applied sciences

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Toxic Metals in Meat Contributed by Helicopter and Rifle Thoracic Killing of Game Meat Animals

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Abstract: Processes of killing wild game meat animals could introduce toxic metals into the animal's meat, which subsequently may pose a risk of consumer exposure to toxins during ingestion. In most cases, toxic metals occur naturally in the environment and may be found in traces in different parts of a game meat animal. However, some of these metals are also introduced to meat animals by bullets used during the hunting and killing of game meat animals. These bullets are generally made from metals such as lead, arsenic, and copper, all of which have strictly regulated limits in food products including meat. Samples of helicopter-killed impala in the area around the bullet/pellets' wound (n = 9) and from animals killed by a single projectile (n = 9) were analysed using inductively coupled plasma mass spectrometry (ICP-MS). The type of bullet used influenced the mean concentration of some of these toxic metals (mg/Kg) in meat samples; helicopter killing resulted in the following levels of As (0.665, SD = 1.95); Cd (0.000, SD = 0.000); Pb (620.18, SD = 1247.6); and Hg (0.017 SD = 0.033) compared to single projectile killing that resulted in the following levels: As (0.123, SD = 0.221); Cd (0.008, SD = 0.021); Pb (1610.79, SD = 1384.5); and Hg (0.028, SD = 0.085). The number of samples per metal with levels above the EU products' limits were Pb = 18/18 samples from both killing methods, As = 2/18 samples from helicopter killing, Cd- = 1/18 from rifle killing and Hg = 0/18. To minimise the risks of toxic metals posed by bullets, the use of lead (Pb) free bullets should be encouraged, and the control of meat animal killing methods must always be performed, especially for meat contamination prevention.

Keywords: toxic metal; arsenic; lead; cadmium; mercury; thorax and helicopter killing

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Citation: Nkosi, D.V.; Bekker, J.L.; Hoffman, L.C. Toxic Metals in Meat Contributed by Helicopter and Rifle Thoracic Killing of Game Meat Animals. *Appl. Sci.* **2022**, *12*, 8095. https://doi.org/10.3390/app12168095

Received: 12 July 2022
Accepted: 11 August 2022
Published: 12 August 2022

1. Introduction

Worldwide, the shortage of animal protein sources as well as the high cost of red meat from traditionally farmed livestock has contributed to a search for sustainable alternatives. Since the birth of the new millennium, game meat, due to its abundance in many countries has been identified as such a source [1,2]. There are, however, some meat safety hazards (biological, chemical, physical) that exist with the production of such meat [1]. Elements such as arsenic (As), cadmium (Cd), and lead (Pb) occur naturally in the environment and may be found in traces in food products including meat, but their dose and concentration must be monitored to prevent health effects since these toxic metals tend to bio-accumulate, and the rate of excretion is generally lower than the intake in the body, thus causing toxic accumulation and health effects [3–5]. These toxic metals, once ingested, in the long run tend to affect the major organs of the body such as the head, thyroid gland, lungs, heart, liver, kidney and skin [6]. Researchers [7–9] conclude that overexposure to these metals could include long term health problems such as carcinogenic effects, non-communicable disease aggravation, blood circulatory effects, and subsequent fatalities of consumers. While different exposure routes exist, ingestion from contaminated food products and inhalation remain the most significant forms of exposure [10,11]. The risk is even greater as some of these health effects could be long-lasting and have a negative effect on baby/child mortalities, reduced IQ, and reduced reading and learning capabilities, especially where pregnant women and babies are exposed [12].

Similar to other countries in the world such as Botswana and Croatia wild life management and game meat production has grown [13,14]. The circumstance around the production of wild/game/bush meat does not differ greatly and the possible presence of these toxic metals in meat continues to be a concern to authorities, especially in areas where wild meat is mostly consumed [15]. Worldwide, a number of studies on toxic metals and the possible effect they may have on the environment and exposed people have been conducted [16,17]. These studies have identified the risks and the eminent need to control these, especially where vulnerable communities are dependent on hunting of game for meat as a source of protein [8]. As a result, the existence of toxic metals in meat and their control remain a well-known and documented challenge; hence it is important to develop toxic metal limits in meat and meat products to protect consumers.

1.1. Occurrences and Accumulation of Toxic Metals in Farmed Animals

Researchers in game meat production have identified As, Cd, Pb, and Hg as the most likely to occur in game meat animals, and as a result, these toxic metals are the most investigated in raw meat [6]. It is important to note that different human exposure limits (daily or weekly) are estimated worldwide [18–20]. For this investigation, the compliance of raw meat derived from the two killing methods is investigated and measured against various international products' limits (Table 1).

Table 1. Maximum limits of toxic metals (mg/Kg) in raw red meat/red meat products.

