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Leaching behavior and ecotoxicological effects of different game shot materials in freshwater

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Abstract – Lead-based game shot used in hunting near waters is considered a main reason for lead-poisoning of waterfowl and aquatic organisms, prompting discussion about alternatives. This study provides a first comparison of the leaching behavior and resulting ecotoxicological impacts of shot exposed to freshwater, comprising lead-based and alternative shots containing bismuth, copper, steel, tungsten, and zinc. Ecotoxicological effect assessment was based on the acute *Daphnia magna* 48 h toxicity test according to the EN ISO 6341:2012 guideline. Strong leaching of copper (up to 4.22 µmol/L) and zinc (up to 41.12 µmol/L) from three types of alternative game shot caused significantly increased immobilization rates of up to 100%. In contrast, even the highest leaching of lead did not significantly impair *Daphnia* mobility. Highest concentrations of dissolved metal ions only matched the declared main components of the respective shots in 3 out of 9 cases. These results demonstrate that metal release from alternative game shot is an underestimated ecotoxicological risk, particularly since release of copper and zinc from alternative shots was demonstrated to be more hazardous for aquatic biota than conventional lead shot. There is an urgent need of managing the use of shot ammunition near waterbodies based on realistic ecotoxicological risk assessments.

Keywords: heavy metal / water pollution / ammunition / lead / *Daphnia magna*

Résumé – **Comportement de lixiviation et effets écotoxicologiques des différents matériaux de la grenaille de tir en eau douce.** La grenaille de plomb utilisée dans la chasse près des eaux douces est considérée comme une des principales causes de l'empoisonnement par le plomb de la sauvagine et des organismes aquatiques, ce qui suscite la discussion sur les solutions de rechange. Cette étude fournit une première comparaison du comportement de lixiviation et des impacts écotoxicologiques résultants de la grenaille exposée à l'eau douce, comprenant des grenailles à base de plomb et des grenailles alternatives contenant du bismuth, du cuivre, de l'acier, du tungstène et du zinc. L'évaluation des effets écotoxicologiques a été basée sur l'essai de toxicité aiguë *Daphnia magna* 48 h selon la norme EN ISO 6341:2012. Une forte lixiviation du cuivre (jusqu' à 4,22 µmol/L) et du zinc (jusqu' à 41,12 µmol/L) provenant de trois types de grenaille de rechange a entraîné une augmentation significative des taux d'immobilisation pouvant atteindre 100 %. En revanche, même le lessivage le plus élevé du plomb n'a pas réduit de façon significative la mobilité des Daphnies. Les concentrations les plus élevées d'ions métalliques dissous ne correspondaient aux principaux composants déclarés des grenailles respectives que dans 3 cas sur 9. Ces résultats démontrent que le rejet de métaux provenant d'autres grenailles est un risque écotoxicologique sous-estimé, d'autant plus qu'il a été démontré que le rejet de cuivre et de zinc provenant d'autres grenailles est plus dangereux pour le biote aquatique que la grenaille de plomb classique. Il est urgent de gérer l'utilisation des munitions de tir à proximité des plans d'eau sur la base d'évaluations écotoxicologiques réalistes des risques.

Mots-clés : métaux lourds / pollution de l'eau / munition / plomb / *Daphnia magna*

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1 Introduction

Metal contamination of aquatic systems and the resulting impacts on biota have been recognized as a subject of great concern for many decades. Especially lead was identified as harmful to both the environment and human health due to its environmental persistence and accumulation potential in tissues of organisms (*e.g.* Spehar *et al.*, 1978; Vinodhini and Narayanan, 2008). Whilst the emission of heavy metals into the environment has substantially decreased in industrialized states (Nriagu, 1996) due to the elimination of major emission sources (*e.g.* the ban of leaded gasoline), the relative importance of other, less well-known sources has increased. This also applies to introductions of metals by hunting activities, which can be a major entrance pathway of lead into aquatic ecosystems by the use of lead-based shotgun ammunition (Jørgensen and Willems, 1987; Ma, 1989). Many studies demonstrated lead poisoning of waterfowl caused by intake of spent lead shot, which can remain at the bottom of the water body (*e.g.* Irby *et al.*, 1967; Locke *et al.*, 1967; Grandy *et al.*, 1968; Kelly *et al.*, 1998; Mateo *et al.*, 2007). Moreover, raptors and scavengers are also prone to lead poisoning due to the digestion of carcasses of animals shot with lead-based ammunition (Pain and Amiardtriquet, 1993; Kenntner *et al.*, 2001; Müller *et al.*, 2007; Krone *et al.*, 2009).

