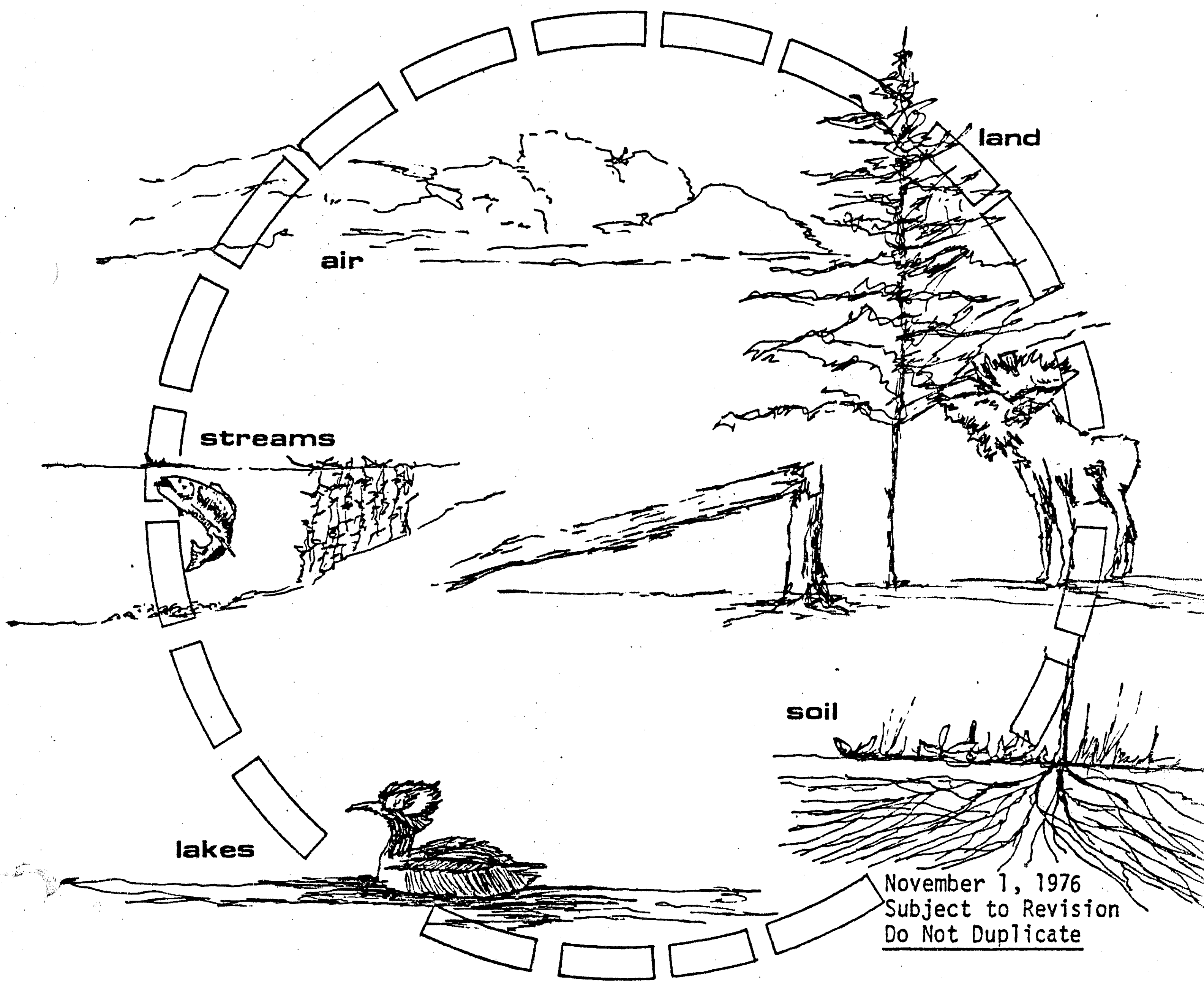
preliminary report

biological sciences

regional copper nickel study

environmental quality council



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Preliminary Report: Biological Science
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INTRODUCTION

The MEQC's Regional Copper-Nickel Study is a complex interdisciplinary effort involving the active participation of several state agencies, federal agencies, a regional agency, university investigators, project consultants, environmental organizations, mining companies, and two project staff groups (one located in Ely and the other in Minneapolis). All of this expertise has been brought together by the MEQC for the common goal of conducting a comprehensive coordinated investigation of the regional environmental, social and economic implications of potential copper-nickel mining and processing in northeastern Minnesota.

Functionally the Copper-Nickel Study has been divided into the study areas of biological sciences, physical sciences, mining and metallurgical technology, and socio-economic planning. Each of these study areas has been subdivided into individual projects which emcompass a unified field of scientific knowledge.

The goals of the Biological Sciences section of the Regional Copper-Nickel Study are two-fold. First, to develop an ecological characterization and baseline for the entire study region and second, to develop predictive measures of the potential impacts on various ecological parameters based on a set of possible perturbations which might be caused by mining activities. As such the biological sciences program has been divided into seven major projects. These projects range from aquatic and terrestrial biology monitoring systems through bioassay, health studies, and tissue analysis programs.

At this stage in the Regional Copper-Nickel Study it is vital that the various projects interact and begin to act synergistically. The regional/multidisciplinary approach to this study makes it unique on a statewide scale. This approach should result in a broad and integrated data base from which impacts can be assessed on a regional and statewide scale. The data base and impact analyses should provide decision-makers with a rational base from which to establish policies on the future of Minnesota's copper-nickel resources.

It is important to keep in mind the scope of the Regional Copper-Nickel Study. General policy decisions will be made on the basis of this program, it is not however a substitute for site-specific environmental impact statements or preconstruction and operational monitoring. In the event that the state decides to allow copper-nickel mining the normal procedures for environmental impact statements will become operative.

The attached reports document the current status and plans of the full array of biological sciences projects currently operating within the framework of the Copper-Nickel Study. During the remainder of the fall and the winter (1976-77) the results of each of these projects will be thoroughly analyzed and plans for the final field season will be made.

TERRESTRIAL STUDIES

INTRODUCTION

To fully acquaint yourself with the original changes, amendments and reasons leading to the current sampling carried on by the terrestrial portion of the Regional Copper-Nickel Environmental Impact Study, interested persons should read the Original Terrestrial Proposal (summer 1976) and the First Review (June 8, 1976). For those interested in the current status of where we are and where we want to be next year, this overview will cover the required ground.

The terrestrial staff is charged with characterizing the vegetation, mammal, bird and insect flora and fauna on approximately a 560 square mile study area known as the Mine Site Area.[1] Our group has not expanded sampling operations beyond their area during the 1976 season, and no current plans exist for doing so during 1977.

With these tasks before us, it was obvious that we had to select and specialize in much smaller categories within these four broad topics listed above. The process of specializing in itself requires a decision making process. Hopefully, you will agree that we made <u>most</u> of the right decisions when you consider the scope of the project, the size of our staff and the time-frame we have to complete the work.

[1] The boundaries of the Mine Site Area have been determined by the Minerals Division of the Minnesota Department of Natural Resources (MDNR).

I. Study of the Vegetation

During the following overview, the term site is used to define the area where various field activities such as small mammal trapping, bird census, insect sampling, vegetation releves and vegetation plots (15M X 15M) were conducted. Due to the highly disturbed nature of much of the vegetation on the Mine Site Area, a single site was often times not large enough or homogeneous enough to conduct all of the above mentioned field sampling.

As a result, the number of sites varied per specific type of sampling. For example, the spring nongame bird census was conducted along 35 transects, each transect having a separate site number. Some of these are the same sites where small mammals were trapped and where insect pit-fall traps were set. However, most of the bird sites, for reasons stated in Section II-A, do not overlap sites used during other field sampling.

If the reader keeps this problem of different numbers and locations of sites for each type of sampling in mind, the sample sizes discussed during this overview will be clear.

I-A. Theory Behind Site Selection and the Number of Sites Sampled for Vegetation, Small Mammals, Nongame Birds, and Insects during 1976

After field examination of the structure of the forest types and the existing road system on the Mine Site Area was completed in May 1976, the following decisions relative to site selection were made: (1) only homogeneous and readily accessible sites would be selected. The original 22 sites would be representative, although not in proportion to area, of the major plant community types on the study area (see Appendix A, sheets 1,2 and 3); (2) the original 22 sites (No. 1-22 incl.) would be used jointly by the terrestrial group and Sagar Krupa's study involving the chemical analysis (heavy metal concentrations) of the soil and selected tree species at each site; (3) although a large proportion of the area consists of recent clear cuts, coniferous plantations, deciduous (aspen primarily) regeneration and forests with mixtures of coniferous and deciduous species, most sites selected and sampled during 1976 were homogenous for a single species (e.g. trembling aspen, paper birch, black spruce, etc.). If mixed forest types and disturbed areas are to be adequately represented in our study, the emphasis will be on additional sites in these types selected for the 1977 field season; (4) nongame bird species were censused on 35 different sites insects by pit-fall trapping on 12 sites vegetation releves and herbarium specimens on 30 sites and small mammal grids on 13 different sites. The degree of overlap of different sampling occurring on the same site (e.g. mammal trapping, bird census, insect and vegetation) and specific - as to the type, age and history of each will be included in future reports.

I-B. Herbarium Specimens

Specimens of herbaceous plant species were collected from 30 different sites primarily during June and July, 1976. Specimens were identified in the fresh state and then properly dried. Herbarium mounts will be made, properly labeled and stored in our herbarium case at the Kawishiwi labs during October through December of 1976. A total of 343 species have been identified to date.

Duplicate specimens of these plants were collected and will be mounted by, and stored at, the herbarium at the University of Minnesota, Duluth Campus. This is part of a cooperative agreement discussed in the First Terrestrial Review (June 8, 1976).

Some additional collecting should be done on these plots in 1977 to obtain some of the more common plants that had already bloomed prior to our major field effort this season. However, unless the number of person months and budgeting are significantly increased, we will not collect additional herbarium specimens if we select additional sites for 1977 on distrubed areas.

I-C. Vegetation Plots and Structural Analysis Along Nongame Bird Sites

Thirty-five sites were established in May and early June (1976) to estimate the number of individuals and species of nongame birds during the breeding season in various forest and shrub, and several clearcut types (Section II, II-A). After completing the spring singing male census in early July, a program of vegetation analysis was planned and initiated on these sites in August and September.

A total of 12, 15M x 15M square vegetation plots were established on each site. These vegetation plots were randomly located along the 500M transect, with 6 plots run on each side of the road or trail used during the bird census.

The data collected included a total tree count by DBH and species on the 15M x 15M plot, total number of shrubs by species and size class (dia. taken at 15CM above the ground) along a 2M x 15M plot, herbaceous species and numbers on a 1M x 1M plot, canopy densiometer readings and vegetation intercept data at each corner of the vegetation plot. Notes as to the occurence of deadfalls and amount of rock out-cropping on the plot were included.

The quantitative vegetation sampling was completed by the end of September. A total of 420 vegetation plots were sampled on the 35 nongame bird census sites. The field sheets are currently being readied for copying.

The vegetation data will be organized into a computer format for analysis during the winter of 1976-77. When a principal component or multi-variate analysis is used on the data base, we expect to show correlations between certain tree, shrub, or structural components of the plant community and the particular bird species present. When carried one step further, destruction of certain susceptible plant species or community structure by certain potential pollutants of copper-nickel mining may cause the reduction or elimination of certain bird species. These predictions or projections hopefully will be made possible by the bird census data and the vegetational analysis techniques.

However, before the above statistics can be run, the data must be checked for homogeneity. If sample sizes are not adequate, additional vegetation plots may have to be run on the same census sites next season.

I-D. Vegetation Plots and Structural Analysis on Small Mammal Trapping Grids

The same methods used for sampling vegetation on nongame bird sites (Sec. I-C) are proposed for the small mammal grids already trapped during the 1976 season. This sampling had to be postponed until 1977 due to the extremely dry conditions that caused earlier than normal leaf fall (especially on herbaceous plant species) and because of the loss of seasonal laborers on the terrestrial staff.

The number of vegetation plots needed for each of the 64 trapping grids has not bee determined, but 4-6 per grid would be a reasonable number (160-240 vegetation plots on the 40 grids trapped in 1976). These decisions will be made after analysis of vegetation data from the bird census sites (Section I-C).

I-E. List of Moss Species

Mosses were collected during two separate sampling periods over the summer (1976) and are presently being identified. This portion of the study is under contract. This is strictly a qualitative approach that will yield a species list for each of the original 22 sites. The list should include a total of 150 species or more. A member of the University of Minnesota's Botany Department has expressed interest in storing and maintaining the collection for future needs on the St. Paul campus. Arrangements are being made along these lines.

I-F. Lichens

We have not sampled, collected or identified lichens during the 1976 season. Since certain species can be extremely suseptible to particular types of pollutants, we hope to initiate some sampling during the 1977 field season. The methods of study are not yet clear, but a literature search is being conducted.

I-G. Archive Collection of Herbaceous Plant Species

The present demand for heavy metal analysis of samples of fish, aquatic plants and invertebrates, small mammals, and perhaps nongame birds has placed a heavy analytical load on the laboratory at Carlos Avery. Thus, the prospect of analyzing a large number of specimens of herbaceous plants which commonly grow in many plant communities throughout the study area seems remote.

However, a dried collection could be preserved and form the baseline data for future studies. If such a program is initiated, the work would be completed during the 1977 field season.

II. Nongame Bird Census

Census techniques were investigated by reading the literature, talking with interested persons and discussions with professors at the University of Minnesota campuses at St. Paul, Minneapolis and Duluth. The final concensus was that nongame bird species could only be enumerated on a study area of this size and with the current staff by using the transect method of counting singing males. We are aware of the problems that exist with this method. However, the alternative method of intensively mist netting a 5-10 hectare site to obtain a populaton estimate (breeding birds plus the free floating population) on 30-40 sites would have taken a staff and a budget far greater than that allocated to this portion of the terrestrial study.

Singing male counts were conducted on 35 different sites in various forest, shrub, plantation and clearcut community types. The greatest emphasis was on forested types (details will be included in future reports). The nongame bird census, as it was conducted, will allow us to obtain individual and species abundance estimates by community types. Findings from these sites can be extrapolated to larger tracts on the Mine Site Area. These types of biological data on the relative richness of a certain community when compared to another are designed to provide a decision making tool to allow legislators to compare sites proposed for various functions of the copper-nickel mining industry.

II-A. Method used for Nongame Bird Census

Transects were established on 35 different sites. Each was 500M long and marked with plastic flagging at 50M intervals. All transects were established along existing improved gravel roads or logging trails. Singing males on either side and within 50 M of the road or trail were recorded on a data sheet by their approximate location within each 50M x 50M area.