		Toxic Elements				
Country	**Type**	**As (Semi-Metal)**	**Pb**	**Hg**	**Cd**	**Source(s)**
South Africa	Regulation	1.0	0.10	-	0.05	[21]
EU countries	Regulation	0.3	0.10	1.0	0.05	[22]
Republic of China	Regulation	0.5	0.5	0.05	0.1	[23]
Australia and New Zealand	Regulation	0.01	0.10	0.002	0.05	[24]
United States of America	Regulation	0.01	0.01	0.01	0.01	[25]
Countries without specified limits	Codex Alimentarius Standards	2.1	0.10	-	0.05	[26]

Some of these metals (Table 1) occur naturally in the environment, while others such as Hg could be introduced to the environment by processes of animal farming, industrial activity, urbanisation, and mining [27,28]. Various studies have identified Pb, Cd, Hg, As, and many others as toxic in nature, and their presence could be dangerous to the animals as well as consumers, while some others can only be dangerous when present in high concentrations or with prolonged exposure [29]. In many developing countries extensively involved with game meat production, there is a significant probability of toxic metals being introduced into the meat, especially due to previous anthropogenic activities (where these animals have been introduced as part of the rehabilitation of the mining sites) as well as different hunting and harvesting methods of game or uncontrolled meat production processes [27,30]. Likewise, researchers agree that toxic metals in ammunition used during game meat animal killing are generally unintentionally introduced, especially through bullets composed of a mixture of toxic metals [7,31,32].

1.2. Composition of Shotgun and Rifle Ammunition

Most hunting bullets are made of different metals such as lead (90–95%), copper, zinc, tin, and other metals to make them hard and effective for hunting and killing purposes. A number of studies have identified toxic metals such as Pb in ammunition used for hunting, and these metals could contribute to the contamination of the game or wild animal [33]. Pellets are generally used in shotgun cartridges when hunting small game and birds or wild animals for meat production but are also used in commercial harvesting or mass culling processes that form part of farm game management plans for sustainable usage of farmland and protein salvaging [32,34]. Other than Pb, pellets are also made from iron, tungsten, zinc, and even copper. Rifle ammunition generally used in hunting is mostly made from lead-antimony, copper-plated, steel and copper jackets, or cupronickel [34]. In most developed countries, the use of these ammunition in processes of food production is discouraged and regulated as these dense or toxic metals may bio-accumulate in the body of consumers, thus posing as a significant health threat [35]. It is noted that these metallic elements could be transferred to game meat animals during the killing process [36,37]. It has been shown that Pb from ammunition could be introduced in meat, especially around the wounds (entry and the exit points) of shot carcasses or around the bullet's trail in the carcass [38]. These contribute to meat contamination at other areas of the carcass generally not suspected [39,40].

Typically, in most developing countries actively producing wild meat, the selection and choice of ammunition for hunting purposes is left to the hunter, although there are some regulations that determine the minimal weight/velocity that a projectile has to be for the hunting of specific-sized animals. Lead bullets are generally selected as these bullets are readily available to hunters and tend to expand upon contact with the animal's body, thus maximizing the damage and increasing the probability of a kill [20,39]. This could subsequently introduce toxic metals into carcasses. In the South African/Namibian context, the two most common methods used to kill game are: (1) through rifle shooting during hunting or killing; and (2) aerial (helicopter) shotgun shooting from the helicopter during harvesting or mass culling of game as part of game farm management [6,41,42]. Figure 1a,b and Figure 2a,b depict examples of the different ammunition used to kill game animals during helicopter and rifle thorax shot killing.

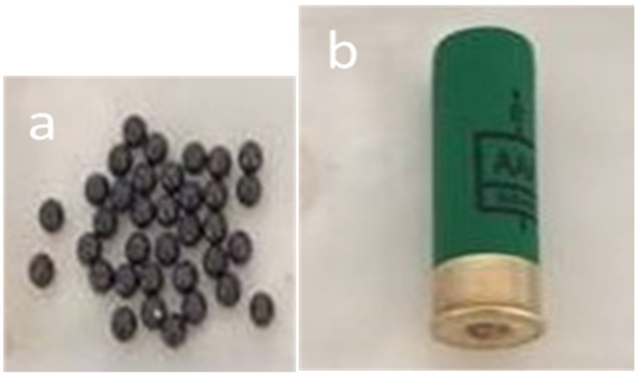

Figure 1. (**a**,**b**) Shotgun cartridge (35 g; 5.2 mm pellets) used for helicopter shooting [43].

