Molybdenosis and Land Reclamation Strategies for Prevention

By

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EXECUTIVE SUMMARY

Molybdenosis is a molybdenum-induced copper deficiency that can occur in ruminants when the copper to molybdenum ratio in forage is less than 2:1, typically due to trace element imbalances in the underlying soil. Ruminants refer to the group of grazing herbivores with multi-chambered stomachs who chew the cud, such as cattle, moose, or deer. Molybdenosis can cause symptoms such as hair loss, depigmentation, gait problems, and death if left untreated.

Regions with high molybdenum content in soils include reclaimed mine tailings sites and waste rock piles where residual molybdenum exists as a by-product of extraction and processing activities. While incidences of molybdenosis have been recorded internationally, this report focuses on molybdenosis associated with copper-molybdenum mines located in the Southern Interior of British Columbia. In these areas, end land-use goals often include grazing land for domestic cattle or wildlife habitat, including for moose and mule deer (whose populations are currently in decline throughout the province for unknown reasons).

Despite an identified risk of molybdenosis in these areas, a framework for the reclamation of molybdenum-contaminated sites does not exist. To address this gap, I have conducted a literature review on molybdenosis and ruminants and land reclamation strategies for molybdenum for the prevention of molybdenosis in affected sites, which include soil amendment and phytoremediation interventions. The literature review serves in the construction of a land reclamation framework designed for use by professionals and landowners in the remediation of molybdenum contaminated land.

INTRODUCTION

Globally, many soils have naturally high molybdenum concentrations due to underlying geology and parent material. Anthropogenic releases of excess molybdenum into the environment are associated with mines, steel mills, incinerators, and oil refineries (Frascoli & Hudson-Edwards, 2018; International molybdenum Association, 2008). Excessive molybdenum in the environment has the potential to negatively impact plants, animals, and human health due to its tendency to accumulate (Haque et al., 2008) and its effects on ruminant health. The ingestion of excess dietary molybdenum from the uptake of forage plants growing in contaminated soil can cause a copper deficiency known as molybdenosis to this group of animals (Giussani, 2019).

In the Southern Interior of British Columbia, land reclamation goals for mining areas include grazing, wildlife habitat, forestry, and recreation (Teck, 2015). There are several copper-molybdenum mines with high molybdenum soil concentrations whose end land use goals include grazing or wildlife habitat, including Highland Valley copper (Logan Lake) and Brenda Mine (Peachland). However, the status of the severity and prevalence of molybdenosis in British Columbia is still not fully understood.

A framework for site-informed land reclamation should exist to minimize the risk to the receiving environment and animal populations (Ali et al., 2013), especially as moose populations continue to decline in the province (British Columbia Ministry of Forests, Lands, Natural Resource Operations, and Rural Development, 2019). A literature review concerning the present situation in the province will be made from available information to determine the current status of the molybdenosis problem. The objective of this project is to assess the state of molybdenosis in the region to establish a land reclamation framework utilizing phytoremediation and soil amendment strategies for the mitigation of the molybdenosis problem and the preservation of soil health.

METHODS

Literature Review

A review of the existing literature was conducted on the incidence of molybdenosis of ruminants in British Columbia and abroad. Soil, vegetation, and animal tissue data from corporate and government reports were examined to inform an assessment of the current status of molybdenosis in the province based on available findings to date and potential for site-appropriate land reclamation strategies.

Framework Development

The secondary objective of this report is to develop a framework for assessing sites with potential molybdenosis risk using environmental risk assessment parameters. Framework development included reviewing and identifying native ruminant forage plants with the ability to extract and stabilize molybdenum and other means of correcting low copper to molybdenum ratios in the soil via soil amendments such as copper fertilization, and dietary copper supplementation when possible as in the case of domestic ruminants.
The construction of this framework was constructed using a literature review of the following topics:

- Molybdenum in the environment;
- Factors affecting molybdenum availability;
- Molybdenosis in ruminants;
- Phytoremediation strategies for molybdenum;
- Soil amelioration strategies for molybdenum; and
- Ruminant health interventions

This framework aims to provide a tool for the assessment, testing, and reclamation of molybdenum-contaminated sites for safe ruminant use. The environmental risk assessment (ERA) procedure (Canadian Council of Ministers of the Environment, 2020) serves as the foundation of a remediation plan for the contaminated site of interest. The four main components of an ERA include the assessment and characterization of potential receptors, exposure routes, hazards, and risks. The conduction of an ERA should occur within a comprehensive site analysis and an effective monitoring program (Jones, 1994).

LITERATURE REVIEW

Molybdenum and Molybdenosis

Molybdenum: uses, bioavailability, and natural occurrence

Molybdenum exists in the environment in several forms, namely as molybdenum disulphide in porphyry molybdenum deposits, and complexed with oxygen as metal molybdates in copper, uranium, and zinc-dominated deposits (Fitzgerald et al., 2008). When metals such as molybdenum enter the environment in excessive quantities, they tend to accumulate as they do not biodegrade. Molybdenum has many important industrial uses, especially in the development of alloys, superalloys, catalysts, lubricants, pigments, and fertilizers (Frascoli & Hudson-Edwards, 2018; International molybdenum Association, 2008). Molybdenum is typically found in igneous rock associated with copper and copper-molybdenum porphyry (high-tonnage, low-grade) deposits. In British Columbia, these deposits are located along the northwest-trending Intermontane Belt of the Western Cordillera and in Vancouver Island's Insular Belt. Molybdenum may be present in rocks and soils in various chemical forms and, therefore, will behave differently in the environment in terms of potential toxicity. Notably, as soil pH increases, molybdenum sorption to soil particles decreases which is the opposite relationship to that of most metals in an alkaline environment (Jones, 1994).

Figure 1: Map of major molybdenum-producing mines in British Columbia (Google Earth, 2020)

Molybdenosis in ruminants

Molybdenosis in ruminants occurs when copper, molybdenum, and sulphur present in forage or feed combine in the animal's anaerobic rumen to form a tightly bound thiomolybdate complex. The formation of this complex renders dietary copper unavailable to the animal's organs (Suttle, 1991; Swank & Gardner, 2004). The mechanism behind the toxicity is due to the unique ruminant physiology of a four-chambered stomach where anaerobic digestion occurs. The anaerobic conditions of the rumen facilitate the binding of molybdenum with copper and sulphur into tightly bound complexes varying among ruminant species (Gooneratne et al., 1989). Molybdenosis has been recorded in several ruminant species, including cattle, moose, deer, yaks, and gazelles in many parts of the world (Shen et al., 2010; Steinke & Majak, 2010; Swank & Gardner, 2004; Xiao-yun et al., 2006).

