

## Lead ingestion as a potential contributing factor to the decline in vulture populations in southern Africa

V Naidoo<sup>1</sup>, K Wolter<sup>2</sup>, CJ Botha<sup>1</sup>

<sup>1</sup>Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria

<sup>2</sup> Vulture Programme (VulPro), Plot 121, Rietfontein 0048, South Africa, South Africa.

Corresponding Author: Vinny Naidoo: [vinny.naidoo@up.ac.za](mailto:vinny.naidoo@up.ac.za); Tel: +2712 529 8082; Fax: +2712 529 8321

### Abstract

Vulture populations in southern Africa have been on the decline for years, which unlike the Asian vulture crisis, has no one specific cause. Reasons attributable are deliberate and secondary poisonings, drowning, power line injuries, electrocutions, traditional medicine (“muti” trade) and calcium deficiencies. However, lead toxicity as a potential causal factor is hardly mentioned. The potential for lead toxicity needs to be considered as substantial game hunting occurs in the region with little regulatory control on bullet types. In this study, we determined the whole blood lead concentrations of captive and wild vulture populations in South Africa and Namibia (n=185). Results were compared to previous published ranges indicative of background exposure (< 10 µg/dL), non-toxic point exposure based upon the range established from captive birds and subclinical exposure. In general, whole blood lead concentrations were higher for wild African White-backed vultures (*Gyps africanus*)(AWBV) than Cape vultures (*G. coprotheres*)(CGV) at  $15.54 \pm 12.63$  µg/dL vs  $12.53 \pm 8.88$  µg/dL (non-significantly different), while in the Bearded vultures (*Gypaetus barbatus*) no indication of exposure was evident. Very similar exposures resulted irrespective of the birds being in captivity or under wild, free-roaming conditions. A proportion of wild birds did, however, appear to be exposed to another source of lead than purely environmental ( $\pm 12\%$  and  $30.6\%$  for AWBV and CGV respectively). One bird, which had a whole blood concentration of 100 µg/dL, died soon after capture. To find the relationship between whole blood lead concentration and likely exposure factors, birds were compared by their rural/urban location, vicinity to mines and surrounding soil lead concentrations. With no relationship being present for the latter factors, we believe that this is evidence that the portion of southern African vultures being exposed to unknown source of lead, which we suggest arises from leaded ammunition remaining from hunting.

**Keywords:** Lead toxicity; southern Africa; African White-backed vulture; Griffon vulture; Bearded vulture; Whole blood

**Abbreviations**

AWBV: African White-backed vultures

CGV: Cape Griffon vultures

NZG: National Zoological Gardens

KD: Krugersdorp

PMB: Pietermaritzberg

## **1. Introduction**

Vultures play an important role in their environment (i.e. ecosystem services) by disposing of carcasses which help not only in nutrient recycling, but also plays a role in reducing the spread of diseases like anthrax. Vulture populations could also indirectly affect population numbers of obligate scavengers (such as feral dogs) thereby mitigating the spread of zoonotic diseases like rabies and dangerous human-animal interactions (Sharp, 2006). Southern Africa is home to eight old-world vulture species (Mundy et al., 1992; IUCN, 2014). Despite being home to this many vulture species, most are endangered with no specific reason being readily evident for their decline when compared to the diclofenac-associated decline on the Asian subcontinent (Oaks et al., 2004; Pain et al., 2008) or the effects of illegal poisoning in Europe (Margalida, 2012). In contrast their endangered status is ascribed to a combination of factors such as poisoning (malicious, accidental and secondary), drowning, electrocutions, power line collisions, traditional medicine trade and poor chick development due to calcium deficiencies to name some of the documented threats (Richardson and Plug, 1986; Ogada et al., 2012; Ogada et al., 2015), with further concerns now being raised over wind turbines (De Lucas, 2012). However, strikingly absent on the probable list of hazards is any mention of lead exposure as a potential cause of vulture mortalities as occur in other European species (Hernández and Margalda 2009). Despite an extensive literature search, only one study contained limited information on lead concentrations in six White-backed vultures (*Gyps africanus*) sampled near Kruger National Park, South African (Van Wyk et al., 2001).

While medical reports from South Africa indicate the most common sources of lead in people results from lead in the paints, soil and the atmosphere (Mathee et al., 2014), lead poisoning in animals can additionally result through the ingestion of lead from sources such as motorcar battery plate; direct intake of lead ammunition and shot; the ingestion of plants growing in a highly polluted areas; or the consumption of meat contaminated with lead fragments (Osweiler et al., 1996). For the vulture, lead shot or bullets and tissue residues remain the likely source as the birds are predominantly carnivorous feeding on predator kills, hunter kills or natural deaths. In terms of residues this would result in exposure following the consumption of edible tissues and bone high in lead as evident in the tissues of cattle feeding on fields contaminated with lead (Alkmim et al., 2014). However, the most important source could be the ingestion of lead ammunition in hunted carcasses, through the consumption of

lead particles that remain in the bullet wound track or in contaminated and disposed offal (Dobrowolska and Melosik, 2008). A study by Hunt et al. (2006), concluded that 95% of the carcasses they sampled in the USA originating from rifle-killed deer (*Odocoileus* spp.), were positive for lead fragments, while 90% of offal piles discarded from these killed animals were also positive for lead fragments. The latter is extremely important in a South African context, as the game hunting industry forms a massive component of the local economy, with an estimated value of US\$700million in 2010 in the provinces of North West, Eastern Cape, Limpopo, Northern Cape and Free State (Van der Merwe et al., 2014). An important feature of the hunting industry is that hunters usually leave behind portions of the carcass especially the gut piles for predator feeding. Also of importance is that South African legislation only limits the use of leaded ammunition, when the intended use is for the hunting of waterfowl (Avery and Watson, 2009).