Morning census periods started at sunrise and ran 3-4 hours, depending upon the environmental factors of rain, wind, cloud cover and temperature. Two separate transects were run simultaneously by two researchers. The route was covered twice, with 20 minutes spent on each leg of the census (40 minute census/plot/morning). Depending on the distance between sites and environmental conditions, an average of 6 total sites (3 per person) were censued each monring. Each site was visited 3 or 4 times during the peak of the breeding season in June. The time at which each site was visited was varied to avoid bias due to timing of the census period relative to sunrise.

Large portions of the Mine Site Area are highly disturbed. It was difficult to find homogeneous stands that were similar over the entire 500M's. Since small mammal grids require an area only 135M x 135M in size (105M x 105M for 8 rows and columns, and a minimum of 15M on all sides as a buffer zone), nongame bird census sites were often located near, but not coincident to these mammal sites. Ideally all sampling would have been conducted on the same site, but the diversity of the area interfered with this goal.

The emphasis on this year's work was to census primarily forested types. Plans for future nongame bird censuses, if the work is done, should include and emphasize recently disturbed types. If these areas cannot be censused in 1977, we may be able to apply the findings of a thesis by Gerald Niemi to the disturbed types in the Mine Site Area.

II-B. Mist Netting of Nongame Birds

Mist nets were set up in July and early August on 16 of the sites where singing males were censused in June. Three, 12M nets were placed in a line perpendicular to the road. Two lines were run on each plot. This amounted to a very superficial coverage of each site, but even this degree of netting required a team of four persons.

The general conclusion was that the mist netting portion of this study should be discontinued, since we do not have the staff to mist net a large area. We do not propose to mist net during the 1977 field season.

III. Game Bird Observations and Census

Observations of game birds (woodcock, ruffed grouse, spruce grouse and waterfowl) encountered while conducting routine sampling were recorded by date, location, species, size of group, etc. (see Data Form A). These observations will be coded and may be tabulated by location and ploted using computer facilities.

III-A. Ruffed Grouse Drumming Census

The MDNR has established a ruffed grouse drumming census route on the Mine Site Area. These routes are established throughout the state, run by Area Game Managers or other DNR employees, and provide a census tool to monitor the status of this game bird. However, this single route is not enough to show differences that may exist in the quality and quantity of the habitat, and thus the density of ruffed grouse over the study area.

Since ruffed grouse are the principal non-migratory game bird on the study area, we plan to establish a much larger drumming route and sample it with terrestrial staff during the spring of 1977.

Winter concentrations of feeding grouse (spruce and ruffed grouse alike) will be recorded. Evidence in the literature suggests that winter cover and perhaps even winter food supplies may be a limiting factor to the winter survival of some grouse species.

III-B. Spruce Grouse

Although spruce grouse are presently hunted as a game bird in Minnesota, they have been a protected species at times in the past. Spruce grouse observations are recorded by location, habitat and other parameters present on Data Form A. Other sources of data for this study include the locations of spruce grouse bagged by hunters and flushing records using trained English Setter bird dogs to search various cover types.

There is no known census technique to enumerate spruce grouse during the breeding season on extensive areas equivalent to the ruffed grouse drumming census. As a result, the distribution of spruce grouse on the study area will continue to come from the types of observations mentioned above, along with possible winter feeding records.

III-C Woodcock Census

Woodcock have received a considerable amount of attention in recent years and the number of hunters persuing this species is rapidly increasing compared to the past. With the exception of early morning and late afternoon flights and aerial displays during the breeding season, this bird is seldom noticed even in areas where it breeds and nests in relatively large numbers.

A trained English Setter was used during September 1976 to locate and quantify (flushes/man hour) woodcock on various portions of the study area. The preliminary findings show that there is indeed a huntable population of this game bird. Since habitat preferences include alder fringes and swamps adjacent to aspen and aspen-birch uplands, we suspect that the southern 1/3 of the study area the largest number of woodcock.

Work near Cloquet, Minnesota, suggest that spring display grounds are often located on firebreaks and old log landings. Certain clearcuts are also used 2 years after the logging operation when these sites support a 0.3-0.6 M aspen stand.

Since there are many locations on the Mine Site Area that fall into these broad cover types, we feel that some enumeration and habitat use pattern during the breeding season should be conducted. This may lead to management recommendatons for increasing the abundance of this game bird on the study area.

III-D. Waterfowl Census

Waterfowl observations are recorded by members of the copper-nickel fishery survey staff during stream and lake censusing. Observations of ducks and geese are also recorded by the terrestrial staff (Data Form A). Our preliminary conclusions are that there is a low density of waterfowl on the Mine Site Area during the breeding season.

An aerial survey of lakes and rivers was initiated in October 1976 to determine the importance of the study area as a resting and stop-over spot for migrating waterfowl. Terrestrial staff members will accompany regularly scheduled flights conducted by the USFS over the Mine Site and adjacent areas. We hope these flights will be successful in locating waterfowl concentrations. If concentrations are found, an attempt to enumerate waterfowl by species will be made by using a canoe or boat.

If the fall survey is successful, and if no other state or federal agencies are involved in aerial surveys during the spring, we propose to continue this type of aerial survey during the spring of 1977.

IV. Raptors

Raptors were observed, identified and recorded on Data Form A during the normal course of field work. These data will be displayed according to area and frequency of observations by species. Secondary information will be utilized on the locations and history of osprey and eagle nesting sites on or near the Mine Site Area. These data will be obtained from state and federal agencies that conduct surveys annually.

Adequate and inexpensive censusing techniques have not been developed for most raptors. However, several species of owls, especially the barred owl and great horned owl, do respond to call-back tape recordings during the breeding season in February and March. The technique can be used to census a strip up to 3.2 KM (1.6 KM on each side of the road) along any road system.

Literature is still being searched, but we feel that a census route for barred and great horned owls should be established during the nesting season in 1977.

IV-A. Other Bird Species

The status, distribution and relative abundance of other bird species not specifically mentioned in Sections II, III and IV will be obtained from secondary information (thesis, state and federal reports, etc.).

V. Small Mammal Census

The census of small mammals (mice, flying squirrels, voles, and shrews), like that of nongame birds, was concentrated in forest habitats. Snap traps were used in a grid arrangement to provide data which can be used to yield population estimates by using regression analysis techniques. In addition, the richness of species and number of total individuals in different forest types varies sharply and is another way of ranking plant communities.

Trapping was conducted during three principal periods. (Period A, June 22-28; Period B, July 27-August 2; and Period C, August 31-September 6). During these three periods, six different forest types were trapped with two replications occurring in each type (a total of 12 small mammal trapping grids were trapped during each period).

The trapping was equally distributed between deciduous (two trembling aspen, two paper birch, two mixed trembling aspen-paper birch) and coniferous types (two red pine, two jack pine, and two black spruce). For the most part, and when sites could be found, one grid was located north and the other south of the Laurentian Divide. One intermediate trapping period was conducted from August 5-11, 1976. Only four grids were run during this period, one in tamarack, one in 1-2 M tall tamarack, one in a mixed tamarack-black spruce type, and one in white cedar.

Over 1000 small mammals were trapped during the summer. Specimens are currently being identified, aged, sexed, weighed, stomach contents saved for possible analysis and the reproductive state determined. This work is being done under a contract to the University of Minnesota.

In addition, some small mammals will be analyzed for levels of heavy metals. These laboratory tests will be conducted at Carlos Avery on the three herbivore species that were most commonly encountered (eastern chipmunk, boreal redback vole and deer mouse) and the largest of the insectivores (shorttail shrew).

V-A. Methods of Small Mammal Trapping

When sites were large enough, a 64 trap grid was established. On smaller sites, as many traps as possible were fit into the site allowing at least a 15M buffer zone between the outermost traps and other cover types or roads. Most trapping grids included 50 or more traps.

Even though the size of the trapping area varied as a function of available homogeneous cover, the number and biomass of mammals caught per unit area can be compared.

Museum Special snap traps were placed at 15M intervals in 8 rows and 8 columns (64 traps). Variations of this arrangement were necessary on certain sites. Trapping stations contained a single trap. Each station was located using a compass line for direction and a split-image range finder for distance. A 9 gauge, 50 Cm long steel stake was used to hold each trap securely in place and was looped at one end to hold a plastic marking flag.

Traps were baited with peanut butter and rolled oats. Prebaiting (traps unset) was conducted for 2 days and trapping followed for 5 consecutive days. No grid was trapped twice during the summer, but the steel stakes were left in the field for the 1977 season to provide reference points for the vegetation plots (Sec. I-D). The number of traps snapped but containing no animals was recorded and is an adequate estimate of traps closed by other causes, especially rainfall.

Each mammal was placed in a plastic Whirl-Top bag at the trap site, with the trap number, date, species name (if known), site number, and researcher's initials included. Specimens were kept on ice the remainder of the day and frozen upon return to the laboratory. Specimens will remain frozen until identification and laboratory procedures are completed. Animals not saved for analysis will be discarded.

V-B. Whitetail Deer

The principal big game animal in Minnesota is the whitetail deer. The distribution and relative abundance of this species on the study area will be compiled largely from our observations and secondary information in the files of state and federal agencies.

A road survey of deer hunters is proposed for the 1976 and 1977 hunting season.

This survey will show the hunter use on the study area, including the frequency of local hunters and those from more distant locations.

V-C. Snowshoe Hare Census

When snowshoe hares are at a high population level, as they are presently on the study area (October 1976), they undoubtedly constitute the largest biomass of a single species of mammal per hectare in the region. Other researchers who have worked on and near the Mine Site Area for a number of years have been recording the number of hares observed along a 14 mile transect along State Highway No. 2. Although not published, these data are available to the terrestrial staff.

We have no additional plans to expand the snowshoe hare census. Although abundant, this species is not presently a desired game species.

Habitat use, expected densities during different population levels, and other natural history information will be obtained from published articles or unpublished information from state and federal files.

V-D. Other Mammal Species

Observations of moose, fox, martens, fishes, wolves, and other medium and large mammals are recorded by date habitat location, group size, etc. (Data Form A). These observations will provide very generalized range maps. Additional information on specific habitat requirements, densities, and natural history will come from secondary sources.

VI. Insects

After much deliberation and many discussions with prominent entomologists and soil ecologists at the University of Minnesota, the terrestrial staff decided that the ground beetles (Carabidae) were a reasonable group of insects to concentrate on during the current study. Reasons for this decision are presented in the original Terrestrial Proposal (Summer 1976).

Pit-fall traps were set in 12 different habitat types and are discussed in Section VI-A. Ten traps were placed at 15M intervals near the centers and in a straight line on each site. Three of the 12 sites would only accomodate 9 traps, thus a total of 117 pit-fall traps were sampled.

Twenty-four hour trapping periods were initiated at weekly intervals in July. So few ground beetles were caught that 48 hour intervals were then used. The result was the same. The entire 117 traps were run continually and checked and emptied once a week during most of August and all of September. Even after this lengthy trapping period, relatively few ground beetles were caught.

We speculate that the record low rainfall this summer severely reduced the population of adult beetles and/or greatly restricted their movements. Whatever the causes, the result is that we will not be able to provide a list of indicator species unique to or most abundant in the various plant community types.

Although we will identify the ground beetles we have trapped this summer, our recommendations at this time are to discontinue pit-fall trapping during the 1977 field season and shift the emphasis to a survey of the dragonfly and butterfly species on the area.

VI-A. Pitfall Trapping

Pit-fall traps were set in 12 different habitats. Holes were dug with a golf course hole digger. After the soil was removed, a galvanized can with both the top and bottom removed was securely packed into the hole so that the top edge was level with the organic/mineral soil interface.. A waxed paper drinking cup was then fit snuggly into the inside of the can. The top of the cup was 2-3 CM below the top of the can. Ethyleneglycol antifreeze, 4-5 CM deep, was poured into the cup as a killing and preserving agent. A 1.2CM X 1.2CM galvanized wire mesh was laid over the mouth of the cup to prevent small mammals and amphibians from falling into the antifreeze. The trap was then covered by a 20 x 20 x 0.5 CM piece of exterior plywood supported by wire legs 5-10CM above the ground. This cover prevented dilution of the antifreeze during rains and helped to prevent leaves from clogging the mouth of the traps.

The entire contents from one trap line (10 traps) were strained from the antifreeze and stored in a single Mason pint jar with 70% denatured ethyl alcohol with glycerine added. Some of the samples have been "picked" to separate the invertebrates from the soil and vegetative material that fell into the traps. This was especially a problem along certain trapping lines where small mammals removed screens from the traps and did a considerable amount of digging around the lip of the can.

Ground beetles will be identified, pinned, and stored in an insect case during the late fall and winter period (1976-77). This work will result in a species list, with little direct application to species/community association.

