Assessment of intake of copper and lead by sheep grazing on a shooting range for small arms: A case study

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1 Abstract

The Norwegian Armed Forces' shooting ranges contain contamination by metals such as lead (Pb) and copper (Cu) and are often used as grazing pastures for livestock. To determine whether the sheep were at risk from grazing at a shooting range in Nord-Trøndelag (the Leksdalen shooting field), a study was conducted wherein the aim was to determine the amount of soil the sheep were eating, the accumulation of Cu and Pb in the livers of lambs grazing on the shooting ranges, and the accumulation of Pb and Cu in the grass.

The grazing behavior of the sheep was mapped using GPS tracking and wildlife cameras. Soil, grass, feces, and liver samples were collected. All the samples were analyzed for Pb, Cu and Molybdenum (Mo), and soil and feces were also analyzed for titanium (Ti). Mean concentrations in grass, soil, feces, and liver was 41–7189, 1.3–29, 4–5, and 0.3 mg/kg Pb, respectively, and 42–580, 4.2–11.9, 19–23, and 273 mg/kg Cu, respectively. The soil ingestion rate was calculated using Ti in feces and soil. From these results, the theoretical dose of Cu and Pb ingested by grazing sheep was calculated.

The soil ingestion rate was found to be 0.1–0.4%, significantly lower than the soil ingestion rate of 5–30% usually used for sheep. Little or no accumulation of Cu and Pb in the grass was found. There was no difference between the metal concentrations in the washed and unwashed grass. According to the calculated dose, the sheep were at little or no risk of acute or chronic Pb and Cu poisoning from grazing on the Leksdalen shooting range. The analysis of liver samples showed that lambs grazing on the shooting range did not have higher levels of Cu or Pb than lambs grazing elsewhere. None of the lambs had concentrations of Cu or Pb in their livers indicating poisoning.

Keywords

Lead; Copper; Shooting range; Sheep; Soil ingestion; Grazing

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2 Introduction

Ammunition from small arms contains potentially toxic metals such as copper (Cu) and lead (Pb). After years of use, the soil on shooting ranges contains high concentrations of metals from spent ammunition. A mean annual deposition of 48, 68, 6, and 5 metric tons of Pb, Cu, Antimony (Sb), and zinc (Zn), respectively, in Norwegian military small arms shooting ranges for the years 2002–2010 has been calculated (Myhre et al. 2013). The shooting ranges are rarely fenced and are often used as grazing pastures for livestock, which is also the case in other countries, such as Switzerland (Tandy et al. 2017). Grazing on shooting ranges has been viewed positively as it prohibits the ranges from becoming overgrown (Gaertner et al. 2010). The ranges are also often considered to have high feed quality, even though they may be highly contaminated by Pb and Cu and to some degree Sb and Zn.

Pb and Cu are two of the most common substances causing poisoning in grazers (Payne and Livesey 2010; Payne et al. 2004) and there are several known cases of sheep being poisoned by these metals while on pasture (Liu 2003; Oruc et al. 2009). In sheep, the most frequent form of poisoning is Cu poisoning (~70%) (Guitart et al. 2010; VIDA 2006; VIDA 2014). This is most commonly caused by intake of plants sprayed with fungicide containing Cu, grazing on contaminated areas, or intake of plants with naturally high Cu content (Perrin et al. 1990). The most frequent cause of Pb poisoning in grazing animals is exposure to leaded batteries and other waste left in the open or abandoned in fields and soil contaminated by Pb (Payne and Livesey 2010; Sharpe and Livesey 2004). The Veterinary Laboratory Agency in the UK investigated 454 incidents of livestock poisoning by Pb and found that 117 were from leaded batteries and 133 from leaded paint, whereas 112 incidents were geochemical (originating from soil or rock), 31 metallic (mainly anthropogenic), and the rest from other or unconfirmed sources (Payne and Livesey 2010).

Pb is not an essential metal for plants or animals and can be toxic even in small amounts. The toxicity of Pb was already known and described more than 2,000 years ago (Lessler 1988). Chronic poisoning of grazers is uncommon (NAS 1980); acute poisoning of cattle, however, is not (Leary et al. 1970). In acute poisoning, the largest concentration of Pb is found in the liver and kidneys, while for chronic exposure it is found in bone (Wilkinson et al. 2003; Zmudski et al. 1983). Signs of acute poisoning include blindness, ataxia, cramps, aggression, muscle tremors, spasms, anorexia, salivation, stomach pain, diarrhea, and constipation (Sharpe and Livesey 2004). Cu is an essential trace element, but it can cause poisoning in high concentrations. Although

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acute Cu poisoning can occur, chronic poisoning is more frequent and can be seen in older animals that have accumulated Cu in the liver over a long period of time (Froslie et al. 1985; Sivertsen and Plassen 2004). Sheep are particularly vulnerable to Cu intoxication as they have limited capacity for Cu storage in the liver (Bradley 1993).