Most research on molybdenosis and ruminants has been centred on cattle, with a paucity of information available on wild ruminants such as moose and deer. Susceptibility to molybdenosis varies among species. Moose are highly susceptible, cattle are moderately susceptible, but goats are reportedly not susceptible to molybdenosis except where molybdenum concentrations in forage exceed 1.0 g kg^{-1} (Frank, 2004). Different ruminant species require various food sources and ratios of these sources. Moose, for example, need a 45:4:1 proportion of shrubs, forbs, and grasses (Swank & Gardner, 2004), while cattle prefer grasses and hay (Neunhauserer et al., 2001) further complicating the ability to accurately determine toxicity levels of molybdenum to wild ruminants using data from domestic ruminants. Wild ruminants have more extensive and more varied range sizes available for grazing than domestic cattle, who typically graze in an enclosed environment. The 2:1 copper to molybdenum ratio recommendation is a general guideline. Copper to molybdenum ratios below 5:1 – 3:1 has been demonstrated to present a risk for molybdenosis to ruminant species in some studies. Also, this recommendation does not seem to apply when molybdenum concentrations in soil exceed 100 ppm (Raisbeck et al., 2006). Thus, there are many unknowns when estimating molybdenum toxicity among non-cattle ruminant species.

Distinguishing between wild and domestic ruminants in terms of land reclamation is particularly significant in British Columbia, as moose are an iconic, charismatic megafauna species and vital to First Nations for ceremonial, social, and sustenance purposes. Hunting of moose by British Columbia residents and non-residents provides a social and economic benefit to the surrounding hunting areas. Moose populations in British Columbia have been steadily declining for unknown reasons (Ministry of Forests Lands Natural Resource Operations and Rural Development, 2019). While domestic cattle in enclosed grazing areas can be treated with copper supplements (Neunhauserer et al., 2001), it would be more difficult to administer these supplements to wild ruminant populations, especially those that are migratory with sizeable ranges, such as moose (Demarchi, 2003).

Figure 2: Cattle grazing at a reclaimed tailings area at Highland Valley copper. Photo credit: Verdict Media Limited, 2020.

Potential impacts on humans

Molybdenosis is considered relatively harmless to humans, who ingest an average of 100 ug molybdenum per day, with some populations ingesting up to an estimated 250 ug per day in regions with naturally high environmental molybdenum. Molybdenum, when ingested by humans, is rapidly transported out of the bloodstream and into the liver where it accumulates until it is readily excreted in the urine, the homeostatic mechanism for regulating molybdenum levels in the human body (Giussani, 2019). Residual endogenous molybdenum storied in the body is readily remobilized and excreted in the urine. Humans can excrete anywhere from 20 – 300 ug per day depending on daily intake, although the average of molybdenum excretion from the human body is thought to be under 50 ug per day. The biggest risk of molybdenum intake comes from ingesting the ruminant liver, which accumulates the highest concentrations of molybdenum (Teck, 2016). However, consuming the liver is generally not advised in the hunting community as moose liver is known to accumulate many different contaminants, which may be harmful to human health (Government of Northwest Territories, 2016).

International occurrence of molybdenosis

Broman et al. (2002) characterized a wasting disease in moose in Alvsborg, Sweden who exhibited symptoms of central nervous system disturbance, lesions in the digestive tract, atrophied lymphoid organs, anorexia, malnutrition, weakness, impaired vision, lack of wariness towards humans, and diarrhea. Coupled with these observed symptoms was the appearance of several dead moose with no apparent injuries. It was determined that the most likely cause of the wasting disease was environmentally-induced: before the presence of the wasting disease, large-scale liming of moose habitat (including wetlands, lakes, and forests) was used to counter the effects of acid rain that had been occurring in the region during the 1980s. The intense application of lime resulting in a pH increase (alkalization) of soils, increasing molybdenum availability while decreasing copper availability was leading to molybdenosis. Between 150 and 180 animals were thought to have been affected by this profound alteration of environmental conditions (Frank, 2004).

An industrial plant in Austria was associated with a high molybdenum burden in the surrounding environment due to decades of emissions. Molybdenum concentrations in the soil ranged from 2.5 to 11 mg kg^{-1}, well above the recommended limit for grazing ruminants. This region was historically used to grow winter hay for cattle, who were then moved to a low copper alpine grazing meadow during the summer, further exacerbating the molybdenosis symptoms. Many animals fell ill, and several died, and the extreme soil contamination was no longer acceptable under the shift towards organic farming in the region. Two different approaches were tested to increase the copper to molybdenum ratio in the area: the first was to immobilize the molybdenum in the soil through the use of amendments that had proven to have immobilization potential in past studies such as iron oxides, humic acids (e.g. manganese humate), organic matter, clay minerals, and ammonium sulphate. This approach was used for moderately contaminated sites within the study area. For the severely contaminated sites within the study site, phytoremediation was tested in conjunction with soil amendments to increase the export of molybdenum from soils into the plant tissue for disposal and then to stabilize the remaining molybdenum in soil. (Neunhäuserer et al., 2000).

It was determined that the application of manganese humate and sewage sludge decreased plant's molybdenum uptake from soil by 25 and 40 %, respectively, increasing the copper to molybdenum ratio for cattle grazing suitability. For the severely contaminated sites, it was found that phosphate fertilization and vermiculate application enhanced phytoremediation by 88 and 84 % in the field, respectively, concluding that either of these two amendments is suitable for increasing molybdenum mobility for plant uptake in soils (Neunhäuserer et al., 2000)

In the Qing-Hai Tibetan Plateau of China, molybdenosis has been observed in domestic yaks *(Bos grunniens*) known regionally as "shakeback disease," characterized by conspicuous trembling of the back. Attributed to naturally elevated molybdenum in the region, yaks displayed symptoms such as emaciation, unsteady gait, a "shivering" back, decreased appetite, pica, anemia, and prone to falls and fractures. Symptoms were rapidly corrected following the oral administration of copper sulphate (Xiao-yun et al., 2006). Molybdenosis has also been observed in the Tibetan gazelle (*Procapra picticaudata*) population in the same region of China (Shen et al., 2010).

