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Assimilation and accumulation of lead through the aquatic food chain of migratory waterfowl

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ASSIMILATION AND ACCUMULATION OF LEAD
THROUGH THE AQUATIC FOOD CHAIN
OF MIGRATORY WATERFOWL

By

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Lead pathologies resulting from direct ingestion of lead shotgun pellets by migratory waterfowl have been thoroughly explored. However, no known research has been undertaken on the possible transfer of lead pellets from hunters' shotguns through the aquatic plant food chain.

The purpose of this study was to determine whether lead, deposited as lead pellets in the rooting environment of aquatic plants utilized by migratory waterfowl in their normal diet, is assimilated and accumulated by the rooted aquatic plants and, if so, at what levels.

Two approaches were employed. (1) Aquatic plants were collected from several areas which were involved in large scale waterfowl lead poisoning cases and analyzed for lead content. (2) Rooted aquatic plants utilized by migratory waterfowl were grown under a greenhouse environment of artificially introduced lead, then analyzed for lead content.

Analyses of plants from both the field and greenhouse indicate that lead is transferred through the aquatic food chain.

Rooted aquatic plants accumulated lead as much as $2\frac{1}{2}$ times the control levels when grown in areas of high lead shot deposition.

Lead seems to be differentially accumulated in the various parts of plants with roots exceeding seeds and the seeds exceeding the shoots (stems and leaves).

Rooted aquatic plants accumulated lead in concentrations capable of causing fatal lead poisoning in migratory waterfowl in 14 percent of plants sampled and subclinical pathologies in 30 percent of all plants analyzed.

Subacute dosages of lead from the food chain may cause increased waterfowl susceptability to hunter-kill and increased mortality from common disease organisms.

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CHAPTER I

INTRODUCTION

Ecologically, lead has received considerable attention in the past decade because of its association with air pollution via leaded gasoline (Blazell, 1971), its continuing role in infant encephalopathy from lead-based paints (Folstad, 1970), and its toll in industrial toxicology.

History of Lead Poisoning in Migratory Waterfowl

Lead poisoning, or plumbism, in migratory waterfowl was documented as early as 1894 by Grinnel (Bellrose, 1959). In a 20-year period (1934-1954), 34 waterfowl die-offs were recorded due to lead poisoning ranging up to 16,000 waterfowl per incident. Die-offs were defined as occurrences of lead poisonings in which large numbers of waterfowl in relatively small areas perished in short periods of time. Plumbism accounts for 2-3 percent of the waterfowl mortality and 4 percent of the mallard (Anas platyrhyncos) mortality annually (Bellrose, 1959). These figures translate to approximately 1 million ducks, swans, and geese per annum (Andrews and Longcore, 1969). In addition to direct fatalities, lead poisoning results in reduced vigor which increases winter mortality and susceptibility to hunter kill

(Jordan and Bellrose, 1951).

Cottam (1949) expressed concern that lead poisoning among migratory waterfowl could well be a stumbling block to effective future management of wild waterfowl populations. By 1965, the problem had reached the point where the director of the U. S. Bureau of Sport Fisheries and Wildlife informed major American and Canadian arms and ammunition manufacturers of possible reduction in seasons and bag limits due to lead poisoning die-offs in certain flyways (S.A.A.M.I. Bulletin, Oct. 1969).

In a special report of the Migratory Wildlife Committee, the International Association of Game, Fish and Conservation Commissioners (1973), indicated that 47 of 48 states were in favor of a lead shot substitute because of lead poisoning losses in their states. Fifteen states reported significant lead poisoning losses. The report stated: "While the number of deaths due to lead poisoning in wild duck populations has not been measured, evidence points to a relatively serious problem over much of the United States for several species of ducks." By 1974, the problem had reached the point where the U. S. Fish and Wildlife Service, in a Draft Environmental Impact Statement (DES 74-76) proposed the substitution of steel shot for lead shot between 1976 and 1978 for hunting ducks, geese, and swans (Anatidae), and coots (Fulica americana) in all flyways.

Jordan and Bellrose (1951) found that one number six lead shot pellet ingested by a wild duck could result in fatal plumbism. Add to this the fact that 5.5 million kilograms of shot per year (Knap, 1967) is being added to the bottom sediments of hunted-over wetlands and the potential of the problem manifests itself.

Correlation Between Lead Pellet Ingestion and Mortality

Bellrose (1959) summarized important waterfowl lead poisonings from the period 1938 to 1957. A few examples are:

1) In the Central Flyway, nearly 11,000 mallards died from lead poisoning over a 10-year span in the Sandlake National Wildlife Refuge of South Dakota. Ten thousand ducks perished in Lubbock County, Texas, in the winter of 1944, during their northward migration.

2) In the Mississippi Flyway, the largest single outbreak of plumbism occurred in the Claypool Reservation, Arkansas, during the winter of 1953-54. Sixteen thousand ducks, mostly mallards, succumbed. Die-offs have been recorded at Squaw Creek Refuge, Missouri; Hove Lake Refuge, Indiana; and in Iowa, Minnesota, Wisconsin, and Michigan.

3) The largest loss to lead poisoning in the Atlantic Flyway was 600 Canada Geese (*Branta canadensis*) and Whistling Swans (*Olor columbianus*) over a 10-year period in North Carolina.

4) Between 1944 and 1954, 4,000 ducks were lost at the Salton Sea National Wildlife Refuge in southern California.

The presence of lead pellets in the gizzards of waterfowl which have succumbed to plumbism has been dealt with thoroughly. As early as 1919 (Wetmore in Bellrose, 1959), lead poisoning from ingested shot pellets was recognized as a serious problem. Bellrose (1959) stated that over 70 percent of the dead mallards exhibiting clinical symptoms of plumbism at Chautauqua National Wildlife Refuge, Illinois, had at least one lead pellet in the gizzard. In addition, he found a 6.6 percent incidence of ingested shot in 35,400 ducks of 17 species. In the West, Morgan (1944) observed an 8.6 percent fatality rate from ingested lead in 3,000 birds which he examined. Yocom (1951) found, over a 3-year examination of 461 birds, a 3 percent incidence of ingested lead shot. Addy (1964) attributed many thousands of waterfowl deaths annually in the Atlantic Flyway to lead shot ingestion.

From an examination of 14,391 ducks of four species--Black duck (*Anas rubripes*), canvasback (*Aythya valisneria*), lesser scaup (*Aythya affinis*), and redhead (*Aythya americana*)--Hunt (1960) found a 3.2 percent lead pellet presence in all ducks (living and dead) and a 4.6 percent presence in ducks killed during the hunting season. In a similar study involving 218 birds of the same four species, a 9.7 percent ingested lead shot rate was observed (Reid, 1948).

The mallard is the species most affected by lead shot ingestion even in the presence of other waterfowl (Bellrose, 1959). Apparently, ducks pick the spent shot off the bottom during feeding and the pellets become lodged in their gizzards. The grinding action of the gizzards breaks down the pellets and digestive juices convert the native lead into toxic lead salts (Jordan and Bellrose, 1951). This damages the gizzard lining and a portion of the lead then enters the bloodstream via the intestine, causing atrophy of liver and kidneys, anemia, loss of fat deposits in the muscles, flabby flight muscles, and edema of the liver (Coburn et al., 1951). Advanced plumbism manifests itself by lowered food intake, followed by flaccid paralysis, "wing drop" and exposure of the sternum from muscle atrophy (Coburn et al., 1951; Locke et al., 1967).

The toxic action of lead comes from the interruption of the sulphur-hydrogen linkage in enzymes resulting in reduced glycolysis, reduced oxygen consumption by tissue, and virtually complete cessation of hydrogen transfer reactions in nerve tissue. Anemia may occur from interruption of hemoglobin synthesis. Additionally, erythrocyte surface impermeability occurs, causing increased destruction of erythrocytes and subsequent compensatory production. This in turn, leads to increased blood bilirubin resulting in added iron pigment (hemosiderin) in tissues such as the liver. Myocardial infarctions associated with fibrinoid necrosis of the media of arterioles was reported in 75 percent of 67 lead-poisoned ducks (Karstad, 1971).

Neuritis and muscle paralysis in the peripheral nerves is another possible effect of lead in waterfowl.

Increased cerebrospinal fluid pressure may also result, giving rise to encephalopathy. Its symptoms include loss of balance, extreme irritability, convulsions, disturbed reflexes and vision, and cerebral edema.

Food Chain Accumulation of Lead--Hypothesis

Even though the link between ingestion of lead shot and plumbism in waterfowl has been thoroughly established, there seem to be several unexplained inconsistencies.

1) The Atlantic Flyway, with the highest incidence of lead pellet presence (10.52%) in the gizzards of hunter-bagged birds, enjoys the lowest level of plumbism die-offs (Jordan and Bellrose, 1951).

2) The diving ducks--canvasback, redheads, ringnecks (*Aythya collaris*)--exhibit the highest frequencies of lead pellet ingestion (11.3%, 14%, and 10.5%, respectively) yet these species are less frequently associated with plumbism die-offs than the mallards and pintail (*Anas acuta*) which are dabblers (Bellrose, 1959).

3) Presence of lead pellets in the gizzard in ducks diagnosed as succumbing to lead poisoning is less than 100 percent. It is

assumed that they expel the pellet(s) prior to death (Bellrose, 1959).

4) Even though 6.98 percent of hunter-bagged birds nationwide, contain lead pellets in the gizzard, only 2 to 3 percent succumb to lead poisoning annually (Bellrose, 1959).

These inconsistencies, coupled with the failure of a literature search to disclose any analysis of lead content in common natural food plants eaten by migratory waterfowl from areas of recorded plumbism die-offs, led to the following hypothesis.

Lead shotgun pellets deposited in the bottom sediment of ponds by hunting activity may become available for absorption by bottom-rooted aquatic plants. Lead absorbed and accumulated in those plants, which form the principal food source of certain migratory waterfowl in some seasons, may provide an important pathway of lead ingestion for those waterfowl.

Fig. 1 illustrates the possible sources of lead ingestion in waterfowl. The direct intake of lead pellets, T1, has been the only demonstrated source of lead. Transfer, T3, forms the basis of the hypothesis.

One of the critical points in demonstrating the feasibility of either T2 or T3 rests on the probability of chemical changes C1, C2, and C3. If native lead does not undergo chemical alteration in the bottom sediments, it is unlikely that it will be absorbed by the aquatic plants. Geochemical phase diagrams disclose that native lead

Fig. 1. Model of possible pathways of lead ingestion by waterfowl.

