Metal accumulation in voles from an acid mine drainage impacted wetland
by Thomas Lindsey Zavitz

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University

Abstract:
Concentrations of cadmium, copper, and zinc in the kidneys of meadow voles (Micrttus pennsylvanicus) and in the whole bodies of shrews (Sorex spp.) from an acid mine drainage impacted wetland and a control wetland were determined. Cadmium and copper levels were significantly elevated in kidneys of voles from the contaminated wetland; zinc levels were not elevated. Shrew whole body cadmium levels were also significantly higher while copper and zinc whole body levels were not significantly different compared to animals from the control area. The elevated levels of cadmium demonstrate the potentially hazardous uptake of this element. However, cadmium and copper levels were not considered to be accumulating to hazardous levels in voles or shrews or to be a risk to animals that prey on them.

METAL ACCUMULATION IN VOLES
FROM AN ACID MINE DRAINAGE IMPACTED WETLAND

by

Thomas Lindsey Zavitz

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Rehabilitation

MONTANA STATE UNIVERSITY
Bozeman, Montana

June 1990

APPROVAL

of a thesis submitted by

Thomas Lindsey Zavitz

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

July 26, 1990 — D. J. Dollhopf

Date — Chairperson, Graduate Committee

Approved for the Major Department

7-26-90 — Leonard C. Gagnon

Date — Head, Major Department

Approved for the College of Graduate Studies

August 9, 1990 — Henry L. Parsons

Date — Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Dean of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature ______________

Date 7-26-90

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Concentrations of cadmium, copper, and zinc in the kidneys of meadow voles (*Microtus pennsylvanicus*) and in the whole bodies of shrews (*Sorex* spp.) from an acid mine drainage impacted wetland and a control wetland were determined. Cadmium and copper levels were significantly elevated in kidneys of voles from the contaminated wetland; zinc levels were not elevated. Shrew whole body cadmium levels were also significantly higher while copper and zinc whole body levels were not significantly different compared to animals from the control area. The elevated levels of cadmium demonstrate the potentially hazardous uptake of this element. However, cadmium and copper levels were not considered to be accumulating to hazardous levels in voles or shrews or to be a risk to animals that prey on them.

INTRODUCTION

Recently, artificial wetlands have become an alternative to conventional chemical treatment of acid mine drainage (AMD). The problems of degraded water quality caused by AMD are widespread and have been very expensive to ameliorate. In Montana, the use of man-made wetlands is being considered by the Abandoned Mine Lands Bureau (AMLB) of the Department of State Lands. An earlier study (Dollhopf et al. 1988) concerning the AMD impacted Swamp Gulch wetland suggested that these wetlands may pose a hazard to local wildlife. Specifically, these wetlands act as accumulators of heavy metals, and the species of wildlife living at high trophic levels may be subject to toxic levels of certain metals through biomagnification.

At the abandoned Carbonate Mine site near Lincoln, Montana (Figure 1), AMD has been entering into a natural wetland for over 40 years. The vegetation in this wetland has been tested and found to have metal concentrations above levels found in a nearby pristine wetland (Table 1) (Dollhopf et al. 1988). The levels of some metals are above maximum tolerable forage levels for cattle ingestion (Table 2) (National Academy of Science 1980). The 1988 study by Dollhopf suggested that an examination of metal levels in tissues of the common herbivores would help determine the extent to which certain metals are present in the food chain.

