THE PERFORMANCE AND CAPABILITIES OF TERRESTRIAL ORGANISMS IN EXTREME AND UNUSUAL GASEOUS AND LIQUID ENVIRONMENTS

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Introduction

by

Alan M. Eshleman

The major conclusion of this study on lead toxicity is that lead enters the Hawaiian environment as an industrial contaminant resulting from the combustion of leaded gasoline. Some of the principle findings in support of this conclusion are listed below:

1. High levels of lead are present in roadside plants (20-1050 μg/g dry matter) and soils (30-4300 μg/g dry matter) from various locations in Honolulu, Oahu. There is a statistically significant relationship between the lead contents of roadside soils and vegetation and their proximity to traffic. Over 400 metric tons of lead as the gasoline additive tetraethyl lead are consumed annually on Oahu. In contrast, roadside plants from the sparsely populated island of Kauai where the annual consumption of leaded gasoline additives is less than 20 metric tons contained 12.5-52.5 μg Pb/g dry matter. Samples of the Hawaiian Silversword, *Argyroxiphium sandwicense* DC, from the slopes of Mt. Haleakala on the island of Maui contained less than 5 μg Pb/g dry matter.

2. The levels of lead in some roadside grasses are high enough to constitute a hazard to grazing animals. In one instance, a horse was discovered grazing on heavily contaminated grass. High levels

of lead found in the isopod *Oniscus* may pose a threat to the birds which feed on this organism.

3. The distribution pattern for lead in the sediments of the Ala Wai Canal and Yacht Harbor in Honolulu suggests either a complicated circulation pattern within the Canal, or, more likely, the existence of more than one source of lead. Some contaminant lead may originate externally to the Canal, perhaps in the combustion of leaded motor fuels.

Continued inputs of lead to the local environment could be halted by a legislative ban on the sale of leaded fuels in the state. Suitable alternatives to leaded fuels exist and it is most unlikely that any oil company would abandon the Hawaiian market rather than supply a line of unleaded fuels. Existing contamination would decrease quite slowly following such a ban, necessitating continued monitoring of exposed areas. Such monitoring would be especially important in contaminated areas that might be put to agricultural uses such as crop production or grazing.

A truly comprehensive picture of the disposition of lead, mercury and other heavy metals in the local environment will require the cooperation of several laboratories and workers. It is this author hope that such a major study can be developed because the Hawaiian Islands offer a unique natural laboratory for trace metal studies. The islands are isolated from mainland North America by over 3200 km of open sea. This isolation makes it much easier to identify inputs to the environment from human activities.

LEAD CONTAMINATION IN URBAN HAWAII

Abstract

Parkland soils and vegetation in Honolulu, Hawaii are heavily contaminated with lead and zinc originating from automobiles. Copper in the same soils and vegetation may also come from automobiles. Some of the lead appears to have entered terrestrial food chains. The lead values for grasses (20-1050 $\mu g\ g^{-1}$) and soils (30-4300 $\mu g\ g^{-1}$) are among the highest reported for exhaust contamination. By comparison, grasses from one of the busiest intersections on the sparsely populated island of Kauai contained 52.5 $\mu g\ g^{-1}$ plant. The background level for lead in Hawaiian vegetation may be at less than 5 $\mu g\ g^{-1}$. The high values on Oahu reflect unusually high traffic densities in the vicinity of the sampling sites, the prevailing winds and the high mean annual temperature.

Introduction

The native soils of the Hawaiian Islands are volcanic in origin and should contain only small amounts of naturally occurring lead. Soils from remote areas of the lightly populated islands Maui and Kauai have a lead content of less than 10 $\mu g\ g^{-1}$. a figure which is close to the probable natural abundance of lead in the earth's crust (*cf.* Bowen 1966, Motto *et al.* 1970, Patterson 1965). Some Hawaiian soils contain amounts of lead which exceed these estimated natural abundance by several orders of magnitude. The source of this lead is the antiknock gasoline additive tetraethyl lead (Jernigan 1968, Eshleman and Siegel 1970, Jernigan, Ray, and Duce 1971).

Jernigan (1968, 1971) measured the amounts of lead present in aerosols at selected sites on the island of Oahu (the City and County of Honolulu) and found a good correlation between these amounts and the average density and proximity of motor vehicle traffic (Table 1). In areas of high traffic density the amounts of aerosol lead were somewhat lower than, but of equal magnitude to, those reported from Los Angeles, California (Eshleman and Siegel, 1970).

In 1970 an investigation was begun to determine the amounts of lead and other metals in soils, vegetation and other biota at various sites in Hawaii. The major part of this study was conducted in the vicinity of the Ala Moana Shopping Center in Honolulu, an area where aerosol lead values as high as 29 $\mu g\ m^{-3}$ have been reported (Jernigan, Ray, and Duce 1971).

Materials and Methods

(A) Plants and other living material

All materials to be analyzed were transferred to the laboratory in sealed polyethylene bags.

Table 1

Lead in Pollution Aerosols from Oahu, Hawaii [a]

Type location	Lead content ($\mu g \cdot M^{-3}$)		
	Minimum	Maximum	Mean
Parking Garage	2.10	29.0	12.45
Urban Curbsides	2.00	13.8	7.73
Other Urban	0.08	6.10	1.64
Rural-Interior and Leeward	0.08	0.29	0.23
Rural-Windward	0.0017	0.0017	0.0017

[a] From Jernigan, Ray and Duce (1971)

Only the leaves of trees or the aerial portions of grasses were chosen for analysis. Root material was avoided because of the difficulty in removing all contaminating soil material from the roots before analysis. Since the metal concentration in surface soils was often many times greater than that in the aerial portions of plants growing in the same soil, a small amount of soil clinging to a root could bias the results of an analysis. Plant and animal materials were either washed with agitation for one minute in each of two solutions, a mild detergent and 1.0 N HCl, followed by two rinses in deionized water or received no washing at all. Unwashed material was assumed to be more representative of the actual amounts of lead which the sample might contribute to a food chain. Biological materials were wrapped in cheese-cloth and transferred to an electric oven where they were dried to constant weight at 75°C (12-48 hours). The dried material was ground in a Wiley Mill with a 20-mesh (1.6 X 10^{-2} cm^2) screen. One-half to two gram portions of the powdered material were wet-ashed in 10.0 ml of a 17:3 nitric perchloric acid solution (both 70% reagent A.C.S.) until dense white fumes of perchloric acid were liberated. At this point deionized water was added to the digest and boiling continued for about 30 minutes to remove any residues of nitric acid. The digests were diluted to a final volume of 50-100 ml and aspirated directly into a Beckman laminar flow burner attached to a Beckman 1301 atomic absorption spectrophotometer.

(B) Soils

Soil samples were also dried to a constant weight at 75°C. Large pebbles or pieces of wood were removed and the dried soil was passed through a 20-mesh screen. Soil samples were wet-ashed in a 17:3 nitric-perchloric acid solution as above. Any solids remaining in soil digests were removed by filtration.

(C) Atomic Absorption

Suitable blanks and standards covering the proper range of concentrations were prepared and digested as though they were samples. A calibration curve was prepared at the beginning and end of each analytic run (10-50 samples). Lead was determined at the 283.3 nm resonance line; zinc at 213.9; and copper at 324.8. The Beckman laminar flow burner was operated in the hot mode with a lead air-acetylene flame. The recoverability of lead and reproducability of standards are discussed in Appendix A.

(D) Statistical Analysis

Polynomial regressions for the Ala Moana transect data were obtained using a program for an IBM 360 computer system prepared by the Health Sciences Computing Facility of the University of California at Los Angeles.

Results

The lead, zinc and copper contents of soils and vegetation in Ala Moana Park are presented in Tables 2, 3, 4, and Figure 1. Tables 5 and 6 present the results of analyses of plant and soil materials from other sites in Hawaii.

In close proximity to sites A and B are stands of the tree *Vitex parvifolia*. One tree, closest to the major road, exhibited pathological signs, notably tip-burn and some generalized necrosis. The lead content of this tree's leaves averages 67 ppm dry matter while the leaves of an apparently healthy individual some 150 meters further from the road contained an average of 34 ppm. The zinc content of the damaged tree's leaves were also higher: 219 as compared to 86 ppm.

The leaf litter in Ala Moana Park is densely populated by the pillbug, *Oniscus* sp. Pooled samples of 12-15 individuals each from site B were selected for analysis. Samples receiving a preliminary wash in detergent, dilute acid and deionized water had a mean lead content of 24 ± 13 ppm dry weight, while samples receiving no washing contained 53 ± 11 ppm.

Discussion

The soils and vegetation (Figure 1) of Ala Moana Park are heavily contaminated with lead and zinc. The copper contents of these same samples are higher than usual (Bowen 1966).