MODEL OF POSSIBLE PATHWAYS OF LEAD INGESTION BY WATERFOWL

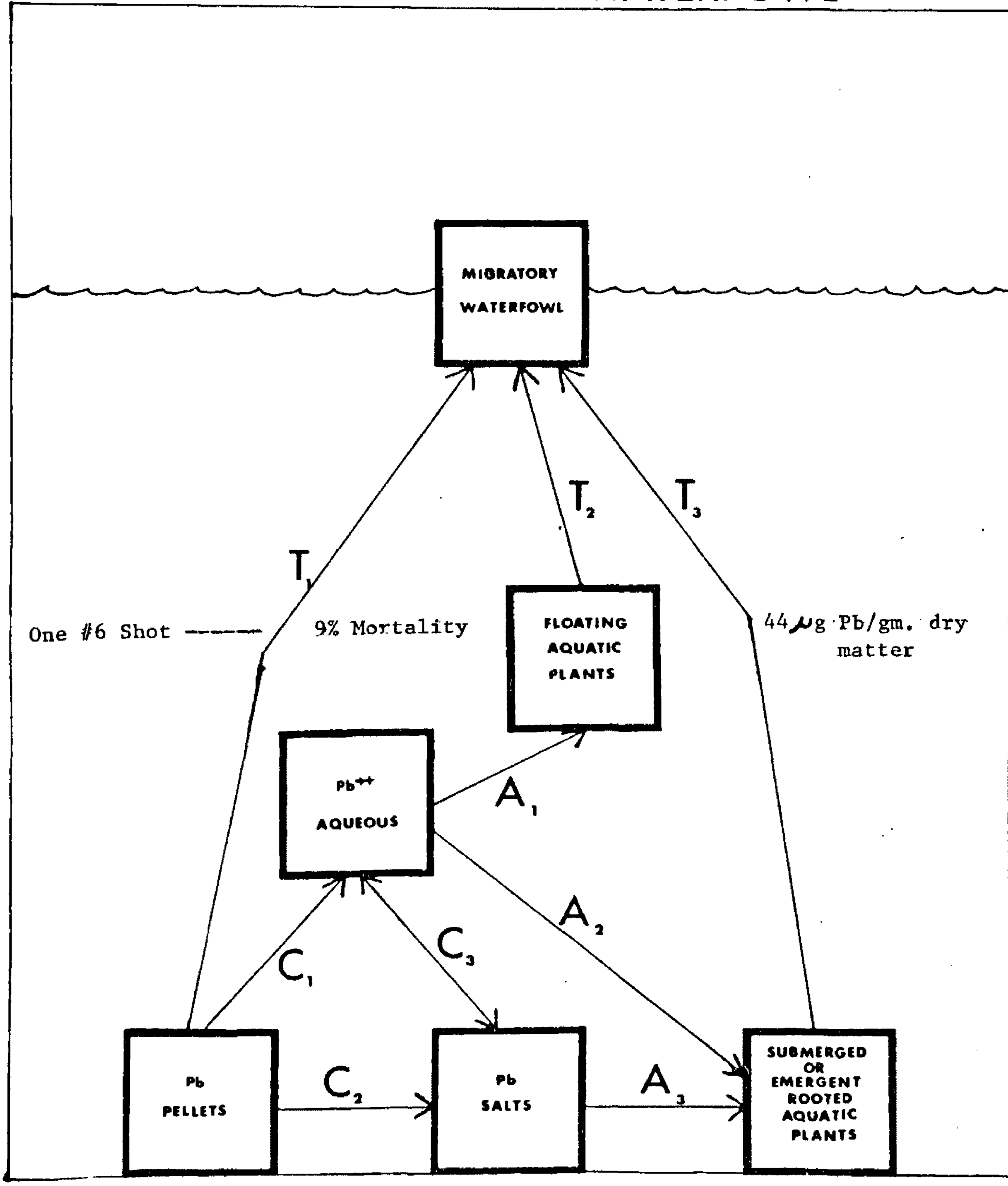


FIGURE 1.

T= Transfer of Pb to waterfowl
C= Chemical change of Pb
A= Absorption and accumulation of Pb by food sources

is unstable in the geochemical environment characterized by bottom sediments of ponds (Garrels and Christ, 1965). Native lead is unstable at less than pH 10 and Eh (Reduction-oxidation potential) values more positive than -0.2 to 0.0 (Bass-Becking et al., 1960), thus a host of lead compounds could be present. The specific compounds formed would depend on the combination of pH, Eh and the activity of sulphur and carbon anions and various oxides. The relatively low concentration of soluble Pb expected does not indicate significant accumulation of lead by floating aquatic plants via transfer A1 (Fig. 1). Rooted aquatics such as Potamogeton spp. and Polygonum spp. present a different situation. It has been demonstrated that one of the principal means of adaptation by plants to the emergent or submerged aquatic environment is an anatomical provision for the transfer of oxygen from the shoot to the root so that the root may function aerobically in an anaerobic environment (Behan, 1963). This oxygen transfer may markedly affect the environment at the root/sediment interface. HuKara (1964, cited by Behan, 1970) demonstrated that the transfer of oxygen to the roots of birch (Betula spp.) grown in an anaerobic root environment was sufficient to oxidize reduced leucomethylene blue die in the immediate vicinity of the root tip. This situation at the root surface of more positive Eh and increased hydrogen ion activity may increase the solubility of native lead and its salts. The likelihood that a root would grow in contact with a lead pellet seems reasonable since Bellrose (1959) cites a study which indicated pellet densities of 2.1 to 28.3 pellets/m^2 in bottom sediments. For these reasons it was assumed that transfer C2 was feasible.

Lead Accumulation in Plants

Lewis (1966) summarized evidence indicating that lead is widely distributed in such root-like materials as beets and carrots and often in concentrations from 2 to 4 μg/g. Suchadoller (1967) documented lead uptake by terrestrial plants along highways. Hammond and Aronson (1964) found lead as high as 272 μg/g in alfalfa-clover hay and corn silage on Minnesota farms. As much as 57 μg/Pb/g dry matter in foliage near a busy highway was reported by Cannon (1960). In a study of lead uptake by bromegrass (Bromus enermis) on contaminated soils, values from 3.0 to 34.5 μg/g were recorded (Marten and Hammond, 1966). Lagerwerff (1971) found in radishes (Raphanus sativus) grown in soils containing 29.9, 165 and 299 μg Pb/g soil lead values of 20.8, 31.0 and 38.4 μg/g dry matter in the roots and 26.1, 33.9 and 40.7 μg/g dry matter in the tops. In a survey of mean lead concentrations in urban woody plants in New Haven, Connecticut, Smith (1972) recorded Pb values as high as 203 μg/g dry weight in the intact shoot (leaves and twigs) of eastern hemlock (Tsuga canadensis).

If lead were present in an acceptable form in the root environment, it would be absorbed and accumulated (Fig. 1, A3) as well by aquatic plants as by terrestrial plants.

Lead Toxicity in Animals and Waterfowl

Kehoe (1966) described some observations in which lead salts were fed to men. A dietary lead intake of 3 mg/day (0.4 mg Pb/kg body weight/day), induced plumbism in 8 months. In another experiment, lead poisoning symptoms occurred after 30 days with an intake of 10 to 15 mg Pb/day. This is equal to 0.12 to 0.12 mg Pb/kg body weight per day.

Hammond and Aronson (1964) suggest that a minimum of 6 to 7 mg Pb/kg body weight/day will give rise to lead poisoning in cattle.

Coburn et al. (1951) determined that between 6 and 8 mg Pb/kg/day in adult mallards would result in 100 percent mortality from lead poisoning in an average of 28 days.

Objective of This Study

The objective of this research was to determine whether lead deposited as pellets in the bottom sediments of aquatic habitats is abosrbed and accumulated by rooted aquatic plants and, if so, at what levels.

Three approaches were employed: (1) Field Investigations. Aquatic plants were collected from several areas exhibiting histories of severe waterfowl die-offs and analyzed for lead content. (2) Controlled Environment Study. Aquatic plants which are known to be utilized by migratory waterfowl commonly involved in lead related die-offs were grown in special aquaria under controlled greenhouse conditions, including artificial introduction of lead. These plants were analyzed for lead content in the same manner as the field samples. (3) Ravalli Test Pond. With the permission of the U.S. Fish and Wildlife Service, a small pond on the Ravalli Wildlife Refuge, Stevensville, Montana, was used for an in vivo experiment to provide field data on lead uptake into aquatic plants while controlling as many variables as possible.

CHAPTER II

MATERIALS AND METHODS

Controls, in the field samples, were those plants growing in areas of the refuges which had no known history of being shot over. Records kept at refuge headquarters and personal communication with refuge staffs provided verification. Treated samples were plants taken from areas which had been hunted over consistently for at least 20 years or longer. Verification was the same as above. The majority of treated samples came from in front of permanent hunting blinds.

Samples were separated into roots, shoots (stems and leaves), and seeds to determine any differential lead accumulation. This was done because different species of waterfowl have dietary preferences for different plant parts (Cottom, 1939; Yocom, 1951).

Normalization, as indicated in Tables 9 and 10, was achieved by taking the control lead contents for each given area and plant part and making it 100 percent. The lead content of each treated plant from that area was then divided by the lead content of the control sample to derive the percent of control lead content that treated plant had accumulated. The mean of all treated plant parts from that area was then determined for comparison with the control sample.

Field Collection Sites

Four areas were chosen for field sampling on the basis of histories of severe plumbism related die-offs or high frequencies of die-offs in the waterfowl population over a period of years as documented by Bellrose (1959).

1) Squaw Creek National Wildlife Refuge, Holt County, Missouri. During the winters from 1945 to 1957, 16,000 mallards succumbed to lead poisoning. This represented 1.1 percent of the total bird population at the Refuge.

The Squaw Creek Refuge was established in 1936 from reclaimed agricultural land. Hunting is not allowed on the Refuge itself so the South Pool was used as the control area (Site 9). Three heavily hunted areas--Maitland Gun Club (Site 6), N.W. corner of Big Lake (Site 7), and the Hall-Forgrove Area (Site 8)--around the periphery of the Refuge, were chosen as sampling sites for plants subjected to lead application. These areas have been subjected to heavy seasonal hunting for over 20 years according to local sources. Most plants were collected 20 to 50 meters out from established hunting blinds (refer to Appendix A for Refuge maps).

2) Chautauqua National Wildlife Refuge, Mason County, Illinois. From 1941 to 1957, 13,000 mallards were lost to plumbism,

representing 0.9 percent of the bird population at the Refuge. Site 11, the control area located in the N.E. corner of the North Pool, Chautauqua Lake, has not been hunted over for 35 years (Gerald Clawson, Refuge Supervisor, personal communication). The lead application sites were the south end of the public hunting area on the Refuge (Site 10), two private hunting areas on Quiver Creek south of the Refuge (Al Martin Hunting Club [Site 12] and Doyle Watkins Hunting Area [Site 13]), and the Crane Lake Hunting Club west of Saidora, Illinois, approximately 20 kilometers S.W. of the Refuge (Sites 14A and 14B).

The control area experiences heavy siltation (15-45 cm annually) while the hunted-over areas all had firm sand bottoms and little siltation with the exception of the public hunting area on the Refuge. This site experiences much the same siltation as the control area.

3) Fountain Grove Wildlife Management Area, Linn and Livingston Counties, Missouri. This area was established in 1947-48 and contains 45 blinds and 16 pits for seasonal waterfowl hunting. The bottom is firm clay with some silting from the Grand River floods.

The control area (Site 17) was a waterfowl resting area west of the upper pool in the Refuge proper. The two lead application areas (Sites 15 and 16) were in front of the blinds on the upper pools.

