# CHAPTER 5 - HAEMATOLOGICAL STUDIES

## 5.1 Results

The results from the haematological studies are summarized in Figures 5.1 and 5.2. All values are represented as mean + standard error and the significant differences were determined using One- way ANOVA and Mann-Whitney U-Test ($p < 0.05$).

### 5.1.1 Leucocyte Count

During the 2002 survey Rietvlei had the highest leucocyte count (Figure 5.1A), followed by Barberspan, Olifantsvlei, Holfontein and then Roodekrans. There was a significant difference ($p < 0.05$) in the leucocyte count between Rietvlei and Roodekrans, Rietvlei and Olifantsvlei, Rietvlei and Holfontein, Barberspan and Roodekrans, Barberspan and Holfontein and Roodekrans and Olifantsvlei. During the 2005 survey Rietvlei had the highest leucocyte count again, followed by Roodekrans and Barberspan having the lowest. The only significant difference was between Barberspan and Rietvlei. At all the sites there was a significant difference between the leucocyte counts when comparing the results from 2002 and 2005. The leucocyte counts for all the sites of the 2002 survey were much higher than the 2005 count. This is attributed to a Type I statistical error and explained in the discussion.

### 5.1.2 Erythrocyte Count

Rietvlei had the highest erythrocyte count in the 2002 survey. Roodekrans had the lowest value, with the other three sites having very similar values (Fig. 5.1B). There was no significant difference between the sites. Rietvlei also had the highest erythrocyte count in the 2005 survey, followed by Roodekrans. The rural site, Barberspan, had the lowest count. There was a significant difference ($p < 0.05$) between erythrocyte counts from Barberspan and Rietvlei and Rietvlei and Roodekrans. At all the sites there was a significant difference ($p < 0.05$) between the erythrocyte counts when comparing the results from 2002 and 2005. The erythrocyte counts for all the sites of the 2002 survey were higher than the 2005 count.

### 5.1.3 Haematocrit

During the 2002 survey the haematocrit was determined at Barberspan, Rietvlei and Holfontein (Fig. 5.1C). The values were very similar with Rietvlei having the highest value and Holfontein the lowest. There was no significant difference ($p < 0.05$) between any of the sites. In the 2005 survey Barberspan had the highest haematocrit value, followed by Rietvlei and Roodekrans having the lowest. There was a significant difference ($p < 0.05$) in the haematocrit value between Barberspan and Roodekrans and Rietvlei and Roodekrans. When comparing the haematocrit values between the two sampling surveys, the haematocrit values are very similar. At Barberspan the haematocrit was slightly higher in 2005 than in 2002, whereas at Rietvlei it was slightly higher in 2002 than in 2005. There were no significant differences ($p < 0.05$) between the values from the two surveys at each site.

### 5.1.4 Haemoglobin Content

The haemoglobin content was the highest at Rietvlei during the 2002 survey. Holfontein had the lowest value (Fig. 5.1D). There was no significant difference ($p < 0.05$) between the haemoglobin content of the sites. During the 2005 survey Rietvlei had the lowest value and Roodekrans had the highest. There was a significant difference ($p < 0.05$) between the values of Rietvlei and Roodekrans. The haemoglobin content was a lot higher in 2002 than in 2005 for all the sites. There was a significant difference ($p < 0.05$) between the 2002 and 2005 values at all three sites sampled both years.

### 5.1.5 Mean Corpuscular Volume

The highest mean corpuscular volume value was at Holfontein in the 2002 survey (Fig. 5.2A). All the sites had similar values, except Roodekrans, which had very low values. There was a significant difference ($p < 0.05$) between the value of Roodekrans and al the other sites. During the 2005 survey Roodekrans had the highest value. The values for all the sites were very close and there was no significant difference ($p < 0.05$) between the sites. The mean corpuscular volume was also higher in the 2002 survey than in the 2005 survey for Barberspan and Rietvlei. However, at Roodekrans the mean corpuscular volume was much higher in 2005 than in 2002. The values for the two surveys were significantly different ($p < 0.05$) at Roodekrans, but not at Rietvlei and Barberspan.