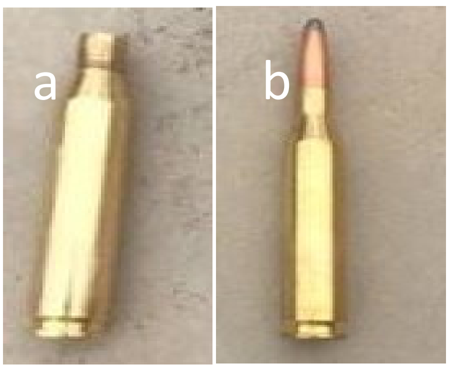

Figure 2. (**a**,**b**) Bullet (30.06 rifle; 150 g) used for rifle thorax shot killing (traditional hunting) [43].

Intensive (animals maintained in small to moderate predator-controlled camps) and semi-extensive farming systems (larger environments to self-sustain the game population) are generally found in game meat producing countries. Farmers have the right to trade, breed, and harvest/hunt game animals as part of their game farm management strategies and subsequently supply local markets with such meat [43]. Some of the strategies employed include the promotion of breeding for trophy hunting (horn size at a pre-determined age) and harvesting of meat from those animals that do not meet trophy standards [44]. In these processes, identified animals are shot as part of the normal management strategy to remove sub-par animals that do not meet the breeding criteria (horn size or colour). This meat subsequently enters the food chain after normal slaughter and dressing at local slaughter facilities or abattoirs [43].

In developed countries worldwide, the risks of toxic metals in food, including meat, and their prevalence in wild ungulates have been investigated [40]. In contrast, the occurrence of toxic metals in game meat derived from developing countries/regions where game is hunted or harvested by means of different hunting regiments is seldom documented unless such meat is intended for export [6]. The presence of toxic metals in game meat harvested for export to European communities is generally monitored [45]. However, concerns regarding meat exported to other regions and meat consumed locally have been raised [46]. Even though the risk of these metals when in higher concentrations is known and their presence in game meat is expected to be controlled, this is generally not carried out in developing countries [30,47]. This study evaluates the occurrence of certain toxic metals in impala (*Aepyceros melampus*) as derived from rifle shots targeting the thorax and aerial (helicopter) and shotgun shots targeting the head and neck.

2. Materials and Methods

This study forms part of a larger study related to strategies to reduce hazards in commercially harvested impala (*Aepyceros melampus*) carcasses. The impala (*Aepyceros melampus*) were shot by one proficient hunter at the same game farm located at Mokopane South Africa (S 24″26′43.73 | E 28″31′25.86). Killing was with a rifle (30.06 150gr Nosler Accubond Points made from white polymer, lead and copper metal) targeting the thorax (n = 36) region and by aerial (helicopter) using a shotgun (12 gauge pump action, 32 g cartridges, with ±45 pellets of 2–4 mm diameter made from lead, steel, or zinc per cartridge) targeting the head and neck area of the impala (n = 12) [37,48]. The approximate distances for rifle shots ranged between 40 and 100 m, and the shotgun shots were between 10 and 15 m height above the animals, respectively [49]. All these animals were bred and raised within the same game farm and the age and gender of helicopter-killed animals ranged between 18 and 30 months (all males) and for rifle-killed, 18–40 months (of which 5 were males and 4 were females).

This paper forms part of a study approved by the Tshwane University of Technology (TUT) Faculty of Science Committees for Research Ethics and Animal Research Ethics Committee (Reference number AREC2019/03/002).

2.1. Sampling and Sample Handling

The carcasses were transported to a game abattoir registered (registration number 2/4G) in terms of the Meat Safety Act, Act 40 of 2000 and located on the same farm where the animals were shot. A total of 18 rifle-killed carcasses, and all 12 shotgun-killed carcasses, were selected and screened for physical hazards with X-ray imaging by the same research team. From these, 9 carcasses of each killing method (n = 18) were randomly selected for heavy metal analysis (Figure 3). After chilling, meat and control samples were taken from the carcass positions as depicted in Figure 4. Per carcass, a pooled sample of 10 g was collected at the entry points of the pellets (Figure 4A) and the rifle bullets (Figure 4B) using sterilized scalpels and tweezers. Each sample was placed into a sterile whirl-pack bag (whirl-pak® flat wire bag 18 oz supplied by Whirl-pack (Merck Pty Ltd., Modderfontein, South Africa). Samples were then placed in cooler bags with ice under 5 °C and taken to the Tshwane University of Technology's Food laboratory for further storage at −18 °C until processing.