As a consequence of these findings, some countries already prohibited the use of lead-based shot for waterfowl hunting several years ago (*e.g.* USA, Canada, Norway). After a public consultation concerning the ban of lead-based shot, the European Chemicals Agency (ECHA) is preparing a restriction proposal for the ban of lead shot in wetlands to prevent waterfowl from poisoning after the ingestion of dispersed lead pellets (ECHA, 2017). On the other hand, possible adverse effects to the environment from alternative materials like copper, zinc, iron, bismuth or tungsten have not yet been sufficiently tested, hampering management recommendations on the best possible alternatives. Thomas and Guitart (2003) already argued for the implementation of a mandatory toxicity screening (like the US Protocol according to USFWS (1997)) of all substitute shots that are commercially available in the EU. Without a screening of all alternatives there would still be several shot types on the European market that are already known to be highly toxic to wildlife and the environment. One example is the documented toxicity of zinc shot to avian wildlife (Levengood *et al.*, 1999; Levengood *et al.*, 2000) challenging the adequacy of this alternative metal.

In addition to harmful effects as a result of direct metal ingestion, acute toxic effects of dissolved heavy metals in aquatic systems (*i.e.* via aqueous exposures) have already been demonstrated in a variety of organisms. This was for example demonstrated for larvae of the white sturgeon *Acipenser transmontanus* (Vardy *et al.*, 2014), the ostracod *Stenocypris major* (Shuhaimi-Othman *et al.*, 2011) and the branchiopod *Daphnia magna* (Khangarot and Ray, 1989). Moreover, dissolved copper was also shown to impair reproduction and growth of the fathead minnow *Pimephales promelas* (Mount and Stephan, 1969), and alter gene expression patterns in delta smelt *Hypomesus transpacificus* (Connon *et al.*, 2011). Likewise, dissolved zinc was demonstrated to inhibit the growth of grass carp *Ctenopharyngodon idella* (Chen *et al.*, 2016).

Thus, some new and alternative materials to lead ammunition from European manufacturers, which are mostly used for hunting waterfowl and ultimately end up in aquatic systems, might also pose a risk to the aquatic biota. However, information on metal leaching behavior of spent shot and possible resulting impacts on aquatic organisms are not available yet. This is especially crucial, as the use of different metal alloys for alternative shot materials (Thomas, 2016) might result in different leaching behavior compared to the ion release of pure metals. Thus, risk assessments based on known dissolved-metal toxicity are potentially insufficient and might not reflect real circumstances. Additional leaching tests are required to investigate ecotoxicological effects of different shot materials under specific conditions.

Therefore, this study was intended to provide first insights into metal leaching behavior of experimentally spent gameshot in a controlled freshwater environment, and to connect this information with resulting toxic effects on an important aquatic model organism with high relevance for pelagic food web integrity. We investigated the leaching behavior of (i) two conventional lead-based shots and (ii) six alternative shots made of bismuth, copper, steel, tungsten or zinc as well as (iii) a coated lead-based prototype at four exposure time points. Subsequently, the acute *D. magna* 48 h toxicity test was utilized to assess possible ecotoxicological effects of the resulting leachates. We hypothesized that the main component, based on manufacturer's data, is the one which is the dominantly released metal during the exposure of game shot to freshwater. Furthermore, due to the common assumption of lead shot posing a high threat to the environment, the exposure of alternative shot was hypothesized to not result in increased immobilization rates in *D. magna* compared to lead shot. The results of this study are intended to guide management and legal decisions on the future use of shot ammunition near waterbodies.

2 Material and methods

2.1 Investigated shots

Nine types of shotgun ammunition (Tab. 1) were selected considering a broad representation of different shot materials available in the EU: lead (Pb), iron (Fe), copper (Cu), zinc (Zn), tungsten (W) and bismuth (Bi). Similar sizes were chosen (size number #2) with the exception of two shots (Fiocchi PL 34 and Eley Bismuth Alphamax) which were only available with a smaller diameter (Tab. 1). The selection of shot ammunition was conducted in close collaboration with the Bavarian Hunting Association (BJV) to consider the most relevant ammunition types and sizes. RUAG Ammotec GmbH disassembled the shot pellets from the cartridges and provided a galvanic tin-coated prototype with a lead core for an investigation of the manipulated Pb-leaching through a galvanic lamination on Pb shot. Unexpectedly, the cartridges of FOB Sweet Copper contained a small percentage of copper coated lead shot pellets. In order to standardize the testing conditions, only the pure copper shots were used in this case for subsequent investigations.