Analyses of the grazing behavior of sheep have been conducted on Pb- and Cu-contaminated areas, such as mining areas in mid-Wales (Abrahams and Steigmajer 2003; Smith et al. 2009) and in Spain (Pareja-Carrera et al. 2014). As far as we know, no studies have previously assessed the risk of Cu and Pb poisoning of animals pasturing at training areas for small arms. Domestic animals pasturing at shooting ranges may be at a particular risk through ingestion of contaminated soil carried by vegetation. A previous study in mid-Wales found a median soil ingestion rate of 7.6% dry matter (dm) for sheep (Smith et al. 2009). Typically, the concentrations of Pb and Cu in soils on a shooting range may be much higher than 1000 mg/kg dm (Bannon et al. 2009; Mariussen et al. 2017; Mozafar et al. 2002). Considering their estimated daily food intake of 1.3 kg (Rupflin and Krebs 2015) and high soil ingestion rate, there is reason to believe that sheep may be subjected to considerable levels of contaminants. To gain further insight into whether grazing on shooting ranges puts sheep at risk of Cu and Pb poisoning, a preliminary case study on a Norwegian military shooting range for small arms was conducted in the summer of 2016. One aim was to improve the risk assessment of such areas, which can minimize expensive and unnecessary remediation measures. The study evaluated grazing behavior and preference. The soil–animal pathway of metal ingestion was evaluated using analysis of the soil concentration and soil ingestion rate. The soil–plant–animal pathway was evaluated by analysis of Pb and Cu accumulation in grass. In the grass, the concentration of molybdenum (Mo) was also analyzed. The Cu/Mo rate is important, as Mo binds Cu and thereby makes it less bioavailable (Buck and Sharma 1969; Hidiroglou et al. 1984). The metal contamination on shooting ranges is not homogeneously distributed and is typically found in hotspots consisting of both contaminated soil and metal particles. Toxic doses of Cu and Pb for livestock used in risk assessments today are typically derived from studies using metal salts rather than from metals in soil as is the case here. These forms of the metals might therefore not have the same bioavailability as the metals present on contaminated shooting ranges. In this study, one of the aims was therefore to compare the calculated metal dose intake of the sheep to the actual metal uptake taken up by measuring the metal content in liver samples from the animals.

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3 Materials and methods

3.1 Study area

The study area was a training area for small arms named Leksdal in Nord-Trøndelag County, in the middle of Norway (see Figure 1 in the supplementary material), which has been operative since 1895. Leksdal shooting range occupies an area of 630ha and has a safety zone of 1,400ha. The vegetation on the range that has not been removed due to shooting activity consists of coniferous forest and marsh. It has been estimated by the Norwegian Defense Research Establishment that 4.6 metric tons of Pb and 39.5 metric tons of Cu from spent ammunition was deposited in Leksdalen between 2007 and 2017. The Norwegian armed forces have phased out Pb in their ammunition, therefore a higher deposition of Pb previous years (before 2007) can be assumed. Three ranges for small arms were selected (out of 10) to be monitored (Figure 1); ranges J, R, and U. Range J was dominated by fine-grained soil and range R by coarse-grained soil species and marsh, while U consisted mainly of partly converted moss. Range R had a dense and lush layer of vegetation. Range U consisted mostly of moss, with some grass. The vegetation on range J was lush but had wounds from shots in the berm. The sheep grazed in the Leksdalen area between late May or early July and early October. The metal contamination of the ranges had not been mapped prior to the study.

