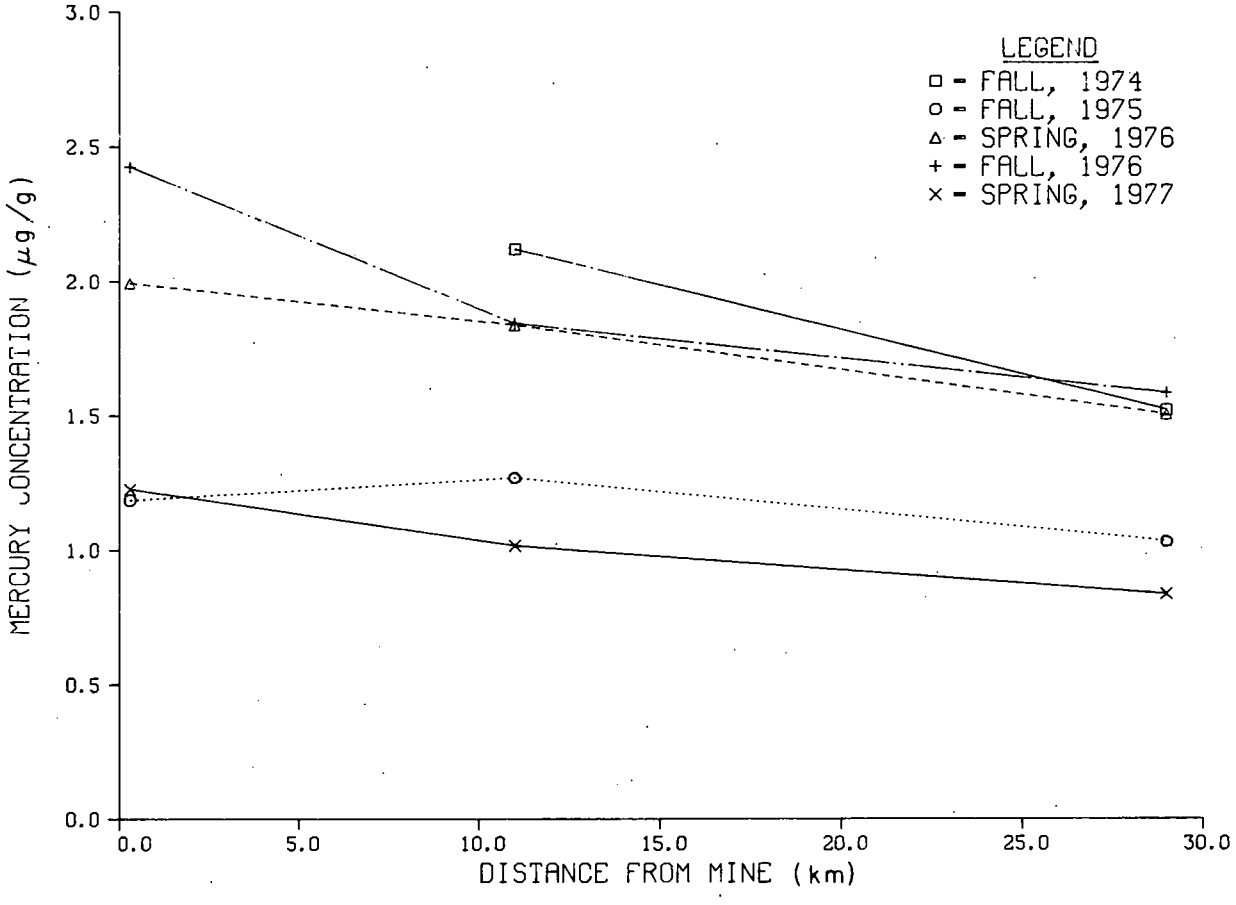

Fig. 5. Mean total mercury concentration in barbo at stations 9, 11, and 12 downstream from the mine effluent for the five sampling dates.