Most of the pools are drained during the summer months.

4) Bear River Migratory Bird Refuge, Brigham City, Utah. Over 300 mallards and whistling swans were lost from 1947 to 1954. The bottom is predominantly clay with a hardpan layer about 15 cm down. The water is brackish and alkaline. The control area (Site 19) has not been hunted for over 15 years (Lloyd Gunther, Refuge Manager, personal communication). The two hunted-over sites, 18A and 18B, experience heavy use and were about 70-80 meters out in front of permanent blinds.

5) Farmington Bay Migratory Bird Refuge, south of Bear River Refuge, Utah. Bottom conditions are the same as at Bear River Refuge. The control area (Site 21) was a small pond near headquarters not hunted since the inception of the Refuge (Lloyd Gunther, Refuge Supervisor, personal communication). The hunted-over area receives moderate use (Site 20).

Greenhouse Investigations

Eight tanks (61 cm x 91 cm x 61 cm) were constructed of plywood and made watertight with polyurethane-catalyst paint. Each was filled with bottom sediment to the average depth of 18 cm. This amounts to 117,918 cm^3 of soil in each tank. The sediment, analyzed and found free of lead, came from along the Blackfoot River near Missoula, Montana. The eight tanks were divided into three groups of three, three, and two tanks. The first tank in each group was designated as the control tank with no lead being added to the sediments. The remaining tanks in each group were treated with the addition of approximately 400 g of powdered reagent-grade lead. The lead was mixed with the bottom sediments prior to filling with water, which was analyzed and found to have no detectable lead. Powdered lead was utilized in the greenhouse environment to speed up the reaction rate of lead into compounds which might be assimilated by the plants being grown. This was done to reduce the research period to a workable time frame.

Four bottom-rooted aquatic plants, Zizania aquatica, Polygonum lapathifolia, Potamogeton pectinatus, and Sparganium sp., were planted. The P. pectinatus resulted in total failure and the P. lapathifolia produced no seeds. For these reasons, it was decided to concentrate on the Z. aquatica and Sparganium since they grew well, yielded all three parts of the plant desired for analysis, and are readily eaten by wild waterfowl wherever available to them.

The seeds for planting were obtained from Game Food Nurseries, Oshkosh, Wisconsin. The Zizania was planted in seed form by broadcasting and the Sparganium in the form of roots placed in predug holes.

Plants in the greenhouse were planted in March and grown for 7 months, harvested, and analyzed. The Zizania was turning brown and the seed heads were beginning to fall from the plants.

Ravalli Pond Investigation

With the permission of the U.S. Fish and Wildlife Service, a small pond, located on the Ravalli Wildlife Refuge near Stevensville, Montana, was used for an *in vivo* experiment to provide actual field data on lead uptake with control of as many variables as possible. This pond, approximately 0.2 hectare in size, was created by shallow excavation of alluvial soil behind an existing dike on the Bitterroot River and was supplied with continuous fresh water from a flow-through ditch. The pond was divided in half using a series of steel fence posts driven into the bottom sediment. One half was the control site with no lead application while the other half received 227.3 kilograms of No. 12 lead shot distributed evenly from shore edge to midpond (121.9 cm deep).

Laboratory Analysis

Field and greenhouse samples of aquatic plants were analyzed for lead content. The plant samples were dried at 90°C and then digested by a wet ash technique modified from Behan and Kincaide (1970) (Appendix C) and analyzed on a Beckman Model 440 Atomic Absorption Spectrophotomer set at 2,170° A (Appendix D).

Since the absorbance curve for lead was only linear from 0.0 to 5.0 mg/1, the sample solutions containing more lead than this were diluted to bring them into the linear portion of the curve.

National Bureau of Standards Sample Analysis

A standard reference sample from the National Bureau of Standards was used as a check against the analysis technique to maintain accuracy and precision.

	NBS sample #1571	Percentage of normal
NBS lead content	44.5 mg Pb/ml	100
Analysis #109	45.5 mg Pb/ml	102
Analysis #120	48.1 mg Pb/ml	108

Both results were within an acceptable margin of error (10%) for such determinations.

To facilitate comparisons and reference back to earlier experiments, final lead content in the plants was expressed as micrograms of lead per gram of dried plant tissue. This figure was derived by multiplying the micrograms Pb per milliliter of analyzed solution times the milliliters of solution analyzed (25) and dividing by the grams of dried plant sample analyzed.

$$\mu g \text{ Pb/g dried plant tissue} = \frac{(\mu g \text{ Pb/ml determined})(\text{ml analyzed solution})}{(\text{g dried plant tissue analyzed})}$$

CHAPTER III

RESULTS

Field Data

Tables 1 through 4 indicate lead accumulations in plants from the five wildlife refuges chosen for sampling.

Roots of the treated *Cyperus esculentus* plants, taken from the Chautauqua and Fountain Grove Refuges (Tables 1 and 2), were higher in lead than the controls in 86 percent of the samples. The treated roots of plants from Chautauqua exhibited a mean 147 percent increase in lead content over the control.

Treated shoots of the *Cyperus e.* exceeded the controls in 67 percent of the cases with a mean increase over the control of 143 percent at Chautauqua and 240 percent at Fountain Grove.

The treated seeds of *Cyperus e.* were higher than their control in 100 percent of the samples from the Chautauqua Refuge with a mean increase of 226 percent over control (Table 5).

Treated samples (whole plant) of *Eleocharis smallii*, from the Chautauqua, Fountain Grove, and Squaw Creek Refuges (Tables 1, 2, and 3), were higher in lead than the controls in 50 percent of the samples with a mean increase over control lead level of 221 percent

Table 1. Lead content of rooted aquatic plants at Chautauqua National Wildlife Refuge, Illinois.

Collection site	μg Pb/g dry plant tissue			
	Roots	Shoots*	Seeds	Whole plant
		Cyperus esculentus		
11 (control)	12.50	4.67	2.92	
10	13.33	6.83		
12	18.33	7.50	2.50	
13	16.67	10.17	8.00	
14A	19.33	4.56	10.00	
14B	24.17	4.33	5.00	
		Xanthum globosom		
11 (control)	5.00	7.17	23.33	
10	8.33	7.17	10.17	
		Eleocharis smallii		
11 (control)				4.00
12				10.00
14A				7.67

*Stems and leaves.

Table 2. Lead content of rooted aquatic plants at Fountain Grove Wildlife Refuge, Missouri.

Collection site	μg Pb/g dry plant tissue			
	Roots	Shoots*	Seeds	Whole plant
		<u>Cyperus esculentus</u>		
17 (control)	16.25	2.08	15.42	
15	17.10	5.00	12.08	
16	6.25		10.40	
		<u>Polygonum lapthifolia</u>		
17 (control)	17.92	6.47		
15	8.75	14.17		
16	12.50	8.75		
		<u>Eleocharis smallii</u>		
17 (control)				16.25
15	44.04			
16				3.75

*Stems and leaves.

Table 3. Lead content of rooted aquatic plants at Squaw Creek National Wildlife Refuge, Missouri.

Collection site	μg Pb/g dry plant tissue			
	Roots	Shoots*	Seeds	Whole plant
		Sagittaria latifolia		
9 (control)		14.94	5.08	
6	32.92			
7			3.30	
8	11.67	5.93		
		Scirpus fluviatilus		
9 (control)	10.00	10.83		
6	12.08	8.30		
7				
8	13.75	8.30		
		Nelumbo lutea		
9 (control)			73.33	
6			75.80	
7			11.04	
		Eleocharis smallii		
9 (control)				6.54
8				5.93

*Stems and leaves.

Table 4. Lead content of rooted aquatic plants at Bear River Refuge and Farmington Bay Bird Refuge, Utah.

Collection site	μg Pb/g dry plant tissue			
	Roots	Shoots*	Seeds	Whole plant
		Scirpus paladosus		
Bear River				
18	30.00	4.17	4.17	
19 (control)	25.42	2.08	6.25	
Farmington Bay				
20	75.51	8.75	8.67	
21 (control)	52.50	7.72	4.40	
		Potamogeton pectinatus		
Bear River				
18A			26.25	23.75
18B				25.42
19 (control)			15.00	15.83
Farmington Bay				
20				138.75
21 (control)				29.17

*Stems and leaves.

Table 5. Normalized* comparison of lead content in aquatic plants from national wildlife refuges in Missouri, Illinois, and Utah.

Collection site	µg Pb/g dry plant tissue							
	Roots	% control	Shoots**	% control	Seeds	% control	Whole plant	% control
				Cyperus esculentus				
Chautauqua								
11§	12.50	100	4.67	100	2.92	100		
10	13.33	107	6.83	146	--	--		
12	18.33	147	7.50	161	2.50	86		
13	16.67	133	10.17	218	8.00	274		
14A	19.33	155	4.56	98	10.00	343		
14B	24.17	193	4.33	93	5.00	171		
		(147%)§§		(143%)		(226%)		
Fountain Grove								
17§	16.25	100	2.08	100	15.42	100		
15	17.10	105	5.00	240	12.08	78		
16	6.25	39	--	--	10.40	67		
		(72%)		(240%)		(73%)		
				Scirpus paladosus				
Bear River								
19§	25.42	100	2.08	100	6.25	100		
18	30.00	118	4.17	201	4.17	67		
Farmington Bay								
21§	52.50	100	7.72	100	4.40	100		
20	75.51	144	8.75	113	8.67	197		
				Potamogeton pectinatus				
Bear River								
19§					15.00	100	15.83	100
18A					26.25	175	23.75	150
18B							25.42	161
								(156%)
Farmington Bay								
21§							29.17	100
20							138.75	476

*Normalization achieved by setting the control sample at 100%.
**Stems and leaves.
§Controls.
§§Mean, treated samples.

at Chautauqua.

With the Scirpus paladosus from the Bear River and Farmington Bay, Utah Refuges (Table 4), the treated roots had more lead than the controls in all samples with increases of 118 and 144 percent, respectively (Table 6). The trend was the same in the shoots with increases of 101 and 113 percent.

The treated Scirpus p. seeds exceeded the controls only 50 percent of the time with a 197 percent increase in lead content over the control sample at Farmington Bay.

In each plant part of the Potamogeton pectinatus sampled at the Bear River and Farmington Bay Refuges, the treated samples exceeded the controls with increases in the treated whole plants of 156 and 476 percent over the controls.

Differential Lead Accumulation--Field Samples

Lead accumulation was greater in 89.3 percent of the roots than all other plant parts analyzed (Table 6). The roots exceeded the seeds in 12 of 14 (86%) of the samples and were higher in lead than the shoots in 89 percent of the cases.

Using mean figures for each part of each species analyzed, the roots of the various plants accumulated lead over the shoots from 133 to 581 percent. In the case of the Scirpus p. and Cyperus e., the increase in mean lead accumulation of roots over seeds was

Table 6. Differential lead accumulation in various plant parts--field sites.

