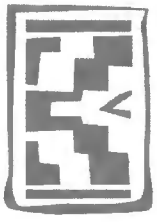

Copper poisoning in the Kruger National Park: Field investigation in wild ruminants

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ABSTRACT

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Chronic copper poisoning was investigated in ruminants within the Phalaborwa area of the Kruger National Park (KNP). Exposure of ruminants to environmental copper pollution resulting form copper smelting operations of a mine in the area was examined by comparing impala faecal copper concentrations in dung heaps and tissue (liver, lung and kidney) copper concentrations of organs collected from impala and buffalo culled within three risk zones (high, moderate and low) of the study area in relation to the distance from the smelter over a period of 4 years. An additional area within the KNP not exposed to the environmental copper pollution from the mine served as control. Tissue copper accumulation was also determined in tracer impala placed in the highest risk zone. The results of this study confirmed the occurrence of chronic copper poisoning in impala and indicated an inverse relationship in extent of impala faecal copper elimination and in tissue copper accumulation in impala and buffalo with distance from the copper smelter. Impala liver copper concentrations were shown to be a reliable indicator of copper accumulation for these ruminants. The presence lung copper concentrations, indicating the exposure to airborne copper were the highest in impala culled in the zone closest to the smelter. Liver copper concentrations above the diagnostic limit of 150 ppm for chronic copper poisoning in domestic sheep were consistently found in impala within the highest risk zone. Clinical pathological measurements suggested that AST activity could possibly be used as an indicator for chronic copper poisoning in impala. It is concluded that, in addition to the environmental and geo-botanical evidence previously reported, the copper smelter of a nearby copper mine is the most likely source of copper pollution responsible for chronic copper poisoning in impala and the occurrence of high copper concentrations in buffalo in the Phalaborwa area of the KNP.

Keywords: Air pollution, buffalo, chronic poisoning, copper, copper smelter, impala

INTRODUCTION

Inefficient smelter emission control associated with copper mining operations has resulted in copper pollution of soils, vegetation and rivers (Hutchinson 1979). Chronic copper poisoning due to pollution from mining operations has been reported in domestic animals (Parada, Gonzales & Bergqvist 1987).

During 1989 chronic copper poisoning was reported in cattle in the Phalaborwa area, Northern Province, South Africa (Gummow, Botha, Basson & Bastianello 1991). Geo-botanical and dustfall bucket data, as well as circumstantial evidence, implicated a copper smelting unit of a nearby copper mine as the most likely source of copper responsible for the cattle deaths.

An initial investigation in the Kruger National Park (KNP), which borders the Phalaborwa industrial complex, was conducted (Gummow *et al.* 1991). Significantly higher ($P \leq 0,05$) copper concentrations in liver samples collected from buffalo (*Syncerus caffer*) culled in the Phalaborwa region of the KNP compared to buffalo culled elsewhere in the KNP, were

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observed. Very high liver copper concentrations were also determined in 16 impala (*Aepyceros melampus*) culled in the same region. Two impala deaths investigated during the same time, were ascribed to chronic copper poisoning based on post mortem evidence (Grobler 1996) and on copper analyses of the livers and kidneys (Gummow *et al.* 1991).

Information regarding chronic copper poisoning in game under natural free-roaming conditions is almost non-existent. Surveys of liver copper concentration of various game species have been conducted in South Africa (Boyazoglu, Barrett, Young & Ebedes 1972) and in Kenya (Howard 1964). Elevated liver copper concentrations in moose (*Alces alces*) in Finland (Hyvärinen & Nygrèn 1993) and in hippopotami (*Hippopotamus amphibius*) in Kenya (Howard 1964) have been reported. In none of these cases chronic copper poisoning had occurred. White-tailed deer (*Odocoileus virginianus*) living near a zinc smelter in Pennsylvania, USA contained extremely high concentrations of both zinc and cadmium in the liver and kidneys, as well as high copper concentrations in the liver. Pathology observed in these deer was ascribed to zinc poisoning (Sileo & Beyer 1985). Reports of chronic copper poisoning in zoo animals have been associated with errors in mixing of rations (Junge & Thornburg 1989).

The objective of this study was to assess the organ tissue concentration of copper in wild ruminants within the study area, using impala and buffalo as indicator species, and to compare the copper concentrations found in the tissues of animals within the different zones in the study area.

MATERIALS AND METHODS

Study area

An area in the Kruger National Park (KNP) adjacent to Phalaborwa and potentially exposed to environmental copper pollution from a copper smelter of a mine in the vicinity was selected as the study area. The study area was divided into three zones, namely high-risk (H-r), moderate-risk (M-r) and low-risk (L-r), based on the extent of tissue copper concentrations derived from an initial impala survey (Gummow *et al.* 1991) and according to known topsoil copper concentrations relative to the distance from the copper smelter (Grobler 1999). The zones also represented a degree of risk for the occurrence of chronic copper poisoning in ruminants within the study area. Additional areas within the KNP not exposed to potential copper pollution from the smelter served as controls.

Impala study

Impala were selected as the most suitable wild ungulate species, partly because of their abundance, but also due to the occurrence of two confirmed chronic copper poisoning deaths in impala during October 1989 (Gummow *et al.* 1991; Grobler 1996). Furthermore, impala being mainly grazers and area-bound for long periods of time are considered more at risk than pure browsers and non-area-bound species.

Impala movements

Three impala herds each were selected within the H-r and M-r zones. From each of the six herds, one ewe was captured and fitted with a radio-telemetry collar (MOD 500 transmitter, Telonics, 932 E. Impala Av., Mesa, Arizona, 85204-6699, USA). The impala ewes were captured with a mixture of 1,5 mg etorphine hydrochloride (HCl) (M99, R&C Pharmaceuticals) and 10 mg xylazine (Rompun, Bayer SA) using a Cap-Chur (Palmer Chemical & Equipment Co. Inc., Douglasville, Georgia, USA) long distance darting system. After the collars were fitted, anaesthesia was reversed by injecting 3 mg diprenorphine HCl (M5050, R&C Pharmaceuticals) intravenously and yohimbine HCl (Yohimbine, Kyron Laboratories) at 0,125 mg/kg, intramuscularly.

Impala herd movements were monitored on a weekly and sometimes daily basis, for 1 year, which included a dry and wet seasonal cycle. The radio collars were removed after approximately 1 year, using the same capture and immobilization procedures as previously described. One radio-collared ewe was killed by a leopard and another was culled.

Impala dung heaps

Faecal samples have previously been used as an indicator of copper status in sheep and their pastures (Suttle 1987a, b), and to determine faecal copper elimination following treatment (Hidiroglou, Heaney & Hartin 1984). Impala are territorial animals and often use the same dung site for prolonged periods (Young 1984).

