



Analysis of fish tissue for trace amounts of lead by furnace atomic absorption spectroscopy
by Dennis Riley Neuman

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:

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Fish samples were collected from the West Gallatin River and the Federal Fish Hatchery. The lead levels are in the p.p.m. range. Tissue from fish within the Gallatin Canyon exhibit the highest lead levels. It is believed that this is the result of leaded vehicle exhaust emissions.

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ANALYSIS OF FISH TISSUE FOR TRACE AMOUNTS OF LEAD
BY FURNACE ATOMIC ABSORPTION SPECTROSCOPY

by

DENNIS RILEY NEUMAN

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

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Chemistry

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ABSTRACT

Trace amounts of lead were determined in fish bone, gill, liver and muscle tissue by a wet digestion and furnace atomic absorption. Sensitivity is 0.27 ng/g. The detection limit is 0.15 ng/g. The accuracy of the method, determined by standard addition, is $99\% \pm 6\%$. Matrix interferences were minimized as evidenced by similar slopes of three analytical standard curves prepared from three different chemical matrices.

Fish samples were collected from the West Gallatin River and the Federal Fish Hatchery. The lead levels are in the p.p.m. range. Tissue from fish within the Gallatin Canyon exhibit the highest lead levels. It is believed that this is the result of leaded vehicle exhaust emissions.

INTRODUCTION

Since the Industrial Revolution there has been an increasing use of lead. Today the U. S. consumption of lead is over one million tons annually.¹ Lead is a natural component in soil, water, animal life, vegetation and the air. It has been mined and used since early times for the making of bronze, brass, pipes to carry water and for "sweetening" wines. Dated snow samples from Greenland indicate that the lead content is increasing and has become an exponential function of time since 1940.² There was a base level of 20 $\mu\text{g Pb/ton}$ of ice up to 1750. By 1860 it was 50 $\mu\text{g/ton}$. In 1940, 80 $\mu\text{g/ton}$; in 1950 120 $\mu\text{g/ton}$; and in 1965 the lead level was 210 $\mu\text{g/ton}$. This sharp increase in the Pb snow level has been attributed to the use of leaded gasolines.

In the past 30 years the increasing use of lead has culminated in an environmental level far above that which would normally exist, with consequent harmful effects on health.^{3,4} The major sources of lead contamination are leaded gasolines and pesticides, the use of lead in manufacturing, the combustion of coal, incineration of refuse, leaded paints and earthenware pottery that has been improperly glazed.

A major study of lead in urban air⁵ was conducted in 1961 and 1962. 3400 air samples in three major U. S. cities were analyzed. The annual average concentration of lead was 1-3 $\mu\text{g/m}^3$. In areas of heavy traffic the lead level ranged from 14 to 44 $\mu\text{g/m}^3$. In a city with a population of over 2.5 million the mean value was 5 $\mu\text{g/m}^3$, while the level dropped to 1.7 $\mu\text{g/m}^3$ in cities with less than 100,000 people. It

was determined that the lead level in urban air is rising approximately 5% annually. Lead in gasoline is the major source of airborne lead in urban areas.⁶

Lead as the tetraethyl and tetramethyl alkyls is added to gasolines to increase their octane ratings. The national level is 2.40 grams of TEL/gallon or 1.54 grams lead per gallon, with a range of 1.28 to 2.56 grams Pb/gallon of gasoline. Organic scavengers, ethylene dichloride and ethylene dibromide, are also added to gasoline. The exhaust emissions contain mostly PbBrCl and smaller amounts of PbCl_2 , PbBr_2 , Pb(OH)Cl and Pb(OH)Br .⁷ The amount of lead discharged to the atmosphere in exhaust gases varies, depending on driving conditions, from 25% to 75% of the lead intake in the fuel.^{1,8} As a consequence the atmospheric concentrations near highways are an exponential function of distance from the roadway and a linear function of traffic volume.⁹ The lead level was measured at various distances (10 to 1750 feet) from highways supporting traffic load of between 19,800 cars and 58,000 cars daily. Figure 1⁹ shows the airborne lead levels as functions of traffic volume and distance from the highway. The lead level near the highway is relatively high, but drops off rapidly during the first few feet (150) from the highway. The concentration of lead was reduced 50% between 10 and 30 feet from the highway supporting 58,000 vehicles daily. In a similar study¹⁰ lead in soils and plants was related to traffic volume and proximity to highways. Soils and plants along heavily traveled highways show that lead concentrations increase

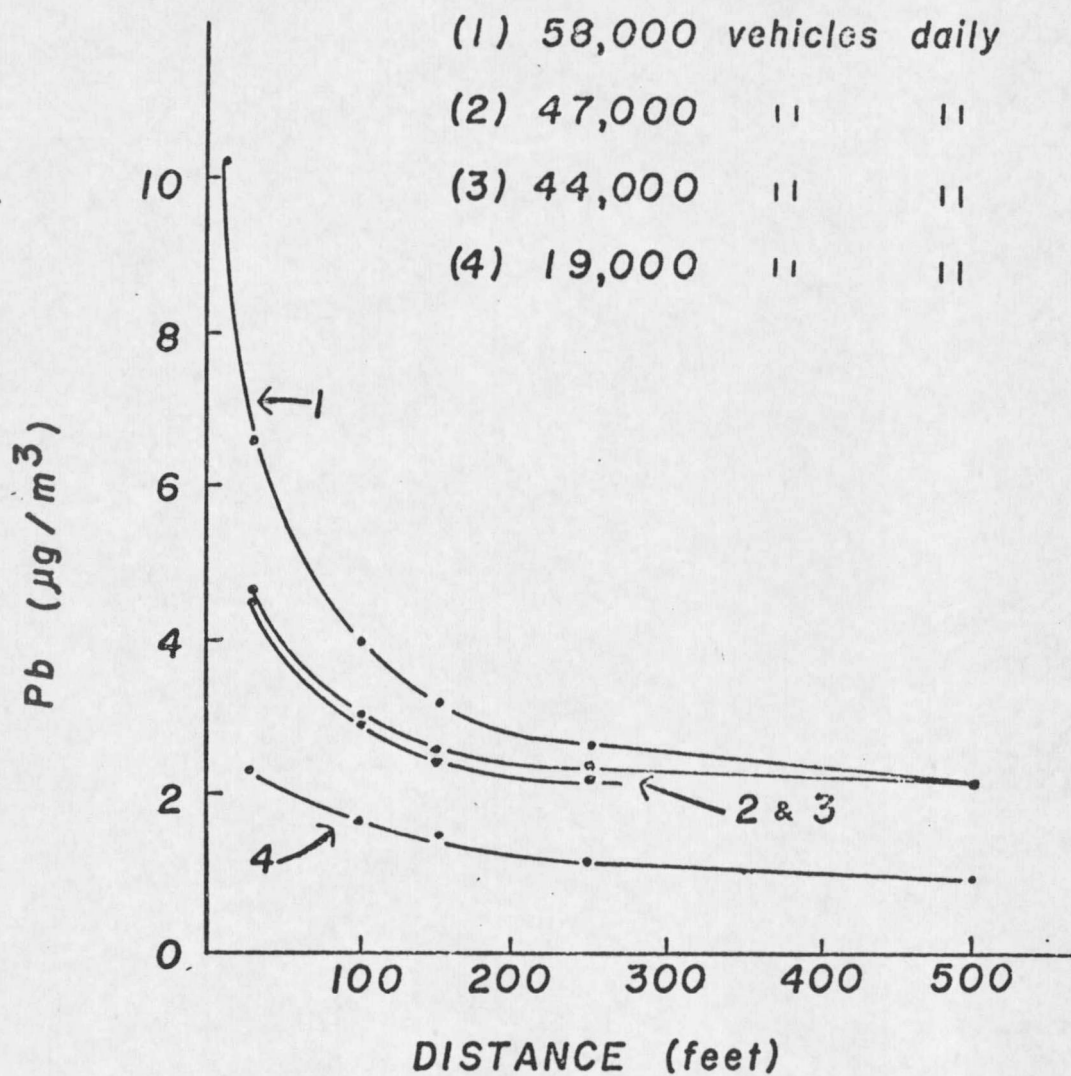


Figure 1. Air-Lead Values as a Function of Traffic Volume and Distance from the Highway

Source: Environmental Science and Technology, 4, 320 (1970).

with traffic volume and decrease with distance from the highway. Much of the lead was a removable surface contaminant on the plants. The major effect of traffic was limited to the surface soil and to a narrow zone within 100 feet of the highway. The average Pb level in non-washed roadside grass at 0 feet from the highway was 255.0 p.p.m. At 255 feet from the highway the Pb level was 46.3 p.p.m. Grass samples that were washed prior to the analysis contained less lead. Their average values at 0 feet and 255 feet were 136 p.p.m. and 40.6 p.p.m. lead, respectively. The average lead content of soil samples taken at 25 feet from the highways were 159.2 p.p.m. (0-6 inches depth), and 105.0 p.p.m. lead (6-12 inches depth). At 225 feet the average values were 92.5 p.p.m. and 71.2 p.p.m. respectively. The higher levels of lead in the air, soil and foliage near highways have lead to disastrous results for some of the animals in New York City zoos.¹¹ A lion's death was directly attributed to lead poisoning. Grass, leaves and soil samples taken from within the zoo cages ranged up to 3900 μg lead/gram. Ralph Strebel of New York Medical College, attributed the high lead concentrations to atmospheric fallout.