Despite a large body of literature describing the clinical and subclinical effects of lead in various bird species, the actual tolerances of the wild African vultures is yet to be established. The closest indication of toxicity was described by Naidoo et al. (2012) in a captive Cape Griffon vultures (*G. coprotheres*) breeding colony, exposed to high lead concentrations within their enclosures at the South African National Zoological Gardens in Pretoria. In this case-study, they described signs of decreased egg hatchability, embryonic death and abnormal chick development concurrent with whole blood lead concentrations ranging between 50 to 100 µg/dL in the adult birds. Thus toxic effects were thus only non-lethal at these concentrations and no clinical signs were observed. This was similar to a model applied by Buekers et al (2009), which predicted that the adult baseline non-toxic concentration would be in the region of 71 µg/dL based upon a no observable effect level of 20 µg/dL and the lowest observable effect level of 110 µg/dL in Turkey vultures (*Cathartes aura*) Furthermore, Garcia-Fernandez et al. (2005) reported that concentration between 20 and 50 µg/dL in the Griffon vultures (*Gyps fulvus*) would not cause physiological toxicity, while concentrations above 100 µg/dL would result in clinical signs of toxicity (neurological signs, muscle wasting, weakness, anaemia and weight loss).

In this study, we evaluated whole blood lead concentration of various African White-backed and Cape Griffon vulture colonies in South Africa from both captive and wild populations.

All results obtained were compared to the whole blood lead concentrations obtained from wild birds sampled at the Etosha National Park in Namibia, which we considered to be a pristine site. To obtain an indication whether environmental exposure alone would explain the wild bird exposures, the distribution of wild bird individual lead concentrations were evaluated in relation to the 95% confidence intervals of the mean of whole blood lead concentrations obtained from the captive birds. Mean whole blood lead concentrations per area of sampling were also compared to previously published soil concentrations around the said area to ascertain if exposure could be explained by only exposure via the soil. In addition concentrations between rural and urban birds; as well as relations with mining activities were compared to ascertain if environmental lead exposure could explain the levels of exposure.

## ***2. Material and Method***

### ***2.1. Sampling***

The sampling of the birds was approved by the Animal Ethics Committee of the University of Pretoria according to the National Code for the Care and Use of Animals for Research Purposes (SANS 10386). Birds were sampled (2010 to 2012) in different parts of South Africa and Namibia (Fig 1) in the non-breeding season (September to April) from captive and wild sites. The sites were selected based on both accessibility and being part of the greater area that previous GPS tracking indicated as potential foraging areas (Figure 1)(Unpublished data).

The captive African White-backed vultures (AWBV) were sourced from the Anne van Dyk Cheetah trust, while the captive Cape Griffon vultures (CGV) were sourced from the Anne van Dyk Cheetah trust (De Wildt), African Birds of Prey Sanctuary (Pietermaritzburg) and the Johannesburg Zoological Gardens (Joburg Zoo). The captive birds were resident at their respective sites for a minimum period of at least three months prior to sampling, and where the meat was screened prior to feeding. For the sampling, birds were manually restrained by a trained handler. Blood was collected from the tarsal vein using a sterile hypodermic needle and syringe. Samples were immediately transferred into EDTA coated tubes and submitted to a commercial laboratory (Ampath, (Pty) Ltd, South Africa) for analysis. Whole blood lead

concentrations were determined using graphite furnace atomic absorption spectrometry following a preparation step with 0.1% Triton X-100.

The wild AWBV were captured in South Africa at Hoedspruit, Kimberley and Dundee; as well as in Namibia at Etosha National Park and Otjiwarongo, while the CGV's were sampled at Dundee, Kimberley, and Pilanesberg in South Africa. The study also included a limited number of wild Bearded vultures (*Gypaetus barbatus*) collected from around the Drakensberg region. In general the wild birds were caught by means of a walk-in baited cage that had a draw curtain to trap the birds. With more birds caught than sampled, sampling was random. An exception to this methodology was applied to the birds sampled in Kimberley, as only injured birds presented for veterinary care were available for sampling. Lead analysis was as mentioned above.

## 2.2. Statistical Analysis

Statistical analysis was undertaken in SPSS statistics 23 (IBM). Results are presented as means and standard deviations where possible. Data were shown to be normally distributed after natural logarithmic transformation. Differences by species and/or wild/captive status were compared with a Student t-test. Pooled results by location, irrespective of species or captive status, were compared by means of a one way ANOVA with post-hoc Bonferroni analysis. Results, irrespective of species, were compared for superiority to the Etosha National Park (natural site) or for inferiority to the National Zoological Gardens (NZG) (contaminated site from a previous study) (Naidoo et al., 2012).