Faecal samples were collected on a quarterly basis from two dung heaps each within the different zones. An additional three dung heaps were sampled at the same time from a control area approximately 60 km north. Approximately 250 g of fresh faeces at the top of each dung heap was collected by hand, placed into plastic bags and identified. The samples were dried at 60 °C in a force dry oven (Memmert, Model 3290) and analyzed for copper content.

Indicator animals

Impala rams (n = 7–25, 1–12 within each risk zone), aged *c.* 2 years and more were routinely culled by shooting every 2–4 months over a period of 4 years (1989–1993) and served as indicators of copper status. At the same time, more or less three impala rams

were culled in the control area. All culling sites were plotted on a map. Necropsies were performed on all culled animals and duplicate liver, kidney and from September 1990 also lung samples collected for determination of copper concentration. Samples of the same organs as well as the spleen, lymph nodes, spinal cord, triceps muscle and testis, were also collected for histological examination. All tissue samples were collected in clean glass jars containing a 10% buffered formalin solution.

An additional group of 12 impala rams, of three different age groups, namely younger than 6 months, approximately 18 months and older than 3 years, were culled in the H-r zone. Equal numbers of animals were included within each group.

During the first year of the study (October and November 1989) blood samples were also collected from 30 of these impala originating from the different zones. Each animal was bled by severing the jugular vein as soon as practicable after being shot and collecting the blood in a 10 mℓ heparinized tube and a 10 mℓ plain glass tube under vacuum (Vacutainer). Following centrifugation, the plasma and serum respectively, were collected and stored in labelled plastic containers at –8 °C. These samples were evaluated for serum gamma-glutamyltransferase (GGT) and aspartate-aminotransferase (AST) activities, as well as plasma copper concentration.

Tracer animals

Radio-collars were fitted to four groups of tracer animals, each consisting of six impala rams ($n = 24$) of 2–4 years of age, at quarterly intervals, starting January 1992. They were captured in the Letaba area, 50 km east of Phalaborwa, where liver copper concentrations in impala were found to be within the normal range (Boyazoglu *et al.* 1972). The tracer impala were released in the H-r zone near the western boundary of the KNP, tracked every month and culled after 3 months. Two tracer animals were killed by predators, while one was poached on the golf course at Phalaborwa, therefore, data from only 21 impala could be obtained. Samples were collected as described above.

Investigation of free ranging impala mortalities

An intensive programme was instituted to locate and examine all impala mortalities within the study area. A higher intensity of game patrols and general vigilance for impala mortalities was maintained, during suspected danger periods. At the end of prolonged dry periods, daily and even twice-daily patrols by foot and on bicycle were carried out. The presence of vultures and other signs, including drag marks left by leopards, hyena sounds and tracks were traced to find carcasses. Blood smears were made and organ samples collected and fixed in 10% buffered formalin of each carcase located. The tissue samples collected were analyzed for copper concentration and examined histopathologically.

Buffalo study

The annual KNP routine culling buffalo programme that had been applied during the study period provided an opportunity for their inclusion in the study. Buffalo are also grazers, tend to be fairly area-bound and are presumed to be equally susceptible to chronic copper poisoning as domesticated cattle (Grobler 1990, personal observation). Collection of organ samples from buffalo commenced in 1989 (Gummow *et al.* 1991) and continued annually until 1993. Organ samples were collected from buffalo routinely culled in the designated Phalaborwa study area, as well as from control buffalo located more than 60 km away from the study area (Grobler 1999) as illustrated in Fig. 1.

Movements of buffalo herds

Four radio-telemetry collars (MOD-600 transmitter, Telonics) were fitted to two cows, each from two different buffalo herds in the Phalaborwa study area. The largest herd consisted of approximately 400 animals, while the other herd comprised only 150 animals. Immobilization was achieved with a drug mixture of 7 mg etorphine HCl and 40 mg xylazine which was administered by means of a Cap-Chur darting system. After the collars were fitted, anaesthesia was reversed by the intravenous administration of 15 mg diprenorphine HCl and 0,125 mg/kg yohimbine HCl.

Buffalo herd movements were monitored on a weekly and sometimes daily basis, for 3 years. At the end of the study period the radio collars were removed following chemical immobilization.

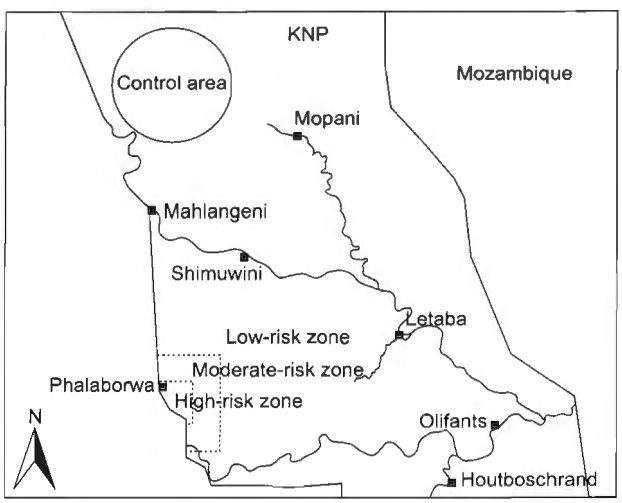

FIG. 1 Phalaborwa and control areas in the KNP

Sample collection

Only liver samples were collected by Gummow *et al.* (1991) in 1989 as part of their study. From 1990 until 1993, liver, kidney and lung samples were collected from buffalo culled in the Phalaborwa area, as well as from the control group buffalo. These were the only organ samples permitted for collection since the animals had been culled for human consumption.

Sample analysis

All tissue and plasma copper concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer Corporation, Norwalk, Connecticut, USA), following wet digestion (WM) (Boyazoglu *et al.* 1972). The various tissues collected in 10% buffered formalin were sectioned and routinely stained with haematoxylin and eosin (HE) for histopathological examination. For verification of analytical results some duplicate samples were submitted to an alternative laboratory.

Statistical analysis

Statgraphics 4.0 programme (STSC 1989, Statistical Graphics Corporation, Maryland, USA) was used for the statistical calculations. Comparisons of the mean copper concentrations of tissue samples collected from impala and buffalo between the different risk zones were performed. One-way analysis of variance was used to compare differences between the various risk zones and control area. Scheffé's multiple separation test (Browne 1985) was used to distinguish individual statistical differences in the case of multiple comparisons.

RESULTS

Movements of impala

Herds generally remained area-bound, with the availability of drinking water being the most important factor determining the distances roamed. During the dry months of August and September in 1990, when seasonal pools dried up, the identified herds moved daily to the Olifants River for drinking water. This resulted in an increase in the area the herd normally roamed by almost threefold, but following good rains the herds returned to their original smaller area, *c.* 7 km^2, for the remainder of the study period.

The three herds originally selected within the H-r zone remained predominantly within this zone for the duration of the study period and occupied areas of *c.* 8,4–11,8 km^2. The two herds within the M-r zone herds used a perennial dam as their water source and utilized a relatively larger area compared to the area occupied by the herds in the H-r zone, *c.* 17,2–19,5 km^2, respectively.