Childhood lead poisoning is a very real disease of the metropolitan ghetto. It affects primarily preschool age children who live in deteriorated housing previously painted with leaded paint. Approximately 8% of all ghetto children suffer from varying degrees of plumbism. A chip of paint the size of an adult fingernail may contain between 50

and 100 mg of lead.¹ A child eating a few of these a day will easily exceed the toxic level of lead. In adults a lead-blood level of 80 μg lead/100 ml of blood is considered to be the minimum toxic level.^{1,8} Children having concentrations of 60-80 μg /100 ml may show clinical signs of plumbism. Above 80 μg the risk of acute lead poisoning rises significantly.

In a major study¹² of lead and its total relationship to man, lead was found in all human organs. Accumulation of lead with age in tissues of American human beings reaches a maximum in the fifth or sixth decade. Some tissues showed a 4 to 10 times increase in concentration over values at birth. In lung tissue at birth the average lead level was 20 p.p.m. This value increased to a high average value of over 70 p.p.m. at age 40-50 years. Bone tissue also showed an average low level of less than 5 p.p.m. at birth, with a high average value of over 30 p.p.m. at age 50-60 years. Similar trends were also indicated in the kidney, pancreas, liver and aorta. The authors determined the lead levels in various sea foods. These values are all in $\mu\text{g/g}$ (wet weight). Frozen shrimp, 0.31; fresh shrimp, 0.45, lobster 2.50; crabmeat, 0.06; clams 0.15; oysters, 0.13; scallops, 0.15; sardines, 0.75; and haddock, 0.27 $\mu\text{g/g}$. In a more recent work¹³ the lead concentrations in fish were determined. Salmon samples contained 1.3 μg Pb/b; clam chowder, 2.2 $\mu\text{g/g}$; tuna, 1.2 $\mu\text{g/g}$; oyster, 4.3 $\mu\text{g/g}$; sardine, 1.3 $\mu\text{g/g}$; and crabmeat 0.85 $\mu\text{g/g}$. The values were established on the

basis of dry weight.

The entrance of Pb into the body is by two routes, the alimentary canal and the respiratory tract. The amount of lead absorbed from a given air concentration varies according to particle size.^{1,8} Particles 1μ or less will be inhaled with 50% being retained in the lungs. Particles 2μ and larger will impinge on mucous linings and either be swallowed or coughed out. Approximately 75% of particulate Pb in vehicle exhaust is less than 0.9μ in mean diameter, a size easily retained in the lungs. Absorption of Pb by respiration in urban areas averages 20-30 $\mu\text{g}/\text{day}$. The mean dietary intake of lead is approximately 300 $\mu\text{g}/\text{day}$.^{1,8,12} Approximately 6-7% of the metal ingested is actually absorbed. This represents 18 to 21 $\mu\text{g}/\text{day}$. The total daily concentration of lead which is retained from both sources, is 38 to 50 μg . Lead is also excreted from the body in urine, sweat, hair and nails. The general population seems to be in "lead balance".¹⁴ That is, the daily amount of lead absorbed (38-50 μg) is matched by approximately the same amount excreted.

More than 90% of the lead in the body is stored in the skeleton. In times of feverish illness or high calcium metabolism, lead may be released from the bone into the soft tissues. Lead in its mobile, soluble form in soft tissues affects chiefly the blood, kidneys and nervous system. Excessive amounts of lead in the blood can cause anemia and idiopathic hemochromatosis.¹⁵ Chronic nephritis and in

severe cases kidney failure may result from high lead concentrations in the kidneys. Juvenile mental retardation,¹⁵ ill defined neurosis, and encephalopathy can result from the effect of acute or prolonged, chronic, exposures to lead.

It has been established that the increasing environmental level of lead is a serious problem. Although the measured levels in biological systems are in the part per million range, any further increase in the environmental lead level will result in a further concentration in these systems. Lead absorbed in trace quantities over a long period of time can accumulate to exceed the threshold levels and produce delayed toxic effects. In geographical locations where the environmental level is not expected to be significant, it should be possible to determine baseline lead levels in biological systems. The importance of establishing these "natural" levels cannot be understated. In the future such questions as, have the "natural" levels increased?, what has caused the levels to increase?, and what should be done about them?, can be more fully understood and more responsibly answered.

The determination of lead in biological samples has been accomplished by colorimetric^{16,17,18} and spectrophotometric methods^{12,18,19} and more recently by conventional flame atomic absorption^{9,10,13,17,20} and flameless atomic absorption.^{21,22,23,24} Before the analysis can be made it is necessary to oxidize the organic matter to CO₂ and H₂O,

leaving only the inorganic salts as residue. In general, there are three approaches to this problem, acid digestion, high temperature dry ashing, and a relatively new method of low temperature oxygen plasma ashing.^{25,26} All three methods will accomplish the oxidation of the organic materials. In the dry ashing technique, oxidation is accomplished by heating the sample to relatively high temperatures, 400° to 750°C. Atmospheric oxygen serves as the oxidizing agent. The time of ashing, depending upon sample size, can easily reach 12 to 16 hours. In acid digestion procedures the temperatures are much lower and liquid conditions are maintained. Oxidation is carried out by oxidizing agents in solution. Nitric, perchloric, sulfuric and hydrochloric acids and combinations of these are usually used to digest the samples. Dissolution times again vary with sample size, but they are considerably less than in dry ashing. In the low temperature oxygen plasma technique, oxidation is accomplished by placing the sample in an oxygen plasma which serves as the oxidizing agent. The plasma is achieved by irradiating oxygen with radio frequency radiation which ionizes the oxygen creating a plasma. Low temperatures, less than 300°C, are maintained. Oxidation times are usually longer than acid digestion yet less than the dry ashing technique. Once the sample is completely oxidized the residue is usually diluted with water and determinations of trace metals made.

Colorimetric and spectrophotometric techniques depend upon the

development of precise colors under carefully controlled pH conditions. In the analysis of lead at the trace level in biological samples, it is usually necessary to preconcentrate and extract the metal using dithionite or ammonium 1-pyrrolidine carbodithionate (APDC) and methyl isobutyl ketone (MIBK). The use of a large number of reagent blanks is necessary. Large sample sizes are required and the total analysis time, because of the extensive sample pretreatment, is quite long.

The most popular method of trace metal analysis in biological samples is conventional flame atomic absorption. Extraction and preconcentration steps are usually used to alleviate chemical and spectral interferences and to achieve better detection limits. Large sample sizes are usually necessary which can represent a real handicap for clinical samples such as blood, urine, spinal and lymph fluids.

Non-flame atomic absorption represents the most promising method for trace metal analysis. The high sensitivities and low detection limits coupled with very small sample sizes make this technique the most versatile method for analysis of trace metals in biological systems. Chemical and spectral interferences resulting from the complicated sample matrix can be eliminated or at least reduced to an acceptable level.

Statement of the Problem

Airborne lead from vehicle exhausts is the main source of lead contamination in urban areas. The lead level near highways is dependent upon the proximity of the roadway and the traffic volume. Vegetation and soil samples near highways show that lead concentrations increase with traffic volume and decrease with distance from the highway. U. S. Highway 191 parallels the West Gallatin River very closely for its entire route within Gallatin Canyon. The average daily traffic volume is approximately 1000 vehicles.²⁷ Although the canyon can hardly be described as urban, the fish within the river have been exposed to lead containing exhaust emissions.

The determination of the lead content of tissues from these fish may indicate to what extent lead has accumulated as a result of this exposure.

In unique locations where fish have not been exposed to lead containing exhausts it should be possible to establish baseline lead levels in their tissues. Fish from the Federal Fish Hatchery, northeast of Bozeman and from the West Gallatin River within Yellowstone Park have not been directly exposed to vehicle exhausts.

A comparison of the lead tissue concentrations of these fish to those from within the canyon may establish to what extent the levels vary in a biological system as a result of exposure to lead containing vehicle exhaust emissions.