Molybdenosis in Saskatchewan, Canada

MacDonald (2009) performed a risk assessment of uranium mining impacts on aquatic ecosystems in the northern Saskatchewan region, which includes the Key Lake, Rabbit Lake, and MacArthur River mines. Northern Saskatchewan has a similar boreal ecology to that of Alvsborg, Sweden, and are both home to sizeable moose populations. The land in Northern Saskatchewan is the traditional territory of the Deneseline First Nation. It is vital for culturally essential activities such as fishing and hunting as well as for recreation. Uranium mining is a significant source of molybdenum contamination in the environment, and Saskatchewan produces 28% of the world's uranium. Molybdenum is released into surface water via leachate from mine overburden, tailings, and milling effluent from the uranium mines in the region. McDonald (2009) notes that under reference surface water conditions throughout Canada, molybdenum levels typically range from less than 0.0001 (undetectable) to 0.5 mg L^{-1} due to natural bedrock weathering. The Canadian recommended limit of molybdenum in the aquatic environment is 0.073 mg L^{-1} and 5 mg kg^{-1} dry weight in agricultural soils for the protection of human and environmental health (CCME, 1999c, 1999a). Molybdenum is typically present as molybdenum disulphide or permanganic acid in alkaline water environments but is present as the molybdate anion in pH less than 7 (acidic) waters. Select aquatic sampling sites in the region exceeded 2 mg L^{-1}, indicating moose could be impacted by molybdenosis in northern Saskatchewan. (MacDonald, 2009).

As molybdenum is a concern as a constituent of mine drainage, notably that associated with uranium mines in northern Saskatchewan, Fitzgerald et al. (2008) conducted a review of toxicity and management criteria for the risks of anthropogenic molybdenum in aquatic and terrestrial receiving environments. This review included the examination of the familiar sources of molybdenum contamination, the environmental fate and transport of molybdenum, and the critical toxicity effects and sensitive receptors compared against existing toxicity thresholds. It concluded that each risk assessment should be conducted specific to the study site or else risk enforcing very conservative discharge limits, which may be difficult for mines to meet. Identified within the report was a suite of factors affecting molybdenum accumulation in plants and animals. Factors included:

- concentration and chemical forms of molybdenum present in the environment;
- receptor species;
- type of exposure;
- surrounding habitat or media of the organism: the chemical and physical characteristics of the identified media such as depth, temperature, and pH;
- timing and extent of the exposure;
- the ability of the receptor to excrete excess molybdenum from tissues; and
- presence of any other constituents that may modify the mobility or toxicity of molybdenum (such as any synergistic compounds or compounds with a similar stereochemistry, e.g. sulphate)

Fitzgerald's report concluded that each of these environmental factors should be carefully assessed and then compared against existing toxicity thresholds and regulations in the study area. In British Columbia, molybdenum toxicity has been tested in numerous studies, and toxicity thresholds vary from 0.78 mg L^{-1} to 300 mg L^{-1} in aquatic environments depending on receptor species (Fitzgerald et al., 2008).

Elevated molybdenum levels in a site impact the potential subsequent uses for that site, notably where this disturbed land is slated to be returned to agricultural use (Jones, 1994) or in an aquatic environment where the water is needed for irrigation uses or to sustain fish habitat. Molybdenum toxicity in the environment varies with pH, climatic, soil moisture level, redox potential, organic matter content, and soil microflora, as well as relative concentrations of other trace elements, such as copper and sulphur (both of which minimize the risk of molybdenosis in high-molybdenum soils). Due to the numerous factors affecting toxicity, it is critical to perform an accurate assessment of the site conditions and any potential receptors (e.g. ruminants, deer or cattle or moose, fish, other wildlife), exposure concentrations, settings, and possible pathways before developing any future use or remediation plan based on the existing literature, e.g. the length of time spent grazing at the site, the life cycle of the plant of concern (Jones, 1994).

For example, the risk of toxicity of molybdenum to the wild moose population in Northern Saskatchewan was determined to be low (Fitzgerald et al., 2008); moose in the region browse mainly twigs and leaves and a very small percentage of aquatic macrophytes (approximately ~ 3 % of moose's dietary intake), and woody shrubs accumulate very little molybdenum in their above-ground tissues (less than 1 mg kg^{-1}) (Fitzgerald et al., 2008). So, although aquatic plants in affected areas are notorious accumulators of molybdenum (332 mg kg^{-1} average), they are such an insignificant constituent of the moose diet they are not of great concern.

Molybdenum in Cape Breton Island, Nova Scotia, Canada

A study by Beazley et al. (2008) set out to investigate the reason for the unexplained absence of moose on southeast Cape Breton Island. In this area, there are no longer moose where they used to be abundantly present. This region has been assessed as prime moose habitat, coupled with strong evidence of moose historically inhabiting the area. Interestingly, moose are indeed present in other areas on the island. There is very high molybdenum in the southwest part of Cape Breton, so the possibility of molybdenum having something to do with the lack of moose was acknowledged. Pre-colonisation, the region was populated with Eastern moose (*Alces alces americana*), which were hunted to extirpation. The subspecies *A. a. andersonii* was introduced from Alberta after the local extinction of the native moose population to bring large herbivores back to the island for the restoration of the trophic regime. Additional hypotheses associated with the lack of moose in southeast Cape Breton Island include possible poaching or heavy metals affecting the palatability of grazing materials. The area is associated with a long-closed coal mine and steel mill, Sidney Mines, which also owes to its high molybdenum concentration in soils. However, there is no confirmation of molybdenum being the cause for the lack of moose in the area, and further investigation is needed in this region.

Molybdenosis in British Columbia, Canada

As different ruminant species have varying thresholds for molybdenum toxicity, there is a concern for molybdenosis at some proposed post-development land uses of affected mines in British Columbia. Cattle grazing and haying, wildlife habitat, forestry, and public recreation are identified post- mine reclamation uses where several ruminant species may frequent the region (Surridge, 2000). Additionally, there is a lack of research on the causes of recent moose decline in the area. Compounded by a lack of data on the safety of molybdenum-contaminated meat, these findings indicate a potential human health and cultural concern as it may affect the inability to harvest and consume moose (Teck, 2015).

In the approximately 2,000 km^2 Wildlife Management Unit 3-18, or WMU 3-18 (Province of British Columbia, 2020), in which High Valley Copper (HVC) is situated, moose populations have been steadily for the past few decades. In 1995, the provincial moose inventory estimated 143 moose were present in WMU 3-18. In 2008, seventy-one moose were counted. In 2014, just thirty moose were counted. While the cause of these moose declines is still unknown, it is believed to have something to do with high calf mortality based on the results of an ongoing tracking study initiated in 2012 (Ministry of Forests Lands Natural Resource Operations and Rural Development, 2019). Regardless of the cause, any threats to the moose population, including the risk of molybdenosis, is undesirable as malnourished animals are susceptible to disease, predation, and accidents (Ministry of Forests Lands Natural Resource Operations and Rural Development, 2019).

Figure 3: Highland Valley copper tailings dam. Photo by Author, 2020.