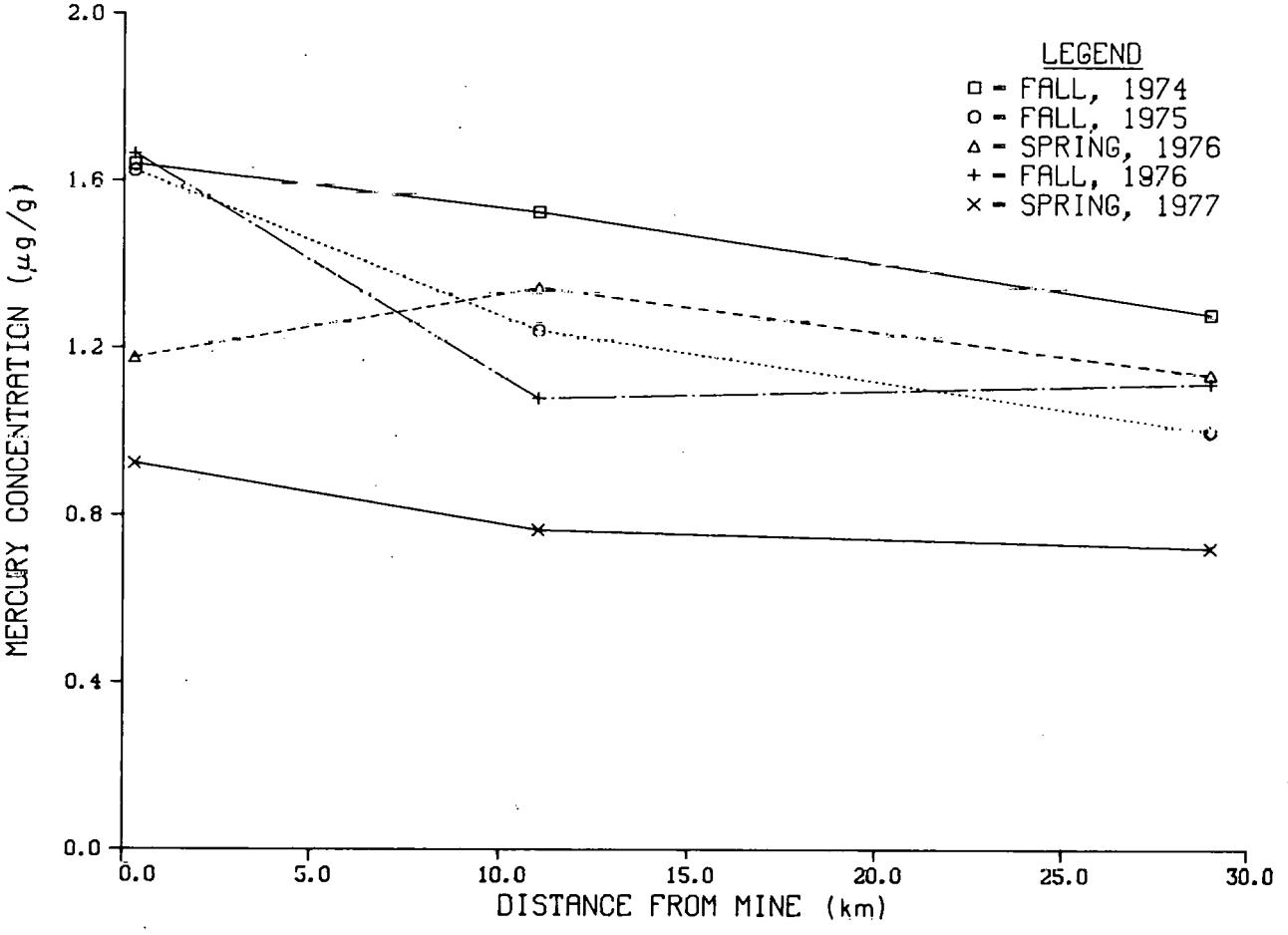

Fig. 6. Mean total mercury concentration in cacho at stations 9, 11, and 12 downstream from the mine effluent for the five sampling dates.

