Selected chemical parameters in the blood and metals in the organs of the Nile crocodile, *Crocodylus Niloticus*, in the Kruger National Park

D. SWANEPOEL[1], J. BOOMKER[2] and N.P.J. KRIEK[2]

ABSTRACT

SWANEPOEL, D., BOOMKER, J, & KRIEK, N.P.J. 2000. Selected chemical parameters in the blood and metals in the organs of the Nile crocodile, *Crocodylus Niloticus*, in the Kruger National Park. *Onderstepoort Journal of Veterinary Research*, 67:141–148

Healthy and sick crocodiles of varying sizes were examined from the Olifants River in the central part of the Kruger National Park, the Sabi River in the southern part and the Shingwedzi River in the northern region. Blood was collected for the determination of certain parameters and samples of fat, muscle, kidney and liver tissue were collected and analyzed for their heavy metal content. The results of the blood analyses are within the range recorded in the literature, but the metal analyses were inconclusive as similar data are not available for comparison. The results of the metal analyses are presented here for use as baseline and reference data.

Keywords: Blood, crocodile, *Crocodylus Niloticus*, Kruger National Park, metals

INTRODUCTION

The numbers of crocodiles, *Crocodylus niloticus*, in especially the Olifants and Letaba Rivers in the Kruger National Park (KNP) seemed to be declining over a number of years. It was postulated that pollution with heavy metals, especially copper and iron, could be a possible cause. Gummow, Botha, Basson & Bastianello (1991) reported accidental copper poisoning of antelope grazing in the KNP near Phalaborwa, and considerable magnetite deposits in the Olifants River (F. Venter, personal communication 1998).

A number of studies on the bioaccumulation of heavy metals in the freshwater fishes *Clarias gariepinus, Barbus marequensis* and *Oreochromis mossambicus* in the Olifants River have indicated that these metals are present in relatively high concentrations which fluctuate seasonally (Van der Merwe, Van Vuren & Du Preez 1993; Seymore, Du Preez & Van Vuren 1995; Robinson & Avenant-Oldewage 1997; Marx & Avenant-Oldewage 1998). As especially *C. gariepinus* is a major food item of larger crocodiles and *O. mossambicus* of smaller ones, it seemed plausible that some of these heavy metals could be a contributing factor to the decline in numbers of crocodiles in both rivers.

During 1996, some six, apparently sick, crocodiles were observed in the river. Typical signs included an emaciated appearance, with protruding scapulae and spinal columns, tails which flopped to one side, and little inclination to move. All the reptiles were stationary to the extent that they could be approached to within a few meters before they fled. No external injuries could be seen on any except one. Their teeth seemed in good condition. All died within 3 weeks of being seen, but the carcasses could not be recovered as they were devoured by healthy crocodiles. The appearance of sick crocodiles stopped as abruptly as it had started and it was only some 18 months later that another observation of a sick animal was made.

[1] Kruger National Park, Private Bag X 402, Skukuza, 1350 South Africa

[2] Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa

Accepted for publication 5 May 2000—Editor

Limited ranges for biochemical and haematological parameters exist for Nile crocodiles. Millan, Janmaat, Richardson, Chambers & Fomiatti (1997) provided some biochemical and haematological data for *Crocodylus porosus* yearlings, and quoted the findings of Foggin (1987), Watson (1990) and Stein (1996) for some values of Nile crocodiles. Since the opportunity to examine sick and healthy crocodiles arose, analyses of selected metals in various tissues were performed and some haematological parameters determined.

MATERIALS AND METHODS

Crocodiles were collected at several localities within, and one outside, the KNP. The collection data are presented in Fig. 1 and Table 1.

The Olifants River originates on the highveld of Mpumalanga, near the coal-mining town of Witbank. On its way to the Indian Ocean, it flows through areas of intense agricultural activity and passes the town of Phalaborwa, which is on the western boundary of the Kruger National Park (KNP), and where phosphates, copper and iron are mined. The river flows for approximately 108 km through the KNP before entering Mozambique to the east. Except for a weir on the western boundary there are no man-made structures in the river in the KNP that could hinder the free flow of water.

