Risk Assessment of Metal Contamination in Shooting Ranges – Investigation of Bioavailability of Pb in Water and Soil

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ABSTRACT

Only a fraction of the total concentration of metals in water and soil is bioavailable and can be taken up by and cause toxic effects to organisms. Knowledge about the bioavailability of lead (Pb) is of importance when performing risk assessments of metal contamination in shooting ranges. The aim of the present study was to assess and compare the bioavailability of Pb in run-off waters and soils from different shooting ranges in Norway. The bioavailability of Pb in the run-off waters was assessed by determining the speciation of the metal by size and charge fractioning. The bioavailability of Pb in the soils was assessed by examining survival and reproduction of earthworms (Eisena fetida). The speciation of Pb in the run-off waters showed that the content of bioavailable Pb varied for the different waters. The effect of Pb contamination on reproduction of the earthworms varied for the different soils, which indicated that the bioavailability of Pb in the soils varied. The survival of adult earthworms was not affected by Pb contamination (up to 1500 mg/kg) in the soils. The pH and content of total organic carbon (TOC) in the run-off waters and soils seemed to have an influence on the speciation of Pb and the effect of Pb on reproduction of the earthworms. The bioavailability of Pb in the particular shooting range should be investigated and taken into consideration when assessing the risk of Pb contamination in shooting ranges.

1.0 INTRODUCTION

Run-off water and soil from shooting ranges commonly contain contaminations of metals such as lead (Pb), copper (Cu), zinc (Zn) and antimony (Sb) originating from ammunition. The contaminations can pose a risk to organisms living in water and soil. The European Union Water Framework Directive (WFD) has established environmental quality standards (EQS) for priority pollutants, including Pb, in inland and coastal waters. The Norwegian Environmental Agency has established limits for Pb in soil based on human health (SFT, 2009), but these limits are not suitable for risk assessment of Pb in soil in shooting ranges. To assess the risk of Pb contamination in shooting range soil, a site-specific risk assessment should be performed.

Only a fraction of the total concentration of Pb in water and soil is bioavailable. The bioavailability of a metal can be defined as the fraction of the metal which can be taken up by and cause toxic effects to organisms (Peijnenburg and Jager, 2003). In 2015, the EQS for Pb in fresh water in the WFD was revised to account for the bioavailable fraction of the metal (WFD, 2015). The bioavailability of Pb depends on water and soil properties, such as the pH, the content of organic matter and ions in the water or soil, the type of organism, and the chemical form of the metal (Fairbrother et al., 2007). The free ion of Pb is considered the most bioavailable form of the metal, while Pb bound to complexes is less likely to be taken up by organisms (Peijnenburg and Jager, 2003, Fairbrother et al., 2007). Knowledge about the bioavailability of Pb is necessary to have in order to make good and realistic risk assessments of metal contaminations in water and soil in shooting ranges.

The aim of the present study was to assess and compare the bioavailability of Pb contamination in run-off water and soil from shooting ranges located in different places in Norway. The bioavailability of Pb in the run-off waters was assessed by determining the speciation of the metal by size and charge fractioning. The bioavailability of Pb in the soils was assessed by examining survival and reproduction of earthworms. The influence of soil and water properties on the bioavailability of Pb was evaluated.

2.0 MATERIALS AND METHODS

2.1 Study Areas

Samples of run-off water were collected from Avgrunnsdalen, Hengsvann, Terningmoen and Steinsjøen shooting range, all located in the south east of Norway. In each shooting range, water samples were collected on four days during one week. The samples in Avgrunnsdalen were collected in November 2016. Samples from the other locations were collected between April and May 2017.

Soil samples were collected from five shooting ranges; Nyborgmoen shooting range in Northern Norway, Vaterholmen shooting range in Central Norway, Ulven and Vikesdalmoen shooting range in Western Norway, and Terningmoen shooting range in the south east of Norway. Samples of contaminated and clean reference soil were collected from each shooting range. The samples were collected between June and August 2018.

