SRAS Final Report

Experimental study investigating the effects of ingested lead (Pb) in captive Nile Crocodile (Crocodylus niloticus)

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Abstract

Lead (Pb) poisoning poses a significant threat to wildlife. While the effects of Pb poisoning in birds and mammals are well established, impacts on reptiles remain poorly documented and difficult to assess in the field. Elevated blood Pb (BPb) concentrations in Nile crocodiles (*Crocodylus niloticus*) from the Lake St. Lucia estuarine system in South Africa have been reported, leading to anemia and tooth loss. However, the long-term physiological effects of elevated BPb and Pb tissue distribution have not been investigated under controlled conditions, and this was the purpose of this study. Captive sub-adult Nile crocodiles were randomly assigned to one of three treatment groups and exposed to Pb through fishing weights, plus a control group. Blood and urine samples were collected at regular intervals, and growth and body condition were monitored. At the end of the experimental period, crocodiles were euthanised, and tissue samples were collected to quantify Pb distribution in the spleen, liver, kidney, brain, thyroid, gonads and bone. The spleen had the highest Pb concentrations, followed by bone, kidney, and liver, whereas the brain, gonads, and thyroid had the lowest. The results demonstrated a dose-dependent increase in BPb concentrations across all treatment groups, with some individuals exceeding 80,000 ng/ml. Elevated BPb concentrations were associated with reduced packed cell volumes, suggesting anemia, although no overt clinical signs of toxicity were observed. This study represents the longest Pb exposure experiment conducted on a reptile to date and revealed that sub-adult Nile crocodiles exhibit remarkable tolerance to exceptionally high Pb concentration, providing novel insights into crocodilian ecotoxicology.

Introduction

Crocodilians, which comprise crocodiles, alligators, caimans and gharials, are top predators and iconic megafauna in aquatic systems throughout the tropical and subtropical regions of the world (Combrink, 2014; Conley & Shilton, 2018). As keystone species and apex predators, crocodilians maintain balance within their environment by regulating prey populations (Ross, 1999). However, because of their extended life span and high trophic status, they are vulnerable to exposure and accumulation of environmental contaminants in their habitat, making them ideal bioindicators of ecosystem health and environmental imbalance (Almli et al., 2005; Rainwater et al., 2007; Lemaire et al., 2024).

The Nile crocodile (*Crocodylus niloticus*) is an important apex predator inhabiting a variety of waterbodies such as rivers, lakes, swamps, impounded wetlands (e.g., dam reservoirs), and estuaries throughout 26 countries in central, eastern, and southern Africa, as well as Madagascar (Isberg et al., 2019; Combrink et al., 2019). The species is currently listed as Least Concern on the IUCN Red List due to its widespread distribution in Africa; however, in South Africa, due to the depletion of wild populations and escalating threats, its conservation status is classified as Vulnerable. Major threats include hunting, illegal killings, habitat transformation, pollution, and water removal for agriculture and mining (Combrink, 2004; Botha et al., 2011; Combrink et al., 2019).

Heavy metals such as lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) are widespread environmental pollutants with profound ecological and health impacts (Jaishankar et al., 2014; Iheanacho et al., 2017). These metals originate from natural and anthropogenic sources, including industrial and agricultural pollution, as well as the use of Pb-based equipment in hunting and fishing activities. Wildlife exposure to Pb occurs primarily through oral ingestion, and elevated concentrations can lead to disorders of the nervous, gastrointestinal, circulatory and reproductive systems. Pb toxicity

is well documented in birds and mammals, but reptiles, particularly crocodilians, remain underrepresented in Pb ecotoxicology research (Warner et al., 2016; Humphries et al., 2022).

Recent studies have shown alarmingly high BPb concentrations in Nile crocodiles in the Lake St Lucia estuarine system, South Africa, with the highest BPb concentration (13,100 ng/ml) ever recorded in free-ranging vertebrates, with seemingly few obvious adverse effects (Warner et al., 2016; Humphries et al., 2022). Despite these findings, no study has ever been conducted to investigate the toxicokinetics of Pb accumulation in Nile crocodiles, including Pb accumulation in different organs, skeletal integrity, and the effects of Pb burdens on reproductive hormones. This controlled experimental study, therefore, addresses major knowledge gaps.