The Phalaborwa Mining Company (PMC) has several sedimentation dams in which water is purified before being re-used or released into the Selati River. The latter is one of the tributaries that join the Olifants River shortly before it enters the KNP. A number of crocodiles have migrated from the Olifants River into the Selati River and into these sedimentation dams where they now permanently reside.

The Letaba River originates on the eastern escarpment, flows through the Park from west to east and joins the Olifants River approximately 7 km before the latter enters Mozambique.

The Sabi River also arises in the mountains of the Mpumalanga escarpment and is one of the two major perennial rivers in the southern part of the KNP. Similar to the Olifants River, it flows through an area of intense agricultural activity before entering the KNP in the east. Several man-made structures occur in this river, which flows south-east to join the Incomati River shortly before the latter enters the Indian Ocean at Maputo in Mozambique.

The Shingwedzi River in the north of the KNP is a typical seasonal Lowveld river that has a limited catchment area outside the KNP. It is fed by several large tributaries, and flows past the Shingwedzi Rest Camp. Two dams, the Silwervis and the Kanniedood Dams, have been built in the river.

Crocodiles were collected on several occasions in the KNP, as is indicated in Table 1. The crocodiles from the Olifants River were shot where they were found; those from the Sabi River and one of the PMC dams were trapped in a baited cage; and those from the Silwervis Dam in a baited pen. All the trapped crocodiles were immobilized by intramuscular injection of gallamine triethiodide (Flaxedil™, 40 mg/mℓ) by means of a pole syringe. Once the drug had taken effect, they were transported to a field laboratory, where they were killed by a single shot through the brain.

Blood specimens were collected in the field from the hearts of those crocodiles shot in the Olifants River, and directly from the bullet wounds of those trapped

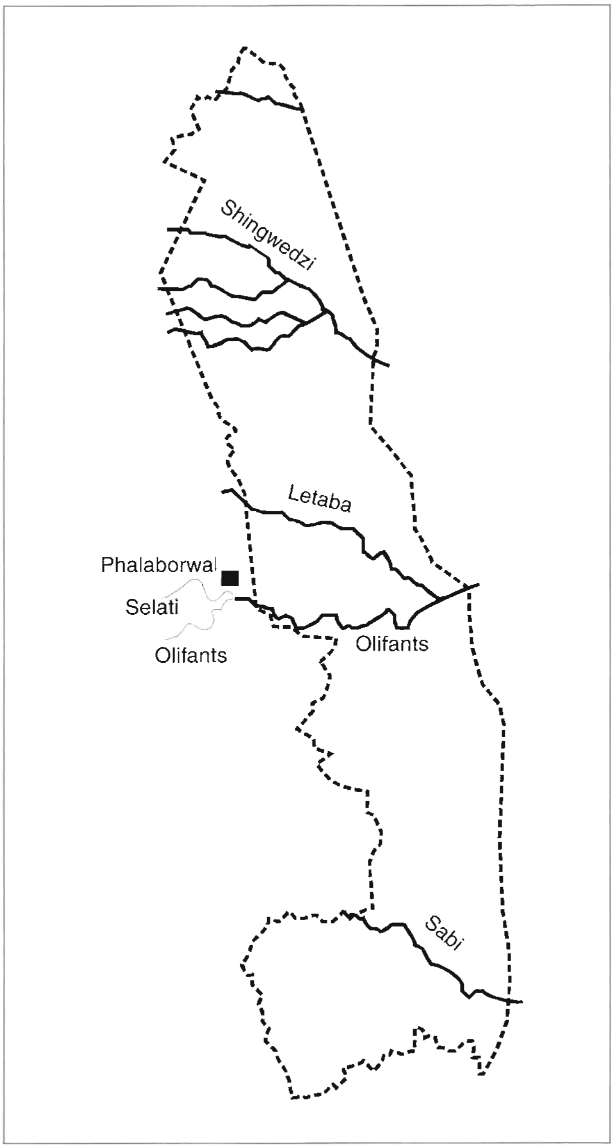

FIG. 1 Map of the Kruger National Park indicating the localities from which the crocodiles were collected