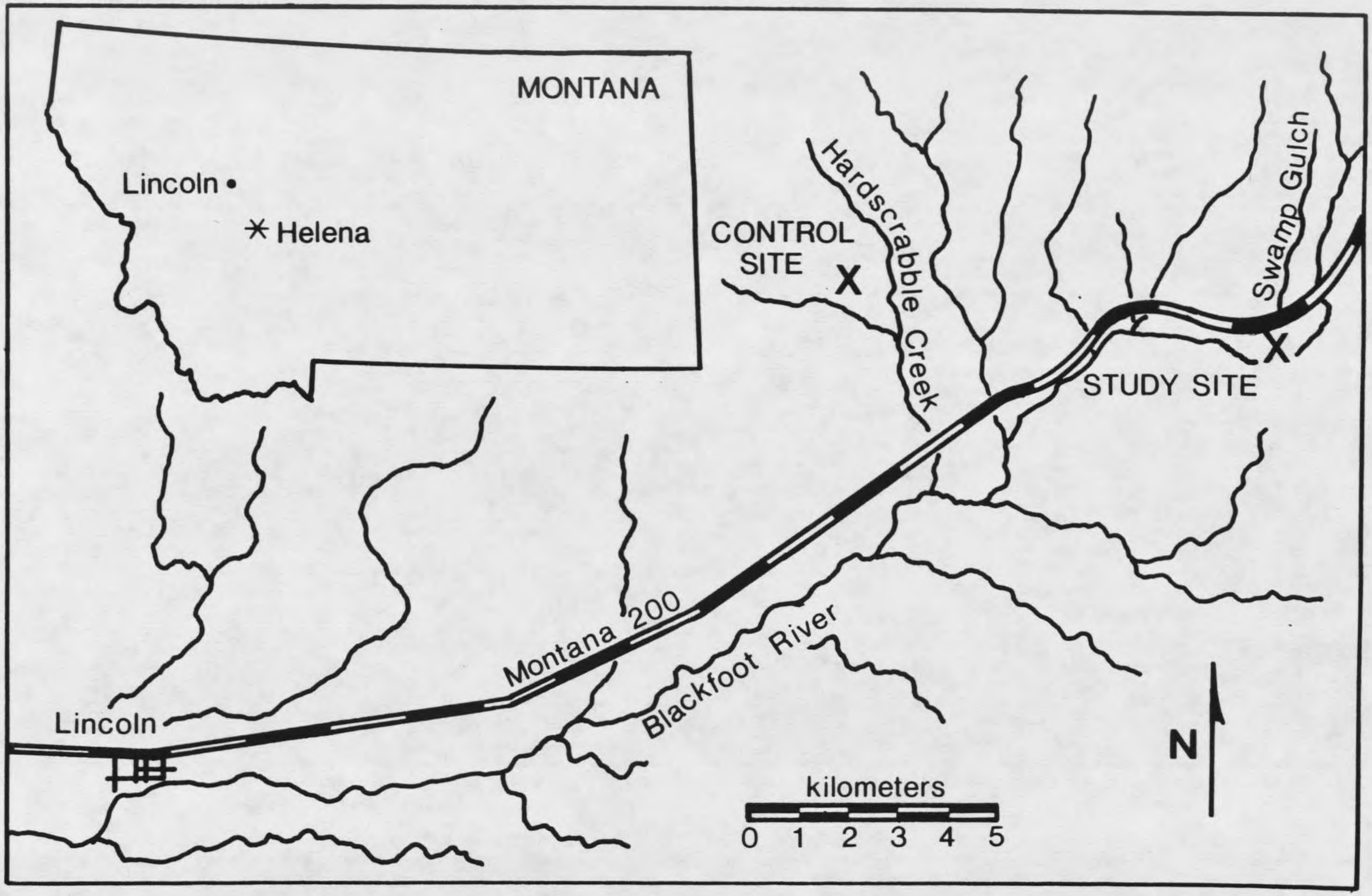

Figure 1. Location of Lincoln, Montana, Swamp Gulch and Hardscrabble Creek.

Table 1. Metal concentrations (μg/g dry weight) in vegetation[t] from the study site (Swamp Gulch wetland) and the background site (Hardscrabble Creek).

Element	Plant species	Hardscrabble Creek		Swamp Gulch	
		n	mean	n	mean
Cd	*Salix boothii*	2	0.6	6	5.6
	Carex rostrata	4	0.8	5	1.5
	Isopterygium pulchellum	1	0.7	4	7
Cu	*Salix boothii*	2	0.35	6	1.3
	Carex rostrata	4	3.8	5	62
	Isopterygium pulchellum	1	15	4	132
Zn	*Salix boothii*	2	130	6	388
	Carex rostrata	4	96	5	134
	Isopterygium pulchellum	1	44	4	1074

[t] Aboveground tissue

Table 2. Maximum tolerable levels[t] of dietary minerals for domestic animals (National Academy of Sciences 1980).