The locations of the shooting ranges are shown in Figure 1.

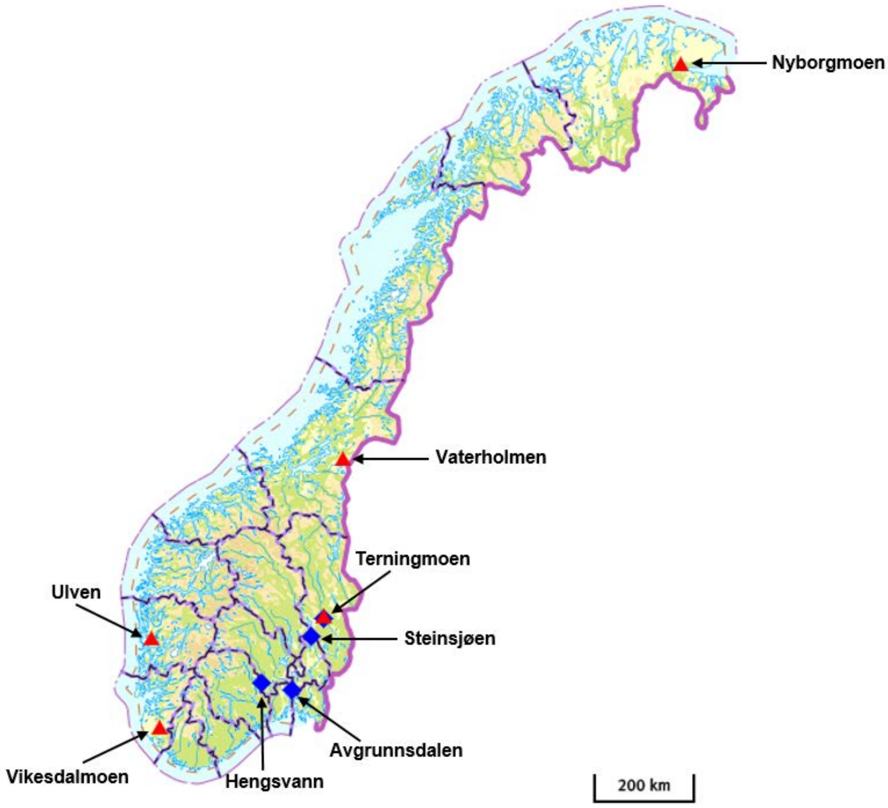

Figure 1: Map of Norway with the locations of the study areas marked. The water samples were collected from the shooting ranges marked by a blue square. The soil samples were collected from the shooting ranges marked by a red triangle. Both water and soil samples were collected from Terningmoen shooting range.

2.2 Fractionation and Analyses of Water Samples

Size fractionation was performed by filtration of the water samples in the lab. The samples were filtered through a 0.45 µm membrane filter, followed by ultrafiltration through 10 kDa Amicon Ultra centrifugation tubes by centrifugation at 4500 rpm for 30 minutes. Charge fractionation was performed by ion exchange chromatography on filtered (0.45 µm) water samples on site. Positively charged elements were separated by the cationic exchange resin Amberlite IR-120 (Merck). Negatively charged elements were separated by the anion exchange resin AG 1-X8 (20-50 mesh, Bio-Rad).

The water samples were preserved with HNO_3 (final concentration 0.65 % HNO_3) and the concentration of Pb was analysed by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo X-series II). Total organic carbon (TOC) in the samples was analyzed by spectrophotometry according to NS-EN 1484 at Eurofins Environmental Testing Norway. pH was measured directly in the run-off waters by a portable field instrument.

