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Soil and Plant Lead of Upland Habitat Used Extensively for Recreational Shooting and Game Bird Hunting in Southern Ontario, Canada

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Since the discovery of cupellation, lead has been widely used in the manufacture of many commercial products such as, batteries, solder, pipes, paints and ammunition. This malleable, relatively inexpensive toxic metal has been used to produce approximately 3 million metric tons of ammunition for hunting and recreational shooting in the 20th century in the USA alone (Craig et al. 1999). Recently in North America and several European countries, the use of lead shotshell has been banned for waterfowl hunting due to the toxic nature of lead (Pain 1992; USFWS 1988; Canadian Wildlife Service, August 19, 1997, News Release). Prior to the Canada-wide ban of lead shotshell for the hunting of all migratory birds on September 1, 1999, an estimated 2000 metric tons per year of lead pellets were deposited on Canadian soil (Scheuhammer and Norris 1995). However, lead shotshell has not been banned for the hunting of upland game birds and small mammals, and no restrictions exist for the use of lead shotshell for clay target shooting. Thus, lead pellets are being deposited in North American upland habitat at historic rates.

Although lead is a toxic metal, it was thought to be relatively inert in soil; thus, unavailable to organisms (Katagishi and Yamane 1981). Indeed, lead pellets recovered from soil have been shown to be covered by a white, gray or brown crust of relatively insoluble lead compounds, such as hydrocerussite ($Pb(CO_3)_2(OH)_2$), cerussite ($PbCO_3$), and anglesite ($PbSO_4$) (Jorgensen and Willems 1987). However, several more recent studies have shown that lead from leaded ammunition is bioavailable in the environment (e.g., Ma 1989; Mozafar et al. 2002) and that the solubility of lead is dependent on soil or water pH (Jorgensen and Willems 1987; Stansley et al. 1992). Moreover, it has been shown in some plant species that an increase in lead concentration in soil results in a decline in nitrate reductase activity, chlorophyll, and protein content but an increase in peroxidase activity (Saxena et al. 2003). In addition, lead in soil may negatively affect the survival of mycorrhizal fungi associated with some plant species (Mozafar et al. 2002). Lead contaminated sediment has also been shown to cause lead poisoning in mallards (*Anas platyrhynchos*) and mute swans (*Cygnus olor*) that ingested contaminated sediment (Heinz et al. 1999; Day et al. 2003). It is clear that lead in soil can have detrimental effects on organisms.

Soil lead data exist for wetland habitats (e.g., Lund et al. 1991; Tsuji and Karagatzides 1998) and shooting ranges (e.g., Bisessar 1992; Manninen and Tanskanen 1993), but little is known with respect to heavily hunted upland habitats (Scheuhammer and Norris 1995; Scheuhammer et al. 2003). More data are needed by policy makers so that they can make an informed decision on whether further restrictions on the use of lead shotshell are required (Craig et al. 1999). Indeed,

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Darling and Thomas (2003) examined the spatial distribution of 135 outdoor shotgun shooting ranges in Ontario, Canada, and identified potential sites of concern with respect to environmental mobilization of lead from spent pellets. In this paper, we determine the distribution of lead in soil and vegetation from a heavily hunted area of upland habitat in southern Ontario, Canada. This study is one of a series (e.g., Tsuji et al. 1997, 2002; Tsuji and Karagatzides 1998, 2001) exploring the environmental consequences of using leaded ammunition to harvest wild game.