Impala faecal analyses

The mean quarterly copper concentrations of faecal samples collected from dung heaps within the various study zones and the control area during the period June 1990 to October 1993 are illustrated in Fig. 2. Mean faecal copper concentrations were significantly ($P \leq 0,05$) higher in the H-r zone in comparison to the other zones. The largest difference was observed between the H-r zone and the control area. No significant differences were observed between the M-r and L-r zones and the control area.

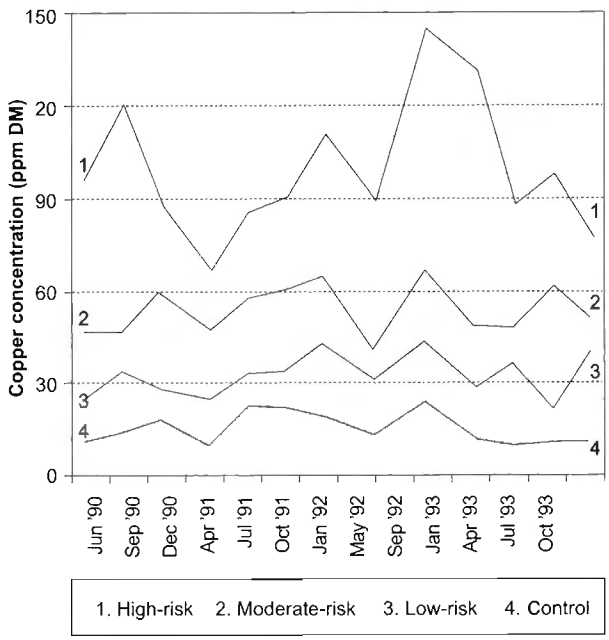

FIG. 2 Comparison of mean faecal copper concentrations (ppm DM) between impala dung heaps within the various risk zones and control area for each study year

Organ copper concentrations in indicator animals

Mean copper concentrations measured in liver, kidney and lung samples collected from impala in the various risk zones and control area during the period October 1989 to December 1993 are summarized in Tables 1, 2 and 3, respectively. The statistical results of the mean organ copper concentrations for the entire study period within each zone and the control area are indicated in the same tables.

A total of 285 impala were culled during the period October 1989 to December 1993 ($n = 100$ for the H-r zone, $n = 78$ for the M-r zone, $n = 56$ for the L-r zone, and $n = 51$ for the control area). The mean copper concentration of liver samples collected over the entire period from impala in the H-r zone was significantly higher ($P \leq 0,05$) than the other risk zones and

TABLE 1 Mean (± SD) liver copper concentrations (ppm WM) of indicator impala in the KNP

Time of collection	Mean ± SD copper concentrations			
	High-risk zone	Moderate-risk zone	Low-risk zone	Control
October '89	217,3 ± 109,1 (n = 8)	147,0 ± 69,9 (n = 9)	45,0–60,0 (n = 2)	19,6 ± 13,1 (n = 6)
November '89	210,0 ± 60,9 (n = 3)	100,3 ± 14,2 (n = 4)	52,8 ± 12,3 (n = 9)	(n = 0)
February '90	152,3 ± 50,4 (n = 3)	90,0 ± 9,5 (n = 3)	51,0–69,0 (n = 2)	25,8 ± 9,2 (n = 3)
June '90	160,2 ± 54,6 (n = 5)	92,0 ± 39,4 (n = 3)	54,0–66,0 (n = 2)	22,0 ± 6,4 (n = 3)
September '90	136,8 ± 24,7 (n = 4)	89,0 ± 29,5 (n = 3)	48,7 ± 7,1 (n = 3)	21,0 ± 5,6 (n = 3)
December '90	91,4 ± 35,4 (n = 3)	60,0 ± 9,9 (n = 3)	52,0 ± 11,2 (n = 3)	21,7 ± 7,6 (n = 3)
February '91	98,8 ± 34,8 (n = 4)	47,3 ± 1,8 (n = 3)	28,0–40,0 (n = 2)	18,0–21,0 (n = 2)
June '91	100,3 ± 22,1 (n = 4)	57,3 ± 29,1 (n = 3)	36,0–40,0 (n = 2)	23,0 ± 7,1 (n = 3)
September '91	137,1 ± 39,5 (n = 4)	94,0 ± 34,6 (n = 4)	46,0–54,0 (n = 2)	23,7 ± 7,5 (n = 3)
December '91	144,7 ± 32,1 (n = 3)	90,8 ± 12,1 (n = 4)	48,3 ± 6,7 (n = 3)	18,0–30,0 (n = 2)
February '92	134,2 ± 52,6 (n = 7)	61,3 ± 24,3 (n = 3)	42,7 ± 9,8 (n = 3)	24,0–36,0 (n = 2)
June '92	233,4 ± 165,6 (n = 10)	57,2 ± 16,9 (n = 6)	43,6 ± 11,6 (n = 6)	26,0–30,0 (n = 2)
September '92	202,7 ± 87,1 (n = 8)	80,5 ± 16,7 (n = 4)	48,6 ± 7,8 (n = 3)	30,0 (n = 1)
December '92	215,3 ± 117,6 (n = 12)	69,8 ± 22,7 (n = 8)	47,3 ± 17,3 (n = 5)	29,0 ± 3,2 (n = 6)
February '93	104,8 ± 34,1 (n = 4)	52,5 ± 12,6 (n = 6)	38,0 ± 4,8 (n = 3)	18,0 (n = 2)
April '93	83,8 ± 13,1 (n = 3)	51,3 ± 20,6 (n = 3)	29,0 (n = 1)	28,7 ± 5,5 (n = 3)
July '93	125,5 ± 27,3 (n = 7)	48,6 ± 8,1 (n = 6)	33,0–36,0 (n = 2)	28,0 ± 5,6 (n = 3)
December '93	140,4 ± 62,3 (n = 8)	79,0 ± 27,5 (n = 3)	44,6 ± 10,8 (n = 3)	27,2 ± 7,8 (n = 4)
Mean ± SD	161,0 ± 90,7[a] (n = 100)	80,1 ± 41,7[b] (n = 78)	46,6 ± 11,3[c] (n = 56)	25,6 ± 8,1[c] (n = 51)

[a, b, c] Mean values with different superscripts are significantly different ($P \leq 0,05$)

TABLE 2 Mean (± SD) kidney copper concentrations (ppm WM) of indicator impala in the KNP