The concentration of Pb in the following fractions of the water samples was determined, by analysis or calculation:

- Pb_{total} = Pb in unfiltered sample
- $Pb_{dissolved}$ = Pb in filtered (0.45 µm) sample
- $Pb_{particular} = Pb_{total} – Pb_{dissolved}$
- $Pb_{low\ molecular\ mass\ (LMM)\ <10\ kDa}$ = Pb in ultrafiltered (10 kDa) sample
- $Pb_{colloidal} = Pb_{dissolved} – Pb_{LMM\ <10kDa}$
- $Pb_{cationic} = Pb_{dissolved}$ – Pb in eluate from cation exchange column
- $Pb_{anionic} = Pb_{dissolved}$ – Pb in eluate from anion exchange column
- $Pb_{neutral} = Pb_{dissolved} – Pb_{cationic} – Pb_{anionic}$

2.3 Soil Samples and Earthworm Toxicity Test

2.3.1 Preparation and Analyses of Soil Samples

Soil samples (800 g, 3-5 replicate samples) with approximately 250, 500 and 1500 mg Pb/kg were prepared by mixing contaminated and clean reference soil (dried and sieved, <2mm) from each shooting range. The samples were added potting soil (5wt%) to ensure sufficiently high organic content for the earthworms. Deionised water corresponding to 50% of the water holding capacity (WHC) of the soil mixture was added to each soil sample.

The content of total organic material (TOM) in the soil samples was determined by measuring loss of ignition (LOI) at 550 °C, which was divided by 1.72 to give TOC. pH was measured in a suspension of soil and deionised water. The concentration of Pb in the samples was analysed according to EN ISO 17294-2 at Eurofins Environmental Testing Norway.

2.3.2 Survival and Reproduction of Earthworms

Survival and reproduction of earthworms was tested according to the procedure described in OECD Earthworm Reproduction Test (OECD/OCDE, 2016), with some modifications. The Norwegian Institute for Bioeconomy Research (NIBIO), Ås, Norway provided earthworms of the species *Eisenia fetida* for the study.

The earthworms were acclimatised in clean reference soil (from each shooting range) for about a week before the start of the experiment. The earthworms were rinsed with deionised water, left on moist filter paper overnight, rinsed with deionised water again and dried on filter paper, before 10 earthworms of approximately equal size (200-300 mg), with clitellum, were added to each soil sample. Once a week, the earthworms were fed by adding horse manure (ca. 3 g) moistened with deionised water (ca. 5 mL) to the soil samples.

Survival of the earthworms was determined after 4 weeks by counting the number of live earthworms in each soil sample. The soil samples without the adult earthworms were kept for another 4 weeks before reproduction of the earthworms was determined by counting the number of live juveniles in each soil sample. This was done by placing the containers with the soil samples in a water bath set to 40 °C, gradually increased to 60 °C such that the live juveniles moved to the surface of the soil where they were counted.

3.0 RESULTS AND DISCUSSION

3.1 Water and Soil Chemistry

The pH and the content of TOC and Pb in the water and soil samples are presented in Table 1 and 2.

Table 1: Water chemistry and content of Pb in the run-off waters (n=4-12 ± SD).

Parameter	Avgrunnsdalen	Terningmoen	Steinsjøen	Hengsvann
pH	5.51 ± 0.11	5.68 ± 0.32	6.05 ± 0.09	6.24 ± 0.10
TOC (mg/L)	12.1 ± 0.9	9.0 ± 0.3	7.2 ± 0.2	7.3 ± 0.3
Pb (µg/L)	18.4 ± 2.1	9.9 ± 0.5	27.4 ± 1.3	8.8 ± 0.6

Table 2: Soil chemistry and content of Pb (n=3 ± SD) in the soil samples from the shooting ranges. Ref. = reference soil, Con. = contaminated soil.

Parameter	Vikesdalmoen		Ulven		Nyborgmoen		Terningmoen		Vaterholmen	
	Ref.	Con.	Ref.	Con.	Ref.	Con.	Ref.	Con.	Ref.	Con.
pH	4.1	4.1	5.2	5.2	4.6	4.2	5.6	5.5	6.9	3.8
TOC (%)	3.5	8.1	3.5	9.9	2.1	2.4	1.9	6.4	2.2	5.0
Pb (mg/kg)	9.8 ± 1.1	10667 ± 577	13.7 ± 1.5	9800 ± 1153	10.2 ± 1.4	6993 ± 551	63.0 ± 14.7	20667 ± 1528	10.6 ± 0.7	3333 ± 379

The run-off waters had low pH and high concentration of TOC compared to typical European fresh waters, which typically have pH >7 and TOC around 5 mg/L (Hoppe et al., 2015). The relatively high content of TOC in the waters reflects the influence from peat from mires within or close by the shooting ranges. The water in Avgrunnsdalen had the highest concentration of TOC and the lowest pH, while the waters in Hengsvann and Steinsjøen had the lowest concentration of TOC and the highest pH.