Plot No.	Type of Plot	General Location	Map No.	Sqr. Mile No.	Technical Description of Plot Location	Ownership
1	Mature Jack Pine Stand	On USFS Road #112 SE of Babbitt	10	346 347	T.60N. R.12W. Sec. 15 NE¼ SE¼ T.60N. R.12W. Sec. 14 E½ NW¼ SW¼	Federal
2	Mature Jack Pine Stand	On USFS Road #130 SE of Hoyt Lakes	21	 739 740	T.57N. R.14W. Sec. 7 SE¼ NE¼ NE¼ T.57N. R.14W. Sec. 7 E½ SE¼ NE¼ T.57N. R14W. Sec. 8 SW¼ NW¼ NW¼ W½ SW¼ NW¼	Federal
3	Mature Red Pine Stand	On USFS Road #112 SE of Babbitt	10	371	T.60N. R.12W. Sec. 23 S½ NW¼ SE¼ NW¼ SW¼ SE¼	Federal
4	Mature Red Pine Stand	On USFS Road #130 SE of Hoyt Lakes	21	757	T.57N. R.14W. Sec. 18 NE¼ SE¼ N½ SE¼ SE¼ N½ SW¼ SE¼	Federal
5	Mature Black Spruce Stand	On USFS Road #173 SE of August Lake	7	257	T.61N. R.10W. Sec. 26 N½ SE¼ NE¼	Federal
6	Mature Black Spruce Stand	On USFS Road #569 SE of Hoyt Lakes	17	628	T.58N. R.14W. Sec. 15 E½ SE¼ SW¼ SW¼ SW¼ SE¼	Federal
7	Mature Paper Birch Stand	On USFS Road #424 2 mi. E of Stony River	11	327	T.60N. R.11W. Sec. 9 E½ NE¼ NW¼	Non-Federal
8	Mature Paper Birch Stand	On USFS Road #128 Skibo Lookout	22	746 764	T.57N. R.13W. Sec. 8 SE¼ SW¼ T.57N. R.13W. Sec. 17 N½ NE¼ NW¼	Federal
9	Mid-Aged Trembling Aspen Paper Birch Mixed Stand	On USFS Road #386 NW of Shamrock Lake	7	278 279	T.61N. R.10W. Sec. 32 SE¼ SE¼ NW¼ SE¼ S½ SE¼ NE¼ T.61N. R.10W. Sec. 33 W½ SW¼ NW¼ W½ NW¼ SW¼ W½ SW¼ SW¼	Federal
10	Mid-Aged Trembling Aspen Paper Birch Stand	On USFS Road #112 W of Hwy. 2	16	456	T.59N. R.11W. Sec. 1 N½ SE¼ SW¼ SW¼NW¼ SE¼ NE¼ NW¼ SE¼ NW¼ NE¼ SE¼	Federal
11	Mature Trembling Aspen Stand	On USFS Road #178 W of Stony River	11	325	T.60N. R.11W. Sec. 7 SE¼ SE¼ *More than 640 acres in Sec.7	Federal

ot No.	Type of Plot	General Location	Map No.	Sqr. Mile No.	Technical Description of Plot Location	Ownership
12	Mid-Aged Trembling Stand-Only Scattered Paper Birch	On USFS Road 128 N of Skibo Vista	22	728 746	T.57N. R.13W. Sec. 5 NW¼ SW¼	Non-Federal
13	Pole Trembling Aspen Stand	On USFS Road #130 (just W of St.Louis River)	17	699	T.58N. R.14W. Sec. 33 S½ NE¼ NW¼ SE¼ NW¼ NW¼ N½ SW¼ NW¼	Non-Federal
14	Mid-Aged Tamarack Stand, Scattered Black Spruce	On USFS Road #424, .5 mi west of Hwy.1	7	277	T.61N R.10W. Sec. 31 NE¼ SE¼ NW¼	Federal
15	Young Tamarack	On USFS Road #424 Approx. 1.25 mi. W of 1431 Intersection	11	349	T.60N. R.11W. Sec. 18 N½ NW¼ SW¼ S½ SW¼ NW¼ *More than 640 acres in Sec.18	Federal
16	Mid-Aged Tamarack-Black Spruce Stand (Co-Dominant in Canopy)	On USFS Road #424, 1.5 mi. E of Junc. w/USFS Road #112	10	324	T.60N. R.12W. Sec. 12 N½ NE¼ NW¼	Federal
17	Mature White Cedar Stand	On USFS Road #424, .5 mi. W of Junc. w/USFS Road #1431	11	349	T.60N. R.11W. Sec. 18 N½ SE¼ NE¼ *More than 640 acres in Sec.18	Federal
18	Mature Trembling Aspen with Dense Fir Understory	On USFS Road #116, Between 2 RR Crossings	15	493	T.59N. R.12W Sec. 18 S½ SE¼ NE¼ N½ NE¼ SE¼ *More than 640 acres in Sec.18	Federal
19	Approx. 6 yr old Red Pine-Jack Pine Plantation	On USFS Road #112 ½ mi. E of Junction w/USFS Road #1431	10	396	T.60N. R.12W. Sec. 25 SE¼ SW¼ E½ SW¼ SW¼	Federal
20	1972 Clearcut	On Spruce Road 1.9 mi. N of Hwy. 1	2	107	T.62N. R.11W. Sec. 26 SE¼ NW¼ N½ NW¼ SW¼	Federal
	Grassland-Skibo Mill	End of USFS Rd#569 approx. 2.5 mi. beyond area where road runs along E side of RR right-of-way	18	708	T.58N. R.13W. Sec. 36 NW¼ SW¼ NW¼	Non-Federal

Plot No.	Type of Plot	General Location	Map No.	Sqr. Mile No.	Technical Description of Plot Location	Ownership
22	Alder-Willow Swamp	Along USFS Rd. #128 Just S of Inter-section w/129, before road takes sharp bend SW	22	746 747	T.57N. R.12W. Sec. 8 NE¼ NW¼ NW¼ T.57N. R.12W. Sec. 5 SE¼ SW¼ SW¼	Federal Non-Federal

CARD #1

TERRESTRIAL BIOLOGY
REGIONAL COPPER-NICKEL STUDY

X = no data
0 = 0

Bird & Mammal Observation
While Driving a Know Distance for a Known Time

1 2 — X NO.
3 4 5 6 — Y NO.
7 8 — YR.
9 10 — MO.
11 12 — DAY
DATE

13 14 — OBSER. #
1 DH 6 JJ
2 LP 7 GN
3 DS 8 DB
4 BB 9 GS
5 NS

15 16 17 18 — START OBSERV.
TO
19 20 21 22 — END OBSERV.

23 24 25 — TOTAL MIN. DRIVING

(________miles)

26 27 28 . 29 KM — DISTANCE TRAVELED

30 — TYPE OF ROAD
1 hard surface
2 improved gravel
3 unimproved gravel

31 32 33 °C
1 +
2 -
TEMPERATURE

34 — PRECIP.
1 none
2 fog
3 drizzle
4 rain
5 lt. snow
6 blizzard

35 — VEG.
1 wet
2 dry
3 frosty

36 — SKY
1 clear
2 scattered(<½o.c.)
3 broken (>½o.c.)
4 completely o.c.
5 obscured

37 — WIND
1 calm
2 0-5mph
3 6-10mph
4 11mph or>

38 — SNOW COVER
1 none
2 present

39 40 41 . 42 CM — SNOW DEPTH

43 — PHENOLOGY
1 trees bare
2 leaves ½ grown
3 leaves fully grown
4 leaves colored
5 leaves falling

RECORD ROAD#
IN SEQ. DRIVEN

REMARKS

RECORD ROAD# IN SEQ. DRIVEN		REMARKS
____	____	____________________
____	____	____________________
____	____	____________________
____	____	____________________
____	____	____________________

78 — OBSERV.
1 see card #2 for observ. data
2 nothing seen, no veg. types recorded
3 nothing seen, see card #2 for veg. type searched

79 80 — # OF CARDS FOR THIS DATA BIT

CARD #2

TERRESTRIAL BIOLOGY
REGIONAL COPPER-NICKEL STUDY
Bird and Mammal Observations

TWNS#	SQM#	40#	TIME SEEN	SPECIES SEEN	# SEEN IN GROUP	SEX OF IND.	AGE OF IND.	#YOUNG/ BROOD-LITTER	BEH.	HABITAT 1st OBS. IN
1 2	3 4 5	6 7	8 9 10 11	12 13 14	15 16 17	18	19	20 21	22	23 24 25
26 27	28 29 30	31 32	33 34 35 36	37 38 39	40 41 42	43	44	45 46	47	48 49 50
51 52	53 54 55	56 57	58 59 60 61	62 63 64	65 66 67	68	69	70 71	72	73 74 75

REMARKS

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SEX OF IND.

1 male
2 female
3 unknown

AGE OF IND.

1 adult
2 yearling
3 young-of-the-year
4 adult female with yearling(s)
5 adult female with young-of-the-year
6 adult female with brood
7 unknown

BEHAVIOR

1 standing
2 fleeing
3 feeding
4 swimming
5 flying
6 perched

PLANT PATHOLOGY/SOILS STUDIES

INTRODUCTION

Where direct anthropogenic activity is limited, atmospheric deposition is the largest source of both beneficial and harmful chemicals to the terrestrial system. The soil-vegetation complex constitutes the largest component of terrestrial ecosystems. Addition of chemicals to this phase occurs through both active and passive processes. For example, vegetation, where canopies are involved, absorbs gaseous and aerosolic pollutants actively in measurable quantities. Thus, tree canopies act as biological sinks. In contrast, dry fallout of particulates and wet scavenging of atmospheric chemicals through rain and snow is a more passive process. In the end, the chemical composition of vegetation may change over time through direct deposition and/or active absorption and, in addition, through uptake of chemicals from the soil.

The chemistry of soil is changed over time with deposition and weathering. However, the mechanism by which the soil affects the chemical composition of the vegetation it supports is dependent upon the concentration of available elements for biological uptake. This is governed by the moisture content of the soil, pH, common ion effects, hydrolytic capabilities of the plant roots in the rhizosphere, capillary capabilities of the roots, etc.

Uptake of soil minerals by the plants themselves is controlled by factors such as the weather, generic, species and varietal aspects of the plants in question; growth rate of the plants, presence or absence of a pre-existing biotic or abiotic stress, the soil physics and chemistry, etc. Thus, the plant--soil interface represents a complex but integral relationship.

Over the years, scientists have used plants as indicators of air quality. In addition to monitoring the pollutant-induced symptom expression, investigations have been performed on the changes in the inorganic chemistry of specific and selected plant species over time. Correlations have been derived between vegetational elemental composition and corresponding availability of such elements in the soil. Most of these studies have been conducted with reference to line and point sources as post-mortem investigations. Few studies exist at this time where pre-operational or background information has been gathered. Furthermore, other than in a few cases, little effort has been devoted toward developing correlations between continuous quantification of atmospheric chemical components and changes in soil and vegetational chemical composition over time. This is particularly true with reference to ecosystems.

Air pollution is a source-transport-effect phenomenon. For many years, man's efforts in understanding air pollution were restricted to urban and pollutant source areas. More recently, however, with the recognition of air pollutant transport processes, greater concern is being directed at rural and other areas partially influenced by anthropogenic activities. The region of potential copper-nickel mining in northern Minnesota falls into this category. Other than minor investigations conducted by the University of Minnesota - Department of Plant Pathology, little information is available on the region's air quality and the latter's impact on terrestrial vegetation. The proposed copper-nickel mining may generate particulate and aerosolic pollutants (cation, cation-anion complexes). While the effects of the particulate pollutants on the soil-vegetation system may be local, aerosolic components are suspected to have a significantly greater geographic influence. It should be pointed out that aerosols are considered to constitute the largest depositors of atmospheric chemicals on vegetation and soil. Recent evidence also indicates that aerosols contain most of the important elements from the point of view of man's environment. Should a smelter be operated, gaseous pollutants such as sulfur dioxide and fluorides are considered to be the important plant pathogenic air pollutants.

For details on the vegetaional effects of the aforementioned pollutants, the reader is referred to the literature survey on the "Impact of air pollutants on terrestrial vegetation" by Krupa _et al_. (1976 Annual Report to the Minnesota Environmental Quality Council).

OBJECTIVES OF THE PRESENT STUDY

The objectives of this study are as follows:

1) To determine over a two year period, the changes in the concentrations of certain inorganic elements in the foliage of selected plant species.
2) To determine over a two year period, the changes in the geologically available concentrations of selected inorganic elements (corresponds to objective 1) at two different depths of soil (0-6" and 6"-12") supporting the vegetation used in objective 1.
3) To attempt to develop an understanding of the relationship between air quality (primarily particulates and possibly aerosols) with regard to atmospheric deposition and changes in soil chemistry over time.
4) To attempt to develop over time, correlations between the biologically available elemental composition of the soil and foliar elemental composition.
5) To attempt to define the relationships between air, soil and vegetational chemistry, for the purpose of suggesting threshold biological tolerance values through atmospheric loading.

CURRENT STATUS OF THE STUDY

A total of 34 field sites have been established for long term studies. Some of these sites are located within the copper-nickel Mine Site Area and are coordinated with the terrestrial biology study. Certain additional sites have been located with reference to the air quality program. Others have been distributed to represent the true regional aspects of the project's goals.

The following plant species were selected for study: jack pine, white pine, red pine, black spruce, paper birch, balsam fir, aspen, tamarack, and white cedar. Grasses were also sampled. Each of these plant species was chosen because of its predominance within the various study sites.

Plot size was approximately 150 feet in radius. Twelve trees within each plot were selected for foliar analysis. Care was taken to select trees that did not exhibit visible symptoms of biotic or abiotic stress. Individual trees were marked, and sampled for foliar analysis during each month of the growing season (June, July, August and September). Sampling was performed on large trees through the use of either a pole pruner or a 22 caliber magnum rifle with hollow-point bullets. The hollow-point bullets tend to go through small branches without leaving metalic residue in the tree and at the same time break the branch for sampling. Samples from all 12 trees on a plot were pooled, by sampling period, and analyzed for the total concentrations of the following elements; Fe, Pb, Cu, Zn, Mn, Cd, Ca, Mg, Na, K, S, Cr, Ni and F. To date analyses have been completed on the June and July samples and some of the results will be discussed at the Regional Copper-Nickel Study Biological Sciences Workshop.

Soil samples were obtained twice during the growing season. These samples were collected during the first and the third sampling periods (June and August). Soil samples were obtained as 1" diameter cores at two different depths of the soil, 0"-6" and 6"-12", at distances of 50 feet, 100 feet, and 150 feet from the center of the plot in four directions (N, S, E and W). All samples from a given depth at each site were pooled by sampling period. Soil samples are being analyzed at this time for biologically available concentrations of the elements discussed under foliar analysis. In addition, pH of the soil samples is being determined.

In order to understand the current status of stress problems on terrestrial vegetation in the study area, a disease survey was performed every month over the entire region. This type of information is vital in not only contributing to our background knowledge but also in determining the possible consequences of mining and smelting on the increase or decrease in the incidence of these stresses in the future. To date, a list of the existing vegetational stress problems in the study region has been compiled for the summer of 1976. The symptoms of these stress problems on various plant species have been photographed and will be discussed at the Regional Copper-Nickel Study Biological Sciences Workshop.

At this time foliar and soil chemical analyses are being continued and the results will be communicated to the study group within the next few months. As the air quality and the weather data start to come in, efforts will be directed toward data anlaysis.

HEALTH STUDIES

INTRODUCTION

The objectives of the health studies are:

1) Delineation of the possible impacts on human health of a copper-nickel mining and processing industry.
2) Design of health surveillance studies of exposed workers, their families, and communities to be implemented after the onset of mining, smelting, and processing.
3) Recommendations for the protection of the health of the workers, their families, and communities.

LITERATURE REVIEW

In order to predict impacts and make study recommendations, it was necessary to conduct a thorough literature review of the effects to human health of copper-nickel mining and processing activities. The literature review has revealed the paucity of good epidemiologic and biologic research regarding human exposures to copper-nickel mining and related activities. Some of the factors responsible for the paucity of information are:

1) the inherent difficulties and expense of studying human subjects;

2) historically, the lack of good laboratory instruments and epidemiologic methods, which today, in light of current knowledge and methodology, makes interpretation of the results of many studies very hazardous and renders some studies invalid, primarily because of lack of controls.