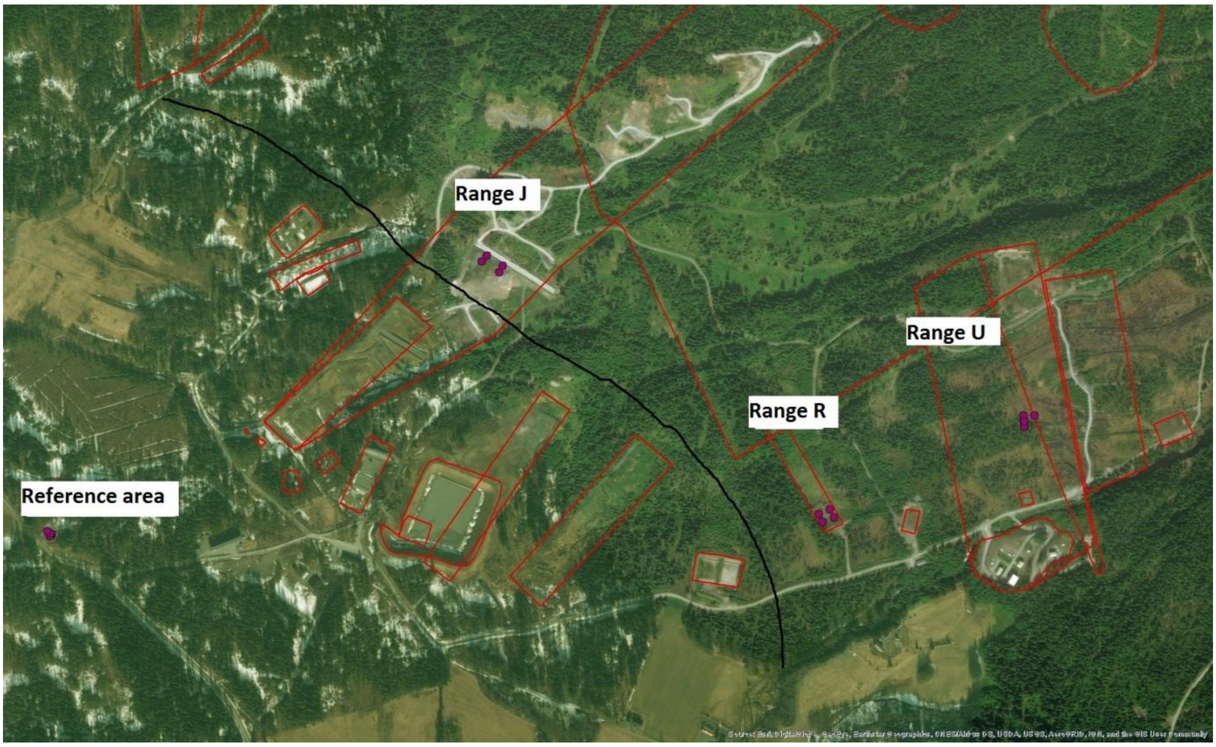

Figure 1 Picture of Leksdalen, the ranges are framed in red. The areas monitored and tested in this study are marked with purple dots. An approximate placement of the fence crossing the shooting range is drawed in black. The figure was produced in ArcMap 10.4.

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3.2 Surveillance of sheep

Wildlife cameras activated by motion were placed on the ranges selected for monitoring (GPS coordinates in supplementary material Table 1; see also Figure 1). Two were placed on range J and one each on ranges U and R. The cameras were active from the 6th of June until the 13th of September. From these images, parameters such as number of pictures daily, percentage of days on which pictures of sheep were taken, number of sheep per picture, average length of "visit", and percentage of "visits" longer than 10 minutes were evaluated. GPS trackers were attached to 14 sheep. Of these, 9 grazed inside the shooting range, 1 at a cultivated pasture on the farm, and 4 at an area outside the shooting range called Vikan (GPS coordinates in Table 1 in the supplementary material). Between 1 and 8 positions were logged each day. The sheep were tracked from late May (the 24th–29th) until September or October (the 08th of September – 25th of October). Some of the sheep grazed on cultivated pastures on the farm before and/or after the grazing period on the range. It is important to note that parts of the shooting ranges were fenced and inaccessible to the sheep. This was true for the lower part of range J and the target area of range R, which could contain unexploded ordinances.

Table 1 **Grass- and soil sample collection. Shows area size and number of increments in each composite sample.**

Range	**Area size (m^2)**	**Number of subsamples**
J	30x13	91
R	13x30	70
U	10x20	50
Reference area	7x7	49

3.3 Sample collection of soil and vegetation

There are two potential routes for the ingestion of Cu and Pb by grazing animals. One is from soil ingestion, either intentionally or unintentionally through either soil stuck to plants or grazing on areas which are overgrazed or have little natural growth of plants. In this study, both plant and soil samples were collected to determine to what extent the two routes caused Pb and Cu exposure to grazing sheep. Soil samples were also washed to determine how much soil was stuck to the plants.

Both soil and grass samples were collected from the three shooting ranges and a reference area outside the ranges (Table 1). The samples were collected in front of the cameras placed on the ranges (Figure 1; GPS points from all the sample areas can be viewed in Table 1 in the supplementary material). Samples were collected on the 22nd and 23rd of July 2016 using a technique called "multi increment sampling (MIS)" (Clausen et al. 2013; Hewitt et al. 2007). This technique involves collecting samples from a defined area called the "decision unit (DU)" and gathering these samples into one composite sample. This produces a good average concentration

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without preparing and analyzing a large number of samples. Clausen et al. (2013) found that the relative standard deviation on the metals measured from a shooting range was 10% using MIS and often greater than 50% using conventional replicate grab samples. The number of samples in one composite sample should be >50, but the more samples, the lower the uncertainty. Soil samples were collected from the top 5 cm of soil. Grass samples were collected by cutting the grass with stainless steel scissors 1–2 cm above the ground. The sample areas contained different species of plants in the Poales order, which included grasses (Poaceae spp), the sedge family (Cyperaceae spp), and the rush family (Juncaceae spp). The sampled grass from range U was primarily from the rush family; the sampled grass from range J was primarily from the grass family, and the sampled grass from range R was primarily a mix of the grass and sedge families. One composite sample of soil and grass was collected from each area.

3.4 Sample collection of feces and liver

On and near each range, feces samples (from sheep) were collected wherever present. Only relatively fresh feces were collected, and care was taken not to collect samples that had been in contact with soil. The samples were counted and gathered into one composite sample for each area. The samples were mixed while collected in the field by collecting approximately the same amount from each fecal deposit. Feces were only found on and near ranges R and J. In all, 2 composite samples of feces were collected one from range J and one from R. The composited sample from range R contained 4 increments and the sample from range J contained 31 increments.

