

ABANDONED COAL MINE LAND RESEARCH PROGRAM

EIGHTH PROJECT REVIEW SEMINAR

**UNIVERSITY OF WYOMING
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STRATEGIES FOR ESTABLISHMENT OF BIG SAGEBRUSH
(Artemisia tridentata spp. wyomingensis)
ON WYOMING MINED LANDS

G.E. SCHUMAN, D.T. BOOTH, AND J.R. COCKRELL, and J. GORES

Strategies for Establishment of Big Sagebrush
(*Artemisia tridentata* spp. *wyomingensis*)
on Wyoming Mined Lands

G.E. Schuman, D.T. Booth, J.R. Cockrell, J. Gores, and P. Stahl

Introduction

Wyoming big sagebrush is one of the most widely distributed and adapted shrub species in Wyoming and the region. Although considerable debate has surrounded its value, and the need for reestablishment during mined land reclamation, the fact remains that reclamationists are required to restore sagebrush to mined lands when wildlife habitat is a critical postmine land use. In light of this, methods for establishment of big sagebrush are an important area of revegetation research and technology development. Sagebrush is well adapted and persistent when mature, but establishment from seed has proven difficult. Problems include low seedling vigor, the seedlings inability to compete with herbaceous species, poor seed quality or ecotypic adaptation, inability of agronomic seeding methods to meet seed microsite requirements, and the effect of altered edaphic conditions on establishment of the vesicular-arbuscular mycorrhizal (VAM) associations that are believed important to sagebrush seedling survival.

A research study was initiated in January, 1991, to test several cultural approaches for obtaining stands of big sagebrush on mined lands. Specific objectives were to determine:

1. Efficacy of direct-applied topsoil for enhanced sagebrush stand establishment,
2. The value of a stubble mulch crop of annual grain for sagebrush establishment through effects of snow catchment and microsite modification,
3. The usefulness of surficially applied hay/straw mulch in improving sagebrush establishment through seed/seedling protection and microsite modification,
4. The effect of competition from concurrently seeded herbaceous species on establishment of sagebrush, and
5. The value of seeding fourwing saltbush as a pioneer species for enhancing soil biological properties leading to the natural recruitment of sagebrush.

The research allowed for the evaluation of primary questions and interactive effects among treatments in a manner to suggest an optimal combination of methods to enhance sagebrush revegetation.

Project Description

The details of the project design and field plot layout can be obtained from the previous Abandoned Coal Mine Lands Research Program report of November 8, 1994. The project is being conducted at the North Antelope Coal Co. Mine south of Gillette, Wyoming.

Progress Report

This project was initiated January 1991 and this report discusses the findings covering the period November 1994 through October 1996.

Establishment Study

Sagebrush seedling counts made in late September 1995, showed about 8% mortality of seedlings from the September 1994 counts. Seedling mortality did not appear to be affected by mulch type, herbaceous competition, or topsoil source. Sagebrush seedling height was also measured in September 1995. Seedling height was affected by herbaceous competition. The average seedling height was 14 and 30% greater on plots not seeded to grass, and on stockpiled and fresh-stripped topsoil, respectively. This implies greater seedling height on fresh topsoil; however, the difference in average height across all competition and mulch treatments for the stockpiled compared to the fresh-stripped topsoil was only 2%.

Pioneer Study

The pioneer study tested two contrasting hypotheses. The "exclusion" hypothesis stated that where fourwing saltbush was seeded at 2.2 kg/ha pure live seed (pls) or greater rates, monotypic stands would develop that would exclude other shrubs, particularly sagebrush. The alternative or "pioneer plant" hypothesis held that poor physical and biological characteristics of mined-land soils is the reason for poor stands of climax plants, such as sagebrush; and, that planting fourwing saltbush would improve mined-land soils and thereby improve sagebrush seedling establishment and survival. The variables in the study included fresh stripped vs stored topsoil and 4 seeding treatments. The plots were seeded the first year of the study and shrub seedlings have been counted each spring for 5 years. Analysis of variance for each year of seedling counts suggests no significant differences among treatments. Sagebrush seedling density on fresh-stripped topsoil is not different than on stored topsoil. After 5 growing seasons, plots seeded to 2.2 kg/ha pls sagebrush for 2 successive years (total of 4.4 kg/ha) have an average of 6.8 shrubs (sagebrush)/m². Plots left fallow for 1 year, then seeded at 2.2 kg/ha sagebrush, have 6.0 shrubs (sagebrush)/m². Plots seeded to 26 kg/ha pls fourwing saltbush the first year, and 2.2 kg/ha sagebrush the second year, have 4.8 fourwing saltbush + 3.5 sagebrush = 8.3 shrub seedlings/m². Plots not seeded to shrubs have essentially no shrub seedlings. Since seedling densities among seeded plots are not different, we reject both hypotheses. It appears that yearly weather patterns may have had a greater influence on shrub establishment and survival than the whether the topsoil was stored or fresh, and whether sagebrush was seeded with or without fourwing saltbush. Data will be collected from these plots in November 1996, and a final report prepared.

Survey Study

Shrub density and cover were measured by species in 1994 on 14 pre-1985 seedings at 8 mines throughout Wyoming. Seedings which used a diversity of shrub seeds generally had greater 1994 shrub densities. Seeding rates between 60 and 1000 shrub seeds/m² had a positive, linear relationship with the 1994 shrub density up to 0.6 shrubs/m². Shrub communities dominated by fourwing saltbush had a mean canopy cover of 5.81%, compared to 5.59% for sagebrush communities. Multiple-species shrub seedings appear to have higher canopy cover, density, and diversity than single-species shrub seedings. We believe that mining companies which rely on pre-1985 shrub reclamation methods are unlikely to consistently establish shrub

stands with 1 shrub/m².

Greenhouse Study

A greenhouse study was conducted to test the following two null hypotheses: 1) mycorrhizal sagebrush seedlings are no more tolerant of soil moisture stress than are non-mycorrhizal sagebrush seedlings and, 2) there is no interaction between seedling age and mycorrhizal status on soil moisture stress tolerance in big sagebrush. To test these hypotheses, different aged mycorrhizal and non-mycorrhizal sagebrush seedlings were subjected to increasing levels of soil moisture stress to determine the degree of soil dryness that would cause death of the seedlings.

Results demonstrated greater moisture stress tolerance in mycorrhizal sagebrush seedlings compared to non-mycorrhizal seedlings. In all of the different aged seedlings tested (30, 45, 60, 90, 120, and 150 days), the degree of soil dryness causing death of mycorrhizal seedlings was significantly greater ($P < 0.001$) than that causing death of non-mycorrhizal seedlings. Non-mycorrhizal seedlings could not survive in soils with soil water potential values less than -3.3 MPa (-33 bars), whereas some mycorrhizal seedlings could survive in soil as dry as -3.7 MPa (-37 bars). Our data also indicate that mycorrhizal seedlings at all ages tested were similarly more water stress tolerant than non-mycorrhizal seedlings and that there was no significant interaction of plant age and mycorrhizal status on moisture stress tolerance.

Publications from Research

Cockrell, J.R., G.E. Schuman, and D.T. Booth. 1995. Evaluation of cultural methods for establishing Wyoming big sagebrush on mined lands. P. 784-795. *In*: G.E. Schuman and G.F. Vance (ed). Decades Later: A Time for Reassessment. 12th Annual Meeting, June 3-8, 1995, Gillette, WY. American Society for Surface Mining and Reclamation, Princeton, WV.

Schuman, G.E., D.T. Booth, and J.R. Cockrell. Cultural methods for establishing Wyoming big sagebrush on mined lands. *J. Range Manage* (submitted).

Booth, D.T., J.K. Gores, G.E. Schuman, and R.A. Olsen. Shrub establishment on pre-1985 reclamation: I. Shrub densities. *J. Range Manage*. (In review).

Gores, J.K., R.A. Olson, D.T. Booth, and G.E. Schuman. Shrub establishment on pre-1985 reclamation: II. Wildlife habitat value. *J. Range Manage*. (In review).

**TOXICOLOGIC EVALUATION OF CHRONIC SELENOSIS
IN WYOMING HERBIVORES**

**M.F. RAISBECK, E.L. BELDEN, D. O'TOOLE,
J.W. WAGGONER, E.T. THORNE**

Toxicologic Evaluation of Chronic Selenosis in Wyoming Herbivores

M. F. Raisbeck, E. L. Belden, D. O'Toole,
J. W. Waggoner and E. T. Thorne

The overall objective of this project was improved understanding of chronic selenosis in economically important herbivore species in Wyoming. Permissible soil and spoil selenium concentrations in reclaimed coal minelands ultimately hinge on potential toxic effects in grazing animals. When our group initially began looking at naturally occurring chronic selenosis in 1989, the prevailing dogma was based upon early (1930-1960) reports from the experiment stations at Wyoming and South Dakota. Personal experience and anecdotal information from ranchers and veterinarians in southeastern Wyoming and the Nebraska panhandle suggested that large pieces of this dogma were inaccurate. Thus, while the experiments of this study were not undertaken to with the specific goal of establishing a magic "level", they do provide a sound experimental basis for extracting such information from soil-plant accumulation experiments, epidemiologic data in animals and the older literature.

Overview

In order to evaluate the toxicity of any substance, one must first define the target. In keeping with the objective of returning reclaimed minelands to livestock and wildlife grazing we selected calves and antelope as our experimental models. These species are: 1) among the most numerous large grazing species in coal mining areas of Wyoming; 2) lend themselves readily to experimentation; and 3) make good experimental surrogates for other large prairie herbivores. Horses were specifically excluded because of expense and the fact that, under reasonable management, aren't as completely dependent on forage for nutrition. Swine, although exceptionally susceptible to selenosis, are rather unlikely inhabitants of the post-mining environment.

Selenium occurs in a variety of chemical forms in the environment. Prior to 1991 most experimental studies utilized inorganic Se salts such as selenite, however the predominant form of Se in palatable forage plant species is as Se analogs of S amino acids, especially selenomethionine (Se-met). As noted by Olson (1977) "evaluation of the toxicity of the plant based on its total selenium hardly possible". Thus, our initial experiments compared of the toxicity of Se-met to inorganic Se salts which have been historically used in toxicology experiments. The results of these experiments were to be validated in a second species (antelope), with similar diets. Finally, the toxic endpoints determined from these studies would be used to evaluate the potential hazard of "naturally" seleniferous forages. In aggregate, these experiments would support or refute existing "toxic levels" and link ongoing and future reclamation studies to the pre-existing selenosis literature.