The following are selected findings of interest in the literature review:

Nickel

1) A study of 15 U.S. copper smelters indicated:
   a) air concentration exceeded OSHA standards;
   b) arsenic induced cancer from high SO_2

2) Workers employed in refining of nickel had increased risk of lung or nasal cancer, probably from the dust of nickel ore and its products. Protective measures have been used, but some risk continued to 1960. Workers in refining process also were at higher risk of chronic respiratory disease (primarily in smokers who smoke cigarettes) and allergic dermatitis (associated with working conditions of extreme heat).
3) Increased risk of sinus and pulmonary cancer in furnace workers, sintering plant and process workers. Studies in Norway indicated greatly increased risk of death from cancer of all sites, nasal, larnyx, lungs, all respiratory organs.
4) "Converter" workers in nickel refinery were at greater risk of chronic bronchitis, particularly among those who smoke.
5) Nickel carbonyl poisoning can occur accidentally. This results in severe physiologic response and possibly death.
6) Nickel dermatitis occurs in a wide variety of occupations and exposures.

Copper

1) Causes permanent damage to testicular tissue and sperm in rats.
2) Smelter workers exposed to arsenic trioxide were at increased risk of respiratory cancer.
3) Risk of much higher mortality rates, compared to general populations, from major causes of death including all cancers and respiratory cancer in miners of all types.
4) Increased morbidity ratio for respiratory conditions in mining occupations compared with foundry workers.
5) Smelter workers were at higher mortality rate of lung cancer than other male populations. Arsenic trioxide exposure said to be high.
6) Silicosis studied in all miners (underground) is more an effect of amount of silica in the dust than type of commodity mined. Proper ventilation and use of respiratory equipment is responsible for the recent decline in rates and is considered necessary for underground miners.
7) Arsenic exposures to workers in a copper mine and processing plant (compared to a control group) produced cases of arsenical melanosis, arsenical dermatosis, and perforation of nasal septum. There was also significant absorption of arsenic in the urine and skin hypersensitivity to a patch test.
8) Chronic long term exposure to large amounts of copper results in a persistent destruction of red blood cells and liver disease.
9) "Metal fume fever" and high serum copper levels attributed to inhalation and absorption of copper dust present in factories.
10) Recent nationwide lung cancer mortality data, by county, indicates counties with copper, lead, and zinc industries had significantly higher rates than the rest of the United States.
11) School children in the neighborhood of a copper smelter had much higher levels of arsenic in hair and urine than a group of control children. Another study indicates an inverse relationship of arsenic concentration and distance from the smelters. In addition, respiratory lung cancer morbidity was more than three times expected.

Current epidemiologic knowledge about cancer indicates that miners, workers in industries related to mining, and workers in dusty occupations are at increased risk of cancer in general, and of cancer of certain sites in specific, as compared with the general population. Also families of these workers are at increased risk, and current environmental epidemiology suggests that communities many miles downstream or many miles downwind may have higher rates of certain cancers compared with non-exposed communities. Current research indicates the occurrence of these cancers are associated with the additional factors of age, sex, race, socioeconomic status, ethnic practices, dietary practices, duration and intensity of occupational exposure, and smoking habits. Cancers are probably related also to biochemical balances of the hormonal, endocrine, and immune-response systems, of which we know very little.

Environmental epidemiology also suggests that miners and processors, and their families and communities, are at increased risk of chronic respiratory disease, compared to the general population or non-exposed groups.

Little is known about the effect of trace metals on chromosomal abberrations and congenital malformations, but this is under discussion by epidemiologists and research is taking place.

The literature review reveals the levels of trace metals found in healthy humans, but little is known about levels of toxicity or the biological mechanism which produces a toxological effect.

FUTURE STUDY PLANS

The following is a discussion of the major studies that may be done as a surveillance of health effects of copper-nickel mining, smelting and processing, and related industries, in workers, their families and communities. Some studies are of higher priority and greater feasibility than others. Some of the methodological considerations include:

1) The population (or the denominator) studied must be large enough so that small numbers in numerator data are meaningful.
2) The characteristics of the population should be defined by age, sex, race, area of residence, socio-economic factors, and other variables, all of which are available only in census data.
3) The population studied (or sample thereof) should accurately reflect the population at risk.
4) Surveillance of a population for differences in rates of disease should not only be compared over time, but should also be compared geographically to an unexposed population, that is similar in characteristics to the population at risk by demographic distribution and additional factors such as socio-economic characteristics, quality of medical care, and ethnicity.

On the basis of these criteria, it seems scientifically invalid to collect data regarding health and disease at this time when we don't know the demographic characteristics of the population, and even if we did, it does not reflect the characteristics and distribution of the population just prior to the onset of mining. (The population would include miners and their families who have just moved into the area for the new mining operations, and they would bring with them prior exposures and greater risks of the health effects of copper-nickel industry.)

A study of high priority and feasibility at the time just prior to the onset of mining would be the determination of body burdens of trace elements. Body fluids and tissue specimens, such as blood, urine, deciduous teeth from seven year olds, possibly umbilical cords or cord blood from newborns, surgical and autopsy specimens (including whole organs)--these would all be obtained, aliquots analyzed, and aliquots saved for many years when new research techniques, knowledge, and more precise laboratory methods make additional study possible. Human body burdens of trace elements vary by age, hour of the day, cycles within and by season, hormonal patterns, dietary patterns, etc. and we need more information about levels of toxicity. Furthermore, trace elements are organ specific. A wide variety of organs, such as bronchus, lung, liver, kidney, bone, and lymph nodes from autopsy or surgical specimens would be necessary to monitor for metals in copper-nickel mining and industry. The Environmental Protection Agency (EPA) recently held a workshop on the problems of studying health effects surrounding point sources of pollution. An expert in determination of trace metals in humans stated that what we don't have in medical or environmental research are measured levels of exposure, and what we need to do is measure and quantify people's exposures.

To make extrapolations from animal experimentation to the expected effects on human health is hazardous because laboratory animals are generally exposed by different routes and in much greater doses than humans. Furthermore, to substitute analysis of free-living wild animal specimens for human specimens is hazardous because such animals and humans are different from each other in area of habitation, route of exposure, and characteristics of tissue and biological response.

Another study of high priority and feasibility is the incidence of cancer morbidity. Rates of cancer morbidity are much more reliable than rates of mortality. Because of the asbestos situation in Duluth, I have been conducting a study which represents the only study of human health effects from the ingestion of asbestos. I am locating all cases of gastro-intestinal and lung cancer diagnosed during 1969-1974 in residents of Duluth, Two Harbors, Silver Bay, and Beaver Bay. These data are compared to data collected in the same manner for the same time period for residents of the cities of Minneapolis and St. Paul. The main sources of data for this study are hospital medical records, autopsy and pathology reports, and death certificates. Within several months, I expect funding from other sources to expand the study to include all cancers in all residents of St. Louis and Lake counties diagnosed in 1969 to the present time and to continue the surveillance for many years. Similar methods will be used in the future to insure comparability of data. The results of this study may help explain why St. Louis and Lake counties have some of the highest cancer mortality rates in the country and will serve the dual purpose of contributing knowledge regarding health effects of copper-nickel mining and processing and the health effects of the asbestos situation in Duluth.

A baseline study of chronic respiratory disease is probably less feasible than the studies I've already mentioned because of cost and methodologic problems. Most of the well designed studies of this health problem measure periodically:

1) Meteorological conditions
2) Ambient air quality
3) Respirable air quality
4) Pulmonary function tests
5) Human factors of such things as daily activities and stress
6) Chest X-rays
7) Smoking history
8) Other medical conditions determined from personal diaries.

If a study of chronic respiratory disease is conducted, extensive consultation is needed.

A study of chromosomal aberrations is also of uncertain feasibility. There is some evidence that chromosomal aberrations may be caused by asbestos, mercury, and lead. The cost of analysing a single blood specimen is over $150.00, and the laboratory techniques and epidemiologic methodologies are uncertain. Major logistical problems are also involved. Less sensitive than this method for the determination of congenital effects from trace elements is a laboratory procedure at about $30.00 a specimen that detects inherited major chromosomal aberrations. An alternative to these two methodologies is surveillance by a congenital malformation registry established in hospital newborn nurseries and physician offices. However, this method fails to detect malformations of internal organs which sometimes are not detected until adult life.

The above discussion outlines some of the important health studies that should be done, and mentions very briefly some of the methodological problems. Surveillance of the same health effects should be conducted in all employees of copper-nickel mining and processing. This would take the form of pre-employment physicals including analysis of body burdens of trace elements, lung function tests, and personal interviews regarding occupational history, smoking history, dietary history (since diet affects impact of trace metals on human health), medical history, etc. These data should be reportable to a person/agency outside the mining industry whose responsbility it is to analyze the data and decide if health effects are greater than expected, and if so, what remedial action should take place. The terms and routes of action should be defined legally.

The future of the health studies is dependent upon further discussions. If these studies are to be done, other human health experts must be involved, such as pathologists, clinicians, geneticists, physiologists, cytologists, toxicologists, radiologists, pulmonary specialists, and other epidemiologists who have specialized in these fields.

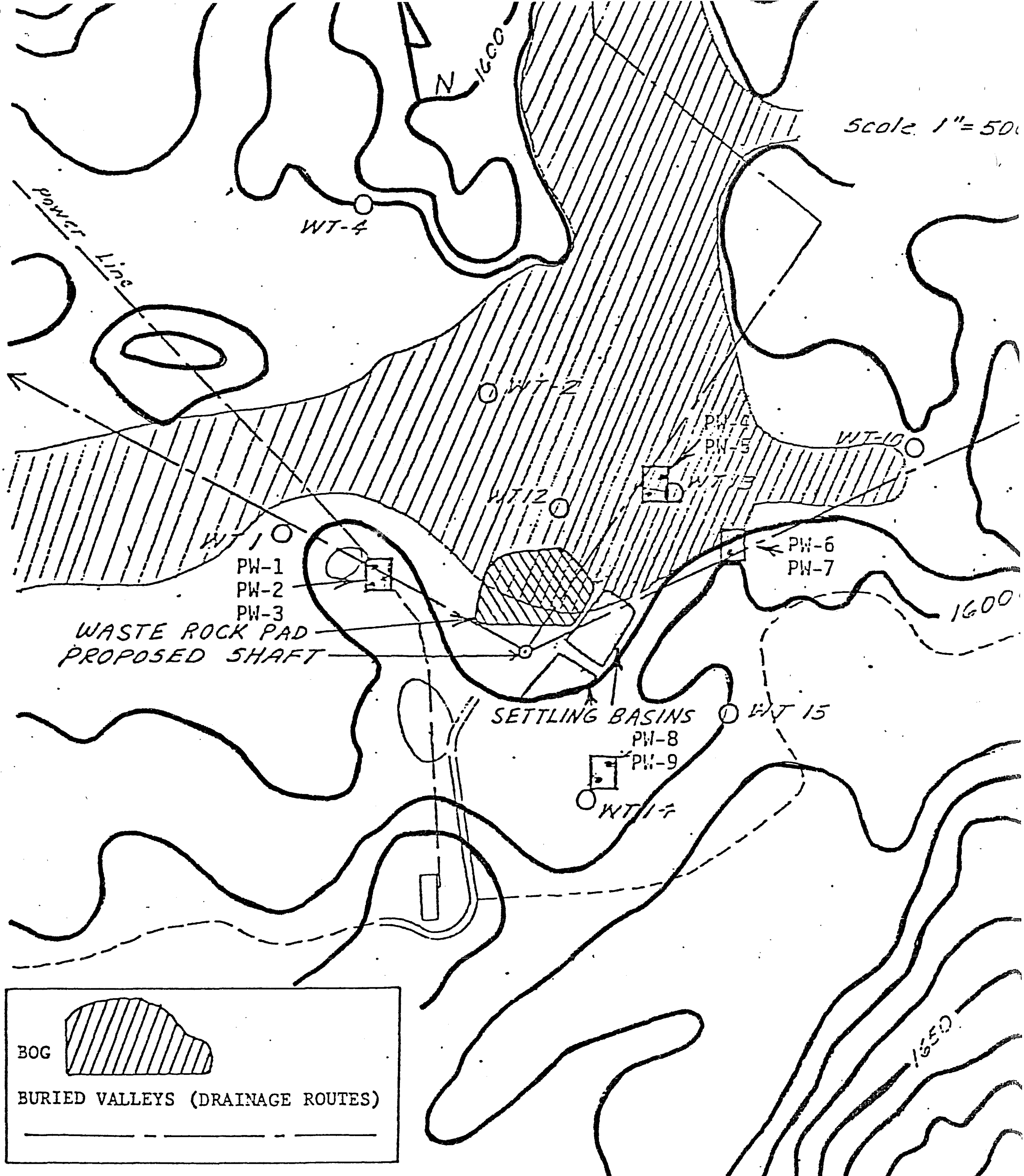
BOG STUDIES

INTRODUCTION

Minnesota is in a unique position of being able to study and collect data on an area before any mining activity has begun. It is the goal of the Regional Copper-Nickel Study to provide an environmental base line and to predict socio-economic and environmental impacts associated with copper-nickel development. This document will then serve as a basis from which the Lesiglature can decide the state policy regarding copper-nickel mining. In order to predict environmental impacts, areas must be selected which will provide meaningful data. One of the areas is the AMAX test shaft. Considerable effort already has been expended both by the company and the state to collect good baseline information. A network of surface and ground water quality monitoring stations, vegetation transects and terrestrial monitoring have been established.

The company has begun work on the test shaft and has begun sinking the actual shaft. Waste rock material from the shaft is being stockpiled, some on upland soils and some in a spruce bog (Figure 1). It is the goal of this study to examine the impacts of this operation, with particular respect to heavy metals on the surrounding area.

Since all the runoff, seepage and leachate from the area will flow into the spruce bog, it is important that this ecosystem be monitored. Peat bogs have been known to concentrate metals, and this fact has been used as a geochemical survey tool. The ion exchange capacity of sphagnum has been documented,and some data exists to suggest that heavy metals can also be



AMAX PAD AND PEAT DEPOSIT

Fig. 1

adsorbed. Given the large expanse of bogs in the copper-nickel study area and the fact that several of the alternate tailing basin sites studied during the Reserve EIS are in or adjacent to bogs, the question of how or if heavy metals are transported through this type of environment is important. It may be that peatlands may act as a mitigating measure for adverse environmental impacts resulting from copper-nickel mining.

Since AMAX is stockpiling waste rock material in and adjacent to a peat bog, this area is excellent for study. If the study is to have predictive value, the integrity of the bog must be maintained. The vegetation and the hydrology are integral parts of the peatland. Changes in vegetation and/or hydrology could drastically alter the water quality and metal transport in the bog.

The importance of this particular bog is that 1) a significant amount of background data exists, and 2) activities will occur which may increase heavy metal concentrations in the area to measurable levels.

PRELIMINARY PROGRAM

Maps showing an approximate outline of the bog, and the project area are shown in figures 1, 2, and 3.