To quantify the extent of excessive exposure in the wild birds, the frequency of distribution of individual concentrations in the following three categories was determined: 95% confidence interval of the mean for the Etosha birds (pristine site), 95% confidence interval of the mean of captured birds, and those above the latter. Lead concentrations per site were also compared to the published soil lead concentrations (Herselman et al., 2007), that were averaged over a 50 km radius around the sample sites of South Africa to determine if whole blood were related to the soil concentration through Pearson's correlation. To ascertain if whole blood lead concentration was related to vehicle and industrial-related sources of lead, average concentrations were compared between rural and urban sites by a Student's t-test

both dependent and independent of species. We've considered sites where the entire population in an area was under 50 000, to be rural (Etosha, Hoedspruit, Otjiwarango, Dundee, and Pilanesberg) while those with larger populations were characterised as urban (Kimberley, De Wildt, Krugersdorp and Pietermaritzburg). Sites were also divided into mining activity and non-mining activity to ascertain if mining activity could explain the results obtained. For the former Dundee, Pilanesberg, Pretoria and Johannesburg sampling sites were within 30km of aggregate, coal, gold or platinum mining activity.

### 3. Results

We evaluated the whole blood lead concentrations of three species of vultures resident in southern Africa from both wild and captive sites to ascertain the extent of exposure of the regions vultures to lead. In total 96 AWBV (6 captive and 90 wild), 84 CGV (35 captive and 49 wild) and 5 Bearded vultures were sampled. The number of birds sampled per location is provided in Table 1. Only one CGV was sampled at Dundee and Kimberley, respectively. All birds sampled were adults. The sexing of the birds was not possible. Results are presented as descriptive statistics in Table 1 and graphically in Figure 2. The whole blood lead concentrations of AWBV ranged from 1.5 to 84  $\mu\text{g/dL}$ , with the highest average concentrations being present in the birds sampled at Kimberley ( $28.0 \pm 21.23 \mu\text{g/dL}$ ), and the lowest concentrations determined in Etosha birds ( $5.27 \pm 1.56 \mu\text{g/dL}$ ). With the exception of these two groups, the remaining wild AWBV had similar blood lead concentrations circa 16 to 18  $\mu\text{g/dL}$ , while the captive birds had lead concentrations of  $19.67 \pm 7.94 \mu\text{g/dL}$ . In the CGV the blood lead concentration ranged from 4 to 46  $\mu\text{g/dL}$  with the mean concentrations between the sites being similar circa 11 to 14  $\mu\text{g/dL}$ . The one CGV sampled at Kimberley had the highest blood lead concentration of 46  $\mu\text{g/dL}$ . One additional CGV blood sample from the Drakensberg was submitted for analysis (not a formally sampled bird), as the bird in question exhibited nervous signs. This vulture had a whole blood lead concentration of 100  $\mu\text{g/dL}$  (unfortunately no necropsy was performed to ascertain tissue concentrations). The results from this bird was not included in the analysis, to prevent bias in the study results as its sampling was not random, but was used as an indication that values around 100  $\mu\text{g/dL}$  are probably in the toxic range for the CGV. Caution should also be applied in the interpreting the result from the Kimberley birds, as the higher concentrations evident in the sampled birds

may have been causal in their injuries that required veterinary treatment i.e. random sampled birds in the population may have resulted in lower overall concentrations being evident. The bearded vultures all had blood lead concentrations below the limit of quantification ( $< 3 \mu\text{g/dL}$ ).

Based on the ANOVA, the different sites evaluated (independent of species) were significantly different to one another ( $p < 0.0001$ ), with vultures sampled at all other sites (except Pietermaritzburg) being significantly ( $p = 0.01$  to  $< 0.0001$ ) higher than the Etosha sampled birds (regarded as a pristine site) and significantly lower ( $p < 0.000$ ) than the values presented by Naidoo *et al* (2012) for the captive birds at the NZG which were showing preclinical signs of lead toxicity ( $56.58 \pm 11 \mu\text{g/dL}$ ). The 95% confidence interval of the mean was  $13.31$  to  $26.02 \mu\text{g/dL}$  for the captive AWBV and  $10.07$  to  $13.36 \mu\text{g/dL}$  for the captive CGV. With regards to the degree of exposure (Figure 3), 45.5% of the wild AWBV were within the confidence interval of the Etosha birds, and a further 42.2% within the confidence interval of the captive birds of the same species. As a result, approximately 12% of the birds had concentrations above what can be explained by only background environmental exposure. For the exposure of the CGV, 61.22% of them were within the range of the Etosha birds, while 30.6% were above the confidence interval of the captive birds of the same species. When comparing whole blood lead concentration of the wild-caught birds by species, while not significantly different ( $p=0.142$ ), the AWBV had higher concentrations of  $15.57 \pm 12.58$  versus  $12.53 \pm 8.88 \mu\text{g/dL}$  with the Kimberley birds included and  $14.53 \pm 11.16$  versus  $11.83 \pm 7.5 \mu\text{g/dL}$  with these bird excluded to prevent bias as this group were non randomly sampled.

To determine if the whole blood lead concentration could be explained by soil exposure, the linear relationship between mean soil concentrations within a 50km radius around the capture site was compared to the mean whole blood concentration. For the latter poor correlation was evident, with the fitted regression line being rather flat ( $r^2=0.005$ ) (Figure 4). No difference ( $p=0.66$ ) was present when the sites of sampling were divided by being rural or urban localities (Figure 5). For the mining comparison, birds sampled from non-mining areas were significantly higher ( $p=0.017$ ) in their whole blood lead concentrations (Figure 6).