Steer Experiments

As outlined in previous reports, Wyoming native yearling steer calves were exposed to measured daily doses of Na_2SeO_3 or selenomethionine which approximated 5, 10 and 30 ppm

dietary selenium exposure. There were no readily evident clinical effects in any treatment group after a 120 day exposure. There were no significant clinical biochemical trends beyond a tendency to accumulate Se in various tissues; however, Se-met accumulated in tissues to a much greater degree than did Na_2SeO_3 (Fig 1). There were, however, subclinical effects. Immunologic evaluation of these calves revealed no Se-related effects in a number of *in vitro* parameters of immune competence measured (eg. lymphocyte

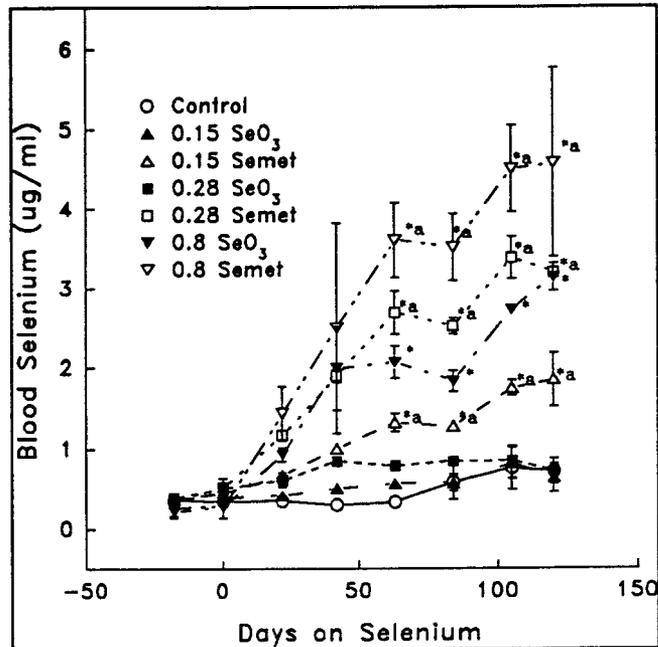


Figure 1: Whole blood Se (mean \pm standard error). "*" indicates significantly greater than controls, "a" greater than corresponding dose of SeO_3 .

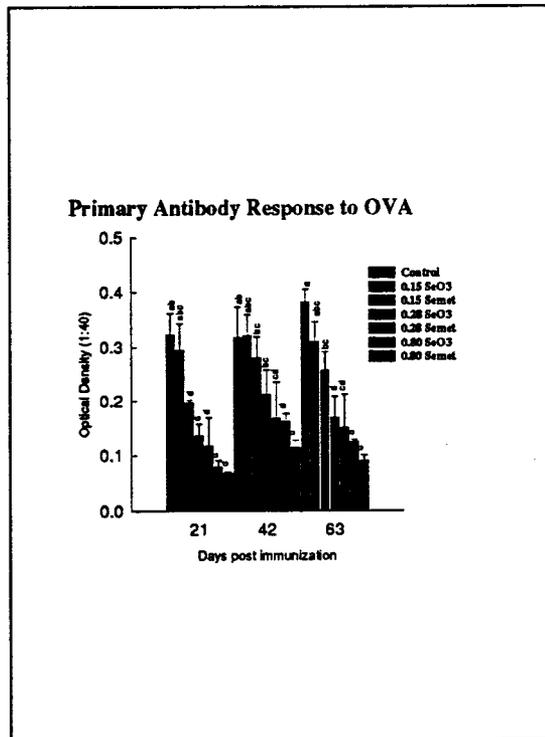


Figure 2 Primary antibody response in calves fed SeO_4 or Se-met.

blastogenesis with PHA, LPS, Con-A, total lymphocyte counts, IL3 and IL4 production, and total immunoglobulin production). There was however, a dose-related decrease in production of specific antibody to a hen egg albumin (OVA) challenge (Fig. 2). The latter result was consistent with the immunotoxic effects of selenocystine, Na_2SeO_3 , and/or Se-met in laboratory rodents (Schamber et al., 1994) and appeared to have a threshold of approximately 10 ppm dietary Se. These results are interesting for several reasons. So far as we know this is the first report of selenium-induced immunotoxicity in cattle, and one of only a handful in any species.

We have seen similar effects in cattle on a moderately seleniferous pasture which suggests

that this is not merely a laboratory curiosity, however the real-world relevance of this finding to grazing mammals under natural conditions will require more focused and extensive experimentation.

Exploratory experiments with purified Se-met and natural (hay) Se produced gross epithelial (hair and hoof) lesions similar to those described previously as "alkali disease" (O'Toole and Raisbeck, JVDI paper). Affected animals developed a mild, transient lameness after several weeks or months on seleniferous diets. Ten days later a hairline crack appears parallel to and 0.5 cm distal to the coronary band and the animal becomes noticeable lame. Hoof separation

exposure data: 1) feeding diets derived from naturally seleniferous hay; or 2) pasturing animals on reclaimed mine sites with varying forage selenium concentrations. We initially opted for the latter as it seemed more representative of the real world situation in the Powder River basin.

Five potential grazing sites were selected to represent the spectrum of Se exposure likely in the Powder River basin. Four were reclaimed pastures at active mines (A,B,C,F), the fifth (E), a ranch with a history of alkali disease, served as a positive control. The latter was necessitated by our inability to find a cooperating mine with toxic vegetation. The owner of two of the sites (C&F) dropped out of the study prior to getting underway. Twelve steers at the High Plains Grasslands Research Station (Site in Cheyenne served as a control (site D). After baseline blood samples were taken, yearling cattle were released on each of the sites. Dietary botanical composition was determined by visual observation at monthly or more frequent intervals. Representative samples of grazed vegetation were collected onto dry ice for transport to the laboratory. Forage samples were frozen at -70 C, then lyophilized, ground and homogenized before Se analysis. Blood and serum were analyzed as previously.

None of the cattle showed any evidence of health problems attributable to Se. Blood Se concentrations in cattle on all sites except E remained below 0.5 ppm, which is typical of cattle in northeastern Wyoming. Blood in group E peaked at slightly more than 2.0 ppm. Forage Se concentration tended to increase later in the growing season. The results of immunologic tests were inconclusive, apparently as a result of reagent failure. Every site had at least on forage specimen which exceeded 5 ppm, but composite samples representative of what the cattle grazed never exceeded 1 ppm on sites A and B. Composite samples from site E reached approximately 8 ppm before cattle were removed in the Fall (Fig 4). It should be noted that the summer of 1995 was extremely wet, and forage conditions were unusually lush on all sites.

Final feeding study

After failing to generate a usable range of dietary concentrations, tissue concentrations and health effects markers from the pasture experiments of 1995, we undertook a final feeding

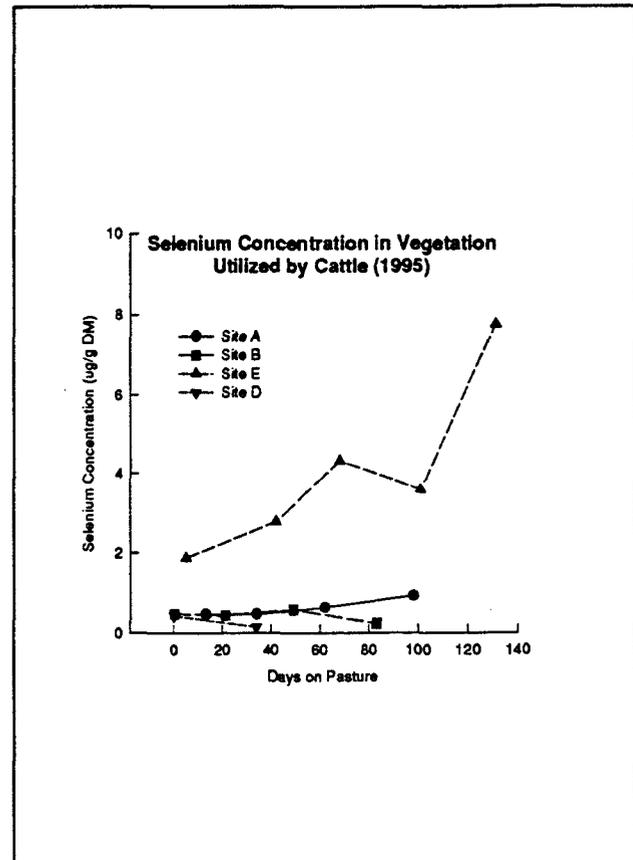


Figure 4 Dietary Se concentration. Each point represents an average concentration derived from observed dietary botanical composition.

experiment with seleniferous hay in steer calves. Twelve yearling steers were obtained from the Wyoming Hereford Fund herd, divided into 3 groups and fed a ground hay ration prepared from seleniferous native grass hay and containing either 3.33, 5 or 6.66 ppm Se. The diets were analyzed and balanced with corn and cottonseed meal to be isocaloric and isonitrogenous. Blood Se concentrations were significantly different between groups, plateaued at 0.5, 0.8 and 1.4 ppm in the low, mid and high Se groups respectively. There were no significant differences in rate of gain between groups, nor did any of the steers exhibit any clinical evidence of disease during the 6 months they were on seleniferous hay.

Publications/technology transfer

Research funded all or in part by the AMLRP have resulted in one Master's thesis, seven refereed publications with another two in preparation and numerous presentations at regional, national or international meetings exclusive of the AMLRP.

Peer-reviewed publications

D. O'Toole, L. Castle, M. F. Raisbeck (1995): Comparison of histochemical autometallography (Danscher stain) to chemical analysis for selenium detection in tissues. *J Vet Diag Invest* 7:281-284.

D. O'Toole and M. F. Raisbeck (1995): Pathology of experimentally-induced chronic selenosis ("alkali disease") in yearling cattle. *J Vet Lab Diag Invest* 7:364-373.

M. F. Raisbeck, D. G. Steward, L. E. Vicklund, G. F. Vance, L. K. Spackman, and J. G. Luther (1995): Selenium and mining in the Powder River Basin, Wyoming: Phase III - Tissue concentrations of selenium in deer mice (*Peromyscus maniculatus*). pp 354-359 in *Decades Later: A Time for Reassessment* (eds. Schuman and Vance) Am Soc Surf Mining Reclam, Princeton, WV.

M. F. Raisbeck, D. T. O'Toole, D. A. Sanchez, R. L. Simieon and J. W. Waggoner (1995): Re-evaluation of selenium toxicity in grazing mammals. pp. 372-383 in *Decades Later: A Time for Reassessment* (eds. Schuman and Vance) Am Soc Surf Mining Reclam, Princeton, WV.

R. A. Schamber, E. L. Belden and M. F. Raisbeck (1995): Selenium immunotoxicity. pp. 384-393 in *Decades Later: A Time for Reassessment* (eds. Schuman and Vance) Am Soc Surf Mining Reclam, Princeton, WV.

M. F. Raisbeck, D. O'Toole, R. A. Schamber, E. L. Belden, L. J. Robinson (1996): Toxicologic effects of a high-selenium hay diet in captive adult and yearling pronghorn antelope (*Antilocapra americana*). *J Wildl Dis* 32:9-16.

D. O'Toole, M. F. Raisbeck, J. Case and T. Whitson (1996): Selenium-induced "Blind Stagers" and related myths. A commentary on the extent of historical livestock losses attributed to selenosis on western US rangelands. *Vet Pathol*, 33:104-116.

and lameness worsen until, by approximately 120 days the animal is unable or unwilling to walk to food or water. Histopathologically, the juncture between new hoof growth and old is characterized by acanthosis and hyperplasia of the lamellar epithelium. Dyskeratotic lesions were also seen in hair follicles of the mane and tail. Even though none of the experimental steers exhibited lameness, and all appeared to be healthy, one high dose Na_2SeO_3 steer, 3 high and one mid-dose Se-met steers had similar, if much milder lesions in hooves and hair follicles. Again, 10-15 ppm appeared to be a dietary threshold for measurable damage.

