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Accumulation of Selenium by the Aquatic Biota of a Watershed Treated with Seleniferous Fertilizer

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Selenium (Se) is an essential trace element. Selenium deficiency and resultant pathologies are a problem in many domestic animal populations. It has been hypothesized that declines in wild animal populations, specifically in several deer herds in California, are attributable to selenium deficiency (Oliver et al. 1991). This study evaluated the bioaccumulation of selenium in aquatic systems in a deer forage range treated with a seleniferous fertilizer. The selenium supplementation program in the Little Antelope Valley was designed to test the general hypothesis that selenium deficiency may be contributing to the decline in deer populations in several areas of California. A pilot study was designed that would evaluate the accumulation and transfer of selenium in a terrestrial food web. The basic hypothesis was that selenium from an aerially applied seleniferous fertilizer would be accumulated by the native vegetation utilized by the deer for browse, and result in elevated deer blood selenium concentrations and increased fawn survival.

The potential exists in such a program for increased selenium exposure to nontarget systems, food webs and organisms. Selenium is a priority pollutant that has degraded freshwater aquatic systems throughout the United States. Aquatic ecosystems have been observed to be very sensitive to elevated concentrations of selenium. The observed impacts on the upper trophic levels of aquatic systems exposed to selenium is the result of selenium transfer through aquatic food webs. The demonstrated ecotoxic problems, chemistry, cycling, transformation, bioaccumulation, toxicity, background concentrations, and environmental risk assessment of selenium in freshwater systems have been reviewed by Maier and Knight (1994), and Lemly (1993).

This investigation was conducted to identify realized or potential aquatic ecotoxicological problems which could be attributed to the application of a seleniferous fertilizer to the winter range of deer. To address this objective four specific questions were formulated. 1) Are the background selenium concentrations in aquatic systems in the Little Antelope Valley low compared with national averages? 2) Is selenium accumulated in the aquatic systems of the Little Antelope Valley the result of seleniferous

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fertilizer application directly to selected portions of the watershed? 3) If selenium is accumulated, at what rate and to what concentration is it accumulated, and what is the residence time in the system? 4) If selenium is accumulated, is there any potential hazard associated with the resultant selenium concentrations in the aquatic systems?

MATERIALS AND METHODS

A commercial seleniferous fertilizer (Selcote™, Agtech Developments, NZ-Ltd., Richmond, NZ) containing 1% selenium by weight as sodium selenite was applied to an experimental area in the Little Antelope Valley, CA. The Little Antelope Valley is located in northern California on the eastern side of the Sierra Nevada mountain range. The treated area contains small (typically <1 m wide and <0.25 m deep), unnamed first and second order streams, which discharge into the Walker River.

The application of 1.2 kg/ha was verified by weighing fertilizer samples collected on plastic sheets placed in the treatment area. The fertilizer was aerially applied directly over the entire snow covered treatment area including the stream system. The direct application of seleniferous fertilizer to the aquatic system was intentionally done in this investigation to determine the environmental fate, bioaccumulation, and environmental risk of selenium used in this manner.

Samples were collected prior and subsequent to the application of the seleniferous fertilizer (Table 1). Samples were collected from three replicate stream sites that were determined during the pretreatment sample collection.

Water, macrophyte, and invertebrate samples were collected from the stream sites. All samples were collected in clean polyethylene bags, transported to the laboratory on ice and processed within 24 hours. Water samples were grab samples which were filtered through a 0.45 µm filter. Suspended particulate matter was not analyzed because of the lack of available material. Aquatic macrophyte samples were obtained by hand and initially rinsed in stream or pond water. Watercress (*Nasturtium* sp.) was the only macrophyte species present in the stream systems. In the laboratory plant samples were rinsed with EPA moderately hard water (USEPA 1975) to remove sediments and attached organisms. Each macrophyte sample contained portions of roots, shoots and leaves. Stream invertebrates were collected using a kick screen (Frost et al. 1971). The dominant stream invertebrates included caddisflies (tricoptera), scuds (amphipoda), snails (*Physa* sp.) and dragonflies (odonata). The invertebrates were rinsed with EPA moderately hard water. Composite samples were formed containing representatives of the dominant taxa in proportions approximating those encountered in the kickscreen. There were no large changes in the macroinvertebrate community apparent during the study.