Time of collection	Mean ± SD copper concentrations			
	High-risk zone	Moderate-risk zone	Low-risk zone	Control
October '89	55,2 ± 57,6 (n = 8)	10,2 ± 2,9 (n = 9)	6,0 (n = 2)	3,0 ± 0,0 (n = 6)
November '89	9,0 ± 3,0 (n = 3)	8,3 ± 3,8 (n = 4)	4,5 ± 1,6 (n = 9)	(n = 0)
February '90	9,3 ± 6,2 (n = 3)	6,7 ± 3,2 (n = 3)	3,5 ± 0,7 (n = 2)	2,8 ± 0,5 (n = 3)
June '90	50,0 ± 35,3 (n = 5)	16,5 ± 7,2 (n = 3)	3,0 (n = 2)	3,0 (n = 3)
September '90	14,4 ± 1,7 (n = 4)	11,0 ± 6,3 (n = 3)	3,7 ± 1,2 (n = 3)	2,7 ± 0,6 (n = 3)
December '90	5,5 ± 2,1 (n = 3)	4,5 ± 2,1 (n = 3)	2,7 ± 0,6 (n = 3)	2,3 ± 0,6 (n = 3)
February '91	5,0 ± 1,6 (n = 4)	3,5 ± 0,7 (n = 3)	4,0 (n = 2)	3,0–5,0 (n = 2)
June '91	3,8 ± 1,1 (n = 4)	4,7 ± 2,5 (n = 3)	3,0 (n = 2)	3,0 (n = 3)
September '91	7,8 ± 1,9 (n = 4)	8,0 ± 2,8 (n = 4)	3,0–6,0 (n = 2)	3,0 ± 1,0 (n = 3)
December '91	13,0 ± 4,4 (n = 3)	8,0 ± 2,5 (n = 4)	2,7 ± 1,2 (n = 3)	2,0–3,0 (n = 2)
February '92	11,5 ± 5,3 (n = 7)	5,3 ± 0,5 (n = 3)	2,7 ± 0,6 (n = 3)	2,0–3,0 (n = 2)
June '92	9,4 ± 4,6 (n = 10)	5,2 ± 1,9 (n = 6)	2,4 ± 0,6 (n = 6)	2,0 ± 1,4 (n = 2)
September '92	6,0 ± 1,7 (n = 8)	3,3 ± 0,9 (n = 4)	3,5 ± 0,8 (n = 3)	3,0 (n = 1)
December '92	5,5 ± 2,8 (n = 12)	3,8 ± 1,2 (n = 8)	3,5 ± 1,7 (n = 5)	3,0 (n = 6)
February '93	4,8 ± 1,9 (n = 4)	3,8 ± 0,8 (n = 6)	4,3 ± 0,7 (n = 3)	3,0–6,0 (n = 2)
April '93	2,8 ± 0,5 (n = 3)	2,7 ± 0,6 (n = 3)	3,0–4,0 (n = 2)	2,7 ± 0,6 (n = 3)
July '93	3,0 ± 0,6 (n = 7)	3,3 ± 0,5 (n = 6)	2,0–3,0 (n = 2)	2,8 ± 2,6 (n = 3)
December '93	5,4 ± 1,3 (n = 8)	3,3 ± 0,5 (n = 3)	3,0 (n = 3)	2,6 ± 0,5 (n = 4)
Mean ± SD	12,3 ± 21,7[a] (n = 100)	6,3 ± 4,3[b] (n = 78)	3,3 ± 1,3[b] (n = 57)	2,7 ± 0,8[b] (n = 51)

[a, b] Mean values with different superscripts are significantly different ($P \leq 0,05$)

control area. Significant ($P \leq 0,05$) differences also existed between the M-r and L-r zone as well as between the M-r zone and control area (Table 1). There was no significant ($P > 0,05$) difference in mean copper concentration of liver samples between the L-r zone and the control area. The differences in the mean liver concentrations collected from impala from the different risk zones and control area are illustrated in Fig. 3.

The mean kidney copper concentration measured in samples collected from impala within the H-r zone (12,3 ± 21,7 ppm WM) was significantly ($P \leq 0,05$) higher compared to the other zones and control area

TABLE 3 Mean (± SD) lung copper concentrations (ppm WM) of indicator impala in the KNP

Time of collection	Mean ± SD copper concentrations			
	High-risk zone	Moderate-risk zone	Low-risk zone	Control
September '90	14,7 ± 5,5 (n = 4)	11,0 ± 4,5 (n = 3)	5,3 ± 4,0 (n = 3)	2,3 ± 0,6 (n = 3)
December '90	8,6 ± 3,9 (n = 3)	5,0 ± 1,4 (n = 3)	3,0 ± 1,0 (n = 3)	1,3 ± 0,6 (n = 3)
February '91	6,5 ± 1,9 (n = 4)	3,5 ± 0,7 (n = 3)	4,0–6,0 (n = 2)	2,0 (n = 2)
June '91	8,5 ± 5,1 (n = 4)	6,0 ± 1,0 (n = 3)	4,0 (n = 2)	2,0 (n = 3)
September '91	14,5 ± 6,7 (n = 4)	7,5 ± 0,8 (n = 4)	5,0–7,0 (n = 2)	1,8 ± 0,6 (n = 3)
December '91	17,7 ± 5,7 (n = 3)	6,8 ± 4,5 (n = 4)	2,4 ± 1,2 (n = 3)	3,0 (n = 2)
February '92	18,2 ± 8,9 (n = 7)	6,3 ± 0,9 (n = 3)	2,6 ± 0,6 (n = 3)	1,0–2,0 (n = 2)
June '92	11,1 ± 8,7 (n = 10)	4,9 ± 1,4 (n = 6)	2,4 ± 0,6 (n = 6)	1,0–2,0 (n = 2)
September '92	8,5 ± 3,8 (n = 8)	4,3 ± 2,7 (n = 4)	3,0 ± 1,4 (n = 3)	2,0 (n = 1)
December '92	8,2 ± 5,8 (n = 12)	4,4 ± 1,4 (n = 8)	2,5 ± 0,7 (n = 5)	2,2 ± 0,5 (n = 6)
February '93	6,7 ± 2,1 (n = 4)	3,3 ± 1,0 (n = 6)	3,0 ± 1,4 (n = 3)	2,0–3,0 (n = 2)
April '93	2,8 ± 0,5 (n = 3)	2,7 ± 0,6 (n = 3)	2,0 (n = 1)	2,0 (n = 3)
July '93	4,4 ± 0,6 (n = 7)	2,5 ± 0,5 (n = 6)	2,0–3,0 (n = 2)	1,6 ± 0,6 (n = 3)
December '93	9,4 ± 4,9 (n = 8)	5,5 ± 1,3 (n = 3)	2,7 ± 0,7 (n = 3)	2,6 ± 0,5 (n = 4)
Mean ± SD	7,9 ± 6,7[a] (n = 81)	3,9 ± 2,8[b] (n = 59)	2,5 ± 1,7[b] (n = 41)	1,7 ± 0,7[b] (n = 39)
% CV*	83,4	72,1	71,5	44,5

[a, b] Mean values with different superscripts are significantly different ($P \leq 0,05$)
* Percentage coefficient of variation