METHODS AND MATERIALS

Samples were collected from upland habitat located on a private island in southern Ontario, Canada. This study area has been heavily hunted with respect to indigenous upland game and imported farm-raised game birds for release-and-shoot hunting, since the 1930s. Sample collection was conducted in July of 2000. Five areas were systematically selected based on hunting activity: the "Shooting Station" has been used primarily for clay target shooting; "Field A" has been heavily hunted on for upland game birds and is tilled annually; "Field B" has been heavily hunted on for upland game birds but has not been tilled for the two years prior to sample collection; "Field C" has been heavily hunted over for upland game birds and has never been tilled; and the "Fly Pen Enclosure" has been used to hold upland game birds prior to being released. At each site, a 0.25 m^2 square area was demarcated and above-ground plant material (if present) was clipped (to within 1 cm of the ground with stainless steel scissors); soil samples were matched for location with plant samples and collected to a depth of 10 cm, using a stainless steel scoop. Four samples were taken for all areas (~ 15 m intervals along a transect), except Field B (n =1) and the fly pen enclosure (n = 1). These soils were under-sampled due to the physical difficulty of excavating the highly compacted soil. Replicate samples were taken in some areas because it has been well documented that spent lead pellets are distributed unevenly in the environment (Tsuji and Karagatzides 1998). All samples were stored frozen at -20°C in marked Ziploc™ bags. Below-ground plant material (>2 mm) was collected from the soil samples after thawing. Soil particles were gently shaken and removed from below-ground plant material. No lead pellets were found adhered to plant material. Plant material was not washed prior to lead analysis.

Soil color was determined using a Munsell color chart, while texture was assessed using samples from the Ontario Institute of Pedology (1985). Organic content of the soil was determined by loss on ignition (550°C for 3 hrs). Calcareous class was estimated by efferescence with 10% HCl. Bromythymol blue was used to estimate soil pH. Prior to lead analysis, soil samples (before air drying) were manually homogenized with a mortar and pestle. Soil samples were air dried and then sieved to pass through a 100-mesh (150 μm) screen and the pellets collected and counted. All pellets collected were lead pellets; only lead pellets have been used for hunting and sport activities in this upland habitat area. Above-ground vegetation was identified to genus. Plant samples were oven dried to constant weight at 95°C and ground in a spice mill with stainless steel blades.

All tissue and soil samples were weighed (0.20 g) and placed into 15-mL test-tubes (Sarstedt). Two mL of HNO_3 (Ultrex, JT Baker) and 2-mL of ultrapure water (distilled double-deionized water [DDW]) were added into each test-tube. Test-tubes were sealed with lid locks (DiaMed) and left overnight in a fumehood to allow initial acid digestion of the sample. Samples were then placed into a 12-hole test-tube heating block (Multi-Blok, Lab-Line) and digested at an initial temperature of 60°C for 1 hr, followed by 5-7 hrs at 80°C. Samples were allowed to cool (approximately 10 min at room temperature). The entire content of each test-tube was filtered

through a Whatman 540 Harden Ashless Filter and subsequently transferred into 15-mL test tubes (Pyrex) with DDW. Samples were vortexed and placed in a test tube rack until the determination of lead by electrothermal atomic absorption spectrometry (EAAS). Certified reference standards (National Research Council of Canada, Mess-3 soil; National Bureau of Standards 1572, citrus leaves) were run with every 10 samples. Recovery of lead was on average within 10% of the expected value for soil and citrus leaves. Duplicate samples for soil (n = 3) and vegetation (n = 3) were also run; duplicates were on average within 10% of the original readings. Blanks were run with each digestion.

Working standards of 0, 2.5, 5, 10, 15, 25, 50 and 100 μg/L lead were prepared from a 2000 μg/L lead intermediate standard prepared by diluting 1 mL of the Lead Reference Standard (1000 mg/L; Ricca Chemical Company) followed by the addition of 0.72 mL of concentrated HNO_3 (Ultrex, JT Baker) and dilution to 500 mL with DDW. Working standards were prepared by adding 0 to 1250 μL of intermediate lead standard, plus 25 μL Triton solution, then dilution to 50 mL with 0.1% HNO_3.

Sample digests were analyzed using a Varian Electrothermal Atomic Absorption Spectrometer (model Spectra AA 220) with graphite furnace. No modifier was used. Prior to the analysis of sample digests and during each sample run, calibration curves were recorded after every 10 samples to ensure that the spectrometer was fully optimized (±10%). Lead levels were determined by reference to the linear equation for the calibration curve recorded for the appropriate 10-sample batch.