μg Pb/g dry plant tissue			Pb concentration ratios
Roots	Seeds	Shoots*	
		Sagittaria latifolia	
19.58		0.67	root/shoot, 4.31
15.33		3.75	
6.50		3.75	
32.92		5.80	
52.50	45.83	15.42	
(25.34)**		(5.88)	
		Scirpus fluviatilus	
12.08		8.30	root/shoot, 2.61
13.75		8.30	
10.00		10.83	
13.83		3.83	
40.00		3.17	
(17.93)		(6.87)	
		Scirpus paladosus	
25.42	6.25	2.08	root/shoot, 5.81
30.00	4.17	4.17	root/seed, 5.62
24.17	8.67	8.75	seed/shoot, 1.04
52.50	4.40	7.72	
(33.02)	(5.88)	(5.68)	

Table 6. (continued)

μg Pb/g dry plant tissue			Pb concentration ratios
Roots	Seeds	Shoots*	
		Cyperus esculentus	
19.33	10.00	4.56	root/shoot, 2.84
24.17	5.00	4.33	root/seed, 1.93
16.67	8.00	10.17	seed/shoot, 1.47
18.33	2.50	7.50	
13.33	--	6.83	
12.50	2.92	4.67	
17.10	12.08	5.00	
6.25	10.40	--	
16.25	15.42	2.08	
(15.99)	(8.29)	(5.64)	
		Xanthum globosom	
8.83		7.17	root/shoot, 0.97
5.00	23.33	7.17	
(6.92)		(7.17)	
		Polygonum lapthifolia	
8.75		14.17	root/shoot, 1.33
12.50		8.75	
17.92		6.47	
(13.06)		(9.80)	

*Stems and leaves.

**Mean values.

562 and 193 percent, respectively, while the seeds of these two species accumulated lead above the shoots at 104 and 147 percent.

The plants from the Ravalli Wildlife Refuge test pond, Stevensville, Montana (Table 7), showed no trends concerning lead accumulation. In each of the five species sampled, the plants from the control half of the pond were nearly equal to or higher than the same species from the treated side of the pond. It should be noted that only 6 months elapsed between treatment of the pond with lead shot and collection of plant samples for analysis.

Greenhouse Data

The first growth of treated greenhouse plants exhibited extremely high lead content in the roots and leaves compared to the controls. There was suspicion of lead powder contamination on the plant surfaces resulting from the application technique; therefore, these results are not reported. A second growth of *Zizania aquatica* and *Sparganium* was initiated in the previously treated bottom soils to resolve the contamination question.

This second growth of plants could not be contaminated above the soil/water interface as the previously introduced lead powder had been carefully worked into the sediments and the water in the tanks changed and checked prior to sowing the seeds.

The second growth of *Zizania aquatica* (Table 8) showed

Table 7. Lead content of plants from Ravalli Wildlife Refuge, Montana.

Collection site	μg Pb/g dry plant tissue			
	Roots	Shoots*	Seeds	Whole plant
		Rumex		
Control	17.50		18.33	
Treated	14.17		24.58	
		Polygonum aquaticus		
Control	10.00	6.67	11.63	
Treated	9.58	7.92	6.00	
		Typha latifolia		
Control	22.83	2.92		
Treated	9.58	7.92		
		Sparganium		
Control	10.00	6.50	8.33	
Treated	12.50	7.08	7.08	
		Eleocharis palustrus		
Control				7.08
Treated				6.67

*Stems and leaves.

100 percent of the treated plants with higher lead accumulations than the controls. The mean increases (Table 9) of treated over controls were 335 percent in the shoots and 382 percent in the seeds.

Table 8. Lead content of rooted aquatic plants--greenhouse investigations.

Tank	μg Pb/g dry plant tissue		
	Roots	Shoots*	Seeds
	Zizania aquatica		
1 (control)	42.33	7.08	9.58
2		37.33	40.50
3		19.17	20.00
4		14.58	49.17
	Sparganium		
Control	130.00	10.83	
Treated	602.50	194.50	

*Stems and leaves.

Table 9. Normalized* comparison of lead content of aquatic plants grown under controlled greenhouse conditions.

Tank	μg Pb/g dry plant tissue					
	Roots	% control	Shoots**	% control	Seeds	% control
			Zizania aquatica			
1§			7.08	100	9.58	100
2			37.33	527	40.50	423
3			19.17	271	20.00	209
4			14.58	206	49.17	513
				(335%)§§		(382%)
			Sparganium			
Control	130.00	100	10.83	100		
Treated	602.50	464	194.50	1,796		

*Normalization achieved by setting the control samples at 100 percent.

**Stems and leaves.

§Controls.

§§Mean, treated samples.

In the Sparganium, although data must be suspect due to the low number of samples, the increase in lead content of over control plants, ranged from 602 percent in the roots to 1,796 percent in the shoots (Table 10). The Sparganium produced no seeds during the experiments.

In more than 85 percent of all plants sampled, field and greenhouse, the root system contained higher lead concentrations than either the seeds or the leaves. Over 50 percent of all seed samples exceeded the leaves in lead content.

Table 10. Differential lead accumulation in various plant parts--greenhouse.

μg Pb/g dry plant tissue			Pb concentration ratios
Roots	Seeds	Shoots*	
		Zizania aquatica	
42.33	9.58	7.08	root/shoot, 5.98
	40.00	37.33	seed/shoot, 1.52
	20.00	19.17	
	49.17	14.58	
	(29.67)**	(19.54)	
		Sparganium	
130.00		10.83	root/shoot, 3.57
602.50		194.50	
(366.25)		(102.67)	

*Stems and leaves.

**Mean values.

CHAPTER IV

DISCUSSION

Variables Affecting the Experimental Data

After field sampling had begun, it became apparent that several uncontrollable variables might affect subsequent Pb content of field plant samples.

1) Lead shot distribution patterns for a given site. Lead shot may ball together and travel far beyond the normal distribution pattern, thereby yielding lead in areas not suspected of containing shot (Lind, 1971).

2) Lead additions from upstream industrial sites. Every refuge sampled was in a flood plain downstream from a major industrial area (e.g., Chautauqua--Peoria, Illinois; Bear River/ Farmington Bay--Salt Lake City, Utah). Mathis and Cummings (1971), in a study of selected metals in the waters of the Illinois River found lead levels as high as 140 ppm ($\mu g/g$) in the bottom sediments of the River below Peoria. This compared with 27 ppm maximum lead in three nonindustrial streams nearby. It would seem that lead might be added to control areas in refuges subject to flooding from a river receiving industrial wastes containing pathological concentrations of

heavy metals.

3) Bottom sediment deposition rates. Bill Watts (Field Staff, personal communication), Chautauqua National Wildlife Refuge, stated that the Refuge receives 7.5 to 35.0 cm of silt deposition a year depending on the severity of flooding. This could easily put a year's Pb shot accumulation out of reach of the geochemical action of the mud/water interface and/or out of contact with the roots of bottom-rooted aquatic plants.

4) Lead shot settling rates. Bellrose (1959) found that pellets settled 2.5 to 5.0 cm below the mud/water interface after approximately 1 year in soft soil and less than 2.5 cm in moderately firm soil. Low and Studinski (1967) and Bachman and Low (1973) found that shot size caused little difference in settling rate and that 75 percent of all Number 4 shot remained in the top 2.5 cm of sediment after 1 year. Wycoff et al. (1971), using three soil types and four shot sizes (#2, #4, #5, and #6), realized no difference in the settling rate of lead pellets due to shot size.

It would seem from variables 3 and 4, that size of shot affects pellet settling rates, and therefore lead shot availability to waterfowl, very little. Sediment type (firmness) and deposition rate, however, appear of prime importance in affecting the availability of lead pellets both for actual ingestion by wild waterfowl and contact for assimilation through the food chain.

Field Studies

The data presented in Tables 1 through 4 contain some erratic accumulation rates. These may, in part, be explained by the above mentioned variables. In addition, different plant species may exhibit varying assimilation rates or tolerance levels to lead accumulation.

The Fountain Grove and Squaw Creek, Missouri, collection sites were subjected to variables 2 and 3, but not 1. Each was in a river flood plain, but not downriver of any significant industrial effluents. Chautauqua, Illinois, was subjected to variables 1 through 3, especially upriver lead input.

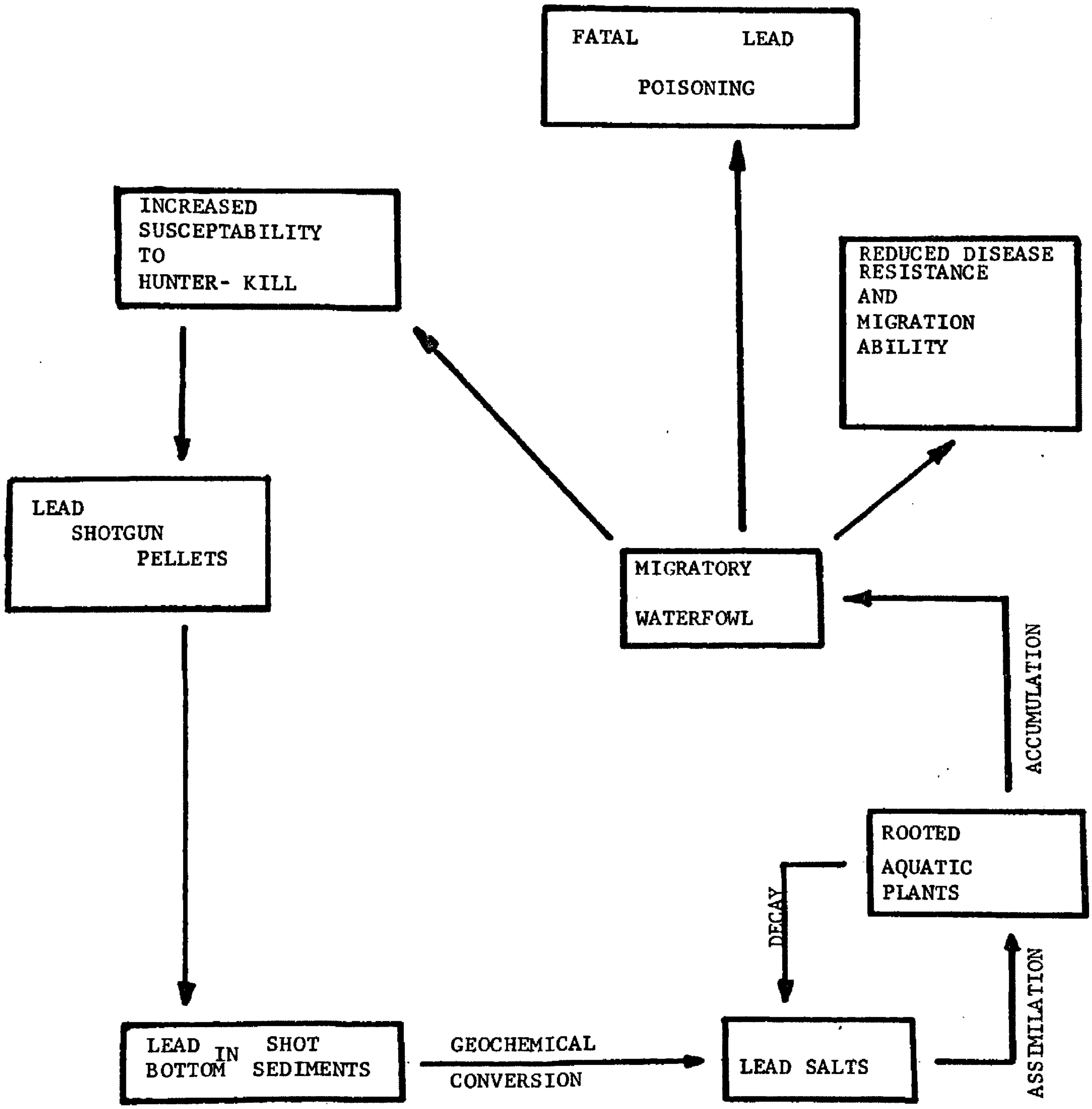
In several cases, plants collected in an area exhibited manifestly higher lead content than neighboring plants of the same species. This could be attributed to root contact with an abnormal amount of lead due to the balling effect mentioned earlier. This is especially true since there is still considerable doubt as to the exact mechanism of assimilation of lead by rooted aquatic plants.