The scope of this research project was four fold:

- 1) To establish the lead content in tissues of fish that have been exposed to leaded exhausts;
- 2) To establish baseline lead tissue levels in fish that have not been exposed to leaded exhausts;
- 3) To determine what effect, if any, the lead emissions have had on this biological system;
- 4) To develop a sensitive, accurate and reliable method of analysis for lead in fish bone, gill, liver and muscle tissues using an acid digestion procedure and furnace atomic absorption.

EXPERIMENTAL PROCEDURES

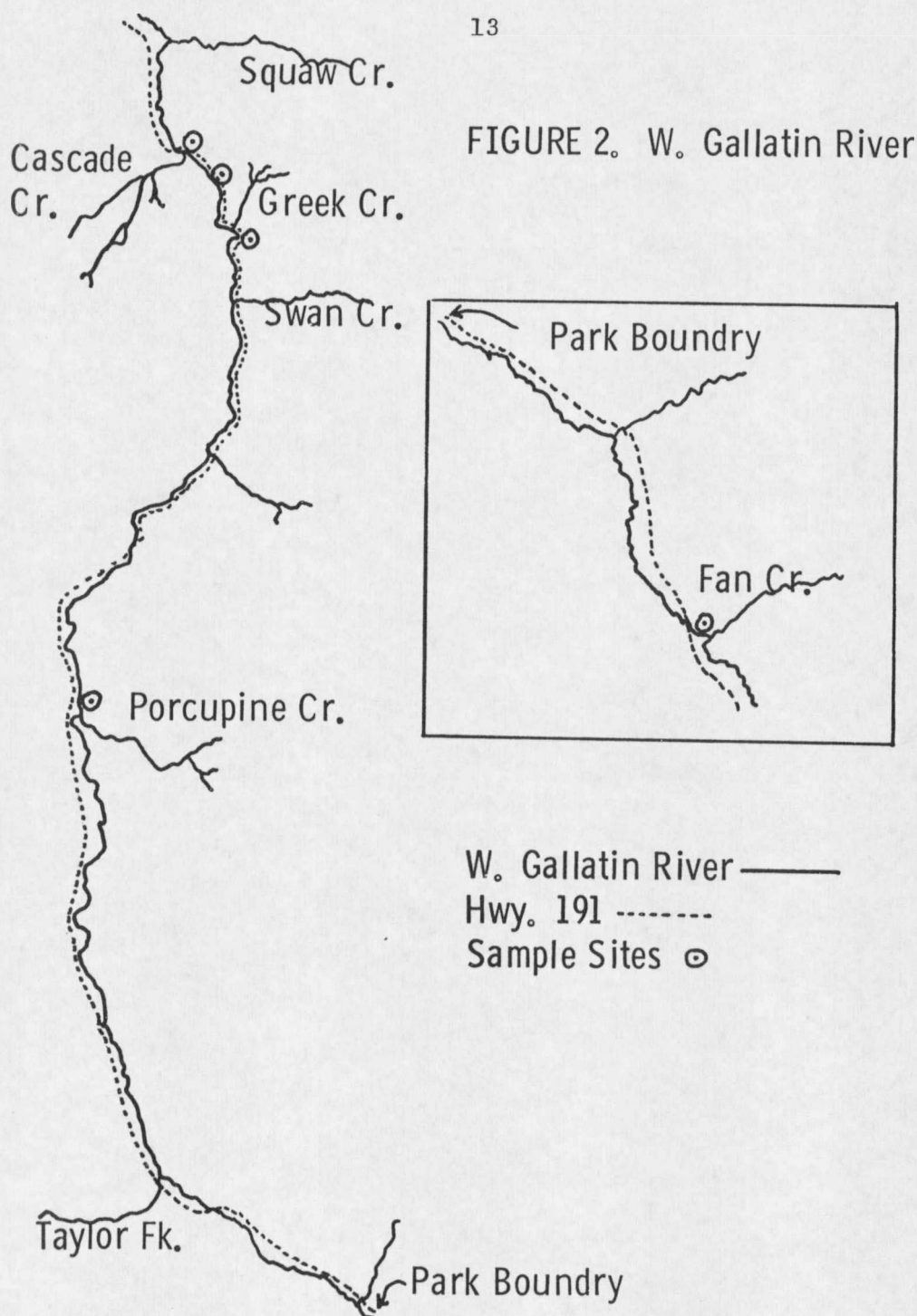
Sample Selection

Fish were caught by the standard hook and line method by G. K. Pagenkopf and D. R. Neuman from six locations along the West Gallatin River. Figure 2 illustrates a map of the West Gallatin River. Four sample collection sites are within Gallatin Canyon: south of Cascade Creek; near the mouth of Cascade Creek; south of Greek Creek and near the mouth of Porcupine Creek. Samples were also taken in Yellowstone Park near the mouth of Fan Creek and north of the town of Gallatin Gateway. All fish taken from the river and analyzed were native. Planted fish have had their dorsal adipose fin clipped prior to being planted. This adipose fin will not grow back.²⁸ Fish samples were also collected from the Federal Fish Hatchery in Bridger Canyon, northeast of Bozeman.

Of a total of 39 fish, 30 were Rainbow Trout (Salmo gairdneri), 4 were Cutthroat Trout (Salmo clarki), 3 were Brown Trout (Salmo trutta) and two were Whitefish (Coregonus williamsi). The fish samples were labeled according to type, date and location of catch and frozen as soon as possible after being caught. Water samples were collected in polyethylene bottles, from the same locations as the fish were taken. The water samples were acidified with nitric acid to assure the solubility of any lead salts.

Glassware Preparation

It was essential that all glassware be immaculately clean to avoid



any contamination. Nine 100.0 ml volumetric flasks, twelve 50 ml pyrex beakers and an assortment of pipettes were bought new and used exclusively for lead analysis. Prior to its initial use all the glassware was washed in doubly distilled water and soaked in hot concentrated HNO_3 . Between usage the glassware was washed and rinsed in doubly distilled water and soaked in hot concentrated nitric acid.

Reagents and Sample Preparation

Redistilled HNO_3 and HClO_4 were used to wet ash the tissue samples. Lead nitrate, that had been dried for two hours, was used to prepare the standard lead solutions. All dilutions were made with doubly glass distilled conductivity water. Sample fish were removed from the freezer and allowed to thaw at room temperature. The total fish weight and length were recorded. Gills, liver tissue and sections of backbone and muscle tissue were removed by dissection and freeze dried by a Thermovac lypholizer. Approximately one gram of the dried tissue was weighed and then digested in a mixture of 7.00 ml of concentrated HNO_3 and 5.00 ml of concentrated HClO_4 . The solutions were slowly heated on a hot plate until all foaming had stopped and dissolution was achieved. At this point the temperature was increased so as to reduce the volume to about 1 ml. This was accompanied by copious fuming of HClO_4 . The maximum temperature was 88°C . The usual precautions were maintained when using perchloric acid to digest organic material. The colorless samples were then analytically transferred to cleaned

100.0 ml volumetric flasks and diluted to volume. A reagent blank solution and standard curve check solution were concurrently digested with all samples. Total analysis time, including lypholization, for 8 samples was approximately 8 hours.

Apparatus

Furnace atomic absorption spectroscopy²⁹⁻³⁴ was used to analyze the fish tissue samples for traces of lead. The furnace design is shown in Figure 3. It is heated by a G.E. arc welder to an internal temperature of 1800°C. Argon gas, at a flow rate of 35 ml/min, was continuously flushed through the furnace to prevent the oxidation of the graphite and to carry out hot residual gases.