To normalize data for pellet density, a log + 1 transformation was performed to eliminate zeros from the dataset, while soil lead data were log transformed. Untransformed, above and below-ground vegetation lead data were normal. Linear regression analysis was used to determine the relationship between log soil lead levels and lead pellet density. One outlier was identified; it had a standardized residual of 2.2 which exceeds the critical level of ±2.0. However, the outlier was not influential on the slope of the line as its DFBETAS of -0.41 did not exceed the critical value of ±0.53 which is derived as $2/\sqrt{n}$ (Neter et al. 1996). Three additional relationships were also explored: above-ground vegetation lead versus log soil lead; below-ground vegetation lead versus log soil lead; and above-ground vegetation lead versus below-ground vegetation lead

RESULTS AND DISCUSSION

The 14 soils were brownish, silty with very fine sand and loam in some sites (Shooting Station 2, 3; Field C 1, 2, 4). The pH ranged from 6.8 to 7.5 (Table 1), neutral to slightly alkaline and the organic content ranged from 9 to 31%. Nine sites were strongly to extremely calcareous based on the effervescence test. The other sites (Shooting Station 2, 3; Field C 1, 4) were non- or weakly calcareous. No data exist for Field C 3 for calcareous class. Soil lead concentrations were in the range of 3 to 199 μg/g ($\bar{x}$±sd, 69±80 μg/g; Table 1); data are comparable to shooting ranges in another area of southern Ontario (Bisessar 1992; range, 41-325 μg/g) and those in California, USA (Hui 2002; range of $\bar{x}$, 6.6-120 μg/g). Only four sites (Shooting Station 1, 177 μg/g; Shooting Station 2, 183 μg/g; Shooting Station 4, 172 μg/g; Field B, 199 μg/g; Table 1) in the present study had lead concentrations in soils that exceeded the 150 μg/g upper limit of normal value established by the Ontario Ministry of the Environment for soil sampled in rural Ontario (Bisessar 1992). The upper limit of normal value is the expected maximum concentration of lead in soil

Table 1. No. of lead pellets per 0.25 m^2, pH of soils, soil-lead concentration, above-ground and below-ground vegetation lead concentration in upland habitat used for recreational shooting and game bird hunting, in southern Ontario, Canada.

Site	pH	no. of pellets ($0.25m^2$)	Soil Pb[a] (μg/g)	Vegetation Pb[a,b] (μg/g dw)	
				Above-ground	Below-ground
Shooting Station 1	7.3	1557	177	-	-
Shooting Station 2	7.3	759	183	-	-
Shooting Station 3	7.0	2051	118	-	-
Shooting Station 4	7.1	1646	172	-	-
Field A 1	7.1	0	8	3.38	2.80
Field A 2	7.1	8	5	2.02	0.72
Field A 3	7.1	0	23	2.15	0.84
Field A 4	7.3	0	11	1.45	-
Field B	7.5	3	199	0.49	-
Field C 1	7.5	12	35	2.64	2.38
Field C 2	7.5	14	4	2.89	1.16
Field C 3	-	1	23	2.00	0.70
Field C 4	6.8	0	3	1.46	1.92
Fly pen enclosure	7.0	1	9	0.37	-

[a]ULN, the upper limit of normal is the expected maximum concentration of lead in soil or vegetation (i.e., 150 μg/g) from unpolluted areas in rural Ontario, Canada (Bisessar 1992).
[b]In remote areas of northern Canada, lead in plants have been shown to be <15 μg/g dw (Dushenko et al. 1996).

or vegetation from unpolluted areas in rural Ontario, Canada (Bisessar 1992). It is noteworthy that three of the four elevated sites for soil lead were used for clay target shooting (Shooting Station 1, 2, 4) and are comparable to shooting ranges. These three sites were also found to have relatively large number of lead pellets per area (range, 759-1646 pellets/$0.25m^2$; Table 1). The fourth site with elevated soil lead was an area heavily hunted over for upland game birds (i.e., Field B). It is interesting that tilled sites with similar hunting activity, in the same general vicinity of the fourth site (i.e., Field A1-4; Table 1), had few lead pellets (range, 0-8 pellets/$0.25m^2$) and relatively lower levels of soil lead (range, 5-23 μg/g). Perhaps, tilling not only reduces the access of game birds to lead shot but also decreases the concentration of lead in the soil by mixing lead-contaminated soil with the soil underneath.