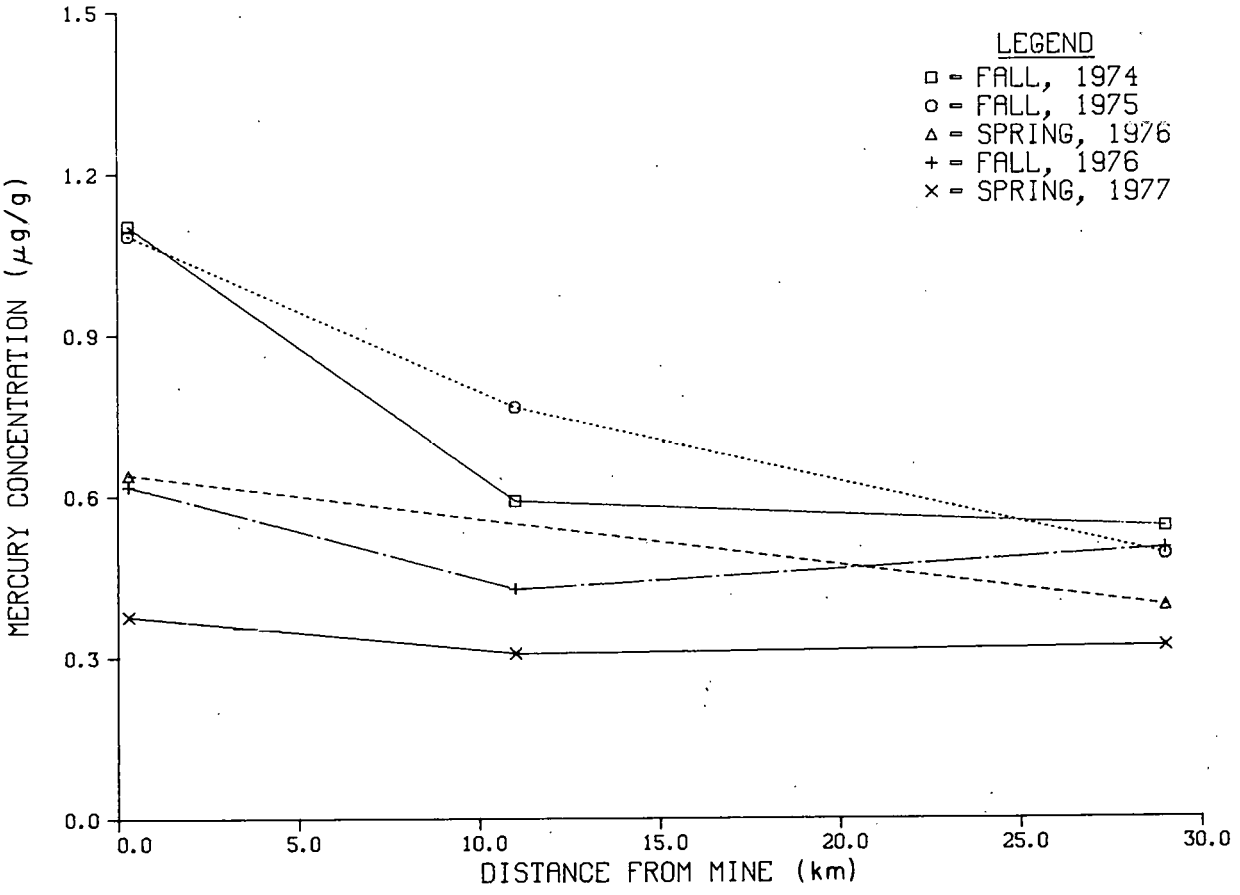

Fig. 7. Mean total mercury concentration in boga at stations 9, 11, and 12 downstream from the mine effluent for the five sampling dates.

The following model was proposed to explain variability in total mercury concentration in barbo, cacho, and boga at stations 9, 11, and 12.

$$[Hg] = a + (\text{date}) + B_1 X_1 + B_2 X_2 = \mu_i + B_1 X_1 + B_2 X_2 \ ,$$

where:

$[Hg]$ = total mercury concentration (μg/g)

a = constant

$(\text{date}) = C_0 W_0 + C_1 W_1 + C_2 W_2 + C_3 W_3 + C_4 W_4$

C_i = constant (i = date 0, 1, . . ., 4)

$W_i = \begin{cases} 1 \text{ if the data are from the } i\text{th date} \\ (\ i = \text{date } 0, 1, \ldots, 4), \\ 0 \text{ if otherwise} \end{cases}$

X_1 = fish length (cm)

X_2 = distance from source (km)

$\mu_i = a + (\text{date})$

The above model was examined for each fish species separately. Residuals were examined to determine the need for data transformation. Transformations were retained in further model analysis where appropriate. The contribution of each variable in the model was examined, and variables were deleted from the model if not significant ($\alpha = 0.05$). Individual observations of total mercury concentration were analyzed by the principal "outlier" procedure (Draper and Smith 1966), and "outliers" were deleted where justified.