Radiation from the hollow cathode tube, set at 12 ma, was chopped at 400 cycles/sec using a PAR model BZ-1 light chopper. A PAR HR-8 lock-in amplifier is tuned on that frequency. The light passed through the heater tube of the furnace and was focussed on the entrance slit (110 μ m) of a Spex 3/4 meter Czerny-Turner spectrophotometer. The amplified signal from the photomultiplier was monitored by a Tecktronics type RM503 oscilloscope and displayed on a Honeywell 6-inch recorder. The Spex monochromator was set at the 2170 Å⁰ lead line. An Eppendorf pipette was used to transfer 20 μ l of the sample into the graphite cups. The cups were placed under an IR light and heated until the solvent had evaporated. The cup was introduced into the furnace through the sample port, and rested at the inner end of the side tube. Its

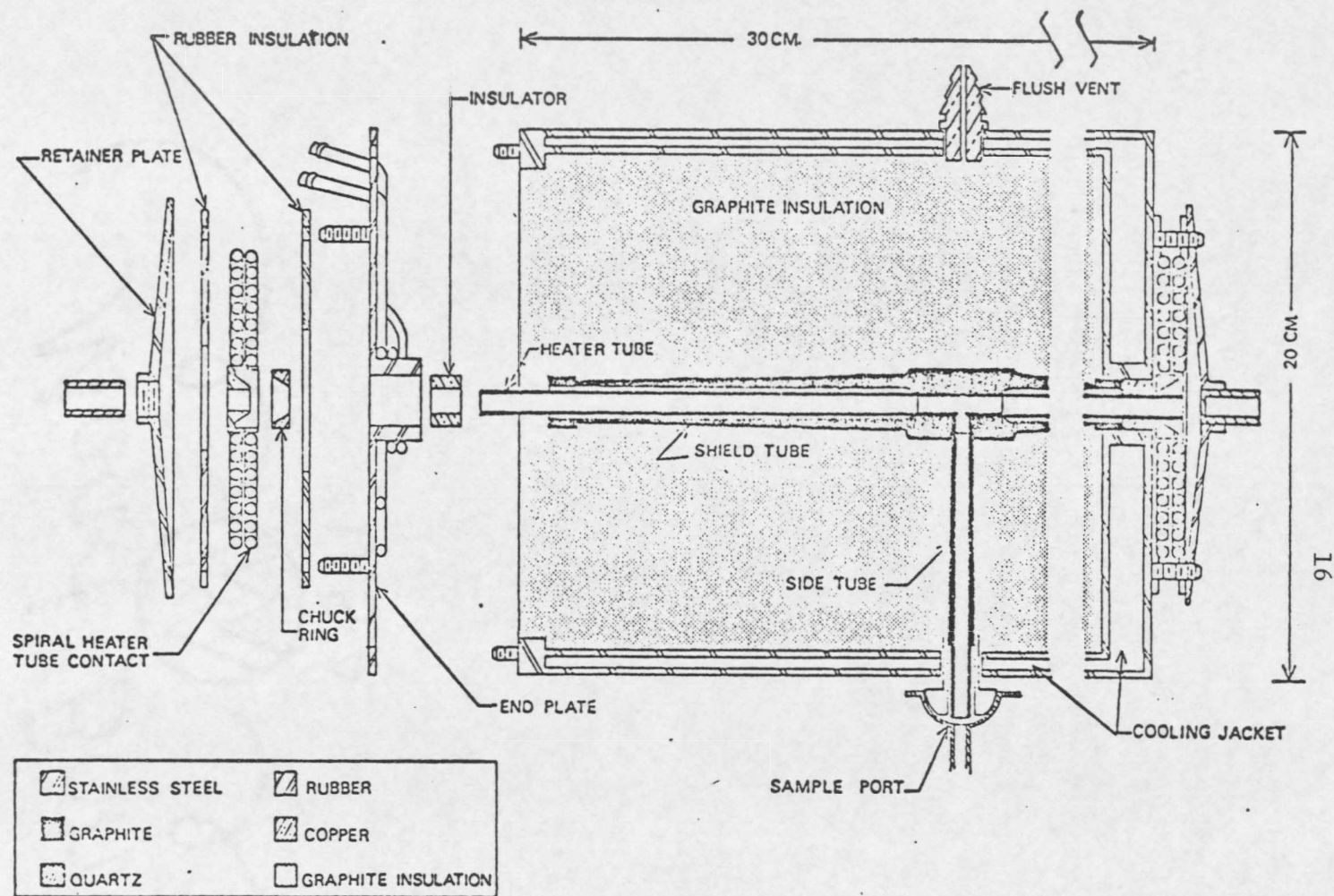


Figure 3. The Furnace

contents were immediately atomized into the heater tube. The peak height was read as a function of absorbance and compared to standard curves. Prior to use each graphite cup was heated to 1850°C in the furnace. This eliminated any absorbance due to contamination.

RESULTS and DISCUSSION

Analytical Method

Before the actual analysis for lead in the tissue samples could be made, it was necessary to determine the internal furnace temperature that would provide the greatest sensitivity for a constant concentration of lead.³⁵ Table I shows the relationship between furnace temperature and absorbance for a given concentration of lead. The table indicates that the maximum sensitivity is achieved near 1800°C.

Table I. Furnace Temperature vs Absorbance for
 8×10^{-10} grams of lead

Furnace Temperature	Average Absorbance
1150°C	.185
1300°C	.370
1450°C	.505
1600°C	.645
1750°C	.680
1850°C	.695
1900°C	.663

Early in the experimental work the analysis of tissue samples were exhibiting severe chemical interferences. The HClO_4 which was used in the acid digestion procedure gave rise to non-specific absorption. If the graphite cups were not heated under the IR lamp for a long enough period or at a sufficient temperature, the HClO_4 would not be completely driven off. When the cups were inserted into the furnace the HClO_4

would "fume," masking the true atomic absorption signal. This caused high, inconsistent absorbance values. Table II shows that three different chemical matrices were used to optimize the time and temperature of drying. The temperature under the drying lamp was varied from 90°C to 190°C. The drying time was varied from 15 to 30 minutes. Higher temperatures were achieved using a muffle furnace. A standard aqueous solution of lead was prepared so that 20 μl gave a concentration of 2.0×10^{-10} g Pb. The absorbance value of this concentration, .170, was unaffected by drying at various temperatures-times. This is illustrated in column three of the table. 20 μl of the reagent blank, which contained the digesting acid HNO_3 and HClO_4 , gave the absorbance values shown in column four. At low temperatures-short drying times these values are high, indicating interference from the HClO_4 . Lead was added to the acid containing reagent blank so that 20 μl provided a concentration of added 2.0×10^{-10} g Pb. This solution gave absorbance values shown in the last column. The values are high at low temperatures-short drying times. At very high temperatures the absorbance values are lower than what is expected from the added lead alone; this indicates that the metal is being lost due to volatilization at these temperatures. At a temperature of 190°C and a drying time of 30 minutes the absorbance value of the reagent blank solution was reduced to .177. Although this value was relatively high, it was consistent, having a deviation of $\pm .003$ at the 95% confidence level. The optimum

Table II. Drying Temperature-Time vs Absorbance

Temperature under drying lamp	Time cups dried under lamp	2.0×10^{-10} g Pb (a)	Absorbance	
			Reagent blank (a)	2.0×10^{-10} g Pb, reagent blank (a)
90°C	15 min	.170	.958	1.000
100°C	15	.170	.886	.920
140°C	15	.170	.782	.744
190°C	15	.170	.769	.657
90°C	20 min	.170	.732	.853
100°C	20	.170	.688	.782
140°C	20	.170	.647	.699
190°C	20	.170	.408	.602
90°C	30 min	.170	.508	.699
100°C	30	.170	.356	.515
140°C	30	.170	.214	.414
190°C	30	.170	.177	.347
* 295°C	15 min	—	.031	.026
* 340°C	15	—	.022	.004
* 400°C	15	—	.017	.031
* 450°C	15	—	.044	.004

* cups placed in muffle furnace

(a) all volumes, 20 μ l

conditions of drying temperature and time were set at 190°C and 30 minutes.

The analytical standard curve is shown in Figure 4. The standard lead solutions for this plot range in concentration from 0.25 to 5.00 $\times 10^{-5}$ g/l. A 20 μ l aliquot of each provided the concentration range, 0.50 to 10.0 $\times 10^{-10}$ g lead. Two additional analytical curves were obtained from standard addition data and lead added to the acid digestion reagents. These are illustrated in Figure 5. The intercept of the lead-acid digestion curve is .177. This is the absorbance value for the reagent blank. The intercept of the plot obtained from standard addition data is .388. This value is higher as it represents the reagent blank value and also the lead naturally occurring in the tissue sample. All three exhibit linearity in this concentration range. The slope of the plot in Figure 4 is 0.88×10^9 . The slopes of the other two are 0.86 and 0.87×10^9 . The lead sensitivity obtained by furnace atomic absorption is many times better than that obtained by conventional flames. The absolute sensitivity, which is the calculated lead concentration that gives an absorbance value of .004 (1%A) is 5.5×10^{-12} g Pb. A detection limit of 0.15 ng/g is calculated as twice the standard deviation at the 95% confidence level for the reagent blank. The excellent sensitivity and low detection limit results in part from the fact that the atoms are confined in a long non-turbulent light path for a greater length of time.