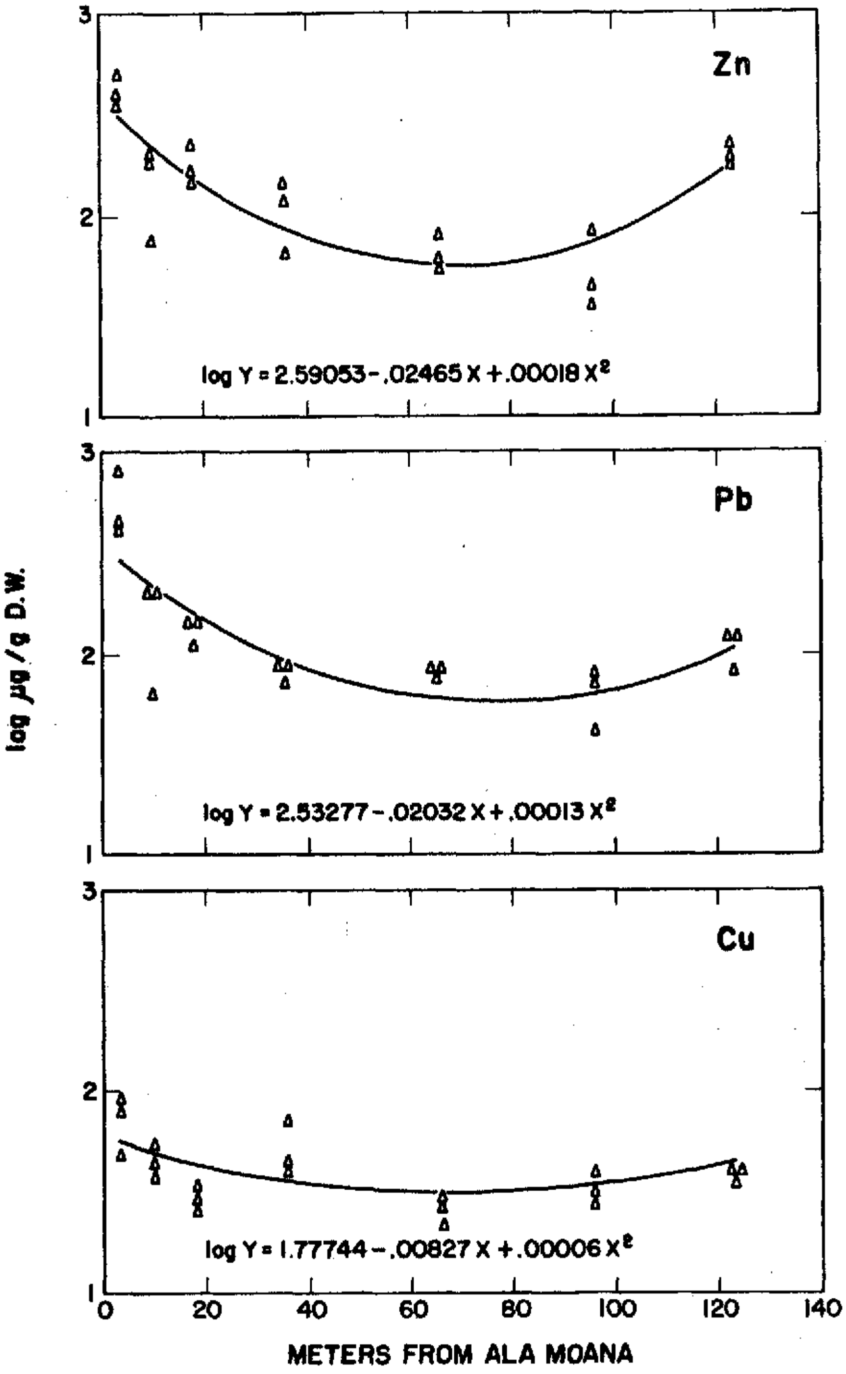

Fig. 1. Lead, zinc, and copper contents of Ala Moana Park grasses along a transect from Ala Moana Blvd. at Piikoi St. to the Ala Moana Park access road, August 1972.

Table 2

Lead, Zinc and Copper in Ala Moana Park Soils and Grasses

Metal content in ppm dry matter

Meters from Ala Moana Blvd.	Pb		Zn		Cu		Grass species
	Soil[c]	Grass[d]	Soil[c]	Grass[d]	Soil[c]	Grass[d]	
[a] 3	4300	567± 202	240	420± 70	200	46±3	Cynodon dactylon
	3100		270		160		
	2800		370		180		
10	1740	153±81	260	153±68	80	73±24	Cynodon dactylon
	1020		280		100		
	2500		520		150		
18	1440	130±17	400	182±43	70	44±8	Cynodon dactylon
	900		530		90		
	980		830		120		
36	50	83±12	60	110±41	45	29±5	Cynodon dactylon
	23		30		30		
	23		35		33		
66	100	83±3	140	65±13	40	51±16	Paspalum sp.
	80		90		30		
	80		120		50		
96	80	63±20	80	53±24	60	26±5	Cynodon dactylon
	40		50		50		
	40		70		60		
[b] 123	460	107±23	250	207±21	50	33±7	Cynodon dactylon
	230		180		50		
	220		180		50		

Table 2. (Continued)

Lead, Zinc and Copper in Ala Moana Park Soils and Grasses

[a] Shoulder of Ala Moana Blvd.

[b] Shoulder of Ala Moana Park Drive

[c] Soil values represent surface 0.0 - 1.0 cm, 5.0 - 6.0 cm, and 9.0 - 10.0 cm.

[d] Unwashed

From the Ala Moana data, the greater amount of lead in the aerial portions of plants relatively close to the roadway appears to be held on the leaf surface as determined by differential washings (Table 3). The proportions of surface held zinc and copper were not determined.

Lead fallout appears to be concentrated toward the surface of the soil, but a close examination of the data reveals that there are several exceptions. The exceptions may reflect the fact that much of the grassy area along the transect line is part of an athletic field which is periodically resodded. Ala Moana Park was created after the Second World War by extensive landfilling over the site of a large garbage dump. To what extent this history has affected the distribution of metals in the park soils is not immediately apparent. Table 4 gives the lead and zinc contents of 10 cm soil cores from sheltered and unsheltered sites near the intersection of Ala Moana Blvd. and the Ala Moana Park access road. These sites are not associated with heavy foot traffic or recreational use. It is interesting to note that at the more contaminated site B the decrease in metal content with depth is not as great as at site A. The extremely high zinc content of the leaf litter at site B has not been explained.

The source of the high lead and zinc contents of Ala Moana Park vegetation and soils is almost certainly the automobile. The sampled transect line runs from Ala Moana Blvd., a major thoroughfare carrying

Table 3

Lead Contents of Washed and Unwashed Grass Samples

Distance from center of roadway (meters)	Lead content (ppm dry weight)	
	Washed [a]	Unwashed [b]
0	350	840±193
17	105	567±202
24	115	153± 81
32	97	130± 17

[a] Collected June 1970

[b] Collected August 1972

Table 4

Lead and Zinc in Ala Moana Park Soil Cores

Site A[a]			Site B[b]		
Depth (cm)	Pb	Zn	Depth (cm)	Pb	Zn
leaf litter	126	105	leaf litter	134	784
0.0-1.0	375	331	0.0- 2.0	3935	774
0.0-2.0	34	79	2.0- 5.0	1460	681
1.0-5.0	23	46	5.0-10.0	459	393

[a] Sheltered site, Ala Moana Blvd., and Park Road.

[b] Exposed site, same as above location.

over 60,000 cars per 24 hours day, to the Ala Moana Park access road, a smaller road carrying less than a tenth the traffic of Ala Moana Blvd. The amounts of copper, lead and zinc ($\log_{10}$ converted) in grasses along the transect line show significant second degree polynomial regressions on the distance from the major thoroughfare (Figure 1, F=mean square due to regression/mean square deviation about regression=14.89 for lead, 22.15 for zinc, and 5.96 for copper all with 2,18 degrees of freedom). This relationship is exactly what would be expected if the source of the metals were associated with automobiles.

Over 400 metric tons of lead as the gasoline additive tetraethyl lead are consumed annually on the island of Oahu. Zinc may originate as zinc dithiophosphate oil additives or zinc oxide and zinc diethyl- or dimethylcarbamate used in vulcanizing tires (Lagerwerff and Specht 1970). Copper may originate as a wear metal which enters the crankcase and is exhausted, or from the simple corrosion of various copper containing parts of an automobile. It is worth noting that corrosion is a serious problem with an automobile in Hawaii, because of a humid, highly saline atmosphere. Anti-corrosion treatments for automobiles are a significant industry in Hawaii.

Though admittedly fragmentary, the data in Table 6 and 7 suggests further that the automobile is a major source of lead. Locations 1-6 (Table 6) show the influences of prevailing winds and distance

Table 5

Lead in Roadside Soils and Grasses

Site and traffic volume as automobiles per 24 hour day	Distance (meters) from roadway	Lead content ppm dry weight		Source
		Soils	Grasses	
Baltimore-Washington Parkway at Bladensburg, Maryland (West of Hwy,) — 56,000	7.6	99	35	Chow, 1970
	15	70	29	
	30	39	33	
Baltimore-Washington Parkway at Bladensburg, Maryland (West of Hwy.) — 56,000	7.6	122	48	*Ibid.*
	15	75	41	
	30	63	24	
Baltimore-Washington Parkway at Bladensburg, Maryland (West of Hwy.) — 48,000	8	540	51	Lagerwerff and Specht. 1970
	16	202	30	
	32	140	19	
U.S. I West near Plant Industry Station, Belts-Ville, Maryland — 20,000	8	522	68	*Ibid.*
	16	378	48	
	32	164	26	

Table 5 (Continued)

Lead in Roadside Soils and Grasses

Site and traffic volume as automobiles per 24 hour day	Distance (meters) from roadway	Lead content ppm dry weight		Source
		Soils	Grasses	
Highway, Northeastern New Jersey — 48,600	7.5	266	454	Motto *et al.*, 1970
	22.5	117	198	
	37.5	104	139	
Ala Moana Blvd. at Piikoi Street, Honolulu, Hawaii — 60,000	3	4300	567±202	This paper
	10	1740	153±81	
	18	1440	130±17	

Table 6

Lead in Vegetation from Various Sites in Hawaii

Location	Species	Lead (μg/g dry matter)
1. East West Road and Maile Way medial strip, University of Hawaii Manoa Campus, Honolulu, Oahu	*Tridax procumbens*	50.0
2. East West Road medial strip opposite entrance to Plant Science parking lot (upwind) of Location I)	" "	25.0
3. Kalanianaole Highway medial strip opposite Kalani High School, Honolulu	*Cynadon dactylon*	96.0
4. 61 m upwind of site #3 (above)	" "	10.0
5. 46 m downwind of site #3 (above)	" "	26.8
6. West Hind Drive at Kalanianaole Highway medial strip, Aina Haina, Oahu (upwind of Kalanianaole Highway)	" "	23.4
7. Intersection of Highways 56 and 57, downtown Lihue, Kauai	*Andropogon* sp.	52.5
8. Shoulder of Rt. 50, 0.4 miles E of Rt. 541 near Hanapepe, Kauai	Grass (sp. unknown)	22.5
9. Ala Kai Swamp Trail at 1-1/4 mile market near Kokee, Kauai	" "	17.5
10. Haleakala Crater, Maui	*Argyroxiphium sandwicense*	5.0

Table 7

Lead in Roadside Soils and Grasses from Aina Haina, Oahu [a]

Distance from center of Highway	Lead (μg/g dry matter) Soil [b]	Grass [c]
North of Highway		
16.5	213.3	57.5
13.0	3708	160.0
0.0	720.8	50.0 [d]
South of Highway		
15.0	1887	105.8
21.0	262.5	83.3
28.5	262.5	41.0
32.0	305.0	56.5

[a] Opposite Hawaii State Library, Aina Haina Branch

[b] Surface 0.0-1.0 cm.