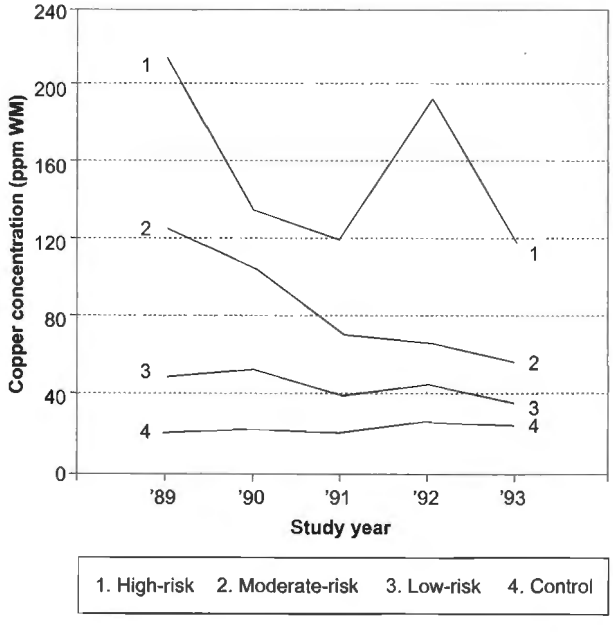

FIG. 3 Mean liver concentrations (ppm WM) from impala culled in the various risk zones and control area for each study year (standard deviation not illustrated due to overlapping of error bars)

(2,7 ± 0,8 ppm WM) (Table 2). No significant ($P >$ 0,05) differences were observed between the M-r and L-r zones and the control area.

The mean lung copper concentrations from impala culled in the H-r zone throughout the trial period was 7,9 ± 6,7 ppm WM and was significantly ($P \leq 0,05$) higher than the mean lung copper concentrations of impala from the M-r, L-r and control areas (Table 3). No significant ($P > 0,05$) difference existed between lung copper concentrations from impala in the M-r zone (3,9 ± 2,8 ppm WM), L-r zone (2,5 ± 1,7 ppm WM) and the control area (1,7 ± 0,7 ppm WM).

TABLE 4 Mean (± SD) tissue copper concentrations (ppm WM) of impala rams of three different age groups (n = 4 per group) culled within the High-risk zone

Age group	Mean ± SD copper concentration (ppm)		
	Liver	Kidney	Lungs
6 months	104,5 ± 47,5	4,3 ± 0,9	7,3 ± 2,6
18 months	91,3 ± 38,9	5,3 ± 0,9	8,3 ± 2,9
3 years	138,7 ± 61,7	4,5 ± 1,3	9,0 ± 3,9

Organ copper concentrations from different age groups

Mean organ copper concentrations collected from impala rams of different age groups within the H-r zone are given in Table 4. There were no significant ($P > 0,05$) differences in liver, kidney and lung copper concentrations between different age groups.

Organ copper concentrations of tracer impala

Mean organ copper concentrations collected from tracer impala which were placed in the H-r zone are given in Table 5.

TABLE 5 Mean (± SD) tissue copper concentrations (ppm WM) of tracer impala (January 1992 to February 1993)

Time of year	No. of impala	Copper concentration (ppm WM)		
	(n = 21)	Liver	Kidney	Lung
January to March	5	54,4 ± 10,8[a]	3,6 ± 0,9	5,8 ± 1,8
April to June	6	79,0 ± 27,5	4,8 ± 0,7	7,5 ± 2,3
July to October	6	99,5 ± 43,1	8,8 ± 5,5	10,0 ± 8,8
November to February	4	104,3 ± 30,1[b]	5,5 ± 1,3	12,5 ± 5,4
Mean ± SD		83,8 ± 22,2	5,7 ± 2,3	8,7 ± 5,6

[a, b] Mean values with different superscripts are significantly different ($P \leq 0,05$)

TABLE 6 Clinical pathology parameters with corresponding liver and kidney copper concentrations (mean ± SD) of impala in the KNP during October and November 1989

Zone/area	No. of impala	Liver Cu (ppm WM)	Kidney Cu (ppm WM)	Plasma Cu (mmol/ℓ)	GGT (U/ℓ)	AST (U/ℓ)	Pathology
High-risk zone	11	223,3 ± 91,5[a]	11,7 ± 2,8[a]	16,3 ± 2,1	38,0 ± 11,4	387,0 ± 256,5[a]	None to severe
Moderate-risk zone	6	77,5 ± 33,3[b]	7,5 ± 2,5	14,6 ± 2,4	37,5 ± 4,3	151,6 ± 22,8[b]	None to severe
Low-risk zone	7	31,0 ± 17,8[b]	4,2 ± 1,6[b]	15,5 ± 1,9	32,0 ± 3,7	167,1 ± 79,1[b]	None
Control area	6	25,1 ± 5,1[b]	3,0[b]	14,7 ± 2,6	37,0 ± 9,9	135,4 ± 28,1[b]	None

[a, b] Mean values with different superscripts are significantly different ($P \leq 0,05$)

The mean liver, kidney and lung copper concentrations of all tracer impala (n = 21) over the four collection periods were 83,8 ± 22,2 ppm WM, 5,7 ± 2,3 ppm WM and 8,7 ± 5,6 ppm WM, respectively. There was a significant increase in the mean liver copper concentration between the initial (54,4 ± 10,8 ppm WM) and final culling (104,3 ± 30,1 ppm WM) of tracer impala. Mean liver and lung copper concentrations of the tracer impala were significantly ($P \leq 0,05$) higher than those of the control animals (Tables 1, 3 and 5). There were, however, no significant ($P > 0,05$) differences in the kidney copper concentration between the tracer and control impala (Tables 2 and 5). Except for mean liver copper concentration of the tracer impala which was significantly ($P \leq 0,05$) lower than that of the H-r zone impala, there were no significant ($P > 0,05$) differences between the organ copper concentrations of tracer animals and those sampled within the H-r and M-r zones. Most animals within the H-r zone remained there, although a few moved into the M-r zone on occasion.

Clinical pathology and pathology

Liver and kidney copper concentrations of samples collected from 30 impala culled during the first year of study (Table 6) were correlated with the plasma copper concentration (plasma Cu), gamma-glutamyltransferase (GGT) activity, aspartate-aminotransferase (AST) activity and occurrence of macropathology recorded in these animals.

Plasma copper concentrations and GGT activity were not significantly ($P > 0,05$) different between impala from the H-r zone and control impala. On the other hand, AST activity was significantly ($P \leq 0,05$) higher in impala from the H-r zone, which also had significantly ($P \leq 0,05$) higher liver and kidney copper concentrations, compared to impala from the M-r and L-r zones and control impala. Impala with severe liver macropathology (n = 8) from the H-r zone had AST activities above 295 U/ℓ. Liver pathology manifested either as necrosis or degeneration (Table 7).

