



Identifying the origin of lead poisoning in white-backed vulture (*Gyps africanus*) chicks at an important South African breeding colony: a stable lead isotope approach

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Abstract

Elevated lead levels in scavenging raptors can originate from a variety of environmental and anthropogenic sources, including soil, water, mining activities and legacy lead from leaded fuel, but has mostly been attributed to fragments of lead-based ammunition embedded in the tissues of carcasses. To identify the origins of lead in the tissues of white-backed vulture (*Gyps africanus*) chicks at Dronfield Nature Reserve, South Africa, we used MC-ICP-MS to compare the isotopic composition of lead in blood samples to those of soil in the chicks' immediate environment, different mining activities in South Africa and lead ammunition commonly used in hunting and game management practices. The isotopic ratios in vulture blood samples ranged widely ($^{207}\text{Pb}/^{206}\text{Pb}$: 0.827–0.911), but fell within those measured for ammunition (0.761–0.938). Dronfield water can be excluded as a significant source, as the lead concentration for water was below detection limits. Uranium, coal, atmospheric Pb, legacy Pb from fuel and Pb mining can also be excluded as significant sources, based on the limited overlap with Pb isotopic ratios measured in vulture blood. Whereas 55% of chicks we sampled displayed isotopic ratios consistent with Dronfield soil, the low local Pb concentration and the low extractable Pb levels in South African soil in general, imply that soil Pb is unlikely the major source of Pb in WBV chicks, especially in birds with elevated blood Pb levels, i.e. $> 20 \mu\text{g}/\text{dL}$. Our results, when considered in the context of vulture feeding ecology and low Pb levels in non-scavenging birds in South Africa, imply the major source of elevated Pb levels in WBV chicks to be fragments of lead-based ammunition embedded in the carrion fed to them by their parents.

Keywords Lead · Vultures · Isotopes · Ammunition · Mining · Soil

Introduction

Lead poisoning is a well-known global threat to wild birds and is widespread in scavenging raptors, terrestrial game birds and waterfowl (Williams et al. 2018; Pain et al. 2019).

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Although lead exposure has been reported as a direct cause of mortality of threatened bird species, the sub-lethal effects on physiological systems, breeding success and behaviour may be equally or more devastating (Bellinger et al. 2013; Golden et al. 2016; Arrondo et al. 2020), making the identification and mitigation of the source of lead poisoning of special conservation concern. Irrespective of the source, lead becomes soluble when it comes into contact with the acidic gastric fluids (Helander et al. 2009) from where it is absorbed into the bloodstream (Franson and Pain 2011). In raptors, physiological impacts of lead increase substantially in severity at blood $[Pb] > 10\text{--}20\ \mu\text{g/dL}$ (Franson and Pain 2011, Finkelstein et al. 2014; Wiemeyer et al. 2017) and are usually attributable to exposure to specific lead sources that elevate circulating levels above background environmental levels. Because raptors are likely exposed to a combination of lead sources, it is important to consider all possible avenues of exposure, as failure to do so may hamper mitigation measures.

Environmental sources of lead are numerous, including exposure near or downstream from current or historic mining areas (Henny et al. 1991, 1994; Strom et al. 2002; Krone 2018), pollution of surface soils by uranium mining (Selvakumar et al. 2018) and environmental heavy metal pollution from coal in countries where electricity generation remains primarily coal-fired (Díaz-Somoano et al. 2009; de Villiers et al. 2010). In addition, lead in paint is typically ingested by captive birds (Krone 2018) when peeling paint contaminate the soil within old enclosures (Naidoo et al. 2012; Pikula et al. 2013), although the ingestion of paint flecks has also been recorded in wild, free-ranging birds (Finkelstein et al. 2003). Another source is legacy lead from leaded fuel, which persists indefinitely in surface soils and may continue to pose health risks for hundreds of years (Clay et al. 2019). However, numerous studies have revealed the most common source of lead poisoning in scavenging raptors globally to be fragments of lead-based ammunition embedded in the tissues of carcasses (Church et al. 2006; Fisher et al. 2006; Helander et al. 2009; Lambertucci et al. 2011; Bellinger et al. 2013; Haig et al. 2014; Carneiro et al. 2015; Jenni et al. 2015; Ganz et al. 2018; Lohr et al. 2020).

One approach to tracing the sources of lead exposure concerns the quantification of stable isotope ratios, with the characteristics of Pb lending themselves to isotopic fingerprinting (Cheng and Hu 2010). Lead has four naturally occurring stable isotopes, ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb (Rabinowitz 1995). Of these, only ^{204}Pb is non-radiogenic, with the radioactive decay of ^{238}U , ^{235}U and ^{232}Th giving rise to ^{206}Pb , ^{207}Pb and ^{208}Pb respectively (Skerfving and Bergdahl 2007). The comparative abundance of the latter three isotopes varies strongly, depending not only on when the lead ore was formed in geological time, but also on the proportions of lead (Pb),

uranium (U) and thorium (Th) in the system (Rabinowitz 1995, Cheng and Hu 2010). Furthermore, as Pb is a heavy element, the fractional mass differences between its different isotopes are small, resulting in minimal fractionation during biological and industrial processes (Cheng and Hu 2010). Consequently, distinct isotope ratios could be associated with many anthropogenic sources of Pb, providing a powerful tool for inferring the origin of Pb in a tissue sample (Patel et al. 2008). Should the isotopic compositions of multiple Pb sources vary, these differences will be evident in the isotopic composition of tissue Pb, providing opportunities to accurately link blood Pb and its potential source(s) (Patel et al. 2008). Due to the proliferation of international trade in lead ores and lead recycling, the isotopic ratios of Pb sources may change unpredictably, becoming increasingly homogeneous globally and complicating source identification through isotopic analysis (Sangster et al. 2000). Lead ammunition, especially, is frequently manufactured from recycled lead from a variety of sources, resulting in variable isotopic ratios which may complicate analysis (Koons and Grant 2002, Franzen-Klein et al. 2018).