The results of application of this model for barbo are given in Table 25. Sampling date was used as the blocking variable. Sampling date and distance from the source both contributed significantly to the

Table 25. Linear model developed to explain variability in total mercury concentration in barbo at stations 9, 11, 12 (see Fig. 1 and text for variable explanation)

Model chosen: $[Hg] = a + (date) + B_2 X_2$
X_2 = distance from source

Source	df	SS	MS	F	P > F
Model	5	39.5743	7.9149	26.76	0.0001
Error	218	64.4875	0.2958		
Total	223	104.0618			

Source	df	SS	MS	F	P > F
R (date)	4	33.8684	8.4671	28.62	0.0001
R[a] (distance|date)	1	5.7060	5.7060	19.29	0.0001

Estimated model: $[Hg] = \mu_i - 0.01384 X_2$
Coefficient of determination (R^2) = 0.3803; standard deviation = 0.5439, constant (μ_i) on i^{th} date:

Date	μ_i = a + (date)
(0) Fall 1974	1.8562
(1) Fall 1975	1.1678
(2) Spring 1976	1.7299
(3) Fall 1976	1.9575
(4) Spring 1977	1.0323

[a]R (a|b) = contribution of a to the sums of squares, given that b is already in the model.

model (α = 0.0001); size of fish did not contribute significantly (α = 0.05). The observed trend of a decrease in mercury concentration in barbo with distance from the source (Figs. 4 and 5) is consistent with the negative sign of the coefficient for distance (Table 25). The model indicates 38.03% of the variance in mercury concentration in barbo at the stations downstream from the source is explained by the sampling date and distance from the source (R^2 = 0.3803). Two individual estimates of mercury concentration in Barbo were deleted from the data set as "outliers."

The results of application of this model for cacho are given in Table 26. The natural logarithim of mercury concentration in cacho was employed to stabilize the variance. Sampling date was used as the blocking variable. Sampling date and distance from the source both contributed significantly in explaining variability of mercury concentration in cacho (α = 0.0001). Size of fish did not contribute significantly to the model (α = 0.05). The general trend of a decrease in mercury concentration in cacho with distance from the source (Figs. 4 and 6) is consistent with the negative sign of the coefficient for distance (Table 26). The significant date effect in the cacho model indicates mercury concentration in cacho decreased during the sampling period as suggested in Fig. 6. This model indicates 46.34% of the variability in mercury concentration in cacho at these stations is explained by sampling date and distance from the source (R^2 = 0.4634). Two "outliers" were deleted from the data set.

The results of application of the linear model for boga are presented in Table 27. The natural logarithm of both mercury

Table 26. Linear model developed to explain variability in total mercury concentration in cacho at stations 9, 11, 12 (see Fig. 1 and text for variable explanation)

Model chosen: $\ell n\ [Hg] = a + (date) + B_2\ X_2$
X_2 = distance from source

Source	df	SS	MS	F	P > F
Model	5	11.24077	2.24815	36.28	0.0001
Error	210	13.01472	0.06197		
Total	215	24.25549			

Source	df	SS	MS	F	P > F
R (date)	4	9.37696	2.34424	37.83	0.0001
R^a (distance|date)	1	1.86381	1.86381	30.07	0.0001

Estimated model: $\ell n\ [Hg] = \mu_i - 0.00852\ X_2$
Coefficient of determination (R^2) = 0.4634; standard deviation = 0.2489, constant (μ_i) on i^{th} date:

Date	μ_i = a + (date)
(0) Fall 1974	0.37026
(1) Fall 1975	0.20776
(2) Spring 1976	0.16220
(3) Fall 1976	0.14066
(4) Spring 1977	-0.24878

[a]R (a|b) = contribution of a to the sums of squares, given that b is already in the model.