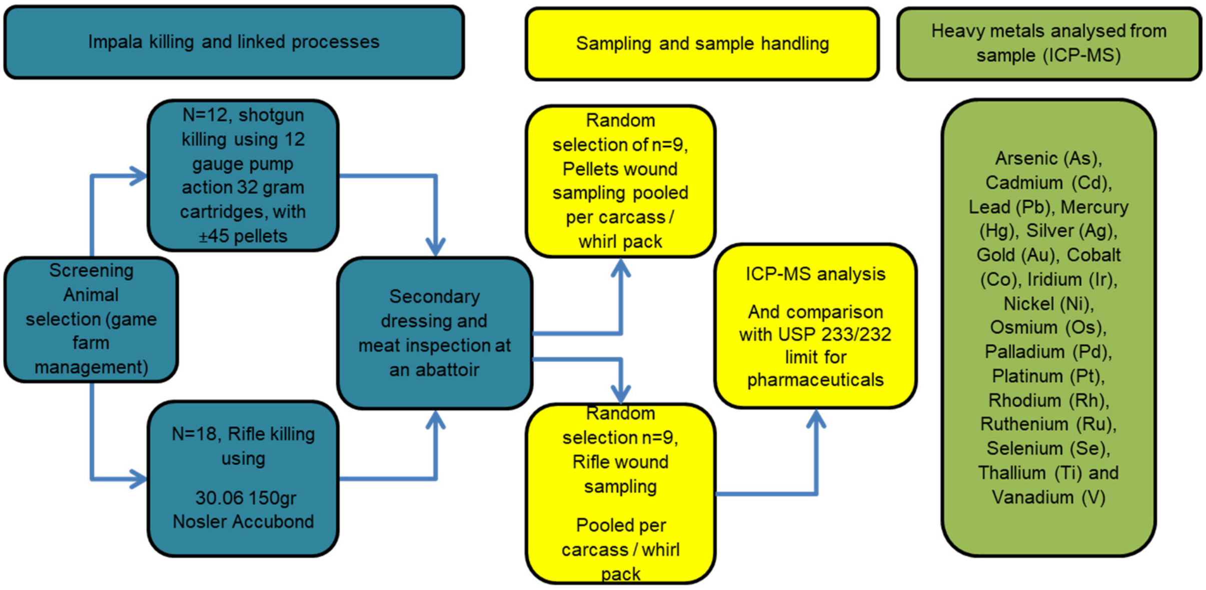

Figure 3. Sampling schematic diagram of the study design. Figure developed from processes followed during a commercial harvesting.

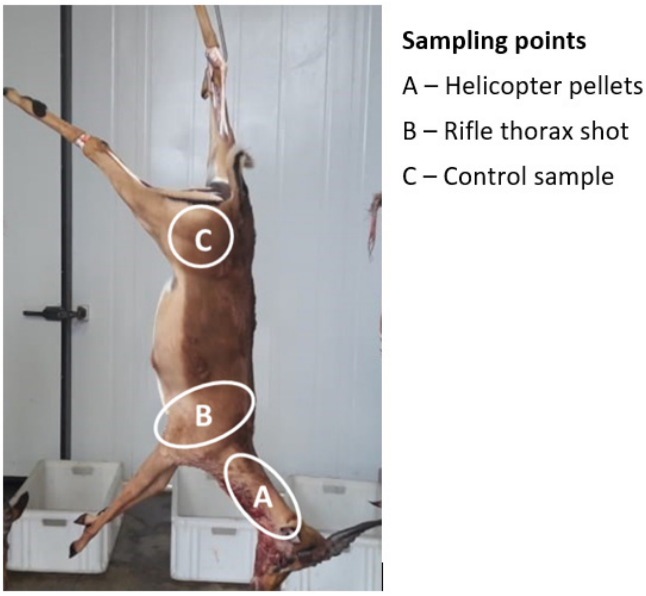

Figure 4. Undressed hanging impala showing positions of sampling points.

2.2. Sample Analysis

Samples were analysed by a laboratory registered with the South African Health Product Regulator Authority (SAHPRA; license number 0000001373-1) which applies the ISO 17,025 requirements during analysis of samples. Small samples from different areas were taken from each bulk sample provided; all samples were weighed (approximately 200 mg) into centrifuge vials and digested with trace metal acid and trace metal deionized water on a heat block. Samples were then filtered using a 0.45 µm nylon filter and analysed on an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Back-calculation of toxic metals concentration was then performed to establish the concentration of toxic metals on the sample against USP 233/232 limit for pharmaceuticals [50]. Trace elements were determined from meat samples as outlined in Figure 1.

From the initial analysis results, the mean concentration of the most prevalent toxic metals in game meat (As, Cd, Pb, and Hg) was used for further reporting. Their limits were measured against USP 233/232 elemental impurities procedures limits for pharmaceuticals [51]. To determine the mean concentration of a specific toxic metal in the sample as being contributed by ammunition used during the killing methods, the intrinsic concentrations of that toxic metal occurring naturally within the carcass (control) was determined by taking a sample away from the bullets or pellets trail where contamination could have been introduced by the bullets. The recorded concentration was the difference between the estimated mean concentration of the sample and the estimated control sample reading indicating the level of intrinsic toxic metals following a formula in conjunction with Figure 4:

$$TM_{con} = A_vA - A_vC \text{ (helicopter shots)}$$

or

$$TM_{con} = A_vB - A_vC \text{ (rifle thorax shots)}$$

TM_{con} = Toxic metal concentration per carcass
A_vA = Average concentration of toxic metal around shotgun pellet entry points at sampling point A
A_vB = Average concentration of toxic metal around rifle bullet entry sampling point B
A_vC = Average concentration of toxic metal in the control sample taken at sampling point C.