2.2 Metal leaching behavior

Since this study should enable a first relative comparison of the leaching behavior and ecotoxicological effects between the

Table 1. Overview of the different types of game shot, the respective size number (#2: 3.75 mm diameter; #3: 3.5 mm diameter) and the main element according to the product specification.

Shot type	Size number	Main element	Source
Fiocchi PL 34 (PL 34)	#3	Pb	www.fiocchiuk.com
RWS Silver Selection (Silver)	#2	Pb	www.rws-munition.de
Coated prototype	#2	Pb	RUAG Ammotec GmbH
Rottweil Steel Game (Steel Game)	#2	Fe	www.rws-munition.de
Winchester Blind Side (Blind Side)	#2	Fe	www.winchester.com
Eley Bismuth Alphamax (Alphamax)	#3	Bi	www.eleyhawkltd.com
FOB Sweet Copper (Sweet Copper)	#2	Cu	www.cartouchesfob.fr
Rottweil Ultimate (Ultimate)	#2	W	www.rws-munition.de
SK Hubertus Zink (Hubertus)	#2	Zn	www.jagdgewehr.de

different materials, we used a preferably simple experimental set-up by focusing on an aqueous exposure, excluding effects of different substrates and redox conditions.

Shot pellets were exposed in a defined freshwater environment, suitable for the *Daphnia* test (water hardness: 250 mg/L $CaCO_3$) consisting of 333 mg synthetic sea salt, 2.3 mL $CaCl_2$-solution (0.8 mol/L), 2.2 mL $NaHCO_3$-solution (0.3 mol/L) and 0.1 mL SeO_2-solution (0.13 mol/L) dissolved in 1 L deionized water (ADaM; Klüttgen *et al.*, 1994). Two pellets of each respective shot type were exposed in each of 12 (3 replicates for 4 different exposure times) medical urine cups (Uritop, Frohnhäuser) filled with 100 mL ADaM. In contrast to existing guidelines like the OECD (2001) that stipulate big volumes of a pre-defined exposure medium, we adjusted exposure volumes to 100 mL to ensure more homogenous exposure conditions. Since cartridges of this calibre contain up to 200 pellets, the pellet-water ratio likely mimics a high-exposure scenario as *e.g.* occurring in shallow water conditions. The cups were closed and the solutions were aerated with pre-cleaned, compressed and water-saturated air to promote aerobic conditions and a circulation in the medium (Fig. 1). Following the OECD (2001) guidelines, we decided to choose a short term (24 h) and long term time point (a maximum of 22d was technically feasible) as well as two intermediate time points (8d, 15d). At exposure termination three cups per each type of shot were removed and samples were completely filtered (0.45 µm) for subsequent metal analysis and *D. magna* immobilization tests.

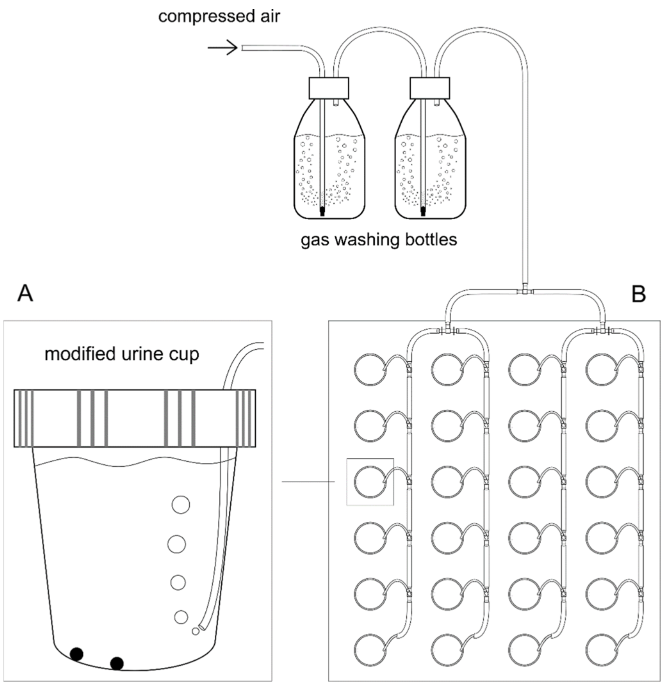

Fig. 1. Experimental setup of the metal leaching test. (A) Single exposure unit and (B) arrangement of the exposure units during the experiment (two shot types tested simultaneously).