Table 27. Linear model developed to explain variability in total mercury concentration in boga at stations 9, 11, 12 (see Fig. 1 and text for variable explanation)

Model chosen: $\ell n\ [Hg] = a + (date) + B_2\ X_2 + B_1\ \ell n\ X_1$

X_1 = size (cm), X_2 = distance from source (km).

Source	df	SS	MS	F	P > F
Model	6	24.9579	4.1597	46.02	0.0001
Error	201	18.1661	0.0904		
Total	207	43.1240			

Source	df	SS	MS	F	P > F
R (date)	4	17.5504	4.3876	48.55	0.0001
R^a(dist|date, ℓn size)	1	5.0534	5.0534	55.91	0.0001
R^a(ℓn size|date, dist)	1	2.0889	2.0889	23.11	0.0001

Estimated model: $\ell n\ [Hg] = \mu_i - 0.01372\ X_2 - 0.55017\ \ell n\ X_1$, coefficient of determination (R^2) = 0.5787; standard deviation = 0.5439, constant (μ_i) on i^{th} date:

Date	μ_i = a + (date)
(0) Fall 1974	-0.60580
(1) Fall 1975	-0.39212
(2) Spring 1976	-0.63574
(3) Fall 1976	-0.67121
(4) Spring 1977	-1.05575

[a]R (a|b) = contribution of a to the sums of squares, given that b is already in the model.

concentration and fish length was deemed appropriate. Sampling date, distance from the source, and fish size contributed significantly in explaining variance in mercury concentration in boga ($\alpha = 0.001$) (Table 27). The trend of a decrease in mercury concentration with distance from the source (Figs. 4 and 7) was confirmed, indicated by the negative coefficient for the distance term in the model. Boga was the only species where fish size (length) explained a significant proportion of the variability in mercury concentration, and the negative coefficient for the size term in the model indicates an inverse relationship.

In summary, analysis of the linear model indicates total mercury concentration in axial muscle of barbo, cacho, and boga decreases with distance from the mercury source. For each species, sampling date contributed significantly to explaining variation in mercury concentration. For one species (cacho), a clear trend of decreasing mercury concentration over the study period is indicated (Table 26 and Fig. 6). Size of fish was found to be a significant variable, explaining variation in mercury concentration for only one species (boga) for which concentration varied inversely with length.

4.4 MERCURY CONCENTRATION IN WATER, SEDIMENT, AND BENTHIC INVERTEBRATES

The sampling effort devoted to collection of water, sediment, and benthic invertebrates was not extensive enough for a rigorous statistical analysis. Table 28 presents summary information obtained for these compartments of the aquatic system in the vicinity of the mine at Almadén. Several trends are indicated in Table 28. Total

Table 28. Mean total mercury (ΣHg) concentration in water and sediment and mean total mercury concentration and percentage methylmercury (MeHg) in benthic invertebrates collected in the vicinity of the mercury mine at Almadén, Spain, 1975-1977. Values reported are $\bar{X} \pm 1$ SE.[a]

Station number	[ΣHg] water[b] (μg/liter)	[ΣHg] sediment (μg/g)	[ΣHg] in benthos (μg/g)	% MeHg benthos
7	1.87 ± 1.21	157 ± 51[c]	-	-
9	3.00 ± 1.30	1085 ± 681[c]	11.15 ± 4.30[d]	2.70 ± 1.60[d]
10	0.37 ± 0.12	153 ± 83[c]	-	-
11	1.64 ± 0.26	515 ± 136[c]	-	-
12	2.25 ± 1.07	178 ± 53[c]	0.68 ± 0.08[d]	11.92 ± 1.18[d]
13	0.75 ± 0.55	203 ± 88[c]	0.14 ± 0.05[d]	51.02 ± 5.63[d]
14	1.00 ± 0.10	-	-	-
15	354.60 ± 147.31	1547 ± 252[e]	-	-
16	72.70 ± 36.82	1343 ± 266[e]	-	-
17	5.07 ± 0.84	970 ± 287[e]	-	-

[a]See Fig. 1 for station identification.