Samples of livers (33 whole livers weighing 43–244.5 g wet weight [ww]) from lambs pasturing in the study area and a reference area were collected by butchers from “Nortura Malvik” (Stavsjøvegen 14, 7550 Hommelvik, Norway). The livers were wrapped in polyethylene plastic bags, frozen at -20 °C, and sent to FFI for chemical analysis.

3.5 Sample preparation and analysis

The soil samples were treated according to Clausen et al. (2013) with some minor modifications. The samples were weighed and dried at 105 ˚C for approximately 24 hours (to constant weight), sieved through a 2 mm sieve (Fritsch) and homogenized by crushing using a ball mill (Retsch RM100) for 5–10 minutes at 300 rpm (to visual homogeneity). Crushing time varied because the soil with high organic content (humus) took longer to homogenize. The fecal samples were treated in the same manner as the soil, except for the sieving. In Teflon vials, a 0.3–0.5 g sample was weighed out in triplicate and 5 ml HNO_3 (Ultrapure 67%, Merck) and 1 ml HF (38–40% puriss, Merck) added. The soil and fecal samples were both digested using a pressurized microwave

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(Ultrawave, Milestone). The samples were then heated to 260 ˚C under pressure and maintained at this temperature for 10 minutes.

The grass samples from each area were divided into two separate samples. One was washed and the other dried without prior washing. The grass was first rinsed for two minutes under running ultrapure water (milliQ) and then placed in plastic containers filled with ultrapure water (MilliQ). The containers were placed on a rotator for 24 hours, and the water was changed twice during this period. The grass was dried at 60 ˚C for 48 hours and homogenized with a ball mill at 400 rpm for 10 minutes. The grass was digested in triplicate using a pressurized microwave (Ultrawave, Milestone). In Teflon vials, 0.2–0.4 g sample was weighed out and 3 ml HNO_3 (Ultrapure 67%, Merck) and 6 ml HCl (30%, Suprapure, Merck) added. The samples were heated to 260 ˚C and maintained at this temperature for 10 minutes.

The outer layer of the livers was removed to minimize the effect of contamination from handling. The liver samples were dried at 60 ˚C for about 72 hours (until stable weight). The livers were homogenized in a ball mill at 300 rpm for 5 minutes. A sample of approximately 0.3 g was weighed out in triplicate in Teflon vials and digested with 7 ml HNO_3 (Ultrapure 67%, Merck) at 220 ˚C for 10 minutes.

All samples were diluted and analyzed for metals using ICP-MS (inductively coupled plasma mass spectrometry) (Thermo x-series 2). A four-point standard curve and an internal standard were used in the analysis. For further quality assurance, four different certified standards were used, three for water (TMDA-53.3, TM-23.4 [Al, Sb, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ti, Zn], and AES-07 [Al, Ca, Mg, K, Na] from Environmental Canada) and one for soil: (GBW07407 [Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Ti, Zn] from the Institute of Geophysical and Geochemical Exploration, Langfang, China), which were digested by the same method as the soil samples.

Certified reference material for plants and liver were not prepared for analysis, which may imply a higher uncertainty in the analysis. However, the plant samples were prepared according to ISO 12914:2012 with some alterations (260 ˚C instead of 175 ˚C), which should indicate satisfactory recovery of the analyzed elements. The liver samples were prepared according to the nitric acid digestion method described in Enamorado-Báez et al. (2013) (with some alterations), which showed good recovery in biological certified reference materials (muscle tissue and milk).

Organic and mineral content of the soil was also found using a loss of ignition (LOI) method described by

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Chambers et al. (2011).

3.6 Calculation of soil intake

One method for calculating how much soil sheep ingests is that described by Thornton and Abrahams (1983) and Smith et al. (2009) (equation 1). This method uses titanium (Ti) as an indicator by comparing Ti in soil and feces, as Ti is abundant in soil but is not taken up by plants.

Equation 1

$$\%\ \text{soil ingestion} = \frac{(1-Pd)Ti_F * 100}{Ti_S - Pd * Ti_F}$$

Pd – plant digestibility (70%)

Ti_F – Ti concentration in feces (mg/kg)

Ti_S – Ti concentration in soil (mg/kg)

3.6.1 Estimation of ingested dose

To calculate the theoretical Cu and Pb dose the sheep ingested, the method described by Johnsen et al. (2016) was applied (equation 2). This method includes soil ingestion rate, weather conditions (dry or wet), and the duration the sheep has spent in the contaminated area. Weather data was collected from the Norwegian

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Meteorological Institute (www.yr.no 2016). Weather conditions are an important factor because research shows that slight rain can rinse plants of soil, while heavy rain can splash soil onto them (Herling and Andersson 1996; Smith et al. 2009). Precipitation exceeding 1 mm per 24 hours was considered to indicate wet conditions. This is a simplified approach, as other conditions also can influence the dose ingested. The method calculates dose per day. This method only considers the metal concentration in the soil. If the metal concentration in the grass is found to be high, grass ingestion should be included in the dose calculation.