Antelope

We were unable to obtain sufficient purified Se-met for the proposed antelope study. We therefore substituted seleniferous native grass hay as our source of Se in the experimental diets. Captive-raised antelope (4 adults, 5 yearlings) were maintained on a diet of 40% alfalfa, 60% high selenium grass hay (15 ppm total dietary Se as fed) for 6 months after a 30 day acclimation period. Low Se native grass hay was similarly ground with alfalfa and fed as a control diet. Although the antelope initially ate the ground grass/alfalfa diet readily, feed refusal of both the control and especially the experimental diets was a recurring problem from day 30 until the end of the study. We thus can't say for certain whether poor weight gain seen in these animals was directly attributable to Se or an indirect result of poor palatability and feed aversion (Fig 3).

Due to the small number of animals and the loss of a control animal to an iatrogenic infection, we didn't try statistical analysis. Clinical pathology and biochemistry did not reveal any obvious differences between the groups, nor deviations from historical normals. Blood Se concentrations were similar to the bovine 0.28 mg/kg Se-met group. Histopathologic evaluation of tissues, including a detailed examination of hooves did not reveal any evidence of Se-induced effects. There were no consistent trends in the *in vitro* immunocompetence assays associated with the high Se diet. It should be noted that the intradose variability in the latter parameters was large, possibly due to logistics problems with collecting and transporting samples and the excitable nature of the species. Nevertheless, the primary antibody response to OVA was less in animals on the high Se hay diet than in controls.

Grazing

The final phase of this project was to be validation of experimental data with natural exposure. There are two general methods by which to develop natural

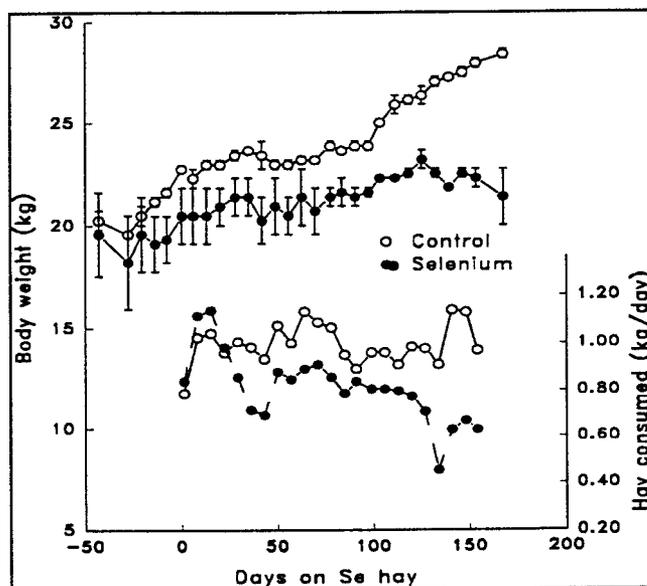


Figure 3: Feed consumption and body weight in antelope fawns on high Se hay. We believe that the lesser feed consumption in the Se hay group accounts for the smaller gain seen.

D. O'Toole and M. F. Raisbeck (submitted 8/9/96): Experimentally-induced selenosis of adult mallard ducks: clinical signs, lesions and toxicology. *Vet Pathol*.

M. F. Raisbeck, D. O'Toole and Belden EL: Experimental chronic selenosis in cattle. in preparation for *Tox Appl Pharm*.

Thesis, other research publications

R. Schamber: *Selenium immunotoxicity*. MS thesis, UW Dept Vet Sci, 1994.

Castle LE, Raisbeck MF, O'Toole D: Comparative analysis of rat tissue selenium concentration by chemistry and histochemistry. *McNair Scholar's Journal*, July, 1994.

M. F. Raisbeck, D. O'Toole, E. L. Belden, D. A. Sanchez and R. A. Siemion (1993): Selenium vs. Sulfur: A comparison of toxic effects in mammals on western rangelands. pp 139-152 in *Planning, Rehabilitation and Treatment of Disturbed Lands*, Montana State University Reclamation Research Unit Publ. #9301, pp. 139-151.

P. K. Carroll, J. G. Luther, M. F. Raisbeck, L. K. Spackman, D. G. Steward, G. F. Vance and L. E. Vicklund (1993): Selenium and mining in the Powder River Basin. pp 160-176 *Planning, Rehabilitation and Treatment of Disturbed Lands*, Montana State University Reclamation Research Unit Publ. #9301, pp. 160-175.

M. F. Raisbeck, D. O'Toole, K. Orsted (in prep): Chronic selenosis in food animals and horses. chapter in *Current Veterinary Therapy*.

Invited presentations

Raisbeck MF, O'Toole D, Belden EL, Sanchez DA, Siemion RA: Progress in selenium research. *Planning, Rehabilitation and Treatment of Disturbed Lands, 6th Billings Symposium*, Billings, MT 3/24/93.

Raisbeck MF, O'Toole D, Belden EL, Sanchez DA, Siemion RA: Selenium vs. sulfur: A comparison of effects in range mammals. *Planning, Rehabilitation and Treatment of Disturbed Lands, 6th Billings Symposium*, Billings, MT 3/25/93.

Raisbeck MF: Selenium toxicity. 3 hr presentation on "*Advances in Science and Medicine*", Am Vet Med Assn, San Francisco, CA 7/12/94.

Raisbeck MF, O'Toole D, Sanchez DA, Siemion RL: Re-evaluation of Selenium Toxicity in Grazing Mammals. ASSMR, Gillette, WY June, 1995.

Schamber RA, Belden EL, Raisbeck MF: Selenium immunotoxicity. ASSMR, Gillette, WY June, 1995.

Presentations

Castle LE, Raisbeck MF, O'Toole D: Comparative analysis of rat tissue selenium concentration by chemistry and histochemistry. UW McNair Scholar's Program, Laramie, WY, 7/28/93.

O'Toole D, Raisbeck MF: Danscher staining in tissue from rats exposed to oral selenium: *Caveat tinctor!* Am Assn Vet Lab Diagn, Las Vegas, NV 10/26/93.

Schamber R, Belden EL, Raisbeck MF: Selenium immunotoxicity. Rocky Mountain ASM Spring 1994 meeting, Boulder, CO. (Awarded best student presentation).

Raisbeck MF, O'Toole D, and Belden EL: Comparative toxicology of selenite and selenomethionine in herbivores. Am Assn Vet Lab Diagn, Las Vegas, NV 10/26/93.

O'Toole D, Raisbeck MF: Comparative pathology of selenite and selenomethionine. Am Assn Vet Lab Diagn, Las Vegas, NV 10/26/93.

M. F. Raisbeck, D. O'Toole, E. L. Belden, D. A. Sanchez and R. A. Siemion (1993): Selenium vs. Sulfur: A comparison of toxic effects in mammals on western rangelands. pp 139-152 in *Planning, Rehabilitation and Treatment of Disturbed Lands*, Montana State University Reclamation Research Unit Publ. #9301, pp. 139-151.

P. K. Carroll, J. G. Luther, M. F. Raisbeck, L. K. Spackman, D. G. Steward, G. F. Vance and L. E. Vicklund (1993): Selenium and mining in the Powder River Basin. pp 160-176 *Planning, Rehabilitation and Treatment of Disturbed Lands*, Montana State University Reclamation Research Unit Publ. #9301, pp. 160-175.

**SPECIFICATION AND RECOMMENDATIONS FOR REPAIR
OF RESIDENTIAL STRUCTURES DAMAGED BY
GROUND MOVEMENTS RELATED TO MINE SUBSIDENCE**

K.D. BASHAM, T.R. CARTER, and W.L. JOHNSON

**THE INFLUENCE OF POST-HARVEST
AND PREPLANTING SEED TREATMENT ON
SAGEBRUSH SEEDLING VIGOR**

D.T. BOOTH, E.E. ROOS, and Y. BAI

The Influence of Post-harvest And Pre-planting Seed Treatment on Sagebrush Seedling Vigor

D.T. Booth, E.E. Roos, and Y. Bai

Introduction

Returning native shrubs to the post-mining plant community remains a challenge for western reclamationists. Since Wyoming big sagebrush is commonly recommended for revegetation of mined lands, and since there is a need to improve sagebrush seeding success, this project was initiated in the fall of 1993 to measure factors affecting sagebrush seed quality, including the effect of post-harvest seed handling and the effect of pre-sowing seed moisture manipulation to improve seedling vigor. The research is a cooperative effort by scientists of the High Plains Grasslands Research Station, Cheyenne, WY (ARS); the National Seed Storage Laboratory, Fort Collins, CO (ARS); the University of Wyoming, Laramie, WY; Wind River Seed Co., Manderson, WY; and the Dave Johnston Coal Mine, Glenrock, WY.

Progress Report - Completed Studies

1. Pre-dispersal seed moisture. Seeds from several locations were collected in late winter. The moisture content and seed weight were determined, and seed germination and seedling vigor were tested. We found that seed moisture ranged from 2.3 to 9.0% and seed weights ranged from 0.022 to 0.032 g/100 seeds. Germination percentages were highest, and seeds germinated most rapidly for collections which had the greater seed weights.

2. Post-harvest seed handling. Sagebrush seeds processed by a 48" commercial debarker were tested to determine the process effects on seed quality. Processing through a debarker did not reduced seed quality and is useful in preparing seeds for market.

3. Seed humidification. Seed humidification refers to holding seed in a high humidity environment. We tested this treatment at 4 temperatures (2, 5, 10 and 15°C) through 15 days and for 2 seed collections. We found that sagebrush seeds hydrate most gradually at 2°C, and most rapidly and to the greatest extent at 10°C. These differences did not appear to influence germination or seedling vigor in laboratory tests.

4. Effect of humidification and pericarp on germination under water stress. We evaluated the effect of water stress on germination in combination with the effect of pericarp removal. Seeds with and without pericarp were hand separated from processed seed and used to measure the rate of moisture uptake, effect of humidification, germination and germination rate under water stress. Water stress was applied using the matric potential control system of Hardegree and Emmerich. Seven water potentials ranging from 0 to -1.5 MPa were used in the test. We found that the greatest number of seeds germinated when there was no water stress (0.00 MPa). Also, that the pericarp reduces water uptake; pericarp removal increased the number of seeds germinating between -0.50 and -1.00 MPa. However, we suggest the pericarp is also important in retaining viable seeds in the soil until moisture conditions are favorable. For that reason we do not recommend increasing the number of naked seeds in a sagebrush seed lot beyond what commonly results from current

seed processing methods.

Progress Report - Ongoing Field Study

Sagebrush seed production. This study addresses the following questions: (1) How does sagebrush seed production (quantity and quality) from reclaimed coal mined lands compare with production from adjacent native communities? (2) Can cultural methods be used to increase the quantity and quality of sagebrush seed produced on mined lands or in native stands? The study was installed at the Dave Johnson Coal Mine in July of 1995.