Lead stable isotope ratios have been used in several studies to identify the source of lead poisoning in raptors, including Griffon (*Gyps fulvus*), Bearded (*Gypaetus barbatus*) and Egyptian vultures (*Neophron percnopterus*, Berny et al. 2015), white-tailed sea eagle (*Haliaeetus albicilla*, Helander et al. 2009), Andean condor (*Vultur gryphus*, Lambertucci et al. 2011), California condor (*Gymnogyps californianus*, Church et al. 2006, Finkelstein et al. 2014), golden eagle (*Aquila chrysaetos*, Jenni et al. 2015), bald eagle (*H. leucocephalus*, Franzen-Klein et al. 2018) and red kite (*Milvus milvus*; Pain et al. 2007). Previous research revealed elevated Pb levels among white-backed vulture (*Gyps africanus*) chicks at Dronfield Nature Reserve (van den Heever et al. 2019), an important breeding colony in South Africa's Northern Cape Province. Because the chicks were all nest-bound at the time of sampling, it was expected that they would display similar blood lead concentrations, likely obtained from lead sources in their immediate environment. However, some chicks displayed blood Pb levels significantly higher than others, implying that these chicks were experiencing additional exposure, most likely via ingestion of regurgitated carrion fed to them by their parents. White-backed vultures feed predominantly on the flesh and viscera of mammalian carrion, mostly originating from wild ungulates and livestock (Piper 2005). Wildlife ranching, the establishment of formal and informal vulture supplementary feeding sites and in situ supplies of offal during hunting and culling operations have provided vulture populations with a reliable source of food, but have also inadvertently created a possible conduit for the ingestion of lead-based rifle and shotgun ammunition.

Here, for the first time in sub-Saharan Africa, we aim to quantify stable Pb isotope ratios in a scavenging raptor to trace the major source of Pb exposure. We hypothesised that fragments of lead-based ammunition embedded in carrion are a major source of Pb poisoning in white-backed vulture chicks at Dronfield Nature Reserve, especially in chicks with high levels of exposure, and predicted that the Pb isotopic values of birds with background exposure (i.e. < 20 µg/dL) are significantly different than those with elevated Pb levels. To test this hypothesis, we compared the stable lead Pb isotopic compositions in blood samples taken from white-backed vulture chicks to those of soil in their immediate environment, different mining activities in South Africa and lead-based ammunition commonly used in hunting and game management practices.

Materials and methods

Sample collection

The 38 blood samples analysed for this study were selected to represent each of the major lead exposure groups as defined by Franson and Pain (2011) (Table 1). The samples were collected from white-backed vulture chicks at Dronfield Nature Reserve (28.659° S, 24.802° E) near Kimberley (Northern Cape Province, South Africa) in 2016, 2017 and 2019, using methods described by van den Heever et al. (2019). Figure 1 with the exception of seven samples with [Pb] > 100 µg/dL (collected in 2019), all samples used in the present study were taken from the dataset previously analysed by van den Heever et al. (2019).

Soil samples ($n=8$) were collected at Dronfield Nature Reserve ($n=3$, including the supplementary vulture feeding site) and several sites within a 120-km radius: Jacobsdal (29.046° S, 24.729° E), Riverton (28.511° S, 24.686° E), Douglas (29.118° S, 23.757° E), Benfontein (28.810° S; 24.811° E) and Mokala National Park (29.155° S; 24.337° E). Samples were taken from topsoil at a depth of ~5 cm and stored in urine specimen jars. Water samples ($n=3$) were collected in urine specimen jars from three livestock water troughs at Dronfield Nature Reserve, including one at the

supplementary feeding site. An additional soil sample was collected from the tailings of a uranium mine near Hartbeesfontein (26.911° S; 26.398° E, North West Province).

Rifle ammunition ($n=48$) representing a range of calibres from brands used for hunting and game management purposes in South Africa were donated by members of the National Hunting and Shooting Association and the Confederation of Hunting Associations of South Africa (Table S1 in supplementary material). Brands included Nosler (USA), Hornady (USA), Lapua (Finland), Swift (USA), Speer (USA), PMP (South Africa), Claw (South Africa), Stewart (South Africa), Sellier & Bellot (Czech Republic), Woodleigh (Australia), Sierra (USA), Norma (Sweden), Highland (Australia) and Hirtenberg (Austria).

Pb concentration

Lead concentrations ([Pb]) in blood samples were determined using ICP-MS, as previously described by van den Heever et al. (2019). Lead concentrations in soil and water samples were measured using an Olympus Vanta handheld X-ray fluorescence (XRF) analyser with a 50-kV Rhodium tube (Innov-X-Africa, Johannesburg, South Africa). The analyser had a 20-mm silicon drift detector (SDD) with a graphene window with a resolution of 134 eV. Tube voltage and current was set at 40 kV and 71 µA, respectively. Samples were decanted into plastic cups and sealed with 6-µm Mylar XRF film. With the analyser mounted in a stand on a desktop surface, the sample was inverted and placed directly above the detector for measurement. Each sample was measured for 60 s, in triplicate. The mean of the three measurements was recorded as the sample [Pb]. The limit of detection (LOD) for Pb is 5 µg/g.