[b]Concentrations are for unfiltered samples (particulate and dissolved).

[c]Means are for the <105-μm sediment size fraction.

[d]Samples collected in the spring 1977. Means were calculated from estimates of concentration in individual taxonomic groups at each station.

[e]Means are for whole sediment (unfractionated).

mercury concentration in water and sediment at stations 15, 16, and 17 clearly demonstrate the Arroyo Azogado as a substantial source of mercury to the Rio Valdeazogues aquatic system (Fig. 1). Total mercury concentrations in water (354.60 ± 147.31 μg/liter) and in sediment (1547 ± 252 μg/g) at station 15, just downstream from where the mine liquid effluent and drainage from the mine tailings enter the Arroyo Azojado, are extremely elevated. Total mercury concentration in water and sediment then decrease downstream to the confluence with the Rio Valdeazogues.

The concentration of total mercury in water and sediment at stations on the Rio Valdeazogues and Rio Zujar downstream from the mercury input from the Arroyo Azogado (9, 11, 12) generally decrease with distance (Table 28 and Fig. 1). Concentrations of total mercury in water and sediment at station 12, 29 km below the mercury input, appear to be elevated compared to those at the control stations (10 and 13).

Limited estimates of total mercury concentration in benthic invertebrates indicate highest concentration occurs at station 9 (11.15 ± 4.30 μg/g), decreasing to 0.68 ± 0.08 μg/g at station 12. Total mercury concentration in benthic invertebrates at the control station 13 (0.14 ± 0.05 μg/g) is considerably lower than that at the other stations. Percentage methylmercury of total mercury in benthic invertebrates ranged from 2.70 ± 1.60% (station 9) to 51.02 ± 5.63% (station 13). The trend of lower percentage methylmercury at station 9 observed for fish species (Tables 21 and 24) appears to be also evident in benthic invertebrates.

4.5 DISCUSSION OF RESULTS OF THE AQUATIC SURVEY

Mean total mercury concentrations in barbo and cacho in the Rio Valdeazogues and Rio Zujar, downstream from the point of entry of the Arroyo Azogado containing the mine liquid effluent (stations 9, 11, 12), are clearly elevated (Table 21 and Fig. 4) with respect to the United States Food and Drug Administration "Action Level" for mercury of 1.0 μg/g (Federal Register, 1979, Vol. 44, No. 14). Total mercury concentrations in boga at stations 9, 11, and 12 are consistently below 1.0 μg/g with the exception of station 9 in 1974 and 1975 (Table 21). Limited sampling of total mercury concentration in water and sediment in the Arroyo Azogado (Table 28) clearly identifies this stream as a point source of inorganic mercury. Most of the mercury in fish tissue in the vicinity of the mine at Almadén is in the form of methylated mercury (Table 23). This observation is consistent with other published information (Noren and Westoo 1967, Zitko et al. 1971, Kamps et al. 1972, Lockhart et al. 1972, Westoo 1973, Bache et al. 1971, Bishop and Neary 1974, Hildebrand et al. 1980).

A postulated sequence of events whereby aquatic biota can accumulate methylmercury in the absence of direct methylmercury discharge to waters includes the following (Jensen and Jernelov 1969, Wood et al. 1968, Jernelov 1968, Landner 1971):

(1) Mercury is discharged as elemental mercury or as mercury (II) chloride and hydroxide complexes.

(2) This mercury is incorporated onto suspended particulates and into the sediments within a short distance from the outfall.

(3) In the sediments, bacterial action converts the mercury to methylated forms. In basic waters dimethylmercury can be volatilized. In acidic waters any dimethylmercury is converted to the much less volatile monomethylmercury ion which is retained in solution.

(4) Monomethylmercury ion can be accumulated directly from the water by the biota.

(5) Fish predators can receive methylmercury both from water and from the food chain.