Given the unusually high spring precipitation received in Wyoming in 1995, we were not expecting differences due to treatments for the 1995 harvest and there were none. There were also no differences in seed quality between reclaimed and native sites. Seed germination ranged from 0 to 80%, with most lots germinating around 50 - 60%. Time to 50% germination ranged from 1 to 3.3 days with most lots averaging about 2.3 days.

Future Plans

We have completed the proposed work on post-harvest processing, seed humidification, imbibition and its interaction with time and temperature. What remains is the field work, as amended. We will seek to extend the project to allow us to evaluate seed crops from the plots at the Dave Johnston Coal Mine for 2 - 3 more years.

Other Significant Information

The monies we received from this grant provided the greatest portion of support for Dr. Y. Bai's position. That position has contributed to our obtaining additional funds from the Saskatchewan Agricultural Development Fund and Ducks Unlimited of Canada to conduct cooperative work with the University of Saskatchewan on winterfat seedbed ecology. Since winterfat is a shrub commonly used in revegetation of Wyoming coal mined lands we have listed publications from that project with those from the sagebrush work.

Publications

Published:

Booth, D.T., Y. Bai, and E.E. Roos. 1995. Wyoming big sagebrush seed quality as influenced by processing with an industrial debarker. Abstracts 1995 Meeting, American Society for Surface Mining and Reclamation.

Booth, D.T., Y. Bai, and E.E. Roos. 1995. Wyoming big sagebrush seed quality related to debarker operation during seed cleaning and storage. pp. 8. IN: Fifth International Rangeland Congress Abstracts. Dept. of Rangeland Resources, Utah State Univ., Logan.

Bai, Y., D.T. Booth, E.E. Roos. 1995. Influences of initial seed moisture and humidification on seed germination of Wyoming big sagebrush. pp 4. IN: Fifth International Rangeland

Congress Abstracts. Dept. of Rangeland Resources, Utah State Univ., Logan.

Booth, D.T., Y. Bai, and E.E. Roos. 1995. Wyoming big sagebrush seed quality as influenced by processing with and industrial debearder. p. 858. IN:Proceedings of the 12th Annual National Meeting of the American Society for Surface Mining and Reclamation. G.E. Schuman and G.F. Vance (eds).
Abstract. ASSMR, Princeton, WV.

Accepted:

Booth, D.T., Y. Bai, and E.E. Roos. Preparing sagebrush seed for market: effects of debearder processing. J. Range Manage.

Bai, Y., D.T. Booth, E.E. Roos. Effect of seed moisture on Wyoming big sagebrush seed quality. J. Range Manage.

Submitted:

Bai, Y., D.T. Booth and J.T. Romo. Developmental stages of winterfat related to survival after freezing. J. Range Manage.

Bai, Y., D.T. Booth, and J.T. Romo. Low temperature exotherm did not mark the death of hydrated winterfat (*Eurotia lanata* (Pursh) Moq.) Seeds. Amer. J. Botany. .

In Preparation:

Bai, Y., S.P. Hardegree, D.T. Booth, and E.E. Roos. 1996. Effect of pericarp removal on seed germination of Wyoming big sagebrush under water stress. J. Range Manage.

Bai, Y., D.T. Booth, and J.T. Romo. Freezing tolerance in winterfat (*Eurotia lanata* (Pursh) Moq.) seeds related to hydration temperature. Amer. J. Botany.

**HANDBOOK OF RECLAMATION TECHNIQUES FOR THE
WESTERN UNITED STATES WITH FIELD GUIDE**

**F. FERRIS, M. STEWARD, R. STOWE, L. VICKLUND
L. KLEINMAN**



**AMAX COAL
WEST, INC.**
A Cyprus Amax Company

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October 31, 1996

Mr. William A. Gern
Vice President for Research
University of Wyoming
P.O. Box 3355
Laramie, WY 82071-3355

Dear Mr. Gern:

Enclosed is the final summary of the "Handbook of Western Reclamation Techniques."
Bob Stowe and I will be at the seminar November 6 to present the final handbook.

Thank you for your support of this project.

Sincerely,

Laurel E. Vicklund
Environmental Engineer

HANDBOOK OF WESTERN RECLAMATION TECHNIQUES
F. K. Ferris, L.H. Kleinman, D.G. Steward, R. R. Stowe, L.E. Vicklund

Introduction

Disturbance from surface coal mining in the Powder River Basin of Wyoming results in hundreds of acres being reclaimed each year. A reclamationist from one of these mines realized that much of the reclamation work occurring was on the forefront of this art. The people who are responsible for the reclamation activities in this area represent years of experience. Methods have been defined, improved and evolved into successful techniques and could not be found in any textbook or journal.

Objective

The idea was conceived to put together some of these reclamation techniques in a handbook. A committee comprised of people involved in the various disciplines of reclamation was formed. The intent of creating a handbook was to present, in an accessible format, economical and successful reclamation techniques that have survived the test of practical application so that others could benefit from these efforts. An important issue during the planning stage of the handbook was that only the techniques in place and functioning at the time of writing would be included.

Results

Nine people edited the resulting 260-page Handbook. Contributions were received from twenty-three authors writing about 97 different techniques in seven different categories. Funds from the Wyoming AML program, in-kind contributions from eight mining companies, four consulting firms, one lab, one vendor, and one university made the Handbook a reality.

The Handbook is divided into the disciplines of hydrology, vegetation, topsoil, topography, wildlife, postmining land use and drilling. Hopefully, the information in this Handbook may be used not only in mined land reclamation, but for reconstruction of endangered habitats, revitalization of damaged environmental systems, and establishment of wetlands. These reclamation techniques may be used to minimize the impact of human development in housing subdivisions, on ski slopes, oil and gas field construction, and in highway construction.

**ESSENTIAL HYDROLOGIC FUNCTIONS OF PLAYAS IN
THE POWDER RIVER BASIN OF NORTHEASTERN WYOMING**

**V.R. HASFURTHER, J.B. BROUGH, G. KERR,
C. BOWMAN, L.C. MUNN, AND M.A. SMITH**

**Abandoned Coal Mine Land Research Program
Final Report**

**Essential Hydrologic Functions of Playas in
the Powder River Basin of Northeastern
Wyoming**

**Victor R. Hasfurther, James Brough, Greg Kerr and Caitlin
Bowman**

**Department of Civil and Architectural Engineering
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**Department of Plant, Soil and Insect Sciences
University of Wyoming**

Michael A. Smith and Everett Bartz

**Department of Range Management
University of Wyoming**

October 31, 1996

INTRODUCTION

This research has as its primary purpose to characterize the unique hydrologic functions of playas within the Powder River Basin of northeastern Wyoming by defining the interrelationships which exist between soils, vegetation and water and how these interrelationships will be important in the restoration of playa areas once they are drastically disturbed by mining activities.

METHODOLOGY USED

Four mining companies participated (physically and monetarily) in the project and allowed the project team access to playas on their properties for intensive study. They are: AMAX Coal West, Inc. (Belle Ayr Mine); Kerr McGee Corporation (Jacobs Ranch Mine); Atlantic Richfield Company (Black Thunder Mine) and Powder River Coal Company (North Antelope and Rochelle Mines).

Field investigations began in June 1994 and have continued through August of 1996. The field investigations were separated into two investigations. The first investigation consists of a reconnaissance level survey of over 50 playas within the Eastern Powder River Basin. The field investigations and sampling for these 50 plus playas were completed in early August 1995. The second investigation involved an intensive study of seven individual playas on the five different mine sites indicated above. The Belle Ayr site has three different playas that were being intensively studied. Installation of necessary wells, water level monitoring equipment, lysimeters and climatic equipment occurred on all playas between October 1994 and May of 1996 and were monitored until August 1996.

Soils

Reconnaissance level soils work for the 50 plus reconnaissance level playas resulted in soil sampling at depths of 0-6 and 6-12 inches in each of the vegetation zones identified for each of the 50 plus playas. These soil samples underwent analysis for color, organic content, texture, electrical conductivity, particle size distribution, some Atterberg limits and pH. Over 500 soil samples were collected. The intensively studied playa soil samples were collected from each soil horizon identified in the field during trench excavations within each of the vegetation zones of the playas and at other points where it was determined necessary. Trench depths were generally between 5 and 8 feet for a length of 5 to 10 feet. These soil samples were analyzed for the same things as indicated for the reconnaissance level playas plus soluble salts, reduced iron and some other metals. From the trench excavations, horizons that are present and their corresponding thicknesses were obtained, as well as, information on surface horizon textural class and dominate subsoil textural class.

The field investigations indicated that playa formation was most probably due to settlement as a result of physical changes in the coal beds (burning, etc) followed by surface runoff and weathering of parent material to form the fine sediment deposits in the low areas of the playas except perhaps for playas in the Teckla Southwest quadrangle area which could be wind erosion related. The soil material available for deposition seems to vary within the entire study area from the analysis of the reconnaissance level playas giving rise to different groupings of the playas within the study area.

Vegetation

The reconnaissance level playas were mapped by identifying the transition zones between different vegetation communities that occurred on each playa and identifying the boundary between upland areas and the playa basin. The transition zones and playa/upland boundaries were staked, and the vegetation communities defined by the three (or four in some instances) most dominant vegetation species that occurred within that zone. A topographic survey of the playas was done making sure to define the boundaries of each of the vegetation zones.

Vegetation zone classification of the reconnaissance level playas was accomplished on the basis of major groups. The major vegetation groups were artemesia, bromus, agropyron, opuntia, rumex, thistle-like plants, taraxacum, lupinus, plantago, mustards, legumes, poa, carex, bouteloua, miscellaneous forbs, bare ground and other. An estimate of the percentage of each group present in the zone was made along with the approximate total cover by grasses. Results of statistical analysis between soils and vegetation show that the soil parameters conductivity, percent clay, and pH do not correlate to the groups of plants present. There was only slight correlations with other parameters. The analyses showed that there was no significant difference in the plant communities between playas except for Taraxacum but there was significant differences between the zones within each playa. It therefore appears that the variance among zones within playas of the vegetation occurs in all cases except for thistle-like plants. This variance among zones is a direct result of standing water duration within each playa.

Vegetation zones were identified for each of the intensively studied playas in a manner similar to the reconnaissance level playas. Then detailed vegetation sampling was performed on each of the seven playas during the summer of 1995. The sampling pattern (radial lines from the center of the playa) used determines the types and amounts of vegetation present in each visibly distinct upland and playa vegetation zone on each of the seven playas. Eighteen different species of plants were identified in the seven playas. Cover and composition data were developed for the top five species in each of the seven playas. Again, the variance in plant species was due to standing water duration within the playa.

Water

From survey data collected on each playa, the playa's length, width, and depth were evaluated and the shape plotted. From this information, the development of geomorphic properties for each of the playas was completed. A relationship between the playa size and configuration and the water quantity which could potentially be stored within the playa throughout the year was produced.

Soil moisture lysimeters were used to collect water samples in the unsaturated soil zone. When a volume sufficient to analyze for all the parameters listed in WDEQ/LQD's Guideline 8 - Hydrology, Appendix 1 - Water Quality Sampling was obtained, all important analyses were performed. When a sufficient volume sample was not obtained, only the major anions and cations (bicarbonate, carbonate, chloride, sulfide, iron, magnesium, potassium and sodium) were analyzed.