2.3. Data Analysis

A total of 17 heavy metals of interest in food and meat products were analysed from all samples. To determine the differences in the average concentrations of the four dominant metals (As, Cd, Pb, and Hg) between the entry wounds and the control point from each killing plan, a t-test of the average concentration of each toxic metal was conducted. In addition, a chi-square test to determine the number of samples that exceeded the safety limit as determined by the EU standards was also calculated. Differences were regarded as significant when $p < 0.05$ (95% confidence level).

3. Results

Toxic metal concentration comparisons between the two killing methods were conducted in the study, and the results show that with the exception of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), all other metals presented as not detected or negligible. To determine the level of toxic metals contributed by bullet particles (sampling point A or B, Figure 4), the natural intrinsic toxic metals were measured (sampling point C, Figure 4) and subtracted from the entry point samples' results. Although toxic metal limits differ for countries and/or regions (Table 1), the EU limits were used as the standard as South African game meat is mainly exported to EU countries.

3.1. Intrinsic Toxic Metals

The results show some levels of intrinsic concentrations of toxic metals taken on the buttock of the carcasses (position C. on Figure 4) were within the EU limits indicated in Table 1, with Cd and Hg not detected (ND). All meat samples were from animals shot/killed for meat purposes. The age, size, and sex of the impala killed by both methods did not influence the concentration or occurrence of any of the analysed toxic metals in the muscles. Given that these animals are raised in a controlled game farm, general varying of intrinsic concentrations of these metals was not expected. All samples were from animals fed with grass and free roaming to feed on natural forage in the farm and were also hunted/harvested in their natural environments.

Figure 5 shows the levels of naturally found or occurring toxic metal from control samples.

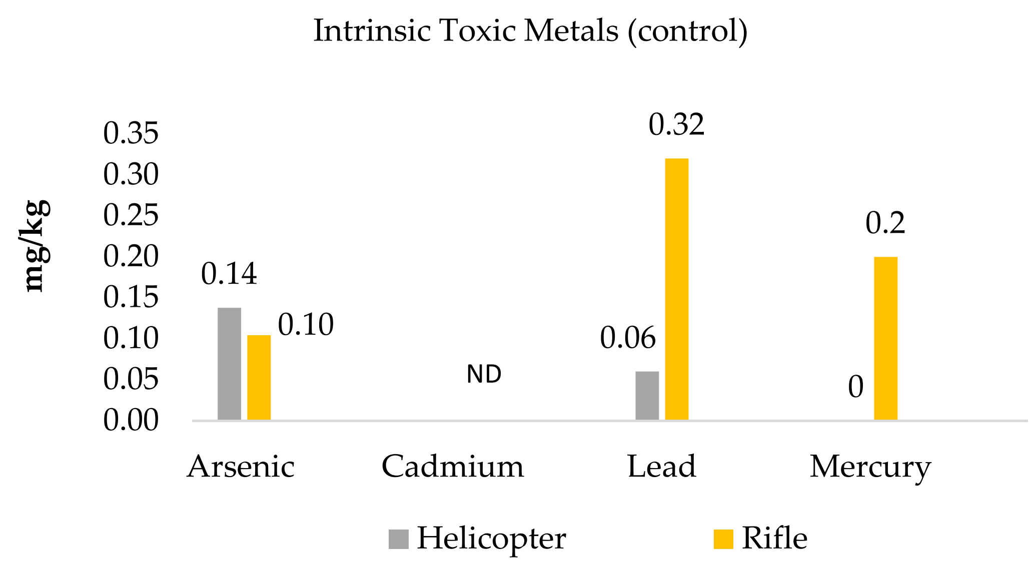

Figure 5. Average intrinsic toxic metals found in control samples.