TABLE 1 Collection data of Nile crocodiles from the Kruger National Park and the Phalaborwa Mining Company Game Reserve

Crocodile number	Locality	Date	Sex	Length (m)	Condition
1	Silwervis Dam (S; E)	10-6-1998	F	2,76	Good
2	Silwervis Dam	10-6-1998	M	2,41	Good
3	Silwervis Dam	10-6-1998	F	3,22	Good
4	Silwervis Dam	10-6-1998	F	2,69	Good
A	Olifants River (S; E)	20-7-1999	M	3,5	Poor, wounded
1	Olifants River	23-9-1997	F	2,10	Good
2	Olifants River	23-9-1997	M	2,60	Good
3	Olifants River	24-9-1997	M	3,50	Good
5	Olifants River	25-9-1997	F	1,40	Good
6	Olifants River	25-9-1997	M	4,15	Good
7	Olifants River	16-3-1999	M	3,80	Emaciated
PMC 1	Sedimentation dam	6-4-1999	M	3,86	Emaciated
PMC 2	Sedimentation dam	16-6-1999	F	2,57	Very good
1	Sabie River	26-9-1997	M	2,41	Good
2	Sabie River	27-9-1997	M	3,91	Good

in the Sabi River and Silwervis Dam. Blood was collected into plain glass tubes (10 mℓ capacity) for the assay of routine haematological parameters, and into glass tubes containing a sodium fluoride—potassium oxalate mixture (5 mℓ capacity) for the determination of glucose. These were kept on ice for variable periods but not exceeding 4 h and transported back to the laboratory, where they were centrifuged at 3000 *g* for 10 min. The supernatants were carefully pipetted off and transferred to clean glass tubes without any additions, where after they were frozen at –4 °C.

Total serum protein, albumin, chloride, urea, creatinine, calcium, sodium, potassium and inorganic phosphate values were determined in the serum of the blood samples collected as such, and plasma glucose in those samples collected in the tubes containing the fluoride/oxalate mixture. All were analyzed on a Technicon RA-1000 analyzer (Miles Inc. Diagnostic Division) using Technicon reagents™. The total protein was determined using the Technicon method no. SM4-0147D91 modification for the RA-1000 of the method described by Skeggs & Hochstrasser (1964); albumin by the Technicon method no. SM4-0131D91, a modification for the RA-1000 of the method described by Doumas, Watson & Biggs (1971); urea by the Technicon method no. SM4-0150D91, a modification for the RA-1000 of the technique described by Tiffany, Jansen, Burtis, Overton & Scott (1972); creatinine by the Technicon method no. SM4-0141D91, a kinetic modification for the RA-1000 of the method described by Rossignol, Rossignol & PetitClerk (1984); total calcium by the Boehringer Mannheim method no. 1 489 216, modified for the RA-1000 based on the methods described by Gitelman (1967) and Gosling (1986); inorganic phosphate by the Technicon method no. SM4-0144D91, a modification for the RA-1000 of the method described by Amador & Urban (1972) and chloride by the Technicon method no. SM4-0162D91, a modification of the method described by Schoenfeld & Lewellen (1964). Sodium and potassium were measured by the NOVA Biomedical Electrolyte Analyzer I, using sodium and potassium selective electrodes. Modification of the NOVA method is based on the description of Eisenmann (1967). Globulin values were calculated from total protein and albumin values, as was the albumin/globulin ratio.

All the crocodiles were necropsied and double sets of specimens of liver, muscle, and kidney tissue collected for mineral analysis. Each tissue specimen weighed approximately 100 g and was placed individually into a clean bottle. The specimens in one set of tissues were preserved in 10 % neutral buffered formalin, and those of the other set was kept on ice until they could be frozen at the laboratory. In those crocodiles where fat was present, approximately 50 g of it was collected. In addition, a 50 mm section of the seventh rib of crocodiles 2, 3 and 4 from the Olifants River was collected, cleaned of all adherent muscle and connective tissue, and periostium, and preserved in 70 % ethyl alcohol.