Methods

The study population consisted of 22 randomly selected sub-adult Nile crocodiles (all < 2.5 m total length at the start of the study) on a commercial crocodile farm (Croc Skin Traders, Limpopo Province, South Africa). All crocodiles were hatched and raised at the same breeding facility. Individuals were housed under similar conditions in single enclosures (2.4 m × 1.2 m), each with a waterbody for full submersion and a dry basking area. Crocodiles remained in the same enclosures throughout the experimental period and were monitored daily by facility staff for signs of Pb toxicity or other clinical issues.

The population was divided into three Pb treatment groups and a control group (Table 1). Lead dosing mimicked field exposure by administering Pb fishing weights packaged in week-old broiler chicken carcasses. Unconsumed chicken carcasses were retrieved, and the weights repackaged into new chicken carcasses and presented to the same individuals the following day, until all Pb weights had been ingested.

Blood Pb analysis and haematology

Blood samples (~10 mL) were collected from the post-occipital venous sinus using an 18-gauge hypodermic needle following the procedure described by Myburgh et al. (2014). Blood was sampled weekly for the first month and monthly thereafter. Approximately 6 mL were transferred into sterile lithium heparin tubes for Pb analysis. Approximately 0.5 g of whole blood was digested with 3 mL nitric acid (65% Suprapur, Sigma Aldrich) using a Multiwave GO Plus microwave digestion system and analysed for Pb by ICP-MS (Agilent 7700), following Humphries et al. (2022).

Packed cell volumes (PCV; haematocrit) were determined at every sampling using the microhaematocrit method. Heparinised capillary tubes were centrifuged at 12,000g for 3 minutes following Weiser (2022). Blood smears were prepared using the coverslip-to-slide technique described by Perpiñán et al. (2006) and examined at weeks 8 and 48 for signs of erythrocyte regeneration, including polychromasia, anisocytosis and basophilic stippling.

Table 1. Administered Pb dosages for experimental treatment and control groups*

Group	Number of Individuals	Pb dosage
Treatment 1	5	2 x 1 oz sinkers (~54 g)
Treatment 2	3	5 x 1 oz sinkers (~135 g)
Treatment 3	6	8 x 1 oz sinkers (~215 g)
Control	4	Zero

*The study initially involved 22 individuals. However, post-mortem examination showed that four of these individuals had ingested fewer Pb weights than expected for their assigned treatment groups, and therefore, they were excluded from the final analysis.

Urine lead (UPb)

Urine was collected approximately every alternate month using urinary catheterisation (FG 10; 3.3 mm × 500 mm) following Myburgh et al. (2012). Samples were transferred into 50 mL tubes and transported on ice to the laboratory, filtered (0.45 μm) to remove urates, and analysed directly by ICP-MS.

Pb sinker erosion

At week 48, crocodiles were humanely euthanised according to the South African National Standards 'stun and cut' method (SANS, 2009; Manolis and Webb, 2016). Crocodiles were electrically stunned for two to four seconds to render them unconscious and insensible, followed by severing of the spinal cord and pithing (Manolis and Webb, 2016). Lead sinkers were recovered from the stomachs and washed, dried, and reweighed. Erosion rate was calculated as the mass loss over 345 days.

Tissue bioaccumulation (post-mortem)

Liver, kidney, brain, thyroid, gonads, spleen and bone (humerus) were collected following euthanasia. Individuals were opened ventrally along the midline of the coelom to expose the internal organs. Samples were placed in individually labelled plastic bags and stored frozen until analysis. Tissue analysis was conducted on 18 crocodiles, although sample sizes varied among tissues: liver and kidney (n = 18 each), brain, spleen and gonads (n = 14 each), bone (n = 13), and thyroid (n = 12).

Soft tissues were freeze-dried, while bones were cleaned, sonicated in Millipore water and dried at 80 °C. Dried samples (0.1–0.2 g) were digested using 3 mL nitric acid (65% Suprapur, Sigma Aldrich) in a Multiwave GO Plus microwave digestion system. Pb concentrations were quantified by ICP-MS (Agilent 7700). Certified reference material NIST SRM 2976 and procedural blanks were used for analytical quality control. Results are reported on a dry weight (dw) basis.

Statistics

All statistical analyses were performed using R software (version 4.2.3; R Core Team, 2023). Normality was assessed using the Shapiro–Wilk test (Shapiro and Wilk, 1965). Depending on the distribution, either one-way ANOVA with Tukey HSD, paired t-tests, Kruskal–Wallis with Dunn's post hoc, or Wilcoxon signed-rank tests were applied. For tissue Pb concentrations, data were log-transformed where appropriate, and Holm and Bonferroni corrections were applied to non-parametric multiple comparisons. Statistical significance was set at $\alpha = 0.05$.