3.2. *Toxic Metals Contributed by Killing Methods*

In meat sampled around the bullet entry points from either pellets or rifle entry points (helicopter = 9; rifle = 9), Figures 6–9 show different toxic metal occurrence patterns. Arsenic ($p = 0.29$): 2 out of 18 samples from rifle killing did not comply with EU standards; Cd ($p = 0.90$): all 17 samples were within the EU specifications with the exception of one sample from the rifle killing. The concentrations of Cd and Hg were below the EU limits; however, 2 of the 18 impala samples from both killing methods tested positive for Hg though in small traces (helicopter: 0.077 and rifle: 0.254 mg/Kg meat). For Pb, all of the meat sample results from both killing methods were above EU limits; all these samples had high standard deviations (SD). Using a t-test to compare the concentration of metals from the different samples as contributed by bullets or killing methods compared to control samples from the same animal, only the Pb concentrations of helicopter shot ($p = 0.001$) and rifle shot ($p = 0.008$) carcasses were significantly different. For Hg ($p = 0.43$), all 18 were in compliance with EU limits of toxic metals in food.

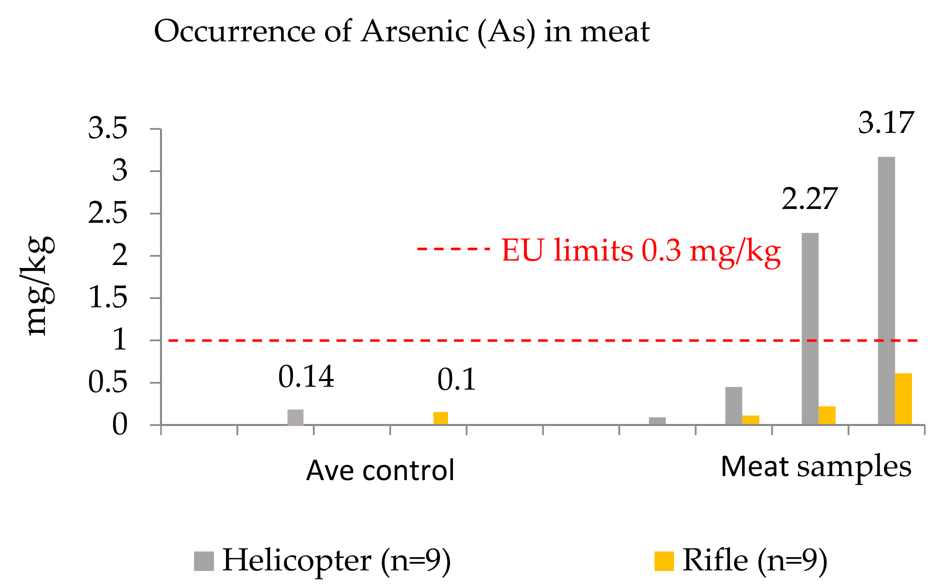

Figure 6. Arsenic concentration in meat samples.

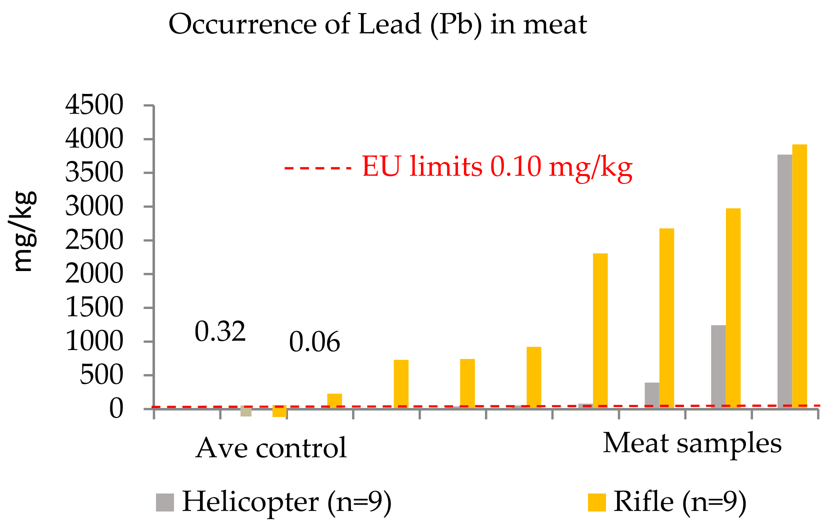

Figure 7. Lead concentration in meat samples.