Even with the nonideal accumulation rates there emerges, in the field samples, a picture of definite lead accumulation by certain rooted aquatic plants which are utilized by wild waterfowl in their normal diet (Fig. 2).

In the plants from the five national wildlife refuges sampled (Tables 1-4), the roots of the treated plants exceeded the controls in

Fig. 2. Lead transfer through the aquatic food chain.

Fig. 2. Lead transfer through the aquatic food chain.



lead content 80 percent of the time. The treated shoots were higher in lead content than the controls in 67 percent of the cases and the seeds from shot over plants exceeded their controls 55 percent of the time.

Table 6 illustrates the tremendous increases in the lead content of treated roots over other plant parts, for example, 581 percent in Scirpus paladosus. This may be due to the fact that the root is the entryway into the plant and is a normal storage area for many nutrients and other constituents. It is also quite possible that these high lead levels in the roots are due to difficulty in removing lead contaminated soils from the surface of the often fibrous root systems of these aquatic plants. If this is true, the lead levels still remain significant because waterfowl feeding on these treated roots (tubers, corms) would ingest the same lead as those concentrations determined by the plant analysis.

Regardless of whether the lead detected in root samples was adhering as difficult to remove surficial deposits or being absorbed into the root tissue, it represents both a significant possible source of pathological lead uptake by waterfowl and a means of reentry of lead into the growth environment of the rooted aquatics upon decay of the plant system (Fig. 2).

As mentioned earlier, the results from the Ravalli Refuge test pond (Table 8) were nonconclusive at the time they were obtained, with the treated plants being higher in lead than the controls in exactly 50 percent of the samples and 60 percent of all the treated plants sampled being within 15 percent of their controls' lead content. These nonconclusive data were probably the result of insufficient time elapsing for the necessary geochemical conversion of the lead shot and the subsequent absorption or adsorption of lead into the rooted aquatic plants sampled from the treated half of the pond. Therefore, the data from this analysis must be viewed as random sampling of different plant tolerances to normal background lead in the soil. Lead transfer into the food chain from the root environment apparently has a minimum time factor which cannot be shortened through increased lead shot in the growth environment.

The Ravalli test pond was set up in order to simulate natural field growth conditions during lead treatment and yet control as many variables as possible of the type mentioned earlier. Subsequent sampling and analysis at the Ravalli test pond by Drs. Mark Behan and Thomas Kinraide (summer 1974), indicate that the lead content of the treated plants has greatly increased.

Greenhouse Studies

The controlled growth studies in the greenhouse appear to strongly support the field data in showing lead accumulation in the treated plants over the untreated. The second growth of *Zizania* aquatica (Table 9) showed 100 percent of the treated plants with higher lead content than the controls. The increased concentrations of treated plants over controls were major, being 335 percent and 382 percent for shoots and seeds, respectively.

The Sparganium exhibited lead accumulation in treated plants greater than the controls, to a greater extent than Zizania, with 194.50 μg Pb/g in the shoot as compared to 37.33 μg Pb/g in the Zizania stems and leaves. This is not conclusive as the sample number is quite small.

All these data appear to support the hypothesis that rooted aquatic plants, when exposed to lead in the form of shotgun pellets in their rooting environment, will assimilate and accumulate the lead.

Potential Toxicity of Accumulated Lead

Moyle (1961) and Sincock (1962) estimated food consumption of the average adult mallard duck (1,350 g) at 682 grams of wet plant material or 181.6 grams dry plant tissue per day.

Coburn et al. (1951) determined that ingestion of 8 mg lead per day would result in 100 percent mortality from lead poisoning (plumbism) in 28 days in an adult mallard duck. Using these figures, plant matter would have to contain 44 mg Pb/g dry plant tissue to induce fatal plumbism in an adult Anas platyrhincos in 28 days.

Of the plants analyzed in Tables 1-4 and 8, 14 percent were at or above the 44 mg Pb level. This may be compared to Bellrose's (1959) findings that 10.4 percent of the mallard drakes and 13.0 percent of the mallard hens found dead or dying from lead poisoning over a six-state area contained no ingested lead shot.

Subacute Lead Intake and Its Affects on Waterfowl

Bellrose (1959) found that mallards dosed with one Number 6 lead pellet were 1.84 times as vulnerable to hunting as lead-free mallards. In mallards dosed with four Number 6 pellets, the vulnerability level rose to 2.12 times as much. In addition, Bellrose found a pronounced affect on the migration rate of mallards after being dosed with only one Number 6 lead pellet. A single Number 6 pellet reduced the daily flight distance by over 25 percent, two pellets reduced the daily mileage by almost 50 percent. Moreover, the ingestion of lead was found to greatly increase the mortality rate of mallards. Using banded birds, Bellrose found that one Number 6 lead pellet increased the mortality rate of adult drake mallards by 15 percent while two pellets raised the death rate to 44 percent over that of undosed controls.

Lead in amounts below the level necessary to produce acute plumbism may reduce wild waterfowl's chances of surviving hunters, disease, and wintertime food deprivations.

Clinical Effects of Subacute Lead Intake

Hemphill et al. (1971) discovered that a dosage of 250 μg of $Pb(NO_3)_2$ daily for 30 days in white mice (Swiss-Webster strain) caused no visible symptoms of plumbism. These mice, however, when challenged by Salmonella typhimurium after the 30-day lead dosing, exhibited 100 percent mortality in 3 days. The lead-free control mice had a 13 percent mortality after 7 days. Seyle et al. (1966), found that a single intravenous injection of lead acetate (5 mg Pb/100 g body weight), a dose normally producing no outward symptoms of lead poisoning, increased the sensitivity of female laboratory rats approximately 100,000 times to mortality and organ damage from concurrent intravenous injections of Escherichia coli endotoxin. Truscott (1970) suggests a similar effect when 2.8 mg of lead acetate per 100 g body weight was introduced into Columbia rock chicks and the chicks then challenged by Escherichia coli endotoxin. The increase in sensitivity to the endotoxin when given with 2.8 mg Pb/100 g body weight may be as high as 1,000-fold. Williams et al. (1954), in studying the case history of a 23-month-old girl, learned that lead causes precipitation of the gamma globulin antibodies. They felt that this inactivation of the antibody system might result in brain damage due to unchecked bacterial invasion rather than, or precedent to, clinically observable lead encephalopathy.

In the Hemphill et al. (1971) study, daily 100 μg Pb injections (in the form of lead nitrate) given over 30 days increased mortality in white mice 41 percent over nondosed controls when challenged by Salmonella typhimarium 24 hours after the 30-day lead dosing.

If these data are extrapolated, a mallard duckling, at approximately 680 g (34 times the weight of the average test mouse), would need to take in 3,400 μg Pb per day to be similarly affected. The duckling, eating approximately 75 percent that what an adult eats, would consume 136 g of plant tissue (dry weight) per day. Thus, plant material would need to contain as little as 25 μg Pb/g tissue (dry weight) to significantly reduce the resistance of mallard ducklings to mortality from bacteria such as Salmonella and Clostridium botulinum, provided these mammals and birds have similar susceptibilities to plumbism. Tom Lyle (personal communication, 1970), assistant manager of the Farmington Bay Bird Refuge, Utah, indicated that some 40,000 waterfowl succumbed to botulism in the summer of 1970. Thirty percent of all field site plants exposed to lead shot exhibited this concentration of lead.

All of the above findings point to an issue not yet considered in migratory waterfowl management. In many cases, even though no lead pellets were ingested, lead may have been assimilated through normal dietary intake in subacute dosages, thereby reducing the bird's resistance to disease, its ability to avoid hunter-kill, and its ability to migrate away from harsh winter weather. Any combination of these factors could be working to produce the increased mortality rate observed by Bellrose.

Lead shot, breaking down in the bottom environment of marshes and wetlands, may be assimilated and accumulated by aquatic rooted plants in concentrations significant to cause clinical lead poisoning outright in some cases and subclinical pathologies in many others.

One final observation seems in order. Bellrose (1959) indicated that the lower incidence of lead poisoning in pintails as opposed to mallards might be a result of diet. He felt that a preference for leafy aquatic vegetation on the part of the pintails reduced the ingestion of lead pellets which he surmized mallards picked up mistakenly while foraging for corms and tubers. The fact that the roots (tubers, corms) of 89 percent of the plants sampled exhibited higher lead concentrations than the shoots (part preferred by pintails) might be a valid alternative reason for the observed differential in plumbism between mallards and pintails. The preference for tubers and corms, with their higher lead content, could explain, in part, the higher lead poisoning incidence in the mallards.

Implications for Wild Waterfowl Management

With up to 5.5 million kilograms of lead shot being introduced annually into the rooting environment of aquatic plants utilized by waterfowl in their normal diet, the accumulation of lead through the aquatic food chain and its possible role in waterfowl pathologies must be carefully considered.

If subsequent research adds support to the findings of this study concerning lead accumulation through the aquatic food chain, local, state, and federal agencies charged with managing the wild waterfowl populations of the nation may wish to take a fresh look at their programs for replacement of lead shot for hunting. The United States Fish and Wildlife Service, in a Draft Environmental Statement on proposed use of steel shot for hunting waterfowl in the United States (1974) indicated, "A better understanding of sublethal effects [of lead] could reveal a problem more serious than that previously perceived, since these effects must result in forms of mortality not generally recognized as being related to lead ingestion."

In addition, if subacute intake of lead through the diet were to be substantially linked to large waterfowl mortalities from botulism or similar bacterial sources, it would seem reasonable to begin developing methods for large-scale removal of lead from the rooting environment of aquatic plants and/or seek ways of reducing the intake of lead by waterfowl during the phasing out period for lead shot.

Suggestions for Further Study

To further substantiate the findings of this study, the Ravalli Refuge test pond, Stevensville, Montana, should be monitored yearly to determine the pattern (if any exists) of lead accumulation over time and whether there is any correlation between the duration of lead shot in the rooting environment of the aquatic plants and the level of lead in the plants. Additional work could be done at Ravalli on differential lead accumulations in the various plant parts and whether the settling of shot or annual siltation affects the availability of the lead shot for uptake into the aquatic plant food chain.