The contaminated soil samples had higher content of TOC than the clean reference soil samples from the same shooting range. All the soil samples had low pH. Unlike the water samples, there was no correlation between the concentration of TOC and the pH of the soil samples. The highest concentrations of TOC were found in soil samples with both low and higher pH. The large difference in the pH between the contaminated and clean reference soil from Vaterholmen can be explained by a greater distance between the points where the soil samples were collected in this shooting range.

The differences in the concentration of Pb in the run-off waters and contaminated soils from the shooting ranges are likely to be related to the type of shooting range, and the impact and type of ammunition, which has been used in the shooting range where the samples were collected. The Norwegian Armed Forces phased out use of ammunition with Pb in 2007, but shooting ranges can still contain high levels of Pb from historic use, and ammunition containing Pb is still in use in some shooting ranges (Utstøl et al., 2019).

3.2 Speciation of Pb in the Run-off Waters

The content (% of total Pb) of particles, colloids and low molecular mass Pb in the run-off waters are shown in Figure 2, and the content (% of dissolved Pb) of anionic, cationic and neutral species of Pb in the run-off waters are shown in Figure 3.

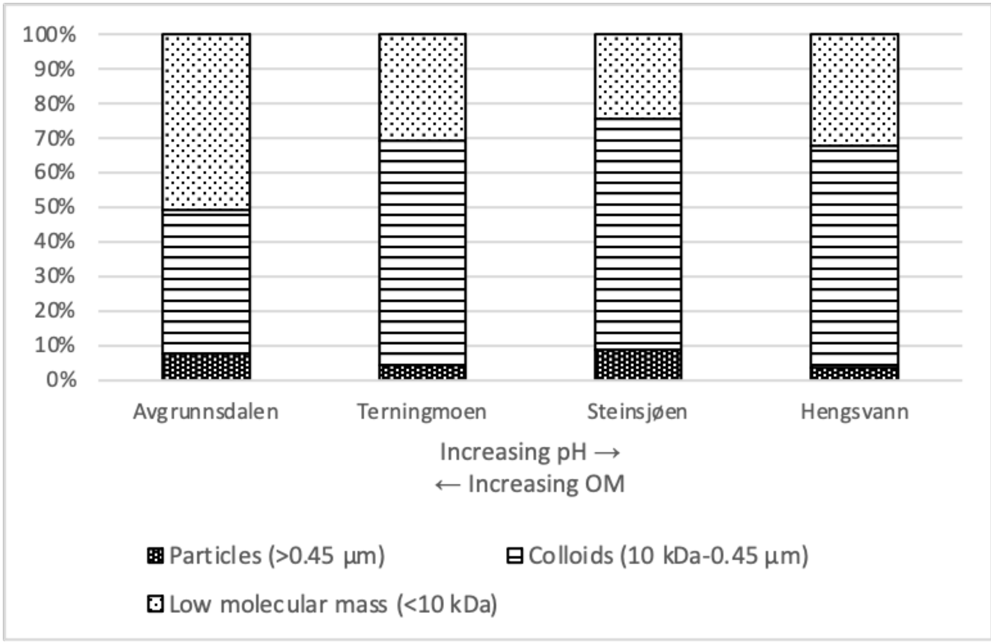

Figure 2: Size fractions of Pb in the run-off waters shown as percentage of total concentration of Pb.