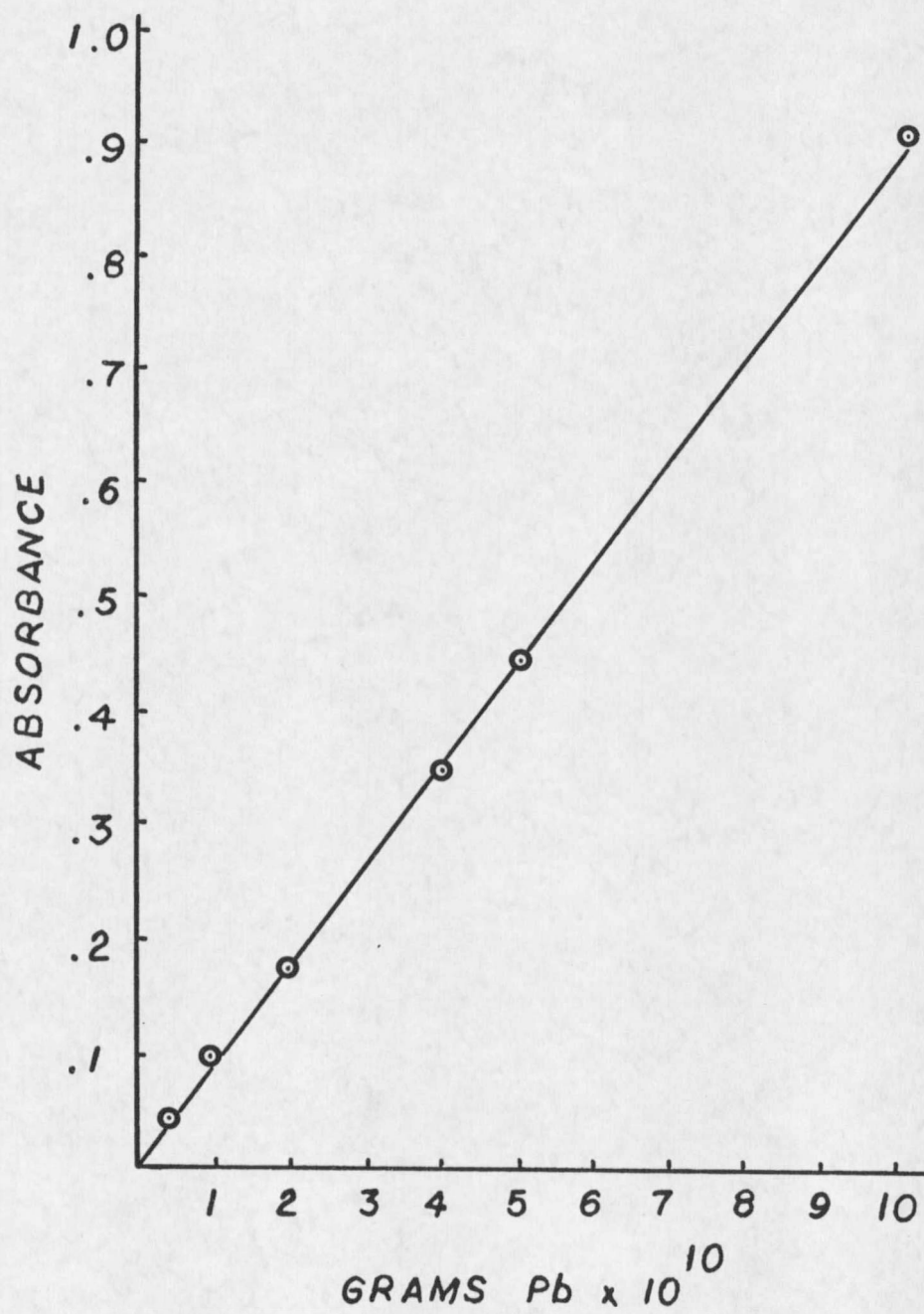


Figure 4. Standard Curve for Lead

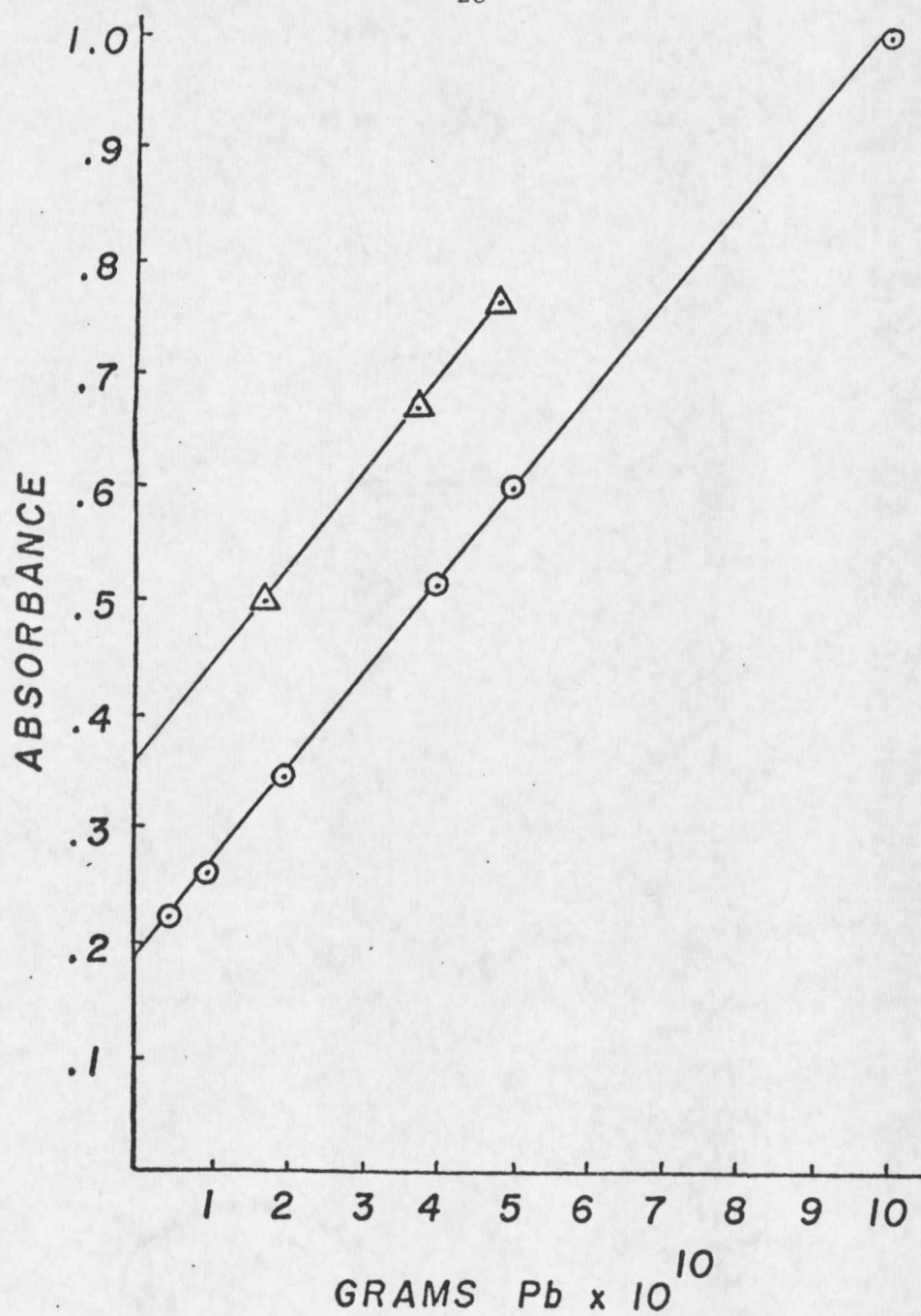


Figure 5. Calibration Curves for Lead Added to Reagent Blank , and from Standard Addition Data

The accuracy of this method has been evaluated using standard addition. A known quantity of lead was added to approximately equivalent samples, 1 g, of dried muscle tissue. The samples were then digested and absorbances determined. The results are presented in Table III. The values are based on at least 5 determinations, and all deviations are at the 95% confidence level. The first column represents the Pb found in muscle tissue in $\mu\text{g/g}$. Predicted lead is the sum of the naturally occurring lead (1st column) and the amount of lead added. The last column represents the ratio of Pb found to Pb predicted, or the fraction recovered. The data in Table III indicate that virtually no lead is being lost during the procedure. The data also show one part intrinsic lead can be determined in the presence of 49 parts added Pb. The lead present naturally in these fish muscle tissues ranges from 0.12 to 1.81 $\mu\text{g/g}$. The percent recovery ranges from 95 to 102 with a central tendency of 99%. The deviation at the 95% confidence level is about $\pm 6\%$. The relative standard deviation for the lead in muscle tissue is $\pm 5\%$. Over this range of lead concentration it is concluded that the accuracy of the method exceeds the obtainable precision. These recovery results are consistent with experiments using ^{212}Pb with the $\text{HClO}_4\text{-HNO}_3$ wet ashing technique of cocoa.³⁶

The method presented in this work has the advantage that the quoted sensitivity and the detection limit can be obtained with real as well as synthetic samples. The linearity and slopes of the three