Highland Valley Copper is one of the largest open-pit copper mines in the world (Surridge, 2000). HVC has been operational since 1986 and is located 80 km southeast of Kamloops, British Columbia, near Logan Lake. Gardner et al. (2003) conducted a study in which 32 calf-cow pairs, consisting of one control group and one treatment group were administered copper bolus medical treatment to prevent molybdenosis while grazing at a reclaimed mine tailings site at HVC. The two groups showed no difference among health status while grazing on a contaminated reclaimed mine site at HVC with very high molybdenum values, indicating that the recommended 2:1 ratio does not apply at this site. However, molybdenum serum and liver levels were shown to increase linearly with time spent grazing on the contaminated site. Majak et al., (2004) found that less than half of 84 calf-cow pairs grazing at a high-molybdenum site HVC for 11 weeks displayed symptoms of molybdenosis, including a stiff shuffling gait, watery diarrhea, and stiff hair coat. The primary source of forage was alfalfa and orchard grass containing 100-400 ppm of molybdenum. The incidence and severity of symptoms seemed positively correlated to field moisture levels. During the third year of the study, the copper boluses were not effective in the prevention or mitigation of molybdenosis symptoms in the treatment group but found by the end of the study, symptoms had resolved themselves in both groups. Thus, is appears that the length of time that ruminants graze at a site in a contributing factor. Also, the vegetation at HVC is primarily grasses and forbs with few shrub "islands" dispersed throughout, meaning that the primary required food source of moose, i.e. woody shrubs, is not particularly abundant in the area, thereby minimizing the risk of molybdenosis to moose.

In 2012, Nlaka'pamux hunters noticed moose harvested in the vicinity of the mine were displaying signs of ill health, including liver discoloration. They brought these findings to the attention mine owners, Teck Resources Ltd.., who launched an investigation to determine the cause of the physiological changes in moose observed by the hunters (Teck, 2015). It included an environmental assessment and an assessment of forage and animal (moose and mule deer as a stand-in for moose), whose populations have been rapidly declining for several years (Kuzyk et al., 2018; Ministry of Forests Lands Natural Resource Operations and Rural Development, 2019). Interestingly, mule deer populations have been rapidly declining in other regions of the province, the HVC area being one exception. An analysis of tissue metal levels was performed to determine whether the metals associated with the mine environment were a potential contributor to these observations. Vegetation (willow, sedge, alfalfa, and crested wheatgrass) content of metals, including molybdenum and Cu, were collected from a remediated tailings pond was compared to vegetation from a reference site (Teck 2016). They found the mean molybdenum concentration of all plant samples exceeded the recommended thresholds for forage consumption by cattle. The copper to molybdenum ratio in most vegetation samples was below the recommended threshold (2:1) for cattle consumption. Tissue metal concentrations of 7 harvested moose and 13 mule deer were tested. In the moose and deer samples, all individuals (except for one moose) displayed tissue molybdenum levels below the cattle grazing safety thresholds. The conclusion of this preliminary study is consistent with the hypothesis that mule deer may be more effective at excreting molybdenum than moose (Teck, 2016). Although domestic animal thresholds tend to be very conservative, it is ultimately *not known* how these thresholds translate to wild ruminants.

Trail cam film footage from Teck (2016) over a three year filming period between May and October showed only three individual moose frequenting the site. The finding that moose do not frequent the site indicates that the probability of grazing ruminants developing molybdenosis if they grazed exclusively at HVC, is low. The low incidence of moose grazing in reclaimed areas in HVC areas compared to undisturbed reference plots has been attributed to the lack of woody structural vegetation in the site (Teck, 2017), although mule deer populations seem to prefer the remediated sites over reference sites. In riparian areas at HVC, it has been noted that all leaf tissue collected to date had an average copper to molybdenum ratio of 0.13:1, well below recommended thresholds, indicating high potential for molybdenosis (Teck, 2018). However, the degree to which ruminants graze in this area is currently not known.

Figure 4: Highland Valley Copper reclaimed area with mine in the background. Photo Credit: Shewchuk, 2020.

Riordan (2003) performed a comparison of both pre- and post-development molybdenum concentrations in vegetation on the Endako mine site near Peachland, BC using data collected from 1950 to 1965 and concluded that mining activities had not impacted the naturally-evaluated molybdenum levels in vegetation. It was concluded that since there were no historical reports of illness in ruminants, the naturally high levels of molybdenum in the region do not pose a significant risk to wildlife, in contrast to the Cape Breton Island case study, where ruminants have disappeared altogether from a high-molybdenum site. Molybdenum concentrations were higher over the ore body than in the post-disturbance reclaimed areas. Consistent with Fitzgerald et al., (2008), Riordan's review found that aquatic plants accumulate significantly more molybdenum ($\sim$ 753 mg kg^{-1}) than terrestrial plants (3-79 mg kg^{-1}). However, as Fitzgerald (2009) concluded, moose ingest a relatively small proportion of aquatic plants compared with woody browse that this is probably not of concern.

Figure 5: *Medicago sativa* (alfalfa), a forage crop commonly grown in the southern Interior of British Columbia. Photo credit: Rankin, 2019.

A study at the Brenda site was undertaken to determine the nutritional status of mule deer and to determine whether the population was displaying any symptoms of molybdenosis. Brenda Mine was a copper-molybdenum mine in operation from 1967-1990 and has now been revegetated. Throughout the study, 32 deer of all life stages were sighted near the mine. Mule deer were observed grazing mainly alfalfa (*Medicago sativa*) and other herbaceous plant species, including white clover (*Trifolium repens*), sweet clover (*Melilotus alba*), birdsfoot trefoil (*Lotus corniculatus*), sanfoin (*Onobrychis viciifolia*), and fireweed (*Epilobium* sp.). During the summer months, mule deer spent summers only in the Brenda Mines area as their winter range was in the Peachland-Westbank region, minimizing the time spent on the site and, therefore, the amount of potentially contaminated vegetation consumed. In addition to the delineation of a summer and winter range for mule deer, the study found the average summer range area size of a female deer is 7 km^2 while a male deer's range is estimated to be around 15 km^2. The Brenda site is less than 7 km^2 in area, inferring that it is unlikely a deer would spend its entire summer on the site. Although the average molybdenum concentration in vegetation grown on tailings was 130 mg kg^{-1} and copper was low (the copper to molybdenum ratio was 0.5), there were no signs of molybdenosis exhibited by the mule deer, excluding a few observations of diarrhea, which is not unusual for this species according to consulted wildlife biologists (Taylor & McKee, 2003).

Figure 6: Mule Deer in Kane Valley. Photo courtesy of Alan Burger, 2020.

Remediation of Molybdenum

Phytoremediation

In this report, we investigate remediation strategies for molybdenum contaminated lands, with a focus on phytoremediation. Phytoremediation is an attractive option for molybdenum remediation because it is a relatively inexpensive and less environmentally damaging method of soil remediation compared to conventional ex-situ soil remediation techniques; performed in-situ, it is an efficient process with a positive public perception that also serves to conserve and improve soil quality and characteristics (Ali et al., 2013; Ghazaryan et al., 2019).