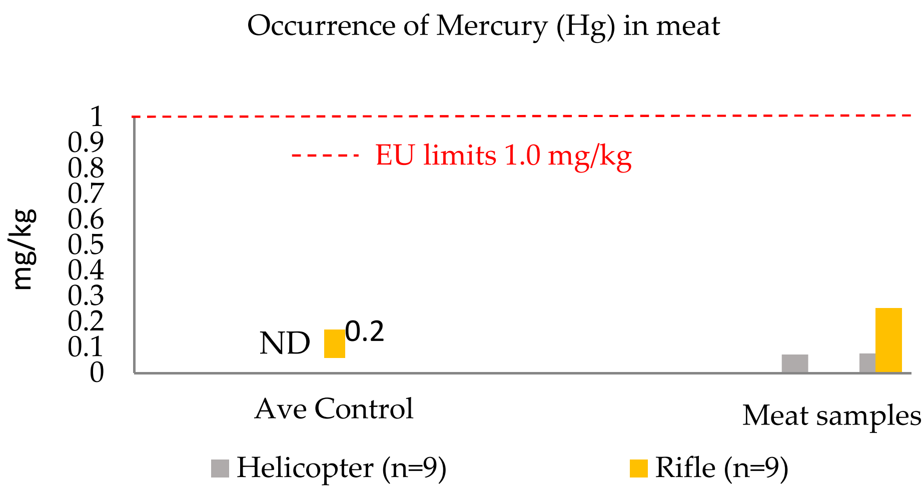

Figure 8. Mercury concentrations in meat samples.

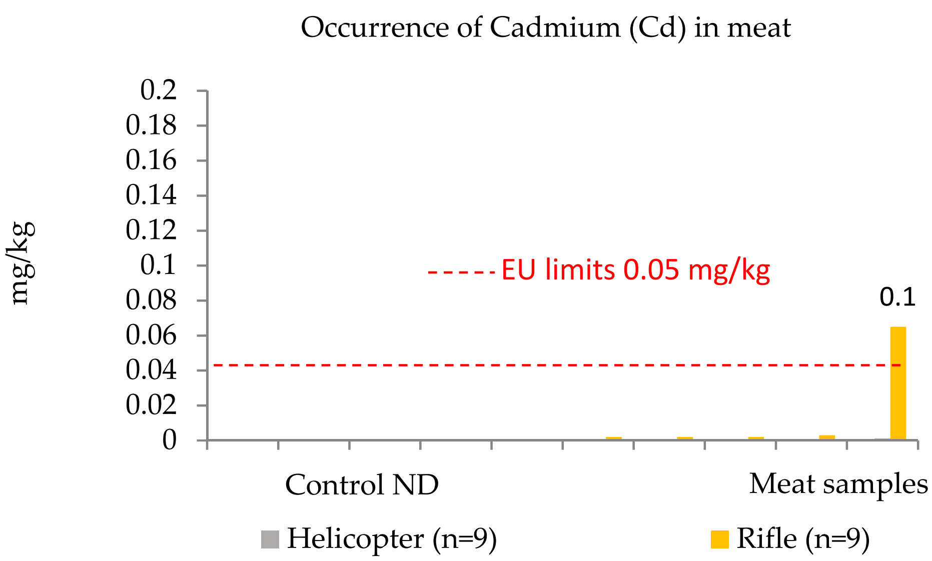

Figure 9. Cadmium concentrations on meat samples, control not detected (ND).

4. Discussion

This investigation confirmed that the major metals of potential health concern found in food, drugs (medicines), and dietary supplements are As, Cd, Pb, and Hg [52]. These non-essential but hazardous toxic metals are generally introduced to meat in higher concentration by processes linked with game animal killing, hunting, or harvesting as well as processing of wild meat [37,53,54]. Other operations that are manmade such as previous mining operations around game meat farms could contribute to an increased intrinsic concentration of toxic metals in animals produced for meat purposes [5]. In most cases, wild animals are bred within the same farm, thus limiting exposure to toxic metals. However, contaminated feed, water, and veterinary medicine could be a good source of animal, and subsequently carcass, contamination [27]. Other processes of meat contamination include uncontrolled meat production practices and the use of bullets made from metals such as Pb and Cu. These could increase the toxic metal concentration levels in meat and later lead to consumer health effects [20,55]. Researchers have highlighted the need to ensure proper environmental screening, monitoring, and testing of food products to ensure their compliance to limits [15,56,57]. This not only assists with monitoring, but it also facilitates processes of certifying meat products as meeting the regulated meat products' limits for daily or weekly exposure or ingestion by consumers.

In developed countries, particularly the EU communities, the need to regulate the presence of toxic metals in game meat has been identified for some time, and these measures are continually promulgated in hunting regulations, meat safety processes, and environmental pollution regulations [7]. For game meat animal production, the four toxic metals of interest remain, As, Cd, Pb, and Hg; these toxic metals are identified as potential threats to game meat production and its sustainability as a safe and cheaper protein option [58].

Arsenic: In this study, the mean concentrations exceeded the limit (0.3 mg/Kg) prescribed by the European Commission. This was caused by other samples recording higher, especially from the rifle-killed carcass concentrations [59]. A study in Croatia [60] revealed the presence of arsenic in tissues of the European hare (*Lepus europaeus*) and highlighted the importance of monitoring arsenic in the environment and also game meat after slaughter, where the occurrence and the introduction of this trace element was considered possible as the result of environmental contamination and slaughter operations.