Table III. Recovery of Lead Added to Fish Samples

<u>Pb in Muscle Tissue</u>	<u>Pb Added^a</u>	<u>Pb Predicted^a</u>	<u>Pb Found^a</u>	<u>Pb Found/ Pb Predicted</u>
1.81 ± .14	0.00 ± .00	—	1.81 ± .14	—
1.81 ± .14	0.43 ± .01	2.24 ± .14	2.28 ± .08	1.017 ± .072
1.81 ± .14	0.90 ± .01	2.71 ± .14	2.72 ± .13	1.004 ± .071
1.81 ± .14	1.60 ± .01	3.41 ± .14	3.33 ± .05	0.977 ± .043
0.77 ± .07	0.00 ± .00	—	0.77 ± .07	—
0.77 ± .07	3.33 ± .01	4.10 ± .07	3.99 ± .01	0.973 ± .017
0.77 ± .07	1.36 ± .01	2.13 ± .07	2.10 ± .16	0.986 ± .082
0.12 ± .10	0.00 ± .00	—	0.12 ± .10	—
0.12 ± .10	2.27 ± .01	2.39 ± .10	2.38 ± .22	0.995 ± .10
0.12 ± .10	4.88 ± .01	5.00 ± .10	5.00 ± .16	1.000 ± .038
0.12 ± .10	8.84 ± .01	8.96 ± .10	8.52 ± .18	0.952 ± .023

^alead in $\mu\text{g/g}$ dry weight, deviations at 95% confidence level

analytical curves indicate that this system is relatively free of any matrix effects. Similar analytical curves using the carbon rod atomizer and the tantalum boat assembly do not exhibit comparable linearity. These techniques are also sensitive to the volume of analyte used. This is not a problem with the furnace since the solvent is evaporated prior to atomization.

The detection limit for lead by the carbon rod method is 20×10^{-12} g Pb.³⁷ The tantalum boat procedure³⁸ reports a detection limit of 1.19 μ g/100 ml and an absolute sensitivity of 0.1×10^{-9} g Pb. The atom reservoir technique has a detection limit of 0.3 ng/g.²³

Many of these other non-flame techniques exhibit severe chemical and spectral interferences^{22,23,24} which arise partly from the complicated chemical matrix of the ashed biological sample and from the method of drying and ashing of the sample. In the carbon rod atomizer method drying, ashing and atomization of the sample all take place on the carbon rod in successive programmed steps. This is an attempt to reduce analysis time by using non-pretreated samples of very small size, 5-20 μ l. In the case of Pb determination in red blood cells²² the matrix elements cannot be removed completely during the ashing cycle and thus a weak nonatomic absorption signal is superimposed on the atomic absorption peak. A deuterium background corrector has been successfully used to alleviate this problem.

In the atom reservoir technique²³ the matrix effects were so

severe that a solvent extraction was necessary to achieve the excellent detection limit. The tantalum boat procedure²⁴ also included a solvent extraction step before the analysis could be successfully carried out.

In this procedure we have separated the dissolution, drying and atomization steps and it is believed that this results in a minimization of any matrix interferences. The advantages of this method include superior sensitivity, accuracy and reproducibility. There is no need for large samples, tedious extraction or preconcentration steps. Since acid digestion is used, the method is readily amendable to the analysis of any type of tissue or bone sample.

Lead Levels in Fish Tissues

The relationship between fish age and length was obtained from the State Fish and Game Department²⁸ and is illustrated in Table IV.

Table IV. Fish Age vs Average Length

<u>Fish Age</u>	<u>Average Length</u>
1 year	5.2"
2 years	7.7"
3 years	9.4"
4 years and older	12.0"

These values are based on fish samples from the West Gallatin River within Gallatin Canyon. The fish samples from the Federal Fish Hatchery and from the Gallatin River in Yellowstone Park were grouped by their length alone.

The values of the lead concentrations in the fish tissues are presented in Table V. The data is arranged according to increasing fish age. The total fish weight, length, location of collection and type are also given. All values were determined on the basis of dry sample weight and are quoted in $\mu\text{g Pb/g}$ sample. The location of collection and fish type are coded. The key is at the bottom of the table. The range of lead values is quite large; from $0.07 \mu\text{g/g}$ in the liver tissue of a Rainbow Trout from the Hatchery to $7.65 \mu\text{g/g}$ in the liver tissue of a Rainbow Trout from the West Gallatin south of Greek Creek.

Table V. Lead Levels in Fish Tissue

Length Inches	Est. Age	Weight grams	Location Type	$\mu\text{g Pb/g sample}$			
				Bone	Gill	Liver	Muscle
6.0	1	73.4	C3/R	1.36	1.32	2.25	—
6.5	1	70.0	C2/R	3.96	1.24	6.61	—
7.0	1-2	70.2	C1/R	—	3.07	—	—
7.5	1-2	95.6	C2/R	4.03	1.94	0.20	—
7.5	1-2	90.4	C3/R	0.57	0.50	7.65	—
8.0	2	89.0	C2/R	4.04	3.04	2.95	—
8.0	2	106.0	C3/R	3.49	5.72	2.10	—
8.0	2	99.6	H/R	0.33	0.42	0.07	—
8.5	2	123.2	H/R	2.45	0.31	0.96	—
8.5	2	116.5	C3/R	1.53	1.16	0.97	—
9.0	2-3	123.4	C4/CT	2.67	2.33	0.80	—
9.25	2-3	155.7	P5/CT	2.25	1.78	1.63	0.74
9.5	3	160.3	C1/R	3.56	2.12	1.96	—
9.5	3	154.6	C1/R	3.33	2.56	2.02	—
9.75	3	152.4	C1/R	6.62	—	1.11	—
10.0	3	201.0	C2/R	6.29	—	—	—
10.0	3	180.0	H/R	0.43	0.71	0.10	—
10.0	3	192.4	H/R	0.61	1.05	1.31	0.12
10.5	3	260.0	P5/CT	2.50	1.65	1.42	—
11.0	3-4	220.6	C3/R	4.81	0.76	2.14	—
11.0	3-4	294.5	C3/R	2.72	2.11	1.27	—

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Table V. (cont.)

Length Inches	Est. Age	Weight grams	Location Type	$\mu\text{g Pb/g sample}$			
				Bone	Gill	Liver	Muscle
11.5	3-4	294.1	C3/R	2.76	2.07	1.30	—
11.5	3-4	307.1	C4/R	3.30	4.21	1.70	1.34
11.5	3-4	172.2	C2/R	2.55	2.43	—	—
12.0	4	390.6	C4/B	5.11	2.53	1.43	1.40
12.0	4	305.0	P5/CT	3.15	3.06	2.89	—
12.5	4	453.4	C2/R	4.97	2.55	—	—
12.5	4	343.5	C1/R	5.56	—	—	0.77
13.0	4-	540.0	P5/W	2.56	2.00	2.43	—
14.0	4-	436.4	C3/W	4.35	1.47	0.21	—
14.0	4-	460.5	G/B	4.45	3.11	3.28	—
14.0	4-	443.0	C1/R	6.08	1.08	—	1.81
14.5	4-	750.0	P5/B	3.20	2.12	2.00	—
15.0	4-	415.6	C4/R	6.20	7.40	2.50	—
15.5	4-	558.2	C3/R	2.78	2.78	0.97	—
18.0	4-	740.0	G/R	4.98	6.55	—	—

C - Canyon
P - Park
H - Hatchery
G - North of Gallatin Gateway

R - Rainbow Trout
B - Brown Trout
CT - Cutthroat Trout
W - Whitefish

1 - South of Cascade Creek
2 - Near Mouth of Cascade Creek
3 - South of Greek Creek
4 - Near Mouth of Porcupine Creek
5 - Near Mouth of Fan Creek

Table VI presents the average lead levels for all tissue samples, arranged by location and tissue type. The ranges and the number of tissues analyzed are also given. Since the lead values are grouped together, regardless of the age of the fish, the ranges are appreciably large. In most cases the standard deviation is larger or at least comparable to the average value. Several of the individual lead values deviate from their means by more than twice the standard deviation. This clearly indicates that further differentiation on the basis of age must be made.