Conventional soil decontamination involves costly and labour-intensive interventions, both chemical (soil washing, soil flushing, in-situ vitrification), and physical, such as removal and disposal to make the site less contaminated. However, none of these methods can preserve soil health, biological integrity, or structure and are also more costly than phytoremediation (Ali et al., 2013; Wang et al., 2017).

As soil is already a critically threatened resource worldwide (International Soil Reference and Information Centre, 2020) and the employment of chemical-based remediation carries the risk of potential secondary pollution, phytoremediation is a better method of soil restoration in terms of cost-effectiveness, soil health, and public acceptance. Phytoremediation has been studied since 1990 and is defined as "the use of plants and associated microbes to decrease the concentration or toxic effects of contaminants in the environment" (Ali et al., 2013), and by Ghazaryan (2019) as "the use of plants to remove contaminants from the environment or render contaminants harmless."

The correct identification of the chemical form of molybdenum present in plant tissues is a crucial indicator of potential toxicity as a food source for ruminants. In some plant species, molybdenum is present in plant tissues as the molybdate ion (MoO_4^{-2}), which is toxic to ruminants. However, in other metal-tolerant plants, the molybdenum combines with organic material to form organometallic complexes which immobilize the molybdenum in the plant tissue. Surridge (2000) attempted to determine the form of molybdenum present in alfalfa because it has a copper to molybdenum ratio below the recommended 2:1 ratio. Based on these findings, and the popularity of alfalfa as grazing material, severe molybdenosis symptoms would be expected, but this was not observed in any cattle grazing at the site. The hypothesis was that molybdenum was present in a non-toxic form in the alfalfa tissue. Although Surridge concluded the molybdenum species in the alfalfa was MoO_4^{-2}, it was later determined by Steinke and Majak (2010), that molybdenum was actually in the form of a molybdenum-malate complex. Due to Surridge's alkalizing experimental conditions, the molybdenum in alfalfa only appeared to exist as the molybdate ion. This molybdenum-malate complex was determined to be extremely sensitive to pH changes and disassociates into the molybdate ion at a pH greater than 8, which the experimental conditions exceeded. Metal-tolerant plants are thought to facilitate the endogenous formation of organometallic complexes to protect themselves from the toxic effects of metals (Steinke & Majak, 2010), indicating that alfalfa is tolerant of molybdenum-rich soils. Molybdenum levels were present up to 400 mg kg^{-1} in vegetation grown on mine tailings at the HVC site, and if taken up by plants at this concentration as the molybdate ion, grazing would likely be correlated with severe molybdenosis. Ultimately, "…the chelation of molybdenum at the site may well explain the lack of long-term clinical side effects in cattle grazing at the site" (Steinke & Majak, 2010).

Interestingly, there is a category of plants that occur naturally around ore bodies rich in metals. These plants are known as metallophytes and have adapted to survive mostly by forming organometallic complexes within their tissue to protect against metal toxicity (Ameh et al., 2019). As a result of their habitat type, most of which have been exploited for extraction purposes, the natural metallophyte population is in decline. Many metallophytes are in the Brassica family. There are three main categories of metallophytic plants:

- *Metal excluders*: Plants which stabilize the metal in and around the root zone, minimizing uptake into the plant's above-ground tissues;
- *Metal indicators*: Plants which are metal-tolerant and typically excellent extractors of the target metal into above-ground tissues and therefore "indicate" the presence of the target metal in their growing media; and
- *Metal hyperaccumulators*: Plants that accumulate the target metal into their above-ground tissues according to specific metal-specific parameters (for example, a plant

must accumulate at least 100 mg kg^{-1} of copper dry weight in order to be classified as a hyperaccumulator of copper).

There are three essential indices used to determine the phytoremediation potential of plant species known as the bioconcentration factor, the translocation factor, and the bioaccumulation coefficient:

$$\text{Bioconcentration factor (BCF)} = \frac{metal\ concentration\ in\ root}{metal\ concentration\ in\ shoot} \quad \text{[Eq. 1]}$$

$$\text{Translocation factor (TF)} = \frac{metal\ concentration\ in\ plant\ shoot}{metal\ concentration\ in\ plant\ root} \quad \text{[Eq. 2]}$$

$$\text{Bioaccumulation coefficient (BAC)} = \frac{metal\ concentration\ in\ plant\ shoot}{metal\ concentration\ in\ soil} \quad \text{[Eq. 3]}$$

A BCF, TF, and BAC of less than 1 indicate the plant species is a candidate for phytoextraction. If the plant has a BCF greater than one paired with a TF of less than 1, this indicates the potential for phytostabilisation (Ameh et al., 2019). The three primary techniques of phytoremediation are phytoextraction, phytostabilisation, and phytofiltration (Ali et al., 2013). Selecting suitable plant species is critical to the success of phytoremediation interventions. The desirable plant traits include tolerance to site conditions, dense rooting systems, rapid growth rate, and extensive biomass production(Ali et al., 2013; Ghazaryan et al., 2019). It is preferable to use native plants, especially given the usually remote locations of mine sites, to preserve biological activity in the region of interest and to minimize the need to soil inputs, as transport to these locales is often very costly (Wang et al., 2017). Oustriere et al. (2017) lists plant tolerance traits to trace element (TE) to include:

- The formation of dense, perennial, self-sustaining vegetative cover;
- Accumulation of target TE mainly in roots (phytostabilizers);
- A low root-to-shoot transfer to limit the entry of TE into the food chain;
- The ability to decrease TE concentration in soil pore water; and
- Tolerance to abiotic stresses such as drought (to ensure the vegetative cover remains alive even if planted in a remote area where regular watering and care may not be manageable).

Phytoextraction refers to the process where plants can take up contaminants and store them in their below or above-ground tissues, such as roots and shoots. Plants that are metal "accumulators" and "hyper-accumulators" of the target metal are the most desired species to be used for phytoextraction. It is also the most labour intensive of the three, as removal of the vegetation is required to prevent the resultant biomass from returning to the soil. This method is feasible only if there is no risk to grazing animals of ingesting the forage, such as in a fenced-off area. If the metal in the soil is abundant and hyperaccumulators are used for phytoremediation, there is sometimes the option to incinerate the green waste and extract the metal for reuse, a process known as phytomining (Ali et al., 2013) which is becoming more widely used as virgin metal deposits are becoming depleted worldwide (Regel-Rosocka & Alguacil, 2013). Phytoextraction can be natural or induced. "Natural" phytoextraction is the process whereby the plant extracts the target contaminate from the soil without the application of soil amendments. Induced phytoextraction aids the extraction process by some form of soil amendment that increases the mobility of the target contaminant for uptake such as EDTA or citric acid, some of which then have the potential for secondary contamination (Ali et al., 2013).