Equation 2

$$D = \frac{S * F * (SI_D * DD + SI_W * WD)}{BW} * G$$

D – Dose per day (mg/kg, bw, per day)

S – Metal concentration in soil (mg/kg)

F – Amount of fodder per day (kg,dw)

SI_D – Soil ingestion rate in dry weather

DD – Percentage of dry days

SI_W – Soil ingestion rate in wet weather

WD – Percentage of wet days

BW – Body weight (kg)

G – Amount of time the animals graze on contaminated area

3.6.2 Statistics

A P-P plot was performed using SPSS to see whether the data had a normal distribution. Where a normal distribution was found, a one-way ANOVA analysis was performed to determine whether there was a significant difference between groups in the study. The ANOVA analysis was performed using Excel with a 95% confidence interval. The statistical analysis was performed on Cu and Pb content in livers of sheep that had grazed inside and outside the shooting range. Because normality could not be assumed for the washed versus unwashed plants, a paired non-parametric test (a related samples Wilcoxon signed rank test) was performed for these using SPSS.

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4 Results and discussion

4.1 Grazing pattern

The pictures taken by the wildlife cameras showed that the sheep were grazing on ranges R and J but very little on range U. From these data, one could conclude that range J was the most popular range, and range U the least popular, from a sheep's perspective (complete results can be viewed in the supplementary material, Table 2).

Of the 14 sheep with GPS attached, 9 grazed inside the shooting range at some point during the grazing period. Out of these, 5 mainly grazed inside the range. The data from the GPS tracking coincided with the data from the wildlife cameras, revealing range J to be the most popular area for the sheep. The 5 sheep that mainly resided inside the range spent approximately 25% of the time on ranges, and 93% of this time was spent on range J. On average, sheep spent 23.5%, 0.9%, and 0.04% of their time on ranges J, U, and R, respectively, according to GPS-trackers.

The GPS and camera results yielded inconsistent data as to whether range R or U was more popular and how often the lanes were visited. Lane U has a road through it, causing sheep to frequently walk through the area. This does not mean that the sheep went from the road to the range, as the results from the wildlife camera suggest. No road passed through range R, and even though sheep appeared on camera 70% of the days, the visits lasted for an average of only 27 minutes. The lane was usually visited by only 1–3 sheep at a time. This is probably why range R did not seem as popular from the GPS results, as GPS signals were only tracked 8 times per 24 hours.

4.2 Soil–animal pathway

4.2.1 Metal concentrations in soil

The background levels of Cu and Pb are 28 mg/kg and 67 mg/kg on average in Norway (Ottesen et al. 2000), while the soil quality guidelines for sensitive land use are 100 and 60 mg/kg, respectively (Vik et al. 1999). Previous studies on shooting ranges for small arms have shown varying levels of Pb and Cu in the soil. For instance: Mozafar et al. (2002) found Cu and Pb in the range of 17–1250 mg/kg and 44–33600 mg/kg in two shooting ranges in Switzerland, Bannon et al. (2009) in the range of 223–2936 mg/kg and 4549–24484 mg/kg on eight ranges in the US, and Mariussen et al. (2017) in the range of 50–5200 mg/kg and 260–13000 mg/kg on six Norwegian ranges.

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Metal concentration and organic content in soil samples are shown in Table 2. The high organic content in soil from range U was expected, as this range consisted mostly of partly converted moss. The metal (Cu and Pb) concentration from the reference area was, as expected, low and within expected background concentrations in soil. The soil on range J also had low Cu and Pb concentrations comparable to that on the reference area which may indicate low shooting activity or may be because part of the range has recently been subjected to remediation measures. The soil on range R had considerably higher Cu and Pb concentrations than that of range J. Range U had soil with Cu and Pb concentrations between that of ranges J and R.

Table 2 Ti, Cu, Pb and Mo concentrations and organic content measured in soil from ranges on Leksdalen shooting range and a reference area outside the shooting ranges. Shown as mean ± SD, n=3 preparational and analytical parallels, not sample parallel.

mg/kg (dw)	Ti	Cu	Pb	Mo	Organic content
Range J	3382 ± 541	42 ± 5	41 ± 6	2.3 ± 0.3	16%
Range R	3696 ± 291	580 ± 44	7189 ± 1827	2.0 ± 0.1	24%
Range U	97 ± 9	279 ± 18	347 ± 26	0.3 ± 0.1	97%
Reference area	1899 ± 82	18 ± 1	55 ± 1	1.6 ± 0.2	23%

In addition to Pb and Cu, the soil was analyzed for Ti and Mo. The Ti concentrations on ranges J and R were quite similar, while that of range U was significantly lower. This has a natural cause, as range U consisted mostly of humus-rich soil or partly converted moss low in inorganic matter. However, the Ti concentrations on all areas were lower than the background level in Norway (6200 mg/kg) (Ottesen et al. 2000). The Mo concentrations on range J, R and the reference area were quite similar and coincided well with the average Mo concentration in soil in Norway (2.2 mg/kg) (Ottesen et al. 2000). Range U had a lower Mo concentration than the other sampling areas, probably because of the high organic content of the soil.

