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Drivers of Pb, Sb and As release from spent gunshot in wetlands: Enhancement by organic matter and native microorganisms

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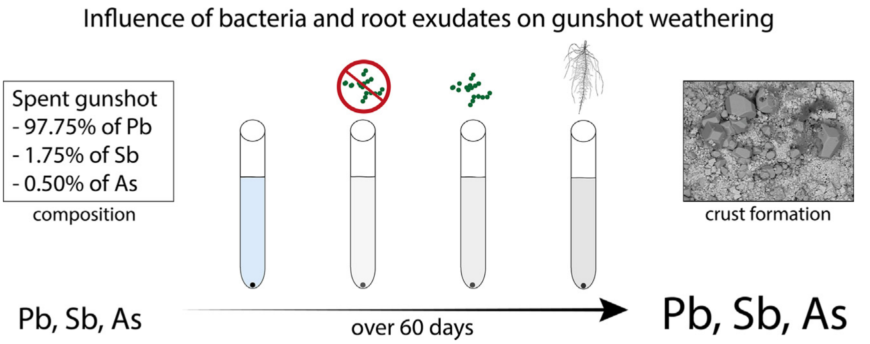
HIGHLIGHTS

- Greatest mobilization of elements occurred in solutions containing organic matter.
- 60-day leaching in organic-rich solutions was 2.69 % (Pb), 1.16 % (Sb) and 1.83 % (As).
- Native microorganisms enhanced Pb and As mobilization from shot.
- Bioweathering resulted in the occurrence of Pb-carbonates on spent gunshot.
- Spent gunshot may result in contamination of wetland habitat.

GRAPHICAL ABSTRACT



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ABSTRACT

In many countries the use of lead-based ammunition is prevalent, and results in exposure and poisoning of waterfowl and other species of birds. In waterfowl hunting areas large quantities of spent shot may be deposited in wetland and terrestrial habitats. These pellets can undergo transformations, which are influenced by various abiotic and biotic factors. In addition to lead (Pb), other elements like antimony (Sb) and arsenic (As) can be leached from Pb shot into the environment. In vitro simulations that included organic matter and microorganisms were utilized to examine elemental leaching from gunshot. We found that leaching efficiency was the greatest in solutions rich in organic matter derived from artificial root exudates (2.69 % for Pb, 1.16 % for Sb, 1.83 % for As), while leaching efficiency was considerably lower in river water (0.04 %). In vitro simulations containing native microorganisms also exhibited greater leaching efficiency (0.49 % for Pb, 0.52 % for Sb, 1.32 % for As) than in ultrapure deionized water and river water. Surface alterations in gunshot included the formation of a weathering crust and secondary phases dominated by carbonates. Spent gunshot is a source of Pb, Sb and As in wetlands that could affect aquatic ecosystems.

1. Introduction

In many countries, waterfowl hunting inherently entails releasing large amounts of spent lead (Pb) gunshot into wetland areas. Density of spent gunshot is related to the intensity of hunting and can range from a few shot to several hundred shot/m^2 (Pain et al., 2019). It has long been recognized that continued deposition of spent Pb shot in a hunted wetland increases the potential for exposure (primary - direct shot ingestion; secondary - consumption of prey containing spent shot) and poisoning of wildlife, and contamination of the environment (Binkowski and Sawicka-Kapusta, 2015; Pain, 1990; Pain et al., 2019; Romano et al., 2016). Despite widespread awareness of this problem, Pb shot remained in use for waterfowl hunting in many countries (Meissner et al., 2020). At the request of the European Commission in 2016, the European Chemicals Agency (ECHA) investigated the use of Pb in ammunition, and proposed restriction

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of its use in many settings, including shotgun hunting in and over wetlands (ECHA, 2021a). In 2021, the European Commission accepted this proposed restriction and initiated legislative efforts (ECHA, 2021b). However, banning the use of Pb ammunition does not fully resolve this environmental problem. Depending upon the rate of fresh sediment deposition and its physical characteristics, Pb shot can reside in the upper sediment of a wetland for extended periods of time and remain bioavailable for decades (Flint and Schamber, 2010; Mateo et al., 1998).

There have been many studies of the fate of Pb at shooting ranges, including the bioavailability of Pb, which is seemingly high, the process of Pb shot weathering and the mobility of Pb in terrestrial environments (Bannon et al., 2009; Cao et al., 2003a; Takamatsu et al., 2010). However, conflicting reports describe the possibility of Pb migration from shooting range to water (Craig et al., 1999; Soeder and Miller, 2003). In wetland settings, much less is known about the form and fate of Pb in and on the spent shot, and in the fraction of Pb released into aqueous media. Pellets deposited in wetlands are almost always submerged, with physicochemical (e.g., temperature, pH, dissolved ions, friction) and biological (e.g., plant litter, organic matter, microbial activity) factors that create different bioweathering conditions than occur in a terrestrial setting (Binkowski, 2017). To address this issue, preliminary chemical and biological information on elemental leaching from shot was sought to assist in the design of more complex field studies.

We undertook a simplified in vitro approach to examine abiotic and biotic factors potentially influencing the bioweathering of shot in aqueous conditions of fish ponds (popular hunting sites for waterfowl), focusing on Pb, Sb and As commonly found in ammunition used for hunting waterfowl in Europe. We sought to determine the extent to which abundant organic matter or the presence of native pond microorganisms affected dissolution of elements from Pb shot. Conditions conducive to dissolving Pb were deliberately chosen to test the importance of organic matter and microorganisms over a relatively short time. In this context, we examined the surface of gunshot and the efficiency and dynamics of gunshot weathering (proxied by pellet weight loss and elemental leaching efficiency) in different environmental conditions over a 60-day period. Such information will assist in the evaluation of the long-term risks posed by spent gunshot deposited on wetlands.