Phytostabilisation refers to the process of the plant taking up and accumulating the target metal in and around below-ground tissues via sorption in the roots or complexation or precipitation in the rhizosphere (Ali et al., 2013). As the plant retains the metals into primarily below-ground plant parts, phytostabilisation is the preferred method when the land is to be used for grazing by wild or domestic ruminants, as it diminishes the chance of re-entry of the contaminant back into the food chain. Potential phytostabilisation is not a long-term fix, for when the plant dies, the target contaminant will be re-introduced to the environment via degradation of the vegetation back into the soil. However, it offers a short-term management strategy that can be used when land is needed for grazing in the short-term, e.g., one grazing season (Ali et al., 2013). Phytofiltration is a similar process to that of phytoextraction in terms of uptake and accumulation of the target contaminant primarily into above-ground tissues but is specific to the phytoremediation of aquatic environments.

One phytostabilizer of molybdenum was identified as corn (*Zea mays*) (Ameh et al., 2019), which demonstrated a BCF greater than 1 and TF less than 1 growing on molybdenum-contaminated soils in the northern Anambra Basin. Yellow sweet clover

(*Melilotus officinalis*) was identified as an effective phytoextractor of molybdenum in molybdenum-contaminated soils at an Armenian mine but is classified as a noxious invasive species in the Southern Interior of British Columbia (Invasive Species Council of British Columbia, 2018), precluding its use as a potential phytoremediation candidate. *Zea mays* may be a phytostabilisation candidate in British Columbia due to its value as a cattle forage, but this is an area of further study. However, a more ecologically sound phytoremediation intervention would be to use only plants endemic to the Interior of British Columbia for remediation purposes, to avoid any unwanted ecological changes – the identification of suitable species meeting these criteria is an additional area of potential further study.

Many species of plants already exhibit a high tolerance to metals in the environment via adaptation through unique uptake and detoxification methods. Interestingly, for some nitrogen-fixing legumes (such as alfalfa), molybdenum is required in higher quantities as molybdenum is a biological component of the metalloenzyme nitrogenase, which is necessary for N fixing in the symbiotic rhizobia nodule (Jones, 1994). Also, providing a vegetative cover to a contaminated site reduces the risk of runoff and erosion of contaminated material and sediments into waterways, thereby actually improving the site and soil quality in terms of organic matter, nutrient concentration, and qualities and characteristics (Wang et al., 2017). Of course, the overarching goal should be to avoid environmental contamination through a comprehensive assessment and mitigation program in the first place, but if contamination has already occurred, then phytoremediation should be considered. With aided phytoremediation, different studies have yielded varying results regarding molybdenum mobility in soils with the addition of organic matter (OM) such as sewage sludges to contaminated sites depending on its contents (Jones, 1994).

An experiment that took place in the Northern Anambra Basin, Nigeria, determined that *Zea mays*, taro (*Colocasia asculenta*), West African mallow (*Corcorus aestuens*), and wireweed (*Sida acuta*) were phytostabilizers of molybdenum. At the same time, amaranth (*Amaranthus hybridus* and *A. Viridis*), okra (*Abelmoschus escueltus*) and squash (*Curcubita maxima*) were efficient phytoextractors of molybdenum (Ameh et al., 2019).

Ghazaryan's 2019 ex-situ phytostabilisation study on contaminated, alkaline soils (pH=7.9) from the Zangezur copper and molybdenum mine found that *Melilotus officinalis* was effective in stabilizing molybdenum in below-ground plant parts without the addition of chelating solutions. In the southern Interior of British Columbia, *Melilotus officinalis* is an introduced invasive species. While it is invasive and a known allelopathic plant, it is an effective stabilizer of molybdenum in similar soil conditions and has the potential for use in well-contained areas to reduce the risk of molybdenosis to ruminants.

One greenhouse study in northern Chile by (Santibáñez et al., 2008) demonstrated that the addition of 6 % biosolids to *Lolium perenne* (perennial ryegrass) in greenhouse pots of mine-contaminated soil could reduce the overall uptake of molybdenum into the plant shoot. The biosolids used had high salinity, neutral pH, and metal concentrations not exceeding the 1993 US EPA limits for land application.

A different greenhouse study tested the phytostabilisation potential of the nickel and cadmium tolerant *Festuca pratensis* (meadow fescue) on a barren, alkaline, steel-mill contaminated technosol (anthroposol) with a high molybdenum concentration while reducing the potential for herbivory exposure in eastern France. The treatment with composted sewage sludge yielded the highest molybdenum uptake in roots, but the shoot concentration of molybdenum still exceeded forage limits for cattle (Oustriere et al., 2016).

The limitations of phytoremediation include adverse field conditions not conducive to growing vegetation, e.g. climate, nutrient availability, plant pathogens, climate, soil type, soil pH, soil structure, and non-uniform contaminant loads throughout the site (Ali et al., 2013). Additional considerations include some plants that may not tolerate the site conditions and fail to thrive; thus, agriculture becomes impossible due to the toxification of edible plant parts (Wang et al., 2017).

Copper fertilization of molybdenum-contaminated soils

Phytoremediation is often performed in conjunction with soil amelioration to adjust pH (as the mobility of molybdenum increases in alkaline soils), enhance soil quality, and stimulate microbial activity. Soil amendments for remediation of molybdenum-contaminated soils include sewage sludge, compost, and copper fertilization (Mullen et al., 2005; Oustriere et al., 2016). The application of copper to grazing pasture has the potential to minimize the risk of molybdenosis in ruminants pasture by correcting for copper to molybdenum ratios below the recommended 2:1 threshold in forage (Government of Western Australia Department of Primary Industries and Regional Development, 2019; Stark & Redente, 1990).

Stark and Redente (1990) tested copper fertilization on retorted oil shale piles in the Piceance basin area of northwest Colorado to correct to the suggested appropriate copper to molybdenum ratio in the soil to restore the land's grazing potential for ruminants. The copper to molybdenum ratio was less than 2, and the average molybdenum concentration in soil was 8 mg kg^{-1}, which is exceptionally high. Copper sulphate was applied to the soil in treatments of 0, 8, 16, and 32 kg ha^{-1} at two soil depths: 30 cm and 90 cm. At the end of the first growing season, every plant species, except for alfalfa, showed an increase in copper concentration. However, molybdenum was so high in this region that all plant species except winterfat (*Krascheninnikovia lanata*) still had copper to molybdenum ratios less than two after all treatments of copper sulphate application.