Element*	Cattle	Rabbit
Cd	0.5	(0.5)
Cu	100	200
Zn	500	(500)

[t] All values are in μg/g. Values in parentheses were derived by extrapolation.
* Other elements were analyzed and reported in Dollhopf et al. 1988.

The objectives of this study were to determine if metal levels in the kidneys of the herbivore *Microtus pennsylvanicus* were higher in animals living in the AMD impacted wetland as compared to levels in animals from a similar, but visibly unimpacted wetland. If metal levels are higher, the second objective would be to determine if these levels represent a threat to the voles themselves or to the animals that prey on them.

This research is limited by the funds available. It is not intended to be the definitive statement about metal accumulation in animals frequenting wetlands. It will, however, provide data about the uptake of metals in such areas and supply information for decision makers about the potential accumulation of metals in wildlife in such areas.

LITERATURE REVIEW

Animals as Indicators of Environmental Metal Contamination

A limited amount of information is available concerning the accumulation of heavy metals in wildlife inhabiting metal contaminated environments. We have not been able to find work concerning metal accumulation in animals living in wetlands impacted by acid mine drainage. However, studies have been done involving the use of small mammals as indicators of possible bioaccumulation of heavy metals from metalliferous mine sites (Roberts and Johnson 1978; Smith and Rongstad 1982; Hunter et al. 1987; Andrews et al. 1984; Cloutier et al. 1986). Voles and mice have been used to show food chain accumulation of lead along roadsides (Jeffries and French 1972; Mierau and Blaise 1975). Metal accumulations have also been shown in wildlife living on land treated with sewage sludge (Anderson et al. 1982; Dressler et al. 1986). Livestock and wildlife living near smelters have also been shown to accumulate metals (Munshower 1977; US-EPA AP-91).

Few studies have been completed concerning the use of small mammals as indicators of environmental contamination. The use of the field vole (*M. agrestis*) as an indicator of heavy metal contamination in the environment was shown to be the most sensitive indicator among three small mammals studied by Beardsley et al. (1978). He concluded, however, that differences in *Microtus* tissue concentrations (whole body and liver) were too small to be considered good indicators. Other work seems to refute this conclusion (Roberts and Johnson 1978; Anderson et al. 1982; Andrews et al. 1984; Hunter et al. 1987). In fact, a review by Martin and Coughtry (1982) of the Beardsley data, suggested that differences in habitat metal concentrations actually were reflected in tissue concentration levels of their animals.

Copper, Zinc and Cadmium Accumulation in Small Animals

The accumulation of metals by small mammals is not clearly understood. Different species of small mammals accumulate metals at dissimilar rates. Interactions between metals (synergistic, additive or antagonistic) affect uptake, body distributions and also play a role in animal tissue metal concentrations. Additionally, metal accumulation can be a function of sex or age (Anderson et al. 1982; Smith and Rongstad 1982). Metals may be accumulated via the digestive system from the diet or from the animal cleaning itself. Metals may also be absorbed by the respiratory system. All of these factors and others influence animal tissue metal concentrations.

The fact that individual species of small mammals accumulate metals at differing rates is probably a result of differing diets or exposures among the different species. Shrews, primarily insectivorous, have been shown to accumulate cadmium at a higher rate than certain species of mice or voles, primarily herbivorous (Roberts and Johnson 1978; Andrews et al. 1984; Hunter et al. 1987). Hunter et al. (1987) showed that the shrew diet of insects increased cadmium intake 43 times from control site to contaminated site, while the cadmium in the *Microtus* diet increased only six fold. They concluded that the shrews living at a higher trophic level were ingesting twelve times more cadmium than the voles per day. Jeffries and French (1972) found different levels of lead among three species of voles and mice living on roadside verges and attributed this, in part, to differences in food consumed. In this study, *Microtus* consumed the most heavily contaminated material, grass, while *Apodemus* and *Clethrionomys* consumed the seeds and kernels of many fruits discarding the husk and pulp which were likely to have the highest lead concentrations. The wide ranging forage behavior of *Apodemus* and *Clethrionomys* also allowed them to feed on less contaminated material farther from the contamination source. Also, Smith and Rongstad (1982) reported *Peromyscus* accumulated lead at an active mine site while *Microtus* did not.