Pb separation

Blood samples

Lead was extracted from blood samples using methods described by Yahaya et al. (2013), Memon et al. (2007) and Gwiazda et al. (1998) at the ultra-clean Wits Isotope Geoscience Laboratory (WIGL) of the University of the Witwatersrand. A blood aliquot of 200 mg was weighed and pipetted into a Teflon beaker, before 550 µL of H₂O₂ was slowly added to each sample to break down the organic matter. The mixture was then heated at 100 °C on a hot plate, allowing the H₂O₂ to evaporate. Once dry, 2 mL of double distilled HNO₃ was added to each sample, and left overnight in capped beakers on the hot plate at 100 °C. The following day, the beakers were opened, allowing the HNO₃ to evaporate, before 0.5 ml of 0.7 M HBr was added, and heated on the hot plate at 100 °C for 1 h. Thereafter, beakers were again opened, allowing the mixture to evaporate,

Table 1 White-backed vulture blood samples classified according to the main lead exposure groups described by Franson and Pain (2011)

Range (µg/dL)	Interpretation	<i>n</i>
< 10	Background exposure	7
10–20	Mild to moderate subclinical effects	4
20–50	Significant subclinical effects	12
50–100	Clinical poisoning	8
> 100	Severe clinical poisoning	7

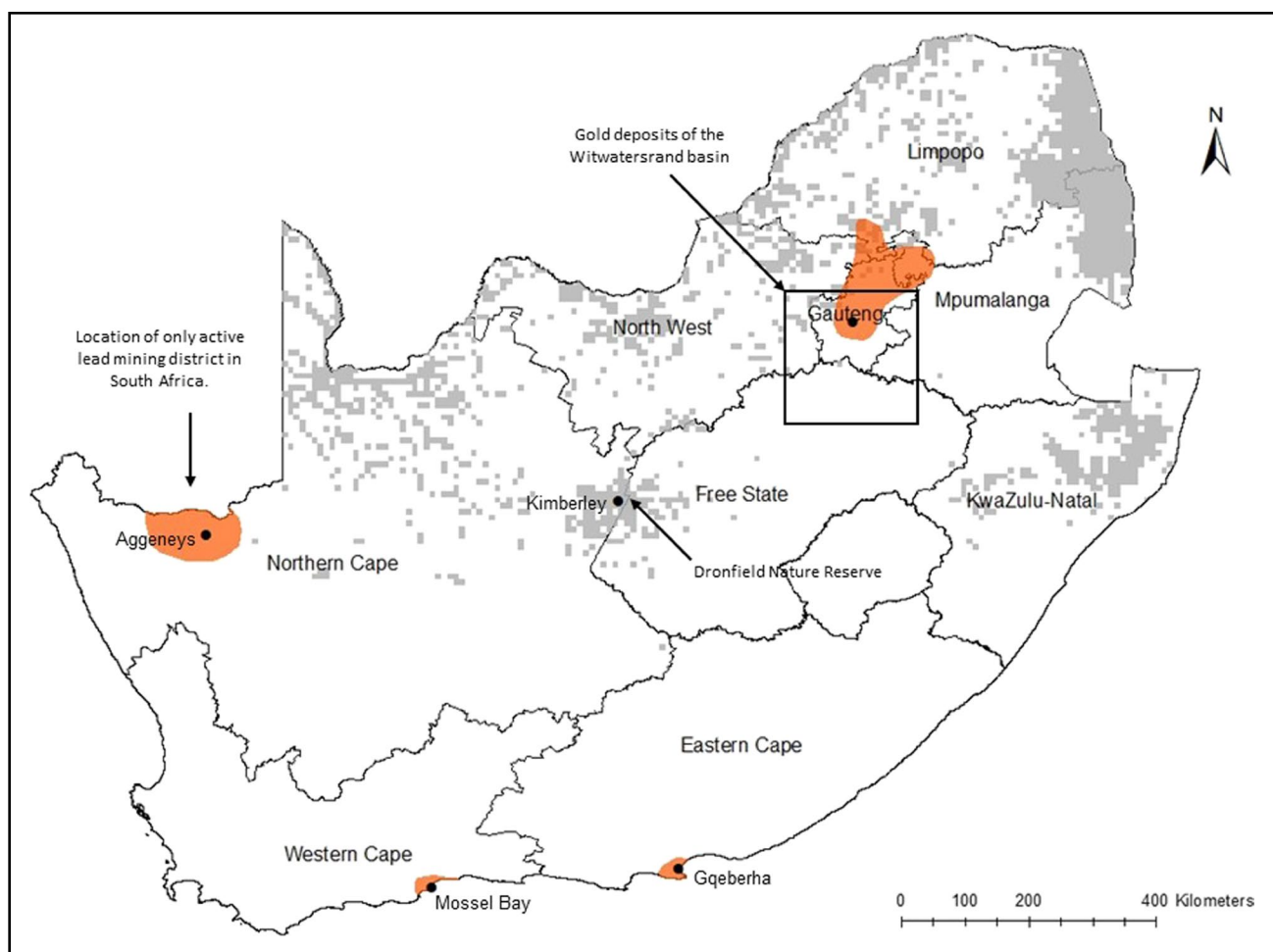


Fig. 1 Locations of the most likely sources of anthropogenic Pb in South Africa (orange polygons), adapted from de Villiers et al. (2010), in relation to the distribution of white-backed vultures (grey). In South Africa, the main source of uranium is the gold deposits of the Witwatersrand basin, which covers the provinces of Gauteng,

western Mpumalanga, northern Free State and eastern North West Province. White-backed vulture distribution data were obtained from the Southern African Bird Atlas Project 2 (Animal Demography Unit, University of Cape Town)

before an additional 0.5 ml of 0.7 M HBr was added to each beaker in preparation of the chromatography. The samples were centrifuged and loaded in Teflon micro-columns on a 1.5 ml bed of AG1-X8 (200–400 mesh) anion exchange resin, flushed and eluted with 3.2 ml of 0.7 M HBr and 0.3 ml of 3 M HCl, before the Pb fraction was collected in 1 ml of 6 M HCl. A blank extraction (without added sample) was carried through the entire procedure.