The liver, muscle and kidney samples preserved in formalin were analyzed by means of the wet ashing technique described by Gorsuch (1970). Briefly, they were minced and dried overnight at 105 °C, after which they were ashed using nitric acid and a mixture consisting of one part sulphuric acid and seven parts perchloric acid. The samples were heated and once decomposition commenced, nitric acid was added until they were fully reduced. After ashing, all minerals except phosphorus were analyzed by atomic absorption spectrophotometric method (Cunnif 1995) Phosphorus was measured colorimetrically using the South African Bureau of Standards' specification 516 of 1957.

The frozen tissues including fat were thawed and the wet mass determined. They were then dried in an oven at 60 °C for 24 h, cooled down in a dessicator and the dry mass determined. One gram of each dried sample was placed in an Erlenmeyer flask and a mixture consisting of one part concentrated perchloric acid and two parts nitric acid, added (Van Loon 1980; Houba, Remacle, Dubois & Thorez 1983). Acid digestion was performed at temperatures ranging from 200–250 °C until the samples were clear. After digestion, each sample was separately filtered, using acid resistant 0,45 µm filter paper. The volumes of the samples were increased to 50 mℓ with distilled water and metals and minerals determined by atomic absorption spectrophotometry. Glassware that was used for the analyses of these samples was soaked in a 2 % Contrad soap solution (Merck Chemicals) for 24 h and then rinsed twice with distilled water. Thereafter it was placed in a 1M HCl solution for 24 h and again rinsed twice in distilled water to remove all traces of metals (Giesy & Wiener 1977).

The ribs were analyzed for calcium, phosphorus and magnesium using the techniques advocated by the American Organization of Analytical Chemists (Cunnif 1995), and fluoride using the method described by Singer & Armstrong (1968).

Statistical analyses were done with the computer software StatGraphics™, Jandel Sigmastats™ and QuatroPro™.

RESULTS

The results of the blood analyses of the crocodiles are listed in Table 2 and those, previously recorded by Leslie (1997), in Table 3. The metals present in the frozen tissues and their concentrations are listed in Table 4 and those in the formalin-fixed tissues in Table 5. In Table 6, the metals are ranked according to their mean concentration in each of the tissues from crocodiles from each locality.

The mean values of the minerals tested for in the ribs of the three crocodiles from the Olifants River were:

Calcium	17,87	± 0,31 %
Phosphorous	7,89	± 1,08 %
Magnesium	0,38	± 0,02 %
Fluoride	713,03	± 197,93 ppm

Iron was the only metal that showed some correlation between its accumulation and size (TL) of the individual crocodiles, the longer (bigger) ones from all localities having higher levels in the muscle samples than the shorter (smaller) ones. The converse was found in the relationship between Fe and fat tissue, the smaller individuals having higher concentrations. The Fe contents in the tissues were significantly different between localities ($P < 0,01$), the highest levels being present in those reptiles from the Olifants River. The amount of Iron also differed between kidney, liver, fat and muscle tissue in the Silvervis Dam samples ($P = 0,005$).

TABLE 2 Blood chemical parameters of Nile crocodiles from different localities in southern Africa