#### **4. Discussion**

Whole blood concentrations were evaluated in birds from captive and wild populations, from different parts of South Africa and Namibia, both in terms of urbanisation and location. Results were also compared to two other sites, one captive (where lead poisoning was confirmed) and one considered pristine. For the former our results were compared to published results from the National Zoological Gardens of South Africa, while for the latter we selected Etosha National Park (Namibia), as a remote park representing a natural foraging area for vultures within a 22 750 km<sup>2</sup> wildlife sanctuary. In addition the park is completely protected with no hunting being permitted. As expected the Etosha sampled birds all had low whole blood lead concentrations of  $5.27 \pm 1.56$  µg/dL, which was well within the 10 µg/dL limit specified by Kelly and Johnson (2011) as low level background exposure. With regards to the other wild birds sampled, 55 and 40% of the AWBV and the CGV populations, respectively, exceeded this 10 µg/dL limit, which would indicate that exposure to further sources of lead had occurred. A small percentage (3.33%) of AWBV also had whole blood lead concentrations that exceeded 50 µg/dL (Figure 3) which would indicate they would be susceptible to subclinical toxicity effects such as decreased fertility, decreased hatchability and abnormal chicks may occur in these birds, as previously reported in a captive breeding population at the NZG (Naidoo et al., 2012).

To ascertain the potential cause of this exposure, the results from the wild birds were compared to the captive birds. While the Etosha birds indicate that low levels of exposure are possible under completely natural conditions, this is not necessary the case for birds in less pristine although natural conditions. This was evident in the captive birds sampled in this study as their average whole blood lead concentrations were double (CGV) or triple (AWBV) that for the Etosha birds. Since the places sampled had a controlled feeding programme in place in addition to confirming that their exposure made use of non-lead paints, their likely exposure would be natural via the soil and air. The latter is not surprising considering that South Africa, as a country is known to have high soil and air lead concentrations. In a study undertaken in 2007, total soil lead concentrations in South Africa varied from 2.99 to 65.8 mg/kg, with the higher concentrations being recorded in Johannesburg and Pretoria, which are mining regions of the country (Herselman et al., 2007). In addition to lead in the soil, another common source of lead exposure in the South African environment has been linked

to the deposition of lead from fuels high in lead or from lead containing dusts arising from mining activity (Mathee et al., 2014). With regards to leaded fuels, which were only phased out from South Africa in 2006 following long-term use, the country had some of the world's highest recorded concentrations of lead in petrol (Thomas et al., 1999).

When comparing the wild birds to the captive birds, a portion of wild birds had higher whole blood concentrations than their captive counterparts which would indicate that they were being exposed to another source of lead. To establish which of these factors would be contributory, comparison was made between vultures by their exposure to high/low soil lead concentration as well as between areas with low and high human population densities. For the former, we assumed that higher soil concentrations would result in higher exposure and thus higher plasma concentrations. This was supported by a previous study by Naidoo et al., (2012) who reported that higher soil concentrations ( $72.48 \pm 21.83 \mu\text{g/g}$ ) resulted in increased plasma concentrations ( $56.58 \pm 11 \mu\text{g/dL}$ ). With the correlation between whole blood lead concentrations and soil lead concentrations being poor, it is unlikely that exposure to soil contaminated carcasses alone is a likely cause for the higher exposures. With regards to air borne exposure, with previous studies demonstrating that lead exposures were much higher in children living closer to highways (von Schirnding et al., 1991), as well as that persons living in mining towns had higher whole blood lead concentrations than those living some distance away (von Schirnding et al., 2003). We evaluated air exposure by looking at the relationship of whole blood lead concentration to population densities (and thus indirectly lead arising from fuel emissions), and to the presence of mining activity in the area of sampling. With no significant difference being present for these comparisons, it is unlikely that air borne exposures alone could explain the reason for the higher exposures seen.

With both soil and air-borne exposure alone being likely sources for the higher concentrations seen, would imply that some vultures in South Africa are being exposed to another source of lead. Based on the hunting history of the country, we believe that leaded bullets could be likely source of exposure, even though the average whole blood lead concentrations from this study were lower than reported for the Cazorla National Park, where mean whole blood lead concentrations were of  $43.07 \pm 3.96 \mu\text{g/dL}$  in Griffon Vultures exposed to lead bullets (Garcia-Fernandez et al., 2005). While the concentrations reported in

this study were not as high on average, individual birds in the specific areas did have concentrations close to or exceeding these levels (Kimberley n=2 and Dundee n=2). One reason for this potential difference would be that the Griffon vultures were resident in a hunting area, while the South African vultures were exposed to both hunted and non-hunted carcasses.

When the species results were pooled the AWBV had marginally higher whole blood lead concentrations than the CGV. While their difference in feeding location could have been contributory, a more likely reason is their smaller size, which would translate into a relatively higher exposure on the consumption of a bullet or its shrapnel or remnants of the bullets' trajectory as opposed to tissue bound lead. This conclusion is made as tissue-bound lead would be more uniformly distributed in the tissue, with the result that food intake and size of the bird would result in a fairly constant intake. However, if the birds were to consume an intact bullet, shrapnel, remnants or the entire bullet tract, the relative exposure per body weight would be higher in the AWBV due to their smaller size (Mundy, 1992).