4.2.2 Metal concentrations in feces

The Cu, Pb and Ti concentration measured in feces collected in and around ranges R and J can be seen in Table 3. Cu and Pb concentration in the feces was not significantly different between the ranges. However, the Ti concentration was higher in feces collected on or near range J than in those on range R. This was not due to different Ti concentrations in the soil on these ranges, as these were in fact similar. However, this could be attributed to the difference in soil quality, which can lead to a difference in soil ingestion rate (Healy 1967). In

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comparison to the findings in Leksdalen, Smith et al. (2009) found Ti concentration in feces from sheep in a mining area in mid-Wales to be between 19 and 2365 mg/kg, with a median of 740 mg/kg.

Table 3 Ti, Cu and Pb concentration in feces from on and around range J and R. Shown as mean ± SD, n=3 preparational and analytical parallels, not sample parallel.

mg/kg (dw)	**Ti**	**Cu**	**Pb**
Range J	46 ± 2	19 ± 2	5 ± 1
Range R	11.9 ± 0.2	23 ± 5	4.0 ± 0.1

4.2.3 Soil ingestion rates

In calculations and risk assessments, different soil ingestion rates have been used: Rupflin and Krebs (2015) used 10–15% for dry weather and 10–30% for wet weather and , while Eriksen et al. (2009) used 5%. Another study, conducted by (Healy 1967), showed that sheep soil ingestion rate varied with soil type. No such studies have, to our knowledge, been conducted in Norway.

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Based on the Ti concentrations in soil and feces (using equation 1) a soil ingestion rate of 0.1% was found for range R and 0.4% for range J. These were comparable to the lowest soil ingestion rates found by Smith et al. (2009). These low soil ingestion rates in Leksdalen can be explained by: an apparent lush pasture partially due to sample collection in July, no sign of overgrazing and dry weather before and during sampling (www.yr.no 2016). A lush pasture can make the soil less available, while overgrazing exposes the soil. Wet weather tends to cause soil particles to stick to the grass. A previous study conducted by Smith et al. (2009) has shown that the soil ingestion rate for sheep can vary from 0.1% to 81.8% (dm) depending on parameters such as time of year and pasture quality. Smith et al. (2009) found the highest median soil ingestion rate in January (15.1%) and the lowest in September (0.4%), with an overall average of 7.6% over the year. Samples were only collected once during the study in Leksdalen, and nothing can be concluded regarding soil ingestion rates during other weather conditions.

4.3 Soil–vegetation–animal pathway

In a study conducted on an old shooting range in Switzerland, a Pb concentration of 3,900 mg/kg (dw) was found in grass. The Pb concentration in soil from the same range was 29,550 mg/kg (dw) (Braun et al. 1997). Cai et al. (2009) found that plants growing on contaminated areas beside a Pb-Zn smelter and a highway had Pb concentrations of 100–200 mg/kg when the soil concentration was approximately 400–500 mg/kg. The metal (Cu, Pb, and Mo) concentration in both washed and unwashed grass from Leksadalen can be viewed in Table 4. No significant difference in the Pb and Cu concentration of washed and unwashed grass was found (for output from the non-parametric test [related samples Wilcoxon signed rank test] see supplementary material Table 3 and 4). This indicates that there was little soil stuck to the grass. This corresponds well with the low soil ingestion rate found. Except for the washed grass on range R, none of the grass samples exceeded the threshold for Pb in fodder (5 mg Pb/kg, 12% water content) of the EU (2002/32/EC 2002). EU does not have a limit for Cu in animal fodder, but the US has a limit of 25 mg/kg for sheep (NAS 1980). None of the plants contained Cu exceeding the US fodder limit (of 25 mg/kg). Both the Cu and Pb concentrations measured in all the grass samples were also within the range considered normal in plants (2–5 mg Pb/kg and 3–20 mg Cu/kg [dw]), except the washed grass from range R (Chaney 1989; Robinson et al. 2008). The washed grass from R probably contained a soil particle from sampling or was contaminated during sample preparation. There was little difference in the Cu and Pb concentrations in the grass from the different ranges, indicating minimal accumulation. Other studies of Cu and Pb in plants on shooting ranges have found considerably higher concentrations than the present study.

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The Cu/Mo rate is very important, as Mo binds Cu and thus makes it less bioavailable. The Mo concentration found in the plants was highest in the washed grass from range J and lowest in the washed grass from range U. If the Cu/Mo rate is low (<6), the sheep can experience Cu deficiency, and if the rate is high (>10), the sheep can experience Cu poisoning even though the Cu concentration in Leksdalen was not considered toxic (Buck and Sharma 1969; Hidiroglou et al. 1984). The Cu and Mo rates varied among the ranges, and were higher than the risk rate of 10 on both ranges R and U. However, on range J, the range preferred by the sheep, the rate was a bit below the recommended rate of 6 (Table 4). The difference between the ranges can be explained by the differences in both soil (such as pH and organic content) and grass type. The difference could also be attributed to the higher Cu concentrations in soil from ranges U and R than from range J and the reference area.