In contrast to the present study, Emerson (1994) found soil-lead concentrations in a shooting range near St. Thomas, southern Ontario, to be substantially higher, up to 6700 μg/g. These results can be explained when it is noted that the soils in the

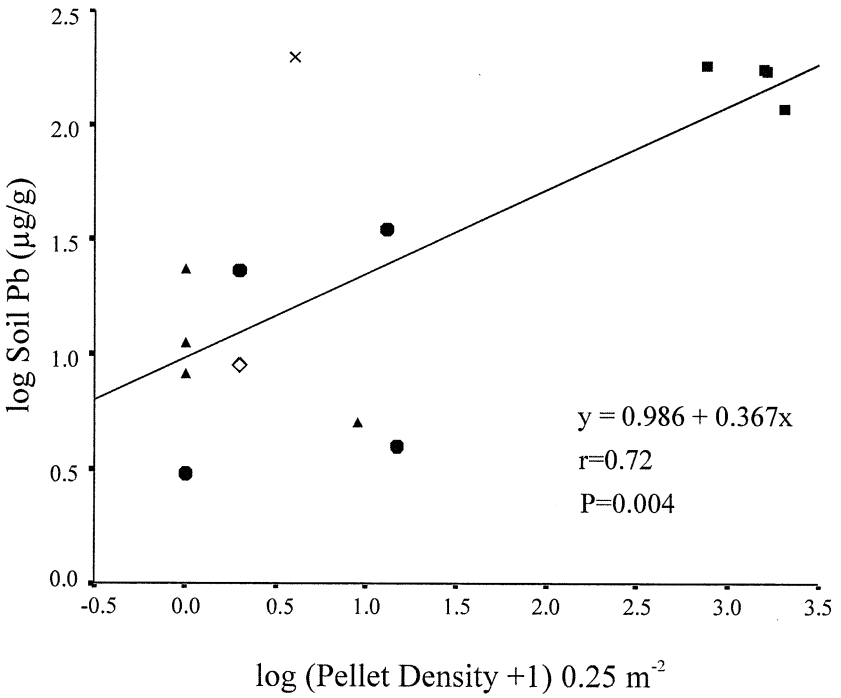

Figure 1. Relationship between pellet density and soil-lead levels (symbols: x, Field B; ▲, Field A; •, Field C; ■, the Shooting Station; and ◇, Fly Pen Enclosure).

Emerson (1994) study were sometimes acidic (pH = 5.5). It has been well documented that pH of soil is a major determinant of mobility and bioavailability of lead (Jorgensen and Willems 1987; Darling and Thomas 2003). Further, Manninen and Tanskanen (1992) have shown that acid rain (range of pH, 4.0-4.7) in Scandinavia was an important factor influencing lead concentrations in soils (47 000-54 000 µg/g) of a shooting range. Thus, the neutral to alkaline soils (range of pH, 6.8-7.5) found in the present study would help explain the relative inertness of the lead pellets noted in the soil. In addition, most sites were extremely calcareous, allowing for the buffering of acid rain that has been reported for southern Ontario (Kumar et al. 2001). Similarly, low levels of lead in soils/sediments contaminated by lead shot have been reported for alkaline wetlands in western Australia (Lund et al. 1991) and northern Ontario, Canada (Tsuji and Karagatzides 1998).