The Bearded vultures had blood lead concentrations that were below the limit of quantification of 3 µg/dL, compared favourably with the study of Hernández and Margalida (2009), who described blood lead concentrations of 5.4 µg/dL and 2.32 µg/dL in adult and fledgling Bearded vultures, respectively. This is in sharp contrast to findings of Kruger (2014) reported finding two dead Bearded vulture carcasses with high lead concentrations in the bones (> 20 µg/g), indicative of chronic lead exposure. A possible explanation could be that high blood lead concentrations are only an indication of acute exposure. As a result these birds could have occasionally been exposed to lead, which was being adequately deposited in bone. Nonetheless, the low blood lead concentration in Bearded vultures represents a lower exposure compared to other vulture species sampled, which may be linked to a diet of predominantly bone with marrow (70%), medium sized mammals like goats and sheep which are unlikely to be hunted in South Africa, with the rest of the diet being rodents and ungulates (Brown et al., 1991; Margalida et al., 2005). The latter does therefore tend to support that dietary exposure via ammunition could be the source of the higher lead exposure seen in the other bird species that were sampled for the following study.

While we were able to establish that whole blood lead concentration in vultures from various areas in southern Africa were higher than merely background exposure would explain, one of the limitations of this study was its static nature as blood lead concentration could increase or decrease in time. This was demonstrated in Turkey vultures, where mean blood lead concentration decreased between the hunting ( $15; \pm 6-170 \mu\text{g/dL}$ ) and non-hunting season ( $7; \pm 6-36 \mu\text{g/dL}$ ) (Kelly and Johnson, 2011). Taking this into consideration the real impact of lead will require sequential sampling from the same location and, if possible, even the same bird. While it is certainly plausible that the concentration could decrease, we don't believe this will be the case as hunting in southern African tends to be limited to private reserves where there is no restriction to a particular season. We were, however, able to compare our results to a previous published result from six wild AWBV in the Moholoholo area undertaken in 1991. The study reported blood lead concentrations of  $7.81 \mu\text{g/dL}$  (Van Wyk et al., 2001). In the current study, a mean blood lead concentration of  $13.76 \pm 5.84 \mu\text{g/dL}$  in the same region was determined, together with an upper range of  $32 \mu\text{g/dL}$ , these are suggestive that a temporal change in exposure has resulted.

Another interesting comparison we were able to make was to compare the results with those from neighbouring Botswana. In a study by Kenny et al., (2015), the blood lead concentrations of AWBV were evaluated through the use of an electrochemical analyser. From this study they were able to demonstrate an overall low exposure in the area with 70% of the vulture population having blood lead concentrations below  $10 \mu\text{g/dL}$ , while only 2.3% had concentrations above  $45 \mu\text{g/dL}$ . This differed from the current study where we were able to demonstrate that blood lead concentrations above  $10 \mu\text{g/mL}$  occurred in more than 50% of the AWBV and 39% of the CGV indicating a clear difference in geographically exposure. One possible reason for this difference can be contributed to the larger hunting industry between the two countries, with South Africa having the larger industry (Lindsey et al., 2007).

## **5. Conclusion**

From this study we were able to demonstrate that both wild and captive vulture populations in South Africa are being exposed to higher than expected sources of lead. For the captive birds, this higher than expected exposure is most likely linked to the historic use of leaded

fuels in country and the country mining activities. For the wild birds, for which some birds had very high whole blood lead concentrations, we suspect that they may have been subjected to a further source of lead, of which leaded bullets would be a likely source. Also of concern was the finding that some birds have concentrations that could potentially interfere with reproduction, which is important if one considers that the species only produces one egg a season. Considering the endangered status of the various populations, attempts need to be put in place to reduce exposure of vulture species to lead. As a first step, research should be focused on finding the source. If leaded bullets are the source as we speculate for the wild birds, correction initiatives could include replacing bullets with non-lead alternatives; removing the bullet and tract of carcasses placed out for feeding; and perhaps even enforcing non-hunting in the areas where the birds breed. If a shortcoming of this study was to be identified, this would be the absence of stratification of results by age, which may differ due to the difference in feeding habits of subadult versus adult birds (Kane et al., 2016).

## **6. Acknowledgement**

This study was made possible by funding by Afgri via the WWF and the Ernst Oppenheimer Trust. Many thanks expressed to the organisation and people that made available samples for evaluation: KZN Ezemvelu, REST Namibia, Mankwe Game Reserve, Namibian Department of Wildlife and National Parks, the Biobank of the NZG, Kimberley Animal Hospital, Anne van Dyk Trust, and the Johannesburg Zoological Gardens. VulPro would like to acknowledge Rand Merchant Bank, who's supporting their conservation programme for several years.

## **7. References**

Alkmim Filho, J.F., Germano, A., Dibai, W.L.S., Vargas, E.A., Melo, M.M., 2014. Heavy metals investigation in bovine tissues in Brazil. *Food Sci. Technol.* 34, 110-115.

Avery, D., Watson, R.T., 2009. Regulation of lead-based ammunition around the world. Ingestion of lead from spent ammunition: Implications for wildlife and humans. *The Peregrine Fund. Idaho*, 161-168.

Brown, C., 1991. An investigation into the decline of the Bearded Vulture *Gypaetus barbatus* in southern Africa. *Biol. Conserv.* 57, 315-337.

Buekers, J., Redeker, E.S., Smolders, E., 2009. Lead toxicity to wildlife: derivation of a critical blood concentration for wildlife monitoring based on literature data. *Sci. Total Environ.* 407, 3431-3438.