[c] Unwashed *Cynadon dactylon*

[d] An automatic sprinkler at this site probably washed the grass hence the proportionately lower lead value.

from the roadway on the lead contents of vegetation. Locations 7-10 are a series of rural sites ranging from Lihue, Kauai (population 4,000) to a site in the Haleakala Crater on Maui, approximately 10 km from a road with a daily traffic load of less than one vehicle.

Table 7 shows the lead enrichment of roadside soils and grasses from Aina Haina, a suburb of Honolulu. Traffic volume at this site is about 16,000 cars per 24 hour day.

Perhaps the most striking feature of the Ala Moana data is the extent to which these plants and soils have been contaminated. Table 5 summarizes some recent studies of road side lead contamination including this investigation. While some of Motto *et al*'s values for lead in grass are comparable to this study, the Ala Moana values are much higher than those in other reports.

The high soil lead values may be explained in part by sampling technique. The soil lead values for Ala Moana Park represent the metal held in the surface 0.0-1.0 cm, a zone of maximum enrichment, while the other studies report the lead content of the surface 0.0-5.0 or 0.0-10.0 cm. Still, the Ala Moana values are much higher. At a site ten meters from the roadway the surface 0.0-1.0 cm had a lead content of 1740 $\mu g\ g^{-1}$ while the soil at 5.0-6.0 cm showed 1040 $\mu g\ g^{-1}$.

Several factors may account for the high amounts of lead found in Ala Moana Park. While the other studies have concerned themselves with large volumes of traffic at relatively high speeds, the present investigation was conducted in an area where traffic flow rarely exceeds 40 km hr^{-1} and is often impeded by high volumes of cross traffic and traffic signals. Under such inefficient driving conditions, the total pollutants emitted by automobiles should be higher than with a more free flow of traffic.

The high volume of traffic on Ala Moana Blvd. is not the only major source of automobile emissions near the study area. The north side of the road is a parking area which can, and frequently does, accomodate over 7,000 automobiles during business hours. Total traffic flow within a one km radius of the study area probably exceeds 200,000 cars per day. The high traffic density of this part of Honolulu is a recent phenomenon. The success of the Ala Moana Shopping Center across the street from the sampling sites has so changed the economic and physical geography of Honolulu that it has become the effective center of Honolulu.

The prevailing NE tradewinds which sweep across Honolulu during 70 percent of the year act to carry the pollution aerosols generated by this heavy concentration of vehicles toward the sea and across Ala Moana Park. Evaporative losses of tetraethyl lead are probably higher in Honolulu than at the mainland sites reported in Table 5 since the mean annual temperature in Honolulu is 23.9°C.

The association of pathological signs in park trees with high lead contents is cause for concern. Though the effect is most likely that of the oxidizing air pollutants, lead gasoline additives have been shown to be powerful defoliants (Siegel *et al.* 1971). Thiele (1971) suspects that tetraethyl lead fumes were responsible for damage to flowers in the vicinity of a service station. The Ethyl Corporation, a major manufacturer of tetraethyl lead gasoline additives holds a patent from an organo-lead defoliant developed for the United States Army (Klare 1970, Wollensak 1971).

The presence of large amounts of lead in the isopod, *Oniscus* sp., is further cause for concern since these organisms are part of the diet of birds in the park. Earthworms from heavy contaminated roadside soils have been reported to contain amounts of lead which could be lethal for birds (Anon. 1972). A study to determine the levels of lead in the bodies and eggs of local birds is presently being planned.

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LEAD AND OTHER METALS IN THE ALA WAI CANAL

Less than 200 meters from the western boundary of Ala Moana Park lie the Ala Wai Yacht Harbor and the Ala Wai Canal. The Canal, which communicates with the Harbor at the Ala Moana Bridge, was dug by the United States Army Corps of Engineers in 1927 to provide drainage for the reclamation of Waikiki marshlands (Gonzalez 1971). The Canal and Harbor separate the Waikiki resort area from the rest of Honolulu by creating a peninsula. In 1969 it was disclosed that the waters in the Canal and Harbor contained concentrations of coliform bacteria which exceeded Hawaii State Department of Health standards (Cox 1969, Gonzalez 1971). In 1971, an analysis of the sediments of the Ala Wai was begun in an attempt to assess the degree of contamination by lead and other heavy metals.

Materials and Methods

(A) Sediments

Sample preparation and digestion techniques for estuarine sediments are identical to those previously described for the analysis of terrestrial soils. Care must be taken with those samples which contain high amounts of carbonate since the large quantities of carbon dioxide liberated during acid treatment are sufficient to cause the sample to overflow a small container. In this preliminary study it was intended to analyze only the surface layer of sediments but this was not always possible. At most of the sampling stations in the Ala Wai the substrate was found to be a soft, anaerobic sludge. The jaws of the heavy bottom sampling apparatus were often completely buried in the bottom sediments, resulting in a sample which represented a composite of the surface 10 cm of sludge.

(B) Water

Water samples were prepared for atomic absorption analysis by chelation with ammonium pyrrolidine dithiocarbamate (APDC) and extraction into methyl isobutyl ketone (MIBK). Samples of 100 ml were filtered through a 0.45 μm membrane filter and their pH was adjusted to approximately 2.4. To each sample was added 0.5 ml of a 1.0% (W/V) solution of APDC. The pH was then adjusted to approximately 2.8. Samples were shaken with 10 ml of MIBK in a 250 ml separatory funnel for one minute. The MIBK fraction was recovered and aspirated directly into the burner of the atomic absorption unit.

Complete recovery of the MIBK fraction is not necessary, nor is an additional MIBK extraction. Recovery of lead with this method ranged from 75% at 10 μg liter^{-1} to 104% at 400 μg liter^{-1}.

Results and Discussion

Before sampling the sediments of the Ala Wai it was suspected that the highest level of lead would be found in the Harbor area, reflecting the large concentration of boats using the leaded paints and leaded fuels. Boat traffic in the Canal proper is essentially nil. The Texaco fuel dock, where leaded fuels are dispensed, was thought to be a prime source of sediment lead. In fact, the lead levels at the Texaco Dock were consistently low when compared to other stations (Table 8). The highest levels of lead were detected in sediments from the following locations:

1. The extreme landward end of the Canal, near Kapahulu Boulevard.
2. Toward the middle of the channel and slightly seaward of the confluence of the Ala Wai Canal and the Manoa-Palolo Drainage Canal.
3. Directly opposite the transmitting tower of Radio Station KPOI, seaward of the McCully and Kapahulu Bridges.
4. One relatively high reading was recorded on July 7, 1971, at the McCully Bridge station, just seaward of the bridge.

Table 8 lists the lead, zinc and copper contents of Ala Wai sediments at various sampling stations in the Ala Wai (Figure 2). There is no clear trend in the distribution of lead values. There are several possible explanations for this lack of a pattern to the data.

Table 8

Heavy Metals [a] in Sediments from the Ala Wai Yacht Harbor and the Ala Wai Canal, Honolulu, Oahu.

Site	Pb [b]			Zn [c]	Cu [c]
	A	B	C		
Harbor Entrance	—	29	—	—	—
Texaco Dock [d]	20	26	29	113	30
Texaco Midchannel	67	33	59	226	76
Hawaii Yacht Club	—	—	55	176	51
Skindivers Hawaii	—	—	67	231	71
Ala Moana Bridge	78	43	56	252	46
KPOI	126	50	85	168	38
Kalakaua Bridge	69	32	38	159	31
McCully Bridge	108	46	19	146	51
Kuamoo Street	—	—	74	251	74
Manoa-Palolo Drainage Canal	99	95	92	189	45
Manoa-Palolo [d] Canal Dock	—	70	—	—	—
Kanekapolei St.	—	—	14	111	33
Paoakalani St.	—	—	58	150	38
Kapahulu Blvd.	193	141	—	—	—

[a] Expressed as $\mu g\ g^{-1}$ dry matter

[b] A: July 7, 1971; B: July 14, 1971; C: March 6, 1972

[c] March 6, 1972

[d] Sediments sampled directly next to this location; other site designations refer to midchannel samplings opposite the named landmark.