2. Materials and methods

This study consisted of four treatments (Table 1) that simulated some conditions that may be encountered in wetland environments. Capped 50 mL falcon plastic tubes (ISOLAB, Germany) were filled with: 1) natural river water (RWA), 2) sterile extract of pond sediments (SPS), 3) extract of pond sediments (APS), or 4) artificial root exudates (ARE). Each tube contained 10 mL of the solution and one number 4 Pb shot, and there were 10 replicates per treatment. Tubes were incubated at 30 °C in an orbit shaker incubator (100 oscillations/min; ES-20/60, Biosan) for a 60-day period to speed up the reaction rate (see Section 4.4 for wider explanation) and to include simulation of wind action on the pond. During the initial 30-day period, the weathering media in each tube was collected (stored at 4 °C for subsequent analysis) and totally exchanged (to maintain good level of nutrients and limit the influence of metabolites on microorganisms) at 10-day intervals (semi-open pass flow-through mode commonly known as a solution renewal), while retaining the original gunshot in the tube. Between days 31 to 60, the media containing gunshot was not renewed, with media and gunshot collected for subsequent analysis at the end of day 60. For all solutions (10 replicates/treatment), pH was measured at the beginning and end of their use (Tables 1, S8). Each shot was gently rinsed with ultrapure water and air-dried (5 days in an unsealed 1 mL Eppendorf microfuge tubes) and then weighed for subsequent observation by electron microscopy. Leachate from each tube was analyzed for the dominant element present in shot, namely As, Pb, and Sb.

2.1. Experiment preparation

Ultrapure water was the matrix in three treatments (RWA excluded) and was obtained from a Millipore Direct 3 unit at a resistivity of 18.2 MΩ·cm. Surface water (RWA) was collected from the Skawa river near Stawy Zatorskie (southern Poland, Europe) feeding wetlands that were sampled (pH 6.8). We confirmed the presence of microbes in RWA by agar plating. Additionally, the presence of microbial metabolites (e.g., aromatic compounds, humic-like compounds, microbial by-products) was confirmed using a three-dimensional excitation-emission spectrofluorophotometer (RF-5301 PC Shimadzu) equipped with a 150 W xenon lamp. Measurements were performed in 1 cm quartz optical cells. Prior to those measurements, samples were filtered with 0.22 μm polyethersulfone filters (PES).

Sediment for two treatments (SPS and APS) was collected on a wetland hunting area (GPS N 50.003013, E 19.477784) that was fed by the Skawa river. It was dried at room temperature and sieved. The dried material (100 g of the <2 mm fraction) was suspended in 1 L sterile mineral salt medium for biostimulation. The medium included glucose (3 g/L), succinic acid (3 g/L), ammonium sulfate (1 g/L) and disodium hydrogen phosphate (0.4 g/L) at a pH of 4.0. The sediment suspension was orbital shaken for 24 h at room temperature. A 100 mL aliquot of the suspension was sterilized using syringe filters (0.22 μm PES) under aseptic conditions to obtain a sterile sediment extract (SPS). Another aliquot was filtered using qualitative Whatmann paper filters (#10010155) under a laminar flow hood to obtain a microbiologically active sediment extract (APS).

The root exudates solution used in ARE treatment was prepared in the laboratory and contained glucose (1.66 g/L), fructose (1.66 g/L), sucrose (1.57 g/L), citric acid (0.88 g/L), lactic acid (0.83 g/L), and succinic acid (0.81 g/L) (Potysz et al., 2018). The pH of ARE solution was adjusted to 4.0 (equal to pH of SPS and APS) with sodium hydroxide. Finally, a 100 mL aliquot of the ARE solution was sterilized using PES syringe filters (0.22 μm) under aseptic conditions to obtain a sterile solution.

At the end of the experiment, a microbial activity test for SPS and APS treatments was run to confirm the absence of microbial conditions in sterile cultures (SPS) and the presence of microbial conditions in the biotic cultures (APS). This was accomplished by spreading an aliquot of each replicate on an agar plate, followed by observation of growth after incubation at 30 °C for 72 h.

Lead gunshot (diameter 3 mm, No. 4 shot, weighing 0.155 g–0.188 g) used in experiments were removed from recently purchased hunting shot cartridges (12 cal., weighing ~34 g). This type of ammunition is commonly used by waterfowl hunters in this region of Europe, where Pb ammunition has not been banned. Before use, shot were gently cleaned using 70 % ethanol, weighed and sterilized at 121 °C for 20 min.

Table 1
Characteristics of four treatments stimulating some conditions that may be encountered in wetland environments to examine abiotic and biotic factors potentially influencing the bioweathering of lead gunshot in fish ponds.

Treatment	Name	Water source	Characteristics of constituents	Presence of microorganisms	pH of solution	Proxy
River water	RWA	River	Inorganic, organic, biological	+	6.81 ± 0.01	Natural conditions
Sterile pond sediment	SPS	Laboratory[a]	Inorganic, organic	−	4.00 ± 0.01	Influence of chemical composition
Active pond sediment	APS	Laboratory[a]	Inorganic, organic, biological	+	4.00 ± 0.01	Biotic influence
Artificial root exudates	ARE	Laboratory[a]	Organic	−	4.00 ± 0.01	Influence of organic compounds

The composition of the SPS, APS and ARE came from adding the demanded reagents (see Experiment preparation in Materials and methods for details).
pH of solution at the beginning of the replacement. pH data from the consecutive sampling dates are presented in the Supplementary materials.
[a] Ultrapure water (resistivity of 18.2 MΩ·cm at 25 °C) obtained in laboratory from Millipore Direct 3 unit.