Variable	Authority					
	This study				Foggin 1987	Watson 1990
	Silwervis ($n = 4$)	Olifants ($n = 6$)	Sabie ($n = 2$)	PMC*($n = 2$)	Zimbabwe	
Alb (g/ℓ)	16,38 ± 2,48	11,72 ± 2,69	14,30 ± 0,71	9,80 ± 2,12	19,00	–
Glob (g/ℓ)	39,59 ± 10,95	39,20 ± 8,75	47,35 ± 6,29	30,70 ± 0,57	31,00	–
A/G	0,43 ± 0,08	0,30 ± 0,02	0,31 ± 0,02	0,32 ± 0,06	–	–
Gluc (mmol/ℓ)	5,30 ± 0,99	5,68 ± 4,01	3,20 ± 0,42	11,45 ± 7,71	4,50	5,90 ± 0,90
Na (mmol/ℓ)	146,75 ± 2,75	141,50 ± 17,17	154,50 ± 7,78	142,00 ± 4,24	–	154,0 ± 1,00
K (mmol/ℓ)	2,53 ± 0,43	4,59 ± 0,70	5,35 ± 0,35	3,95 ± 0,49	–	3,80 ± 0,50
Ca (T) (mmol/ℓ)	3,89 ± 1,52	3,74 ± 2,28	3,16 ± 0,03	2,60 ± 0,20	2,60	2,97 ± 0,09
Mg (mmol/ℓ)	1,32 ± 0,25	2,24 ± 2,58	1,51 ± 0,07	1,23 ± 0,01	–	0,90 ± 0,10
SIP (mmol/ℓ)	1,96 ± 0,84	1,17 ± 0,50	0,88 ± 0,3	1,43 ± 0,29	–	–
Urea (mmol/ℓ)	1,08 ± 0,21	2,62 ± 5,54	0,60 ± 0,71	0,45 ± 0,21	–	–
Creat (mmol/ℓ)	36,50 ± 3,32	77,67 ± 39,64	97,00 ± 84,85	76,00 ± 48,08	–	–
Cl (mmol/ℓ)	112,25 ± 4,50	113,40 ± 11,95	120,50 ± 21,92	88,50 ± 0,71	–	–

* PMC = Phalaborwa Mining Company

TABLE 3 Some blood chemical parameters of Nile crocodiles of different ages at St. Lucia, KwaZulu-Natal (After Lesley 1997)

Variable	Hatchlings	One-year olds	Juveniles
Na (mmol/ℓ)	142,7 ± 3,3–150,0 ± 2,0	141,8 ± 1,40–153,8 ± 2,1	146,5 ± 15,5–160,7 ± 12,9
K (mmol/ℓ)	3,2 ± 0,1–4,3 ± 0,2	3,4 ± 0,10–4,1 ± 0,1	4,1 ± 0,40–5,8 ± 0,1
Cl (mmol/ℓ)	110,0 ± 0,6–115,0 ± 2,0	109,0 ± 1,30–118,6 ± 2,5	86,0 ± 1,20–111,3 ± 3,8

TABLE 4 Concentrations of selected metals in frozen tissues of Nile crocodiles in the Kruger National Park

Locality/organ	Silwervis Dam				Olifants River			
Variable	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat
Al	367,8 ± 63,1	487,6 ± 212,2	360,6 ± 98,6	367,6 ± 80,7	147,2 ± 116,9	363,9 ± 85,7	135,4 ± 122,8	111,1 ± 120,8
Cu	7,9 ± 0,4	23,2 ± 8,5	13,9 ± 2,6	7,6 ± 0,6	10,5 ± 2,7	27,1 ± 8,2	5,3 ± 2,3	6,1 ± 0,7
Cr	90,5 ± 8,0	69,0 ± 19,7	82,0 ± 21,3	105,4 ± 29,7	9,8 ± 8,0	5,1 ± 7,8	5,1 ± 8,6	14,6 ± 13,1
Fe	156,0 ± 19,9	690,8 ± 55,2	438,3 ± 177,6	188,1 ± 62,9	399,6 ± 216,8	12 851,3 ± 4 192,3	520,0 ± 419,0	297,7 ± 327,9
Mn	17,8 ± 0,5	15,5 ± 3,3	17,6 ± 2,8	18,7 ± 3,7	0,1 ± 0,03	0,1 ± 0,03	0,12 ± 0,06	0,1 ± 0,03
Ni	24,9 ± 0,6	23,0 ± 1,9	28,9 ± 5,7	31,2 ± 7,7	10,3 ± 1,5	8,25	8,1	12,8 ± 7,4
Pb	3,7 ± 1,8	14,1 ± 6,3	9,7 ± 3,6	8,5 ± 4,2	20,3	19,85	NA	1,9
Sr	26,7 ± 12,9	24,4 ± 22,0	32,5 ± 32,2	23,2 ± 10,4	6,7 ± 1,1	6,6 ± 1,5	8,3 ± 4,6	6,9 ± 2,1
Zn	109,7 ± 43,8	61,5 ± 15,0	54,2 ± 18,5	11,2 ± 3,0	39,4 ± 3,2	100,7 ± 23,9	94,4 ± 56,2	27,5 ± 29,8