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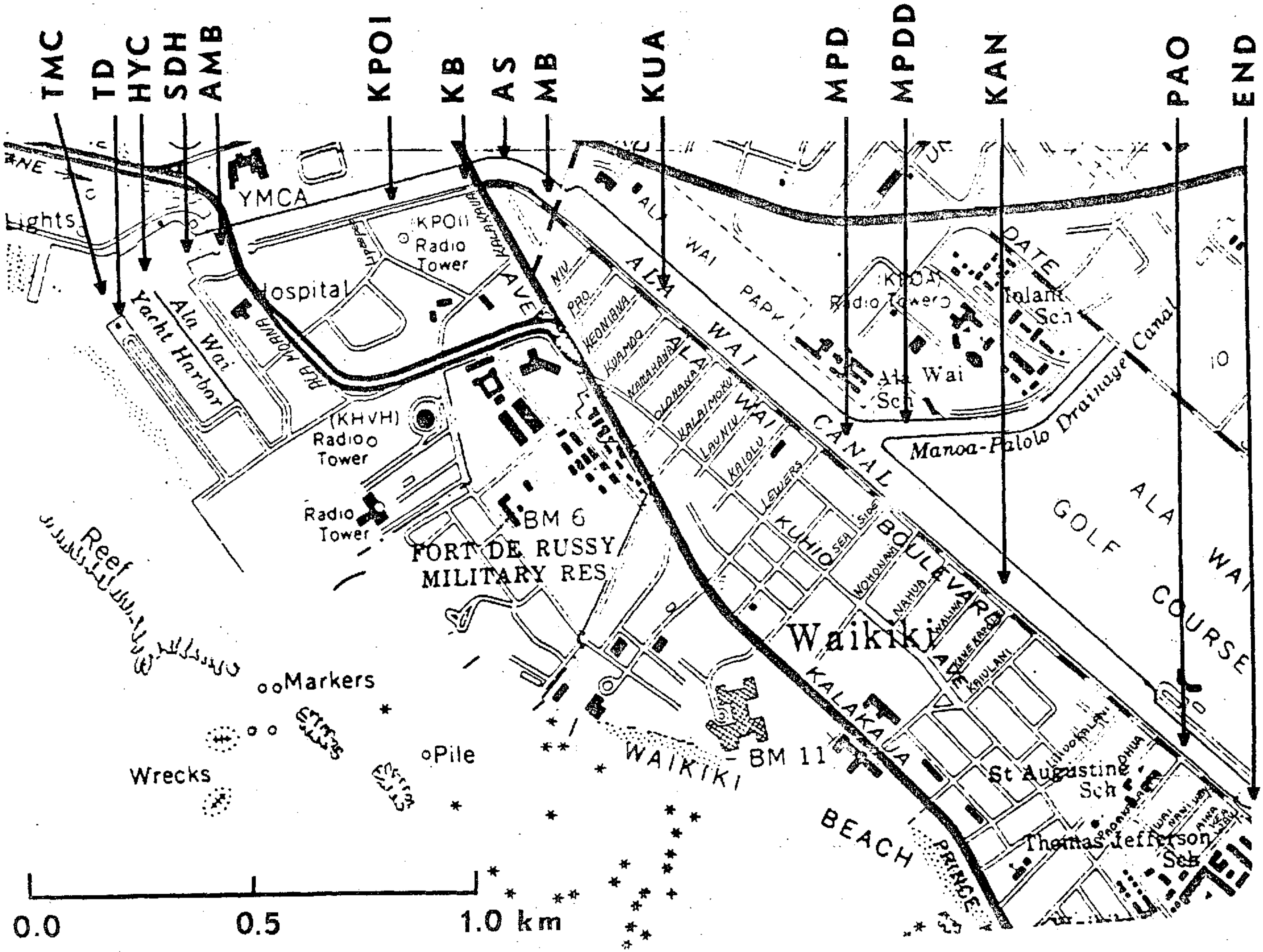
Fig. 2. Sampling Stations in the Ala Wai Canal and Yacht Harbor. Station codes are TWC, midchannel opposite Texaco Fuel Dock; TD, at the Texaco Fuel Dock; HYC, midchannel opposite the Hawaii Yacht Club; SDH, midchannel opposite the dock with a large sign reading "Skindivers Hawaii"; AMB, midchannel by Ala Moana Bridge; KPOI, midchannel opposite the transmitting tower of radio station KPOI; KB, midchannel by the Kalakaua Bridge; AS, the entrance of Apukehau Stream; MB, midchannel by the McCully Bridge; KUA, midchannel by Kuamoo Street; MPD, the confluence of the Manoa-Palolo Drainage Canal and the Ala Wai Canal; MPDD, a dock on the Manoa-Palolo Canal about 100 m upstream of the Ala Wai; KAN, midchannel opposite Kanekapolei Street; PAO, midchannel opposite Paoakalani Street; END, the extreme landward end of the Canal near Kapahulu Blvd.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Iolani Sch
DATE
Ala Wai Sch
Manoa-Palolo Drainage Canal
ALA WAI GOLF COURSE
Waikiki
St Augustine Sch
Thomas Jefferson Sch
PRINCE
0.0
0.5
1.0 km

The low values in the harbor might be explained by the observation that the harbor is periodically dredged to allow safe passage for boats. Perhaps such dredging removes appreciable amounts of lead held in surface sediments. The upper reaches of the Canal are dredged less frequently. Gonzalez (1971) has reported that the Canal was last dredged in mid-1966.

There may be significant differences among substrate affinities for lead at the different sampling stations. For example, the samples from the Texaco Dock station are unique in consisting almost entirely of crushed coral. This probably reflects dredging activities. The substrate at all but two of the other sampling stations is a soft, anaerobic sludge with a distinct odor of hydrogen sulfide. At the extreme landward end of the Canal (END) the sediment is a mixture of sludge and crushed coral. The McCully Bridge Station (MB) yielded a substrate of irregular pebbles about 0.5 cm in diameter.

Perhaps the best explanation for the lack of clear trends in the data is lead is entering the Ala Wai from more than one source. Some lead undoubtedly enters the Canal and Harbor as direct fallout from automobile exhaust. Automobile traffic in this part of Honolulu is heavy. Further lead may enter the Canal as part of the sediment load of the Manoa-Palolo Drainage Canal and Apukehau Stream. The Manoa-Palolo Drainage Canal is the major source of suspended sediments in the Ala Wai (Gonzalez 1971).

It is of interest to note that soluble lead in both the Harbor and Canal never exceeded 10 μg liter^{-1} (limit of detection) on any of the sampling days. The U.S. Geological Survey reports less than 10 μg liter^{-1} soluble lead in the Manoa-Palolo Drainage Canal discharge during October 1970 (Durum *et al.* 1971).

It seems unlikely that the major source of lead in the Ala Wai is in the Harbor area. Mercury, another heavy metal contaminant of the Ala Wai probably originates in the Harbor area as an ingredient in marine anti-fouling paints. The distribution pattern of mercury in Ala Wai sediments is quite different from that of lead (cf. Figure 5). There is a significant linear relationship between the $\log_{10}$ of the amounts of mercury in Ala Wai sediments and their distance from the Harbor area. The pattern for mercury has persisted despite the possible effects of substrate affinities and dredging activities, indicating that these variables have probably not obscured the distribution pattern of lead and that the two metals may have different sources.

THE EXTENT OF LEAD CONSUMPTION ON OAHU

In Hawaii, only two major industries consume appreciable amounts of lead. It has been estimated that over 400 tons of lead in the form of solder is consumed by the pineapple canning factories on the island of Oahu. There is little wide spread loss of lead from this industry. Most of the cans leave the islands for sale on the mainland U.S., (Eshleman and Siegel, 1970). On the other hand over 400 tons of lead in the form of tetraethyl lead gasoline additives are consumed annually on Oahu. Eighty percent of this lead is released directly to the environment following combustion. (Chow 1970).

In 1971, 196,569,793 gallons of gasoline were sold on Oahu (Table 9). Roughly two-thirds of this total was supplied by the Standard Oil Company of California which operates a refinery on Oahu. Standard Oil markets three grades of gas for motor vehicle use. Each grade contains a characteristic amount of lead (Table 10). From this data, the amounts of lead added to Standard's products during 1971 are estimated to be between 272.05 and 292.45 metric tons or 299.26 and 321.70 short tons. Assuming that other oil companies' products contain comparable amounts of lead, the total lead consumption in motor vehicle fuels on Oahu in 1971 was between 407.70 and 438.65 metric tons or 448.87 and 482.52 short tons.

Table 9

Gasoline Sales for Highway Use on Oahu, Hawaii 1953-1971 *

Year	Gallons of gasoline sold per year Calender year	 Fiscal year
1953	74,643,048	n.a.
1954	75,170,691	75,269,560
1955	79,987,371	76,969,936
1956	84,702,302	82,446,702
1957	88,504,519	86,751,759
1958	91,118,212	89,516,535
1959	97,820,001	94,195,803
1960	106,933,112	102,100,506
1961	111,894,823	110,197,143
1962	116,124,189	114,343,948
1963	118,876,230	116,934,694
1964	126,051,172	121,818,547
1965	133,028,140	128,525,878
1966	139,558,752	136,280,003
1967	149,059,930	145,828,302
1968	156,551,638	152,536,973
1969	175,955,391	167,384,198
1970	184,669,410	180,003,627
1971	196,569,793	190,670,721

* Figures supplies by the Hawaii State Department of Taxation, Tax Research and Planning Office and Records of the Territory of Hawaii Department of Taxation.

Table 10

Quantities and Lead Contents of Motor Vehicle Fuels Marketed by Standard Oil of California on Oahu in 1971.

Grade	Quantity sold[a] (U.S. gals)		Total lead consumed (metric tons)	
	Maximum	Minimum	Maximum	Minimum
Unleaded	2.6×10^6	1.3×10^6	0.0	0.0
Low lead (Regular)	46.1×10^6	39.5×10^6	23.05	19.75
Premium[b]	90.0×10^6	83.0×10^6	272.70	249.00

[a] Standard Oil estimates that low lead gasoline accounts for 30-35% of sales, Premium for 63-69% and unleaded for 1-2%. Standard's share of the market is estimated as two-thirds of the total or 130 X 10^6 U.S. gallons.

[b] Lead content varies between 2-3 grams per gallon. These figures are based on 3 grams per gallon, the current amount being added to fuels sold on Oahu. Low lead fuels contain 0.5 grams per gallon. Unleaded fuels contain no lead.

Low lead and unleaded motor fuels were first marketed on Oahu in late 1970. To assess the possible reduction in lead consumption due to these new fuels, the estimated lead usage for 1969, the last year when no low-lead fuels were marketed on Oahu, was calculated. This estimate ranged from 386.22 to 433.73 metric or 424.84 to 477.10 short tons. Though the two ranges show considerable overlap, the actual lead consumption is probably on an increase. Standard Oil's public relations staff on Oahu has admitted that consumer acceptance of unleaded gasoline has been "nil" and "insignificant" (personal communication with the author). As this is being written, Shell Oil is attempting to market a "super regular" fuel containing 0.5 g lead per gallon.

Further indication that lead consumption is increasing is given by Figure 3, per capita gasoline consumption on Oahu by year. The 50 percent increase in individual gasoline consumption in 16 years is equivalent to a compounded rate of increase of 2.75 percent *per annum*. Actual gasoline consumption (Figure 4, Table 9) shows a compounded annual rate of increase of over six percent. Further, the average efficiency of gasoline has been slowly declining (Figure 5) due to higher speed driving and larger engine displacements. The trend toward small imported cars has not significantly affected the rates of gasoline consumption of Oahu or in the United States in general.