2.2. Element analyses

We measured concentrations of As, Cu, Pb, Sb, Sn, and Zn in gunshot and As, Pb and Sb in leaching solutions using inductively coupled plasma atomic mass spectrometry (ICP-MS Nexion 300D PerkinElmer; ^{75}As, ^{63}Cu, ^{208}Pb, ^{121}Sb, ^{118}Sn, and ^{66}Zn) with ^{209}Bi (for Pb), ^{115}In (for Sn and Sb) and ^{45}Sc (for As, Cu and Zn) as internal standards. At the sampling step, each solution was passed through 0.22 μm PES and acidified with nitric acid (65 %, Suprapur, Merck) to obtain a 2 % solution. We also measured concentrations of the elements in the gunshot ($N = 6$) that were acid digested (15 mL of nitric acid 65–67 %, Baker Instra-Analyzed, JT Baker) in capped 100 mL glass volumetric flasks (Simax, Czech Republic) at room temperature. The tubes were shaken on an orbit shaker (100 oscillations/min; SK-O330-Pro, Chemland) for 3 h, topped up with ultrapure water, and shaken again for 1 h. Such prepared solutions were filtered with quantitative filter paper (type 389, Ahlstrom Munktell, Germany).

An ICP multi-element standard (solution IV Certipur, Merck) was used in the calibration. The calibration curve ranged within 0–50 μg/L for analysis of leachates and 0–500 μg/L for analysis of shot. Samples were analyzed at different dilutions to fit the working range of the method as well as to determine data accuracy and precision. The limits of quantification were typically <0.01 to 0.02 μg/ L for these 6 elements (Table S6). Quality control included systematic analysis of control solutions and spikes as well as certified reference materials (CRM BCR-288 Lead with added impurities, IRMM, JCR, Belgium and CRM TMDA-70 Fortified water, Environment Canada). Spiking with known quantities of analytes (ICP standards, Merck) was done to randomly chosen samples of water from each treatment (recoveries fitted typically 96–104 %). CRM BCR-288 samples were dissolved in nitric acid (65–67 %, Baker Instra-Analyzed, JT Baker) and CRM TMDA-70 samples were prepared for analysis following the same procedure as water samples from the treatments (Table S6).

2.3. Scanning electron microscope (SEM-EDS) observations

After the termination of the experiment, dry gunshot were examined using a scanning electron microscope (JSM IT-100, JEOL) coupled with the x-act energy dispersive X-ray spectrometer (Oxford Instruments). The sample chamber pressure was set at a low vacuum working mode to avoid sample coating with carbon and the accelerating voltage was set as 15 kV.

2.4. Statistical analysis

Elemental leaching from shot (μg of element released from a gram of gunshot; μg/g) was calculated by multiplying the elemental concentrations in solutions (μg/ L) by their liquid to solid ratio (based on the initial weight of each pellet) (Piatak et al., 2015).

Since dissolution data were determined on a concentration basis, we suspected the observations would be log-normally distributed, and this was confirmed by graphical data inquiry. Thus, all inferential analyses were conducted on log transformed data, which improved homoscedasticity and goodness of fit. For this reason, descriptive statistics included the geometric mean (GM) followed by the arithmetic mean (AM), standard deviation (SD), relative standard deviation (RSD), and extreme values (min-max). The statistical investigation examined total leaching, leaching during various sampling times (dynamics), and correlation among leached elements. Data on total leaching of each element over a 60-day period were compared among treatments by ANOVA followed by the Tukey HSD post-hoc test. For leaching dynamics, data from each sampling day (i.e., 0, 10, 20, 30, and 60) were compared among treatments with repeated measurements ANOVA. For both total leaching and dynamics, parametric model assumptions were validated based on graphical inquiry of residuals. Finally, the leaching correlations among elements at day 60 were studied without division to treatments with Pearson coefficients.

Calculations and statistical analyses were conducted using Excel (ver. 365) for Mac (Microsoft), GraphPad Prism (ver. 7) for Mac (GraphPad Software), Statistica (ver. 13.3) for Windows (TIBCO Software) and R (ver. 4.02 for Mac; R Core Team 2020) equipped with R Studio for Mac (ver. 1.3.1056; RStudio PBC). The significance level was set as 0.05 for all comparisons.

3. Results

The microbial activity test performed after the experiment revealed growth on APS and RWA plates indicating that microbial activity was maintained. In contrast, no microbial growth on SPS and ARE plates was observed, indicating that sterile conditions were maintained during the experiment.

3.1. Composition of gunshot pellets

Gunshot were predominantly composed of Pb (977.46 mg/g), accompanied by a relatively high content of Sb (17.47 mg/g) and As (5.00 mg/g). Other elements (e.g., Cu, Zn) were present at much lower concentrations (<0.03 μg g^{-1}) (Table S7). Since Pb, Sb and As dominated the composition of the gunshot, we focused on these elements.