The data collected from water level measurements, climate data and lysimeters was used for a water balance analysis. The results of this analysis indicate that most of the stored water in the playas does not reach the shallow aquifers and that the amount of infiltration between

playas is not equal.

SUMMARY

Playas in the Eastern Powder River Basin are formed mainly due to settlement. As a result, the soils within the playas are the same as those in the surrounding area. The soils indicate that the landscape is relatively "stable" in geologic terms. The soils at and near the surface in the low lying areas of the playas are derived from the local shale beds through hydrological transport processes and some wind erosion. The vegetation within a playa was found to be a function of the amount and duration of standing water during the year within the playa area causing the resulting vegetation zones. Eighteen different plant species were found and the dominant plants in each zone were identified. No exotic plants were identified.

Effective restoration of the playas should not be difficult since the materials within the playas come from the parent soil materials. No special handling of the soils is indicated. To accelerate the process, a thick clay seal (from the local soil materials) could be placed in the low area of the depression in proportion to the area of the catchment. Vegetation consistent with the plant species found is needed to establish stability of the soils and transpire water.

The project will have produced three theses when all are complete. One thesis has been completed by Mr. James Brough entitled "Essential Hydrological Functions of Playas in the Powder River Basin of Wyoming".

Two others will be completed during this academic year (FY1996-1997) by Caitlin Bowman and Everett Bartz.

**DETERMINATION OF CONTRIBUTION TO CUMULATIVE
GROUNDWATER IMPACTS FROM COALBED METHANE
DEVELOPMENT AND SURFACE COAL MINING**

**L. BORGMAN, J. KERN, K. PEACOCK, M. BROGAN, J. MEYER
T. DOBSON, R. SHAFER**

ABANDONED COAL MINE LANDS RESEARCH PROGRAM

Semi-annual Progress Report

October 31, 1996

Determination of Contribution to Cumulative Groundwater Impacts From Coalbed Methane Development and Surface Coal Mining

Leon E. Borgman¹

John W. Kern²

Kenneth Peacock³

Michael Brogan³

Joseph Meyer³

Michael Brenneis¹

Trey Dobson¹

Concerns over the cumulative effects of coalbed methane and surface coal mining on water quantity have increased in recent years. As surface coal mines must satisfy certain constraints imposed by the Surface Mining Control and Reclamation Act (SMCRA), the question of determination of responsibility for aquifer impacts is a critical issue. This project was funded for the purpose of developing methods to make such determinations. The 3 primary objectives for the project are to

- *Investigate the existence of anisotropy of hydraulic conductivity in the coal aquifer for the purpose of evaluating modeling methods used by Peacock and Kern (1995), and to verify the presence or absence of inter-aquifer communication at these test sites.*
- *Develop methods to differentiate groundwater changes due to surface coal mining activities from those associated with non-mining related aquifer stresses.*
- *Acquire and assess all available data from Permit to Mine Applications using all available means for the four active mines nearest the Marquiss CBM development.*

Current Progress:

Significant progress has been achieved toward objectives one and three. Four multiple well pump tests have been conducted to date with analyses completed. Further, the authors identified a lack of statistical methods available for quantifying the precision of estimates of the ratio and orientation of the principal transmissivity. We derived variance formulas for the ratio and angle of orientation for the principal axes of transmissivity (Kern and Dobson 1996) allowing development of statistical tests of isotropy against the alternative of anisotropy. Methods were also developed for construction of confidence intervals on the ratio and angle of orientation. For accurately identifying sources of impacts, anisotropy may be a significant factor. These methods were applied to 3 of the 4 aquifer tests with results presented in Table 1. The fourth

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pump test did not have sufficient monitoring wells to allow variance estimates. A detailed account of the pump test design and analysis is given in Dobson and Kern 1996.

Various controlling factors have been proposed for the anisotropy observed in the Wyodak Anderson Coal, including formative cleat sets, or alternatively tectonic fracture networks. To investigate this question, fractures and lineaments identified by Denson (1980) were digitized and the mean fracture orientation was calculated. It was found that the first 3 aquifer tests resulted in a mean orientation of the principal transmissivity of N43°W, while the mean lineament and fracture orientation was found to be N36°W. This consistency provides some evidence in favor of the tectonic theories. In contrast, the fourth test which had significantly larger well spacings and longer duration resulted in a principal axis oriented N108°W, nearly 90° off the mean orientation of the other 3 tests. It is not currently known whether this represents spatial variability in the principal axes or if short duration small scale tests tend to be influenced by local lineaments and faults. To investigate this issue a fifth test is currently under way in which observation wells will be simultaneously monitored at long and short well spacings. For the modeling effort, lineaments and faults will be investigated through inverse modeling to determine if aquifer compartmentalization should be incorporated into the hydrogeologic design.

Progress on objective 3 has included review and commitment of aquifer test data to the CPR data base for permanent storage and retrieval. Substantial progress has been made in efforts to enter geologic data from well logs into an electronic data base, followed by geostatistical modeling of the coal isopac and estimation of the extent of partings in the Wyodak Anderson coal. This effort is nearing completion. Other data sources gathered include mine permit water level monitoring, coalbed methane water production and BLM monitoring data.

Continuing Work:

Future direction of the project will include implementation of a proposed modeling framework in which

- a conceptual hydrogeologic model is developed,
- aquifer parameters and statistical distributions are estimated from data,
- parameter estimates and hydrogeology are refined through inverse modeling, and
- forward modeling is conducted to predict impacts spatially and temporally

Allocation of individual contribution to impacts will be based on a validated model containing all potential stressors. This model will provide estimates of total impacts at any point in time and space. To determine individual contributions;

- The model will be rerun once for each potential stressor not included in the model.
- Impacts associated with that stressor are given by the difference between total impacts and those observed with that stressor excluded from the model
- Uncertainty in predicted impacts are assessed through stochastic modeling.

Table 1. Results from 4 multiple well pump tests conducted in the Powder River Basin in Wyoming.

Test Site	Total Wells	Wells Pumped	Orientation of Principal Axis and 95% Interval	Ratio of Anisotropy and 95% Interval	Overburden Drawdown	Q and Total Time
Powder River Coal PRCC-4	3	3	N27°W (N1°W: N52°W)	2.26 (1:3.66)	None	2700 ft ³ /day 365 min
Powder River Coal PRCC-8	3	3	N50°W (N : S)	2.15 (1:4.45)	None	2900 ft ³ /day 300 min
Belle Ayr Mine	5	1	N52°W (N47°W: N56°W)	2.1 (1.64:2.60)	None	2500 ft ³ /day 240 min
BLM Closed In Test #1	4	1	N108°W NA	5.7-9.5 NA	NA	5005 ft ³ /day 6060min
BLM Closed In Test #2	NA	NA	NA	NA	NA	NA

**CHRONIC SELENOSIS IN WATERFOWL: DEVELOPMENT OF
DIAGNOSTIC HISTOLOGICAL AND TOXICOLOGICAL
CRITERIA AS AN AID FOR RECOGNITION AND CONTROL**

D. O'TOOLE AND M. RAISBECK

Chronic selenosis in waterfowl; development of diagnostic and toxicological criteria as an aid for recognition and control

Donal O' Toole MVB PhD MRCVS MRCPATH and Merl F. Raisbeck DVM PhD The Wyoming State Veterinary Laboratory, Department of Veterinary Sciences, University of Wyoming, #1174 Snowy Range Road, Laramie, WY 82070.

Final semi-annual report on WY DEQ AML Report on Project UW #5/39528. Project Period: 05/10/95 - 05/30/96.
Submitted October 31, 1996

Abandoned Coal Mine Lands Research Program, Office of Research, University of Wyoming, P. O. Box 3355, Room 305 Old Main, University of Wyoming, Laramie, WY 82071-3355

Purpose: To develop corroborative clinical, morphological and toxicological criteria for the diagnosis of selenosis in adult waterfowl.

Rationale: Bioaccumulation of selenium from natural sources occurs in wetlands of the western United States and is most often attributed to run-off from agricultural irrigation. Declines and die-offs of waterfowl populations are attributed to selenosis in at least three states (WY, UT, CA) and are suspected to occur in another four (CO, NM, NV, MT). Wildlife biologists currently rely on chemical analysis of tissues from waterfowl and fish as the primary technique to establish whether mortalities are attributable to selenium poisoning. The costs for remediation of selenium contaminated sites are substantial, and carry significant implications for irrigators and coal companies, as well as state and federal agencies. One aspect of research into spontaneous and experimentally-induced selenosis of waterfowl is the heavy emphasis on contamination and toxicity thresholds. Thresholds for selenosis in waterfowl expressed as wet weights include > 3 ug/g in eggs (reproductive impairment), > 9 ug/g in eggs (terata), > 10 ug/g in liver (adverse sublethal effects), and > 20 ug/g in liver (mortality in young and adult birds)(Heinz, G., Selenium in birds *In: Environmental Contaminants in Wildlife*, eds. Beyer, W.N., Heinz, G.H., Redmon-Norwood, A. W., pp. 447 - 458. CRC Press 1996). Detailed morphological studies of deformed, sick and dead birds are not part of the investigative protocol of Fish and Wildlife Service (USFWS) personnel working at contaminated sites. Few detailed morphological studies of avian selenosis have been published. This is due in large part because wildlife biology has a tradition of underusing histopathology in disease investigations, and because from a regulatory standpoint numerical thresholds are more attractive. Various lesions are described in integument, liver, lungs, heart, pancreas and body cavities. We were interested in performing a collaborative toxicologic-clinicopathologic study on avian selenosis because the present preoccupation with selenium tissue concentrations may not always correctly identify selenium as the cause of problems for the following reasons:-

- Avian selenosis was thought likely to occur at several selenium-contaminated sites on the basis of contamination and toxicity thresholds, yet biological studies at these sites do not clearly identify a selenosis-like syndrome.
- Avian species vary in their sensitivity to the toxic effects of selenium in the diet, and some species - particularly marine birds - accumulate high tissue concentrations without adverse effects. There is also some evidence that experimentally-exposed avian species (domestic chickens, mallards, quail, screech-owls and black-crowned night herons) vary somewhat in their sensitivity to excess dietary selenium.
- By analogy with the history of field investigations into spontaneous diseases of herbivores that were initially attributed to selenosis, avian risk thresholds at the low end of the toxicity range may be misleading when concurrent morphologic and microbiological studies are not performed routinely.
- Confounding factors for the use of these thresholds in field situations are interactions between selenium and other environmental contaminants may increase or decrease the likelihood of selenosis. A complex range of factors influences the bioavailability and toxicity of selenium, including the chemical form of selenium (organic, inorganic), the nature of the aquatic environment (pH, salinity, hardness), soil characteristics (clay content and texture, redox potential, organic content), and the presence of other chemical constituents (sulfate, mercury, lead, cadmium, silver, arsenic and copper).
- Low concentrations of some dietary constituents in some feeds (eg, cyanogenic glycosides) protect birds from

the toxic effects of excess dietary selenium, yet tissue selenium concentrations remain abnormally high.