Soil and ammunition samples

Soil samples were prepared using methods described by Ettler et al. (2004). Samples were air dried and passed through a 1-mm sieve. About 200 mg of the sieved sample was dissolved in PFA beakers using a mixture of concentrated HF and double distilled concentrated HNO₃, and left on a heating mantle at 110 °C for a week. After evaporating the

mixture to complete dryness, each sample was redissolved in 2 mL of double distilled HNO₃ and then processed as the blood samples described above. Ammunition samples were prepared using an adapted version of the method described by Sjøstad et al. (2014). Bullet tips were cleaned using disposable paper tissue soaked in 96% ethanol. Disposable swabs were soaked in 2% HNO₃ and scrubbed on the bullet tip until a discoloration of the swab was observed. The swab was then swirled in 1 mL concentrated Suprapure HNO₃ in a centrifuge tube for a few seconds, from which 5 µL was collected and diluted for analyses.

MC-ICP-MS analysis

Pb isotope measurements were performed in low-resolution mode on a Nu Plasma II multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS; Nu Instruments,

Wrexham) housed at the Analytical Facility of the University of Johannesburg. This MC-ICP-MS is equipped with 16 Faraday detectors and five ion counters. Isotope ratios were measured using the following detector set-up: H2 = ^{208}Pb , H1 = ^{207}Pb , Ax = ^{206}Pb , L1 = ^{205}Tl , L2 = ^{204}Pb , L3 = ^{203}Tl , L4 = ^{202}Hg , L5 = ^{201}Hg .

At the start of each measurement session, the instrument was optimised for sensitivity and stability on a solution containing 250 ppb Pb and 62.5 ppb Tl. Samples and standards were introduced in a 2% HNO_3 solution using wet plasma, with a self-aspirating 200 $\mu\text{l}/\text{min}$ Glass Expansion MicroMist U-series nebuliser. The nebuliser was coupled with a Peltier cooled quartz Glass Expansion Twister Spray Chamber, at 7 °C. An intensity of 4 V was typically obtained for ^{208}Pb on a 250 ppb SRM981 standard solution. Plasma settings included an RF power of 1300 W, coolant gas flow of 13.0 L/min, auxiliary gas flow of 0.88 L/min and nebuliser setting of 36 psi.

Analyses consisted of one block of 40 cycles, each with a 10-s integration time. Background was measured at a magnet offset of half a mass unit away from the measurement position for 30 s directly before commencement of the measurement, and subtracted from the signal during measurement using the Nu Plasma NICE Editor. Automatic peak centring was performed prior to each measurement. Washout between samples was carried out using a 2% HNO_3 solution, monitoring the signal on ^{208}Pb to drop to below 1×10^{-3} V on detector H2, which typically took 2 min.

Although the Hg content was minimal, masses 201 and 202 were monitored to evaluate Hg content. Hg interference on ^{204}Pb was removed using the background-corrected signal on mass 202, and a $^{204}\text{Hg}/^{202}\text{Hg}$ ratio of 0.2296, before further data processing. To correct for lead mass bias, all samples and standard solutions were spiked with Tl. A ratio of $^{205}\text{Tl}/^{203}\text{Tl}$ of 2.3889 was used to correct for mass bias according to the exponential law. Total procedure blanks were between 3 and 4 pg of Pb during the course of the study.

Additional sources

Lead isotope ratios of water from uranium tailings from a mine in South Africa ($n = 10$) and in Namibia ($n = 10$) were obtained from Kupi et al. (2020), who did not report the locations of the two mines. Isotopic ratios of lead (II) sulphide (PbS, galena) of the Bushmanland Group mined at Broken Hill, Big Syncline, Gamsberg and Black Mountain (near the town of Aggeneys, Northern Cape Province) were obtained from Reid et al. (1997). To account for legacy lead from leaded fuel, phased out in South Africa in 2006, lead isotopic compositions were obtained from Monna et al. (2006), representing the major petrol companies operational at the time, i.e. Total, Caltex, Zenex, Engen, Shell and BP. Atmospheric Pb measurements for Windhoek (Namibia), East London, Maseru (Lesotho), Pretoria and Cape Town

were obtained from Bollhöfer and Rosman (2000). Isotopic ratios for South Africa's major coal deposits were obtained from Díaz-Somoano et al. (2009) and Monna et al. (2006).

Statistical analysis

All statistical analyses were carried out in the R 4.1.0 (R Core Team, 2021) environment, using R Studio 1.4.1717 (RStudio, Inc.). Data were tested for normality using the Shapiro–Wilk test. Because normality could not be achieved through data transformation, and the homogeneity of variance assumption was violated, the non-parametric Kruskal–Wallace test was employed to test for significant differences between the isotopic ratios of the different lead exposure groups. Graphics were produced using the ggplot2 (Wickham 2016) and ggbreak (Xu et al. 2021) packages. Where possible, statistical analyses and comparisons with previous studies focused on $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ only. The use of ^{204}Pb was avoided to prevent analytical biases in blood samples with low lead levels (Arrondo et al. 2020). The exception was comparisons with lead isotopic values presented for Namaqualand lead (II) sulphide by Reid et al. (1997), where only $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ were available. In all instances, the level of significance was set at $p < 0.05$.