Impala culled during October and November 1989 were grouped into three classes according to liver copper concentrations analyzed for each animal, namely > 150 ppm WM, between 80 and 150 ppm WM and < 80 ppm WM. Liver copper concentrations above 150 ppm WM represent concentrations known to cause chronic copper poisoning in sheep and those lower than 80 ppm WM are considered to be safe (Suttle 1987a). The mean kidney copper concentrations and severity of histopathological lesions for impala within each class were correlated with the extent of liver copper concentration (Table 7).

Most macroscopical changes observed in impala culled within in the study area involved the liver and only three impala revealed marked kidney pathology. The liver of impala affected exhibited marked yellow to yellow-brown discolouration, varying from a mottled appearance to diffusely yellow. In some cases the liver appeared glistening and slightly enlarged and the consistency was friable. The majority of cases with liver copper concentrations greater than 150 ppm WM had pathological lesions. Histopathology revealed diffuse haemosiderin accumulation,

TABLE 7 Correlation of liver copper concentration with concentrations in the kidney and severity of hepatic and kidney histopathology

Categories of liver [Cu]	Animals (*n*)	Kidney [Cu]	Severity of histopathological lesions							
			Liver				Kidney			
			Hs	Deg	Hd	Nec	Deg	Hs	Hd	Casts
> 150	1*	141	4+	4+	4+	2+	2+	3+	2+	3+
	2	3–9	2+	2+	0	1+	1+	1+	0	0
	7	> 9	3+	3+	1+	2+	2+	2+	1+	1+
80–150	2*	90; 113	4+	4+	4+	2+	2+	3+	2+	2+
	6	3–9	1+	1+	0	0	1+	0	0	0
	3	> 9	2+	2+	0	1+	1+	1+	0	1+
< 80	15	3–6	1+	1+	0	0	0	0	0	0

[Cu] Copper concentrations (ppm WM)
0–4+ Severity of lesions (0 = none; 1+ = slight; 2+ = moderate; 3+ = severe; 4+ = very severe)
Hs Haemosiderin accumulation in renal epithelial cells or liver macrophages
Deg Swelling of cell (hydropic degeneration)
Hd Hyaline droplet degeneration
Nec Single cell necrosis throughout the liver
Casts Granular or hyalin
* Confirmed chronic copper poisoning cases

varying in severity, in the cytoplasm of most hepatocytes, with scattered necrosis of individual and groups of hepatocytes. Liver cells were swollen, the cytoplasm appeared totally deliquescent and contained large amounts of accumulated haemosiderin. Centrilobular fatty degeneration and hydropic degeneration manifested as a homogenic eosinophylic appearance of the cytoplasm of hepatocytes.

Kidneys of culled impala generally showed no macroscopical lesions except in three cases associated with the presence of yellow discoloured livers, where the kidneys appeared congested. Microscopical renal changes included a mild to severe hyaline droplet degeneration of the proximal tubular epithelium, mild to severe accumulation of haemosiderin in the tubular epithelium and mild to severe congestion of the glomeruli. In a few cases there was mild mesangial cell proliferation in the glomeruli, indicating a mesangial proliferative glomerulonephritis.

Haemosiderin pigmentation was also observed in the lungs, spleen and mesenteric lymph nodes of various impala.

Skin lesions of 2–11 patches of alopecia, present mainly on the back, shoulders and hindquarters and varying in size from 20 x 40 mm to 100 x 100 mm, were observed in four impala. No ectoparasites were present, nor could any micro-organisms be isolated from the lesions. The hair from these animals had a dull and rough appearance and the skin appeared thickened and hard, almost without any elasticity. Microscopically, a marked hyperkeratosis associated with mainly perivascular eosinophylic infiltration in the dermis was seen. A deep-brown granular pigment in the sweat glands was also noticed.

Impala field mortalities

In the three confirmed cases of chronic copper poisoning, the livers appeared a dirty dark yellow, almost khaki-like in colour. Icterus was not observed in any of these cases. Histopathology revealed similar but more severe changes, with regard to haemosiderin accumulation in the cytoplasm of most hepatocytes and necrosis of individual and groups of hepatocytes, than the culled impala. The liver cells were swollen and contained large amounts of accumulated haemosiderin.

The kidneys of the impala that had died were dark red to brown and enlarged. Dark, red urine was observed in two cases. Marked histopathological changes included severe general hyperaemia, severe hyaline droplet degeneration of tubular cells and the presence of haemoglobin and hyaline casts within the tubular lumens and glomeruli. Pronounced haemosiderin pigmentation was present. Other pathological changes noted were severe congestion of the spleen, mild ascites and hydropericardium.

During 1991 an outbreak of anthrax occurred in the northern region of the KNP. Impala that had died of the disease ($n = 5$) were found within the study area. Blood smears were made and examined with light microscopy in the field to confirm anthrax mortalities. Thereafter these affected carcasses were burnt at the site where found. A severe drought was experienced towards the end of 1992 resulting in impala dying of starvation ($n = 5$), all being pregnant ewes. Although the liver copper concentrations in all cases were above 150 ppm WM, the kidney copper concentrations were within normal limits. Deaths due to pneumonia following rain and an unexpected cold

spell occurred during December 1991 (n = 2) and January 1993 (n = 4). Predation of impala by lion, leopard, cheetah or wild dog occurred within the study area. Deaths (n = 7) due to impala accidentally running into the western boundary fence were also encountered. Vehicle accidents on tourist roads resulted in the death of four impala, while poachers were responsible for the loss of a tracer impala that roamed onto the Phalaborwa golf course.

Movements of buffalo herds

The movement of buffalo occurred throughout the study area. Following good rains in 1990, the two selected herds merged and remained together for the remainder of the study period. Although buffalo moved over the entire study area, they preferred the *Combretum apiculatum* mixed veld along in the L-r zone the Mulalani stream (Fig. 1), where they spent an estimated 60% of their time. Throughout the study period, buffalo only stayed in the H-r zone for brief periods (*c.* 1 month), always during the summer months.

Copper monitoring in buffalo organs

Organ copper concentrations of buffalo culled in the Phalaborwa study area are presented in Table 8 and the copper concentrations of buffalo culled elsewhere in the KNP are given in Table 9.

Owing to the severe drought encountered during late 1992, the buffalo population decreased almost by half in the KNP, as determined by the annual aerial census (Whyte 1992). Buffalo culling was therefore discontinued and no further samples from buffalo in the Phalaborwa area were collected after July 1992.

Tissue analyses from 256 buffalo were used in the study, 157 from the study area within the KNP, and 99 from areas elsewhere in the KNP, the latter serving as controls.