Furthermore, by the end of the second growing season post-fertilization, there was a 30 % average decrease among the plant species that had initially been shown with an increased uptake of copper after the copper sulphate treatments were applied. It was concluded that although the application of copper sulphate to correct low copper to molybdenum ratios in affected soils was a short-term correction, and it needed to be supplemented with a combination of soil amendment and phytoremediation strategies to correct the metal imbalances for ruminant grazing safety. An interesting note is that *K. lanata* is an excellent source of high-protein winter forage for many ruminant species such as pronghorn, elk, and mule deer. *K. lanata* appears throughout the Canadian prairies, and west from the Yukon to Mexico (Banerjee & Schellenberg, 2001). However, it is not a native species in British Columbia, potentially precluding its use as a phytostabilisation plant to avoid unwanted environmental cascade effects.

Over-fertilization of agricultural land in Western Australia has resulted in high molybdenum levels that do not normally occur naturally (Government of Western Australia Department of Primary Industries and Regional Development, 2019). In this situation, copper sulphate application is practical for correcting insufficient copper-molybdenum ratios for the prevention of molybdenosis in domestic ruminants (sheep and cattle) unless the molybdenum concentration in the soil exceeds 5 mg kg^{-1}. A single application of 3.3 to 10 kg ha^{-1} copper sulphate (depending on site characteristics and soil type) was found to prevent molybdenosis in sheep and cattle for at least eight years (Government of Western Australia Department of Primary Industries and Regional Development, 2019).

Future Areas For Investigation

A more thorough assessment is suggested to include a lack of existing information regarding metal concentrations (mg kg^{-1}) in native plant species growing in the Southern Interior of British Columbia, to identify potential native plant phytostabilizers or extractors of molybdenum that could be recommended for an introduction to molybdenum-contaminated sites for grazing without negatively impacting ecological regimes. Studies to determine suitable native plants that exhibit either phytostabilisation or phytoextraction properties for either the suppression (if feasible in terms of cost and labour) or removal of excess molybdenum in areas with severe molybdenum contamination. Currently, few species are known that extract very high concentrations of molybdenum from the soil and are acceptable for use in British Columbia.

Summary of Literature Review and Objectives

The extent and seriousness of the molybdenosis in British Columbia remain unknown, although it can be surmised that there is likely a low risk to both domestic and wild ruminant populations. Once the conditioned is recognized, due to the ease of administering copper sulphate to quickly treat symptoms observed in domestic ruminants grazing in an enclosure and given the extensive ranges and diversified forage types utilized by wild ruminant populations. However, there is a lack of evidence of the effects of molybdenum on wild ruminants and particularly moose and mule deer, whose populations are drastically declining in select areas throughout the province for still-unknown reasons. A lack of data in the context of forage safety of threatened populations is therefore problematic, as animals in compromised health are more susceptible to nutrient deficiency, disease, and injury. The precautionary principle recommends taking preventive action in the face of uncertainty. Considering the uncertainties identified, the most prudent course of action to prevent the risk of molybdenosis through careful assessment, monitoring, and site remediation of areas of concern.

The review of the literature concerning molybdenosis in ruminants highlights the need for a land reclamation framework to mitigate the risk of molybdenosis to ruminants grazing in high-risk areas. The framework presented in the next section will serve to inform the reclamation and land-management decisions of regions with molybdenum-contaminated soil, water, and vegetation to conserve ruminant populations while improving cultural, recreational, and agricultural land capability in these regions throughout the province.

LAND RECLAMATION FRAMEWORK FOR MOLYBDENOSIS PREVENTION

Framework

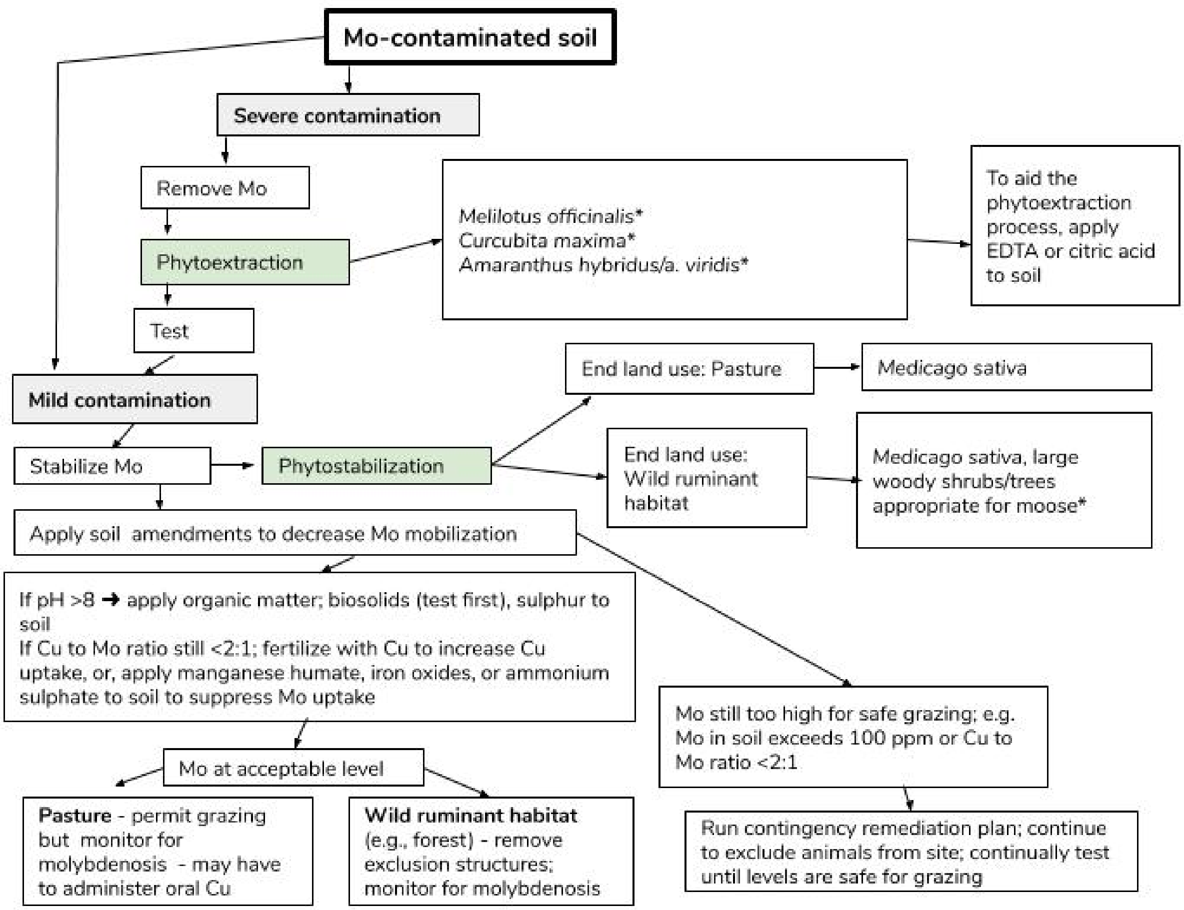

Figure 7: A decision-making framework for the remediation of molybdenum-contaminated sites to inform safe wild or domestic ruminant grazing, including phytoremediation and soil amendment strategies.