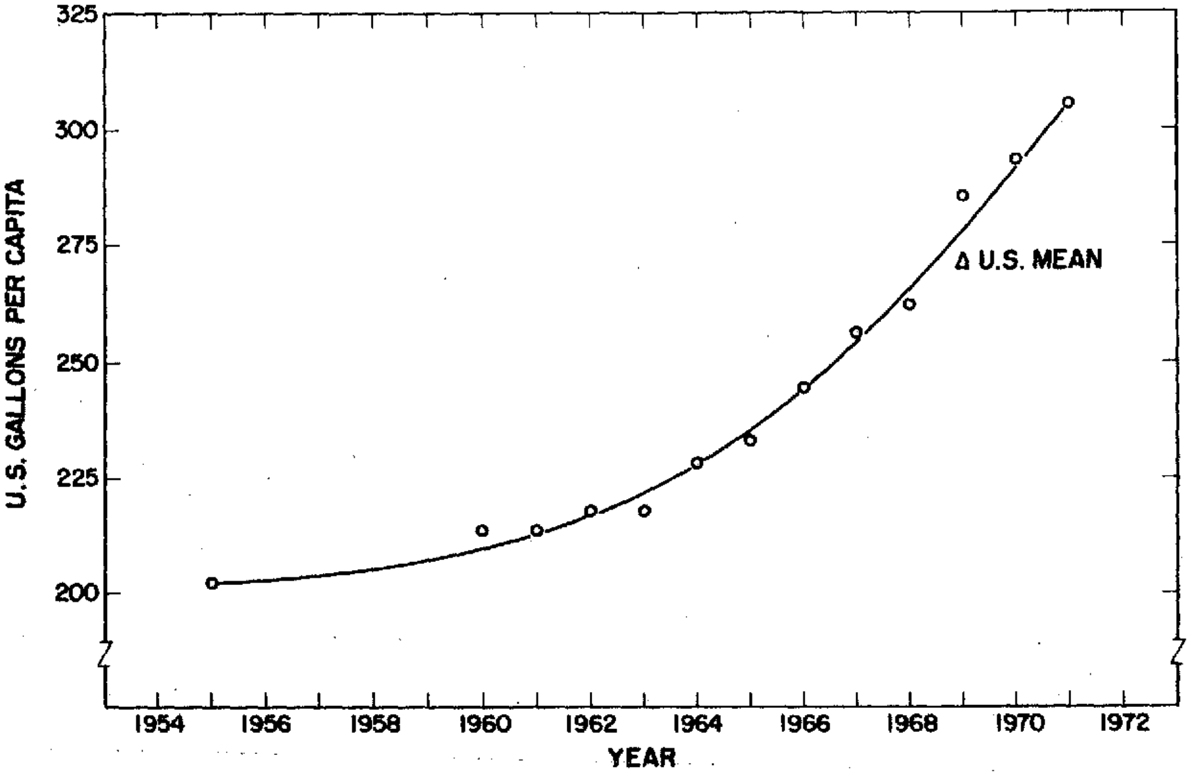

Fig. 3. *Per Capita* gasoline consumption for Honolulu, Oahu (1955-1971).

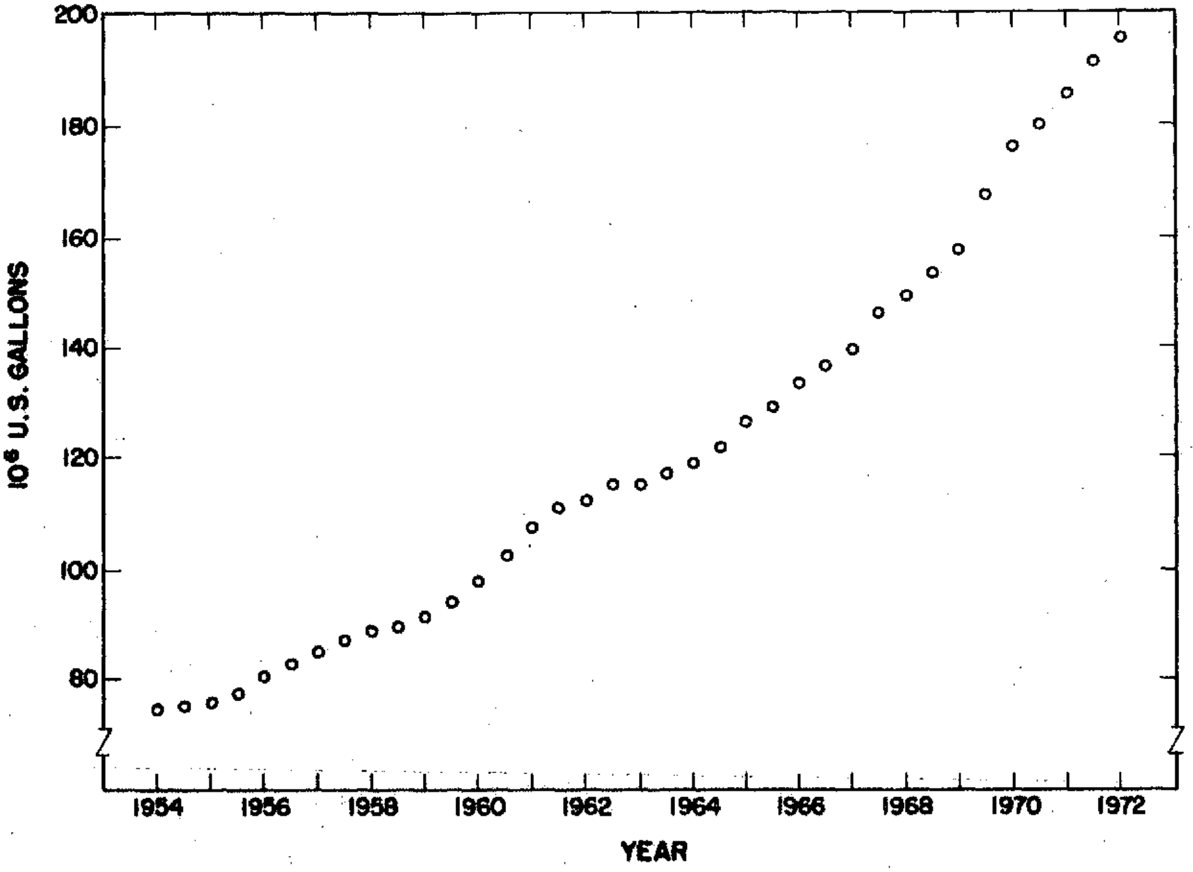

Fig. 4. Gasoline consumption for Honolulu, Oahu (1953-1971).

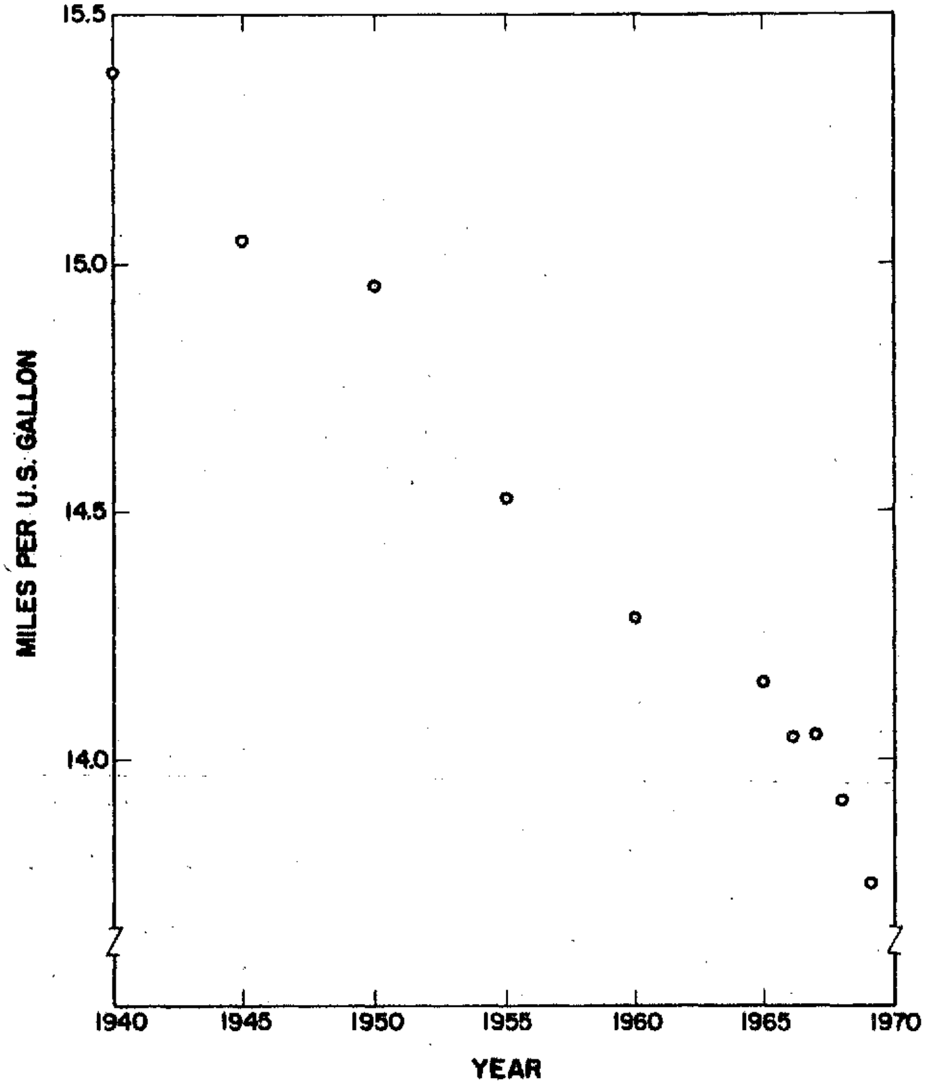

Fig. 5. Miles per gallon of American passenger automobiles 1940-1970 drawn from data in *A Statistical Abstract of the United States*, United States Department of Commerce 1971.

LEAD UPTAKE BY *Aspergillus clavatus* Desm.