Locality/organ	Sabi River			
Variable	Muscle	Liver	Kidney	Fat
Al	73,5 ± 43,5	175,2 ± 24,0	40,8 ± 8,2	55,6
Cu	12,6 ± 0,04	30,6 ± 10,0	3,5 ± 0,07	6,5
Cr	18,4	5,5	0,7	30,3
Fe	615,0 ± 40,3	9 427,5 ± 31,8	131,3 ± 14,4	292,0
Mn	0,1 ± 0,07	0,1	0,2 ± 0,07	0,2
Ni	9,1	7,3	BD	20,6
Pb	BD	0	BD	BD
Sr	8,2 ± 0,3	8,5 ± 0,9	8,0 ± 0,1	8,2
Zn	44,7 ± 1,8	122,5 ± 76,4	66,4 ± 38,3	7,6

BD Below detection
NA Not available

TABLE 5 Concentrations of selected metals in formalinized tissues of Nile crocodiles in the Kruger National Park

Locality/organ	Olifants River		Sabi River	
Variable	Liver	Kidney	Liver	Kidney
Ca	45,84 ± 9,00	71,20 ± 20,60	56,70 ± 14,60	98,20 ± 44,80
P	2 919,80 ± 445,60	1 979,30 ± 131,70	2 925,10 ± 164,80	2 133,90 ± 73,60
Mg	110,60 ± 19,90	47,10 ± 24,90	106,40 ± 18,50	86,40 ± 24,10
Co	2,20 ± 0,4	2,30 ± 0,80	2,70	2,40
Cu	15,90 ± 7,20	3,60 ± 1,80	12,70 ± 0,60	4,050 ± 0,10
Fe	5 264,20 ± 4 181,80	80,00 ± 19,50	2 747,20 ± 380,50	82,90 ± 22,20
Mn	2,20 ± 0,30	2,20 ± 0,90	2,10 ± 0,70	2,50 ± 0,20
Se	3,10 ± 0,40	2,90 ± 0,20	3,10 ± 0,40	2,90 ± 0,10
Zn	64,80 ± 19,50	51,40 ± 9,40	61,20 ± 6,10	58,40 ± 18,00
K	645,60 ± 412,60	129,60 ± 69,20	554,10 ± 35,70	209,10 ± 27,00
Na	159,30 ± 53,00	163,80 ± 61,30	215,50 ± 52,50	262,00 ± 18,60

TABLE 6 Comparison, ranked from 1 to 9, of the distribution of the heavy metals tested for in frozen organs of crocodiles from different localities in the Kruger National Park

Metal	Liver			Kidney			Muscle			Fat		
	SV	O	S	SV	O	S	SV	O	S	SV	O	S[a]
Fe	1	1	1	1	1	1	2	1	1	2	1	1
Al	2	2	2	2	2	3	1	2	2	1	2	2
Zn	4	3	3	4	3	2	3	3	3	7	3	6
Cr	3	8	5	3	7	6	4	7	4	3	4	3
Sr	5	7	6	5	4	4	5	8	7	5	6	5
Cu	6	4	4	8	6	5	8	5	5	9	7	7
Ni	7	6[a]	7	6	5[b]	8	6	6[c]	6	4	5	4
Mn	8	9	8	7	8	BD	7	9	8	6	8	8
Pb	9	5[a]	9	9	BD	BD	9	4[b]	BD	8	9	9

a Data of only one crocodile
b Data of one crocodile; the remaining four's values were below detection
c Averaged data of two crocodiles; the remaining one's values were below detection
BD Below detection
SV Silwervis Dam O Olifants River S Sabi River