3.2. Total leaching, dynamics and correlations

Elements were leached in proportion to their concentration in shot, with quantity of Pb being greatest, followed by Sb and As (Fig. 1). Treatment affected the total quantity of Pb, Sb and As leached from shot ($F_{3, 36}$ consecutively 2891, 1161 and 2040, $p < 0.001$). Dissolution of Pb from shot differed among all treatments (Tukey's HSD, $p < 0.001$). With the exception of the SPS and APS ($p > 0.999$) treatments, leaching of Sb and As differed among the other treatments ($p < 0.001$). Greatest leaching was noted in the ARE treatment, reaching 2.69 % of the total quantity of Pb, 1.16 % of the total quantity of Sb, and 1.83 % of the total quantity of As (Table 2).

Because sampling intervals differed in this study (i.e., 10-day intervals for sampling times on days 10, 20 and 30, and a 30-day interval for the sampling time on day 60), estimated leaching was adjusted to a daily basis, and expressed as μg of element leached per g shot per day (μg/g/day) and also in terms of % of the total quantity of each element in the shot per day (%/day). There was an interaction between treatment and sampling time for leaching dynamics of all three elements (repeated measures ANOVA, $p < 0.001$, Table 3). In general, greatest leaching rates, adjusted to a daily basis, occurred in the ARE treatment (Table 4; specific comparisons presented in supplementary materials Tables S9-S11).

Although treatments strongly affected the total quantity of each element leached from shot, leaching rates among elements for a given treatment were more comparable (Table 2, Leached [%]). One would presume that Pb, Sb and As were homogeneously distributed in shot, and this was verified in part by findings of significant ($n = 40$ pooled observations for each element across treatments, $p < 0.001$) and strong relationships of total leaching among elements by Pearson correlation analysis (Pb vs. As $r = 0.904$; Pb vs. Sb $r = 0.961$; Sb vs. As $r = 0.980$).

3.3. Weathering features

During the experiment, the weight of shot decreased, with the greatest loss observed in the SPS and ARE treatments (Table 5). The greatest change was observed in the SPS treatment (-1.99 ± 1.51 %) and the smallest change in the RWA treatment (-0.20 ± 0.20 %).

Using SEM, fresh gunshot exhibited an even surface without cracks and alterations (Fig. 2). After the 60-day treatment period, shot displayed visible alterations of the surface, with a weathering crust coating being the most common feature. Regardless of the treatment conditions, EDS spectra of the shot surface indicated that carbonates were the dominant secondary phase. The size of cracks and surface alterations visibly differed between treatments. External morphology of gunshot from RWA treatment was found to be the least changed and the surface remained relatively unchanged, with occasional presence of precipitates. Surface alteration of

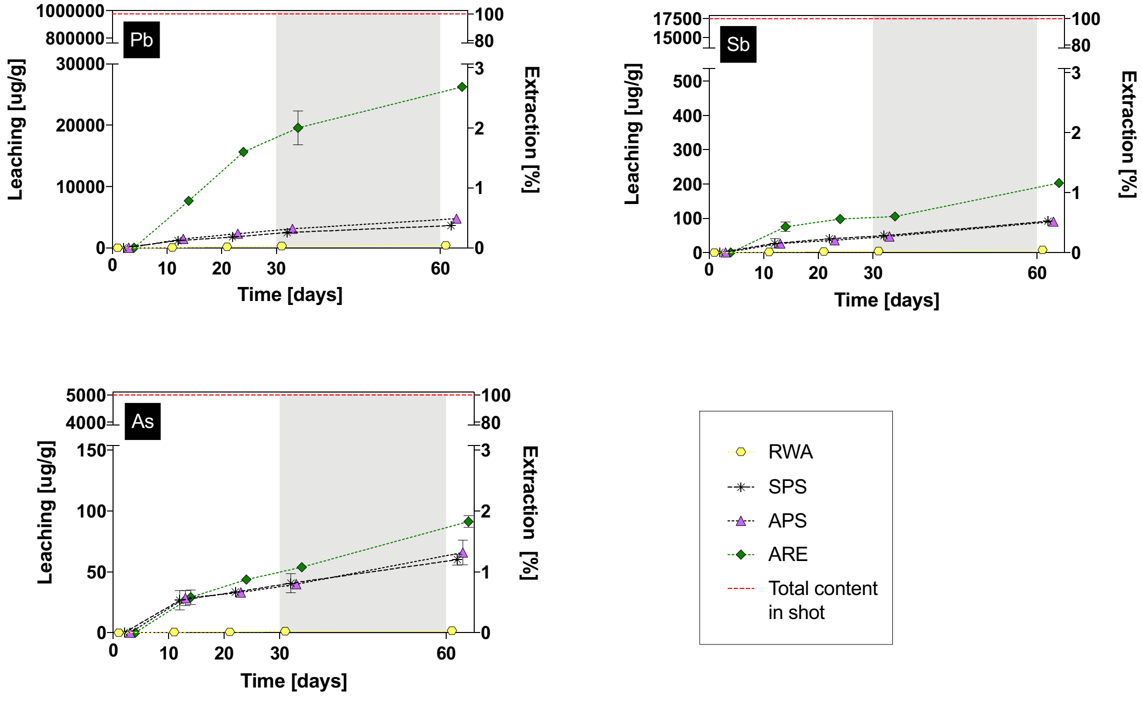

Fig. 1. Cumulative leaching data (mean ± SD) of elements from gunshot pellets exposed to river water (RWA), sterile extract of pond sediment (SPS), active extract of pond sediment (APS) and root exudate solution (ARE) over 60 days. Samples were collected at 10-day intervals for days 10, 20 and 30. A 30-day interval was used for the 60 day sample, indicated by the shaded area. Note that the scale of the y-axis is different for each element.