The mean liver copper concentration of all age groups of buffalo from the study area was 54,7 ± 29,8 ppm WM. This was significantly higher ($P \leq 0,05$) than the mean liver copper concentration of 19,8 ± 9,4 ppm WM from the control animals for each study year and is illustrated in Fig. 4. Mean liver copper concentrations in the Phalaborwa herd decreased significantly ($P \leq 0,05$) from 80,4 ± 44,9 ppm WM in 1989 to 31,1 ± 5,2 ppm WM in 1991. The mean annual liver copper concentration of control buffalo did not differ significantly ($P > 0,05$) throughout the study period. No pathology related to chronic copper poisoning was observed in buffalo organs or samples collected for analysis.

TABLE 8 Mean (± SD) organ copper concentrations (ppm WM) of buffalo culled in the Phalaborwa study area over a 4-year period grouped according to age

Age group	Year of collection	No. of animals	Mean ± SD copper concentrations (ppm WM)		
			Liver	Kidney	Lungs
Juvenile (< 1 year)	1989	5	91,2 ± 33,6	Not collected	Not collected
	1990	5	51,0 ± 18,0	6,6 ± 1,4	9,0 (n = 1)
	1991	5	43,6 ± 22,0	8,2 ± 5,2	8,6 ± 6,4
	1992	14	53,7 ± 17,7	3,5 ± 1,1	2,2 ± 1,3
	Mean ± SD		57,9 ± 26,0	4,4 ± 3,3	2,9 ± 3,8
Subadult (1–3 years)	1989	22	76,5 ± 39,9	Not collected	Not collected
	1990	10	76,1 ± 91,6	5,2 ± 3,1	3,8 ± 4,3
	1991	5	24,6 ± 12,1	7,4 ± 0,9	7,4 ± 2,3
	1992	19	47,6 ± 18,1	4,4 ± 0,9	3,1 ± 2,1
	Mean ± SD		62,0 ± 49,1	3,5 ± 2,6	3,6 ± 2,8
Adult (4–12 years)	1989	7	86,6 ± 32,8	Not collected	Not collected
	1990	12	38,0 ± 8,9	3,3 ± 2,3	30,0 (n = 1)
	1991	16	29,8 ± 13,7	7,2 ± 3,8	8,6 ± 6,4
	1992	15	50,3 ± 20,6	4,2 ± 0,9	2,2 ± 1,3
	Mean ± SD		45,8 ± 25,9	4,4 ± 3,2	3,9 ± 3,9
Old (> 12 years)	1989	3	77,0 ± 9,2	Not collected	Not collected
	1990	9	66,4 ± 81,5	10,7 ± 16,3	12,0 ± 5,6
	1991	7	29,6 ± 20,3	8,2 ± 5,3	7,9 ± 5,4
	1992	3	49,6 ± 17,6	5,7 ± 0,6	2,7 ± 1,5
	Mean ± SD		53,9 ± 55,1	7,9 ± 10,9	3,9 ± 4,7

TABLE 9 Mean (± SD) organ copper concentrations (ppm WM) of buffalo culled in the elsewhere in the KNP over a 4-year period grouped according to age

Age group	Year of collection	No. of animals	Mean ± SD copper concentrations (ppm WM)		
			Liver	Kidney	Lungs
Juvenile (< 1 year)	1989	5	29,4 ± 17,7	Not collected	Not collected
	1990	3	20,0 ± 3,4	3,0 ± 1,0	1,3 ± 0,6
	1991	4	18,5 ± 8,5	2,5 ± 1,1	1,3 ± 0,5
	1992	9	19,2 ± 5,3	2,2 ± 0,7	1,2 ± 0,4
	Mean ± SD		21,6 ± 10,3	2,1 ± 0,9	1,2 ± 0,4
Subadult (1–3 years)	1989	4	21,0 ± 15,3	Not collected	Not collected
	1990	3	11,0 ± 1,8	3,0 ± 1,0	1,4 ± 0,6
	1991	4	18,3 ± 11,9	2,3 ± 0,9	1,2 ± 0,8
	1992	13	22,2 ± 8,9	2,2 ± 0,7	1,1 ± 0,3
	Mean ± SD		19,9 ± 10,2	2,1 ± 0,8	1,1 ± 0,3
Adult (4–12 years)	1989	9	23,3 ± 10,1	Not collected	Not collected
	1990	5	11,4 ± 8,3	2,6 ± 0.9	1,6 ± 0,9
	1991	7	22,4 ± 8,1	2,4 ± 0,5	1,2 ± 0,4
	1992	17	20,0 ± 8,9	2,7 ± 0,7	1,2 ± 0,4
	Mean ± SD		20,1 ± 9,4	2,2 ± 0,9	1,2 ± 0,5
Old (> 12 years)	1989	3	27,0 ± 7,9	Not collected	Not collected
	1990	3	19,0 ± 1,7	1,7 ± 1,2	1,7 ± 0,6
	1991	3	16,0 ± 4,6	3,0 ± 0,5	1,7 ± 0,6
	1992	7	19,1 ± 5,5	3,0 ± 0,5	1,6 ± 0,8
	Mean ± SD		20,0 ± 6,1	2,4 ± 1,1	1,5 ± 0,6

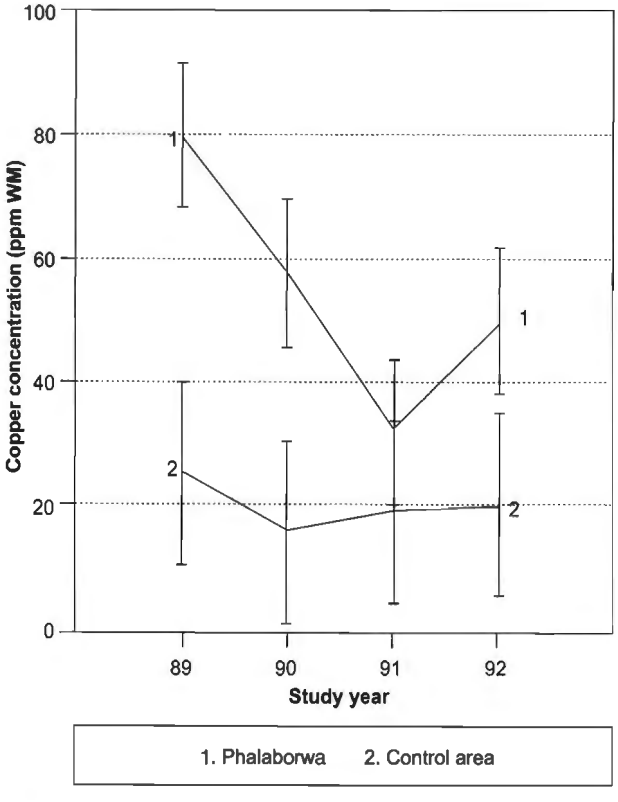

FIG. 4 Mean (± SD) liver copper concentrations (ppm WM of buffalo from the control and Phalaborwa study areas for each study year

During 1991, a significant ($P \leq 0,05$) difference occurred between the mean kidney copper concentrations of old (8,2 ± 5,3 ppm WM) and adult (7,2 ± 3,8 ppm WM) buffalo from the study area, compared to old (3,0 ± 0,5 ppm WM) and adult (2,4 ± 0,5 ppm WM) buffalo in the control (Tables 8 and 9). The mean lung copper concentration of all buffalo culled within the study area over 4 years was 5,6 ± 4,4 ppm WM, with the highest mean lung copper concentrations recorded during 1991 (8,1 ± 0,5 ppm WM). In comparison, the mean lung copper concentrations of all the control buffalo (1,4 ± 0,4 ppm WM) were significantly lower ($P \leq 0,05$) than those recorded in the samples collected from buffalo in the study area.