Application of Framework

This framework was developed based on the findings outlined in the literature review to mitigate the risk of molybdenosis in both wild and domestic ruminants. Before applying this framework to the site of interest, a comprehensive site assessment (including future land reclamation goals for the area) should be performed as a starting point. Land reclamation strategies suggested in this framework can be broadly divided base on two groups:

1) land reclamation for domestic ruminant (cattle) grazing and pastureland; and
2) land reclamation for wild ruminant (moose, mule deer) habitat and forage (e.g. forest)

The preferred approach to preventing molybdenosis would be the prevention of anthropogenic-sourced molybdenum, particularly from mine sites, from entering the environment in the first place. However, recommendations to accomplish this goal are beyond the scope of this report. For molybdenum contaminated land that has an end land use goal including ruminant grazing, whether that be agricultural cattle grazing or wild ruminant habitat, land reclamation plans should begin with a site-specific environmental risk assessment informed by clearly stated future land use goals, sources of molybdenum into the environment, potential receptor species, and the potential routes and nature of contaminant exposure. Careful environmental monitoring and assessment between steps are recommended for the results of remediation interventions and to inform the next steps.

If ruminants are presently utilizing the land as grazing habitat and if they are exhibiting symptoms of molybdenosis or soil tests reveal a low copper to molybdenum ratio, the first course of action is to immediately correct that ratio via the administration of oral copper or by copper fertilization of the pasture land. If it is domestic cattle or other domestic ruminants grazing on the site, symptoms are expected to quickly resolve with the oral administration of copper or copper sulphate.

For longer-term land reclamation interventions, the land itself must be modified to alter the availability of the molybdenum in soil by altering the copper to molybdenum ratio in soil, which can be achieved in several ways. Domestic and wild ruminants should be excluded from the area with appropriate fencing until remediation is complete to prevent the ingestion of molybdenum contaminated vegetation. For severe contamination, phytoextraction should first be employed. *Melilotus officinalis*, *Curcubita maxima*, *Amaranthus hybridus,* and *A. Viridis* are viable options for the phytoextraction of molybdenum – however, none of these species are native to the Southern Interior of British Columbia and may not be site-appropriate for use. The application of EDTA or citric acid can aid the phytoextraction process by increasing the bioavailability of molybdenum in soil. Soils should be tested through the phytoextraction process to monitor molybdenum levels; once desirable levels are achieved, plants should be harvested and appropriately disposed of.

For mild contamination of molybdenum, which could be the initial site state or occur after phytoextraction, the availability of molybdenum should be suppressed and stabilized. Sewage sludge (biosolids) or manganese humate should be applied to soils to decrease plant uptake of molybdenum. Then, phytostablizing plant species that are known to accumulate molybdenum only in below-ground tissues should be planted to prevent toxic levels of molybdenum from entering the food chain. Alternatively, plant species that transform molybdenum into non-toxic compounds in above-ground tissues can also be planted to stabilize molybdenum. Selection of appropriate species will be dictated by end-land use; *Medicago sativa* is suggested for pasture or domestic ruminant grazing, whereas additions of woody shrubs and trees that are appropriate for moose should be selected for wild ruminant habitat. Alfalfa is ideal for use in areas with ruminant grazing as it is highly palatable forage and converts molybdenum into a non-toxic organometallic complex. Other plants with phytostabilisation potential include *Zea mays*, *Melilotus officinalis*, and *Krascheninnikovia lanata*. However, these are agricultural species that may render them inappropriate for use in reclamation that seeks to create pasture or forest habitat. Where molybdenum contamination is still a concern, despite low soil concentrations, large-scale copper fertilization can be implemented to correct for low copper to molybdenum ratios in soils.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Molybdenum contamination from copper-molybdenum mine tailings and waste rock piles increase the risk of molybdenosis, a disease that causes hair loss, muscle spasms, and death, in ruminant populations exposed to forage growing on high-molybdenum forage or where the copper to molybdenum ratio is less than 2:1. Low copper concentrations in soil, high pH (greater than 8), and increased precipitation are three factors that increase the risk of molybdenosis by increasing molybdenum bioavailability and/or decreasing copper availability. Several molybdenum remediation techniques have been demonstrably effective in various locations, such as the application of copper fertilizer in Australian rangelands and the application of biosolids in Austria. They have been identified as having high potential for success in the Interior of British Columbia. A framework was developed from a thorough literature review that incorporated end land use goals, environmental factors, and disturbance type, to guide landowners and land reclamation practitioners to select appropriate remediation strategies for molybdenum-contaminated lands.

Conclusions

The expression of molybdenosis in ruminants is highly variable and depends on several species-specific and environmental factors, including pH, precipitation, soil copper content, and plant species utilized as forage. There are several remediation strategies identified to minimize the risks of molybdenosis to ruminants. Remediation strategies include the application of soil amendments to decrease the availability of molybdenum in the soil; phytoremediation plants that either stabilize molybdenum in and around the root zone and prevent uptake into the food web or extract high concentrations of molybdenum into tissues for later harvest and disposal; and copper fertilization to correct low copper to molybdenum ratios in soil. Depending on site and species considerations, the combination of remediation techniques may vary. In British Columbia, phytostabilizing amendments such as organic matter and biosolids can be utilized alongside the establishment of *Medicago sativa* (alfalfa) to reduce plant uptake and, therefore, ruminant ingestion of molybdenum.

Recommendations

- Mining companies should aim to prevent molybdenum contamination from occurring

- If molybdenum contamination is severe, consider the use of aided phytoextraction to remove excess molybdenum from soils, test results, and continue if necessary or proceed to phytostabilisation strategies
- If molybdenum contamination is not severe, aim to stabilize the molybdenum in the soil using phytostabilisation and soil amendments to suppress the uptake of molybdenum
- If the molybdenosis is caused by a lack of copper rather than a large excess of molybdenum, consider the application of a copper fertilizer to adjust the copper to molybdenum ratio at the site
- Vegetation should be selected to suite end land use goal, with a focus on non-invasive and native vegetation or forage species if applicable – this is an area of further study, as several plants identified in this report as potential phytoremediators of molybdenum are exotic

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