Table 2
Descriptive statistics of cumulative leached elements from gunshot pellets (μg/g) reached at day 60, followed by the leached fraction of initial gunshot content.

Element	Treatment	GM	AM (SD)	Min-max	Leached [%] (SD)	Leached adj. [%]
Pb	RWA^{a}	400.69	401.46 ± 26.46	367.64–447.10	0.04 (0.00)	–
Pb	SPS^{b}	3597.17	3626.71 ± 488.18	2840.72–4389.31	0.37 (0.05)	0.13
Pb	APS^{c}	4750.36	4757.52 ± 278.44	4421.61–5318.41	0.49 (0.03)	0.17
Pb	ARE^{d}	26,082.28	26,255.72 ± 3150.53	21,742.66–30,965.50	2.69 (0.32)	0.92
Sb	RWA^{a}	7.88	7.89 ± 0.23	7.38–8.26	0.05 (0.00)	–
Sb	SPS^{b}	89.76	91.55 ± 20.47	70.30–140.80	0.52 (0.12)	0.18
Sb	APS^{b}	89.57	90.34 ± 12.86	78.74–114.54	0.52 (0.07)	0.18
Sb	ARE^{c}	202.50	203.17 ± 17.51	182.62–233.55	1.16 (0.10)	0.40
As	RWA^{a}	1.77	1.77 ± 0.10	1.57–1.91	0.04 (0.00)	–
As	SPS^{b}	58.99	59.87 ± 11.33	46.87–82.75	1.20 (0.23)	0.41
As	APS^{b}	65.14	65.96 ± 11.17	49.15–88.58	1.32 (0.22)	0.45
As	ARE^{c}	91.18	91.37 ± 6.23	83.46–103.71	1.83 (0.12)	0.63

GM – geometric mean, AM – arithmetic mean, SD – standard deviation, RWA - natural river water, SPS - sterile extract of pond sediments, APS - extract of pond sediments; ARE - artificial root exudates.
Leached – leached fraction of element included in the shot.
Leached adj. – values adjusted to the pH 7 scenario (see Environmental significance in the Discussion).
Differences were observed between all treatments for all elements (ANOVA on log transformed data).
Differences between each group separately within each element were marked with a different letter.

the shot in sterile conditions (SPS) was less pronounced than under biotic conditions (APS), where more severe changes of the surface (e.g., fractures, cracks) and formation of secondary phases commonly occurred. The most pronounced change in surface morphology of gunshot was in the ARE treatment, where significant surface perforation was observed even if secondary phases were formed (Fig. 2).

Table 3
Statistical results (F statistic followed by *p* value) of the comparison for leaching dynamics (RM-ANOVA carried out on logged data).

Element	Pb	Sb	As
Treatment (F$_{3,\ 36}$)	1047, <0.001	1439, <0.001	2833, <0.001
Sampling (RM) (F$_{3,\ 108}$)	81, <0.001	220, <0.001	160, <0.001
Treatment ∗ sampling (F$_{9,\ 108}$)	15, <0.001	38, <0.001	24, <0.001

4. Discussion

We found that bioweathering of Pb gunshot was markedly influenced by treatments, some of which simulate natural environmental conditions. Presence of abundant organic matter seemed to be the principal factor triggering leaching of elements, with surface alternations being more pronounced when treatment conditions changed from river water to complex sediment solutions containing microorganisms. The extent of surface alterations of shot observed by SEM seemed to parallel the degree of Pb, Sb and As dissolution.

4.1. Native microorganisms and organic matter as bioweathering factors

Microorganisms have deteriorative abilities on metal-bearing minerals (Gadd, 2007; Rhee et al., 2012; Uroz et al., 2022) and slags (Yin et al.,

Table 4
Daily leaching (mean ± SD) of Pb, Sb and As from shot during various sampling intervals.[a]

Element	Treatment	Days 0–10		Days 10–20		Days 20–30		Days 30–60	
Pb	RWA	6.40 ± 0.77	(0.0007)	12.08 ± 2.98	(0.0012)	10.95 ± 1.34	(0.0011)	3.57 ± 0.40	(0.0004)
Pb	SPS	116.82 ± 7.79	(0.0119)	59.42 ± 9.83	(0.0060)	74.97 ± 15.12	(0.0075)	37.15 ± 8.62	(0.0037)
Pb	APS	146.23 ± 20.44	(0.0148)	84.48 ± 11.73	(0.0086)	81.23 ± 9.32	(0.0083)	54.6 ± 8.17	(0.0055)
Pb	ARE	764.86 ± 72.88	(0.0779)	798.83 ± 66.68	(0.0814)	391.34 ± 276.14	(0.0262)	223.51 ± 8.73	(0.0229)
Sb	RWA	0.18 ± 0.02	(0.0010)	0.12 ± 0.02	(0.0007)	0.14 ± 0.01	(0.0008)	0.12 ± 0.01	(0.0007)
Sb	SPS	2.71 ± 1.40	(0.0142)	1.29 ± 0.20	(0.0073)	0.92 ± 0.19	(0.0052)	1.41 ± 0.33	(0.0079)
Sb	APS	2.62 ± 0.90	(0.0143)	0.99 ± 0.25	(0.0055)	1.11 ± 0.21	(0.0062)	1.44 ± 0.20	(0.0082)
Sb	ARE	7.56 ± 1.40	(0.0426)	2.22 ± 0.54	(0.0123)	0.78 ± 0.08	(0.0044)	3.25 ± 0.37	(0.0185)
As	RWA	0.05 ± 0.01	(0.0009)	0.04 ± 0.00	(0.0007)	0.05 ± 0.01	(0.0010)	0.01 ± 0.00	(0.0003)
As	SPS	2.66 ± 0.79	(0.0516)	0.67 ± 0.15	(0.0132)	0.73 ± 0.78	(0.0114)	0.64 ± 0.15	(0.0125)
As	APS	2.85 ± 0.61	(0.0557)	0.46 ± 0.08	(0.0091)	0.68 ± 0.20	(0.0131)	0.87 ± 0.34	(0.0164)
As	ARE	2.92 ± 0.60	(0.0574)	1.44 ± 0.28	(0.0283)	1.03 ± 0.28	(0.0201)	1.25 ± 0.16	(0.0248)