Table VI. Average Lead Levels in Tissues by Location

Canyon Fish			
<u>Tissue</u>	<u>Average</u> ^(a)	<u>Range</u>	<u>Number of Samples</u>
Bone	3.86 ± 1.64	0.57 - 6.62	24
Gill	2.47 ± 1.66	0.50 - 7.40	22
Liver	2.11 ± 1.97	0.20 - 7.65	19

Park Fish			
<u>Tissue</u>	<u>Average</u> ^(a)	<u>Range</u>	<u>Number of Samples</u>
Bone	2.73 ± .41	2.25 - 3.20	5
Gill	2.12 ± .55	1.65 - 3.06	5
Liver	2.02 ± .59	1.42 - 2.89	5

Hatchery Fish			
<u>Tissue</u>	<u>Average</u> ^(a)	<u>Range</u>	<u>Number of Samples</u>
Bone	0.95 ± 1.00	0.33 - 2.45	4
Gill	0.62 ± .33	0.31 - 1.05	4
Liver	0.61 ± .62	0.07 - 1.31	4

(a) lead values in $\mu\text{g/g}$, dry weight

However, some general tendencies are indicated. The highest lead level occurs in bone tissue of fish from within the Canyon. This value, $3.86 \pm 1.64 \mu\text{g/g}$, is higher than the lead bone content of fish from the Park, $2.73 \pm .41 \mu\text{g/g}$ and samples from the Hatchery, $0.95 \pm 1.00 \mu\text{g/g}$. This same general trend is also indicated in the gill and liver tissues. That is, the average lead concentration in gill tissue is greatest in Canyon samples at $2.47 \pm 1.66 \mu\text{g/g}$, while those from the Park show a gill lead content of $2.12 \pm .55 \mu\text{g/g}$. The average lead level in gill tissue of Hatchery fish is $0.62 \pm .33 \mu\text{g/g}$. In liver tissue the average lead concentrations are 2.11 ± 1.97 , $2.02 \pm .59$ and $0.61 \pm .62 \mu\text{g/g}$ for Canyon, Park and Hatchery samples respectively.

Two other important trends are indicated from the data. First, the average values of the lead tissue concentrations of Hatchery fish are on the order of three to four times lower than values in similar tissues of fish from the Gallatin River. Secondly, within each designated location the lead concentration in bone tissue is the highest, followed by gill, and the lowest values are exhibited by liver tissue. This implies that more of the metal is being stored in the skeleton than in the other organs. These lead concentrations are in good agreement with other values of lead in fish samples, in that they are all in the p.p.m. range. Values reported for salmon, Tuna, oyster and sardine are 1.3, 2.2, 1.2, and $4.3 \mu\text{g/g}$ respectively.¹³ Others are lobster, $2.50 \mu\text{g/g}$, fresh shrimp, $0.45 \mu\text{g/g}$ and scallops, $0.13 \mu\text{g/g}$.¹² These

last three values are on the basis of wet weight and reflect lower lead concentrations.

In order to look more closely at the data, Tables VII, VIII, and IX present the average lead tissue concentrations grouped according to location, tissue type and age bracket. Within each age group, fish from the Canyon area exhibit the highest lead concentrations in bone tissue, Table VII. For example, in the 2-3 year-old bracket the average lead level is 4.49 ± 2.10 $\mu\text{g/g}$, while bone tissue of Park samples show a 2.37 $\mu\text{g/g}$ level. The Hatchery fish bone tissue content is much lower at 0.52 $\mu\text{g/g}$. The data also indicate that there is an accumulation of lead with age.

Table VII. Lead in Bone Tissue

<u>Est. Age</u>	<u>Canyon</u>		<u>Bone Tissue</u>		<u>Hatchery</u>	
	<u>Average</u>	<u># samples</u>	<u>Average</u>	<u># samples</u>	<u>Average</u>	<u># samples</u>
1-2	2.71 ± 1.49	7	—	—	1.39	2
2-3	4.49 ± 2.10	5	2.37	2	0.52	2
3-4	3.96 ± 1.44	8	3.15	1	—	—
4-	4.85 ± 1.60	4	2.88	2	—	—

In samples from the Canyon area the average value of lead in 1-2 year old fish is 2.71 ± 1.49 $\mu\text{g/g}$. This value is 4.85 ± 1.60 $\mu\text{g/g}$ in fish 4 years and older. Although the samples in the 2-3 year old group are higher than those in the 3-4 year old bracket, the standard deviation is large indicating that some of the values may be out of line for this

age group. In bone tissues from Park fish the trend of increasing lead levels with increasing age is also demonstrated. However, there is only 1 bone tissue sample in the 3-4 year old group. Its value, 3.15 $\mu\text{g/g}$, does not fit this trend, but since it is only one sample the value may not be representative. In Hatchery samples there appears to be a decreasing concentration of lead with increasing age. However, there are two tissue samples in each age group. The value of 1.39 $\mu\text{g/g}$ in the 1-2 year old age bracket is an average of 0.33 and 2.45 $\mu\text{g/g}$. The high value of 2.45 $\mu\text{g/g}$ may indicate an anomaly in an individual fish.

The accumulation of lead with age has been well established in humans,¹² with the highest values occurring in the fifth to sixth decade of life in human organs.

In gill tissue, within each age group, the lead concentrations are again greatest in fish from the Canyon. This is illustrated in Table VIII. This general trend is broken in the 3-4 year old age bracket, where the gill tissue of one sample from the Park shows a higher lead level than the average value for Canyon fish. The lead level of 3.06 $\mu\text{g/g}$ is within the range of the lead content of Canyon samples. The lead concentration in the gill of this one fish may not be indicative of the true level as it is the same fish that exhibited the high lead bone level of 3.15 $\mu\text{g/g}$. The Hatchery tissues show a much lower lead concentration at 0.36 and 0.88 $\mu\text{g/g}$ in respective age

groups. The gill tissues in Table VIII demonstrate a slight tendency to accumulate lead with age. The Canyon Samples increase from $2.25 \pm 1.39 \mu\text{g/g}$ in young fish to $3.18 \pm 2.90 \mu\text{g/g}$ in older fish. The Hatchery show the same trend. There is a definite increase from 0.36 to $0.88 \mu\text{g/g}$ in their gill tissues. The lead levels in Park fish appear to increase with age. The one value, $3.06 \mu\text{g/g}$, from the 3-4 year old age group is out of line as indicated before.

Table VIII. Lead in Gill Tissue

Est. Age	Canyon		Park		Hatchery	
	Average	# samples	Average	# samples	Average	# samples
1-2	2.25 ± 1.39	7	—	—	0.36	2
2-3	$2.33 \pm .21$	3	1.71	2	0.88	2
3-4	2.38 ± 1.01	7	3.06	1	—	—
4-	3.18 ± 2.90	4	2.06	2	—	—

Table IX illustrates the lead concentrations in liver tissue. In the 2-3 year old age bracket the lead levels of Canyon fish and Park samples are nearly equivalent, $1.47 \pm .61 \mu\text{g/g}$ and $1.52 \mu\text{g/g}$ respectively. Hatchery samples in the same age group exhibit a level of $0.70 \mu\text{g/g}$ which is approximately one-half of the above values. Lead accumulates with age in Hatchery samples and fish from the Park. There appears to be reversal of this trend in fish liver tissue from the Canyon. In the 1-2 year old age group the level is $3.39 \pm 2.81 \mu\text{g/g}$. Two values,

6.61 $\mu\text{g/g}$ and 7.65 $\mu\text{g/g}$ are included in this average. These values, especially in this age group, seem unreasonably high. If they were to be ignored the average would drop to $1.28 \pm 1.10 \mu\text{g/g}$. This may represent a more realistic lead level for fish liver tissue in this age bracket. The older fish from the Canyon follow the trend more closely.

Table IX. Lead in Liver Tissue

<u>Est. Age</u>	<u>Liver Tissue</u>		<u>Liver Tissue</u>		<u>Liver Tissue</u>	
	<u>Canyon</u>	<u>Park</u>	<u>Canyon</u>	<u>Park</u>	<u>Hatchery</u>	<u>Hatchery</u>
	<u>Average</u>	<u># samples</u>	<u>Average</u>	<u># samples</u>	<u>Average</u>	<u># samples</u>
1-2	3.39 $\pm$ 2.81	7	—	—	0.51	2
2-3	1.47 $\pm$.61	4	1.52	2	0.70	2
3-4	1.57 $\pm$.35	5	2.89	1	—	—
4-	1.23 $\pm$ 1.16	3	2.21	2	—	—

In order to obtain more information about the fish lead environment, water samples from the River and Hatchery were analyzed for the metal. The water used at the fish Hatchery is from natural hot and cold water springs. The water is stored in large holding tanks which are covered. There is a mixing tank for the warm and cold water so that a constant temperature is maintained. The water is piped to troughs where the fish are maintained and fed. Water samples were collected from locations along the W. Gallatin and from different area within the hatchery complex. These values are presented in Table X.