A significant positive relationship ($r=0.72$, $P=0.004$, Figure 1) was noted for lead concentration in soil and lead pellet density. Thus, approximately 52% of the variation in soil lead levels was attributable to variation in lead pellet density. Similarly, Hui (2002) has reported a significant positive relationship ($r=0.95$, $P \leq 0.05$) between soil lead concentrations and lead pellet densities at a skeet range.

Ten of the 14 sites were monospecific stands containing only grass *(Gramineae)*, while the other plots also contained wheat (*Agropyron* spp.; Field A 3), burdock (*Arctium* spp.; Field B), and thistle (*Sonchus* spp.; Field C 3, 4). Lead concentration in below-ground (range, 0.72-2.80 µg/g dw; $\bar{x} \pm$ sd, 1.50±0.86 µg/g dw; Table 1) and

above-ground (range, 0.37-3.38 µg/g dw; $\bar{x}\pm$sd, 1.88±0.97 µg/g dw) vegetation were comparable to those reported by Hui (2002) for plants on a skeet range (beard grass, *Polypogon* spp.; $\bar{x}\pm$ sd, 2.77±0.63 µg/g dw) and Dushenko et al. (1996) for remote northern Canadian sites (<15 µg/g dw). Further, present values were well below the upper limit of normal (150 µg/g; Bisessar 1992) and lower than the range (10-30 µg/g dw) considered acceptable for consumption of plant tissue (Mozafar et al. 2002). Non-significant relationships were found between log soil lead content and concentration of lead in below-ground vegetation (P=0.98, n=7), and concentration of lead in above-ground vegetation (P=0.35, n=10). The relationship between lead concentration in below-ground vegetation and above-ground vegetation was also not significant (P=0.24, n=7). However, it should be noted that sites with the higher soil-lead concentrations did not contain any vegetation. Four of five samples were from the Shooting Station sites and the other sample was the only one collected in Field B. Physical damage due to the impact of pellets may have contributed to the lack of vegetation at the Shooting Station sites.

The results of the present study are the first to show that a heavily hunted upland game bird area can be contaminated to the extent where soil lead concentration (199 µg/g) approaches the generic soil-lead limit (≥200 µg/g requires environmental review) established by the Ontario Ministry of Environment and Energy ([OMEE] 1996) for residential and parkland use. However, present data also suggest that lead pellets remain relatively inert in the alkaline upland habitat studied compared to more acidic areas in southern Ontario. Nevertheless, this situation could change dependent on future deposition of lead pellets on these sites and the ability of the calcareous soils to buffer acid rain.

REFERENCES

Bisessar S (1992) Phytotoxicological survey report: Scarborough Rod and Gun Club - Uxbridge 1992. Phytotoxicology Section, Ontario Ministry of Environment and Energy, Toronto, Canada

Craig JR, Rimstidt JD, Bonnaffon CA, Collins TK, Scanlon PF (1999) Surface water transport of lead at a shooting range. Bull Environ Contam Toxicol 63:312-319

Darling CTR, Thomas VG (2003) The distribution of outdoor shooting ranges in Ontario and the potential for lead pollution of soil and water. Sci Tot Environ 313:235-243

Day DD, Beyer WN, Hoffman DJ, Morton A, Sileo L, Audet DJ, Ottinger MA (2003) Toxicity of lead-contaminated sediment to mute swans. Arch Environ Contam Toxicol 44:510-522

Dushenko WT, Grundy SL, Reimer KJ (1996) Vascular plants as sensitive indicators of lead and PCB transport from local sources in the Canadian Arctic. Sci Total Environ 188:29-38

Emerson R (1994) Contamination of soil from gun shot: St. Thomas Gun Club 1993. Phytotoxicology Section, Ontario Ministry of Environment and Energy, Toronto, Canada

Heinz GH, Hoffman DJ, Sileo L, Audet DJ, LeCaptain LJ (1999) Toxicity of lead-contaminated sediments to mallards. Arch Environ Contam Toxicol 36:323-333

Hui CA (2002) Lead distribution throughout soil, flora, and invertebrate at a wetland skeet range. J Toxicol Environ Health Part A 65:1093-1107