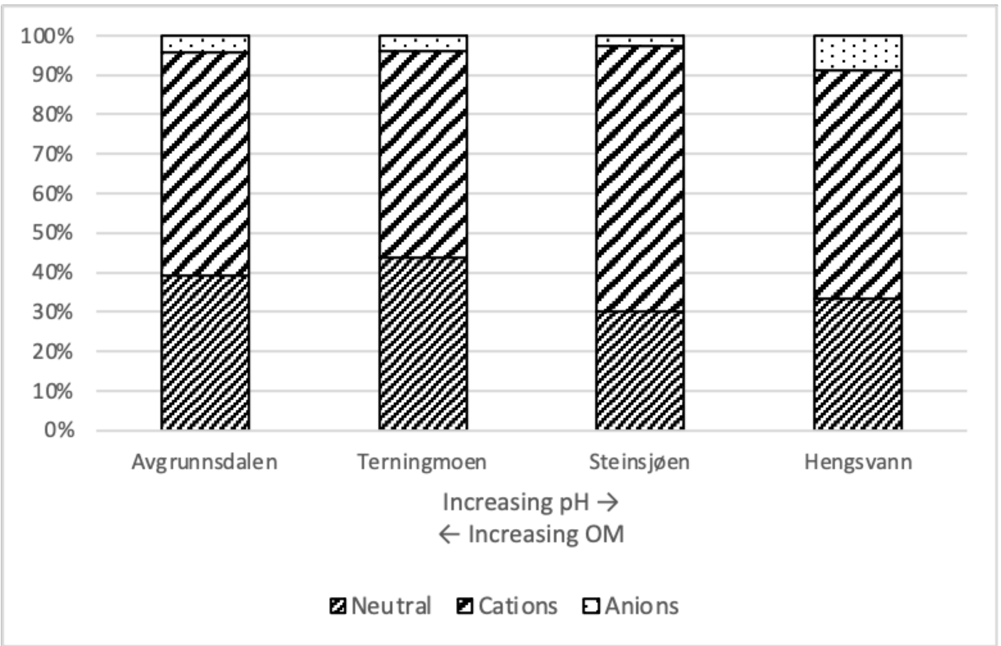

Figure 3: Charge fractions of Pb in the run-off waters shown as percentage of concentration of dissolved Pb.

The size fractioning showed that a major fraction (42-67%) of the Pb in the run-off waters existed as colloids (10 kDa-0.45 µm), indicating that the Pb was largely associated with other substances in the waters. This was as expected since Pb^{2+} has a strong affinity for organic acids and anionic species in water (Powell et al., 2009). The waters also contained a large fraction (24-51%) of low molecular mass (<10 kDa) Pb. The bioavailability of Pb existing as complexes depends on how strongly Pb is bound to the complex, and if the complex itself can be taken up by organisms. Generally, smaller compounds of Pb are more bioavailable than larger compounds (Fairbrother et al., 2007). Only a minor fraction (5-9%) of the total concentration of Pb in the run-off waters existed as particles (>0.45 μm), which are normally not bioavailable (Fairbrother et al., 2007). The charge fractioning showed that the Pb in the run-off waters mainly existed as cationic (53-65%) and neutrally charged (29-45%) species. Only a minor fraction (3-9%) existed as anionic species. Cationic Pb is considered as the most bioavailable form of Pb (Peijnenburg and Jager, 2003).

In Avgrunnsdalen, significantly (t-test, $p<0.05$) more Pb was present as low molecular mass species, and significantly less as colloids, compared to the other locations. This was not expected as the water in Avgrunnsdalen had the highest content of TOC of the waters, which should imply higher content of Pb bound to complexes (Powell et al., 2009). Low pH of the water in Avgrunnsdalen may have contributed to a lower degree of complexation of Pb with TOC in this water, as Pb^{2+} has lower affinity to TOC at low pH (Reddy et al., 1995, Logan et al., 1997). More Pb present as complexes of small size in the run-off water in Avgrunnsdalen indicated that the Pb in this water was more bioavailable than the Pb in the run-off waters from the other shooting ranges. The charge distribution showed that more Pb existed as neutral species in the run-off waters from Avgrunnsdalen and Terningmoen compared to Steinsjøen and Hengsvann. This could be related to the higher content of TOC and lower pH of the waters in Avgrunnsdalen and Terningmoen. The results indicated that the Pb in the run-off waters in Avgrunnsdalen and Terningmoen was less bioavailable than the Pb in the run-off waters from the other shooting ranges. This was in contrast to the results seen from the size distribution, which indicated that Pb was most bioavailable in the run-off water from Avgrunnsdalen.