Attempts will be made to answer the following questions:

1) Will heavy metals be removed by the bog?
2) What is the total capacity of the bog?
3) Will the metals be bound irreversibly? What will be the effect of this metal on the vegetation and water quality in the bog?
4) How can this study be used to predict impacts in other areas?

Water Flow

Using available data, a one foot contour map of the bog has been constructed. From this map, aerial photography, and field observations, two water routes from the shaft have been established. Water inflow to the bog has also been identified. A series of ground water wells are located in the peatland (Figure 2). Background data have been collected since 1974.

Water Quality

Thirteen monitoring stations have been established in the bog. Eleven of these are along the major flow routes leading from the shaft area and two are control stations. The parameters that are being measured are shown in Table I. Runoff is diverted around the site by two ditches. These ditches will also carry seepage from the settling basins. Samples will be collected and analyzed for the parameters shown on Table II.

Vegetation

A series of vegetation transects have been established (Figure 3). More are planned for this summer. The purpose of these transects is to monitor any change resulting from activities in the area of the shaft.

Peat Samples

To better define the type and extent of the peatland, core samples will be collected and peat stratigraphy and depth recorded. Sampling will be more intensive in the immediate shaft area, along the water tracks, and around the ground water wells. Additional sampling will also be conducted on transects through the bog.

Metal concentrations in the live mosses and at various peat depths will be measured. Some of these data have already been collected as part of a soil geochemistry survey, but additional sampling is needed. Sampling will be concentrated in the water tracks and the immediate shaft area.

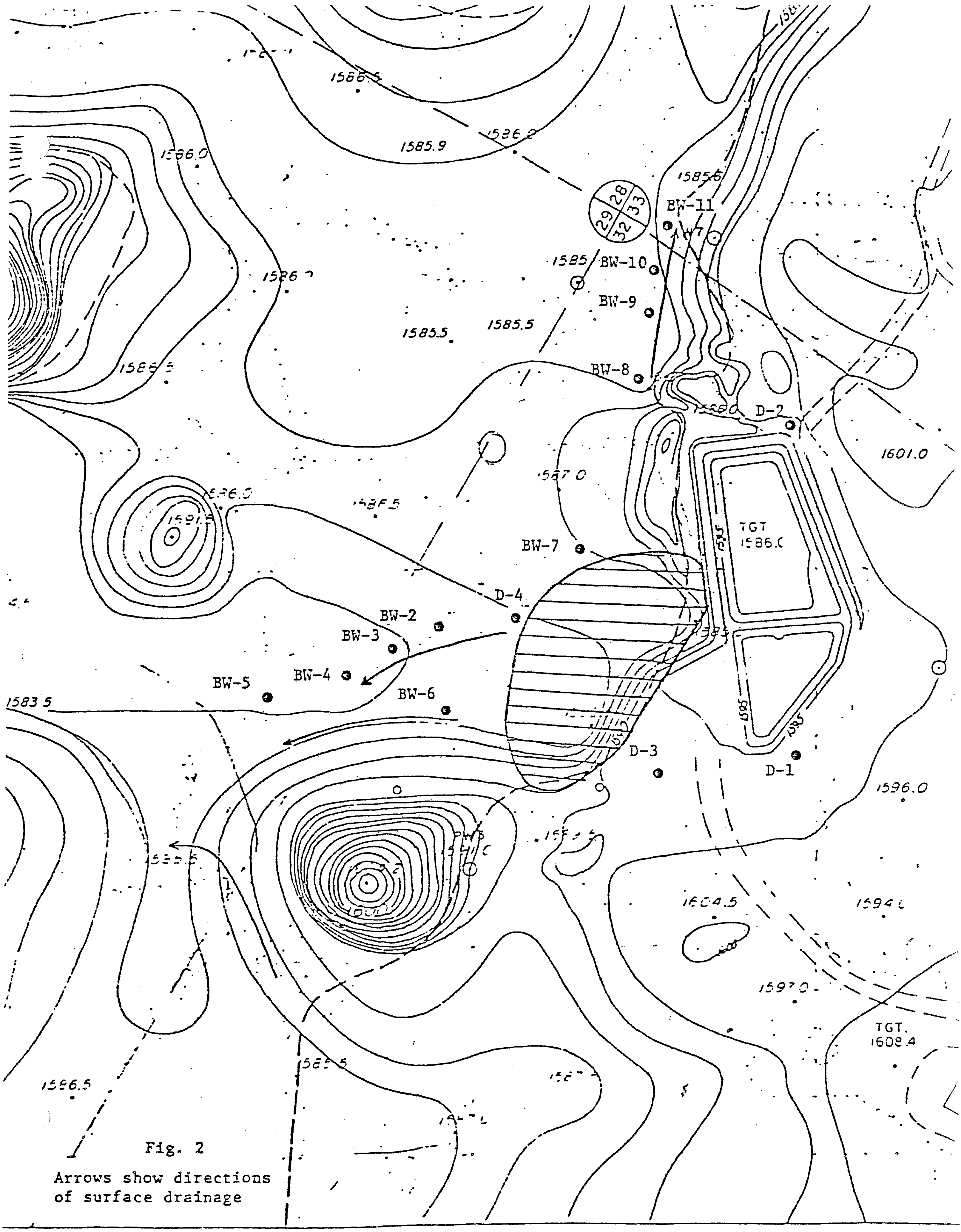


Fig. 2

Arrows show directions of surface drainage

TABLE I	TABLE II
BW1-11	Stations D1,D2,D3,D4
pH	Acidity
Conductivity	Alkalinity
Ca	Hardness
C_1	C_1
SO_4	SO_4
Cu	NO_3
Ni	NH_3
	Cu
	Ni
	Oil
	Suspended solids
	Turbidity
	Fe
	Pb
	Zn
	pH
	Conductivity

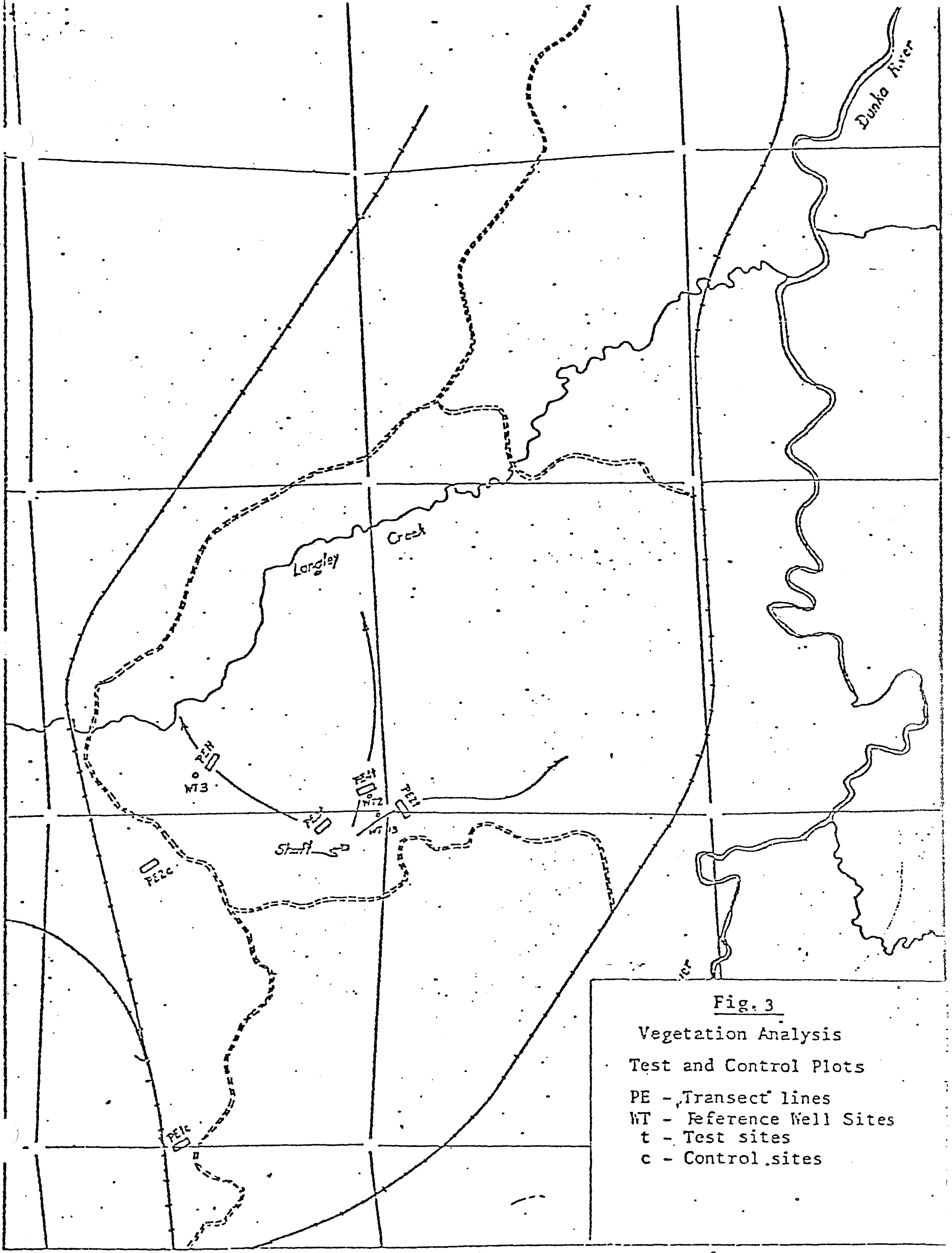


Fig. 3

Vegetation Analysis

Test and Control Plots

PE - Transect lines
WT - Reference Well Sites
t - Test sites
c - Control sites

Some survey work on other bogs in the copper-nickel region will be conducted. The purpose will be to identify similarities and differences between bogs so that results of this study can be extended to other area. In particular the bog adjacent to Erie Mining Company's Dunka Pit is are being examined.

LABORATORY STUDIES

A preliminary leaching study has been designed to estimate the amount of reaction that might occur when the waste rock is placed in the bog. This test would use water collected from the bog as the leaching agent. Tests to date have only used distilled water, so the effect of organic material on the leaching process has not been studied. It is important to estimate the amount of reaction so that sample locations can be chosen. The impacts from this project are likely to be small, and if these impacts are to be documented, samples must be collected before significant dilution occurs.

Studies will also be conducted on the adsorbing capacity of the peat and results compared to field results.

AQUATIC BIOLOGY STUDIES

INTRODUCTION

The aquatic biology study has the responsibility of collecting baseline data on aquatic biota (except fish). Hopefully, these data can be analyzed within the context of the environmental impact study to:

1) identify areas of existing impact
2) identify areas which should not be subjected to any disturbance or which cannot tolerate additional impact
3) determine areas which have a high assimilative capacity
4) determine the potential environmental impact of copper-nickel mining
5) help in the design of mitigating measures to lessen the impact on aquatic communities

Future monitoring on a regional basis should indicate changes which may have occurred. Sampling procedures developed during the study will serve as models for future site specific monitoring.

Three major aquatic biology studies are underway. They are: 1) stream monitoring and survey; 2) lake monitoring and survey; and 3) Erie Mining study. These studies were implemented in May; and the first season of sampling will be completed by November 1, 1976. There will also be some winter sampling in February.

The aquatic biology study's primary emphasis has been placed on streams. Stream monitoring is intended to reflect overall conditions in major watersheds of the region. Because it is not feasible to biologically monitor lakes on a regional basis, a small number of lakes were selected for sampling. Selection of a lake involved a combination of factors: probability of impact from various sources and consideration of how the lake relates to the regional picture in terms of fish populations, lake morphometry, watershed, lake chemistry, and access.

The Erie Mining study is a joint effort of staffs from aquatic biology and Erie Mining Company. Possible effects of elevated levels of copper and nickel on aquatic biota of a small creek (Unnamed Creek) and Birch Lake are being studied and provide data for the impact assessment portion of the Copper-Nickel study.

STREAMS

Sampling Stations

Aquatic biology sampling stations have been selected to coincide closely with the water qualtiy sampling stations. This will facilitate correlation of physical and biological data. The following streams are being sampled: Kawishiwi River, Bear Island Creek, Filson Creek, Keeley Creek, Stoney River, Dunka River, Unnamed Creek, Partridge River, St. Louis River, and Embarrass River.

An attempt was made to locate sample sites in a riffle area upstream from any bridge or dam structure. Primary, secondary, and tertiary designations have been assigned to indicate sampling priority.

Primary sites are overall monitoring sites for major watersheds in the region. There are four quantitative and qualitative sampling periods at primary sites.

Secondary sites are located in areas which are already impacted, are in upstream portions of watersheds which may be impacted by copper-nickel mining development, or are in areas of high potential impact. Two sets of quantitative samples, plus qualitative samples are collected at secondary sites. Semiannual qualitative surveys are made at the tertiary sites. Although these sites have the possibility of being impacted at a later date, they are considered less important in the overall region.

Sampling Methods and Parameters

In order to standardize and simplify quantitative field sampling, artificial substrate samplers have been developed. Artificial substrates make possible the application of equal sampling effort at all stations under most conditions. They also reduce field time and minimize the possibility of human error under adverse conditions. Artificial substrates, which may or may not mimic the natural substrates, are colonized by aquatic organisms. These colonizing organisms are subjected to all the natural fluctuations in chemical and most physical parameters. Because the sampling effort at each station is similar, changes in water quality, as related to the biota, can be observed. The major drawback in using artificial substrates is their selective nature. Regardless of substrate type, a substrate will not be colonized by all organisms found in a given ecosystem. On the other hand, sampling the natural substrate will not produce a more reliable estimate of the actual community structure than artificial substrate samples. For these reasons, artificial substrates were the primary method of collecting quantitative data on periphyton and benthic invertebrates at the primary and secondary stream sites. In addition, qualitative samples of periphyton, benthic invertebrates and macrophytes were collected in order to establish a species list. These samples were collected from various habitat types.

Periphyton--In streams, periphyton or attached algae are essentially the only primary producers. In addition to the production of oxygen, periphyton provide food and habitat for the invertebrate fauna.

By measuring the production (chlorophyll a) and the community structure (diversity and species types) of the periphyton, changes in water quality and subsequent biotic changes can be monitored. Because periphyton are stationary they integrate all physical and chemical factors during the sampling period and thus act as biological monitors of water quality.

The primary sampling technique for periphyton was the use of artificial substrates consisting of glass slides suspended in the streams for three week colonization periods. In addition, a qualitative survey of the naturally occurring periphyton was carried out.

Macrophytes--Macrophytes were qualitatively sampled at all primary and secondary stream sites during August. Samples were split upon returning to the laboratory. The first portion of the sample was retained for identification while the remainder of the sample was shipped to the laboratory of Dr. Sagar Krupa for processing and heavy metals analysis. Plants have been identified, pressed, and will be placed in a collection as a permanent record of species found in the study area.