Jorgensen SS, Willems M (1987) The fate of lead in soils: the transformation of lead pellets in shooting-range soils. Ambio 16:11-15

Katagishi K, Yamane I (1981) Heavy metal pollution in soils of Japan. Japan Science Society Press, Tokyo, Japan, 302 pp

Kumar AJ, Gough WA, Karagtzides JD, Bolton KA, Tsuji LJS (2001) Testing the validity of a critical sulphur and nitrogen load model in southern Ontario, Canada, using soil chemistry data from the Monitoring Acid Rain Youth Program. Environ Monitor Assess 69:221-230.

Lund M, Davis J, Murray F (1991) The fate of lead from duck shooting and road runoff in three western Australian wetlands. Aust J Mar Freshwater Res 42:139-149

Ma WC (1989) Effect of soil pollution with metallic lead pellets on lead bioaccumulation and organ/body weight alterations in small mammals. Arch Environ Contam Toxicol 18:617-622

Manninen S, Tanskanen N (1993) Transfer of lead from shotgun pellets to humus and three plant species in a Finnish shooting range. Arch Environ Contam Toxicol 24:410-414

Mozafar A, Ruh R, Klingel P, Gamper H, Egli S, Frossard E (2002) Effect of heavy metal contaminated shooting range soils on mycorrhizal colonization of roots and metal uptake by leek. Environ Monitor Assess 79:177-191

Neter J, Kutner MH, Nachtsheim CJ, Wasserman (1996) Applied linear regression models. Third edition. Irwin, Chicago, USA, 720 pp

OMEE (1996) Surface soil and groundwater criteria for agricultural, residential/parkland, industrial/commercial land use for a potable ground water condition. Standards Development Branch, Ontario Ministry of Environment and Energy, Toronto, Canada

Ontario Institute of Pedology (1985)

Pain DJ (1992) Lead poisoning in waterfowl: Summary of national reports. Int Waterfowl Wetlands Res Bur Spec Pub 16:86-99

Saxena A, Saxena DK, Srivastava HS (2003) The influence of glutathione on physiological effects of lead and its accumulation in moss *Sphagnum squarrosum*. Water Air Soil Pollut 143:351-361

Scheuhammer AM, Norris SL (1995) The environmental impacts of lead shotshell ammunition and lead fishing sinker products in Canada. Canadian Wildlife Service, PQ, Canada

Scheuhammer AM, Bond DE, Burgess NM, Rodriques J (2003) Lead and stable lead isotope ratios in soil, earthworms, and bones of American woodcock (*Scolopax minor*) from eastern Canada. Environ Toxicol Chem 22:2585-2591

Stansley W, Widjeskog L, Roscoe DE (1992) Lead contamination and mobility in surface water at trap and skeet ranges. Bull Environ Contam Toxicol 49:640-647

Tsuji LJS, Karagatzides JD (1998) Spent lead shot in the western James Bay region of northern Ontario, Canada: soil and plant chemistry of a heavily hunted wetland. Wetlands 18:266-271

Tsuji LJS, Karagatzides JD (2001) Chronic lead exposure, body condition, and testis mass in wild mallard ducks. Bull Environ Contam Toxicol 67:489-495

Tsuji LJS, Nieboer E, Karagatzides JD, Kozlovic DR (1997) Elevated dentine lead levels in adult teeth of First Nation people from an isolated region of northern Ontario, Canada. Bull Environ Contam Toxicol 59:854-860

Tsuji LJS, Karagatzides JD, Hanning RH, Katapatuk B, Young J, Nieboer E (2002) Dentine-lead levels and dental caries in First Nation children from the western James Bay region of northern Ontario, Canada. Bull Environ Contam Toxicol 70:409-415

USFWS (1988) Final Supplemental environmental impact statement: Issuance of annual regulations permitting the sport hunting of migratory birds. US Department of the Interior, Washington, DC, USA