2.3 Metal analysis

After filtering, aliquots of 12 mL were taken from each water sample for metal analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES; Genesis, Spectro Kleve). All eluates were analyzed for the concentration of dissolved As, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Sn and Zn and corrected for the background concentration of ADaM. Samples of the shot types Alphamax and Ultimate were additionally investigated for their content of dissolved Bi and W concentrations in the same way. The determination of detection limits and limits of quantification (LOQ) was conducted by measuring 10 blank values. According to DIN 32645: 2008-11 (DIN, 2008) limits were calculated by adding the mean concentration of blank values and the threefold respectively tenfold standard deviation for detection limit respectively limit of quantification. For better comparison of the metal leaching behavior all concentrations are given in µmol/L.

2.4 *Daphnia magna* immobilization test

A culture of the *D. magna* clone K34J has been maintained at the Aquatic Systems Biology Unit at the Technical University of Munich in Freising, Germany, for more than 7 years. *Daphnia* were cultured in 920 mL beakers filled with the same water that was used for the shot leaching experiment (ADaM) at a density of 10 *Daphnia*/L. Adult *Daphnia* were transferred into new beakers containing new water 3 times a week. At the same time the green alga *Scenedesmus obliquus* was provided *ad libitum* as food and juveniles were removed. In order to avoid overaging of *Daphnia* mothers, new cultures

Table 2. Mean effect concentrations (EC_{50} values, 48 h immobilization; µmol/L) of dissolved metals on *D. magna* at water hardness 240 mg/L $CaCO_3$ (Khangarot and Ray, 1989). EC_{50} for Bi at water hardness 72 mg/L $CaCO_3$ (Okamoto *et al.*, 2015).

As	Bi	Cr	Cu	Fe	Mn
83	>62	34	1.46	129	151
Ni	Pb	Sb	Sn	W	Zn
124	17	3478	182	486	8.57

were started every third week using juveniles from the precursor culture. Thus, test-daphnids were collected from different broods during the first three weeks of reproduction. Cultures were placed in a climate chamber set to 20 °C (19.9 ± 0.2 °C; mean ± SD of reference measurements) and a light:dark cycle of 16:8 h. The toxicity tests were conducted according to the EN ISO 6341:2012 guideline (DIN, 2013). Two or three shot types were investigated simultaneously resulting in a total test period of 16 weeks. For the toxicity tests the three filtered water samples from each shot exposure time were pooled and 6 beakers (100 mL) were filled with 40 mL of the test solution. Thus, water conditions were equal in the replicates. The remaining solution was used for pH measurement. Another six beakers filled with ADaM served as control. Five juvenile daphnids (<24 h, not first brood) were transferred into each beaker. Daphnids were not fed during the test. The test was terminated after 48 h and the number of immobile daphnids was counted. Subsequently, dissolved oxygen and pH were measured. Tests were conducted under the same temperature and light conditions as the culturing.

2.5 Statistical analyses

Dissolved metal concentrations where compared with literature data (Khangarot and Ray, 1989; Okamoto *et al.*, 2015) on metal toxicity to *D. magna* (48 h immobilization) at comparable water hardness to identify exceedances of mean effect concentrations (EC_{50}-values, Tab. 2). Normality of data was tested using Kolmogorov-Smirnov's test. As data were not normally distributed, Kruskal-Wallis tests were used to test for differences in *Daphnia* immobilization among treatments. Pairwise Mann-Whitney-U tests with subsequent Bonferroni adjustment were applied between shot treatments and the control in case of significance ($P < 0.05$).