Results

BPb concentrations and haematological responses

BPb concentrations increased rapidly in Pb-exposed crocodiles following ingestion of Pb fishing weights and continued to rise throughout the 48-week study (Figure 1). Significant differences were observed between treatment groups at week 48 ($\chi^2 = 9.0$, $p = 0.011$), with treatments 2 and 3 showing significantly higher BPb concentrations than treatment 1 ($p = 0.022$ and $p = 0.0057$, respectively). The highest individual BPb concentration recorded was 82,800 ng/mL in treatment 3.

PCV decreased in Pb-exposed crocodiles, with significant differences between groups at weeks 8, 16, 24, 32 and 48 ($p < 0.05$). Treatment 3 had significantly lower PCV than controls at weeks 8 ($p = 0.005$), 16 ($p = 0.031$), 24 ($p = 0.040$) and 48 ($p = 0.026$). Blood smears also revealed increased polychromasia, enlarged erythrocytes and basophilic stippling in Pb-exposed crocodiles.

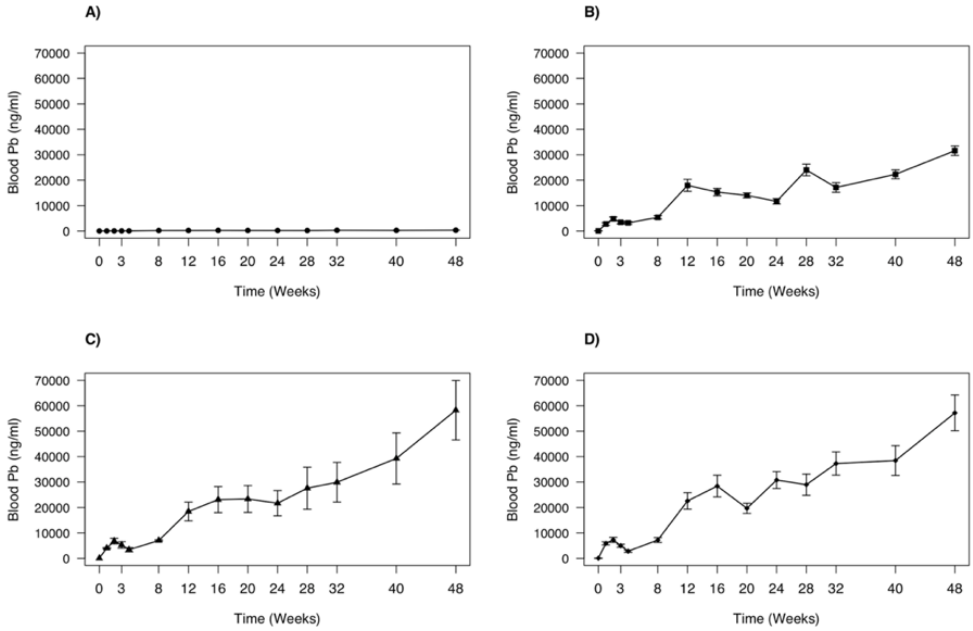


Figure 1. Mean blood Pb (BPb) concentrations (ng/ml) for **A** Control: no lead (Pb) exposure (n = 4); **B** Treatment 1: 2 × 1 oz sinkers (~ 54 g Pb, n=5); **C** Treatment 2: 5×1 oz sinkers (~135 g Pb, n=3); **D** Treatment 3: 8×1 oz sinkers (~215 g Pb, n=6) over the duration of the study. Data are presented as mean ± standard error of the mean (SEM).

Urinary Pb concentrations and sinker erosion

Urinary Pb concentrations increased significantly during the first two weeks following exposure ($p < 0.001$), with significant differences between treatment groups at weeks 2 ($p = 0.0299$) and 16 ($p = 0.0140$). Lead sinkers recovered after 48 weeks showed visible erosion, with significant differences in percentage mass loss among treatment groups ($p = 0.047$). Complete dissolution was estimated to take approximately 5.8–7.3 years.

Growth and body condition

None of the crocodiles displayed obvious clinical signs of Pb toxicity. However, body mass increased by 12.2% in controls compared to 7.5%, 3.5% and 2.6% in treatments 1, 2 and 3, respectively. Weight gain was significantly lower in treatment 3 than in controls ($p = 0.010$). Total length growth did not differ significantly between groups ($p = 0.53$), while snout–vent length ($p = 0.086$), tail girth ($p = 0.79$) and mid-body girth ($p = 0.324$) showed no significant overall changes. Neck girth growth was significantly lower in treatment 3 than in controls ($p = 0.011$). Body condition index (BCI = body mass [kg]/[total length (cm)]^3) increased significantly in controls between weeks 0 and 48 ($p = 0.005$), but not in Pb-exposed groups.

Tissue-specific Pb bioaccumulation

Pb concentrations differed significantly among treatments in the spleen ($p = 0.036$), bone, kidney and liver (all $p < 0.001$). Mean concentrations (mg/kg dw) for the control and Treatments 1–3 were 4.6 ± 0.4, 240.2 ± 51.9, 390.8 ± 144.4 and 368 ± 60.6 in the spleen; 2.1 ± 0.6, 141 ± 31.6, 235 ± 75.2 and 265 ± 59.0 in bone; 3.8 ± 2.5, 121 ± 14.3, 125 ± 23.9 and 173 ± 19.7 in the kidney; and 0.75 ± 0.08, 82.7 ± 3.7, 121 ± 13.8 and 131 ± 13.6 in the liver. All exposed groups differed significantly from controls in bone, kidney and liver, whereas only Treatment 3 differed from controls in the spleen ($p = 0.037$). In the liver, Treatment 3 also exceeded Treatment 1, while no significant differences occurred among exposed groups in bone (Figure 2).

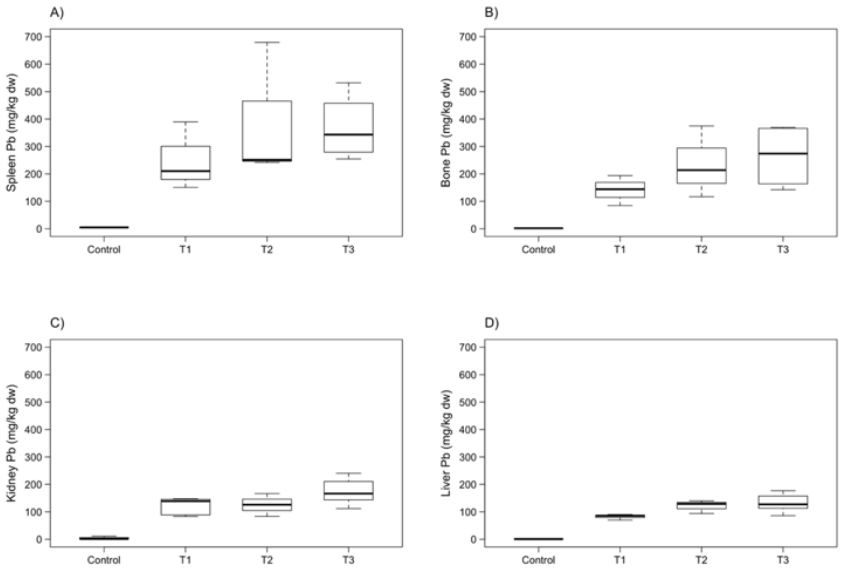


Figure 2. Boxplots showing Pb concentrations (mg/kg dw) measured in four organs, A) Spleen, B) Brain, C) Kidney, and D) Liver across the four exposure groups. Treatments included Control: no Pb exposure (n = 4); T1: 2 × 1 oz sinkers (~54 g Pb, n = 5); T2: 5 × 1 oz sinkers (~135 g Pb, n = 3); and T3: 8 × 1 oz sinkers (~215 g Pb, n = 6). Data are presented as individual values with median, interquartile range, and full range.

Pb concentrations differed significantly among treatments in the brain ($p < 0.001$), thyroid ($p = 0.036$) and gonads ($p = 0.029$) (Figure 3). Mean concentrations (mg/kg dw) for the control and Treatments 1–3 were 2.3 ± 1.0, 34.6 ± 5.5, 41.5 ± 7.7 and 51.2 ± 16.8 in the brain; 1.0 ± 0.5, 8.6 ± 2.8, 30.4 ± 13.7 and 16.1 ± 6.2 in the thyroid; and 0.63 ± 0.55, 19.2 ± 4.4, 12.2 ± 3.5 and 8.8 ± 1.9 in the gonads. All exposed groups had significantly higher brain Pb concentrations than controls, with no significant differences among exposed groups. For the thyroid, only Treatment 2 differed significantly from controls, while in the gonads, only Treatment 1 differed significantly from controls. No other pairwise comparisons were significant.

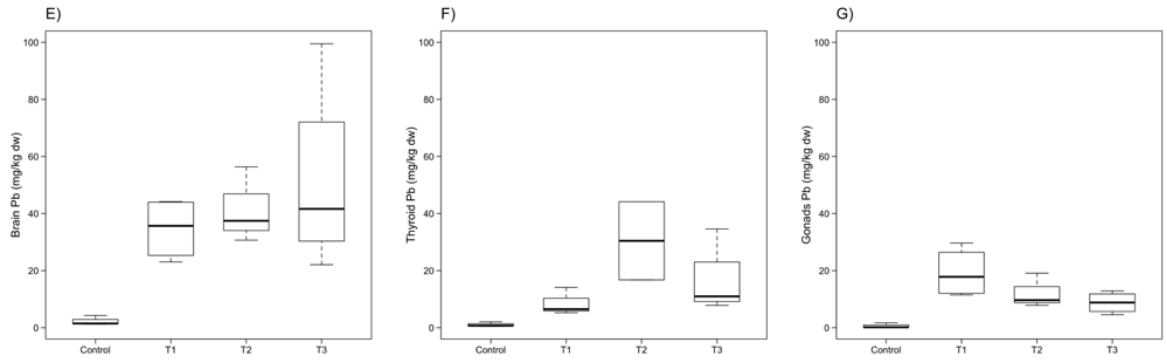


Figure 3. Boxplots showing Pb concentrations (mg/kg dw) measured in E) Brain, F) Thyroid, and G) Gonads across the four exposure groups. Treatments included Control: no Pb exposure (n = 4); T1: 2 × 1 oz sinkers (~54 g Pb, n = 5); T2: 5 × 1 oz sinkers (~135 g Pb, n = 3); and T3: 8 × 1 oz sinkers (~215 g Pb, n = 6). Data are presented as individual values with medians, interquartile ranges, and full ranges.

Discussion

This study represents the longest Pb dosage experiment ever performed on a crocodilian. Despite exposing crocodiles to large quantities of Pb (up to 215 g), none of the individuals died over the 48-week duration of the study or displayed any obvious clinical signs typically associated with Pb toxicity. In birds, BPb concentrations between 500 and 1000 ng/ml are associated with clinical poisoning (Pain 1996), whereas in mammals, BPb concentrations > 600–800 ng/ml are often associated with clinical signs of toxicity, including anemia, neurological impairment, and reproductive failure (Ma 1996). Our findings are consistent with recent studies on lizards (Blanchette et al. 2025; Moore et al. 2025), suggesting that reptiles are relatively tolerant to the acute effects of high concentrations of Pb. Although none of the crocodiles in this study displayed obvious signs of Pb poisoning over the study period, increases in BPb in our experimental animals were generally associated with lower PCVs. Lead has potent inhibitory effects on key enzymes integral in the production of heme, a molecular component of haemoglobin (Warren et al. 1998). Additionally, Pb disrupts cellular membrane integrity by generating reactive radicals and interfering with enzymes that maintain membrane stability (Wani et al. 2015), both of which lead to cellular damage and destruction. Blood smear analysis at weeks eight and 48 indicated a high level of RBC regeneration in Pb-exposed crocodiles. This suggests that increased RBC production may be compensating for the loss of RBCs due to destruction.

Out of the organs analysed, Pb concentrations were highest in the spleen. The extremely high concentrations observed in the spleen are likely due to its role in erythrocyte turnover. Findings from experimental Pb studies, such as increased red pulp-to-white pulp ratios (Huyen et al. 2023) and hemosiderosis and congestion (Ekanem et al. 2015) in the spleen, support this occurrence. Similarly, compensatory red blood cell clearance by the spleen has been documented in experimentally Pb-dosed mammals (Pain 1996; De Francisco et al., 2003). There may also have been some level of extramedullary erythropoiesis occurring in the spleen. While Davhana et al. (2025) showed that the dosed crocodiles in this study were mildly anaemic, they appeared proficient at regenerating erythrocytes. Given the spleen's important role in immune function, the high Pb concentrations in this organ may have important consequences for the animals' ability to fight infection.

Mineralised tissues, such as bone and teeth, are known to accumulate considerable amounts of Pb and together can account for approximately 95% of the body's total Pb burden (Cailto et al., 2017). In contrast, the present study found that bone accounted for only about 24% of the total Pb burden across tissues analysed, suggesting that a substantial portion of Pb was sequestered in soft tissues, particularly the spleen. In birds, bone Pb concentrations >20 mg/kg dw are associated with lethal toxicity (Mateo et al., 2003; Jenni et al., 2015). Although Pb stored in bone is considered less harmful and stored in an inert form, Pb can be released back into the bloodstream during egg laying, pregnancy, lactation, bone fractures, calcium deficiency, and physiological stress, exposing soft tissues, including the brain, kidneys, and liver (De Francisco *et al*., 2003; Caito *et al*., 2017). Humphries et al. (2022) documented anemia in Lake St Lucia crocodiles with BPb exceeding 6000 ng/ml. Despite much higher BPb concentrations in our study, PCVs did not decrease to the low levels reported in wild crocodiles at St Lucia. This discrepancy likely reflects differences in exposure duration. It is possible that a crocodile's ability to regenerate RBCs may decline with prolonged Pb exposure. While the underlying reasons are speculative, one possibility involves impacts on bone marrow. It is well known that Pb is stored in bone during chronic exposure, potentially increasing the likelihood that Pb affects RBC precursors in the bone marrow and leads to non-regenerative anemia. It has been suggested that heavy metal exposure may be associated with aplastic or non-regenerative anemias in humans (Jain et al. 2023). Additionally, many diseases affecting the kidney and liver have been associated with non-regenerative anemia (Divers 2000; Saggese 2009).

The liver and kidneys are known target organs for Pb. In treatment group 3, Pb concentrations reached 131 ± 13.6 mg/kg dw in the liver and 173 ± 19.7 mg/kg dw in the kidney. These concentrations are considerably greater than those previously reported in wild crocodilians (e.g., Swanepoel *et al.*, 2000; Burger *et al.,* 2000; Almli *et al.,* 2005; Xu *et al.,* 2006), including American alligators fed Pb-

contaminated nutria, which showed kidney Pb concentrations of 1.14 – 5.60 mg/kg dw and liver Pb concentrations of 0.37 – 0.46 mg/kg dw (Camus et al., 1998). These findings suggest that crocodiles may tolerate kidney and liver Pb concentrations that are typically lethal to mammals and birds, the effects of Pb on those organs in crocodiles remain poorly understood. Although the brain exhibited low Pb concentrations, these concentrations are still likely to be biologically significant. Pb can cross the blood-brain barrier (Sanders et al., 2009) and affect the central nervous system, particularly brain development. Notably, even low concentrations of Pb can have a substantial impact on the brain (Eisler, 1988). The results of this study show that urinary excretion of Pb is insignificant relative to the body burden. Based on the percentage reduction in the weight of the Pb sinker over the 48-week period, we estimated that it would take between 5.8 and 7.3 years for complete dissolution of Pb fishing weights in the stomach of the crocodiles used in this study. The suggestion by Humphries et al. (2022) that crocodiles at St Lucia could have been subjected to long-term Pb exposure due to sinkers retained in their stomachs for several years, or even decades, is supported by the erosion rates calculated in this study. It is evident that a longer-term study is necessary to fully understand the chronic effects of lead poisoning in crocodilians.

Conclusions

This study demonstrated that sub-adult Nile crocodiles can tolerate exceptionally high Pb exposure over 48 weeks without displaying obvious clinical signs of toxicity. However, increasing blood Pb concentrations were associated with lower packed cell volumes, suggesting mild anemia despite evidence of red blood cell regeneration. Pb accumulation was tissue-specific, with the highest concentrations in the spleen, followed by bone, kidney, and liver, and lower concentrations in the brain, thyroid, and gonads. Although crocodiles appeared relatively tolerant to Pb exposure, these findings raise concerns about potential long-term physiological, neurological and reproductive effects that require further investigation.

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References

Almli, B., Mwase, M., Sivertsen, T., Musonda, M.M. & Flåøyen, A., 2005. Hepatic and renal concentrations of 10 trace elements in crocodiles (Crocodylus niloticus) in the Kafue and Luangwa rivers in Zambia. Science of the Total Environment, 337(1–3), 75–82.

Apostoli, P., Kiss, P., Porru, S., Bonde, J.P. and Vanhoorne, M., 1998. Male reproductive toxicity of lead in animals and humans. ASCLEPIOS Study Group. Occupational and environmental medicine, 55(6), pp.364-374.

Blanchette A, Su KJ, Frick AJ, Karubian J, Gunderson AR (2025) Unprecedented lead tolerance in an urban lizard. Environ Res. https://doi.org/10.1016/j.envres.2025.122531

Botha, H., Van Hoven, W. & Guillette Jr, L.J., 2011. The decline of the Nile crocodile population in the Loskop Dam, Olifants River, South Africa. Water SA, 37(1).

Burger, J., Gochfeld, M., Rooney, A.A., Orlando, E.F., Woodward, A.R. & Guillette Jr, L.J., 2000. Metals and metalloids in tissues of American alligators in three Florida lakes. Archives of Environmental Contamination and Toxicology, 38, 501–508.

Caito, S., Lopes, A.C.B.A., Paoliello, M.M. & Aschner, M., 2017. Toxicology of lead and its damage to mammalian organs. Metal Ions in Life Sciences, 17, 9783110434330–016.

Camus, A.C., Mitchell, M.M., Williams, J.E. & Jowett, P.L., 1998. Elevated lead levels in farmed American alligators (Alligator mississippiensis) consuming nutria (Myocastor coypus) meat contaminated by lead bullets. Journal of the World Aquaculture Society, 29(3), 370–376.

Combrink, X. 2004. Population survey of Crocodylus niloticus (Nile crocodile) at Lake Sibaya. South Africa, M.Sc. Dissertation, University of KwaZulu-Natal. Pietermaritzburg.

Combrink, X. 2014. Spatial and reproductive ecology and population status of the Nile Crocodile (Crocodylus niloticus) in the Lake St Lucia estuarine. South Africa. Unpublished PhD thesis, University of KwaZulu-Natal. Pietermaritzburg, South Africa.

Combrink, X., Lippai, C. & Fergusson, R. (2019). Nile Crocodile Crocodylus niloticus. In Crocodiles. Status Survey and Conservation Action Plan. Fourth Edition, ed. by S.C. Manolis and C. Stevenson. Crocodile Specialist Group: Darwin. 28pp.

Conley, K.J. and Shilton, C.M., 2018. Crocodilia. In Pathology of Wildlife and Zoo Animals, Academic Press, pp.855–870.

Davhana, F., Humphries, M., Hunter, G., Seoraj-Pillai, N. & Combrink, X., 2025. Exposure of sub-adult Nile crocodiles (Crocodylus niloticus) to extreme lead concentrations: a 48-week experimental study with implications for wild populations. Archives of Environmental Contamination and Toxicology, 1–14.

De Francisco, N., Ruiz Troya, J.D. & Agüera, E.I., 2003. Lead and lead toxicity in domestic and free-living birds. Avian Pathology, 32(1), 3–13.

Divers SJ (2000) Reptilian renal and reproductive disease diagnosis. In: Fudge AM (ed) Laboratory medicine. Avian and Exotic Pets. WB. Saunders Company, Pennsylvania

Eisler, R., 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service, Washington, DC.

Ekanem, A.U., Kwari, H.D., Garba, S.H. and Salami, H.A., 2015. Effect of lead acetate on spleen and blood parameters in albino rats. IOSR J. Dent. Med. Sci. Ver. I, 14(3), pp.43-49.

Gandhi, J., Hernandez, R.J., Chen, A., Smith, N.L., Sheynkin, Y.R., Joshi, G. and Khan, S.A., 2017. Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. Zygote, 25(2), pp.103-110.

Humphries, M., Myburgh, J., Campbell, R. & Combrink, X., 2022. High lead exposure and clinical signs of toxicosis in wild Nile crocodiles (Crocodylus niloticus) from a World Heritage site: Lake St Lucia estuarine system, South Africa. Chemosphere, 303, 134977.

Iheanacho, E.U., Ndulaka, J.C. & Nuh, C.F., 2017. Environmental pollution and heavy metals. Environmental Pollution, 5(5), pp.2321–9122.

Isberg, S., Combrink, X., Lippai, C. & Balaguera-Reina, S.A. 2019. Crocodylus niloticus. The IUCN Red List of Threatened Species 2019: eT45433088A3010181.

Jain A, Wadhera S, Malhotra P, Singh C, Lad DP, Khadwal AR, Prakash G, Varma N, Reddy P (2023) Association between heavy metal exposure and idiopathic aplastic anemia in adolescents and adults: a case control study from Northern India. Blood 142(Suppl 1):7323

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. & Beeregowda, K.N., 2014. Toxicity, mechanism, and health effects of some heavy metals. Interdisciplinary Toxicology, 7(2), pp.60–72.

Jenni, L., Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H. & Jenny, D., 2015. The frequency distribution of lead concentration in feathers, blood, bone, kidney and liver of golden eagles (Aquila chrysaetos): insights into the modes of uptake. Journal of Ornithology, 156, 1095–1103.

Lemaire, J., Bustamante, P. & Shirley, M.H., 2024. Preliminary assessment of blood mercury contamination in four African crocodile species. Environment International, p.108877.