Elevated dietary or environmental levels of metals are not always reflected by accumulation in small mammals. This is apparently due to the ability of higher animals to metabolically regulate the retention of some metals.

There is a lack of effective homeostatic control for cadmium in animals (Underwood 1977) so it is not surprising that cadmium has been shown to accumulate in tissues of small mammals living in environments of elevated cadmium concentrations. Andrews et al. (1984) reported elevated total body cadmium levels in voles and shrews living on metalliferous mine waste. Smith and Rongstad (1982) reported elevated cadmium levels in *Microtus* and *Peromyscus* living on an active zinc-copper mine site. Other studies have shown similar results on different types of metal contaminated sites: sewage sludge treated fields (Anderson et al. 1982), smelter waste heaps (Johnson and Roberts 1978), and smelter fallout areas (Munshower 1977). Hunter et al. (1987) reported some regulation of cadmium accumulation in *Microtus* at low levels of exposure. *Sorex* living at the same site indicated no regulation at these levels.

In higher animals, the ability to regulate zinc retention has been well documented (Vallee 1959). Studies concerning zinc retention in small mammals have shown varying results. Roberts and Johnson (from two reports in 1978) have shown that zinc was not accumulating in small animals living on mine sites having elevated zinc concentrations. They attributed this to the possibility that zinc is effectively regulated by these animals. Also, zinc concentrations in meadow voles living in wheat and old field plant communities that received applications of sewage sludge (high in zinc concentrations) were the same as those found in animals from control sites (Andrews et al. 1982). However, Smith and Rongstad (1982) showed a significant increase in zinc accumulation in animals from mined sites compared to control sites. Zinc levels were found to be higher in the femurs of cottontail rabbits living on sewage sludge treated fields than in rabbits inhabiting non-treated fields (Dressler et al. 1986). Significant differences were also found in kidneys of meadow voles fed sorghum herbage containing 34 μg zinc/g and herbage containing 65.6 μg zinc/g (Williams et al. 1978).

In all animals the continued ingestion of excess copper leads to some accumulation in the tissues (Underwood 1977). Accumulation has been shown in animals from environments containing high copper levels. Kidney and liver copper concentrations were elevated in *Microtus* from sewage sludge treated fields in Ohio (Anderson et al. 1982). Also total body concentrations of copper were higher in *Microtus* and *Peromyscus* living at an active zinc-copper mine in Ontario (Smith and Rongstad 1982). Again, however, other studies involving environments high in copper indicated that some homeostatic control within small mammals was occurring and copper was not accumulating. In *Microtus*, *Apodemus* and *Sorex* studied from copper contaminated environments, total body concentrations remained constant despite large scale increases in copper ingestion (Hunter et al. 1987). As with zinc, this was attributed to efficiency of homeostatic mechanisms controlling copper distribution in animals. Cloutier and Clulow (1986) reported no kidney or liver variation in copper levels among *Microtus* from mine tailings containing elevated levels of copper and animals from a control site. In a similar study of *Microtus pennsylvanicus* from waste water irrigated old fields and nonirrigated old fields, no variations were found in the liver and kidney metal levels of voles from both sites (Anthony and Kozlowski 1982). The variation in findings among these studies may reflect other less well understood factors involved in the uptake of metals in animals.

The potential toxicity of certain metals is difficult to determine because of the interactions that may occur if metals are available simultaneously. Cadmium has been shown to decrease iron concentrations in tissues of Japanese quail (Fox et al. 1971). Elevated dietary cadmium caused a zinc deficiency in poults (Weber and Reid 1969). Marcus (1981) reported that a zinc treatment given to male mice slowed the flow of calcium from blood to kidney. Underwood (1977) also indicated that high amounts of zinc, copper and cobalt inhibited the uptake of cadmium in animals.