Cadmium: With an exception of one sample from the rifle-killed carcass, the mean concentrations of Cd were below the limit (0.05 mg/Kg) prescribed by the EU [59]. It was not clear how the one sample had concentrations in excess of 1 mg/Kg. The results of this study, in general, confirmed findings [40] where Cd was found in traces, but not significant enough to render meat products unsafe for human consumption. Similar to another study [18], the averages of Cd from rifle thorax shots (0.008 mg/Kg) and helicopter killing (0.000 mg/Kg) conducted on free range game meat derived from a non-contaminated environment was inconsequential when compared to limits stipulated in regulations relating to maximum levels of toxic metals in developing and developed countries.

Lead: The mean concentrations exceeded the limit (0.1 mg/Kg) prescribed by the EU. On comparing the two killing methods and with reference to high lead contamination around the bullet entry points, it is important to highlight that this could have been because Pb base pellets were used during these processes [47,61]. The results of this study were similar to those noted in Europe [39], where Pb was found to be high around bullet entry points in wild boar (69.7 mg/Kg–1095.9 mg/Kg) and red deer (137.7 mg/Kg–324.6 mg/Kg) shot with bullets used for meat animal hunting or killing purposes. These results were over the legal product limit [21,24,59]. The results of this study confirmed findings in [62], where a conclusion was drawn that while visible affected areas could be removed during dressing and inspection, extensive traces of Pb could be picked up from areas that were generally not affected by bullets thus posing a risk to consumers.

Mercury: The mean concentrations were below the limit (1 mg/Kg) prescribed by the EU (European Commission) [59]. Similar to an earlier study [35], Hg concentrations are generally not significant in meat unless there has been some environmental contamination where the killed animal could have been grazing. The results of Hg were lower than the EU limit of 1.0 mg/Kg in meat products. De Oliveira, de Castro [63] explains that the natural occurrence of Hg in the wild is unlikely. Studies have found that Hg could be introduced to meat animals by the processes of meat production. However, environmental contamination remains the most significant source of wild animal contamination. This type of contamination is generally recorded in environments such as mine rehabilitation, where wildlife is introduced as part of the processes and ecotourism [63–65].

5. Conclusions

The control and monitoring of chemical hazards linked with processes of meat production remain a challenge in game-meat-producing countries such as South Africa. This is mainly caused by the lack of regulations controlling bullets used during the killing of game meat animals. This shortcoming is exacerbated by poor monitoring of feed and environmental contamination and infrequent testing for toxic metals in game meat products. While there are limits for maximum levels of some metals in foodstuffs, these limits do not include game meat and game meat production including killing processes. The responsibility lies with processing plants including game abattoirs to establish, implement, and maintain Food Safety Management Systems that could identify, evaluate, and control hazards likely to be present in meat products. Good slaughter procedures must always be followed, and all animals should be subjected to meat inspection. During meat inspection, the affected parts should be trimmed, especially around the bullet entry point as it will be observably bruised with excess blood. The risks of toxic metals present in game meat production must be mitigated or always controlled for the protection of consumers. These could include the encouragement of the use of lead-free bullets during hunting. Compulsory monitoring of toxic metals at game farms and during game meat production periods, and training hunters/harvesters, slaughter operators, and meat inspectors on mitigation plans to reduce meat contamination and other potential strategies that could be implemented to minimize the food safety risk must be developed. The adoption of other measures of meat animal killing, such as the use of bows and arrows, could also be utilized, though these may not be practical in cases of mass game meat production and animal welfare compliance. Regulators must also look at developing regulations that evaluate the type of bullets used to kill meat animals. This may include the use of less fragmenting bullets and the use of bullets made free of toxic metals (Pb-free bullets). These regulations must be enforced for all stakeholders in the production of game meat.

Author Contributions: D.V.N. conducted the research as part of his Doctorate degree in Environmental Health. This study was supervised by J.L.B. and L.C.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research is supported by the South African Research Chairs Initiative (SARChI) and partly funded by the South African Department of Science and Technology (UID number: 84633), as administered by the National Research Foundation (NRF) of South Africa, and partly by the Department of Trade and Industry's THRIP program (THRIP/64/19/04/2017) with Wildlife Ranching South Africa as partner and by Stellenbosch University. Any opinions, findings and conclusions or recommendations expressed in this material are that of the author(s) and the National Research Foundation does not accept any liability in this regard.

Institutional Review Board Statement: The study was conducted in accordance with the Tshwane University of Technology and approved by the (Animal Research Ethics Committee) of Tshwane University of Technology (Proposal code AREC 2019/03/002, approved on the 12 of March 2012).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The authors thank Ramaco game ranch in Makopane, South Africa, for the provision game meat animals, professional hunters, slaughter of facilities for the purpose of this study.

Conflicts of Interest: The authors declare no conflict of interest.

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