- Diseases of waterfowl that are likely in arid parts of the western United States, such as poisoning by sodium and magnesium salts, may be incorrectly attributed to selenosis when analytical chemistry is the principal diagnostic approach.
- Laboratory analysis of selenium is difficult, particularly when graphite furnace atomic absorption spectrophotometry - the method of choice for USFWS studies - is used. There are reported interlaboratory differences of 7 fold as well as absolute errors of -100% and +12,158% in samples containing low concentrations of selenium (see Final Report to the United States Bureau of Reclamation (1995), *Monitoring selenium, nickel and chromium in agricultural commodities of the Western San Joaquin Valley 1984* and Bureau (1985), Laboratory performance for selenium analysis of reference water samples. *Preliminary report to Central Regional Water Quality Control Board*, Dept. of Land, Air and Water Resour., Univ. of Calif., Davis, CA).
- It is widely assumed that the teratogenic effects of selenosis in birds (beak and skeletal abnormalities, microphthalmia, exencephaly, abnormal plumage) represent a highly specific syndrome that is essentially diagnostic for selenium. Unfortunately, this spectrum of abnormalities can be reproduced by other dietary deficiencies or excesses (riboflavin, nicotinic acid and biotin), physical insults (environmental hyperthermia), viral infections (Bunyaviridae) and inherited diseases.
- The cut-off used by some USFWS personnel as an index for background rate of spontaneous overt terata in waterfowl at uncontaminated wetlands is <0.2% (Dr J Skorupa, *pers. comm*). The finding of more than 2 avian embryos per 1,000 with overt terata is the threshold that some regulatory agencies consider as evidence of exposure to a significant environmental teratogen. This cut-off is lower than the expected rate of background malformations in chicken embryos (<5%) or mammalian species (eg., rat <1.9%).

Experimental design: Two studies were undertaken between June 1995 and January 1996. In experiment 1, 48 flighting male mallards (*Anas platyrhynchos*) from a commercial source were housed individually and fed a proprietary waterfowl ration that was either unsupplemented ($n = 12$ controls) or else supplemented with selenium as seleno-L-methionine (10, 25 and 60 ppm Se; $n = 12$ /group). Birds were scheduled to be killed after 50, 100 and 150 - 152 days of dietary exposure. In experiment 2, 12 adult male mallards were offered either unspiked feed ($n = 3$) or else a morning ration of spiked (120 ppm Se) and afternoon ration of unspiked feed for 150 days ($n = 9$). Blood was collected monthly from all 60 birds for chemical analysis. At necropsy, 54 tissues from predetermined organs or sites were collected from each bird and examined histologically.

Results: In study 1 and 2, birds in the control and 10 ppm groups ate most or all offered feed (>95 %). Birds in the 25 and 60 ppm groups ate approximately 80 and <10% of the diet, respectively. This behavior was evident by the second day post-exposure (DPE) of study 1 and persisted for the remainder of the trial. In study 2, birds in the 120 ppm group that were offered a choice of unamended feed (mornings) and high selenium feed (afternoons) ate all of the former and little (<5%) of the latter, also beginning at 2 DPE. Control birds ate most or all of both daily offerings of unsupplemented feed. The 12 birds in the 60 ppm group of study 1 rapidly lost body weight and died or were killed at the following times: 23 days (2 birds), 39 days (1 bird), 48 (1 bird) and 50 DPE (8 birds)(Fig. 1). In addition to emaciation, the principal histological lesions in these birds were hepatopathy, atrophy of lymphoid tissues, and degeneration of the germinal epithelium of feather follicles. Mild to moderate hepatopathy was the most distinctive lesion, affected 50% of birds fed the 60 ppm diet, and resulted in bronze-colored livers at necropsy. Histological lesions in the liver included hepatocellular necrosis, bile duct hyperplasia, marked iron accumulation in Kupffer cells, and karyomegaly. With the exception of some of the hepatic lesions, most of the changes in this group could be accounted for by low dietary intake (starvation). Selenium in whole blood increased from baseline concentrations (< 0.4 ug/ml) to means of 4.5, 8.9 and 16.0 ug/ml in the 10, 25 and 60 ppm groups, respectively (Fig.2). After 75 days of dietary exposure, some birds fed 25 ppm Se developed distinctive alopecic lesions on the scalp and dorsal midline of the neck, and deformed and/or necrotic maxillary and digital nails. Similar lesions developed in principals on the split feed trial. This necessitated the euthanasia of 1/12 birds in the 25 ppm group in study 1 and 0/12 birds in study 2. Other birds survived until they were euthanized according to protocol. Histologically, integumentary changes corresponded to a range of degenerative abnormalities affecting stratified squamous epithelium producing hard keratin. Electron microprobe analysis (EPM) and keratin immunohistochemistry (ICC) were undertaken in an attempt to better characterize of cutaneous lesions of chronic selenosis and the distribution of intracellular selenium and abnormal keratin. Both techniques were unsuccessful due to overfixation (ICC) or low sensitivity of the technique

(EPM). No hepatic lesions developed in the intermediate dose group. None of the low dose birds fed 10ppm selenium as seleno-L-methionine developed clinical signs or lesions attributable to selenosis. No lesions developed in the control birds. Chronic toxicosis was associated with hepatic and blood selenium concentrations of >10 ug/g and >7 ug/ml (Fig.3).

Management implications: This study indicates that selenosis in birds causes distinctive lesions of keratinizing epithelium, in addition to hepatotoxicity and emaciation. The integumentary lesions are essentially similar to those that develop due to selenosis in cattle, pigs, people, and long-tailed macaque monkeys (*Macaca fascicularis*). Epithelial necrosis in keratinizing tissues is however a more prominent feature in mallards. Lesions of the plumage were also reported in the Kesterson site in California. A recently published study of the gross lesions of selenosis in mallards at the Patuxent environmental science center confirms that lesions of the digital nails and plumage are the principal finding in mallards with chronic selenosis. Lesions did not develop in all birds in the medium dose group, and were not necessarily correlated with the highest tissue and blood selenium concentrations in individual birds. The presence in waterfowl of emaciation, hepatopathy, necrosis of the tip of the beak and digital nails, alopecia of the scalp and dorsal cervical midline should be considered suggestive of selenosis at sites where selenium contamination is suspected. Given that emaciation is a common, nonspecific finding in waterfowl that die of various natural causes and that lesions of selenosis are not invariably present in intoxicated birds, examination of >3 birds per species is recommended to ensure that toxicological and morphological samples are representative. In field situations where selenosis is suspected, investigators should determine the body score of dead birds, evidence of gross abnormalities of the liver and digital nails, and plumage quality with particular attention paid to evidence of alopecia.

The following is a recommended minimum list of tissues that should be collected in order to confirm a diagnosis of selenosis in waterfowl where threshold concentrations of selenium in water or waterfowl foods are found:-

- *For histopathology:* formalin-fixed pieces of liver, kidney, spleen, digital nail, skin of the scalp and dorsal cervical midline, maxillary beak, and the foot including digits II - IV.
- *For toxicology:* unfixed samples of liver, kidney and primary flight feathers. If live birds are captured, whole blood in trace-element grade plastic tubes should be collected.

Citations of resulting publications, book chapters and presentations supported by WY DEQ AML Project UW #5/39528

Submitted article:

1. O' Toole, D., Raisbeck, M.F.: Experimentally-induced selenosis in adult mallard ducks: clinical signs, lesions and toxicology. Vet Pathol. Submitted 9 August 1996. In revision.

Invited chapter:

2. O' Toole, D., Raisbeck, M.F.: Magic numbers, elusive lesions: comparative aspects of selenium toxicosis in herbivores and waterfowl *In:* Environmental Chemistry of Selenium, eds. Frankenberger, W.T., Engberg, R.A. Pub. Marcel Dekker Inc.

Abstracts:

3. O' Toole, D., Tynan-Cuisinier, G., Raisbeck, M.F.: 1995, Selenium poisoning in waterfowl due to agricultural drainwater contamination: the obscenity analogy. 8th annual meeting, American Water Resources Association/Wyoming Section. Nov 1-2, 1995. p:16, Proceedings. Laramie, WY.
4. O' Toole, D., Raisbeck, M. 1996, Integumentary lesions of experimentally-induced chronic selenosis in waterfowl: a distinctive pterylopathy-onychopathy. 40th Annual Congress, Association of Veterinary Teachers and Research Workers, April 1996, Scarborough, Yorkshire, U.K. P. 26, Proceedings.
5. O' Toole, D., Raisbeck, M. F.: 1996, Experimental chronic selenosis in adult waterfowl : 1. Clinical signs and lesions. 39th Annual meeting, American Association of Veterinary Laboratory Diagnosticians and US Animal Health Association. Little Rock, AR, Oct 12 - 18, 1996.
6. Raisbeck, M. F., O'Toole, D., Sanchez, D.: 1996, Experimental chronic selenosis in adult waterfowl : 2. Toxicology. 39th Annual meeting, American Association of Veterinary Laboratory Diagnosticians and US Animal Health Association. Little Rock, AR, Oct 12 - 18, 1996.

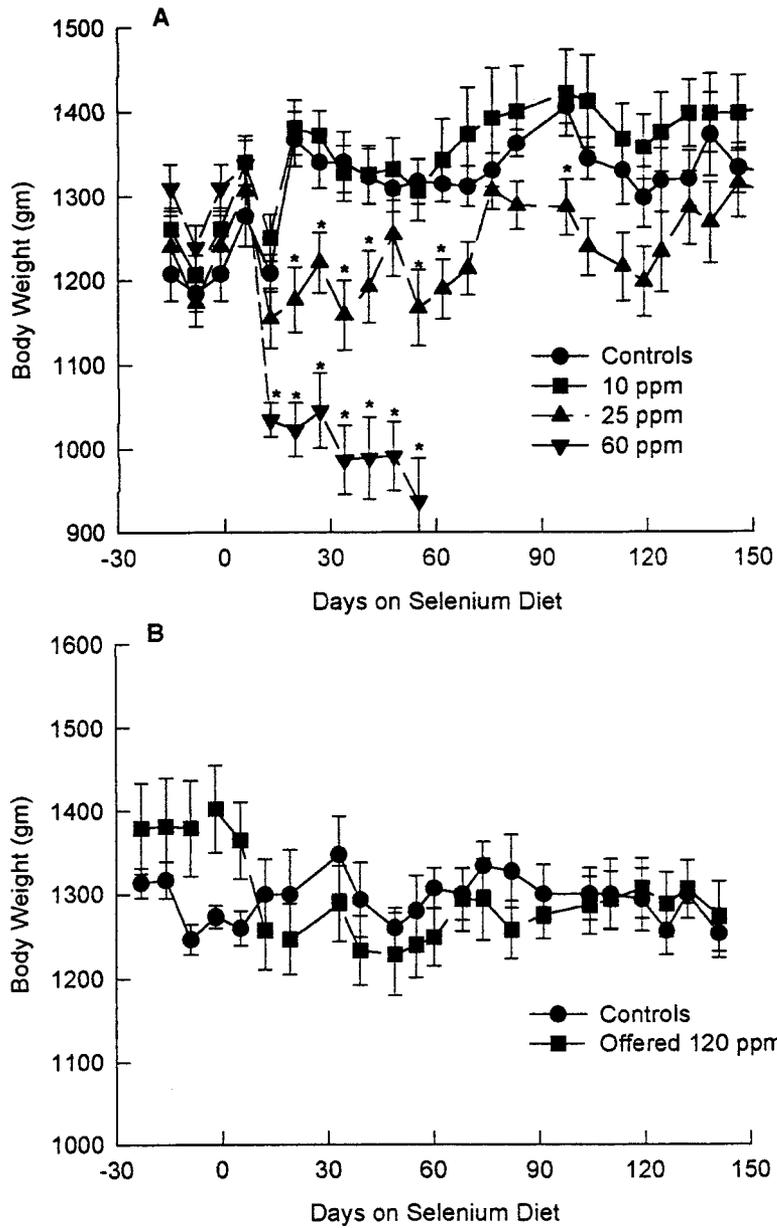


Fig 1: Body weight of ducks in study 1 (A) and study 2(B). Each point represents the mean /- the standard error of the indicated group. An asterisk (*) indicates a significant difference from the control means ($p < 0.05$)