The only information we have concerning sources of methylmercury to fish species at Almadén consist of potential food (benthic invertebrates, Table 28) and sediment. No analyses of methylmercury concentration in water were attempted. Limited analyses of methylmercury in sediment from the Almadén area performed at ORNL indicate that 0.001 to 0.03% of total mercury in sediment is in the methylated form. An evaluation of the mechanism of methylmercury accumulation in fish in the vicinity of Almadén is not possible, except to postulate that fish most likely accumulate methylmercury directly from the water and their food (invertebrates).

Mercury concentration in boga was consistently lower than mercury concentration in barbo and cacho (Fig. 4). Although we did not examine food habits of these three species, barbo and cacho most likely feed predominately on benthic invertebrates, while boga feed predominantly on benthic algae (Muus and Dahlstrom 1971). If the concentration of mercury in benthic algae in the Rio Valdeazogues and Rio Zujar is lower than the concentration of mercury in benthic invertebrates in these

streams, then a lesser accumulation of mercury by boga from the food source may account for the above-observed differences.

The observed trend suggesting percentage methylmercury in fish species is lower at station 9 where exposure condition to inorganic mercury appears highest (Tables 23, 24, 28) deserves note. Methylmercury is accumulated from water at a higher rate and eliminated at a slower rate in fish species compared to inorganic mercury (de Freitas et al. 1974, 1977). It is probable that the high exposure level of inorganic mercury at this station (Table 28) is responsible for the lower percentage methylmercury observed in muscle tissue at this station.

Limited measurements of mercury concentration in benthic invertebrates at Almadén indicate this potential fish food source contains methylmercury. Percentage methylmercury in benthos ranges from 2.7 to 51% (Table 28). This range of percentage methylmercury is consistent with other published studies (Jernelov and Lann 1971, Cox et al. 1975, Hildebrand et al. 1980). Our estimates of mercury concentration in benthic invertebrates are for the whole animal, so mercury contamination by sediment in the gastrointestinal tract and surface contamination is likely.

Estimates of mercury concentration in fish at our control stations (10, 13) are consistently lower than those at stations influenced by the mine (Table 21). However, mercury concentrations at the control stations are elevated compared to concentrations in fish from areas remote from known mercury inputs (0.05 µg/g range, Huckabee et al. 1974). Obviously the high "geologic background" concentrations of

mercury and possible airborne contamination of control stations in this region account for this observation.

Our analysis of factors affecting mercury concentration in fish species downstream from the point source indicates that distance from the source and sampling date explain a significant proportion of the variance for all three species studies (Tables 25-27). The trend of decreasing mercury concentration over time observed for cacho (Fig. 2 and Table 26) may reflect a decreasing exposure condition. During the study period, a new water treatment plant was brought on line. Whether or not this new treatment facility is responsible for reducing exposure conditions remains speculation.

Mercury concentration was significantly related to size (at stations 9, 11, 12) only in boga (Table 27), and the relationship was inverse. Many investigators observed a positive relationship of mercury concentration to fish size/age (Scott 1974, Koirtyohann et al. 1974, Kelly et al. 1975, Olsson 1976). Two major factors may have masked a concentration/size relationship in this study. Both relate to probable episodic environmental exposure conditions. Variability in mercury input to the Rio Valdeazogues due to the operational history of the mine is unknown but probably occurs. Potentially significant also are the seasonal differences in hydrology of the area. General observations indicate that the Rio Valdeazogues is free flowing during the period December through July. From July to late fall, the river consists of a series of pools. Fish tend to be concentrated in these pools in the fall. Because our collecting periods included both hydrologic conditions, exposure conditions could have been different.

No adequate hydrologic data are available to explore this possibility. In addition, any migration of fish in the system could affect exposure conditions. Size of fish reflects age and thus duration of exposure to mercury. If mercury exposure conditions are episodic at Almadén due to hydrology and mine operation, the failure to detect a consistent size effect is not surprising.

Distance from the source, sampling date, and fish size explained a maximum of approximately 50% of the variance in mercury concentration in fish downstream from the mercury source. Considerable variability remains unexplained. The hydrologic factors and mine operation discussed above may be a major cause of this remaining variability.