Table 4 Cu, Mo and Pb concentrations measured in washed and unwashed grass from ranges on Leksdalen shooting range and a reference area outside the shooting ranges. The table also shows calculated Cu/Mo rate in washed grass samples. Shown as mean ± SD, N=3 preparational and analytical parallels, not sample parallel.

mg/kg (dw)		**Cu**	**Mo**	**Pb**	**Cu/Mo**
Range J	Washed	8.2 ± 0.4	1.9 ± 0.1	1.6 ± 0.3	4.3
	Unwashed	6.8 ± 0.7	1.3 ± 0.02	1.4 ± 0.3	5.2
Range R	Washed	11.9 ± 0.9	1.5 ± 0.1	29 ± 2	7.9
	Unwashed	4.3 ± 0.5	0.24 ± 0.05	1.26 ± 0.02	17.9
Range U	Washed	4.2 ± 0.2	0.16 ± 0.03	1.3 ± 0.1	26.3
	Unwashed	4.9 ± 0.2	0.15 ± 0.03	1.3 ± 0.1	32.7
Reference area	Washed	5.3 ±0.3	0.17 ± 0.02	1.4 ± 0.1	31.2
	Unwashed	6.4 ± 0.7	1.6 ± 0.06	1.5 ± 0.2	4.0

4.4 Estimation of ingested dose

To calculate the theoretical dose of metal ingested in 2016 by the sheep at Leksdalen, equation 2 was used. Fodder intake of 1.3 kg per day was assumed (Rupflin and Krebs 2015). Soil ingestion rates of 0.4% (range J) and 0.1% (range R) were used for dry days and 30% for wet days. This is a very conservative rate, which was used to create a safety margin, as there is no known soil ingestion rate for wet days in this area. The percentage of wet days over the whole period was 40% (www.yr.no 2016). A body weight of 75 kg and dry matter intake of 1.3 kg per day was used (Rupflin and Krebs 2015). The amount of time spent on each range was found using the

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GPS-tracker data (J: 23.5%, U: 0.9%, R: 0.04%). The calculated average dose was 0.033 mg/kg, body weight (bw), per day for Pb and 0.021 mg/kg, bw, per day for Cu. Compared to the chronic toxic dose of 6 mg/kg, bw, per day for Pb (Payne and Livesey 2010) and 0.26–0.35 mg/kg, bw, per day for Cu (Oruc et al. 2009), the doses found are not considered toxic.

Under the assumption that all the ranges in the area was contaminated equally to range R (580 mg Cu/kg and 7189 mg Pb/kg), a potential ingested dose of 0.31 mg Cu/kg, bw, per day and 3.8 mg Pb/kg, bw, per day was calculated. This is at the limit of the Cu dose which is considered chronically toxic to sheep. The theoretical Pb dose was still below the dose considered chronically toxic.

For comparison, a calculation was performed to see what the theoretical Cu and Pb dose from plants might be. In this calculation, the concentration in washed grass was used, and 100% grass ingestion was assumed. The other parameters used in the calculation corresponded with what was used for soil. The total theoretical amount of Cu and Pb a sheep could ingest during one day under these conditions was found to be 0.031 mg/kg and 0.0007 mg/kg, respectively. Both calculated doses were well below the doses for chronic toxicity, and more than 40 times lower than the calculated dose from soil for Pb. The calculated daily dose from Cu, however, was similar to that from soil. Using the same approach as for soil, a dose was calculated for the theoretical case in which a sheep grazed on range R for 25% of the time. The potential doses ingested from grass under these conditions were 0.052 mg Cu/kg, bw, per day and 0.13 mg Pb/kg, bw, per day. This is lower than the toxic doses from literature and the calculated theoretical dose from soil. All doses calculated can be viewed in Table 5.

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Table 5 Theoretically calculated acute dose of Cu and Pb for sheep grazing in one area for 14 days. Theoretical calculated chronic dose was calculated using percentage of time spent on each range found from GPS tracking. In the calculations of dose from grass, a 100% grass ingestion rate was assumed. All calculations used fodder intake of 1.3 kg (dw) per day, body weight of sheep 75 kg and 40% wet days. Acute and chronic toxic dose for Cu and Pb from literature is also included.