Aluminium content of muscle tissue differed significantly in the crocodiles from the three localities ($P < 0,05$) and only in the Olifants River, between muscle, liver and kidney ($P < 0,05$). Al and Cr were higher in crocodiles from the Silvervis Dam than in those of the Olifants and Sabi Rivers, and the values for Fe ($P < 0,05$) and Mn ($P < 0,05$) were significantly different for the kidney samples from all the localities. Copper was not different between the various localities ($P = 0,60$) but differed significantly between kidney, liver, muscle and fat tissues in the Olifants River crocodiles ($P = 0,003$) while Pb differed significantly between muscle and kidney ($P < 0,05$) in crocodiles from the Silwervis Dam.

DISCUSSION

Blood

The data obtained from the Nile crocodiles in the KNP fall within the ranges of those reported for Nile crocodiles occurring elsewhere (Foggin, 1987; Watson 1990; Stein 1996; Leslie 1997) as well as for other crocodilians, *Crocodylus acutus*, *Crocodylus porosus* and *Alligator mississippiensis* (Millan *et al.* 1997).

All the serum samples were slightly milky in appearance, probably due to cholesterol and triglycerides. However, these were not tested for. Despite some of the crocodiles being cachectic, there was no macroscopic difference in the turbidity of their serum. This implies that, although the reptiles are not feeding, they may still be lipaemic.

No references in the literature as to the presence or absence of urea in the blood could be found and the values obtained in this study should perhaps be treated with reserve.

Organs and tissues

The presence of pollutants in water, such as heavy metals, is reflected in the accumulation in, and effects on, aquatic animals. Once metals have accumulated in the tissues of fish, for example, they may cause biochemical, physiological, morphological and genetic transformations, which can ultimately influence specific performances, such as the ability to survive, develop, grow and reproduce (Nagel 1991).

Although Nile crocodiles are less dependent on water for their existence than fish, pollutants may affect them indirectly as they may be acquired through ingestion of the fish rather than absorption from the water.

With the exception of strontium, lead and possibly aluminium, the elements tested for in this survey are essential macro- or micronutrients in mammals but their role in biochemical and metabolic pathways in Nile crocodiles are poorly documented and understood (Frey 1991).

Although all these minerals and trace elements are more than likely required by reptiles (Frye 1991), their roles in the biochemical and metabolic pathways of Nile crocodiles are poorly documented in the literature and no comment can therefore be made.

In Table 6 the values of the various metal assays obtained in the survey are ranked. Iron was present in kidney, liver and muscle tissue, but occurred in especially high levels in the liver. Al and Zn were the next most abundant, while the values of the other metals tested for fluctuated. The levels of Fe, Al and Zn especially in those crocodiles from the Olifants River, are thought to be due to bioconcentration from an environment where the levels of them are seasonally high. Similar results have been obtained in fishes from several survey points in the Olifants River (Robinson *et al.* 1997; Van der Merwe *et al.* 1993; Van Vuren, Van der Merwe & Du Preez 1994; Du Preez, Van der Merwe & Van Vuren 1997).

The differences in the values of the metals in the frozen and formalinized tissues cannot be explained, as they were collected at the same time, and from the same site in the respective organs. A 10 % neutral buffered formalin solution, prepared with distilled water, was used throughout for all the samples preserved in it but it is possible that residues of the various metals may have been present in the concentrated formaldehyde solution.

In conclusion, as there are no reports in the literature concerning normal numerical values of the tissues and organs of Nile crocodiles, no comparisons can be drawn. The values presented here are intended to be used as baseline and reference data. Taking into consideration that bio-accumulation of especially Fe, Al and Zn has been reported in fish collected from the Olifants River, the metals in the various organs of the crocodiles were in all probability not responsible for the emaciation and deaths of some individuals.

ACKNOWLEDGEMENTS

We thank the staff of the Zoology Department, Rand Afrikaans University and the Department of Animal Health and Production, Medical University of Southern Africa, for the assays of the various metals, and Mrs Elsabe Myburg for the determination of the haematological parameters.

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