Abstract

The fungus, *Aspergillus clavatus* Desm., absorbs appreciable amounts of lead from nutrient broths containing either lead oxide (PbO) or lead nitrate ($Pb(NO_3)_2$). Lead, as the nitrate, appears to interfere with the hydration of the cytoplasm. Both salts depress the amounts of conidiospores formed while apparently having no effect on their viability. A preliminary electron microscopic examination of the material suggests that much of the absorbed lead oxide is sequestered in vacuoles. It is suggested that fungi may form important links in a natural lead cycle.

Introduction

Each year in the United States some 2×10^8 kg of lead are emitted from the combustion of leaded motor fuels (NAS, 1972). This represents some 99% of the total lead input to the environment from industrial sources. Some of this lead has been deposited in soils, sediments, plants and animals including man. There is reason to suspect that this lead can enter natural biogeochemical cycles as has been observed with other toxic industrial materials such as mercury and the chlorinated hydrocarbon pesticides. The possibility exists that lead might be concentrated by cyclic processes, eventually reaching levels toxic to a variety of biota (Eshleman and Siegel 1970, NAS 1972, Tornabene and Edwards, 1972).

There are ample reasons for suspecting the fungi of occupying an important position in a natural lead cycle. The fungi include many species with unusually high tolerances to a variety of toxic substances including several metals.

Species of *Aspergillus, Penicillium,* and *Neurospora* are capable of growth in the presence of high levels of boron, copper, arsenic, selenium, tellurium and mercury. *Penicillium notatum,* is able to solubilize and concentrate elementary boron and copper (Roberts and Siegel 1967, Siegel 1972). *Aspergillus clavatus* can solubilize mercuric oxide (Siegel *et al.*, 1971). Some fungi have the biosynthetic capability to produce volatile alkyls of arsenic, selenium, tellurium and mercury (Challenger 1955, Landner 1971). Roberts and Siegel (1967) observed the production of a volatile form of boron by *P. notatum. P. simplicissimum* is able to leach significant quantities of magnesium, aluminum, silicon, and iron from a variety of rocks (Silverman and Munoz 1970). The mechanism in the latter case appears to be the production of citric acid which attacks the rock. Whether *P. simplicissimum* concentrates the metals leached from rock is not known.

There is little information concerning fungal uptake of lead. Brown and Slingsby (1972) have reported that the lichen *Cladonia rangiformis* can accumulate lead from industrial wastes. Presumably the fungal component of the lichen is the most important accumulator. Tornabene and Edwards (1972) have shown that the bacteria *Azotobacter* sp. and *Micrococcus luteus* are capable of growth and lead uptake in media containing high concentrations of lead halides. This study concentrates on one possible stage in a natural biogeochemical lead cycle, the uptake and concentration of lead by *A. clavatus*.

Materials and Methods

Preliminary experiments showed that both *A. clavatus* and *P. notatum* were able to grow in and accumulate lead from nutrient broths containing as much as 10 mg PbO/ml. *A. clavatus* was chosen for more detailed study because of its relatively better growth and more copious production of conidiospores. The original culture of *A. clavatus* was obtained from the U.S. Dept. of Agriculture, Northern Research Utilization and Development Division, Peoria, Illinois (NRRL No. 8) and subcultured on 5% Czapek solution agar. During the lead uptake studies, *A. clavatus* was grown in a broth containing glucose (1.0%), peptone (0.5%) and yeast extract (0.5%). The initial pH of the broth was 6.5. All broth cultures were inoculated with 5 ml aliquots from a suspension of conidiospores in a 0.1% solution of the wetting agent, Tween 80.

(A) Uptake of Lead Oxide (PbO)

In this series, *A. clavatus* was cultured in 2800 ml Fernbach flasks containing 1000 ml of steam-autoclaves nutrient broth. Following broth sterilization, 1.0 g of gas-sterilized reagent grade PbO was added to the broth. Lead oxide was presented both freely mixed with the broth and in suspended dialysis membranes. Cultures were harvested after 30 days, rinsed with distilled-deionized water, weighed, dried to constant weight, reweighed and digested in a 17:3 mixture of nitric and perchloric acids. Lead content of the digests was determined by atomic absorption. The remaining broth was filtered through a 0.45 μm membrane filter and its lead content determined by atomic absorption.

(B) Uptake of Lead Nitrate.

A series of nutrient broths containing 0.0, 1.0, 10.0, and 50.0 mg Pb (as $Pb(NO_3)_2$) per liter were prepared and sterilized by filtration through a 0.45 μm membrane filter. Steam sterilization was avoided because of the possibility that elevated temperatures might create unexpected lead species. Cultures were grown in 250 ml Erlenmeyer flasks containing 100 ml of broth. The cultures were harvested after 7 days and prepared for atomic absorption analysis as previously described.

(C) Electron Microscopy

Fresh hyphal tissue was washed in distilled water and then fixed with 2% glutaraldehyde in a 0.05 M phosphate buffer (pH 7.2) for three hours. Following fixation, samples were washed in phosphate buffer and post-fixed for one hour in a 1.0% osmium tetroxide. After an additional phosphate buffer wash, samples were dehydrated with a graded series of ethanol, treated with propylene oxide and imbedded in epoxy resin. Thin sections were obtained with a Porter-Blum Mt-2B ultramicrotome fitted with a diamond knife. Sections were placed on copper grids and routinely stained for 5 minutes in aqueous 2.0% uranyl acetate. Some sections were also stained with lead citrate (Reynolds 1963). Sections were examined and photographed with a Hitachi HS-8-1 electron microscope operated at 50 kv.

Results and Discussion

(A) Growth and Uptake: Lead Oxide

Table 11 summarizes the data for *A. clavatus* cultured in the presence of lead oxide. Lead was readily detectable in all treated materials at levels between 250 and 1100 ppm dry weight. When PbO was presented mixed with the nutrient broth there was a significant inhibition of growth relative to both the cultures where PbO was contained by a dialysis membrane ($P < 0.001$, Student's "t" test) and controls ($P < 0.005$). There was no significant difference

Table 11.

Growth and Lead Uptake by *Aspergillus clavatus* in PbO Culture medium

				Pb ppm			Concentration Ratio	
Treatment	FW g.	DW g.	H_2O/DW	FW	DW	Broth	FW	DW
PbO 1.0 g/liter mixed with culture medium	8.2	0.62	12.2	30	400	36	0.83	11.11
	10.7	0.81	12.2	83	1100	52	1.60	21.15
	15.3	1.15	12.3	45	600	12	3.75	50.00
	15.1	1.14	12.2	45	600	17	2.65	35.29
	13.7	1.03	12.3	30	400	19	1.59	21.05
	14.2	1.07	12.3	20	250	19	1.05	13.15
PbO 1.0 g/liter in dialysis membrane	21.2	1.60	12.3	31	405	2.0	15.50	203
	22.6	1.71	12.2	32	420	4.7	11.85	156
	26.3	2.00	12.2	40	525	4.0	10.00	131
	25.6	1.93	12.3	31	405	2.0	15.50	203
	21.6	1.62	12.3	20	270	3.1	6.45	87
Control	33.4	2.52	12.3	—	—	—	—	—
	36.3	2.74	12.2	—	—	—	—	—
	33.1	2.50	12.2	—	—	—	—	—
	14.6	1.10	12.3	—	—	—	—	—

FW = Fresh weight
DW = Dry weight

between growth in the dialysis membrane cultures and controls. The differences may be explained by a toxic effect of the generally higher levels of soluble lead in the nutrient broth where PbO was not contained by a dialysis membrane.

There was a significant difference between the fresh weight concentration ratios in the two PbO treatments. Uptake is closer to passive (ratio of 1.91 ± 1.10) in cultures where the compound was contained by a dialysis membrane. Perhaps these differences reflect an effect of PbO colloids in the broth. Such an effect might also interfere with nutrient uptake, helping to explain the differences in growth between the two treatments.

Both PbO treatments depressed conidiospore production. Conidiospore from these lead-treated cultures were, however, viable and produced mycelial masses of normal appearance.

(B) Uptake of Lead Nitrate.

Lead introduced to the nutrient broth as $Pb(NO_3)_2$ was readily absorbed by *A. clavatus* (Table 12). Owing to the greater solubility of $Pb(NO_3)_2$ much smaller amounts of this compound produced an equal or greater concentration of lead in the fungus than did the oxide. Increasing the concentration of $Pb(NO_3)_2$ in the nutrient broth resulted in increased concentrations of lead in the fungus (Figure 6), decreased production of dry matter (Figure 7), reduced conidiospore production and a lowered ratio of lead in the organism to that in the broth.

Table 12

Growth and Lead Uptake by *Aspergillus clavatus* in $Pb(NO_3)_2$ Culture Medium

				Pb ppm		Concentration Ratio	
Treatment	FW g.	DW g.	H_2O/DW	FW	DW	FW	DW
Pb as $Pb(NO_3)_2$ 1.0 ppm broth	5.2	0.40	12.00	7.7	100	7.7	100
	5.1	0.38	12.42	9.8	132	9.8	132
	6.2	0.42	13.76	*	*	—	—
	7.0	0.42	16.07	7.3	122	7.3	122
Pb as $Pb(NO_3)_2$ 10.0 ppm broth	5.6	0.36	14.55	36	556	3.6	56
	5.4	0.37	13.59	41	595	4.1	60
	4.8	0.37	12.24	54	703	5.4	70
	4.7	0.36	12.06	26	333	2.6	33
Pb as $Pb(NO_3)_2$ 50.0 ppm broth	3.1	0.32	8.69	142	1375	2.8	28
	3.0	0.32	8.38	113	1063	2.3	21
	3.0	0.33	8.09	157	1424	3.1	28
	2.6	0.29	7.97	142	1275	2.8	26
Controls	6.5	0.41	14.85	—	—	—	—
	7.1	0.42	15.90	—	—	—	—
	7.5	0.43	16.44	—	—	—	—
	7.6	0.40	18.00	—	—	—	—