- Dobrowolska, A., Melosik, M., 2008. Bullet-derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). *Euro. J. Wildlife Res.* 54, 231-235.
- Garcia-Fernandez, A., Martinez-Lopez, E., Romero, D., Maria-Mojica, P., Godino, A., Jimenez, P., 2005. High levels of blood lead in griffon vultures (*Gyps fulvus*) from Cazorla Natural Park (southern Spain). *Environ. Toxicol.* 20, 459-463.
- Hernández, M., Margalida, A., 2009. Assessing the risk of lead exposure for the conservation of the endangered Pyrenean bearded vulture (*Gypaetus barbatus*) population. *Environ. Res.* 109, 837-842.
- Hunt, W.G., Burnham, W., Parish, C.N., Burnham, K.K., Mutch, B.R.I.A.N., Oaks, J.L., 2006. Bullet fragments in deer remains: implications for lead exposure in avian scavengers. *Wildlife Soc. B.* 34, 167-170.
- IUCN, 2014. The *IUCN* Red List of Threatened Species. Version 2014.3. Accessed on the 07/04/2015.
- Kelly, T.R., Johnson, C.K., 2011. Lead exposure in free-flying turkey vultures is associated with big game hunting in California. *PLoS One.* 6, e15350.
- Kenny, D., Reading, R., Maude G., Hancock, P., Garbett, B., 2015. Blood lead levels in White-backed vultures (*Gyps africanus*) from Botswana, Africa. *Vulture News.* 68, 25-31.
- Kruger, S.C., 2014. An investigation into the decline of the bearded vulture: *Gypaetus barbatus* in Southern Africa (Doctoral dissertation, University of Cape Town). Available online: <http://hdl.handle.net/11427/13211>
- Lindsey, P., Roulet, P., Romanach, S., 2006. Economic and Conservation Significance of the Trophy Hunting Industry in Sub-Saharan Africa. *Biol. Conserv.* 134, 455-469.
- Margalida, A., Bertran, J., Boudet, J., 2005. Assessing the diet of nestling Bearded Vultures: a comparison between direct observation methods. *J. Field Ornithol.* 76, 40-45.
- Margalida, A., 2012. Baits, budget cuts: a deadly mix. *Science*, 338, 192-192.
- Margalida, A., Martina, C., José, A., Sánchez-Zapata, J.A., Donázar, J.A, 2012. Good news for European vultures. *Science.* 335, 284-284.
- Mundy, P.J., 1992. *The Vultures of Africa*. Acorn Books, South Africa.
- Naidoo, V., Wolter, K., Espie, I., Kotze, A., 2012. Lead toxicity: consequences and interventions in an intensively managed (*Gyps coprotheres*) vulture colony. *J. Zoo Wildlife Med.* 43, 573-578.
- Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M. and Mahmood, S., 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature.* 427, 630-633.
- Ogada, D.L., Keesing, F., Virani, M.Z., 2012. Dropping dead: causes and consequences of vulture population declines worldwide. *Ann. NY Acad. Sci.* 1249, 57-71.
- Osweiler, G.D., 1996. *Toxicology*, Williams and Wilkins, Philadelphia.

Thomas, V.M., Socolow, R.H., Fanelli, J.J., Spiro, T.G., 1999. Effects of reducing lead in gasoline: an analysis of the international experience. *Environ. Sci. Technol.* 33, 3942-3948.

von Schirnding, Y., Bradshaw, D., Fuggle, R., Stokol, M., 1991. Blood lead levels in South African inner-city children. *Environ. Health Persp.* 94, 125.

von Schirnding, Y., Mathee, A., Kibel, M., Robertson, P., Strauss, N., Blignaut, R., 2003. A study of pediatric blood lead levels in a lead mining area in South Africa. *Environ. Res.* 93, 259-63.

Kane, A, Wolter, K, Naser, W, Kotze, A, Naidoo, V, Monadjem, A, 2016, Home range and habitat selection of Cape Vultures *Gyps coprotheres* in relation to supplementary feeding, *Bird Study*, 2016, <http://dx.doi.org/10.1080/00063657.2016.1214105>

Table 1: Descriptive statistics of blood lead concentrations ( $\mu\text{g/dL}$ ) obtained from African white-backed Vultures (AWBV) and Cape Griffon vulture (CGV) sampled across South Africa.

Species	Location	Urbanization	Sample Size	Mean	Median	Mode	SD	Min	Max	95% confidence Interval	
										Lower	Upper
AWB	DeWildt*	Urban	6	19.67	19	20	7.94	10	34	13.31	26.02
	Dundee	Rural	23	16.00	10	8	16.25	4	84	9.36	22.64
	Etosha	Rural	11	5.27	6	6	1.56	3	8	4.35	6.19
	Kimberley	Urban	7	28.00	20	4	21.23	4	58	12.27	43.73
	Moholoholo	Rural	25	13.76	14	14	5.84	4	32	11.47	16.05
	Otjiwarango	Rural	24	18.17	16	10	9.90	6.	38	14.20	22.13
Cape	DeWildt*	Urban	21	11.14	10	8	4.54	4	22	9.20	13.09
	Joberg Zoo*	Urban	6	14.67	16	16	4.32	6	18	11.21	18.12
	Pietermaritzburg*	Urban	8	11.00	8	6	6.23	6	22	6.68	15.32
	Pooled Captive		35	11.71	12	6	4.97	4	22	10.07	13.36
	Dundee	Rural	1	6.00	6	6		6	6		
	Kimberley	Urban	1	46.00	46	46.		46	46		
	Krugersdorp	Urban	25	12.80	10	10	7.98	4	46	9.67	15.93
Pilanesberg	Rural	22	11.00	8	8	7.05	3	32	8.05	13.95	
Bearded	Dundee	Rural	5	< 3.00	< 3	< 3					

\*Represents the captive birds. Site Description: Towns were characterised as urban if the population in the region exceeded 50 000 in total. LOQ < 3  $\mu\text{g/dL}$ .