Toxicities and Target Organs

Cadmium is selectively accumulated in various organs of higher animals. Highest concentrations are found in the kidneys and liver, particularly the kidneys (Flick et al. 1975; Underwood 1977); however, it is not restricted to these organs (Flick et al. 1975). The presence of the metal binding protein metallothionen in the kidneys and liver is responsible for the accumulation of cadmium there. Similarly, in voles, highest concentrations of cadmium are found in the kidneys (Johnson et al. 1978; Andrews et al. 1984). In *M. agrestis* living on a metalliferous mine site, the kidneys contained 22.2% of the total body burden of cadmium (Johnson and Roberts 1978). They also found that only the liver and kidney cadmium levels differentiated between polluted and uncontaminated environments. However, Andrews et al. (1984) found significant differences in muscle and bone tissue cadmium concentrations in animals from mine and control sites.

Cadmium is toxic to every system in the animal body (Luckey and Venugopal 1977). It also accumulates with age (Luckey and Venugopal 1977) and has a long half life; approximately 30 years in man (Friberg et al. 1974) and 200 days in rats (Moore et al. 1973). The effects of cadmium toxicity are growth retardation, impaired kidney function, poor reproductive capacity, hypertension, tumor formation, hepatic dysfunctions, poor lactation and lowered hematocrit levels (Luckey and Venugopal 1977). Minimum toxic levels or maximum dietary cadmium levels cannot be given with precision because dietary intakes of other elements with which it interacts, notably zinc, copper, iron, and selenium (Underwood 1977) obscure the precise statement of a chronic or toxic concentration.

Low dietary cadmium levels do not seem to produce critical adverse physiological effects. Levels of 1 to 10 μg/g in the diet of mice had little or no effect on digestion of dietary nutrients or on growth rates after three weeks (Weber and Reid 1969). Concentrations of 412 μg cadmium/g in the diet caused a marked reduction in growth and 8% mortality. At levels of 2060 and 4120

μg/g in the diet, mortality was 59% and 75% respectively after three weeks. Five μg cadmium/g in drinking water of mice increased kidney cadmium levels to 2.9-6.4 μg/g and caused increased mortality at 21 to 24 months. No effect on growth, mature weights or mortality were seen at 18 months (Schroeder et al. 1963). In studies reviewed by Doyle (1977), dietary cadmium levels of 0.1 to 10.0 produced hypertension in rats. However, similar studies reported by him showed no induction of hypertension. In swine, no evidence of renal dysfunction was noted until renal cadmium concentrations reached 200 μg/g wet weight (Cousins et al. 1973).

Metal Movement Through Food Chains

Information concerning the uptake and distribution of metals through food chains, particularly higher trophic levels, is limited. Again, there are several factors that cause specific metals to be potentially mobile or nonmobile through these trophic levels. Some of these include homeostatic control mechanisms, target organs of accumulation, and dietary habits and feeding ranges. Cadmium is considered to be highly mobile in food chains (Hunter et al. 1987; Smith and Rongstad 1982) due to the lack of homeostatic control of cadmium in animals, and the fact that cadmium accumulates primarily in the soft tissues. Zinc and copper are less likely to be mobile in food chains due to homeostatic control mechanisms that regulate the retention of these metals. Ingested lead is also unlikely to move through food chains because a large part of it is immobilized in skeletal tissue (Johnson and Roberts 1978). Skeletally incorporated lead is relatively unavailable for intestinal absorption (Roberts and Johnson 1978).

MATERIALS AND METHODS

Selection of an Indicator Species

Microtus pennsylvanicus was chosen as an indicator species for this study because of their abundance and distribution at the Swamp Gulch site (Dollhopf et al. 1988) and they meet criteria for small mammal indicators as presented by Beardsley et al. (1978). These authors indicated that the ideal biological indicator should be small and easily caught, have a territory of limited range, an unselective but herbivorous diet, and a short life span so that individuals could be assumed to be fairly closely adjusted to their environments. The natural history of *M. pennsylvanicus* matches these criteria very closely.