FW = Fresh weight
DW = Dry weight
* = Digest accidentally destroyed

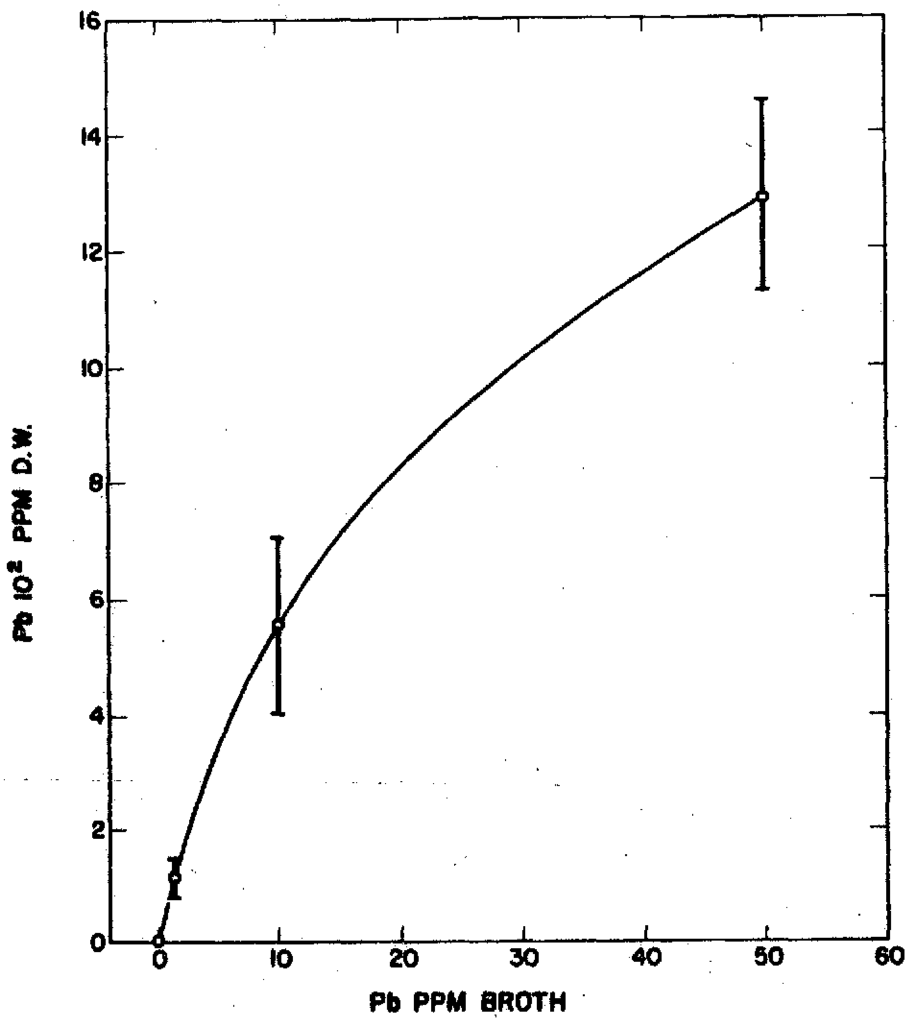

Fig. 6. Mean lead content of *Aspergillus clavatus* as a function of lead (as $Pb(NO_3)_2$) content of its nutrient broth. Vertical bars represent standard deviations.

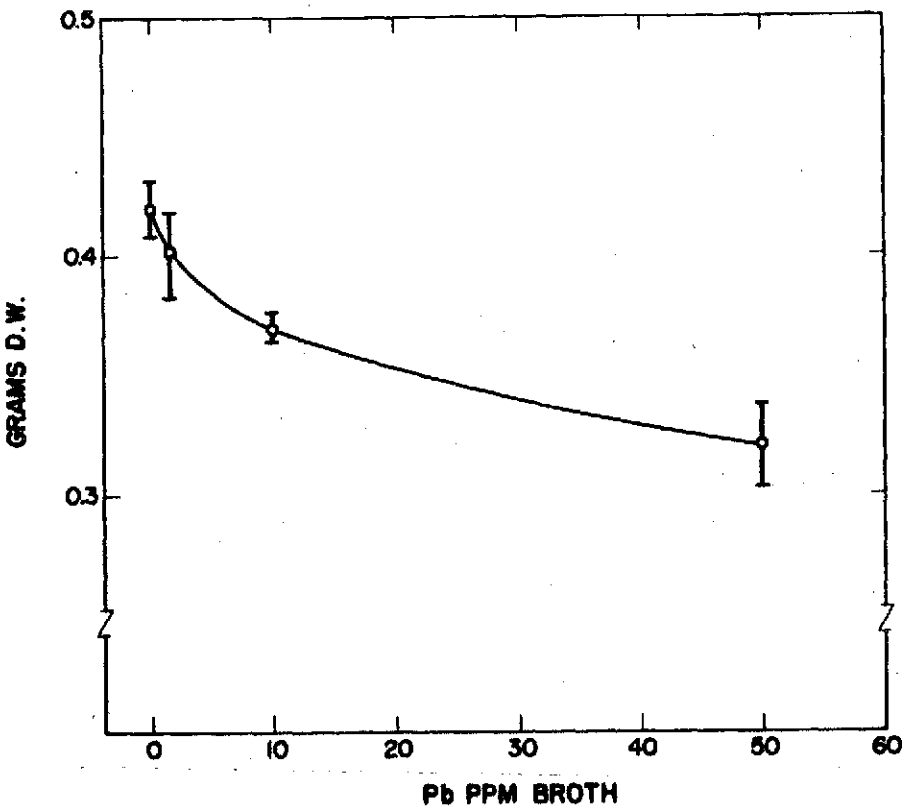

Fig. 7. Mean dry matter content of *Aspergillus clavatus* as a function of the lead (as $Pb(NO_3)_2$) content of its nutrient broth. Vertical bars represent standard deviations.

The degree of hydration of the cytoplasm (Figure 8), expressed as the ratio g water content:g dry weight, was inversely related to the concentration of $Pb(NO_3)_2$ in which it was cultured. Such an effect was not found in the PbO series where the ratio was a nearly constant 12.2-12.3. These differences may be explained by the observation that $Pb(NO_3)_2$ will generate ionic species whereas the oxide and its hydrates exist in largely neutral form. These neutral species should readily permeate the cell membrane, but, having done so, interact weakly, if at all. Ionic lead, on the other hand, may be capable of altering the configuration of the membrane thereby altering its permeability.

(C) The Cellular Location of Lead

Electron microscopy revealed irregular, electron-opaque masses in the vacuoles of *A. clavatus* grown in high (*ca*. 10 mg per ml) concentrations of PbO (Figure 9). Similar masses were quite rare in lead nitrate treated material and absent entirely in controls (Figure 10-11). Further studies are planned to determine the relative percentages of lead held by the various subcellular fractions. Such data may clarify the electron microscopic data.

(D) General

While much remains to be known about the effects of lead on *A. clavatus*, it is clear that the organism posses a high degree of

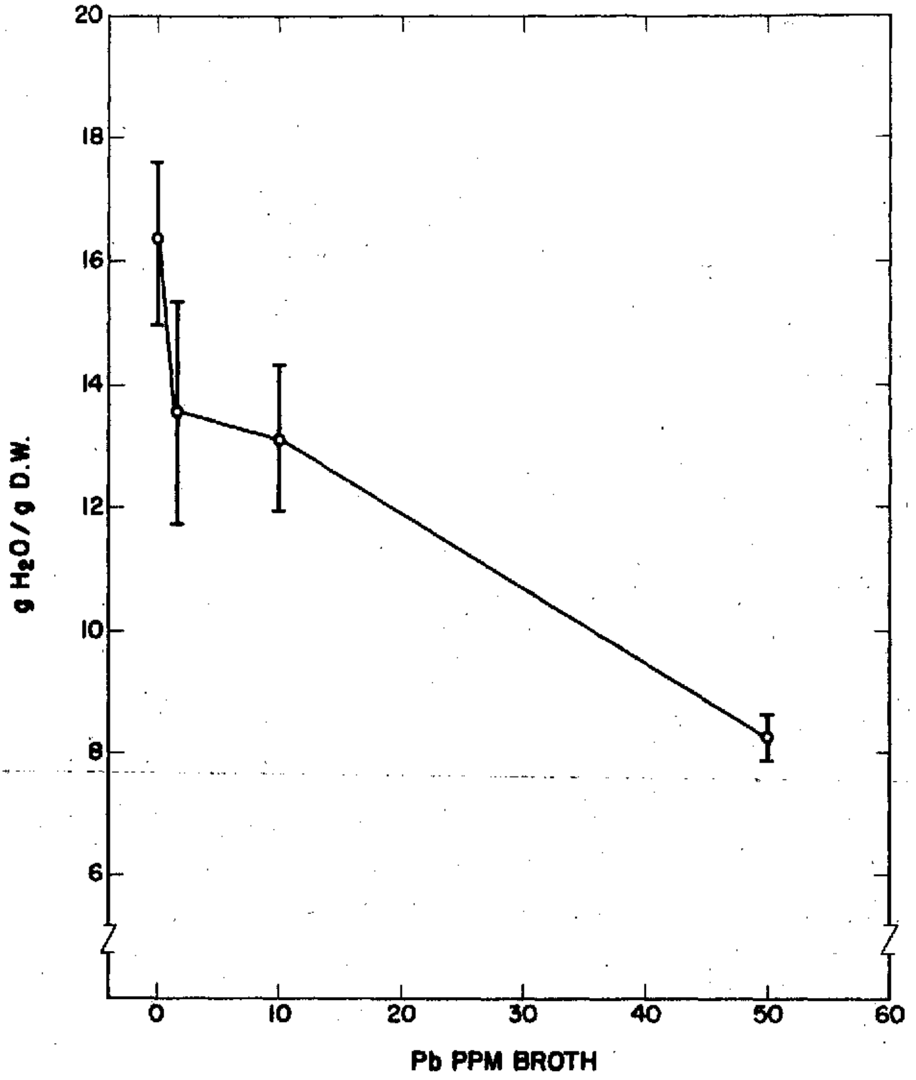

Fig. 8. Hydration of the cytoplasm of *Aspergillus clavatus* (expressed as the ratio of grams lost on drying : grams dry matter) as a function of lead (as $Pb(NO_3)_2$) in nutrient broth. Vertical bars represent standard deviations.