The prepared samples were placed in preweighed digestion tubes and the wet weights calculated. All samples were predigested for >48 h in the nitric-perchloric acid digestion mixture. The samples were then digested using a standard nitric-perchloric acid digestion procedure. Selenium analyses were conducted using an automated fluorimetric method (Bern 1981). Selenium concentrations were converted from a wet weight basis to a dry weight basis for comparison to available data. Invertebrate and plant dry weight selenium concentrations were calculated by multiplying wet weight selenium concentrations by factors of 5 and 10, respectively (Saiki and Lowe 1987). Appropriate quality control and assurance measures were conducted. Blanks were below the detection limit (1.0 µg Se/L). Analytical standards (ranging from 2.0 to 10 µg Se/L) were measured at 96%±5% (avg±SD) recovery. The average difference between analytical duplicates was 4%. The measured concentration of selenium in standard tuna tissue (certified as 3.6±0.4 µg Se/g DW) was 3.7±0.3 µg Se/g DW (avg±SD).

Stream water, macrophyte and invertebrate selenium concentrations in samples collected during this study were statistically analyzed using a one-way ANOVA and Tukey's (HSD) test for comparison of means. For all tests $n = 3$ and $p \leq 0.05$. There was only one pretreatment sampling period due to various project constraints. However pretreatment and post treatment comparisons appear valid because pretreatment selenium concentrations were consistent with selenium concentrations reported from a variety of uncontaminated aquatic systems (Maier and Knight 1994).

RESULTS AND DISCUSSION

Water selenium concentrations (Table 1) from streams in the treatment area collected prior to the application of the seleniferous fertilizer were below 1.0 µg Se/L for all samples. The accepted detection limit for dissolved selenium using a fluorimetric method is currently 1.0 µg Se/L. The treated area stream water samples collected three hours after the fertilizer application contained 10.9 µg Se/L. In all subsequent treated area stream water samples, selenium concentrations were below the detection limit (1.0 µg Se/L).

Pretreatment stream macrophyte samples contained selenium concentrations of 0.62 Se/kg dry wt. Selenium concentrations increased rapidly (11 days) after fertilizer application. Treated area stream macrophyte selenium concentrations increased to 1.16 mg Se/kg dry wt. Macrophyte selenium concentrations increased 87% in the treated area stream systems compared to pretreatment selenium concentrations. Over the next six months treated area stream macrophyte selenium concentrations decreased to 0.43 mg Se/kg dry wt. Selenium concentrations increased in the final macrophyte samples to 0.94 mg Se/kg dry wt.

Table 1. Stream system selenium concentrations.

Date	Time	Water	Plant	Invertebrate
12/15	Pretrt	BD ^{B}	0.62±0.11 ^{C}	1.67±1.65 ^{B}
1/9	3 h post	10.9±0.7 ^{A}	NS	NS
1/20	11 d post	BD ^{B}	1.16±0.32 ^{A}	4.74±1.73 ^{A}
3/17	~2 m post	BD ^{B}	0.71±0.13 ^{B}	4.02±0.68 ^{A}
5/4	~4 m post	BD ^{B}	0.51±0.44 ^{B,C}	4.99±1.47 ^{A}
7/24	~6 m post	BD ^{B}	0.53±0.11 ^{C}	4.21±3.13 ^{A,B}
9/29	~8 m post	BD ^{B}	0.53±0.21 ^{C}	4.30±0.95 ^{A}
12/18	~11 m post	BD ^{B}	0.94±0.16 ^{A}	4.54±0.52 ^{A}

Values are averages ± one std. deviation (n=3)
Values with different letters within columns are statistically different
NS is not sampled
BD is below the detection limit of 1.0 µg Se/L
Water selenium concentrations in µg Se/L
Plant and invertebrate concentrations in mg Se/kg dry wt.

Invertebrates Composite invertebrate samples (hereafter referred to as invertebrates) collected before fertilization of the treatment area contained 1.67 Se/kg dry wt. The treated stream invertebrate selenium concentration increased 184% to 4.74 mg Se/kg dry wt. 11 d after the fertilizer application. Selenium concentrations in the treated area stream invertebrates ranged from 4.02 to 4.99 mg Se/kg dry wt. over the duration of this study.