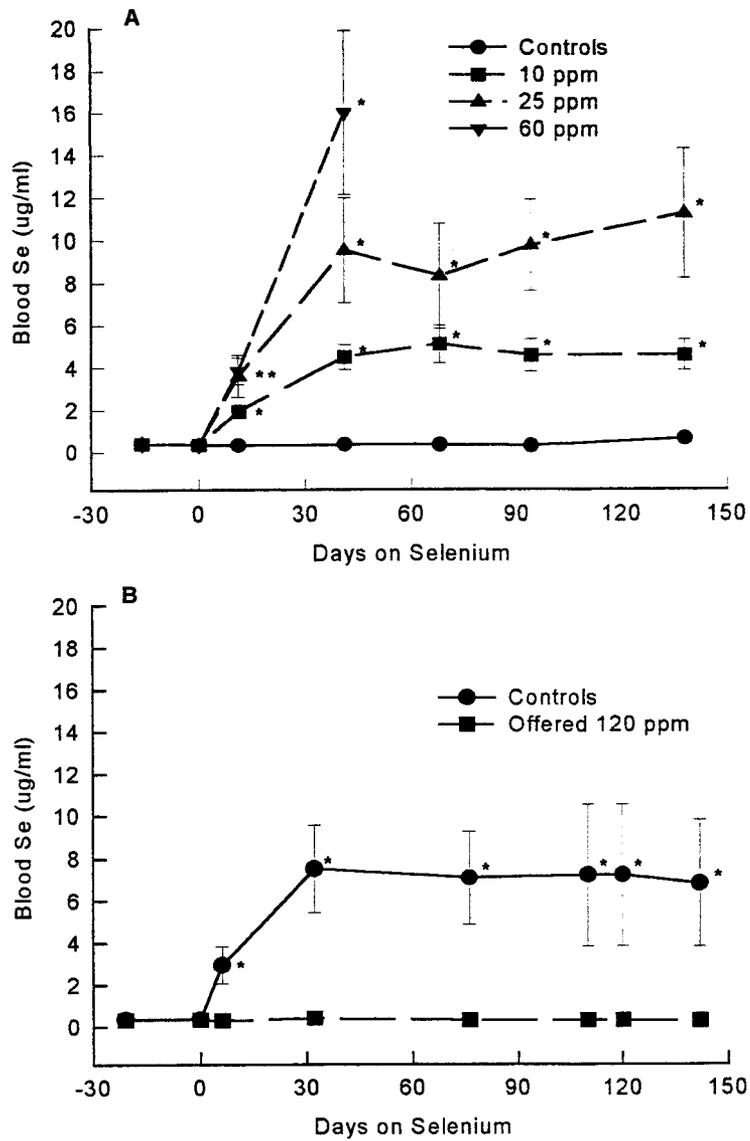


Fig 2: Selenium concentration in whole blood of whole blood of mallard ducks fed seleno-L-methionine for up to 152 days in study 1 (A) and 2(B). Standard error of the control group means was too small to depict on this scale. An asterisk (*) denotes a significant difference from the corresponding control means ($p < 0.05$).

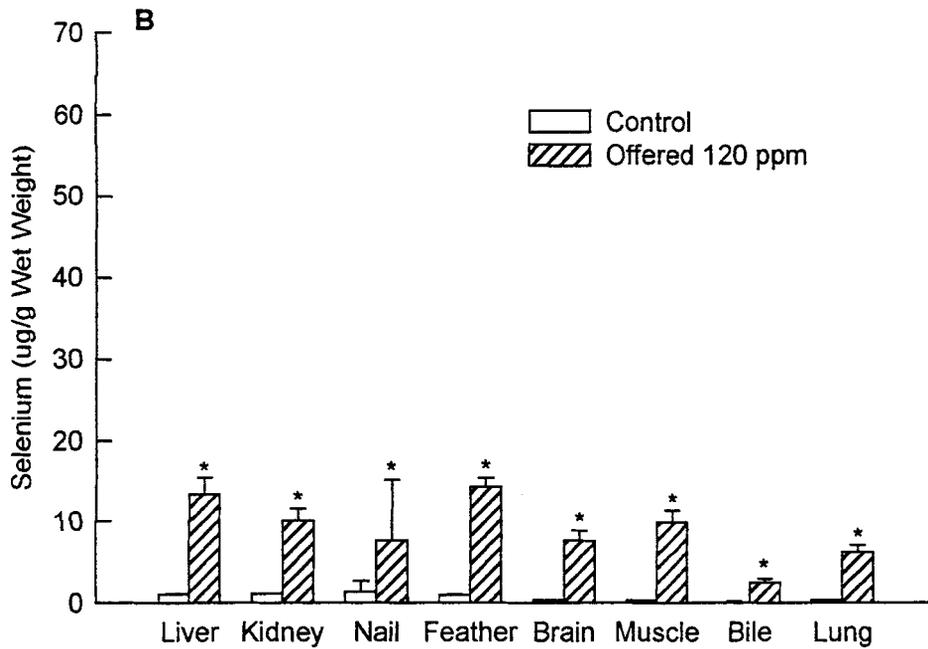
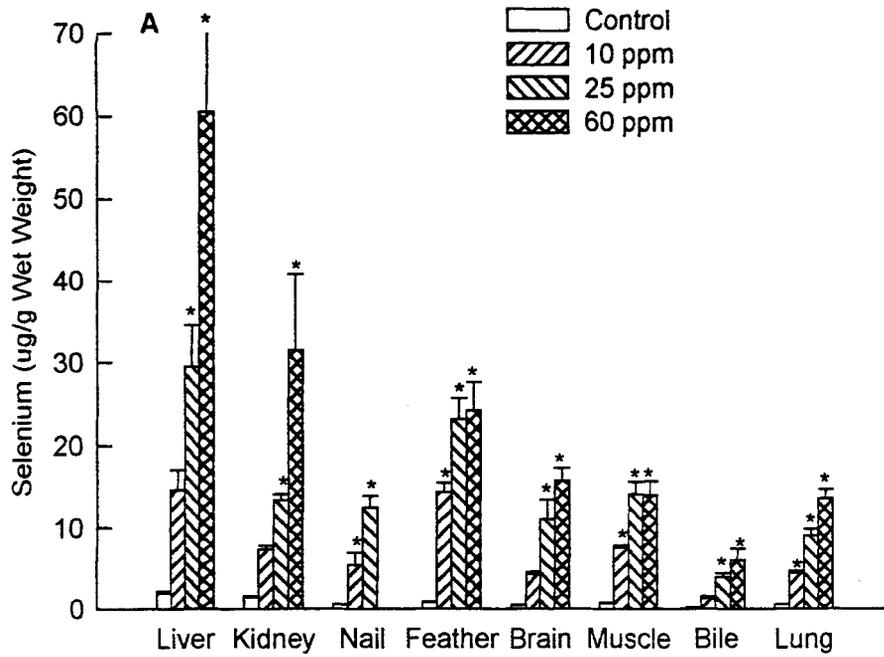


Fig. 3. Selenium concentrations in tissue and other samples from mallard ducks fed seleno-L-methionine for 150 days in study 1 (A) and 2 (B). Each bar represents the mean \pm the standard error for the indicated group. An asterisk (*) indicates a significant difference from the corresponding control means ($p < 0.05$). All concentrations are calculated as received (wet weight)

**METHODOLOGY FOR THE DESIGN AND DEVELOPMENT OF A GIS-
BASED SPATIAL DECISION SUPPORT SYSTEM
FOR ASSESSING THE HYDROLOGIC IMPACTS
OF COAL MINING AND MINE LAND RECLAMATION IN WYOMING**

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**Project Review Seminar
University of Wyoming
Abandoned Coal Mine Land Research Program (ACMLRP)
November 6, 1996 Gillette**

**“Methodology for the Design and Development of a GIS-Based Spatial Decision Support System for Assessing the Hydrologic Impacts of Coal Mining and Mine Land Reclamation in Wyoming”
Semi-annual Interim Progress Report**

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INTRODUCTION

This report gives a brief project background and summarizes the work that has been completed or initiated to date. With a start date of August 1, 1996, initial progress during the past three months has been directed towards project design and data development. These tasks have provided the framework and foundation from which a GIS-Based Spatial Decision Support System can be built.

PROBLEM STATEMENT

The coal permitting process places heavy demands on both permit applicants and regulatory authorities relative to the management and analysis of hydrologic data. While certain, specific data management and analysis issues are currently being addressed by the ongoing Cumulative Hydrologic Impact Assessment (CHIA) development effort at the University of Wyoming, a broader need still exists for the development of computer application tools capable of :
1) managing large quantities of spatial and non-spatial digital hydrologic data;
and 2) providing an efficient means for utilizing such information in an integrated hydrologic impact analysis/modeling environment.

STUDY OBJECTIVE

The primary objective of this research is to develop an integrated, modular spatial decision support system (SDSS) for assessing the hydrologic impacts of coal mining activities in the Powder River Coal Basin of northeastern Wyoming. Components of the System will include existing surface water and groundwater models (HEC1, MODFLOW), a geographic information system (ARC/INFO GIS) , and a relational database management system (ORACLE RDBMS).

The study which will be conducted over a two and half year period, culminating in the final development of a GIS-based SDSS "software application toolbox". The System development will utilize data completed for the Antelope Creek watershed in the Powder River Basin of northeastern Wyoming. However, the products developed will be potentially applicable throughout the coal mining regions of Wyoming.

STATEMENT OF WORK

Model integration within the SDSS will require completion of a series of tasks, ranging from study area database creation, to input parameter format conversion, output capability development, and graphical user interface design and construction.

Task 1: Study Area Database Development. Development of required base data layers for inputs to selected models for the Antelope Creek Watershed Study Area (Figure 1). This watershed is currently the next region of focus for the ongoing Wyoming Cumulative Hydrologic Impact Assessment and has been identified as Cumulative Impact Area (CIA) 1 in the process. The Antelope, North Antelope, and Rochelle coal mines currently are active in the watershed.

Task 2: HEC1/GIS Interface Development. Essentially a GIS interface and "applications toolbox " for HEC1; will require two major tasks:

- 2a. Develop graphical user interface for model input parameter generation, including creation of necessary derived GIS coverages, hydrologic response unit (HRU) delineation, and parameter estimation;
- 2b. Provide GIS output capabilities of modeling results for calibration and validation purposes.