Selection of Trapping Area

Within the Swamp Gulch wetland, trapping was done in areas of greatest predicted cadmium accumulation. The Dollhopf et al. (1988) study of these wetlands did not map specific areas of cadmium accumulation in the upper, undecomposed organic matter layer (acrotelm). However, zinc contamination was mapped and because of its naturally close association with cadmium (Hammons et al. 1978) this map was used to delineate areas where voles would be trapped (Figure 2). Within the 3.9 ha of AMD impacted wetland, the zone of zinc accumulation greater than 3000 mg/l in the wetland acrotelm was chosen

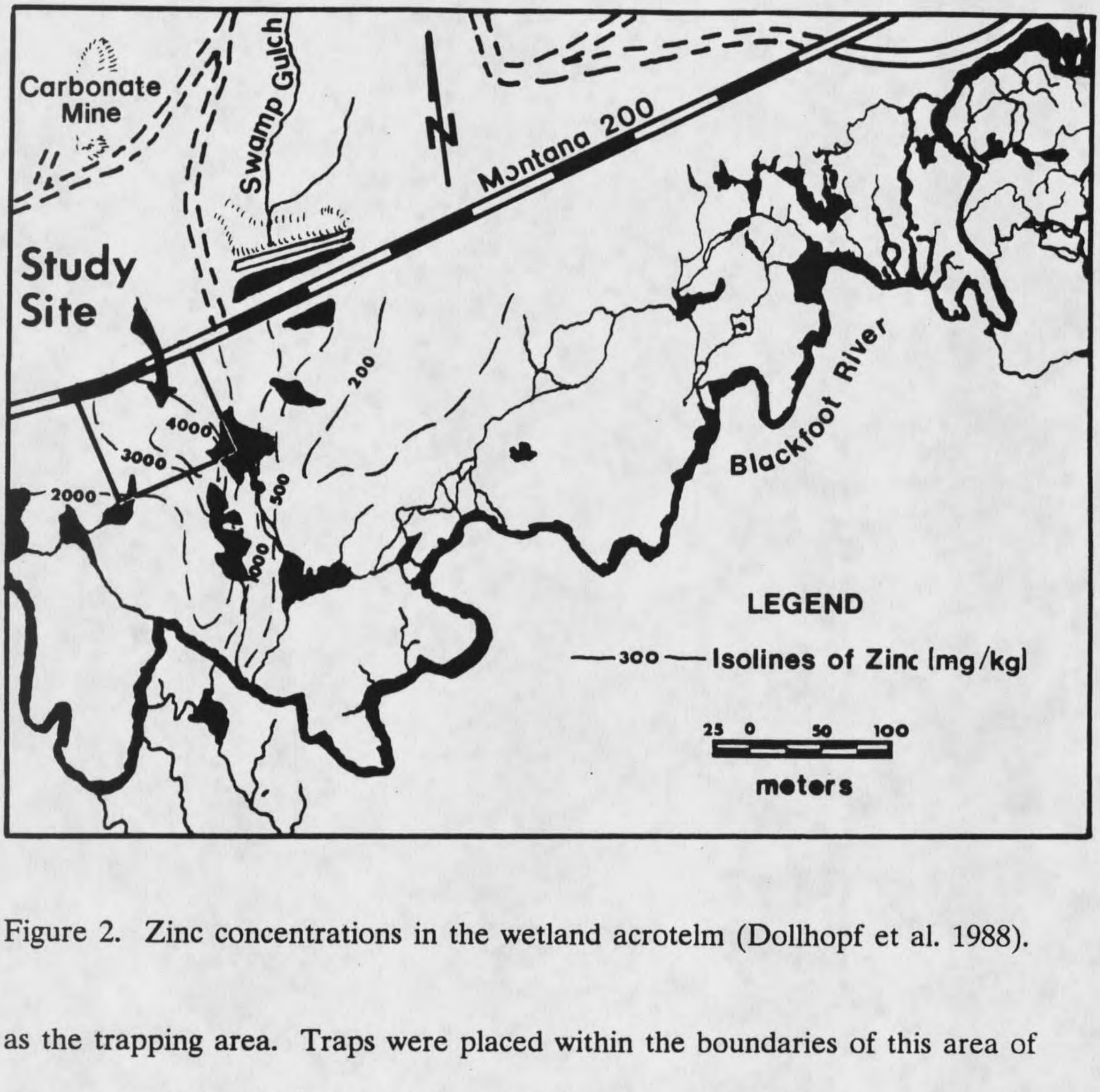

Figure 2. Zinc concentrations in the wetland acrotelm (Dollhopf et al. 1988).

as the trapping area. Traps were placed within the boundaries of this area of approximately 2500 m^2 (Figure 3). This did not include the entire area delineated by 3000 mg/l zinc accumulation due to part of the area being submerged and therefore unable to support voles. After nine days, trapping was terminated due to the low numbers of voles being taken and because enough animals had been taken for a competent statistical comparison.