DISCUSSION

The mean liver copper concentration of 25,6 ± 8,1 ppm WM for the control impala were similar to impala results reported by Boyazoglu *et al.* (1972) of 26,9 ± 11,9 ppm WM. It was therefore apparent that the

high concentrations of copper had accumulated in the livers of impala within the study area. Liver copper concentrations above the diagnostic copper poisoning concentration of 150 ppm WM for sheep (Suttle 1987a) were consistently measured in impala within the H-r zone.

A significant ($P \leq 0,05$) decrease in liver copper concentration collected from impala in the H-r zone through to the L-r zone occurred indicating that the risk of liver copper accumulation was progressively reduced in zones further from the mining complex. There was no significant ($P > 0,05$) difference in the liver copper concentration of impala culled in the L-r zone when compared with the control area.

Evaluation of the annual liver copper concentration data of impala over the entire study period of 5 years (Fig. 3) revealed significantly ($P \leq 0,05$) higher concentrations during 1989 ($n = 8$; mean liver copper concentration 217,3 ± 109,1 ppm WM) and 1992 ($n = 37$; mean liver copper concentration 203,3 ± 83,6 ppm WM) compared to the other study years (Fig. 2). This trend was only seen within the H-r zone, and not in the other two zones or control area. The higher liver copper concentrations of culled impala during 1989 and 1992 within the H-r zone is partially explained by periods of severe drought experienced prior to and during culling when an accumulation of copper on plant material had probably occurred. This was further exacerbated by poor grazing conditions, resulting in a negative energy balance and a high level of stress in the impala. Macroscopical signs of suspected chronic copper poisoning i.e. yellow discoloration, mottled and fatty appearance of the liver, were often seen during these two periods ($n = 23$). Histopathology confirmed the presence of haemosiderin casts indicating periodic breakdown of red blood cells which is also expected with chronic copper poisoning.

Three impala were confirmed to have died of chronic copper poisoning. Two of these cases were reported by Gummow *et al.* (1991) whereas an additional case of chronic copper poisoning was confirmed in a ram during November 1989. The macroscopic lesions and microscopic changes were consistent with the toxicological results obtained.

Kidney copper concentration of impala in the H-r zone was significantly ($P \leq 0,05$) higher than the kidney copper concentration determined from impala within the other zones and control area. This emphasized the possibility that multiple, periodic haemolytic crises had occurred in these animals within the H-r zone, thus lowering or depleting the copper stores of the liver. Small amounts of copper leaching from liver copper stores into the bloodstream also might also have been possible (Bath 1979).

Lung copper concentrations were analyzed to indicate whether the presence of airborne copper had resulted in copper accumulation in the lungs. Lung copper concentrations determined within the H-r zone were significantly ($P \leq 0,05$) higher than that measured elsewhere. The high concentration of copper in the lungs of impala confirms the presence of airborne copper in the Phalaborwa area. The progressive decrease in lung copper concentrations measured in impala within the M-r zone through to the L-r zone, gives an indication of the area of suspected air pollution. Impala culled within the control area had low lung copper concentrations which never exceeded 3 ppm WM. When comparing the lung copper concentrations of impala within the H-r zone for each year, it would seem that less airborne copper pollution occurred during 1993 compared to the previous 2 years. Impala herds remain fairly localized and it is most likely that impala within the H-r zone had probably remained there for prolonged periods. This localized movement was also seen with tracer animals released within the H-r zone, as only three impala wandered into the M-r zone. Increased liver and lung copper concentrations were observed in the tracer impala when compared to the control impala, further indicating that these animals had been exposed to an extraneous source of copper pollution.

The highest faecal copper concentrations were measured at dung heaps from impala within the H-r zone, which were significantly ($P \leq 0,05$) higher than within the control area.

Clinical pathology parameters indicated that AST activity could possibly be used as an indicator of chronic copper poisoning. AST activity was significantly ($P \leq 0,05$) higher in H-r zone impala when compared to control animals. However, care should be taken in evaluating high AST activities on their own, as liver damage can be due to other causes. AST activity seemed to be an accurate indicator of the pathology developing within the liver cells if done in conjunction with liver copper concentration and combined with the histological results. AST activity in conjunction with histology examination of liver samples collected through biopsies would appear to be a more acceptable method to evaluate the copper status of a group of animals, without the necessity of sacrificing any animal. In sheep, liver biopsies were used with satisfactory results to determine copper status (Donald, Paull & Langlands 1984). Unfortunately, in impala it would be impractical and expensive and was therefore not utilized. In domestic ruminants a GGT activity increase in conjunction with that of AST is regarded as indicative of liver damage. The position with GGT in impala seems to differ. GGT activity and plasma copper concentrations did not show any significant difference ($P \leq 0,05$) between impala from the study area and control animals.

The mean liver copper concentration of buffalo in the Phalaborwa area was significantly ($P \leq 0,05$) higher

than control buffalo culled elsewhere in the KNP, indicating that buffalo in the Phalaborwa study area accumulated more copper than the control buffalo. It was reported that buffalo were potentially at risk of copper poisoning when a mean copper concentration of 80,4 ± 44,9 ppm WM was determined during 1989 (Gummow *et al.* 1991). These concentrations decreased during the following years, as can be seen in Fig. 3. Buffalo moved over much greater distances when compared to impala and rarely spent prolonged periods within the H-r zone, thus minimizing their exposure to high copper concentrations.

Lung copper concentrations of buffalo in the Phalaborwa area were significantly ($P \leq 0,05$) higher than in buffalo culled elsewhere, confirming again the presence of airborne copper pollution in the Phalaborwa area.

The results of this study therefore confirm that impala and buffalo within the Phalaborwa area were exposed to high copper concentrations in their habitat and that air pollution was the most likely source of contamination. Impala, being more area bound, are more at risk of accumulating copper and therefore developing chronic copper poisoning. Sheep are very much more susceptible to chronic copper poisoning than cattle (Suttle 1987; Gummow *et al.* 1991). The impression was gained that an equivalent position exists with impala in relation to buffalo. Critical liver copper concentration required in impala for the occurrence of chronic copper poisoning is not known. Further controlled studies would need to be performed to determine these concentrations.

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