Again utilizing the Ravalli test site, captive waterfowl could be fed aquatic plants from both treated greenhouse growths and the treated half of the test pond. The birds could then be monitored to determine if clinical lead poisoning can be induced outright or if waterfowl mortality rates or resistance to disease are affected as has been suggested from subclinical intake of lead.

CHAPTER V

SUMMARY

Lead pathologies resulting from direct ingestion of lead shotgun pellets by wild waterfowl have been thoroughly explored. The role of ingested lead pellets in major waterfowl die-offs has been documented for over 80 years. However, no known research has been done on the possible cycling of the lead from hunters' shotgun pellets through the aquatic plant food chain.

The following approaches were employed to determine whether lead, as lead pellets deposited in the rooting environment of aquatic plants utilized by migratory waterfowl in their normal diet, is assimilated and accumulated by the rooted aquatic plants.
(1) Aquatic plants were collected from four wildlife refuges involved in large scale waterfowl lead poisoning cases. (2) Rooted aquatic plants utilized by migratory waterfowl were grown under controlled greenhouse conditions with artificial introduction of lead into the rooting environment. (3) Four species of rooted aquatic plants were grown in a small test pond at a wildlife refuge, which was half control and half lead treated, to simulate actual field conditions while controlling as many variables as possible.

The results of these studies were as follows:

1) The geochemical environment of bottom sediments in most marshes is conducive to the conversion of lead pellets into a form which can be assimilated by rooted aquatic plants.

2) Lead shot appears to transfer through the aquatic plant food chain under natural and laboratory conditions.

3) Rooted aquatic plants accumulate lead as much as 240 percent over control amounts when grown in areas of high lead shot accumulation.

4) There appears to be differential lead accumulation in various plant parts of lead-treated plants. The roots exceed the seeds by as much as 562 percent while the seeds exceed the shoots (stems and leaves) in lead content at the 147 percent level.

5) Rooted aquatic plants accumulated lead in concentrations capable of causing fatal lead poisoning in 14 percent of plants sampled and subclinical pathologies in 30 percent of all plants analyzed.

6) While fatal lead poisoning via food chain accumulation of lead is not a certainty in hunted-over areas, wild waterfowl may conceivably be exacerbating existing lead toxicity from ingested lead shot by adding to the bodies' lead burden from plants in their normal diet.

7) Subclinical dosages of lead from the food chain may be causing increased susceptibility to hunter-kill and increased

sensitivity to mortality from challenge by common disease organisms. At the same time, this may result in decreased migration capability, navigational ability, and resistance to wintertime food deprivations.

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APPENDIX A

DETAILED MAPS OF FIELD COLLECTION SITES

The following maps show the actual sample sites located as closely as possible within each chosen refuge.

Map 1. Squaw Creek National Wildlife Refuge, Missouri

Map 2. Chautauqua National Wildlife Refuge, Illinois

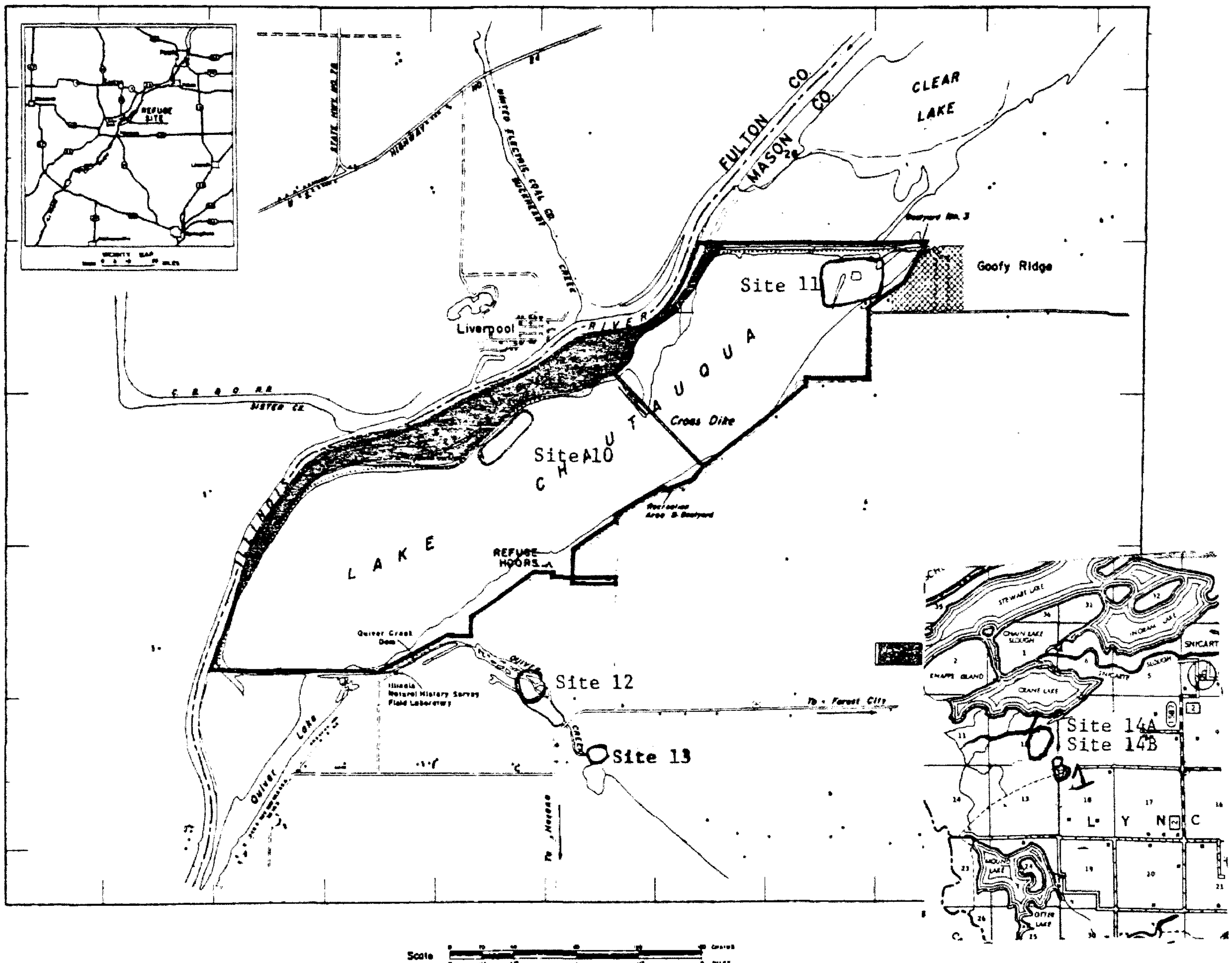
Map 3. Fountain Grove Wildlife Management Area, Missouri

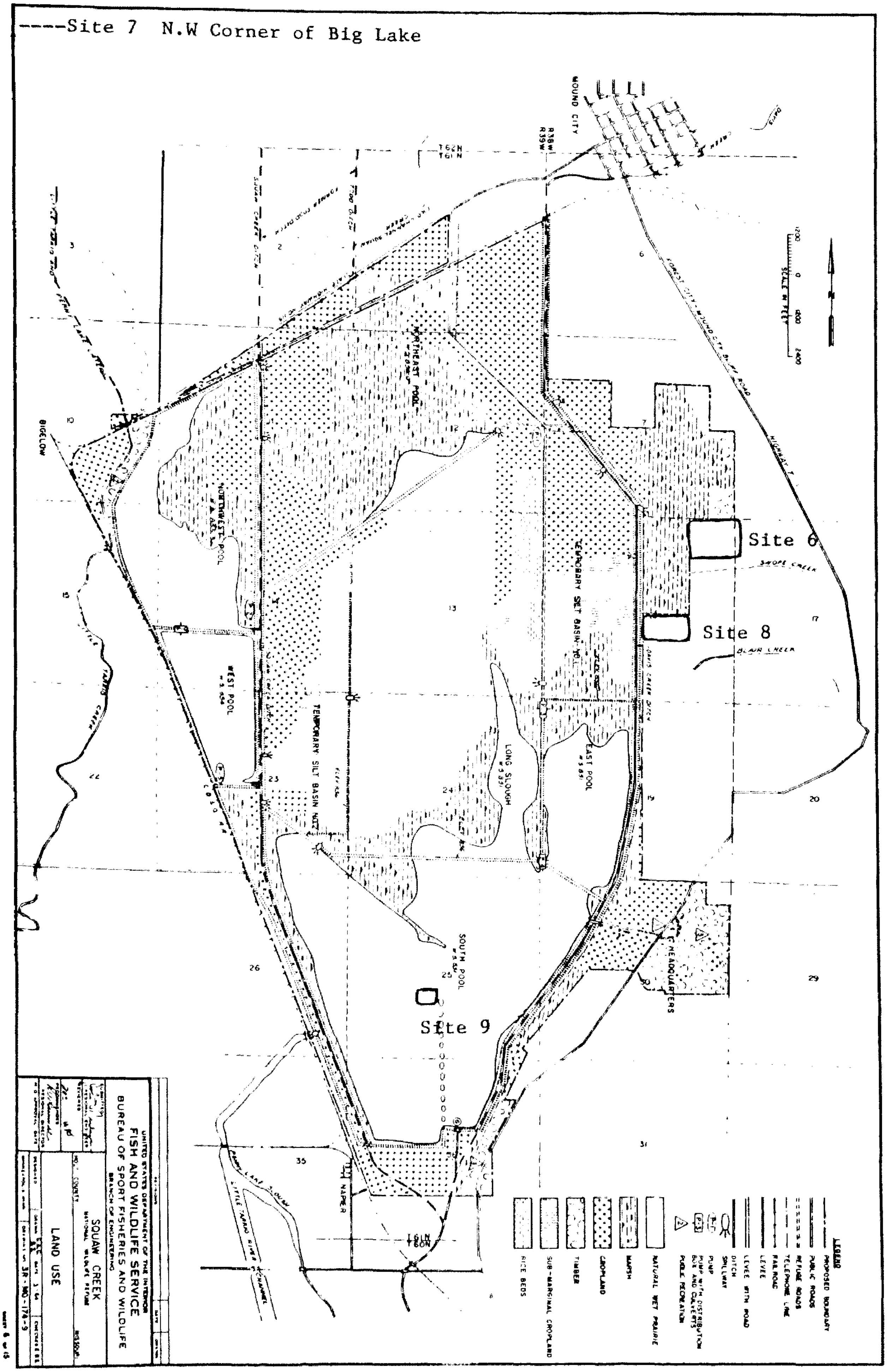
Map 4. Bear River Migratory Bird Refuge, Utah