Benthic Invertebrates--Benthic invertebrates form one of the primary food sources for fish in aquatic ecosystems. They are a link between the primary producers and fish. For this reason it is important to monitor changes in the structure of benthic invertebrate communities. Because benthic invertebrates are relatively immobile within a stream, they like periphyton,tend to integrate the stream conditions at a given point. Changes in community structure and species composition may indicate changes in environmental conditions which might not be discovered through direct water quality sampling. Hester/Dendy samplers are the type of artificial substrate employed for collection of benthic invertebrates. These samplers provide a known area for colonization which is difficult to duplicate using other types of artificial substrates. They are easy to collect and process. These samplers were suspended in the streams for a six week colonization period.

Drift--Drift is comprised of invertebrates who leave the bottom substrate either voluntarily or involuntarily and move downstream with the current. Collection of these organisms over 24 hours was found to be a simple method for estimating the productivity and community structure of the benthic invertebrates. The percentage composition of drift organisms may not be the same as the benthic invertebrate community because of the greater susceptibility to drifting of some organisms. Three drift nets with an upstream opening of 0.025 square meter and length of 2.4 meters were randomly placed in the streams for 24 hours. Because maximum drift occurs between sunset and sunrise, the exact starting time is not critical as long as it is during daylight hours. Since Hester/Dendy samplers and drift nets are selective in their sampling, qualitative invertebrate samples were collected in order to compile a species list of the entire natural community.

Heavy Metal Tissue Analysis--Since macrophytes, periphyton, and molluscs tend to concentrate heavy metals, samples of each of these groups were collected at the primary stations and four secondary stations in August. All samples were split with a portion held at the Kawishiwi laboratory for identification and the remainder shipped to laboratories for heavy metal identification. Macrophyte samples, in water collected from the collection site, were shipped in coolers with ice packs. After arriving in the laboratory, the plants were dried. Molluscs, in contrast, were frozen before shipment and remained frozen until analysis. Dr. Krupa's laboratory is handling macrophytes and Bob Glazer's laboratory will analyze molluscs.

LAKES

Gabbro, White Iron, Colby, Seven Beaver and Birch Lakes were the lakes selected for biological monitoring. Located on each lake were two stations (coinciding with water quality stations). An exception was Birch Lake, where there were four stations (also coinciding with water quality stations). Stations were located in the approximate center of each lake, one at the inlet and one at the outlet. In Birch Lake, stations were located west of Dunka Bay, north of Dunka Bay, between Bob Bay and Stoney River, and south of the South Kawishiwi River.

Sampling Schedule

Lakes were sampled for chlorophyll *a*, phytoplankton, zooplankton, macrophytes in May, June, August, September, and October. Dissolved oxygen, temperature and Secchi disk readings were taken at every sampling. Benthic invertebrates were sampled in May and September. Sampling was not accomplished in July because equipment was being used by the water quality lake survey. During September, Seven Beaver Lake was not sampled because of the fire danger.

Parameters

Collection for chlorophyll a and phytoplankton--At each station water was collected using an integrated sampler. This was constructed of one and one-half inch diameter PVC pipe. The pipe was in two two-meter sections and had the capability of sampling a depth of four meters. This should assure that the entire photic zone was sampled. Seven Beaver Lake, with a maximum depth of 1.7 meters, was the only lake where less than a four-meter sample was collected. After the integrated sampler was removed from the lake, water was drained into a five-gallon carboy and mixed. From this mixed water sample, three, one-liter samples were withdrawn. These samples were kept in a cooler for transport to the laboratory.

Chlorophyll a--Each one-liter water sample was filtered through 0.45 μm Gelman Type A glass fiber filter. These filters were then frozen in acetone awaiting final analysis.

Phytoplankton--Three 120-milliliter phytoplankton samples were withdrawn from the five-gallon carboy at each station. Taxonomic analysis will be performed by Ecology Consulants Inc., Fort Collins, Colorado.

Zooplankton--At each sampling site, zooplankton were collected by taking three vertical hauls from approximately 0.5 meters above the bottom to the surface. Collections were made with a Standard Wisconsin-style plankton net, with an 80 μm mesh size and 13 centimeter mouth opening. The length of each haul was recorded; so that the number of organisms/meter^3 of water could be calculated. Taxonomic analysis of zooplankton samples will also be performed by Ecology Consultants Inc.

Macrophytes--Macrophytes were collected using scuba gear during late July. Sampling was done along transects from shore to maximum depth of the littoral zone. One transect was located near each regular sampling station. Each macrophyte sample was split. The first portion was retained for taxonomy and the remainder was shipped to St. Paul for heavy metals analysis. An effort was made to ship representatives of each recognizable species.

Benthic Invertebrates--The benthic fauna was quantitatively sampled with a ponar (small version, 15.2 x 15.2 cm) dredge. Three replicate samples were collected at each station. Qualitative invertebrate samples were collected along the shoreline in the vicinity of the regular stations. Additional invertebrates (clams, snails, crayfish and leeches) were collected during the macrophyte survey. These samples were split with one portion retained for identificiation and the remainder shipped to Bob Glazer's laboratory for heavy metals analysis.

Other Parameters--During each collection, dissolved oxygen and temperature profiles were recorded using a dissolved oxygen and temperature meter. Secchi disk readings were also recorded.

ERIE MINING STUDY

Sampling Stations

Three stations were located on Unnamed Creek's main channel where elevated levels of copper and nickel have been found. A fourth station was located on a small tributary which received mine drainage but had low levels of copper and nickel. This fourth station was selected as a control for the stream study, but during the first sampling period the tributary dried up and the station had to be abandoned. Two stations were located on Dunka Bay of Birch Lake, and four stations were located on Bob Bay of Birch Lake. The Dunka Bay stations were selected as controls for the Bob Bay stations. Elevated levels of copper and nickel were expected in the sediments and water of Bob Bay. All stations coincide with leaching study stations.

Sampling Schedule

Two six week sampling periods were used in Unnamed creek beginning in mid-July and completed in mid-October. Three collections were made in Birch Lake: September 1st, October 1st, and October 29th.

Parameters

Unnamed Creek Qualitative and quantitative periphyton and benthic invertebrates were collected following the sample procedures employed in the regular stream monitoring program. Six replicate Hester/Dendy samplers and glass slides for taxonomy and chlorophyll a were placed in the creek. Up to six replicate drift nets were used, depending on the discharge at the time of sampling.

Birch Lake--Chlorophyll a and phytoplankton samples were collected at each station by compositing four one-meter, integrated water samples. From this composite sample, three replicate one-liter samples were withdrawn for chlorophyll a analysis and three 120-ml samples were withdrawn for taxonomic analysis. Six replicate dredge samples were collected for benthic invertebrates at each station.

Heavy Metals--Using scuba gear, macrophyte and mollusc samples were collected in July for heavy metal analysis in Dunka and Bob Bays. Sampling was along two transects. Macrophytes were also collected along Unnamed Creek in August.

Sample Analysis

Taxonomic analysis of periphyton and benthic invertebrate samples will be done by consultants contracted by Erie Mining Company. A final decision on the consultant has not been made. There are presently no plans for analysis of phytoplankton samples. A decision on whether these samples will be analysed will depend on results of other analyses. Chlorophyll a measurements were made by aquatic biology staff members. All samples were assigned serial numbers, so that bias could be eliminated at all points in the analysis.

Chlorophyll a analysis is now complete and data are being coded for computer analysis. These data should be ready by December 1st and will be included in the December 15th report.

Taxonomic analysis began October 1st. Qualitative samples will be analyzed first. Mark Johnson proposes a Janaury 1st deadline for completion of all qualitative periphyton and invertebrate samples. A preliminary report discussing results of these data will be ready between Janaury 15th and February 1st.

Between October and Janaury the quantitative inverbetrate samples (drift and artificial substrates) will be picked. At this time, Johnson has arranged for Frank Pafko and John Hanson to pick invertebrate samples for two months after their appointments to fisheries and water quality, respectively, are finished. Additional help has been arranged through the Vermilion Community College from September through April.

Continuation of the Erie Mining study will depend on the results from this year's study and Erie Mining Company's willingness to continue with the study.

Additional studies proposed for next year are fish food habits and intensive sampling at several locations to determine how effective our present level of sampling is in estimating population size and community diversity. An overall schedule for the aquatic biology program is attached.

ANALYSIS PRIORITIES

Within the analysis program outlined above, priorities are being assigned to stations and organisms. During the qualitative analysis stage, primary station samples shall be analyzed first, secondary stations second, and finally tertiary sites (see attached priority schedule).

Periphyton shall be identified to the lowest taxonomic level possible (should be genus or species). The diatom analysis listed in the work plan will be given highest priority in the quantitative periphyton analysis phase. Diatoms have been given this rating for several reasons. First, they should comprise approximately 90-95 percent of the species and individuals of periphytic communities colonizing glass slides in the study area. Secondly, diatoms are more easily identified to the species level than other algae groups; and data from diatom analyses are more easily interpreted. Finally, there will be a permanent record of the diatoms. Once the diatom analysis is completed, sedimentation counts, as described in the work plan, will begin.

Analysis of qualitative and quantitative invertebrate samples will concentrate on the insect portion of the samples. The insect community as a whole generally forms a good indicator base of environmental conditions and changes that will occur. Insects will be identified as precisely as possible while other invertebrates will be scrutinized less intensively. In the future, if time and budget permit, additional work will be done on snails and clams because of their sensitivity to heavy metals.

Identification of macrophytes has been completed. In most cases macrophytes have been identified to the genus or species. Low priority has been assigned to macrophytes in field sampling and laboratory analysis time. This decision was based on the difficulty in quantitatively sampling macrophytes and their poor applicability to monitoring studies. Macrophytes are generally good concentrators of heavy metals and will be used primarily for tissue analysis.

DATA INTERPRETATION

Qualitative aquatic biological data will be more useful in the impact analysis stage of the Copper-Nickel study. Quantitative data presently being collected will serve as a baseline for comparison with future studies to determine the actual regional impact of copper-nickel mining and associated developments. Quantitative data will be useful in the present impact assessment only as it expands the qualitative data base.

As the data become available through taxonomic analyses, parameters listed below will be generated. Hopefully, the use of these parameters will allow analysis of the program and allow adjustments to be made for next year, in additon to fulfulling the objectives listed in the work plan. These parameters are:

1) species list
2) number of organisms per unit area or volume
3) relative abundances
4) Diversity Index $\bar{d} = \Sigma\ (Ni/N) \log Ni/N$
   N = number of individuals
   Ni = number of individuals in the i^{th} species
5) Redundancy $r = \dfrac{\bar{d}max - \bar{d}}{dmax - dmin}$
6) species richness $\dfrac{S-1}{lnN}$
   S = total species
   N = total individuals
7) coefficients of similarity $C = \dfrac{2(\Sigma P_1)}{\Sigma Pi+\Sigma Pj}$
   p(prominence value) = density x frequency at all stations
   ΣP_1 = sum of lower of two P values the two stations have in common
   ΣPi = sum of P values at i^{th} station
   ΣPj = sum of P values at j^{th} station
   $B = 1/K \dfrac{K \min (Xia, Xib)}{\Sigma \max (Xia, Xib)}$
   $CC = \dfrac{P}{P + M}$
   P = number of matches
   M = number of taxa present at one station and absent at another

Depending on the magnitude of the variability in the parameters listed above, statistical and graphical interpretation methods will be employed to characterize the streams and lakes sampled. Attempts will be made to compare stations within watersheds and between watersheds. Lastly, it may be possible to categorize the region's streams and lakes based on their biological and chemical characteristics.

TENTATIVE SCHEDULE FOR AQUATIC BIOLOGY
(As of October - 1976)

October 1, 1976	- All heavy metal I.D.'s complete - Field work essentially complete - Taxonomic analysis of qualitative samples
December 1 , 1976	- Qualitative samples finished - Begin preliminary report of qualitative data
December 15, 1976	- Preliminary report completed - Literature search completed - Quantitative analysis begins
April 1, 1977	- Completion of quantitative analysis - Begin final draft of overall report and data interpretation
May 1, 1977	- Completion of report on overall 1976 monitoring program - Complete plans for 1977 monitoring program
May 15, 1977	- Begin 1977 field sampling - Continue taxonomic analysis work
October 1, 1977	- Completion of field work - Aquatic biology staff moves to Minneapolis - Qualitative and quantitative analysis work continues
February 1, 1978	- All literature review work completed - Data interpretation in progress
April 1, 1978	- All field samples analyzed - Staff reduction - Begin final preparation of aquatic biology section of EIS and monitoring reports
July 1, 1978	- Draft EIS completed - Staff reduction
January 1, 1979	- Final EIS completed - Regional Copper-Nickel Study complete

ANALYSIS PRIORITIES

A. Periphyton
  1. Qualitative samples
    a. Primary
    b. Secondary
    c. Tertiary
  2. Quantitative samples
    a. Diatom slides - primary collection period (1st three weeks of sampling period)
      1) Primary sites
      2) Secondary sites
    b. Diatom slides - secondary collection period (2nd three weeks of sampling period)
      1) Primary
      2) Secondary
    c. Sedimentation counts - primary collection period
      1) Primary
      2) Secondary
    d. Sedimentation counts - secondary collection period

B. Invertebrates
  1. Qualitative samples
    a. Primary
    b. Secondary
    c. Tertiary
  2. Lake dredge samples
  3. Lake qualitative samples
  4. Quantitative samples
    a. Hester-Dendys
      1) Primary
      2) Secondary
    b. Drift
      1) Primary
      2) Secondary

FISHERIES STUDY

INTRODUCTION

The fisheries study is a vital part of the Regional Copper-Nickel Study. In order to permit decision makers to rationally determine if, when, where, and how copper-nickel mining should be permitted in northeastern Minnesota, the fisheries resources of the region must be inventoried thoroughly. We must understand what will be irretrieveably lost or damaged in terms of present and potential recreational and economic resources and esthetic values. In order to site mines properly, fishery as well as other natural and recreational resources must be taken into account.

Considerable fishery information is available (from Minnesota Department of Natural Resources (MDNR) files) for many of the major lakes in the Regional Copper-Nickel Study Area. However, additional information is needed on many small lakes and on most of the streams of the region. The goal of the fish program is to update and expand the existing information on fish resources of the area and to evaluate and summarize the information into a useful format for decision makers.

In addition to collecting information on the fisheries resource of the region, the fisheries staff is responsible for the collection of fish to be analyzed for heavy metals. The data from these tissue analyses will provide a baseline for the design of future studies and for comparisons with other regions.