Although this aquatic survey indicates the liquid effluent from the mercury mine at Almadén results in elevated levels of mercury in fish species, the actual levels observed are not drastically different from levels in fish from other environments experiencing anthropagenic mercury inputs. Hildebrand et al. (1980) report the results of a study on the North Fork Holston River in Virginia and Tennessee, USA. In this study the source of mercury consisted of leachates from waste ponds of an abandoned chloralkali plant. Total mercury concentrations in fish species were highest immediately below the source (1-2 μg/g) then decreased downstream. Mean total mercury concentration in fish species at Almadén (highest recorded = 2.43 μg/g, Table 21) are not drastically elevated compared to those in the Holston River. Thus, even though the mercury mine at Almadén is potentially one of the world's major sources of mercury to the environment, mercury levels in fish are not alarming. It is clear, however, that levels above

1.0 μg/g are common in the Rio Valdeazogues and Rio Zujar. Fish from these systems, therefore, do constitute a potentially significant source of mercury to humans if consumed.

5. SUMMARY

Summarized below are the major observations, results, and conclusions of the ecological survey of mercury in the environment at Almadén, Spain. The reader is referred to the text of this report for a more detailed analysis of the data collected.

1. This study was initiated in the fall of 1974 and sample collection was completed in 1977.
2. The purpose of this study was to determine the concentration of mercury in select ecosystem compartments in the environment at Almadén, and to determine the distribution of mercury in these compartments with distance from the mercury mine complex.
3. Ecosystem compartments sampled included plants, mice, and birds (house sparrows) in the terrestrial environment, and water, sediment, fish, and benthic invertebrates in the aquatic environment.
4. Sample-collecting periods during this study were fall 1974, fall 1975, spring 1976, fall 1976, and spring 1977.
5. Approximately 5000 individual chemical analyses for total mercury or methylmercury concentration in the ecosystem compartments were performed during this study.
6. The majority of chemical analyses were performed by the Laboratorio de Minas de Almadén. Oak Ridge National Laboratory performed all methylmercury analyses, as well as total mercury analyses on approximately 10% of the samples analyzed in Spain for quality control.

7. Correlation coefficients (r) for total mercury analyses between Minas de Almadén Laboratory and Oak Ridge National Laboratory were 0.94 for house sparrows, 0.93 for fish, 0.79 for mice, and 0.57 for plants.
8. Total mercury concentration in terrestrial plant species is generally highest near the mine complex (values > 100 μg/g in moss) and lowest 20 km distant from the mine (< 1.0 μg/g).
9. Highest total mercury concentration in plant species was observed in moss. Herbaceous plant species (grass and forbs) accumulate higher levels of mercury than woody plants.
10. All estimates of mercury concentration in plants at Almadén greatly exceed other reported values.
11. Total mercury concentration in muscle tissue of mice was highest near the stream receiving the liquid effluent from the mine (0.181 μg/g). Kidney tissue showed the highest total mercury concentration (> 4 μg/g) at this location.
12. Methylmercury concentration averaged up to 29% of total mercury concentration in muscle tissue of mice.
13. Total mercury concentration in muscle tissue of sparrows was highest near the mine complex (0.115 μg/g) and decreased with distance from the mine.
14. Percentage methylmercury of total mercury in sparrow tissue was in the range of 1 to 4%.

15. Total mercury concentration in fish species in the Almadén area was highest near the mine liquid effluent, then decreased downstream.
16. Total mercury concentration in fish species at stations downstream from the mine effluent was higher than total mercury concentration in fish at control stations.
17. Total mercury concentration in fish species 29 km below the mine liquid effluent was above the United States Food and Drug Administration guideline of 1.0 µg/g.
18. The highest mean total mercury concentration in fish species was 2.43 µg/g (barbo in fall 1976 nearest the mine effluent).
19. A linear model was developed to explain variance in total mercury concentration in fish species. From 38 to 58% of the variability was explained by sampling date, distance from the source, and size of fish (boga only).
20. The majority of mercury in fish muscle is in the form of methylated mercury.
21. Elevated levels of mercury in water and sediment in the Arroyo Azogado confirm this stream as a major point source of mercury to the aquatic environment.
22. Fish species and possibly young asparagus plants, if consumed, may be a significant source of mercury to humans in the Almadén area.

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