Results

Values for $^{207}\text{Pb}/^{206}\text{Pb}$ varied widely among white-backed vulture chicks at Dronfield Nature Reserve (0.827–0.911), which was also the case for the ammunition samples analysed in this study (0.761–0.938) (Table 2). The $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of all blood and soil samples (with the exception of the uranium mine tailing sample from Hartbeesfontein) also fell within the range of the ammunition sampled in this study (Fig. 2). Twenty-one (55%) blood samples fell within the range measured for all soil samples (excluding Hartbeesfontein), while 17 (45%) fell within the range of Dronfield soil. Three (8%) blood samples fell within the upper limit of water from tailings dams at a South African uranium mine and coal deposits previously reported by Kupi et al. (2020) and Díaz-Somoano et al. (2009), but showed no overlap with those reported for tailing dams of uranium mined in Namibia (Fig. 3). The ratios recorded for the uranium mine at Hartbeesfontein ($^{207}\text{Pb}/^{206}\text{Pb}$: 0.327, $^{208}\text{Pb}/^{206}\text{Pb}$: 0.392) fell far below those measured for vulture blood. There was no overlap between the $^{207}\text{Pb}/^{204}\text{Pb}$ or $^{206}\text{Pb}/^{204}\text{Pb}$ isotopic values of vulture blood and those reported for lead (II) sulphide (galena, PbS) ores mined near the town of Aggeneys (Northern Cape Province) (Fig. 4). There were no significant differences in the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios between the different lead exposure groups ($H(3) = 4.35$, $p = 0.23$, Table 2). Soil samples had

Table 2 Mean, standard deviation and range of $^{207}\text{Pb}/^{206}\text{Pb}$ in analysed white-backed vulture (*Gyps africanus*) whole blood, ammunition and soil samples. Blood samples were allocated to lead exposure categories following Franson and Pain (2011)

	N	Mean	Median	SD	Range
Blood					
< 20 $\mu\text{g}/\text{dL}$	11	0.855	0.849	0.015	0.836–0.877
20–50 $\mu\text{g}/\text{dL}$	12	0.846	0.843	0.016	0.827–0.872
50–100 $\mu\text{g}/\text{dL}$	8	0.871	0.872	0.033	0.827–0.911
> 100 $\mu\text{g}/\text{dL}$	7	0.850	0.840	0.021	0.833–0.884
Cumulative	38	0.854	0.848	0.022	0.827–0.911
Ammunition	48	0.844	0.831	0.038	0.761–0.938
Soil (excl. Hartbeesfontein)	7	0.829	0.828	0.011	0.815–0.851

a mean lead concentration ([Pb]) of $7.4 \pm 0.9 \mu\text{g}/\text{g}$. [Pb] in water samples collected from Dronfield Nature Reserve was below the limits of detection.

Discussion

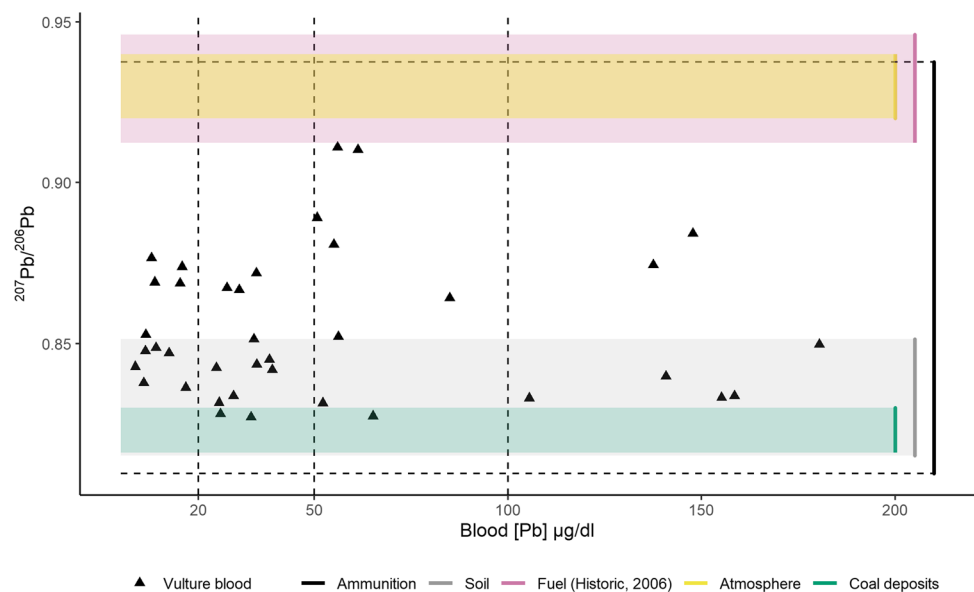
Our results revealed a wide range of Pb isotopic ratios for lead-based rifle ammunition used in South Africa, supporting the findings of previous studies (Scheuhammer and Templeton 1998, Thomas et al. 2009). Consequently, the Pb isotopic ratios of ammunition overlap completely with those of soil and those previously reported for fuel, atmospheric Pb and South African coal deposits (Fig. 2). As a result, Pb isotope ratios alone do not permit unambiguous identification of the sources of elevated Pb in white-backed vulture blood samples. As with any toxin, however, exposure is related to the

dose and concentration in the source material, and the Pb source concentrations can potentially be used to infer the contributions of multiple sources (Komárek et al. 2008; Kim et al. 2021). This reasoning allowed us to exclude Dronfield water as a potential source of Pb in white-backed vulture chicks, as the [Pb] fell below the level of detection.