Fig 1: Map of areas sampled in southern Africa (Map Data: ©AfriGIS Google). A- Etosha National Park; B- Otjiwarongo; C- Pilanesberg National Park; D- Krugersdorp; E- De Wildt; F- National Zoological Gardens (Pretoria Zoo); G- Moholoholo; H- Johannesburg Zoological Gardens; I- Kimberley; J- Dundee; K- Pietermaritzburg. The smaller map on the right, indicates ranging movements of 10 adult Cape Griffon vultures tagged with GPS trackers, as an indication of site selection (Naidoo, unpublished observations).



Fig 2: Mean whole blood lead concentrations for vultures based on the area of sampling in South Africa. Results are presented independent of species and captive status of the birds.

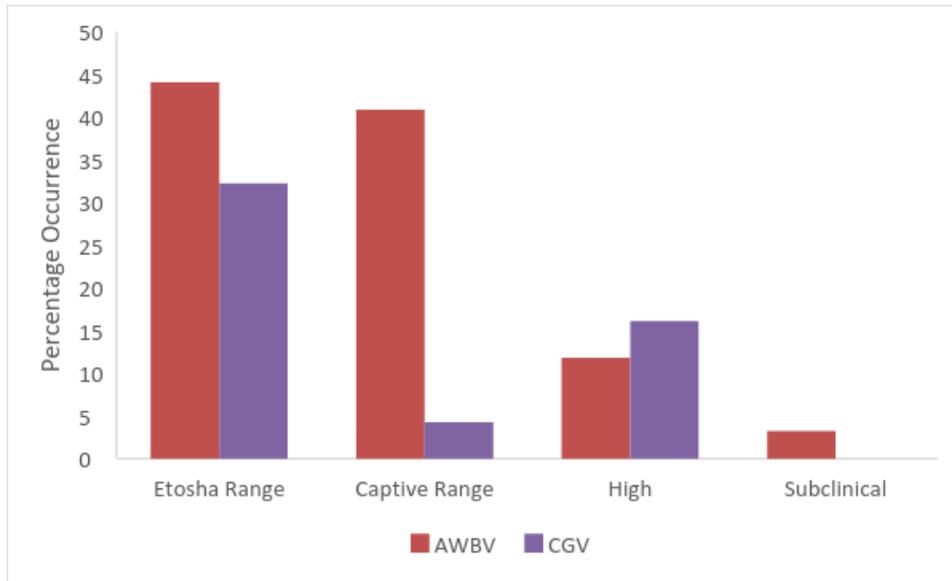


Figure 3: Percentage of birds within each category of whole blood lead concentration. The range for the Etosha bird was 3.80 to 6.20  $\mu\text{g}/\text{dL}$ . The range for the South African group of captive AWBV and CGV was 13.31 to 26.02  $\mu\text{g}/\text{dL}$  and 10.07 to 13.36  $\mu\text{g}/\text{dL}$  respectively. Subclinical was defined concentrations above 50  $\mu\text{g}/\text{dL}$ .