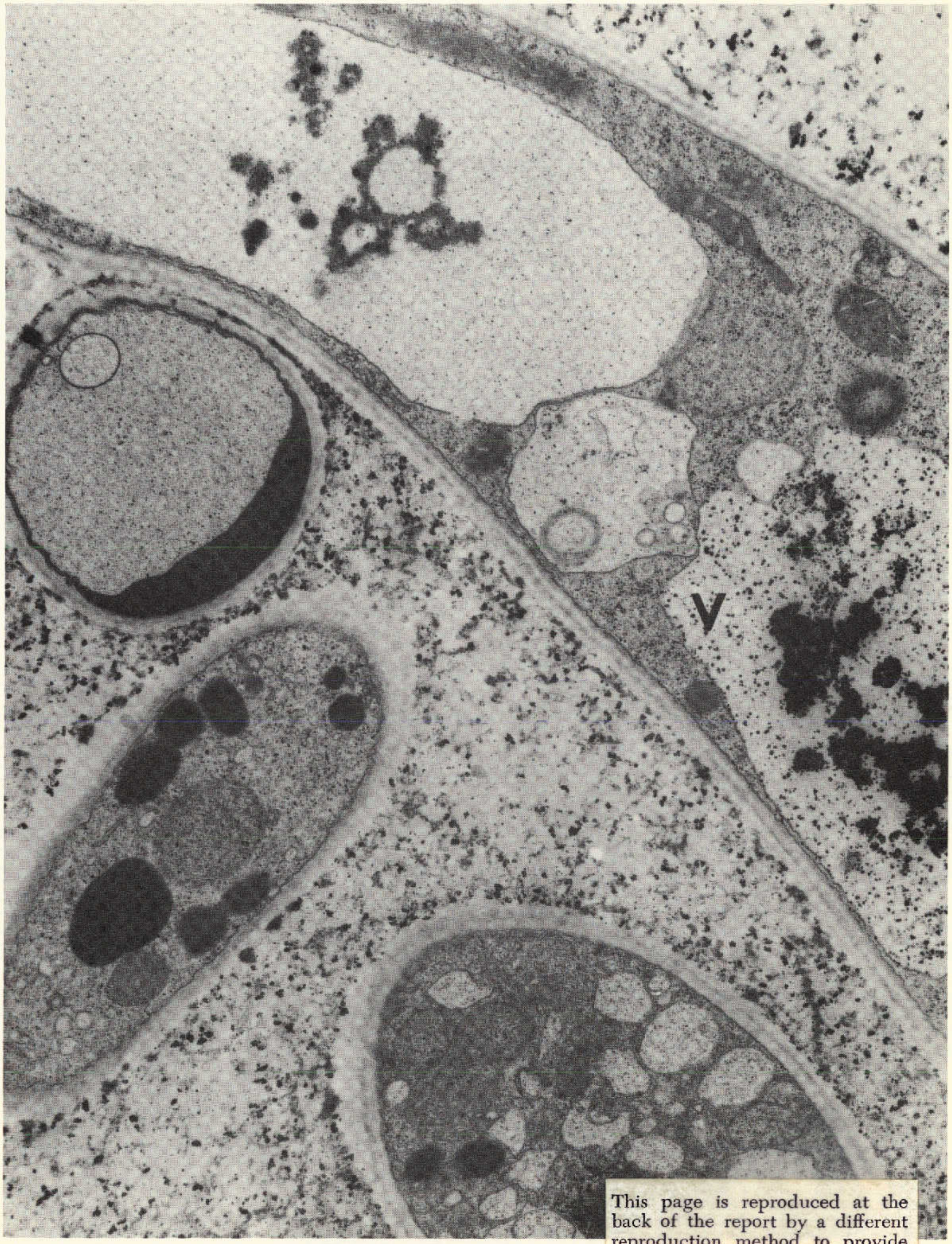

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Fig. 9. Hyphal material of *Aspergillus clavatus* grown in a nutrient broth containing PbO (10 mg/ml). Note the irregular electron-opaque masses in the vacuole (V) which are typical of this material.

Fig. 10. Detail of a vacuole of *Aspergillus clavatus* grown in a nutrient broth containing lead (as $Pb(NO_3)_2$) at 50 ppm. Note the electron-opaque mass and the generally more electron-opaque appearance than in Figure 11.

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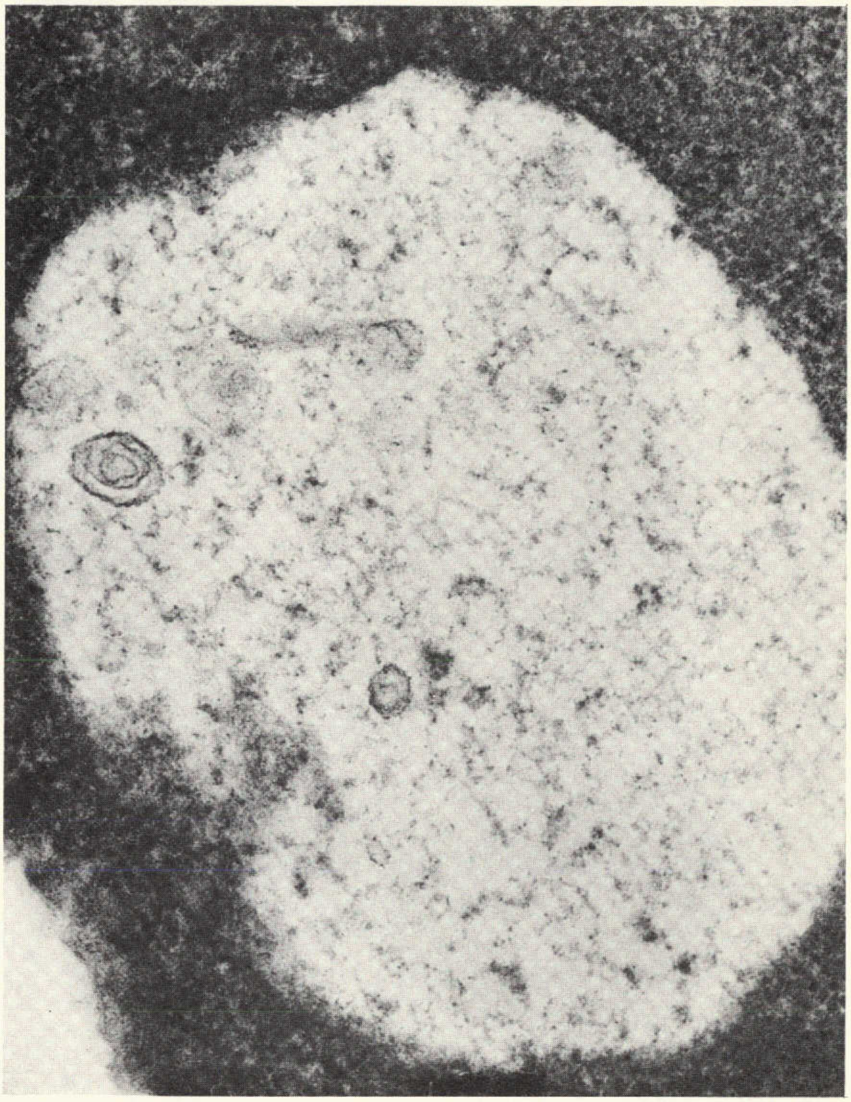

Fig. 11. Detail of a vacuole of *Aspergillus clavatus* grown in an unleaded nutrient broth. Note the absence of electron-opaque masses.

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tolerance to the metal. Although the cells contained large concentrations of lead — over 0.1% of dry matter in some cases — the fungus was able to grow and reproduce. It is likely that this tolerance to lead exists in many other species of the fungi. Fungi may have an important role in a natural biogeochemical lead cycle as concentrators. The possibility also exists that some of the forms of lead concentrated by fungi may undergo biotransformations rendering them more soluble or biologically active or both. The fungal components of mychorhizae have long been recognized as being able to transform nutrititive compounds into forms more readily assimilable by vascular plants. It is quite possible that they could do the same for toxic materials including lead. In view of this possibility, the observation that the majority of contaminant lead deposited on soils is tightly bound by organic matter and incapable of much movement in food chains may face reevaluation.

Acknowledgments

We wish to thank Dr. Roger Goos for assistance in obtaining the original culture of *A. clavatus*, Ms. Ada Chu for performing some of the atomic absorption analyses and Dr. Wm. Sakai for the electron microscopy.

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APPENDIX

Table I

Reproducability of Lead Standards*

Pb Standard (μg/ml)	Absorbance Trial 1	Trial 2	Trial 3	Mean
1.0	0.0123	0.0123	0.0123	0.0123
2.0	0.0236	0.0241	0.0246	0.0241
3.0	0.0348	0.0357	0.0353	0.0353
5.0	0.0521	0.0535	0.0550	0.0535
10.0	0.1124	0.1146	0.1180	0.1143
20.0	0.2388	0.2526	0.2510	0.2474

* Standards digested in 17:3 nitric-perchloric acid solution and brought to final volume with deionized water.

Table II

Recovery of Lead from $HNO_3/HClO_4$ Digestion

μg Pb added	Expected final concentration (μg/ml)	Water control Absorbance	Water control Pb μg/ml	Water control % Recovery
100	1.0	0.0106	1.0	100
500	5.0	0.0540	5.0	100
1000	10.0	0.1180	10.0	100
2000	20.0	0.2434	20.0	100

$HNO_3/HClO_4$ (17:3) Absorbance	$HNO_3/HClO_4$ (17:3) Pb μg/ml	$HNO_3/HClO_4$ (17:3) % Recovery	$HNO_3/HClO_4$ (2:1)* Absorbance	$HNO_3/HClO_4$ (2:1)* Pb μg/ml	$HNO_3/HClO_4$ (2:1)* % Recovery
0.0114	1.1	110	0.0123	1.2	120
0.0535	5.0	100	0.0535	5.0	100
0.1096	9.4	94	0.1146	9.7	97
0.2518	20.5	103	0.2472	20.3	102

* Standards prepared by technicians in the Dept. of Soil Sciences, University of Hawaii, Honolulu.