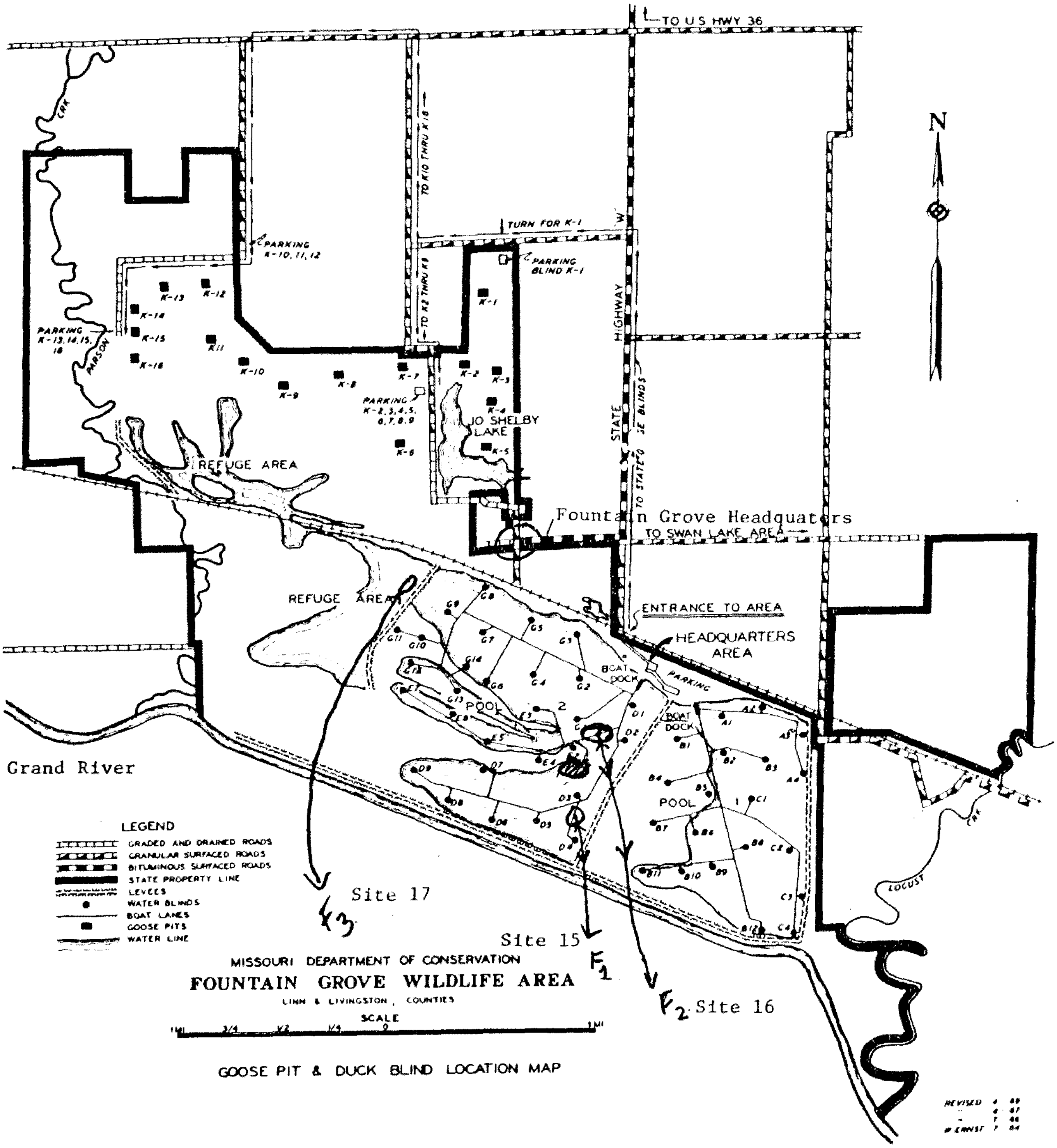
Map 5. Ravalli Wildlife Refuge, Montana

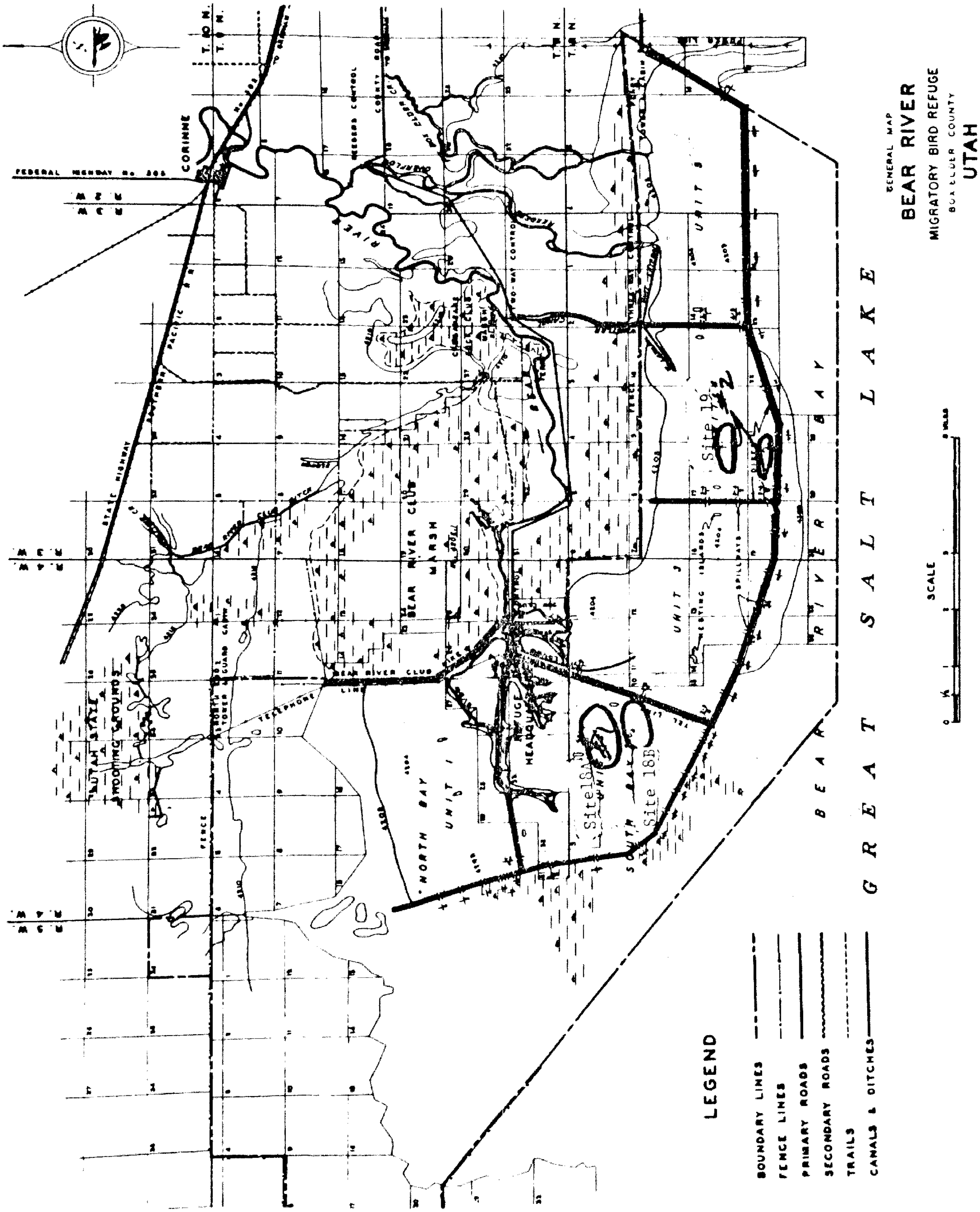
CHAUTAUQUA NATIONAL WILDLIFE REFUGE

MASON COUNTY, ILLINOIS




----Site 7 N.W Corner of Big Lake
Site 6
Site 8
Site 9
MOUND CITY
BIGELOW
NAPIER
HEADQUARTERS
NORTHEAST POOL
NORTHWEST POOL
WEST POOL
EAST POOL
LONG SLOUGH
SOUTH POOL
TEMPORARY SILT BASIN NO. 1
TEMPORARY SILT BASIN NO. 2
SHOPE CREEK
BLAIR CREEK
SCALE IN FEET
LEGEND
PROPOSED BOUNDARY
PUBLIC ROADS
REFUGE ROADS
TELEPHONE LINE
RAILROAD
LEVEE
LEVEE WITH ROAD
DITCH
SPILLWAY
PUMP
PUMP WITH DISTRIBUTION BOX AND CULVERTS
PUBLIC RECREATION
NATURAL WET PRAIRIE
MARSH
CROPLAND
TIMBER
SUB-MARGINAL CROPLAND
RICE BEDS
UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
SQUAW CREEK
NATIONAL WILDLIFE REFUGE
LAND USE


TO US HWY 36
N
TO K10 THRU K18
TURN FOR K-1
PARKING K-10, 11, 12
PARKING BLIND K-1
TO K2 THRU K9
HIGHWAY
STATE
PARKING K-13, 14, 15, 16
PARSON
CRK
REFUGE AREA
JO SHELBY LAKE
PARKING K-2, 3, 4, 5, 6, 7, 8, 9
Fountain Grove Headquaters
TO SWAN LAKE AREA
ENTRANCE TO AREA
HEADQUARTERS AREA
REFUGE AREA
BOAT DOCK
PARKING
POOL 2
POOL 1
Grand River
LOCUST
Site 17
Site 15
Site 16
F1
F2
LEGEND
GRADED AND DRAINED ROADS
GRANULAR SURFACED ROADS
BITUMINOUS SURFACED ROADS
STATE PROPERTY LINE
LEVEES
WATER BLINDS
BOAT LANES
GOOSE PITS
WATER LINE
MISSOURI DEPARTMENT OF CONSERVATION
FOUNTAIN GROVE WILDLIFE AREA
LINN & LIVINGSTON, COUNTIES
SCALE
1 MI 3/4 1/2 1/4 0 1 MI
GOOSE PIT & DUCK BLIND LOCATION MAP