Table X. Lead Levels in River and Hatchery Water

<u>Location</u>	<u>Lead Concentrations ng/ml</u>
W. Gallatin River	
North of Gallatin Gateway	2.93
North of Cascade Creek	2.56
South of Cascade Creek	2.94
North of Greek Creek	2.65
South of Greek Creek	2.96
Porcupine Creek Area	2.81
Fan Creek Area	2.65
Fish Hatchery	
Hot Springs	1.38
Mixed Hot and Cold Springs	1.42
Mixed Hot and Cold Springs	1.41
Fish Tank	1.39
Fish Tank	1.44

The lead concentrations in the River are at the low range of values exhibited in other rivers in the U. S.⁴⁰ The national average lead level in major rivers is 23 ng/ml, with a minimum value of 2ng/ml. The average value of lead in the water from the West Gallatin is 2.81 ng/ml. These lead water levels are three orders of magnitude lower than the lead concentration that will have damaging effects on the fish. A lead concentration of 25 μ g/ml will retard Brook Trout growth.⁴¹ At 10 μ g/ml there is no effect on their growth.

SUMMARY AND CONCLUSIONS

Analytical Method

The analytical method presented in the project demonstrates that lead can be analyzed at trace concentrations in biological samples using an acid digestion procedure and furnace atomic absorption. The analysis was direct - there was no need for extraction or preconcentration steps. From standard addition data the accuracy of the method was evaluated at $99\% \pm 6\%$. Matrix interferences were absent. This was indicated by the similar slopes of the three analytical standard curves. The use of small samples makes the method applicable to the analysis of other types of biological samples where size may be the most critical consideration. Since acid digestion was used, the method is amenable to the analysis of other metals, both those considered toxic to biological systems and those which are necessary for the optimal function of growth, healing and the activity of many metabolic processes. The excellent sensitivity and low detection limit makes the furnace atomic absorption technique one of the most advantageous methods for the analysis of trace metals in biological systems.

Lead Levels in Fish Tissues

Several general trends in the lead tissue concentrations of fish have been established as a result of this study. First, Canyon fish exhibit higher lead concentrations in their bone, gill and liver tissues than fish from the Park and the Fish Hatchery. Second, Hatchery lead tissue levels are approximately three to four times lower than

similar tissues from the Park and the Canyon fish. This is undoubtedly the result of their closely controlled environment. Third, lead appears to accumulate with age in all three tissues studied. Fourth, the lead concentrations are highest in bone tissue, followed by gill tissue and liver tissue. This indicates that more lead is stored in the skeleton than in the other two organs.

All of the values are in the p.p.m. range which indicates that there is not a significant stress in any of the fish due to lead. The data from Table VI show that the lead bone tissue concentration in Canyon fish is $1.41 \pm .63$ times higher than bone tissue from fish in the Park. In gill tissue the Canyon samples have a lead level $1.16 \pm .84$ times higher than Park fish. The Canyon fish have a liver lead concentration 1.05 ± 1.03 times higher than Park samples. It is important to note here that the standard deviations are large as this data was compiled without regard to fish age. It would be advantageous to have a very large number of samples so that a more complete statistical picture could be drawn. However, it is felt that if there were a large number of samples, the statistics would change, but the general trend would remain. That is, fish samples from within the Canyon have slightly higher lead levels.

In two recent studies, experimental animals were exposed to airborne lead concentrations equivalent to those found in urban areas. It was determined that the inhalation of urban air containing on the

average of $2.5 \mu\text{g Pb/m}^3$ can result in significant elevation of bone lead levels.¹⁷ However, their studies did not disclose any adverse health effects in the animals because of this exposure. Mice⁴² that were exposed to lead levels between 2.6 and $15.6 \mu\text{g Pb/m}^3$ for a 15-month period showed lead bone levels between $14.3 \mu\text{g/g}$ and $20.9 \mu\text{g/g}$ (dry weight). These values are significant when compared to an average value of $10.2 \mu\text{g/g}$ in control animals exposed to an airborne lead level of $0.32 \mu\text{g/m}^3$.

The fish within the Canyon are exposed to lead exhaust emission from the traffic load of 1000 vehicles daily. The slightly higher lead levels in the tissues of these fish is believed to be the result of this exposure. The actual effect of the exposure is minimal at this time. If the traffic load were to increase greatly then it would be expected that the tissue concentrations would increase also.

In conclusion, an important aspect, perhaps the most important, is that the lead levels in a biological system essentially free from the urban environmental level, have been established. These levels may be used as the basis of further study and evaluation of the lead ecological problem.

LITERATURE CITED

1. S. K. Hall, Environ. Sci. Technol., 6, 30 (1972).
2. P. Bryce-Smith, Chem. Brit., 7, 56 (1971).
3. C. C. Patterson, Arch. Environ. Health, 11, 344 (1965).
4. C. C. Patterson, T. D. Salvia, Sci. Citiz., 10, 66 (1966).
5. "Survey of Lead in the Atmosphere of Three Urban Communities," U. S. Department of Health, Education and Welfare, Public Health Service Publication No. 999-AP-12 (1965).
6. M. T. Sterbar, Symposium of Environmental Lead Contamination, Public Health Service Publication No. 1440 (1966).
7. G. L. TerHarr, M. A. Baynard, Nature, 232, 533 (1971).
8. A. L. Mills, Chem. Brit., 7, 160 (1971).
9. R. H. Daines, H. Motto, D. M. Chilko, Environ. Sci. Technol., 4, 318 (1970).
10. H. L. Motto, R. H. Daines, D. M. Chilko, C. K. Motto, Environ. Sci. Technol., 4, 231 (1970).
11. R. J. Bazell, Science, 173, 130 (1971).
12. H. A. Schreoder, et al., J. Chron. Dis., 14, 408 (1961).
13. J. C. Meranger, E. Somers, Bull. Environ. Contam. Toxicol., 3, 360 (1968).
14. R. A. Kehoe, Inst. Publ. Health Hygiene, 24 (1961).
15. H. A. Schreoder, A. P. Nason, Clin. Chem., 17, 461 (1971).
16. E. B. Sandell, Colorimetric Determination of Traces of Metals, Interscience Pub. Inc., p. 388 (1959).
17. R. G. Smith, J. Szajnar, L. Hecker, Environ. Sci. Technol., 4, 333 (1970).
18. Official Methods of Analysis, 10th ed., Assoc. Off. Arg. Chem., p. 367 (1965).

19. H. A. Schreoder, J. H. Tipton, Arch. Environ. Health, 17, 965 (1968).
20. J. B. Willis, Anal. Chem., 34, 614 (1962).
21. H. T. Delves, Analyst, 95, 431 (1970).
22. J. P. Matousek, B. J. Stevens, Clin. Chem., 17, 363 (1971).
23. D. A. Segar, J. L. Gilio, Abstracts, Pittsburgh Conference on Analytical Chemistry, Cleveland, Ohio, March 1972, paper number 35.
24. J. Y. Hwang, S. B. Smithe, et. al., ibid, paper number 37.
25. R. Bersin, et al., Symposium on Trace Characterization, National Bureau of Standards, October 1966.
26. C. Berkley, et al., Transactions of the New York Academy of Sciences, 3, 331 (1967).
27. E. Reiser, Traffic Volume of Highway 191, private communications, Montana State Highway Department, Bozeman, Montana.
28. E. R. Vincent, Project Biologist, Fish Length and Age, private communication, Montana State Fish and Game Department, Bozeman, Montana.
29. R. Woodruff, G. Ramelow, Spectrochim. Acta., 234, 665 (1968).
30. R. Woodruff, R. Stone, Appl. Opt., 7, 1337 (1968).
31. R. Woodruff, R. Stone, A. Held, Appl. Spectrosc., 22, 408 (1968).
32. R. Woodruff, B. R. Culver, K. W. Olsen, Appl. Spectrosc., 24, 250 (1970).
33. R. Woodruff, D. Shrader, Anal. Chem., 43, 1918 (1971).
34. R. Woodruff, J. Lech, Anal. Chem., 44, 1323 (1972).
35. J. Lech, Furnace Temperature in Lead Analysis, private communications, Chemistry Department, Montana State University, Bozeman, Montana.
36. T. F. Gorsuch, Analyst, 84, 135 (1959).

37. M. D. Amos, et al., Anal. Chem., 43, 211 (1971).
38. H. T. Selves, Analyst, 95, 431 (1970).
39. R. O. Becker, J. A. Spadaro, E. W. Berg, Bone and Joint Surg., 50A, 326 (1968).
40. J. F. Kopp, R. C. Kroner, Federal Water Pollution Control Association, Division of Pollution Surveillance, Cincinnati, Ohio (1970).
41. D. Dorfman, W. R. Whitworth, J. Fish. Res. Bd. Can., 26, 2493 (1969).
42. F. G. Heuter, G. L. Couter, et al., Arch. Environ. Health, 12, 553 (1966).

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