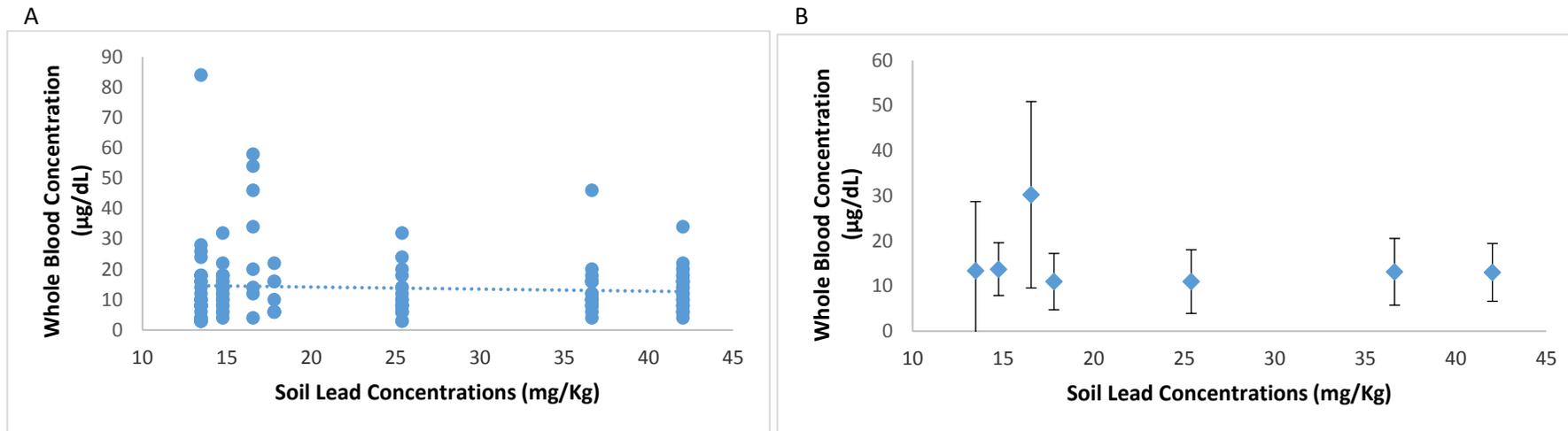


Figure 4: Relationship between average soil lead concentrations in a 50 km radius around each sampling site in comparison to the whole blood concentration at the specific sampling point by individuals (A) or average (mean  $\pm$  SD) per site (B).  $r^2 = 0.005$ . The points on the x-axis from left to right are Dundee, Hoedspruit, Kimberley, Pietermaritzburg, Pilanesberg, Pretoria and Johannesburg.

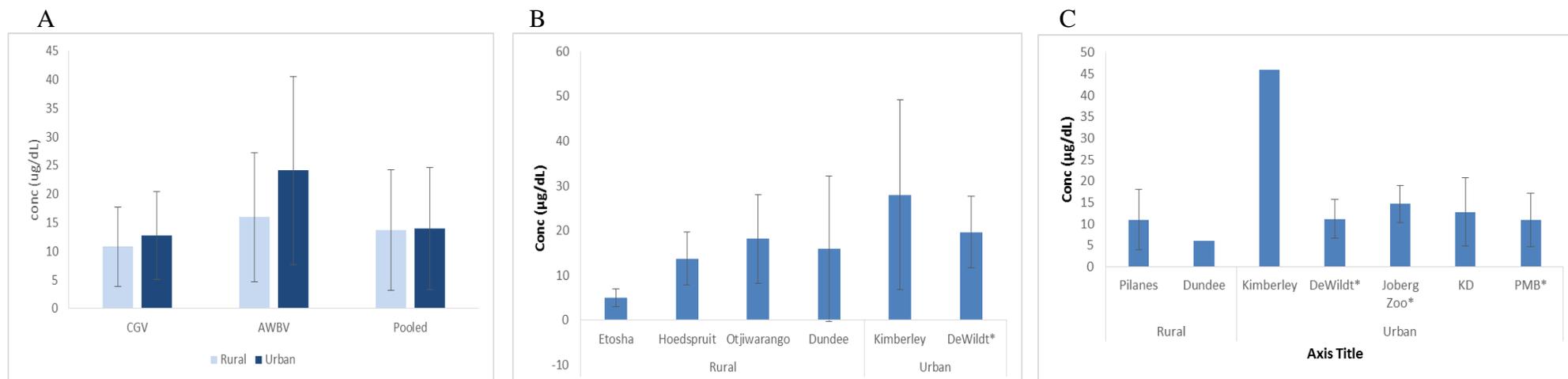


Figure 5: Whole blood lead concentration (mean  $\pm$  SD) proportioned by their rural or urban area pooled (A); by individual site for AWBV (B); and CGV (C). Asterisk indicates the captive locations. For the pooled results the Etosha results were omitted (Pilanes-Pilanesberg; KD-Krugersdorp; PMB-Pietermaritzberg)

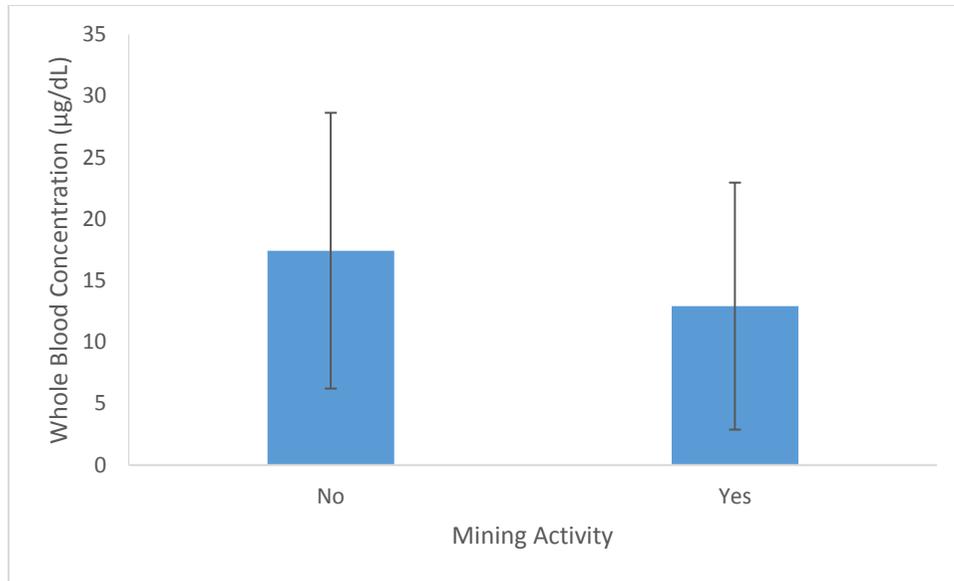


Fig 6: Relationship between mining activities in the sampling area in comparison to whole blood concentrations (mean±SD). The mining areas were Dundee, Pilanesberg, Pretoria and Johannesburg