3.3 Survival and Reproduction of Earthworms in Soils Contaminated with Pb

The survival of the earthworms in the soil samples from the different shooting ranges and with different concentrations of Pb is shown in Figure 4.

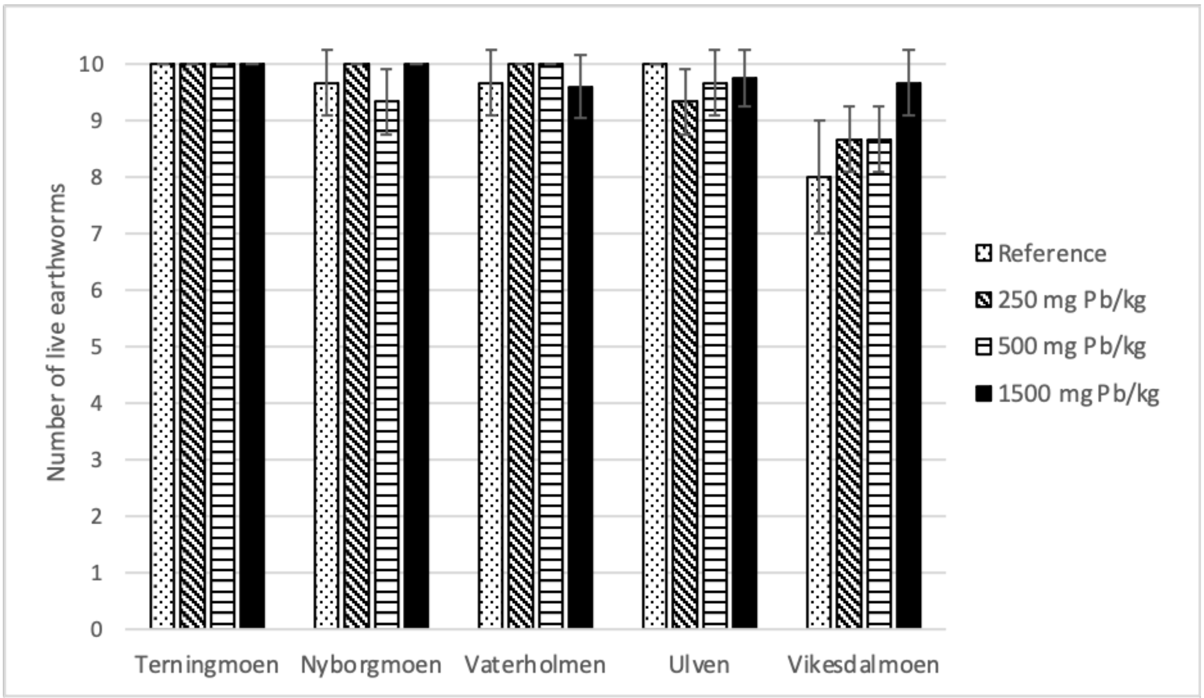

Figure 4: Survival of earthworms as a function of Pb contamination in soil samples prepared from soil from different shooting ranges (n=3-5 ± SD).