STUDY DESIGN

Types of Data Necessary for the Study

In order to evaluate the regional impact of potential changes in the fisheries of the region, information is needed on the common and rare species present, the habitats that they utilize within the region, and on fisherman usage and fish management activities in the region. Many of these data exist either in MDNR files or in the collective/knowledge of the Cu-Ni Study and in MDNR. The fisheries staff's study staff will summarize the available data and relate the data they collect and recent MDNR data to the broad regional picture. In general, if good, post-1960 surveys are available, they will not be repeated unless there are obvious changes in the stream or watershed studied.

Date Collected in the 1976 Field Season

In a study of this kind, priorities must be set as to areas sampled. For the first year of the study, the major drainage basins of the area were assigned a fish study priority rating based on the likelihood of potential impact by copper-nickel mining and associated municipal developments (Table I). Reconnaissance surveys were carried out on the high priority streams to evaluate fish populations and potential for food, cover, and spawning, and to determine potential and existing usage of stream sectors. Access routes to sites for collections for heavy metals analyses and to stream sectors for qualitative fish population surveys were identified. Field sketches were made noting locations of various types of fish habitats. Within the constraints of availability of equipment and access, fish populations were sampled in representative habitats of individual streams to determine the presence and relative abundance of various species.

These data will also be used to check what is present in areas that appear to be potential habitats for species of recreational interest. Evidence of use by fishermen and canoeists was recorded.

SAMPLING TECHNIQUES

Stream surveys and resurveys are carried out following the traditional stream survey methods used by the Fisheries and Wildlife Section of MDNR.

Fish capture whenever possible is accomplished with electrofishing equipment. When this is not possible, fish are sampled using the more traditional means of sampling; gill nets, trap nets, minnow traps and seines. Physical characteristics (such as depth, current, accessibility, and specific conductance) of a body of water or stretch of stream determine to a large extent the appropriate sampling devices. Details of fish sampling techniques at each sample site are recorded on field data sheets. Direct current or pulsed direct current is used as much as possible (as opposed to alternating current) since direct current seems to cause less damage to the fish. Numbers and species of fish sampled are recorded in the field and fish are returned to the water except where needed for laboratory identification, quality control, or tissue sample collection.

EXPECTED RESULTS OF FISH SURVEYS

The results of fish sampling for the open-water season of 1976 will be complete or partially complete survey reports for the South Branch of the Kawishiwi, the Partridge, the St. Louis, the Embarrass rivers and a number of their tributaries. Repeat sampling of at least some of the same electrofishing stations may be done in 1977 since much of this year's sampling was done during extreme drought and may not be representative.

These data on fish populations will be summarized and will be made available to other Copper-Nickel study groups. They form the beginnings of an updated picture of the fish resource of the area, which will enable judgements to be made as to the relative abundance of various species and the fish habitats within the region.

The Dunka River system was not sampled.

SAMPLING FOR FISH USED FOR HEAVY METAL ANALYSES

Fish have been collected at sites and times described in Table II, and shown on the map in Figure 1. Two of the sites, Filson Creek and Bob Bay, are known to have elevated levels of metals in the water, and are therefore of particular interest. The North Kawishiwi River should be an uncontaminated site.

FUTURE PROJECT PLANS--LAKES AND STREAMS

Updating of lake surveys will be carried out, if possible, primarily by the MDNR fisheries personnel as part of their existing program. If the lakes being studied by other sections of the Regional Copper-Nickel Study have not been surveyed recently and cannot be covered by the area personnel of MDNR, then the fish study group will try to cover them.

The analysis of stomach samples to determine the diet of selected species of fish in the area may be undertaken next year, and if so, the fish study group will be responsible for collecting the fish. No decisions have as yet been made on this.

Summarization of field data obtained will continue, and examination of information contained in regional and area DNR fisheries offices will begin.

Continued fish tissue collection for metals analyses will begin again in May, 1977, coordinating with DNR Chemistry Lab activities and with other fisheries field work. The study of water quality problems, heavy metals, and water quality requirements for fish life and production will continue. Interaction with other Copper-Nickel Study groups should increase. Field data obtained by the fish study group will be made available to other study disciplines.

TABLE I.
FISH STUDY PRIORITIES
ASSIGNED TO
MAJOR DRAINAGE BASINS

	Potential Impacts[2]		
River System[1]	Copper-Nickel Mining	Municipal Development	Fish Study Priority
Embarrass	Low	Medium	Low
St. Louis	Medium	Medium	Medium
Partridge	High	High	High
Dunka	High	Low	High
Bear Island	Low	Low	Low
Stony	Medium	Low	Medium
Isabella	Medium	Low	Medium
Kawishiwi	High	High	High

1 Each river system has a number of tributaries which will be studied

2 Subject to revision as further information is developed

TABLE II.
REGIONAL COPPER-NICKEL STUDY
REVISED FISH TISSUE COLLECTION SCHEDULE
CALENDAR YEAR 1976

Sample Site Stream/Lake	Location T.	R.	S.	Code	Date of Collection	Species*
1. Bob Bay Birch Lake	61	12	36	LBBB	July 21	NP, WS, C
2. Keeley Creek Bay, Birch Lake	61	11	17	LBKCB	July 27	NP, WS, C, CM
3. Dunka Bay Birch Lake	60 61	12 12	4 33	LBDB	August 3	NP, WS, C, CM
4. Stony River Bay, Birch Lake	61	11	29	LBSRB	August 10	NP, WS, C, CM
5. N. Kawishiwi River	63	9	19,20	upstream of K-6 & 8	August 24	NP, WS, C, CM
6. "Bob Bay Creek"	61	12	36	BB-1 vicinity	September 14	CM
7. Dunka River	60	12	4	S-7(AMAX)	September 13	CM
8. Kawishiwi River	62	11	23	K-7 vicinity	October 19	NP, WS, CM, Perch
9. Filson Creek	62	11	24	F-1 vicinity	October 19	CM
10. Partridge River	58	15	12	P-1	September 20	NP, WS, C, CM
11. St. Louis River	58	15	21	SL-1	October 5	NP, WS, CM

* Abbreviations used: NP = Northern pike
WS = White sucker
C = Centrarchids (sunfish family)
CM = Composite Minnow/Forage Species

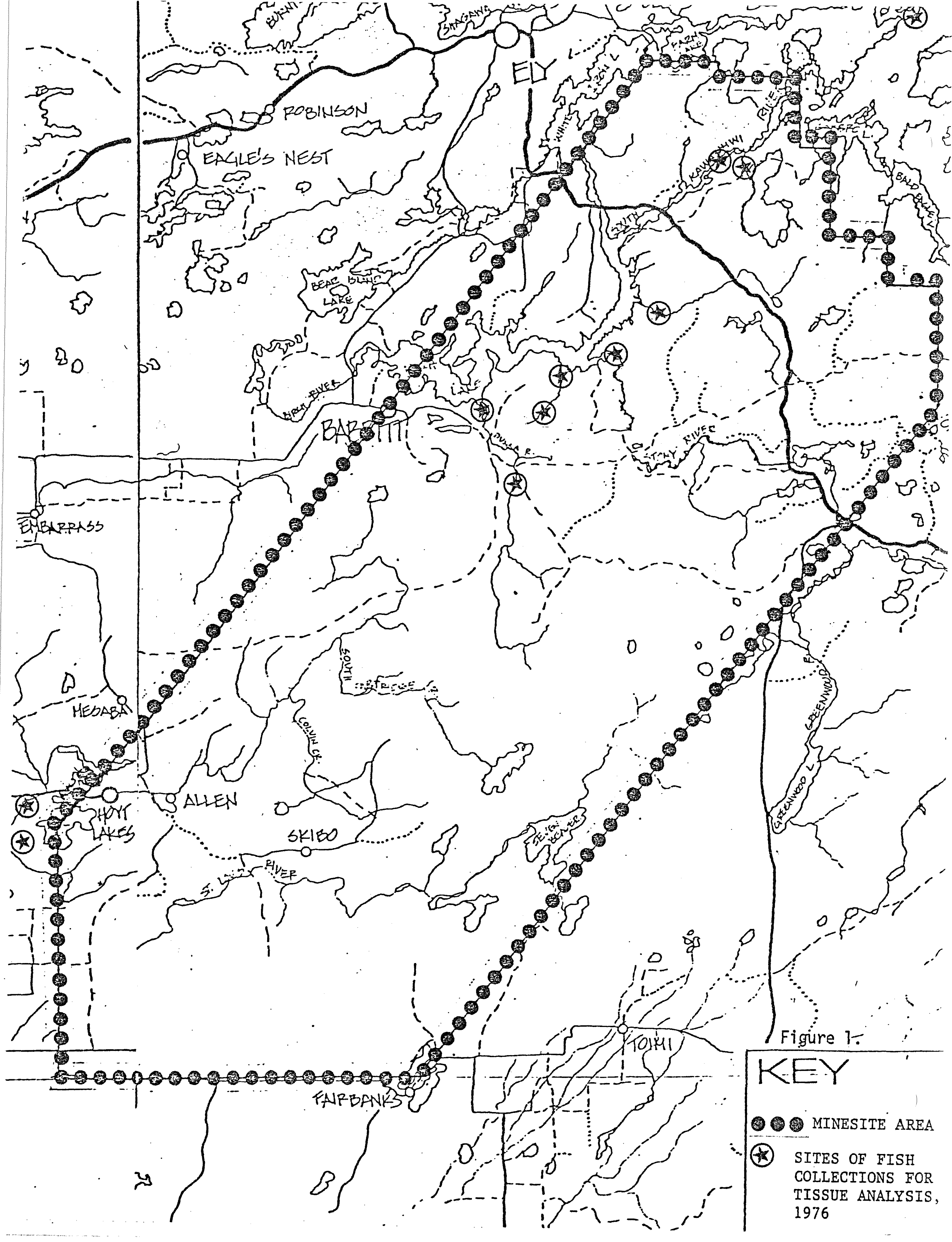

ELY
ROBINSON
EAGLE'S NEST
BABBITT
EMBARRASS
MESABA
HOYT LAKES
ALLEN
SKIBO
FAIRBANKS
TOIMI
COLVIN CR.
KEY
MINESITE AREA
SITES OF FISH COLLECTIONS FOR TISSUE ANALYSIS, 1976

Figure 1.

BIOASSAY STUDIES

INTRODUCTION

The objective of the copper-nickel aquatic bioassay program is to enhance our ability to predict the toxic effects of discharges from copper-nickel mining and processing operations in northeast Minnesota on local aquatic organisms.

The nature and magnitude of the prediction problem is influenced by a number of factors. In the first place, copper-nickel deposits vary in composition within the mine site area, and a variety of mining and processing techniques may be used, so that it is difficult to predict the nature of discharges which may occur. Copper and nickel will undoubtedly be the greatest toxic components of mining discharges, however, cobalt, lead, zinc, cadmium, and silver are also present in the Duluth gabbro in lesser amounts and could contribute to effluent toxicity. In order to be able to predict the toxicities of a range of possible effluents, we must determine the effects, both individually and in mixtures, of what we expect will be the most important toxic components.

Characteristics of the receiving water (temperature, oxygen concentration, pH, hardness, and the concentrations of inorganic and organic complexing substances) may modify the toxic effects of heavy metal discharges. The number of potential modifying factors and our poor understanding of their effects and interactions indicate that bioassays in natural waters from the mine site area are necessary before we can predict the toxicity of heavy metal discharges with any accuracy.

Another factor which complicates the problem of predicting mine discharge toxicity involves the choice of test organisms and test procedures. In choosing a test organism we must consider several factors including: economic, esthetic, and ecological importance to the area most likely to receive mine impacts; relative sensitivity to the pollutants most likely to be present; and adaptability to laboratory experimentation. We must also choose the types of responses to evaluate in toxicity tests. Since most toxicants produce detrimental effects on aquatic organisms at sublethal concentrations the determination of sub-lethal effects can be difficult and time-consuming.

EXPERIMENTAL CONSIDERATIONS

Two approaches may be used in conducting bioassays in natural water:

1) Natural water from a number of sites could be shipped to a laboratory for bioassays. Large volumes of water would be required if test solutions were to be renewed frequently. Furthermore, characteristics of natural water samples may changes during shipping, storage, and testing. Complexation studies have shown that the copper-complexing capacity of stored samples of natural water from the mine site area tends to decrease with time.
2) The alternate approach, which we have chosen, is to conduct flow-through bioassays in a mobile laboratory, drawing test water directly from streams and lakes. There are few locations in the mine site area suitable for such an installation, because of the need for access to surface water and electric power. It may be necessary to generate our own power, or to move outside the mine site area, in order to take water from a variety of locations. Water from less accessible locations may be transported to the mobile laboratory for static bioassays, if procedures can be developed whose results agree well with those of flow-through tests. The test organisms we select for this type of experimentation must exhibit consistent responses to toxicants at all times of year, and a supply of disease-free organisms of a specific age must be continuously available.

Taxonomic groups which might be considered for bioassays are listed below:

- Fish have been studied more intensively by toxicologists than any other group of aquatic organisms. The resulting data have often been contradictory, but it can be generalized fairly safely that the 96-hour median lethal concentration (LC 50) of copper in soft water is between 20 μg/liter and 100 μg/liter for most fish species. Published 96-hour LC 50's of nickel range from 5 mg/liter for the fathead minnow in soft water (Pickering and Henderson, 1966) to 46 mg/liter for the banded killifish in soft water (Rehwoldt et al., 1971). In chronic bioassays, detrimental sublethal effects have been demonstrated at copper levels as low as 17 μg/liter (brook trout; McKim and Benoit, 1971) and at a nickel level of 700 μg/liter (fathead minnow; Pickering, 1974). Chronic bioassays with fish are time-consuming, although embryo-larval tests show promise as sensitive shorter-term indicators of sublethal effects (McKim and Benoit, 1974).

- Amphipods (Gammarus) are probably more sensitive to copper than are fish, although the work of different investigators does not agree well (Arthur and Leonard, 1970, Rehwoldt et al., 1973). The 96-hour LC 50 of nickel for Gammarus (Rehwoldt et al., 1973) appears to be similar to that of fish. Cannibalism among Gammarus has been observed during toxicity tests.

- Cladocerans (Daphnia) are apparently more sensitive than fish to both copper and nickel. The 48-hour LC 50's of copper and nickel for unfed Daphnia magna (Biesinger and Christenson, 1972) are similar to the highest no-effect levels found in chronic tests with fish. Cultures of D. magna and D. pulicaria are maintained at ERL-Duluth. Three-week chronic bioassays of copper, nickel, and other metals with Daphnia were conducted by the above authors, with analysis of survival and reproduction. The addition of food, obviously necessary in long-term tests, markedly decreased the toxicity of copper.