Many chicks ($n = 21$) had isotopic values that overlapped with both ammunition and soil. It is probable that the Dronfield nestlings (aged 60–120 days) were exposed to Pb from the soil continuously since hatching, either via inhalation during respiration or ingestion/inhalation while preening feathers. They could also have ingested soil-covered carrion gathered from the supplementary feeding site at Dronfield by their parents. If soil was the only source of lead, blood $^{207}\text{Pb}/^{206}\text{Pb}$ would match local soil values of 0.828–0.848, yet only 44.7% ($n = 17$) of the chicks had blood $^{207}\text{Pb}/^{206}\text{Pb}$ in this range. Moreover, if soil was the primary source of lead for all these chicks, one would expect them all to show similar blood [Pb] values (Beyer et al. 2013, 2014). The highly variable blood [Pb] thus implies a source beyond Dronfield that can only originate from food provisioned to the chicks.

Vultures can travel great distances in search of food, and it is likely that the Dronfield chicks are also ingesting carrion covered in soil collected hundreds of kilometres from the breeding site. The inclusion of five additional soil samples from Douglas, Mokala National Park, Benfontein, Jacobsdal and Riverton increased the number of potentially exposed birds to 21 (55%). Since it was not feasible to analyse soil samples from all regions within the foraging range of white-backed vultures, site selection was limited to < 120 km of the Dronfield breeding site. Adult birds are certainly capable of foraging further than this, even during the breeding season, so the isotopic range measured for soil may be greater than measured in this study.

Fig. 2 $^{207}\text{Pb}/^{206}\text{Pb}$ values as a function of blood [Pb] in white-backed vulture (*Gyps africanus*) chicks from Dronfield Nature Reserve. Also shown are isotopic ranges of ammunition, soil samples analysed in this study as well as those reported for fuel, atmospheric Pb and coal deposits in previous studies (Bollhöfer et al. 2000, Monna et al. 2006, Díaz-Somoano et al. 2009)



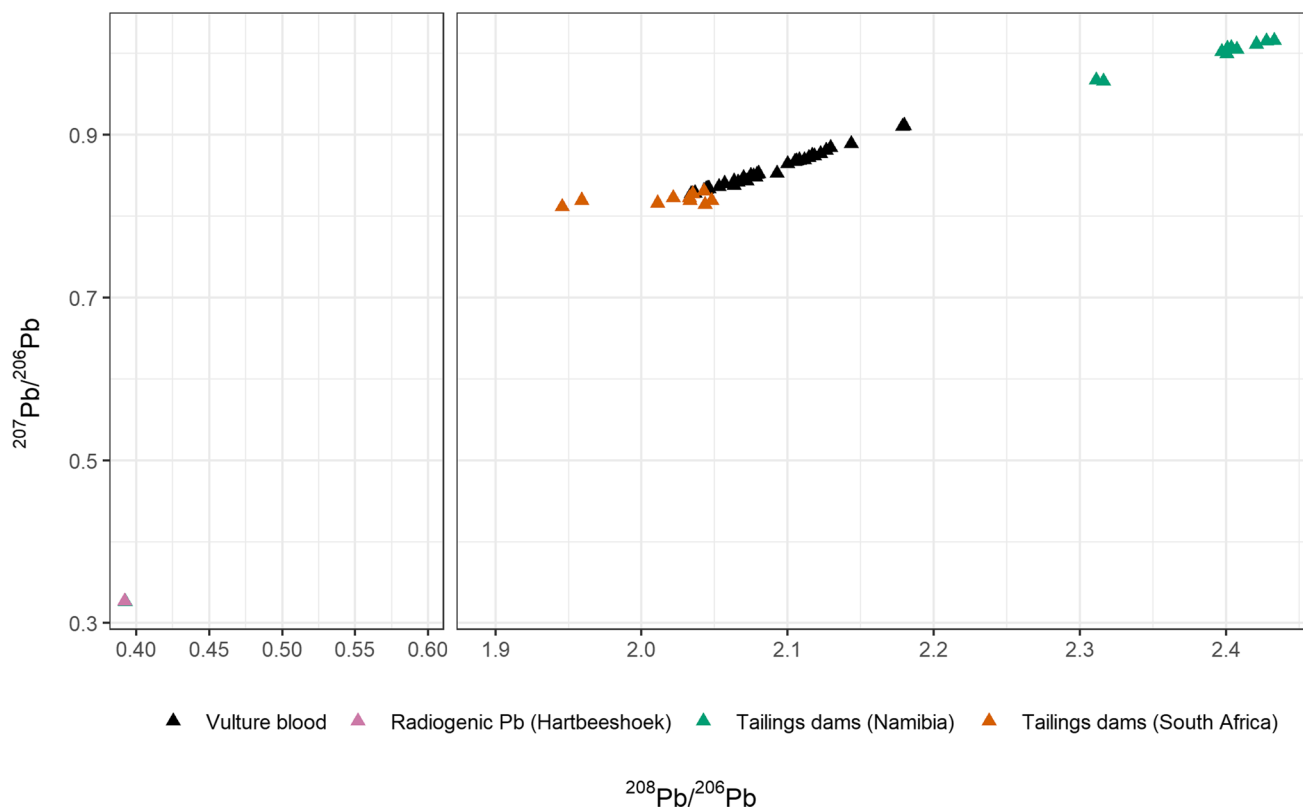
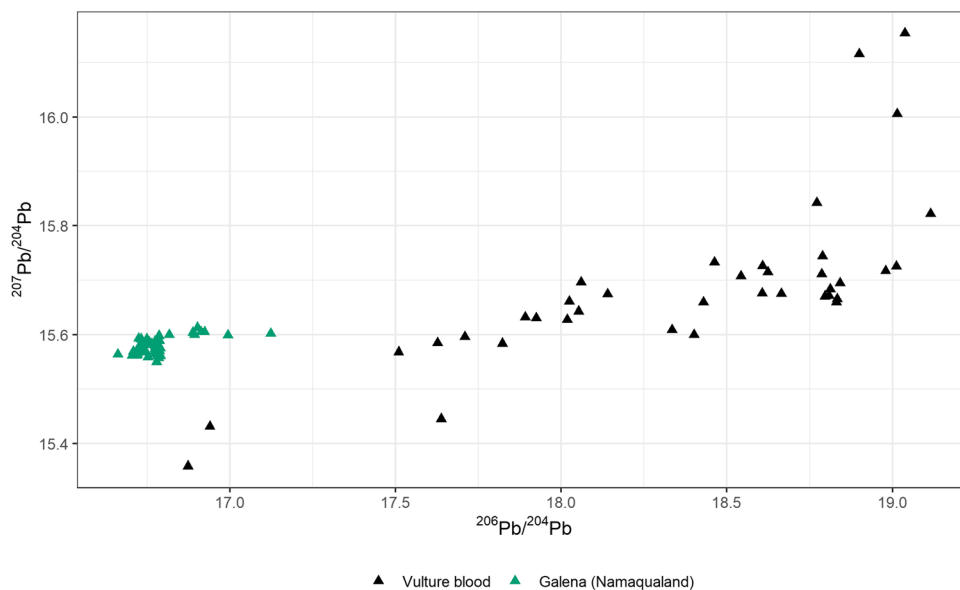