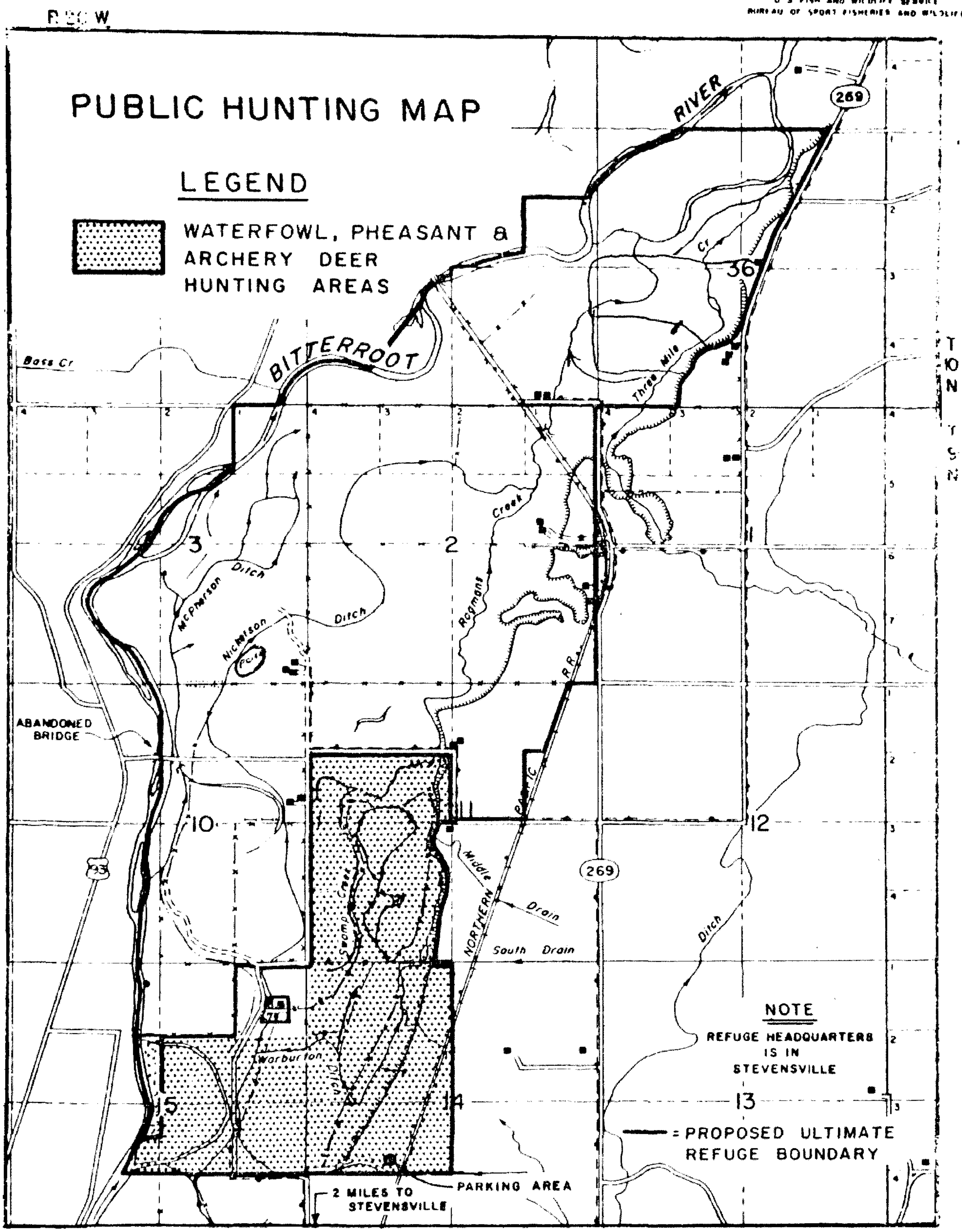

GENERAL MAP
BEAR RIVER
MIGRATORY BIRD REFUGE
BOX ELDER COUNTY
UTAH
LEGEND
BOUNDARY LINES
FENCE LINES
PRIMARY ROADS
SECONDARY ROADS
TRAILS
CANALS & DITCHES
SCALE
GREAT SALT LAKE
BEAR RIVER BAY
CORINNE
FEDERAL HIGHWAY No. 30S
STATE HIGHWAY
UTAH STATE SHOOTING GROUNDS
BEAR RIVER CLUB MARSH
BEAR RIVER CLUB
TELEPHONE LINE
NORTH BAY UNIT 1
SOUTH BAY UNIT 2
UNIT 3
UNIT 5
REFUGE HEADQUARTERS
Site 17
Site 18A
Site 18B
Site 19

RAVALLI NATIONAL WILDLIFE REFUGE

RAVALLI COUNTY, MONTANA

UNITED STATES
DEPARTMENT OF THE INTERIOR

U. S. FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE



COMPILED IN THE BRANCH OF ENGINEERING FROM SURVEYS BY AERIAL PHOTOGRAPHY, F&WS, BLM, USGS, AND MONTANA STATE HIGHWAY DEPARTMENT

PORTLAND, OREGON AUGUST 1966

PRINCIPAL MERIDIAN

Scale 0 10 20 40 60 80 CHAINS; 0 1/8 1/4 1/2 3/4 1 MILES

TOWNSHIP DIAGRAM

MEAN DECLINATION 1962

1R MONT 666 41

APPENDIX B

SAMPLE COLLECTION AND PREPARATION

1. Plant samples placed in new plastic bags.
2. Bags held in ice cooler until taken to laboratory.
3. Plants separated into roots, shoots (stems and leaves) and seeds.
4. Plant parts triple washed in distilled H_2O.
5. Root samples triple rinsed in 1 N HNO_3 to remove lead contaminated soil.
6. Dried at 90° C. for 48 hours in forced-draft oven.
7. Ground in Wiley Mill through a 40-mesh screen.
8. Ground samples placed in acid cleaned glass jars. Jars triple rinsed in 4 N HNO_3.
9. Samples digested according to modified wet-ash procedure from Behan and Kinraide (1970).

APPENDIX C

WET-ASH DIGESTION PROCEDURE

1. 3 g dried plant sample (weighed on analytical balance).
2. Place sample in 300 ml Erlenmeyer flask.
3. Add 50 ml of 4/1 concentrated reagent grade $HNO_3/HClO_4$ solution.
4. Place flask on hooded hot plate.
5. Solution is heated (with some refluxing) until the HNO_3 is evaporated off.

   Caution: NO_2 is released and must be safely vented outside the lab. Also, heated, concentrated $HClO_4$ is an extremely powerful oxidizer. All organic matter must be oxidized by the HNO_3 before the $HClO_4$ begins to increase in temperature or an explosion may result.
6. Solution is allowed to cool.
7. Solution is then filtered (common asbestos fiber).
8. Sample solution brought up to 50 ml with distilled H_2O in a volumetric flask.
9. Sample solution then transferred to pre-rinsed glass bottles for

analysis on the Atomic Absorption Spectrophotometer.

Note: Samples were run within 72 hours to preclude possible leaching of lead from the glass bottles by the strong acid solution within.

APPENDIX D

ATOMIC ABSORPTION SPECTROPHOTOMETRY ANALYSIS

The Atomic Absorption analysis was carried out on a Beckman Model 440 Atomic Absorption Spectrophotometer using the following parameters:

Wavelength	-	2170 Å
Lamp Current	-	20 ma
Acetylene Pressure	-	4 p.s.i.
Support Gas Pressure (compressed air)	-	20 p.s.i.
Lamp	-	Neon/Lead
Slit Width	-	9

The following curve shows the linearity of absorbance versus mg/ml Pb in standard solution (1 N HNO_3). Linearity was quite good up to 5.0 mg/ml Pb in all cases. Also included are the results from analysis of National Bureau of Standards standard reference sample #1571 on two separate occasions. The same procedure as in analysis of field and greenhouse samples was used both times.

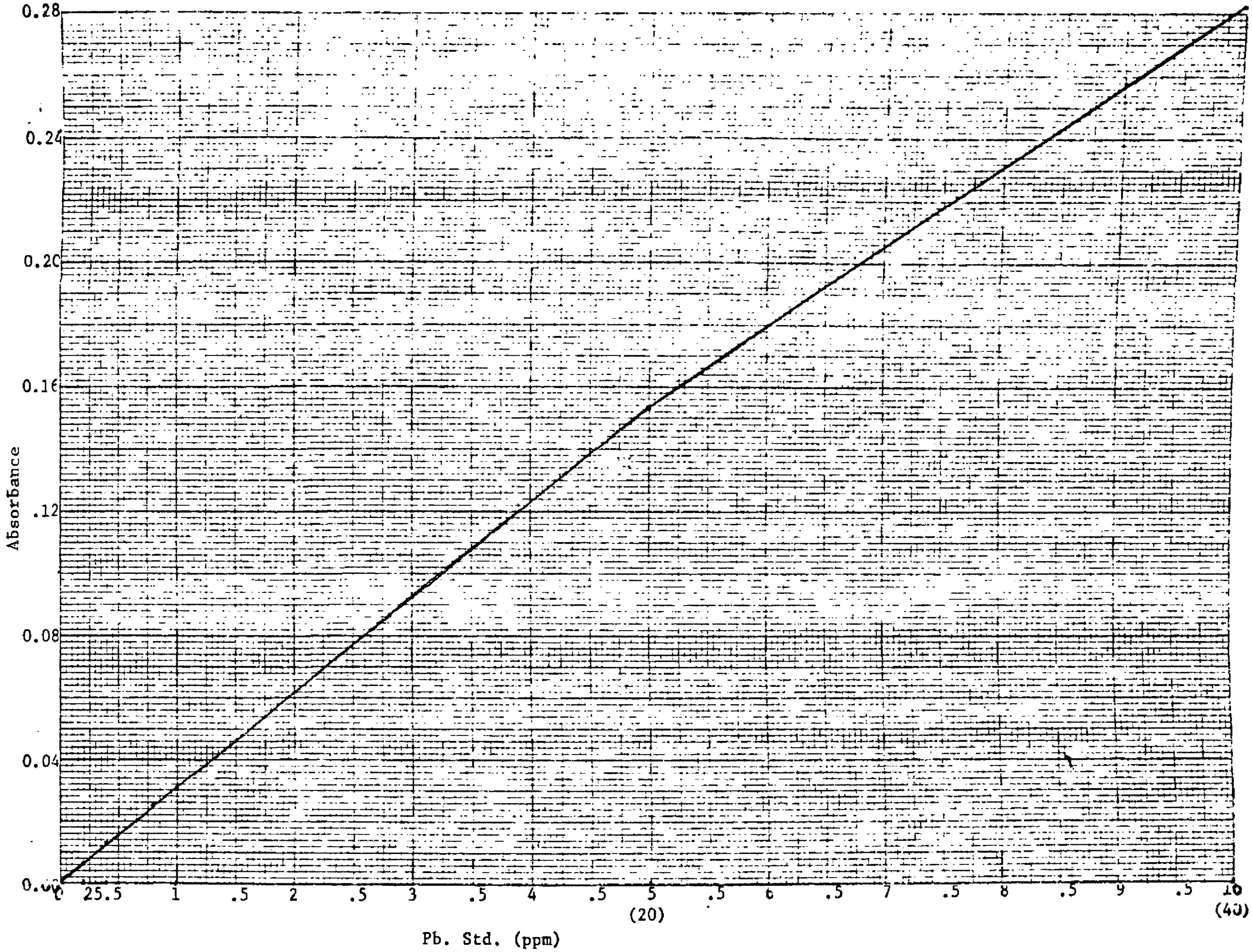
Run #109	NBS 1571	45.5 mg Pb/ml
Run #120	NBS 1571	48.1 mg Pb/ml
Actual	NBS 1571	44.5 mg Pb/ml

Both readings are well within an acceptable margin of error (10%) for such determinations.

The National Bureau of Standards standard reference sample #1571 is orchard leaves collected and analyzed using two distinct techniques: 1) isotope dilution, and 2) polarography.

The N.B.S. reference sample was used as a check against the analysis techniques to maintain accuracy and precision.

RUN #110



0.28
0.24
0.20
0.16
.12
0.08
0.04
Absorbance
25.5
1
.5
2
.5
3
.5
4
.5
5
(20)
.5
6
.5
7
.5
8
.5
9
.5
10
(40)
Pb. Std. (ppm)