RWA - natural river water, SPS - sterile extract of pond sediments, APS - extract of pond sediments; ARE - artificial root exudates.

[a] Values are expressed as μg element leached/g shot per day and (% element leached/total quantity in shot per day).

2014), and thus their effect on weathering of Pb gunshot was not unexpected. Theoretically, microorganisms likely affect spent Pb shot in several ways. First, they may initiate redox reactions on the shot surface by chemolithoautotrophy (obtaining energy from the oxidation of inorganic compounds and using it for fixing organic carbon from inorganic carbon). This process is incompletely understood, and occurs with some elements (e.g., Fe, S, Mn; Berben et al., 2019; Ishii et al., 2015; Yu and Leadbetter, 2020), but this process has not been documented for Pb or Sb. Secondly, microorganisms may release organic metabolites, that could enhance ligand-promoted dissolution (Gadd, 2010, 2007; Jones, 1998). Thirdly, those metabolites may affect pH and other physicochemical parameters (Ratzke and Gore, 2018), and thus change the local environment around spent shot to enhance leaching rate (Binkowski, 2017). It is worth mentioning that microorganisms such as fungi may cause biocorrosion of Pb in aqueous solutions, leading to formation of immobilized secondary phases such as pyromorphite (Rhee et al., 2014). In the present study, we observed a small but significant difference in Pb leaching efficiency between SPS and APS treatments, which indicates that the microbial activity of APS may slightly accelerate dissolution (Fig. 1).

In addition to the presence of microorganisms, consideration should be given to the chemical nature of the treatments (i.e., ranging from relatively simple to a complex matrix) present in the aqueous solution. The influence of the complexity of the solution was proxied by the difference between SPS and ARE treatments, with all the elements seemingly leached more readily by ARE than SPS (Table 2, Fig. 1). The most remarkable difference was noted for Pb (2.32 %), followed by Sb (0.64 %) and As (0.63 %). The differences between leaching in ARE and SPS were 2.32 % points for Pb, but only around 0.64 % points for Sb and for As. A similar tendency (greater dissolution in the presence of a more complex matrix) was observed in our previous work (Binkowski, 2017). Thus, we conclude that the presence of organic matter affected the mobility of elements in soils and soil-water conditions (Jordan et al., 1997; Kalbitz et al., 2000; Lisin et al., 2022).

However, ARE treatment does not only represent the organic-rich matrix but also proxies rhizosphere conditions which contain a wide range of functional groups rendering organic molecules highly reactive for metal complexation (Dudal and Gérard, 2004). They can result in micro-acidification (e.g., associated with local organic enrichment), leading to the formation of secondary phases on the gunshot surface (Pb carbonates, see Fig. 2) (Cao et al., 2003b). Research on topsoil layers at shooting ranges not only confirmed the significance of organic compounds in elemental leaching, but also identified pH as the most important factor which can even hamper elemental mobilization in organic-rich soils (Jørgensen and Willems, 1987).

Table 5
Initial gunshot weights and weight changes after 60 days (mean ± SD) of exposure to four treatments.

Treatment	Initial weight [g]	Weight change [%]
RWA	0.1724^{a} ± 0.0092	−0.20^{a} ± 0.03
SPS	0.1688^{a} ± 0.0036	−1.99^{b} ± 1.51
APS	0.1678^{a} ± 0.0037	−1.07^{ab} ± 1.35
ARE	0.1682^{a} ± 0.0029	−1.68^{b} ± 1.45

RWA - natural river water, SPS - sterile extract of pond sediments, APS - extract of pond sediments; ARE - artificial root exudates.
Differences were marked with a different letter superscript.

4.2. Bioweathering in natural conditions

In our study, the RWA solution proxied leaching under natural conditions. Compared to other treatments, element leaching in RWA was consistently the weakest. In the study, the principal aim was to evaluate the influence of matrix complexity and microorganisms on leaching, which was done by adjusting treatment conditions. It should be noted that pH in the SPS, APS, and ARE treatments (all pH 4.0) was much lower than the RWA treatment (pH 6.8), which resulted partially from the chemical composition of those treatments, but also our intention to speed up leaching in the experiment. Thus, we suspect that the differences in leaching between RWA and other treatments were caused principally by pH. The observations of significant differences in Pb leaching related to pH are well documented (Binkowski, 2017; Zhang and Lin, 2015). Additionally, we can assume that the chemical and biological composition of the APS and RWA treatments are relatively similar, which supports the difference in leaching efficiency may be attributable to differences in pH (Table 2).