3 Results

3.1 Metal analysis

The highest molar metal concentrations were measured in water samples of the steel shot Blind Side and Hubertus zinc shot with values up to 34.88 µmol Zn/L and 41.12 µmol Zn/L, respectively, exceeding the EC_{50} (*D. magna* immobilization) of zinc at most time-points (Tabs. 2 and 3). For the alternative shot type Sweet Copper, leaching of its main element at concentrations from 0.79 to 4.22 µmol Cu/L (exceeding the EC_{50} from day 8 on) were found, similar to the concentrations from PL 34 with detected Pb-concentrations of up to 2.35 µmol/L (below EC_{50}). The shot Steel Game released manganese resulting in a concentration up to 0.49 µmol/L. The chemical analysis of the shot types Alphamax, Ultimate and the coated prototype either only resulted in leachates with metal concentrations below the limit of quantification or no element exceeded the detection limit. Furthermore, some shot types released different than the expected metal ions (Alphamax: Sn, Ni, Pb; Ultimate: As, Ni, Sn; Blind Side: Zn; Silver: Ni) likely resulting from coatings or alloy components. For instance, leaching of the Pb-based shot type Silver resulted in concentration ranges of 0.49 µmol/L to 1.70 µmol/L for Ni. Only Hubertus, PL 34 and Sweet Copper released the respective main compounds as expected from their declaration. The only leached metal of the coated lead prototype was Sn with a concentration below the LOQ, which was only detected after 8 days. For detailed information of all measured metal concentrations see Table S1, supplementary data.

3.2 *Daphnia magna* toxicity test

Immobilization of *D. magna* was significantly increased as compared to the control in test solutions of three shot types at every time point (Fig. 2): Sweet Copper (Mann-Whitney-U: $P = 0.016$ (1d), $P = 0.008$ (8d), $P = 0.012$ (15d), $P = 0.006$ (22d)), Hubertus (Mann-Whitney-U: $P = 0.012$ (1d), $P = 0.006$ (8d), $P = 0.006$ (15d), $P = 0.006$ (22d)) and Blind Side (Mann-Whitney-U: $P = 0.006$ (1d), $P = 0.006$ (8d), $P = 0.004$ (15d), $P = 0.004$ (22d)). No other shot-leachate caused a significantly increased immobilization at any shot-exposure length. Immobilization rates caused by leachates from Sweet Copper and Hubertus were consistently high (81–100%) at every time-point. In contrast, immobilization rates caused by leachates from Blind Side increased by 61% from day 1 to day 15, and decreased by 18% at day 22. For the lead shot PL 34 leachate, there was an increase of *Daphnia* immobilization at day 1 (not significant), with a high variation caused by a mortality of 100% in one test-beaker (SD: 39%). At the following time points, immobilization rates were clearly reduced by 58% (days 8 and 15) and 90% (day 22). Mean control mortality was <10% in all tests (range: 0–8%). Dissolved oxygen was in the range of 7.2–10.0 mg/L at the end of the test in all test beakers. pH-values did not change by more than 0.6 units during the test and ranged from 7.3 to 8.0 and 7.5 to 8.1 at test beginning and end, respectively, over all treatments.

4 Discussion

This study provides first information on metal leaching behavior from different types of gameshot in freshwater systems directly linked with resulting ecotoxicological impacts. Whilst the results clearly suggest that some

Table 3. Molar concentrations [µmol/L] and standard deviations of the mainly released metal ions after 1, 8, 15 and 22 days of shot exposure in ADaM. Bold numbers indicate exceedance of *D. magna* 48 h immobilization EC_{50}-values of respective metals (according to Tab. 2).

	Exposure day			
	1	8	15	22
PL 34	Pb 2.35 ± 0.33	Pb 1.27 ± 0.19	Pb <LOQ[a]	Pb 1.16 ± 0.13
Silver	Ni 0.49 ± 0.10	Ni 0.69 ± 0.24	Ni 1.70 ± 0.39	Ni 0.97 ± 0.07
Coated prototype	n.d.[b]	Sn <LOQ	n.d.	n.d.
Steel Game	Mn 0.12 ± 0.01	Mn 0.49 ± 0.05	Mn 0.39 ± 0.25	Mn 0.41 ± 0.11
Blind Side	Zn 6.13 ± 1.36	Zn **20.65 ± 2.92**	Zn **34.88 ± 1.10**	Zn **34.53 ± 3.38**
Alphamax	Sn <LOQ	Bi, Ni <LOQ	Fe, Pb <LOQ	Pb, Sn <LOQ
Sweet Copper	Cu 0.79 ± 0.18	Cu **3.03 ± 0.29**	Cu **4.22 ± 0.90**	Cu **4.00 ± 1.11**
Ultimate	As <LOQ	Ni <LOQ	Ni <LOQ	Mn, Sn <LOQ
Hubertus	Zn **26.46 ± 10.86**	Zn **41.12 ± 5.70**	Zn **34.42 ± 0.62**	Zn **26.54 ± 1.17**

[a] LOQ = limit of quantification.
[b] n.d. = not detected.