Fig. 3 Pb isotopic ratios of white-backed vulture (*Gyps africanus*) blood compared to those reported by Kupi et al. (2020) for water from tailings dams at uranium mines in South Africa and Namibia,

as well as radiogenic Pb from uranium mined at Hartbeeshoek (North West Province). An axis break was inserted to preserve scale

Fig. 4 Pb isotopic signatures of white-backed vulture (*Gyps africanus*) blood compared to those reported by Reid et al. (1997) for lead (II) sulphide (galena, PbS) ores mined near the town of Aggeneys (Northern Cape Province)



The effect of Pb in ingested soil is not merely determined by its concentration, but also by the chemical form in which Pb was ingested (Petruzzelli et al. 2020), its clay content, organic matter and cation exchange capacity (Poggio et al. 2009) as well as other factors such the developmental stage,

digestive processes and feeding state (i.e. fast or fed) of the animal affected (Yan et al. 2017). This means that only a fraction of ingested Pb may be available for biological uptake (Oomen et al. 2006; Poggio et al. 2009). The mean total [Pb] of $7.4 \pm 0.9 \mu\text{g/g}$ measured for soil was towards the lower end of

baseline concentrations found in surface soils in South Africa (2.99–65.8 µg/g), and far below the recommended total maximum threshold level of 100 µg/g (Herselman et al. 2005; de Villiers et al. 2010). Previous studies found that, of 942 soil samples taken across South Africa, only 12% had extractable Pb levels above 0.4 µg/g, and only 3% were above 1 µg/g, which is much lower than those observed in developed countries with more extensive industrialisation (de Villiers et al. 2010). So, of the total [Pb] measured in soil in this study, only a fraction may be available to vultures for uptake, although the effect may be exacerbated by their extremely acidic stomachs.

De Villiers et al. (2010) identified only four regions in South Africa with significantly elevated levels of extractable soil lead (Fig. 1). The most prominent of these is centred on the province of Gauteng, the most industrialised region in South Africa, which is also characterised by intensive mining activity. Elevated soil lead levels are also found around the industrial city of Gqeberha (formerly Port Elizabeth), Mossel Bay (the location of the country's largest oil refinery) and the border area south of Namibia, near the towns of Pofadder and Aggeneys, where South Africa's only active lead mining district is located. White-backed vulture distribution, which is mostly limited to the north and north-eastern parts of South Africa (Fig. 1), does not overlap with the polluted areas of Gqeberha and Mossel Bay. White-backed vultures are also largely absent from the high-altitude grasslands of Gauteng (Piper 2005). Although the Namaqualand region south of Pofadder and Aggeneys does not fall within the traditional range of White-backed vultures, the ongoing drought in the western parts of the country has attracted increasing numbers of vultures to the area in recent years. However, a comparison between the lead isotopic ratios found in vulture blood samples, and those recorded by Reid et al. (1997) for lead (II) sulphide ore mined in Namaqualand, suggests that it is an unlikely source of lead poisoning in the white-backed vulture chicks at Dronfield Nature Reserve (Fig. 4).

Dust and tailings from coal and uranium mining may potentially yield high concentrations of Pb. In South Africa, the main source of uranium is the gold deposits of the Witwatersrand basin, a vast area covering the provinces of Gauteng, northern Free State, western Mpumalanga and eastern parts of North West Province (Wilson and Anhaeusser 1998). Although North West Province falls within the range of white-backed vultures in South Africa, the $^{207}\text{Pb}/^{206}\text{Pb}$ value (0.327) of uranium mine tailings tested near Hartbeesfontein as part of this study, falls far below the minimum value measured for blood Pb (0.827), excluding uranium mined in this region as a possible source of Pb poisoning in the Dronfield chicks (Fig. 3). The limited overlap between vulture blood isotopic ratios and those reported by Kupi et al. (2020) for water from tailings dams at uranium mines in South Africa, suggests that these mining activities cannot significantly contribute to lead poisoning in Dronfield chicks; a conclusion that is supported by the limited occurrence of white-backed vultures within the Witwatersrand basin (Fig. 1). Uranium mining processes in Namibia, which forms an important part of white-backed

vultures' range in southern Africa, can also be excluded (Fig. 3). The limited overlap between vulture blood isotopic ratios and those reported by Díaz-Somoano et al. (2009) for South African coal (Fig. 2) likewise excludes coal as a significant source. This is supported by de Villiers et al. (2010), who highlights that South African coal is very depleted in Pb (4.2–11 µg/g), compared to global average values (Wagner and Hlatshwayo 2005).