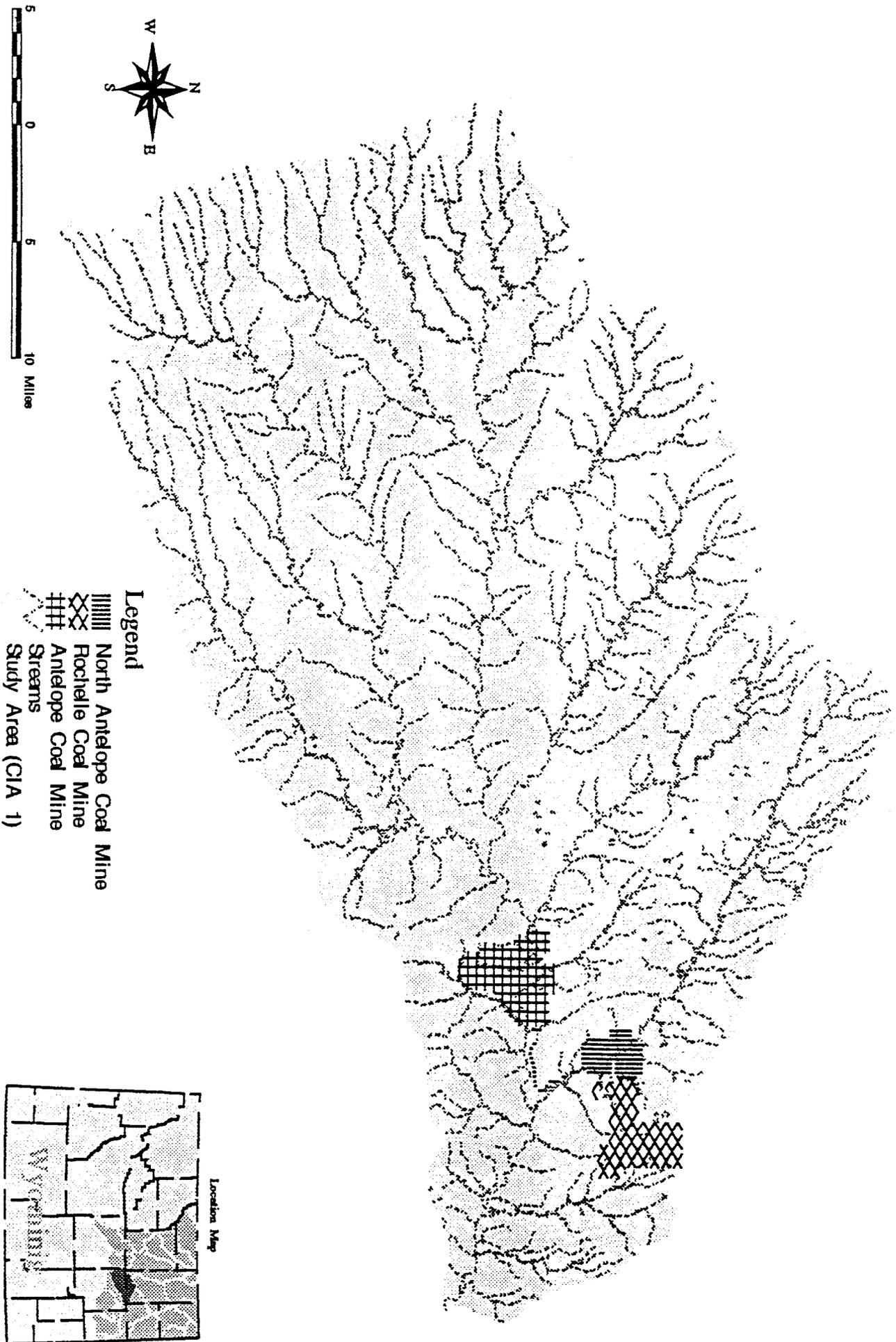
Task 3: MODFLOW/GIS Interface Development. Interface for GIS-based MODFLOW parameter input, analysis, and output generation: three major subtasks are necessary:

- 3a. Develop graphical user interfaces for creating necessary derived GIS coverage model inputs; grid generation and grid parameter population;
- 3b. Create capabilities necessary to directly convert data layers into readable MODFLOW arrays;
- 3c. Provide GIS output capabilities for portrayal of modeling results for calibration and validation purposes.

Task 4: CHIA Modeling Support and Beta Testing. This task will involve providing support and training to modelers associated with the Wyoming CHIA development effort, focusing on the impact analysis in the Antelope Creek Watershed study area (CIA1).

Figure 1

Antelope Creek Watershed Study Area (CIA 1)



Task 5: Probable Hydrological Consequences Modeling Support and Beta Testing. Working in cooperation with Powder River coal Company, the SDSS will be beta tested for PHC-type applications in an active coal mining area of the Antelope Creek watershed study area.

Task 6: User Guide Development. Development of a user guide for the completed SDSS, including an overview of component module functionality.

Task 7: Report Generation. Involves completion of three semi-annual reports, Final Executive Summary Report and the Final Technical report (based on user guide developed in Task 6).

COMPLETED WORK TO DATE

This project officially began on August 1, 1996 and is still in the initial start-up phase. The majority of work completed has been focused on data collection and development for the Antelope Creek Watershed (Task 1). Currently all base data and model input layers have been completed for the region. This included; digitizing hydrography features from all 32 USGS 7.5 minute quadrangles that encompass the study area, assembling all additional pre-existing digital data necessary for modeling and display efforts (e.g. roads, soils, surficial and bedrock geology, digital elevation models, ect.) , and driving digital data from these sources (e.g. slope, aspect, groundwater storage coefficient ranges, ect.).

With data development concluded, a literature review of GIS/MODFLOW and GIS/HEC1 integration was completed. This background information aided in the pre-design of the System and provided valuable examples of previous programming efforts. Although many of these existing systems have established a link with GIS to MODFLOW or HEC1, they are not directly applicable to the Cumulative Hydrologic Impact Assessment efforts associated with this project. Future and current work has been and will be directed toward creating this link specifically for the methods employed in this Assessment. Several individual ARC/INFO programs have been developed within the last month and perform a variety of modeling input and output tasks. These and additional programs will be included within the broader, "user-friendly" GIS-based Spatial Decision Support System being created.

**INTERACTIONS INFLUENCING SELENIUM TOXICITY IN
AQUATIC SPECIES ON RECLAIMED MINELANDS**

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Interactions Influencing Selenium Toxicity in Aquatic Species on Reclaimed Minelands

Interim Report to the ACMLRP
11/6/96

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Problem summary

Selenium, sulfur and coal are intimately related in coal-producing areas of the Powder River Basin. The chemical similarities of Se and S virtually guarantee that environmental disturbances which mobilize one will mobilize both. Indeed, much of the existing surface water in the basin is heavily sulfate-contaminated. The probability of Se mobilization from shales associated with surface coal mining poses a valid concern for the environmental health of land and water after reclamation. Artificial wetlands and impoundments are integral parts of reclamation plans on many mine sites in the Powder River Basin. Such wetlands will attract migratory waterfowl, which are among the species most sensitive to selenosis. Unfortunately, at present *it is not known what influence the main surface water contaminant of the region will have on the toxicity of Se*. The purpose of this project is to determine whether sulfate concentrations typical of surface waters in the Powder River Basin potentiate or antagonize the reproductive and/or immunologic toxic effects of Se. Such knowledge is critical to determining acceptable regulatory thresholds of these elements in wetlands where both elements are present.

Planned approach

For logistic reasons the project is being conducted as two replicated experiments during the late Spring and Summer, one year apart. Winter stress increased mortality in experimental studies at Patuxent, MD (Heinz GH, personal communication) an area with a much milder climate than Wyoming. To avoid such artifacts, and to take advantage of the natural breeding season photoperiod, the animal experiments will be conducted from May until October in both years.

Game farm mallards were chosen as the experimental model for several reasons. In addition to being one of the species at greatest risk, we have considerable experience with the species, the bulk of previous Se/waterfowl research was conducted in mallards and there is thus a considerable knowledge base to draw upon. Immunodeficiency and terata are reported to be

the most sensitive indicators of Se toxicity in this species (Fairbrother and Fowles, 1990; Heinz et al., 1989). These effects will be used in conjunction with tissue Se concentrations as endpoints to evaluate the impact of S on Se uptake, elimination and toxicity.

Selenomethionine (Semet) in aquatic plants and invertebrates is commonly accepted as the major culprit in naturally occurring waterfowl selenosis (Ohlendorf, 1986; Fairbrother and Fowles 1990; Heinz et al., 1989). The L Semet isomer predominates in terrestrial systems (Olson, 1977) and experimental freshwater microcosms (Huckins, J, USFWS, personal communication). Experimental diets were prepared by manually adding an appropriate amount of Se-met in water to duck chow (Mazuri 20% Layer Pellets). The purity of purchased Semet was verified by thin layer chromatography, hydride-ICP and fluorometry in our lab and by total elemental analysis (Desert Analytics, Tucson, AZ). Sulfur was supplied in drinking water as an equimolar mixture of Na, K, Ca and Mg sulfate salts to minimize the effects of individual cations on the experiment.

In our experience and others (Heinz and Sanderson, 1990; Raisbeck et al., 1995) conditioned aversion and feed refusal is a recurring problem in chronic Se feeding studies. We thus included pair-fed controls for the Se treatment group to control for nutritional effects. Waterfowl die as a result of starvation when they lose 35-50% of body weight (Hagar, 1950). Birds which loose more than 30% of body weight will be humanely killed and organs collected for analysis.

birds were inspected daily for general condition and behavior. Other endpoints included complete blood count with differential (CBC), delayed type hypersensitivity (DTH) to *Mycobacterium bovis*, primary antibody response (PAR) to bovine serum albumin (BSA), blood, egg and hepatic Se concentrations, feed consumption, number of viable embryos (eggs hatched) and number of terata produced.

Preliminary results

Ducks receiving 15 ppm Se ate approximately 10% less ration than did control birds. Accurate assessment of feed consumption was complicated by the fact that ducks have extremely non-fastidious eating habits. Selenium treated birds and pair-fed birds gained slightly less weight and looked generally rougher than did controls. Se-treated birds' plumage was generally less water repellent than controls, and Se-treated ducks tended to molt later than non-Se-treated birds. High SO_4 appeared to slightly decrease blood Se concentrations, but did not ameliorate Se effects on plumage.

Selenium treated birds laid more eggs than did controls, however, fewer of these eggs were viable. Terata in eggs from Se-treated birds were similar to those reported previously, ie. failure of the abdomen to close, marked beak deformities, and missing limbs. We did not see internal organ abnormalities (eg. heart defects) reported previously. Ducklings hatched to hens on high SO_4 diet often exhibited a neurologic condition characterized by opisthotonos, incoordination and falling over backwards. Most birds showing these signs died within 48 hr. The cause of death appears to be dehydration; ducklings which were force fed water tended to recover. However, those ducklings which did survive weighed less than their pen mates at 14

days.

The remainder of calendar 1996 will be devoted to chemical, immunologic and statistical analysis.

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