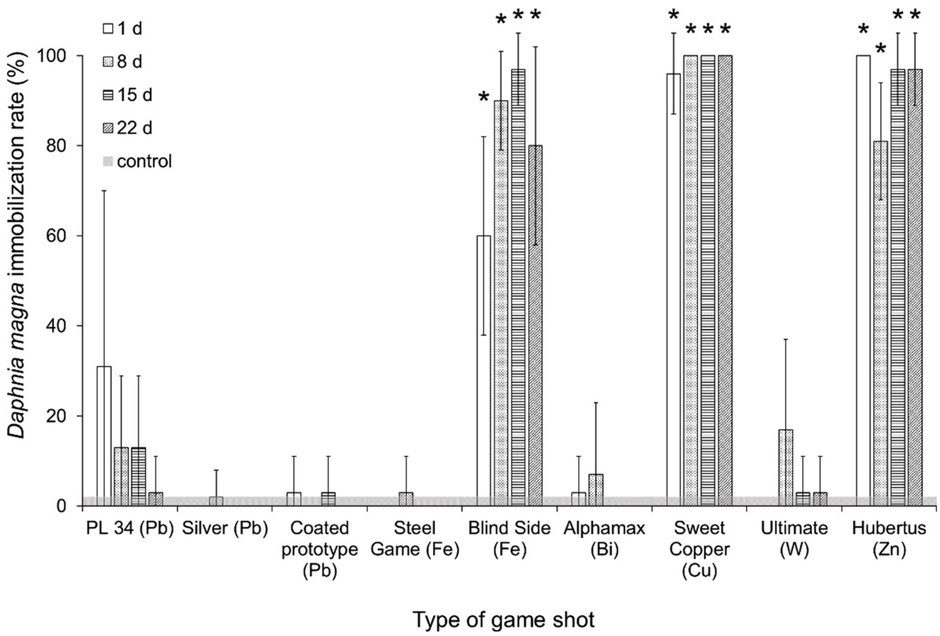

Fig. 2. Mean immobilization rates and standard deviations in the 48 h acute toxicity test with *D. magna* after 1, 8, 15 and 22 days of shot exposure. Control resembles mean immobilization rate of untreated *Daphnia* over the whole test phase. Asterisks indicate significant differences ($P < 0.05$) to the control. The declared main component of the shot is given in brackets.

alternative gameshots pose a higher ecotoxicological risk for aquatic organisms than classical Pb-based ammunition, further research is needed to assess the actual exposure risk in the wild, including a range of different taxa. In addition, abiotic factors like pH, water hardness or organic carbon can substantially change both metal leaching behavior as well as toxicities of the leachates, requiring further testing under likely environmental conditions. Thus, the present study should be primarily seen as a proof-of-concept that lead-free gameshot can release metals of ecotoxicological concern.

Leaching of copper and zinc resulted in significantly increased immobilization rates up to 100% of *D. magna,* which was already observed after 24 h of shot exposure. In contrast to the common assumption that lead would be the most toxic and problematic metal in game shot, none of the tested lead shots significantly increased immobilization within this relative comparison at any time point. Instead, especially the leaching of copper and zinc from new alternative shot types suggests severe impacts on aquatic biota as demonstrated in *D. magna* which is a keystone species of freshwater systems. Measured concentrations of copper (up to 4.22 μmol/L) and zinc (up to 41.12 μmol/L) indeed mostly exceeded 48 h *D. magna* immobilization EC_{50} values found in the literature (Khangarot and Ray, 1989; Tab. 2) in treatments with significantly increased *Daphnia* immobilization. These findings are in line with various studies demonstrating a high sensitivity of aquatic organisms like fungi, green algae, dragonflies and snails exposed to these metals at comparable or even lower concentrations (Knauer *et al.*, 1997; Tollett *et al.*, 2009; Azevedo and Cássio, 2010; Khangarot and Das, 2010). However, in our study, mean immobilization rates of daphnids were often below 100%, which might be due to the formation of non-bioavailable compounds. A clear determination of metal-speciation was not possible in our study because of using the ICP-AES Method. In contrast, even the highest lead concentrations did not significantly increase *Daphnia* immobilization in the present study, as the measured concentrations remained below the respective EC_{50} values (Khangarot and Ray, 1989), possibly due to the formation of insoluble oxide compounds. However, lead concentrations exceeded the 7d LC_{50} value (0.54 μmol/L) determined with the amphipod *Hyalella azteca* (Borgmann *et al.*, 2005) indicating potential acute impacts of dissolved lead from shots on other aquatic organisms. As sensitivities of aquatic organisms can substantially vary, *e.g.* depending on route of uptake or detoxification processes, possible adverse effects of the remaining metals at measured concentrations for other organisms than *Daphnia* cannot be excluded. Moreover, chronic effects of leached metals at these concentrations are likely to occur, especially due to the bioaccumulation potential of heavy metals like lead, as aquatic organisms are not able to actively regulate metal concentrations in tissues (McGeer *et al.*, 2003).