The minimum $^{207}\text{Pb}/^{206}\text{Pb}$ value reported for atmospheric lead in 2000 (0.922) is higher than the maximum measured for blood Pb in vultures (0.911) and, as expected, is similar to ratios reported for leaded petrol prior to its phasing out in 2006 (Fig. 2, Bollhöfer et al. 2000, Monna et al. 2006). We acknowledge that heavy metal content of the atmosphere can be highly variable, depends on prevailing weather systems and industrial activity (as suggested by Monna et al. (2006)), and that atmospheric Pb compositions may have changed substantially in the intervening decades. However, it is unlikely that atmospheric [Pb], which may also significantly impact human health, could be responsible for the blood lead levels observed in Dronfield vultures.

The isotope ratios of all blood samples we examined fall within the range measured for ammunition (Fig. 2). The Pb isotopic ratios of lead shot ($^{207}\text{Pb}/^{206}\text{Pb}$: 0.85–0.88, as reported by Binkowski et al. (2016)) is not isotopically distinct from those measured for rifle ammunition, and may consequently be included as a potential source. Contrary to our expectations, the Pb isotope ratios of chicks with elevated blood [Pb] (i.e. > 20 µg/dL) were not significantly different from those with background exposure (i.e. < 20 µg/dL), although contamination from ammunition Pb has been reported in raptors at all exposure levels (Helander et al. 2021). Five chicks with blood [Pb] < 20 µg/dL had isotopic values consistent with ammunition only, while six had isotopic values consistent with soil and ammunition. Although blood [Pb] may decrease over time, Pb isotope ratios likely change very little as lead isotopic values are only gradually replaced with that of low-level environmental lead (Church et al. 2006). Chicks with background exposure, but isotopic values similar to ammunition, may have been exposed at a younger age, allowing time for their blood [Pb] to subside, but not for the isotopic values to be replaced by those of soil lead. Ongoing monitoring at Dronfield has revealed that lead poisoning is a persistent problem in > 65% of birds (van den Heever et al. 2019), so it is probable that most chicks are exposed to periodic intake of ammunition fragments from their first feeding.

Our results suggest ammunition and soil are the principal sources of lead in white-backed vulture chicks from Dronfield Nature Reserve, although the relative contribution of each remains unclear, especially in birds with low blood [Pb]. The residence time of lead in blood following acute exposure is relatively short, ranging from several weeks to several months, whereas chronic exposure may elevate blood lead

proportionately longer (Franson and Pain 2011). For instance, Cape vultures chronically exposed to Pb-contaminated soil ([Pb]: 72.5 µg/g) in a zoo enclosure displayed blood [Pb] of 56.6 µg/dL (Naidoo et al. 2012). It is reasonable to conclude that the mean [Pb] found in Dronfield soil and elsewhere (i.e. 7.4 ± 0.9 µg/g) is insufficient to explain the elevated lead levels observed in these 60–120 day old chicks which, in some cases, displayed blood [Pb] as high as 180 µg/dL. The impact of low-level soil exposure should be weighed against the impact of ammunition fragments, which consists of > 99% lead (Randich et al. 2002). These findings may explain the large variation in blood [Pb] among *Gyps* vultures at Dronfield as well as elsewhere in southern Africa (Kenny et al. 2015, Naidoo et al. 2017, Garbett et al. 2018, van den Heever et al. 2019).

Conclusion

The homogeneous nature of industrial lead used in ammunition manufacture yields isotopic ratios that range over a wide spectrum, making it increasingly difficult to use in source apportionment studies. Nevertheless, when interpreted in conjunction with Pb concentration and avian feeding ecology, Pb isotopic ratios can still find useful application in excluding other potential sources. This approach allowed us to exclude uranium and Pb mining, water, atmospheric Pb, coal and legacy Pb from fuel as potential sources of Pb in white-backed vulture chicks. Although soil cannot be excluded as a source of Pb in vultures, the low total [Pb] measured for soil, as well as the low levels of extractable lead found for South African soils in general (particularly within the range of white-backed vultures) implies that soil is unlikely the major source of lead poisoning in birds with elevated lead levels. Our findings further suggest that high blood [Pb] is better explained by lead-based ammunition as a source, supporting the findings of Arrondo et al. (2020). These conclusions are further supported when viewed in the context of vulture feeding ecology, known to be a determinant of avian Pb exposure (Slabe et al. 2020). Elevated Pb levels are a feature of South Africa's obligate avian scavengers only (i.e. Cape and white-backed vultures), while other raptors generally have low blood [Pb] suggestive of background exposure (van den Heever et al. 2019). Whereas all birds are exposed to low-level environmental Pb, the scavenging lifestyle of vultures makes them susceptible to the periodic intake of other, acute sources in the form lead ammunition fragments. The unprecedented decline of Africa's vulture populations in recent decades has been attributed primarily to poisoning (Ogada et al. 2016; Botha et al. 2017; Safford et al. 2019), including lead poisoning. The persistent and wide-spread problem of lead poisoning in

southern Africa's vultures will only be alleviated once better carcass management practices become prevalent, reinforced by the use of lead-free ammunition.

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Data availability The datasets and materials used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval White-backed vulture sample collection was approved by the BirdLife South Africa Animal Research Ethics Committee (refs. 2016/04/B, 2016/06/B, 2019/05/B), the Animal Ethics Committee of the University of Pretoria (refs. EC012-17, NAS215-2019) and the SANBI Research Ethics and Scientific Committee (refs. P18/41, P2020-21a).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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