Selenium concentrations in water from natural freshwater systems typically range from 0.1 to 0.4 µg Se/L (Maier and Knight 1994, Central Valley Regional Water Quality Control Board, 1990). Before the application of the seleniferous fertilizer water selenium concentrations at all study sites were below 1.0 µg Se/L. The detection limit for dissolved selenium using the methods in this study was 1.0 µg Se/L. By modifying the fluorimeter, the sensitivity of the analytical technique was increased and the selenium concentrations were determined to be approximately 0.25 µg Se/L. Water selenium concentrations of 0.25 µg Se/L are within the typical range reported for natural freshwater systems.

Three hours after the application of the fertilizer the concentration of selenium in the treatment area stream water samples averaged 10.9 µg Se/L. Subsequent water samples from the treatment area streams contained below detectable concentrations of selenium (<1.0 µg Se/L), indicating that a pulse or slug of selenium was present or moved through the system. The pulse of increased selenium concentration is typical for a

fertilizer treatment of this type. There are two parameters of concern when evaluating a pulse of a toxicant: amplitude (highest concentration) and duration (length of time the toxicant is present in the system). The amplitude or highest selenium concentration in the water during this event is unknown due to the limited sampling frequency. The duration is better defined. Initiation of the pulse was rapid (less than 3 h) indicating that the selenium leaches from the fertilizer quickly. The waterborne selenium concentrations returned to below detectable limits (1.0 µg Se/L) within 2 wk after treatment. This study did not determine whether the selenium concentrations returned to background concentrations (0.25 µg Se/L) within this time.

Presently there are no available studies examining the bioaccumulation or effects of pulsed selenium exposure on aquatic ecosystems.

Typical selenium concentrations in aquatic macrophytes from natural freshwater ecosystems are less than 0.60 mg Se/kg dry wt. Selenium concentrations in the initial macrophyte samples collected from stream sites were approximately 0.60 mg Se/kg dry wt. After selenium fertilization of the treatment area macrophyte selenium concentrations increased to 1.16 Se/kg dry wt. Macrophyte selenium concentrations in samples collected during subsequent sampling showed a decrease with an increase at the final sample date. Selenium was accumulated to the highest concentrations observed in the stream system within two weeks of seleniferous fertilizer application to the treatment area, indicating that selenium accumulation by aquatic macrophytes is rapid.

We hypothesize that selenium is accumulated rapidly during the initial exposure with resulting elevated tissue selenium concentrations. As vegetative growth occurs and selenium is limited, tissue selenium concentrations decrease due to a redistribution of selenium to the new vegetative material. The increase in selenium concentration at the final sample date is an interesting event. There is no simple explanation for this increase. What may be happening is that during the fall macrophytes, both aquatic and terrestrial, are senesing and selenium is being released, resulting in increased bioavailability and accumulation of selenium by the remaining plants or that

Plants from selenium impacted areas can accumulate significant concentrations of selenium, however studies evaluating the toxicity of selenium to aquatic macrophytes are limited. Based on the available data the macrophyte selenium concentrations observed do not appear to cause acute toxicological effects. The effects of chronic exposure of macrophytes to selenium has largely been ignored. Primary producer selenium accumulation is important due to the transformation and subsequent transfer of selenium in aquatic food chains. Selenium transfer through aquatic food chains is the primary route of exposure resulting in the observed impacts on the upper trophic levels of aquatic systems exposed to selenium (Maier and Knight 1994).

Selenium concentrations in invertebrates from uncontaminated freshwater ecosystems typically range from 0.5 to 1.5 mg Se/kg dry wt. (Ohlendorf 1989, Eisler 1985). Selenium concentrations in the pretreatment stream invertebrate samples were 1.67 Se/kg dry wt. Eleven days after seleniferous fertilizer application to the treatment area the stream invertebrate selenium concentrations increased to 4.74 mg Se/kg dry wt. and ranged from 4.02 to 4.99 for the duration of the study. These data indicate that selenium accumulation by the resident invertebrates was rapid, occurring by 11 d. Also the selenium concentrations in the stream invertebrate populations remained elevated, suggesting that the residence time of selenium in the stream system is a year or longer or that there may be a continuous low concentration input of selenium into the stream system. The addition and processing of terrestrial organic material in stream systems is usually significant. If this material, containing relatively low concentrations of selenium, were to enter the treated area stream then it could result in continued elevated invertebrate selenium concentrations.