4.3. Environmental significance

On a global scale, the pH of natural ponds can vary widely, including ponds in forest locations with pH even lower than 4.0 (Sherman and van Munster, 2012; Spyra, 2017). The pH of fish ponds in Poland and elsewhere is regulated to a level healthy for fish, with values generally ranging from 6 to 9 (Binkowski and Rzonca, 2014; Freda, 1986; Tucker and D'abramo, 2008). In the present study, pH of treatments averaged 4, which based on our own studies markedly enhances leaching rates compared to circumneutral pH. Using our previously described findings (Binkowski, 2017), the ratio of Pb dissolution at pH 4 versus pH 7 is 2.9. Applying this adjustment factor to the leaching observed in the SPS, APS and ARE substantially decreases Pb concentration values (Table 2). Using those results, one can make preliminary estimates of the leaching rate occurring in fish ponds and other wetlands commonly used for hunting (for detailed calculations, see Table S12). For a moderately hunted site, spent shot deposition is about 6.9 shot per m^2 (Bellrose, 1959). Using the aforementioned adjustment factor, Pb concentration from leaching for the ARE treatment at pH 7 (9.76 μg/L; calculated with the assumption of 1 m depth pond giving 1000 L per m^2) is predicted to exceed the Pb freshwater chronic criteria for aquatic biota (2.5 μg/L; USEPA, 2022). The USEPA threshold refers to dissolved metal in the water column, and is value is based on Pb in solution. For the intensive hunting condition (300 shot per m^2, (Pain et al., 2019)),

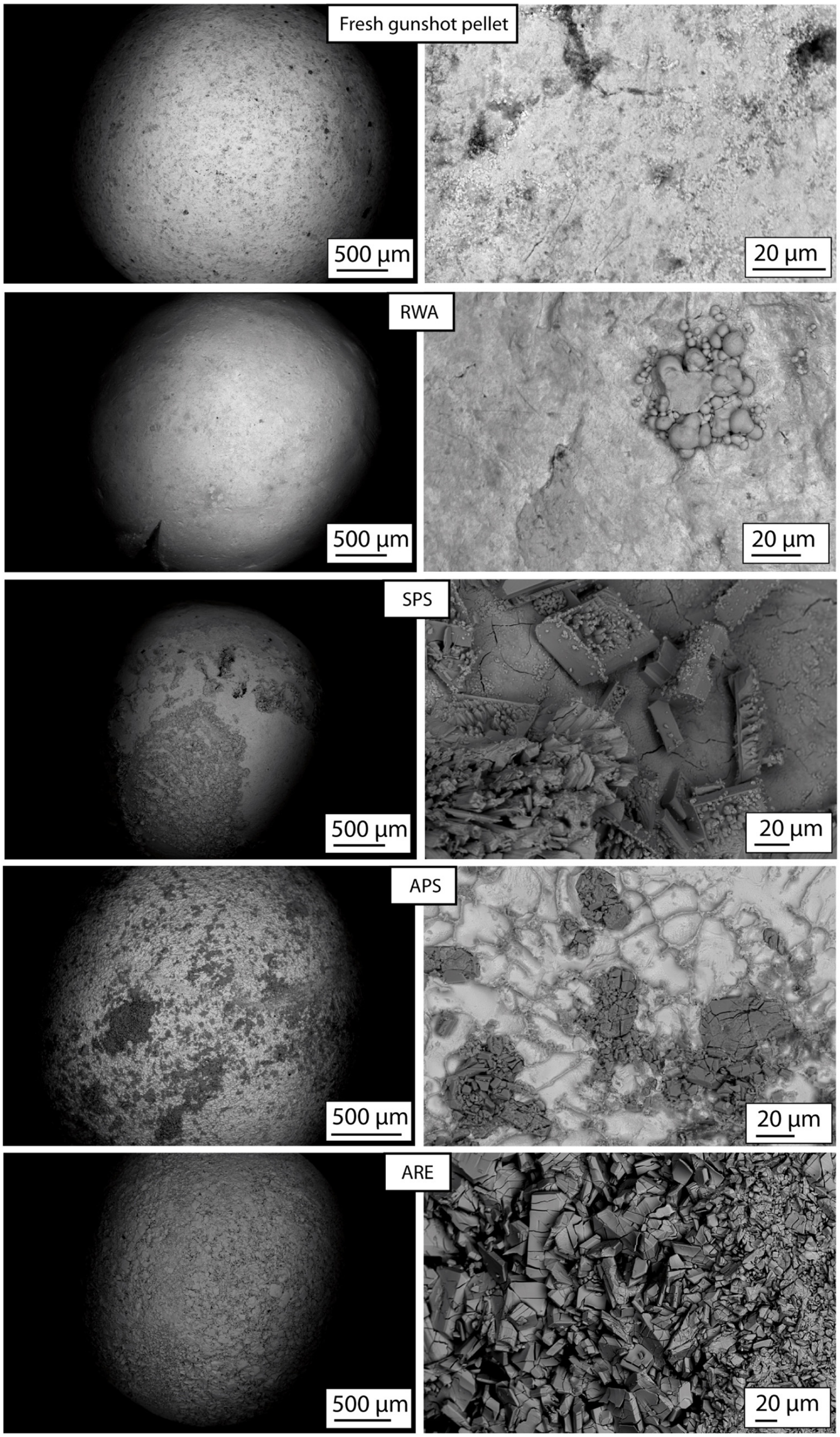

Fig. 2. Scanning electron microscopic observations of fresh and weathered gunshot pellets from various 60-day treatments studied. Fresh gunshot exhibits a relatively smooth surface; RWA gunshot - smoother surface than that of fresh gunshot indicating that incipient weathering took place; SPS gunshot –signs of dissolution notable and secondary phases formed indicating severe weathering of gunshot; APS gunshot – more dissolution features than in the case of SPS and secondary phases formed proving intense weathering; and ARE gunshot –entire surface reveals weathering signs in the form of a weathering crust. RWA - natural river water, SPS - sterile extract of pond sediments, APS - extract of pond sediments; ARE - artificial root exudates.

this threshold was exceeded in all the treatments (including the RWA treatment with the lowest value of 18.45 μg/L; based on the same volume assumption as for ARE). This indicates that Pb shot may pose a risk not only when ingested by waterfowl, but perhaps also to aquatic biota when Pb is released during weathering and leaching into pond water. To better assess the environmental risk associated with leaching of elements from Pb shot in fish ponds, systematic studies should use pond water and associated matrices at a variety of pH levels. It is well recognized that pH can enhance Pb leaching and mobility (Mattson, 1999; Rooney et al., 2007; Soeder and Miller, 2003; Zhang and Lin, 2015), and it is certainly possible that some hunting areas (with low water/soil pH and high prevalence of spent gunshot) currently face significant pollution problems with elements present in gunshot.

While there are no specific regulations classifying spent Pb gunshot as a hazardous waste material, the European Union and USEPA have regulations on the application of waste materials (e.g., sewage sludge) containing Pb and other metals to land (e.g., amending agriculture fields) that may serve as a tentative guideline. Limit values for landfill wastes indicate that the release of up to 10 mg kg^{-1} (for Pb) categorizes the waste as non-hazardous, whereas when these values exceed 50 mg kg^{-1} (EC, 2002, 1999), the waste is classified as hazardous. The values between these threshold levels indicate a ‘potentially hazardous’ waste classification (EC, 2002, 1999). Our data (adjusted to pH 7) revealed that within 60 days (for all conditions studied) the hazardous character of gunshot in the landfill context can be characterized. In the USA, some risk assessments use permissible limits of Pb in sewage sludge for application to arable fields as a threshold value (US DOI, 2013, 1997, 1987). Based on the USEPA document, the cumulative pollutant loading value for Pb equals 300 kg ha^{-1} (US EPA, 1994). That approach is based on a number of deposited shot (not leaching values) and sets a deposition value of about 176 gunshots per square meter as the maximum acceptable threshold. Such deposition has been described in the scientific literature in heavily hunted wetlands (Mateo et al., 1997).

The European Union has proposed restricting the use of Pb shot for hunting waterfowl in wetlands (ECHA, 2021b), and when implemented by member states, the addition of new spent shot to the environment will be reduced. However, in some wetlands the current number of deposited shot is high, and it may be beneficial to consider these areas for remediation.

4.4. Limitations of this study

In contrast to studies and published information on Pb mobility from chat at mining sites, and from spent ammunition at shooting ranges (Cao et al., 2003a, 2003b; Jørgensen and Willems, 1987; Murray et al., 1997), there is a paucity of data on mobility of spent Pb shot in wetlands. There is a need then to identify the mechanisms influencing Pb turnover in such areas. Due to the complexity of the problem, we started with a simple laboratory approach that has some shortcomings. First, the pH was used in our experiment lower than usually found in most (but not all) wetlands to speed up the weathering. However, using our previous findings, we were able to estimate leaching efficiency at neutral pH (Binkowski, 2017). Second, the tubes were oscillated at 100 times per minute, which would most likely physically break up crusts that might form and hasten weathering. Dissolution would likely be slower if shot remained undisturbed for 60 days. Third, sampling intervals were not equal (10 days for the first 3 samplings, and for logistical reasons only one 30-day interval to examine more prolonged responses). However, we expressed leaching data on a per day basis in an attempt to normalize comparisons. Fourth, we used fresh Pb shot from new cartridges to obviate surface and shape effects that may occur during ammunition discharge. Summarizing these limitations, the study includes some artificial conditions to better examine the importance of organic matter and microorganisms for dissolution rather than to mimic environmental conditions. Translating these in vitro findings to actual environmental conditions and potential exceedance of water quality criteria for lead is challenging, as many factors need to be considered including lead shot density, and the volume and pH of the pond. This might be best addressed by a combination of in vitro and field studies.

5. Conclusions

The study reveals that the leaching of elements from spent gunshot can vary greatly depending on in vitro conditions, and likely natural environmental conditions. It is well known that elemental leaching efficiency varies with pH, and the present study highlights that other inorganic, organic and biotic factors also influence leaching. Those observations were confirmed by leaching data as well as by visible weathering of the surface of gunshot. Native microorganisms may play a stimulating role in weathering, but in comparison to other constituents of the matrix, this influence seems to be rather small. We suggest conducting experiments over a range of pH values and with more aggressive microbes to further elucidate processes. In general, the results demonstrate that spent Pb shot has the potential to result in long-term contamination at some heavily hunted sites. Notably, some fish ponds in Europe used for waterfowl hunting are dredged. The dredging spoils are piled on pond banks and islands, with some exposed shot undergoing weathering and potentially available for accidental ingestion by foraging waterbirds. In view of this situation, detailed monitoring of such sites is warranted.

CRediT authorship contribution statement

Anna Potysz: Conceptualization, Investigation, Writing – original draft, Visualization, Writing – review & editing. **Łukasz J. Binkowski:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Jakub Kierczak:** Conceptualization, Writing – review & editing. **Barnett A. Rattner:** Validation, Formal analysis, Writing – review & editing.

Data availability

Data generated during this study are not currently available as a data release from the National Science Center of Poland. Contact the corresponding author, Lukasz Binkowski, for further information.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159121.

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