Depending on the shot type, high amounts of metals were already leached during the exposure within the first 24 h (Tab. 3). Surprisingly, the metal, which was mostly released into the water, was not necessarily the declared main component of the shot, as demonstrated in 6 out of 9 types of shot in the present study. This might be caused by an additional coating of the shot surface as observed for the steel shot Blind Side where high concentrations of zinc were detected in the water samples. Furthermore the Pb-shot Silver Selection is obviously coated with nickel. Another reason for the leaching of additional metals can be the use of alloys, as in the case of Steel Game, where Mn is an obvious alloy component of this iron-based shot. These findings are surprising on first glance, but they also match previous results of the leaching behavior of rifle bullets in different percolation tests (Schwarz *et al.*, 2015). Similarly, Thomas (2016) identified different possible alloying elements in tungsten-based shot (*e.g.* nickel) affecting the leaching behavior. Therefore, the ecotoxicological risk assessment of game shot should not be limited to the toxicity of its declared main component or metal, but rather be based on its leaching behavior under realistic and pre-defined environmental conditions. Concerning coating techniques, a leaching of lead could be inhibited for undeformed shot in aquatic systems through a special lamination demonstrated by the coated prototype. Because the damage of such a "shot-jacket" cannot be excluded, these results need to be verified in further investigations testing the deformation of coated shot pellets during firing action. However, the poisoning of waterfowl, raptors and scavengers following the digestion of lead ammunition (Irby *et al.*, 1967; Locke *et al.*, 1967; Grandy *et al.*, 1968; Kelly *et al.*, 1998; Kenntner *et al.*, 2001; Mateo *et al.*, 2007; Müller *et al.*, 2007; Krone *et al.*, 2009) is attributed to the release of Pb ions as a result of the grinding activity and the acidic environment of the gizzard (Scheuhammer and Norris, 1996). Thus, an intoxication of birds following the digestion of new coated lead shots cannot be excluded and should be investigated in the near future.

In conclusion, the results of this study clearly indicate that some European game shot substitutes, which can accumulate in freshwater systems, are an insufficiently considered ecotoxicological risk. According to our results, shot types leaching copper or zinc pose a high risk to the aquatic biocenosis, whereas game shot made of tungsten, bismuth or steel without a toxic coating might be alternatives that are less harmful to aquatic wildlife. The observed discrepancies between the expected and observed leaching behavior of different game shot and the ecotoxicological effects on *D. magna* should be verified also considering other species and more realistic environmental conditions. In particular, metal leaching and speciation and thus bioavailability is influenced by physicochemical parameters of the water matrix (*e.g.* acid/calcareous water) which have to be considered in further investigations concerning the fate of game shot in natural waters. Moreover, investigations of possible chronic effects on aquatic organisms are required for an adequate assessment of the environmental risk of gameshot in freshwater systems. Consequently, the ban of lead shot in order to protect humans and other vertebrate species like waterfowl as currently discussed on European Union level has to be critically evaluated in light of available substitutes which must not be more hazardous to aquatic systems. Based on the findings of this study, manufacturer statements that their ammunition is "lead-free" or represents a proper and environmentally safe lead substitute clearly not mean that it is devoid of toxic effects. Hence, these aspects have to be pursued by introducing rigorous legal shot testing criteria at the national and European Union levels. Specifically, a better consideration of the toxicity for aquatic key species is necessary in addition to the well-known toxicity to avian wildlife. Management of aquatic ecosystems should restrict the use of critically assessed shot substitutes that contain zinc, copper and zinc coatings on a legal basis. Moreover, licensed use of shot types near water bodies should be based on a thorough ecotoxicological risk assessment that ideally comprises both the already well-known risk to avian species, and the less well documented risk to aquatic or terrestrial species.

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