As stated earlier there are no currently available studies evaluating the bioaccumulation of selenium in ecosystems exposed to pulses of this element. Published “safe” water concentrations of selenium in aquatic systems range from 0.1 to 5 µg Se/L. Skorupa and Ohlendorf (1991) have stated that waterborne selenium concentrations below 0.5 µg Se/L will be necessary to avoid selenium poisoning in sensitive bird populations *via* biomagnification. In a recent technical report the San Francisco Bay Regional Water Quality Control Board (1992) recommend an acceptable water column selenium concentration between 0.1 and 0.8 µg Se (as selenite)/L.

The stream invertebrate selenium concentrations were greater than 4 mg Se/kg dry wt. after seleniferous fertilizer application to the study area. Four mg Se/kg dry wt. in the dietary items of fish and birds is a concentration of concern as defined by the California Department of Fish and Game. Skorupa and Ohlendorf (1991) determined that birds feeding in aquatic habitats with foodborne selenium concentrations greater than 2.9 mg Se/kg dry wt. experienced reduced egg hatchability. Malchow et al. (1995) determined that selenium concentrations above 2.5 mg Se/kg dry wt. resulted in significantly decreased growth rates in larval midges (*Chironomus decorus*) fed a diet of seleniferous algae (*Selenastrum capricornutum*) during laboratory food chain studies. Although we did not observe any acute toxic effects in the study systems there is the potential for an ecotoxic problem based on available laboratory data and published concentrations of concern by regulatory agencies. Additional studies to adequately evaluate the bioaccumulation and toxicity of selenium after field level supplementation are recommended.

The results of this study support the following conclusions. 1) The selenium concentrations in the aquatic systems in the treated area of the Little Antelope Valley are within the typical ranges reported for natural

freshwater systems. 2) Selenium seems to be accumulating in the invertebrate components of the aquatic ecosystems in the Little Antelope Valley as a result of the application of seleniferous fertilizer directly to selected portions of the watershed. 3) Selenium was bioaccumulated rapidly by the aquatic invertebrates (< 2 wk) and the residence time of selenium in the stream invertebrate populations appears to be longer than 1 yr. 4) No acute toxic effects were noted in the invertebrate communities; however, the selenium concentrations observed in the treated stream invertebrate samples have been demonstrated to be toxic to invertebrates in laboratory studies and have been identified as concentrations of concern by the California Department of Fish and Game and other regulatory agencies.

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REFERENCES

Bern EM (1981) Determination of selenium in the environment and in biological material. Environ Health Perspect 37:183-200

Central Valley Regional Water Quality Control Board (1990) Trace element concentrations in selected streams in California: A synoptic survey. California Regional Water Quality Control Board, Central Valley Region, Sacramento, CA.

Eisler R (1985) Selenium hazards to fish, wildlife and invertebrates: A synoptic review. US Fish Wildl Serv Biol Rept 85(1.5). US Fish and Wildl Serv, Dept of the Interior, Washington, DC.

Frost S, Hun A, Kershaw W (1971) Evaluation of a kicking technique for sampling stream bottom fauna. Can J Zool 49:167-170

Lemly AD (1993) Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. Environ Monitor Assess 28:83-100

Maier KJ, Knight AW (1994) Ecotoxicology of selenium in freshwater systems. Rev Environ Contam Toxicol 134:31-48

Malchow DE, Knight AW, Maier KJ (1995) Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum.* Arch Environ Contam Toxicol 29:104-109

Ohlendorf, HM (1989) Bioaccumulation and effects of selenium in wildlife. In: Selenium in agriculture and the environment. Soil Science Society of America Special Publication no. 23.

Oliver MN, Ros-McGauran G, Jessup DA, Norman 99, Franti CE (1991) Selenium concentrations in blood of free-ranging mule deer in California. Trans West Sect Wildl Soc 26:87-90

Saiki MK, Lowe TP (1987) Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch Environ Contam Toxicol 16:657-670

San Francisco Bay Regional Water Quality Control Board (1992) Technical Report: Derivation of site-specific water quality criteria for selenium in San Francisco Bay. San Francisco Bay Regional Water Quality Control Board, California Environmental Protection Agency, Oakland, CA

Skorupa JP, Ohlendorf HM (1991) Contaminants in drainage water and avian risk thresholds. In: Dinar A, Zilberman D (eds) The Economy and Management of Water and Drainage in Agriculture. Kluwer Academic Publishers, Not-well, MA, pp 345-368

United States Environmental Protection Agency (1975) Methods for acute toxicity tests in fish, macroinvertebrates, and amphibians. EPA 660/3-75-009. Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR.