# Heavy metals in muscle tissue of healthy crocodiles from the Kruger National Park, South Africa

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## Introduction

Mass mortality of Nile crocodile *(Crocodylus niloticus*) occurred in the Olifants River of the Kruger National Park (KNP) in 2008 from pansteatitis, a condition in which body fat becomes yellow and inflamed. Much research has been carried out, but the cause(s) of the outbreak in the KNP remains unknown (Ashton, 2010; Ferreira & Pienaar, 2011; Woodborne *et al*., 2012; Bouwman *et al*., 2014). Anthropogenic factors such as heavy metal pollution have been suggested as a potential cause (e.g. Bouwman *et al*., 2014). We found only three studies from Africa on heavy metals in wild crocodiles (Table 1). The aim of this study was to assess the concentrations of heavy metals and metalloids in wild and apparently healthy crocodiles in the KNP.

## Materials and methods

We participated in a wider South African National Parks (SANParks) programme to assess the health of wild crocodiles in the KNP, with appropriate ethical clearance. Six major rivers run through the KNP (Smith, Van Zyl & Bouwman, 2000), each draining large catchments with different anthropogenic and natural sources of minerals and contaminants (Fig. 1a). In August 2010, we collected muscle samples from the tails of twelve crocodiles (CS1-CS12) from rivers representing seven catchments (Sabie, Olifants, Crocodile, Levuvhu, Shingwedzi, Nwaswitsontso and Letaba rivers). More about these crocodiles can be found in Osthoff *et al*. (2014).

**Table 1** Results (mg/kg dry mass) of heavy metal analyses of Nile crocodiles from various rivers in the Kruger National Park and comparable data from elsewhere in the world

| Species | Tisue | Year | Sample | Location | Al | Cu | Hg | Pb | Cr | Ni | Co | Cd | Zn | Se | As | Ti | Mn | Fe | V | Pt | Au | Ag | U | Sn | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nile crocodile (CS1) | Muscle | 2010 | Dry | Crocodile River, (KNP) South Africa | 19 | 0.31 | 1.5 | 0.15 | 0.09 | 0.024 | 0.0061 | 0.005 | 1.4 | 0.9 | 0.041 | 0.13 | 0.25 | 115 | 0.26 | 0.0094 | 0.084 | 0.17 | 0.025 | 0.038 | **This study** |
| Nile crocodile (CS2) | Muscle | 2010 | Dry | Crocodile River, (KNP) South Africa | 16 | 0.042 | 2.8 | 0.1 | 0.08 | 0.027 | 0.005 | 0.0079 | 0.92 | 1.2 | 0.042 | 0.13 | 0.14 | 301 | 0.13 | 0.0078 | 0.026 | 0.073 | 0.01 | 0.022 | **This study** |
| Nile crocodile (CS3) | Muscle | 2010 | Dry | Olifants River, (KNP) South Africa | 15 | 0.7 | 0.86 | 0.0089 | 0.094 | 0.027 | 0.0013 | 0.0033 | 1.4 | 0.46 | 0.033 | 0.12 | 0.17 | 295 | 0.054 | 0.0079 | 0.017 | 0.037 | 0.012 | 0.01 | **This study** |
| Nile crocodile (CS4) | Muscle | 2010 | Dry | Olifants River, (KNP) South Africa | 19 | 0.67 | 0.27 | 0.0082 | 0.14 | 0.04 | 0.01 | 0.016 | 5.8 | 0.69 | 0.049 | 0.3 | 0.25 | 335 | 0.081 | 0.0063 | 0.019 | 0.022 | 0.014 | 0.007 | **This study** |
| Nile crocodile (CS5) | Muscle | 2010 | Dry | Shingwedzi River, (KNP) South Africa | 33 | 0.95 | 1.5 | 0.074 | 0.11 | 0.035 | 0.01 | 0.0041 | 1.9 | 1.62 | 0.061 | 0.0035 | 0.45 | 150 | 0.53 | 0.0056 | 0.0068 | 0.015 | 0.13 | 0.0098 | **This study** |
| Nile crocodile (CS6) | Muscle | 2010 | Dry | Levuvhu River, (KNP) South Africa | 8.8 | 0.059 | 0.22 | 0.02 | 0.096 | 0.02 | 0.0007 | 0.0037 | 0.27 | 0.42 | 0.039 | 0.098 | 0.14 | 77 | 0.25 | 0.006 | 0.004 | 0.005 | 0.012 | 0.0037 | **This study** |
| Nile crocodile (CS7) | Muscle | 2010 | Dry | Levuvhu River, (KNP) South Africa | 1.7 | 0.014 | 0.022 | 0.006 | 0.052 | 0.012 | 0.0004 | <0.00007 | 0.29 | 0.04 | 0.029 | 0.036 | 0.032 | 18 | 0.052 | 0.00045 | 0.0004 | 0.0005 | 0.0053 | <0.00002 | **This study** |
| Nile crocodile (CS8) | Muscle | 2010 | Dry | Nwaswitsontso River, (KNP) South Africa | 22 | 1.5 | 0.14 | 0.088 | 0.06 | 0.033 | 0.0002 | 0.0016 | 1.8 | 0.57 | 0.037 | 0.25 | 0.35 | 305 | 0.57 | 0.0048 | 0.0063 | 0.01 | 0.0068 | 0.0064 | **This study** |
| Nile crocodile (CS9) | Muscle | 2010 | Dry | Nwaswitsontso River, (KNP) South Africa | 31 | 2.8 | 0.21 | 0.13 | 0.091 | 0.028 | 0.0023 | 0.0026 | 3.7 | 0.99 | 0.06 | 0.21 | 0.33 | 301 | 0.18 | 0.0079 | 0.0078 | 0.02 | 0.055 | 0.0086 | **This study** |
| Nile crocodile (CS10) | Muscle | 2010 | Dry | Letaba River, (KNP) South Africa | 28 | 1.3 | 1.4 | 0.048 | 0.069 | 0.035 | 0.0031 | 0.0071 | 2.1 | 1.3 | 0.043 | 0.0018 | 0.42 | 175 | 0.54 | 0.0039 | 0.005 | 0.01 | 0.0083 | 0.0067 | **This study** |
| Nile crocodile (CS11) | Muscle | 2010 | Dry | Letaba River, (KNP) South Africa | 26 | 1.4 | 2.4 | 0.43 | 0.074 | 0.027 | 0.0006 | 0.0094 | 4.7 | 1.7 | 0.11 | 0.0021 | 0.19 | 523 | 0.39 | 0.0033 | 0.019 | 0.0073 | 0.033 | 0.0054 | **This study** |
| Nile crocodile (CS12) | Muscle | 2010 | Dry | Sabie River, (KNP) South Africa | 16 | 0.27 | 0.22 | 0.063 | 0.075 | 0.023 | 0.0014 | 0.0022 | 2.1 | 0.49 | 0.038 | 0.12 | 0.26 | 261 | 0.26 | 0.0057 | 0.016 | 0.013 | 0.014 | 0.006 | **This study** |
| Nile crocodile | Muscle | 2000 | Wet | Crocodile farm, South Africa | 7 | | | | | | | | 11 | | | | | 3 | | | | | | | Hoffman, Fisher & Sales, 2000; |
| Nile crocodile | Muscle | 2000 | Dry | Olifants River, (KNP) South Africa | 147.2 | 10.5 | | 20.3 | 9.8 | 10.3 | | | 39.4 | | | | 0.1 | 399.6 | | | | | | | Swanepoel, Boomker & Kriek, 2000; |
| Nile crocodile | Muscle | 2000 | Dry | Sabie River, (KNP) South Africa | 73.5 | 12.6 | | 0 | 18.4 | 9.1 | | | 44.7 | | | | 0.1 | 615.4 | | | | | | | Swanepoel, Boomker & Kriek, 2000; |
| Nile crocodile | Muscle | 2000 | Dry | Silvervis Dam, (KNP) South Africa | 376.8 | 7.9 | | 3.7 | 90.5 | 24.9 | | | 109.7 | | | | 17.8 | 156 | | | | | | | Swanepoel, Boomker & Kriek, 2000; |
| Nile crocodile | Liver | 2004 | Wet | Kafue River, Zambia | | 5.7 | 3.5 | 8.7 | | | 0.02 | 0.04 | 18 | 1.8 | 0.008 | | 1.4 | | | | | | | | Almli *et al.*, 2005; |
| Nile crocodile | Liver | 2004 | Wet | Luangwa River, Zambia | | 4 | 3.7 | 3.3 | | | 0.05 | 0.04 | 31 | 2.3 | 0.049 | | 1.1 | | | | | | | | Almli *et al.*, 2005; |
| Alligator | Muscle | 2011 | Dry | Louisiana, USA | | 8.8 | | | | | | | 52.8 | | | | | 41.9 | | | | | | | Guillory *et al.*, 2011; |
| Chinese alligator | Muscle | 2006 | Dry | Anhui Province, China | | 6.4 | 0.193 | 0.73 | 0.155 | | | 0.155 | 120.97 | | 0.306 | | 2.68 | 67 | | | | | | | Xu *et al.*, 2006; |

| Species | Tisue | Year | Sample | Location | Al | Cu | Hg | Pb | Cr | Ni | Co | Cd | Zn | Se | As | Ti | Mn | Fe | V | Pt | Au | Ag | U | Sn | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Estuarine crocodile | Muscle | 2011 | Dry | Alligator Rivers Region, Australia | 89.9 | 1.14 |  | 0.308 | 0.413 | 0.507 |  |  | 81.4 | 0.993 |  | 6.22 | 0.706 | 88.7c |  |  |  |  |  |  | Jeffree, Markich & Twining, 2011; |
| Alligator | Muscle | 1985 | Dry | Lake Rodman, Florida USA |  | 0.28 | 0.51 | 0.07 | 0.05 |  |  | 0.02 | 19 |  |  |  |  | 7.42 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Muscle | 1985 | Dry | Lake Hancock, Florida USA |  | 0.34 | 0.1 | 0.1 | 0.05 |  |  | 0.01 | 25.5 |  |  |  |  | 8.45 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Muscle | 1985 | Dry | Lake Orange, Florida USA |  | 0.39 | 0.37 | 0.04 | 0.05 |  |  | 0.06 | 27.88 |  |  |  |  | 17.7 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Muscle | 1985 | Dry | Lake Newnans, Florida USA |  | 0.41 | 0.27 | 0.12 | 0.05 |  |  | 0.02 | 36 |  |  |  |  | 15.38 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Muscle | 1985 | Dry | Lake Apopka, Florida USA |  | 0.4 | 0.11 | 0.07 | 0.03 |  |  | 0.03 | 14.2 |  |  |  |  | 4.56 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Muscle | 1985 | Dry | Lake George, Florida USA |  | 6.03 | 0.04 | 0.09 | 0.06 |  |  | 0.03 | 2.17 |  |  |  |  | 22.76 |  |  |  |  |  |  | Delany, Bell & Sundlof, 1988; |
| Alligator | Liver | 2014 | Dry | Lake Apopka, Florida USA | 13 | 25.2 | 1.76 | 2 | 0.261 |  | 0.0285 | 0.0869 | 84.5 | 5.46 | 0.111 | 0.0022 | 2.38 | 1770 | 0.672 |  |  |  |  | 0.0661 | Horai *et al.*, 2014; |
| Alligator | Liver | 2014 | Dry | Lake Woodruff, Florida USA | 18.6 | 26.7 | 7.77 | 0.349 | 0.385 |  | 0.0334 | 0.0955 | 86.5 | 11.2 | 0.122 | 0.00221 | 2.97 | 3690 | 0.385 |  |  |  |  | 0.0609 | Horai *et al.*, 2014 |

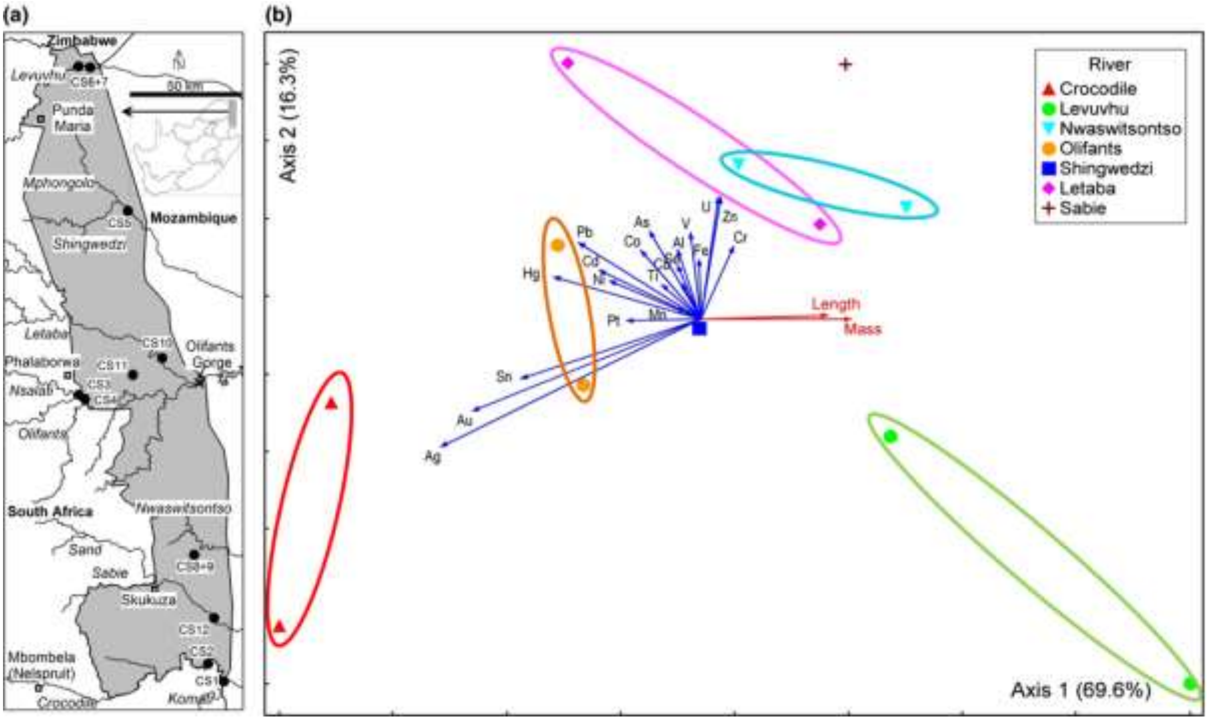


**Figure 1.** (a) Crocodile sampling sites within the borders of the Kruger National Park. (b) Nonmetric multidimensional scaling of relativized concentrations of twenty metals and metalloids in Nile crocodile muscle tissue. Ellipses indicate different rivers.

Muscle samples were stored at −20°C. Twenty metals and metalloids were analysed at North-West University using the revised EPA 3050B method with ICP-AES, using 2 g of dried sample in a 50 ml mixture of $HNO_3$, $H_2O_2$, HCl and deionized water. We only used multivariate analyses due to small sample numbers. Nonmetric multidimensional scaling (NMS) was performed on data relativized per sample as compositional fingerprint, with crocodile mass and length used as descriptive variables.

## Results and Discussion

CS2 from the Crocodile River had the highest concentration of Hg; CS4 from the Olifants River had the highest concentrations of Cr, Cd, Zn and Ti; CS1 from the Crocodile River had the highest concentrations of Pt, Au and Ag; and CS12 from the Sabie River had the highest Cu concentration (Table 1). The higher concentrations are also reflected in Fig. 1b, where the most of the respective longer vectors associated with the Olifants and Crocodile rivers. Gold and silver are often mined together and have a strong association with Crocodile River crocodiles, while Pb, Cd, Ni, Hg and Mn were distinctly associated with Olifants River crocodiles. The compositional fingerprints from the Letaba and Shingwedzi rivers were dominated by U, Zn and Cr. (Table 1), and they ordinated away from the other rivers (Fig. 1b). Despite the few samples, the distinction in compositional fingerprints based on metals and metalloids may be due to mining and other anthropogenic activities in the catchments of the different rivers. The Olifants River catchment has activities such as coal, platinum, chrome, vanadium, iron and copper, and water quality seems to be under severe pressure (Ashton, 2010). The Crocodile River catchment has large irrigation and forestry schemes, as well as wood and paper mills. However, very little is known about pollution in this river. Very little is also known about the pollution status of the Sabie, Letaba, Nwaswitssontso and Shingwedzi catchments, but an association of the first four with U, Cr and Zn is of concern. Most of the Nwatswistsontso and Shingwedzi catchments are protected and within the KNP borders. The concentrations therefore most likely reflect geological background. Luvuvhu River crocodiles had the lowest heavy metal concentrations (especially Fe), probably due to the lack of any major mining or industry in the region. The interpretation of the pollution differences above should be cautioned by the fact that crocodiles are mobile – however, the large distances between the rivers probably reduce the likelihood of relocation of individuals between the major catchments.

One would expect that if metals and metalloids played a role in the deaths of the crocodiles, the pollution would be systematic of the catchment; therefore much higher in the Olifants River and therefore also in its crocodiles. By comparing concentrations within the KNP and with data from elsewhere (Table 1), it can be deduced that this was not the case – the concentrations from the Olifants River crocodiles were within the same orders of magnitude than samples from the KNP or elsewhere with no pansteatitis. Provisionally, we conclude that the metals and metalloids we measured is an unlikely primary cause of pansteatitis.

Longer and heavier crocodiles had lower concentrations of metals in their muscle tissue than smaller crocodiles (Fig 1b). This can be the result of dilution due to growth, depuration with age and/or a larger component of terrestrial prey that may have lower concentrations of metals than aquatic prey (Cott, 1961; Hutton, 1987).

The crocodiles we sampled in 2010 all had relatively lower concentrations than those sampled in 2000 (Table 1). The samples from 2000 had some of the highest concentrations measured anywhere in the world, so it seems that contamination levels have declined markedly for most metals and metalloids over a period of 10 years. However, Hg had the highest concentrations in crocodile muscle tissue sampled in 2010 from anywhere in the world and is a matter of concern.

## Conclusions

The crocodile samples by and large had distinctive and catchment-specific heavy metal fingerprints. These fingerprints are likely a combination of anthropogenic contamination, underlying geology, the mobility of crocodiles and a greater dependence of larger crocodiles on terrestrial herbivores rather than fish. The concentrations seem to have declined with time, but relatively high concentrations in the Olifants and Crocodile rivers and mercury in general are of concern. Overall, the heavy metal concentrations in crocodile muscle from the Olifants River are in the same orders of magnitude compared with other KNP crocodiles or crocodiles elsewhere in the world. Heavy metals therefore are unlikely to have been a primary cause of pansteatitis.

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