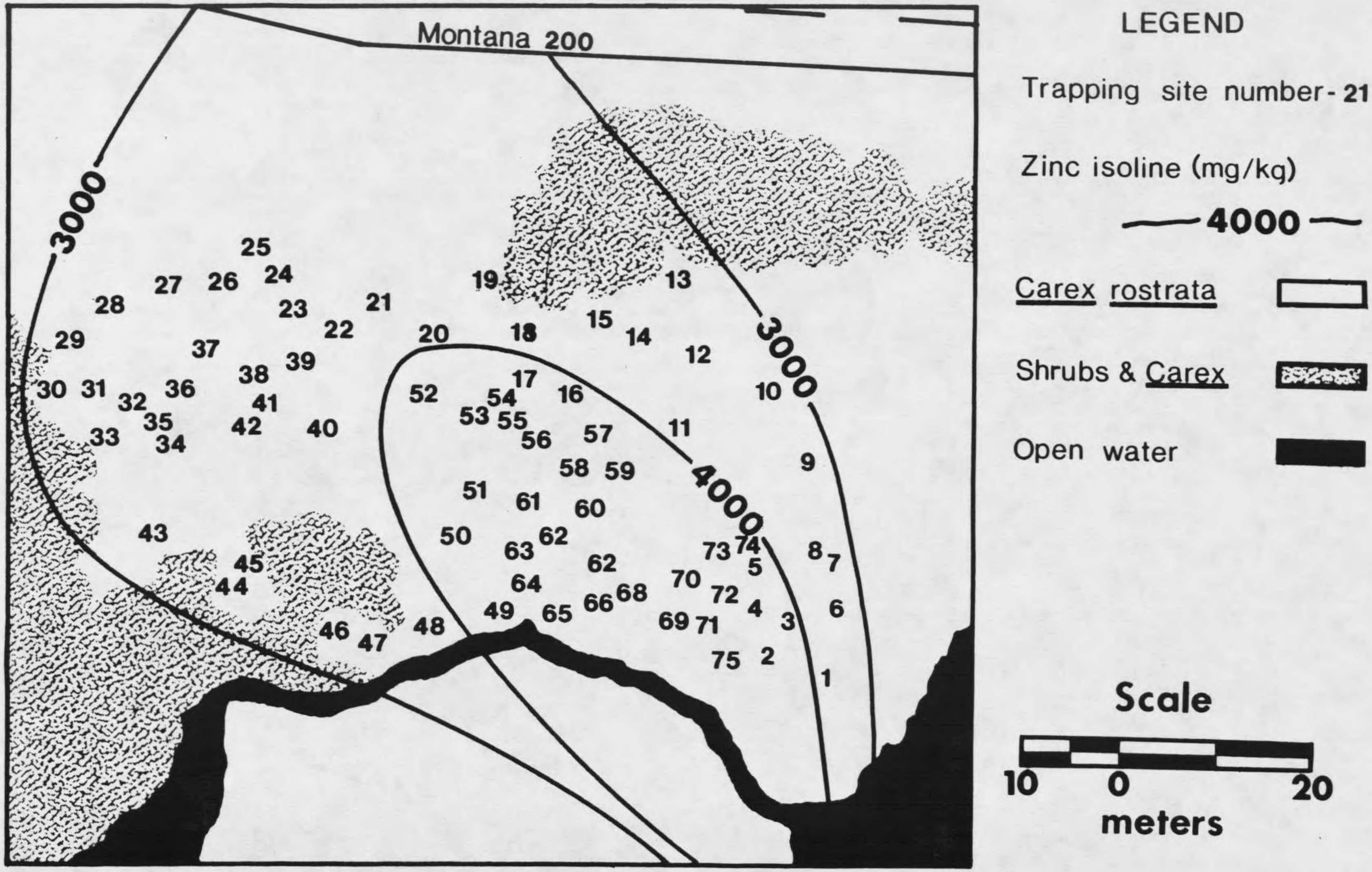

Figure 3. Swamp Gulch study site trap locations.