	Chronic dose (mg/kg, bw, day)			
	Soil		**Grass**	
mg/kg bw	**Cu**	**Pb**	**Cu**	**Pb**
Range J	0.02	0.02	0.03	0.007
Range R	0.0005	0.006	0.00008	0.0002
Range U	0.005	0.007	0.0007	0.0002
Total	0.021	0.33	0.031	0.0074
Toxic dose (from literature)	0.26-0.35	6	0.26-0.35	6

4.5 Measured levels of Cu and Pb in livers

Livers from 32 sheep were collected, of which 23 grazed inside the shooting range and 9 elsewhere (some on cultivated pasture). The concentrations of Pb and Cu in the livers are shown in Table 6. P-P plots proving normal distribution can be viewed in the supplementary material Figure 2 and 3 for Cu and Pb, respectively. One-way ANOVA analysis was conducted in Excel to determine whether there was a difference in Cu and Pb concentrations in the livers of sheep grazing inside and outside the Leksdalen shooting range (see the supplementary material Table 5 and 6 for Cu and Pb, respectively). A significant difference in the Cu concentrations in livers was found between the two groups ($p<0.05$). The livers containing the highest concentration of Cu came from sheep that had grazed outside the shooting range. This could have been caused by several factors in the area: high natural Cu concentration, less Mo in plants, Cu contamination from an unknown source, or higher soil ingestion rate. Cu was found in concentrations above what is considered normal (>300 mg/kg [dw]) in the livers of 8 (out of 23) sheep that had grazed on the shooting range, and in 6 (out of 9) sheep that had grazed elsewhere. None of the lambs had Cu concentrations in their livers compatible with Cu poisoning (>1000 mg/kg [dw]) (NAS 1980). Sivertsen and Plassen (2004) measured Cu in the livers of 599 lambs from six locations in Norway. They found that the national average Cu concentration in lamb liver was 69 mg/kg (ww) and the average from Nord-Trøndelag (where Leksdalen is located) was 124 mg/kg (ww).

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Accounting for the dry matter content, the Cu concentrations found in sheep livers in this study (shooting range: 82 mg/kg [ww]; Vikan and cultivated pasture: 111 mg/kg [ww]) were within the same range as or somewhat lower than the average levels found by Sivertsen and Plassen (2004) in this part of Norway. The Pb concentration in the liver was found to be very low in all cases, and none exceeded the concentration considered to be normal in sheep livers (0–3 mg/kg [dw]) (NAS 1980). No significant differences were found in the Pb concentrations in the livers of the sheep that had grazed inside and outside the range ($p>0.5$). Near a metal smelter, Liu (2003) found Cu and Pb concentrations in fodder comparable to that found in grass in this study (14 mg Cu/kg [dw] and 4.75 mg Pb/kg [dw]) and Cu concentrations in the livers of sheep (248.1 ± 35.9 mg/kg [dm]) similar to those in the livers of sheep grazing on Leksdalen shooting range. However, the Pb concentrations found in the livers of sheep near the metal smelter (15.3 ± 1.14 mg Pb/kg [dw]) were considerably higher than those found in the livers of sheep in Leksdalen. This could be attributed to a difference in Pb bioavailability between Leksdalen and the area of the metal smelter. Johnson et al. (2004) found no elevated Pb levels in woodchucks on a shooting range, indicating that mammals avoid contaminated areas, or that little of the Pb on shooting ranges are bioavailble.

Table 6 Average Cu and Pb in liver from slaughtered lambs (dw). Shown as mean ± SD.

Area	N	Cu (mg/kg)	Pb (mg/kg)	Dry matter (%)
Leksdalen shooting range	23	273 ± 111	0.3 ±0.16	30
Vikan and cultivated pasture	9	381 ± 166	0.19 ± 0.08	29

5 Conclusion

Camera surveillance showed that the sheep spent considerable amounts of time near stop butts, which can be highly contaminated areas at shooting ranges. The range that was most popular among the sheep (range J) was also the range least contaminated with Cu (42 ± 5 mg/kg [dw]) and Pb (41 ± 6 mg/kg [dw]), but the sheep also grazed on range R, which was contaminated with high levels of both Cu (580 ± 44 mg/kg [dw]) and Pb (7189 ± 1827 mg/kg [dw]). The frequent observation of the sheep on the stop butts indicates some attraction to these sites, which can be highly contaminated by Pb and Cu. However, there were no indications of any attraction toward contaminated areas per se. It can therefore not be concluded from this study whether sheep are especially attracted to areas on shooting ranges contaminated with Cu and Pb. It can, however, be concluded that the sheep

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did not shy away from such areas.

The most significant pathway for intake of Cu and Pb in this area seemed to be the soil–animal pathway, as the plants analyzed contained low concentrations of Cu and Pb. There was also no accumulation of Cu and Pb found in plants growing on contaminated soil.

The estimated soil ingestion rate was lower than the soil ingestion rate that has previously been used in risk assessments. However, the soil ingestion rate found was based on only one day of sampling. Therefore, further work should focus on collecting samples from several periods throughout the whole grazing season. To get a better sense of soil ingestion rates in Norway, investigations should also be done in several different locations.

From the dose calculation, it can be concluded that there is no risk of chronic poisoning from Cu or Pb via grazing at Leksdalen from either soil or grass ingestion. If the sheep had grazed on only the most contaminated site (range R) for more than 14 consecutive days, there would be a theoretical chance of acute Cu poisoning. According to the results from wildlife cameras and GPS trackers this is very unlikely. Liver analysis showed no elevated levels of Cu and Pb compared to sheep grazing outside the shooting range. From this experiment, it can therefore be concluded that sheep grazing in Leksdalen shooting range are not at risk from Cu or Pb poisoning.

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