Ma WC (1996) Lead in mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife: interpreting tissue concentrations. CRC Press, Boca Raton, pp 281–296

Manolis SC, Webb GJW (2016) Best management practices for crocodilian farming, version 1. IUCN-SSC Crocodile Specialist Group, Darwin

Mateo, R., Beyer, W.N., Spann, J., Hoffman, D. & Ramis, A., 2003. Relationship between oxidative stress, pathology, and behavioral signs of lead poisoning in mallards. Journal of Toxicology and Environmental Health, Part A, 66(14), 1371–1389.

Moore MM, Foster EG, Amer A, Fraire L, Head A, Blanchette A, Hankison SJ, Gunderson AR, Gangloff EJ (2025) Urban wall lizards are resilient to high levels of blood lead. Environ Res 264:120248

Myburgh JG, Huchzermeyer FW, Soley JT, Booyse DG, Groenewald HB, Bekker LC, Guillette LJ Jr, Iguchi T (2012) Technique for the collection of clear urine from the Nile crocodile (Crocodylus niloticus). J S Afr Vet Assoc 83:1–6

Myburgh JG, Kirberger RM, Steyl JC, Soley JT, Booyse DG, Huchzermeyer FW, Guillette LJ Jr, Lowers RH (2014) The post-occipital spinal venous sinus of the Nile crocodile (Crocodylus niloticus): its anatomy and use for blood sample collection and intravenous infusions. J S Afr Vet Assoc 85:1–10

Pain, D.J., 1996. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H. & Redmon-Norwood, A.W. (eds), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Boca Raton, FL, 251–264.

Perpiñán D, Hernandez-Divers SM, McBride M, Hernandez-Divers SJ (2006) Comparison of Three Different Techniques to Produce Blood Smears from Green Iguanas, Iguana iguana. J Herpetol Med Sur 16(3):99–101

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rainwater TR., Wu TH., Finger A.G., Canas JE., Yu L., Reynolds KD., Coimbatore G., Barr B., Platt SG., Cobb GP., Anderson TA., & McMurry ST. 2007. Metals and organochlorine pesticides in caudal scutes of crocodiles from Belize and Costa Rica. Science of the Total Environment 373, pp.146–156.

Ross, J.P. 1999. Biological Basis for the Sustainable Use of Crocodilians. In: Fang, T., O. Montenegro & R. Bodmer (Eds.) Manejo y conservación de la fauna silvestre en América Latina: pp.275–280.

Saggese MD (2009) Clinical approach to the anemic reptile. J Exotic Pet Med 18:98–111

Sanders, T., Liu, Y., Buchner, V. & Tchounwou, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Reviews on Environmental Health, 24(1), 15–46.

SANS (South African Bureau of Standards Division), 2009. South African National Standard: Crocodiles in captivity. SABS Standards Division, Pretoria, South Africa. Available at: https://law.resource.org/pub/za/ibr/za.sans.631.2009.html

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52(3–4):591–611

Swanepoel, D., Kriek, N.P.J. & Boomker, J.D.F., 2000. Selected chemical parameters in the blood and metals in the organs of the Nile crocodile (Crocodylus niloticus) in the Kruger National Park.

Vallverdú-Coll, N., Mougeot, F., Ortiz-Santaliestra, M.E., Castaño, C., Santiago-Moreno, J. and Mateo, R., 2016. Effects of lead exposure on sperm quality and reproductive success in an avian model. Environmental science & technology, 50(22), pp.12484-12492.

Wani, A.L., Ara, A. & Usmani, J.A., 2015. Lead toxicity: a review. Interdisciplinary Toxicology, 8(2), 55–64.

Warner, J.K., Combrink, X., Myburgh, J.G. & Downs, C.T., 2016. Blood lead concentrations in free-ranging Nile crocodiles (Crocodylus niloticus) from South Africa. Ecotoxicology, 25, 950–958.

Warren MJ, Cooper JB, Wood SP, Shoolingin-Jordan PM (1998) Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. Trends Biochem Sci 23:217–221

Weiser G (2022) Laboratory technology for veterinary Medicine. In: Thrall MA, Weiser G, Allison RW, Campbell TW (eds) Veterinary hematology, clinical chemistry, and cytology. Wiley, Hoboken

Xu, Q., Fang, S., Wang, Z. & Wang, Z., 2006. Heavy metal distribution in tissues and eggs of Chinese alligator (Alligator sinensis). Archives of Environmental Contamination and Toxicology, 50, 580–586.