There was a high rate of survival of the earthworms in all the soil samples regardless of the shooting range and concentration of Pb. The results indicated that concentrations of Pb up to 1500 mg/kg did not influence the survival of the adult earthworms. Other studies of earthworms in polluted soil collected from industrial sites (Spurgeon and Hopkin, 1995) and shooting ranges (Amundsen and Joner, 2011) have shown similar high survival rates, while higher mortality has been observed in cases where artificial soil has been added Pb in form of salts such as $Pb(NO_3)_2$ (Žaltauskaitė and Sodienė, 2010). The results indicate that Pb in shooting range soils is less bioavailable than Pb in form of salts. However, high mortality of earthworms has been observed in a study of shooting range soil with >2000 mg Pb/kg (Luo et al., 2014). Another study showed earthworm mortality ranging from 0 to 100% in different field soils added 2000 mg Pb/kg in form of $Pb(NO_3)_2$, indicating different bioavailability of Pb in the soils (Bradham et al., 2006). Mortality of earthworms has been considered a relative insensitive parameter to assess Pb contamination, as the earthworms can store an amount of Pb before it results in toxic effects (Morgan and Morgan, 1998). Based on the results from this study, survival of earthworms is not a suitable parameter to assess when assessing the bioavailability of Pb in soils from shooting ranges.

The reproduction of the earthworms in the soil samples is shown in Figure 5.

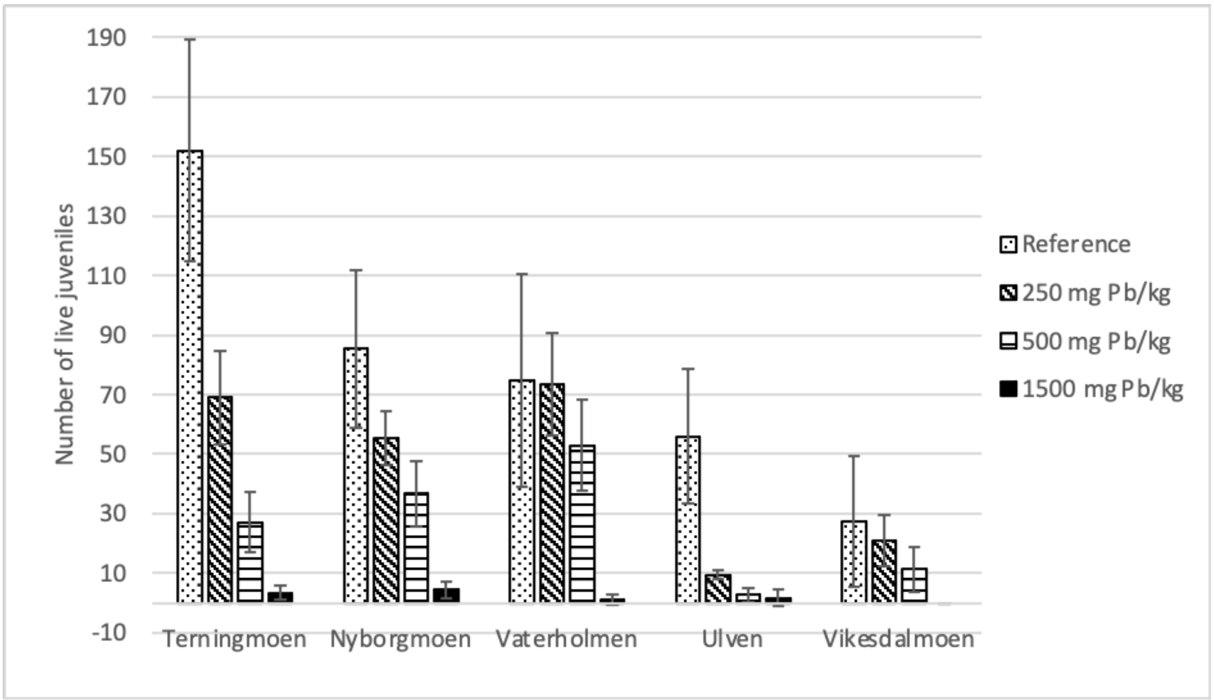

Figure 5: Reproduction of earthworms as a function of Pb contamination in soil samples prepared from soil from different shooting ranges (n=3-5 ± SD).