- Planktonic algae are similar to fish in their resistance to copper and nickel, although green algae in lakes near Sudbury, Ontario have been shown to have adapted to copper levels 15 times higher and to nickel levels three times higher than those tolerated by laboratory cultures of the same species (Stokes, 1975). Mixtures of copper and nickel have a synergistic effect on green algae (Hutchinson and Stokes, 1975). To conduct algal assays of copper in natural waters, Stokes and Hutchinson (1976) found it necessary to filter the water samples and add nutrient media. Both procedures probably affected the capacity of the samples to complex and adsorb metals.

EXPERIMENTAL DESIGN

Nature of discharges - We intend to examine first the toxicity of copper and nickel, the metals most prevalent in the rock and most likely to be present in discharges. The toxicity of copper-nickel mixtures will also be studied to determine the manner in which toxicities of the individual metals act when in combination. We also intend to study, later in the project, the contributions of other metals to the toxicity of mixtures already containing copper and nickel through laboratory and literature research. The toxicity of ore-concentrating chemicals may also be examined.

Effect of receiving water - As discussed earlier, receiving water characteristics may greatly influence the toxicity of heavy metals, notably copper, which is complexed (and apparently detoxified) by a variety of organic and inorganic substances present in natural water. In view of this, the need to conduct bioassay studies in natural water is obvious.

The effect of natural water characteristics on heavy metal toxicity can best be measured by comparing the results of on-site bioassays with results of identical experiments conducted in water with low complexing capacity. The U.S Environmental Protection Agency's (EPA) Environmental Research Laboratory-Duluth (ERL-Duluth), which draws its test water from Lake Superior, will provide laboratory space for the copper-nickel aquatic bioassay staff to conduct these experiments during fall and winter, 1976-77. Bioassays of ore-concentrating chemicals may also be conducted at this facility. In addition, cultures of test organisms for the entire copper-nickel aquatic bioassay program will be maintained at ERL-Duluth.

To study the toxic effects of individual metals and mixtures of metals in different types of water, we must perform identical serial sets of experiments--first at ERL-Duluth in Lake Superior water, and then in different field locations.

In choosing test organisms we must consider the factors mentioned earlier: importance, sensitivity, and adaptability to laboratory experimentation. The species of fish which is probably best suited for these experiments is the fathead minnow (Pimephales promelas), a "standard" test species whose response to a variety of pollutants is fairly well-known. A culture of fathead minnows is maintained at ERL-Duluth which can supply weekly cohorts of any particular age. The fathead minnow itself is not common in the copper-nickel study area, yet other members of the minnow family are abundant and constitute an important food source for game fishes of the area. With respect to its sensitivity to copper, for which the most information is available, the fathead minnow is a good representative of other important fish species.

Acute (short-term lethality) bioassays with this species can rapidly generate information on the effect of natural water characteristics on copper and nickel toxicity. Test water temperature must be controlled to provide reproducible results. If a temperature of 25°C is employed, refrigeration of test water in midsummer will not be necessary. This temperature is also consistent with that used in published studies of copper and nickel toxicity to fathead minnows.

Long-term bioassays to investigate sublethal effects of lower toxicant levels are also being considered as a supplement to acute toxicity tests. Scientists from ERL-Duluth have expressed interest in conducting 30-day fish embryo-larval bioassays of copper and nickel in natural water, using the copper-nickel mobile bioassay laboratory. Difficulties which might be encountered in this type of experiment are 1) the necessity of stripping eggs from fish at the trailer site so that the eggs can be placed in toxicant solutions before they water-harden, 2) meeting the food requirements of fish larvae, and 3) keeping all mobile laboratory systems running continuously for a 30-day period.

We also wish to conduct acute toxicity bioassays of copper and nickel at ERL-Duluth with locally important fish species not previously studied, and compare test results with those already obtained under similar conditions with the fathead minnow. The assumption will have to be made that the effect of differences in water chemistry on copper and nickel toxicity to these species would be the same as for the fathead minnow.

The second major test organism which will be used in both laboratory and field bioassays is the genus Daphnia. This choice was made because it is common in the copper-nickel study area, is an important member of the aquatic food web, has been shown to be sensitive to heavy metals as was discussed earlier, and can be cultured in the laboratory. The species *Daphnia pulicaria* has been recommended as a test species by ERL-Duluth personnel and is one of the species of *Daphnia* found in the study area. Most bioassays with *Daphnia* have been conducted in static (unrenewed) test solutions. This should be satisfactory for our work if the test water is not stored for long periods, although static bioassay results can be checked against those of flow-through tests. Two-day bioassays will be conducted at a test temperature maintained at 18^{o}C in a chilling bath for consistency with previous studies.

SCHEDULE

October 25, 1976-April 15, 1977:

1) Construction of test apparatus and acute bioassays of copper, nickel, and copper-nickel mixtures with fathead minnows and *Daphnia pulicaria* in Lake Superior water.
2) Fish embryo-larval bioassays of copper and nickel in Lake Superior water in cooperation with ERL-Duluth personnel.
3) Review of literature pertaining to the effects of heavy metals on aquatic organisms.
4) Construction of test apparatus for the mobile bioassay laboratory.

April 15-May 31, 1977:

Assembly and testing of mobile laboratory facilities.

June 1-August 15, 1977:

Bioassays in South Kawishiwi River water identical to those conducted in Lake Superior water.

August 16-October 15, 1977:

Second set of field bioassays at a site yet to be selected.

<u>October 16, 1977-May 31, 1978</u>:

1) Analysis of data and beginning of write-up.

If ERL-Duluth facilities are again available:

2) Acute bioassays of ore-concentrating chemicals with fathead minnows and *Daphnia pulicaria*.
3) Acute bioassays of copper and nickel in the presence of chloride, excess sulfate, and ore-concentrating chemicals with fathead minnows and *Daphnia pulicaria*.
4) Bioassays of mixtures of copper, nickel, and other metals with fathead minnows and *Daphnia pulicaria*.
5) Bioassays of copper and nickel with locally important fish species for which suitable information does not exist in the literature.

<u>June 1-July 31, 1977</u>:

Third set of field bioassays at a site yet to be determined.

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TISSUE ANALYSIS PROGRAM

INTRODUCTION

One of the basic environmental concerns of any metal mining operation is the effect of trace metal contamination on biological systems. Considering the fact that the mining of low grade copper-nickel ore requires large amounts of water, it is understandable that aquatic systems are in danger of contamination from target as well as non-target mining metals. The complex role of trace elements in biological systems is not fully understood and is probably unique from situation to situation. One fact is clear, and that is that certain trace elements in high concentrations are toxic to living organisms. At lower concentrations certain trace elements are essential to the physiological function of particular organisms or to the ecological system as a whole. In fact, deficiently low concentrations of essential trace metals are in many cases as detrimental as toxic levels. The difference between deficiency and toxicity is often very small. Great variability exists in the ability of particular organisms, either as species or individuals, to physiologically tolerate a given toxicant.

Because aquatic organisms can accumulate certain trace metals from their environment, and in some instances are physiologically bound to do so, they can act as excellent long term indicators of trace metal change in their environment. Fish have previously considered to be good indicators and are often chosen as a prime tissue source for the study of trace metals. Fish are often chosen for study because of several of their basic attributes including: they provide an abundant source of readily available tissue; are found in most permanent natural aquatic ecosystems; have species associated with different levels of the food web, have well defined, quantifiable and structured populations; are economically important as a human food source; and are among the highest form of aquatic organism available both in structure and physiology. Although fish may not accumulate certain trace metals as well as some of the lower aquatic organisms, they provide a convenient and reliable source of tissue for study to date. Pilot tissue studies on small mammals, aquatic invertebrates and terrestrial insects are currently being planned. If these pilot experiments prove useful the tissue analysis program will be modified to allow for full scale studies of any or all of these taxa.

SAMPLING METHODS

To date whole fish and invertebrate samples have been received by the laboratory contained in labeled,plastic bags packed on ice (Ice paks) or dry ice. Subsamples of tissues (liver and muscle) are promptly disected from the fish using care to avoid metal contamination. To this end all disection tools are surgical stainless steel and/or teflon coated with all contact surfaces protected by plastic, non-metalic surfaces. Plastic gloves are used in handling and all implements are washed and rinsed before each disection. Subsamples of tissue (≃10-20 gm) are placed in pre-weighed and labeled weighing boats and wet weights determined to within .001 gram. The weighing boat and sample are then transferred to polyethylene (Whirl-pak) bags, frozen and transported to the Meat Science Laboratory of the University of Minnesota for freeze drying. The tissue is freeze dried in the weighing boat and then transferred to a clean polyethylene bag minimizing tissue handling and possible contamination. Dried tissue is then reweighed and calculations are made for the conversion of dry weight to wet or assumed live weight. Portions of the muscle tissue (usually about ½) and all of the liver tissue are then homogenized (powdered) in a mortar and pestle and placed in labeled polyethylene bags for future analysis. One-tenth of a gram of homogenized tissue is then digested using high purity, Ultrex brand, nitric acid plus heat and pressure in a closed system. The resulting liquid is diluted to the right concentration range with distilled, deionized water and anlayzed using atomic absorption spectrophotometry. In most instances, where trace metal levels are very low, the more sensitive flameless graphite furnace attachment will be used. Where applicable in instances of higher concentrations the more time efficient flame attachment will be used. The machine is calibrated to record data on both a printed and graphical display. Because mercury is the most volatile metal being studied, a separate digestion and special cold vapor analysis will be required.

We are also storing and maintaining an archive of freeze-dried tissues for future reference and analysis.

DATA TYPES AND EXPECTED RESULTS

The function of tissue analysis is to produce reliable data which will, in turn, be evaluated by other study groups. The data we report will be directly related to basic field data such as ID numbers, species, weight, length, method of collection and storage and location. These data will help us project expected body burdens from station to station and species to species and facilitate analysis, standardization, and tracing of possible contamination sources. The majority of tissue to be analyzed will be freeze dried and therefore freeze-drying techniques must be documented. Results of duplicate analysis made by other laboratories (quality control sample splits) and comparisons to values found in a review of the literature are another important factor in this program. Since we are obligated to use proven and accepted methods of analysis we have conducted a review of literature as a basis for the development of techniques.

Data to be generated by this laboratory include, in addition to determinations of individual metal burdens; wet and dry tissue weights, tissue subsample location and conditions of analysis. A sample of the laboratory analysis bench sheet is appended to this report. Finally, in order to satisfy any legal obligations, all fish samples and/or subsamples are handled on a chain-of-custody basis. This includes the disposition of samples from field to lab, lab to lab, etc. and is affirmed through the proper filing of chain-of-custody forms.

The results of the laboratory analysis for trace metals will be expressed as micrograms of metal per gram of tissue (μg/g = PPM) on a freeze dried and/or wet weight basis. Measureable levels of all metals of interest are expected to be found. Physiologically controlled essential trace metals such as copper and zinc are usually characterized by relatively small variations within a species and between locations. These transition metals are commonly found in the PPM (μg/g) range in fish from fresh waters. On the other hand, non-essential heavy metals such as mercury can be bioaccumulated and magnified in fish tissues. Expected levels of these metals in fish from unpolluted natural waters are usually in the sub PPM range and tend to be variable within a given species and from location to location depending on natural or pollution sources. Concentrations of most metals are also expected to vary between muscle and liver tissues since excess concentrations are known to be stored in the liver with its higher fat reserves. Finally, we may possibly find higher levels of some metals in invertebrate samples due in part to their feeding habits, ability to accumulate and tolerate metals, and differences in physiology. A good example would be higher body burdens of copper in clams due to the presence of the copper based respiratory pigment, Hemocyanin.

The metals to be studied and the detection limits of our instrumentation are indicated in Table 1.

FUTURE PLANS FOR TISSUE ANALYSIS

Our goal is to complete the analysis of all tissues now prepared, before the next field sampling season. Our capabilities are such that we can provide alternative analytical services to do other tissue analysis such as invertebrates or small mammals. Our laboratory facilities are limited and we cannot drastically expand the scope of our assignment. However, we are flexible and will adjust the needs of the project as they arise.

TABLE 1.
EXPECTED SENSITIVITIES (PPM) OF STUDY RELATED METALS UNDER NORMAL CONDITIONS USING VARIOUS TECHNIQUES AND THE PERKIN-ELMER MODEL 603 ATOMIC ABSORPTION SPECTROPHOTOMETER

	Flame	Graphite Furnace	Hydrite or Cold Vapor
Transition Metals			
Zn	.018	.001	
Cu	.09	.05	
Ni	.15	.14	
Heavy Metals			
Hg	7.5		.007
Cd	.025	.001	
Pb	0.5	.01	
Semi-Metals			
As	.8	.020	

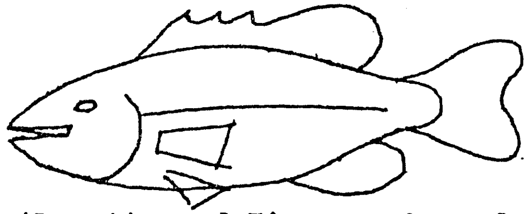
Copper-Nickle Regional Study
Fish Tissue Analysis

Serial No.______________

Sample No.______________

Chain of Custody See Reverse

Side of This Form



*Location of Tissue Subsample

Date / / 19

Time__________ AM PM

Investigator__________

Witness__________

Field Data

Species______________ Date Collected / / 19 Location ____________ Station No.__________

Means of Collection______________ Total Length______mm Weight______gms Collector__________

Means of Preservation and Temporary Storage for Transport______________________________

Subsampling Data

Tissue Sample Location (see diagram above*): Right / Left Side - Dorsal / Ventral - Anterior / Posterior - Entire Fish - Liver

Subsample Weight: Wet______gms Dry______gms Deposited in Archives: Yes No Archives Cat. No.______

Conditions of Freeze-Drying: Time______hrs______min Temperatures______________ Vacuum__________mm Hg

Conditions of Analysis

Weight of Tissue Analyzed__________gms Dry Wet Homogenized Whole Amount Left in Archives__________gms

Digestion Type______________________ Digestion Time ____hr____min Extraction Method__________

pH______ Carrier Solvent__________ Flame: Yes No Gas Mixture__________ Type of AAS__________

Flameless: Furnace (HGA-2100) Cold Vapor (Hg,As)

Metal Species	ul Sample	Wavelength	uv	Slit	mm	Lamp	% Absorption Standards S				Absorbance Standards S				Concentration Standards				Ave.Conc.	Conc. Corrected
							1	2	3	S	1	2	3	S	1	2	3	s		
Cu																				
Ni																				
Pb																				
Cd																				
Zn																				

Figur. .. Laboratory data sheet for fish tissue analysis, Copper-Nickel Regional Study.