On contrary to the survival, the reproduction of the earthworms was greatly affected by Pb contamination in the soils. The reproduction decreased with increasing concentration of Pb, and no or very few live juveniles were found in the soil samples with 1500 mg Pb/kg. The reproduction only accounts for live juveniles in the soil samples, and does not provide information about unhatched cocoons or survival of the juveniles in the soil over time. The effect of Pb contamination on reproduction was slightly higher than what has been found for similar concentrations of Pb in some other studies, both for naturally contaminated (Amundsen and Joner, 2011, Spurgeon and Hopkin, 1995) and artificial soils added $Pb(NO_3)_2$ (Žaltauskaitė and Sodienė, 2010). This could indicate that the Pb in the soils in the present study was more bioavailable, but could also be due to other factors not taken into account.

The largest effect of Pb contamination on reproduction of earthworms was seen for the soil from Ulven, and the least effect was seen for the soil from Vaterholmen. The results indicate that the Pb was most bioavailable in the soil from Ulven, and least bioavailable in the soil from Vaterholmen. The soil samples from Ulven had the highest content of TOC, while the soil samples from Vaterholmen had the highest pH of the soils. High content of organic matter may be related to a higher content of soluble organo-Pb complexes in the soils, thus higher bioavailability, while high pH may contribute to less extractable Pb, thus lower bioavailability (Luo et al., 2014).

There were large variations between the reproduction of the earthworms in the soils without Pb contamination, which indicated that the soil properties, and not only the bioavailability of Pb, influenced the reproduction of the earthworms. High content of organic matter should generally benefit reproduction of earthworms (García and Fragoso, 2002). Low pH may explain the low reproduction of earthworms in the soil from Vikesdalmoen. Reproduction of earthworms has been shown to decrease with decreasing pH of soils (Luo et al., 2014). However, the soil from Nyborgmoen also had low pH, but higher reproduction of earthworms.

3.4 Bioavailability of Pb and Risk Assessment

The concentration of low molecular mass (<10 kDa) and/or cationic Pb can give an indication of the concentration of bioavailable Pb in water. The concentrations of these species can be compared with the environmental quality standard (EQS) for Pb in the Water Framework Directive (WFD), which applies to bioavailable concentration. Although speciation of metals can give an estimation of the concentration of bioavailable metal, it does not provide a definite answer of the bioavailable concentration, or information about the toxicity of the species. The earthworm toxicity test, on the other hand, gives information about the toxicity of the metal contamination to earthworms, but does not provide information about the concentration of bioavailable metal in the soil. The reproduction of earthworms in soil with Pb compared to the reproduction in soil without Pb from the same location can be used to calculate effect concentrations, such as EC_{10} and EC_{50}, of Pb for earthworms. The effect concentrations can be used in risk assessments of Pb in shooting range soils. This study only focused on Pb, but contaminations of other metals, such as Cu and Zn, are also likely to influence the reproduction of earthworms in soils (Lukkari et al., 2005).

4.0 CONCLUSION

The results from this study showed that the bioavailability and toxicity of Pb in run-off waters and soils from different shooting ranges varied. Both the pH and the content of TOC in the run-off waters and soils seemed to influence the bioavailability of Pb. The charge distribution of Pb in the run-off waters indicated that a lower content of TOC and a higher pH resulted in higher bioavailability of Pb (higher content of cationic Pb). On contrary, the size distribution indicated that a higher content of TOC and a lower pH resulted in higher bioavailability of Pb (higher content of Pb present as smaller species). Soils with concentrations of Pb up to 1500 mg/kg showed no effect on survival of the adult earthworms, and survival was not considered as a suitable parameter to assess bioavailability of Pb in the soils from the shooting ranges. The reproduction of earthworms decreased with increasing concentration of Pb in the soils. The largest effect of Pb contamination on reproduction of earthworms was found for the soil with the highest pH, and the soil with the highest content of TOC. In risk assessments of contamination of Pb in run-off water and soil from shooting ranges, the bioavailability of Pb in the particular shooting range should be taken into consideration.

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