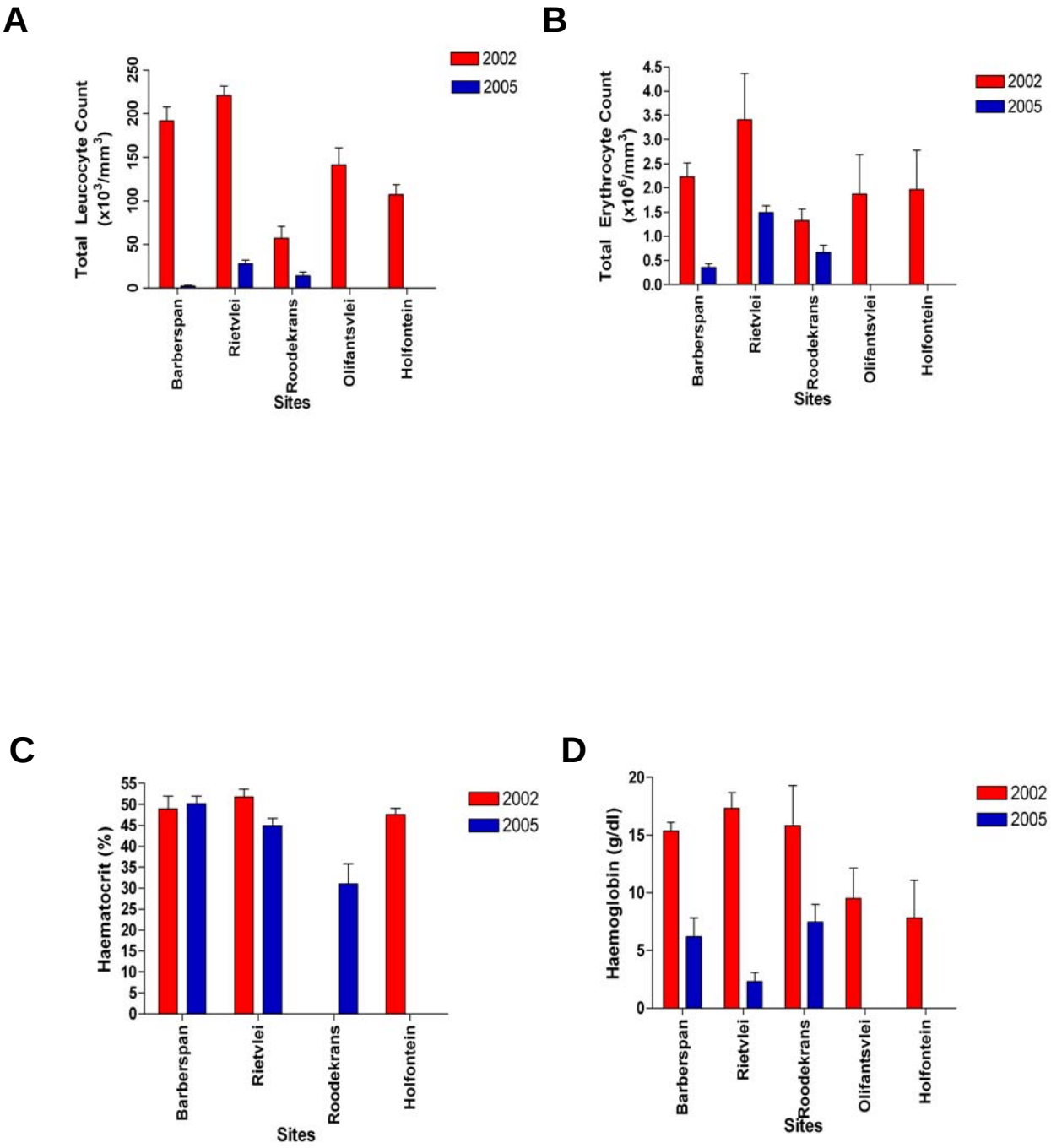


Figure 5.1 The total leucocyte count (A), Total Erythrocyte Count (B), Haematocrit (C) and Haemoglobin (D) for all the sites from the different surveys.

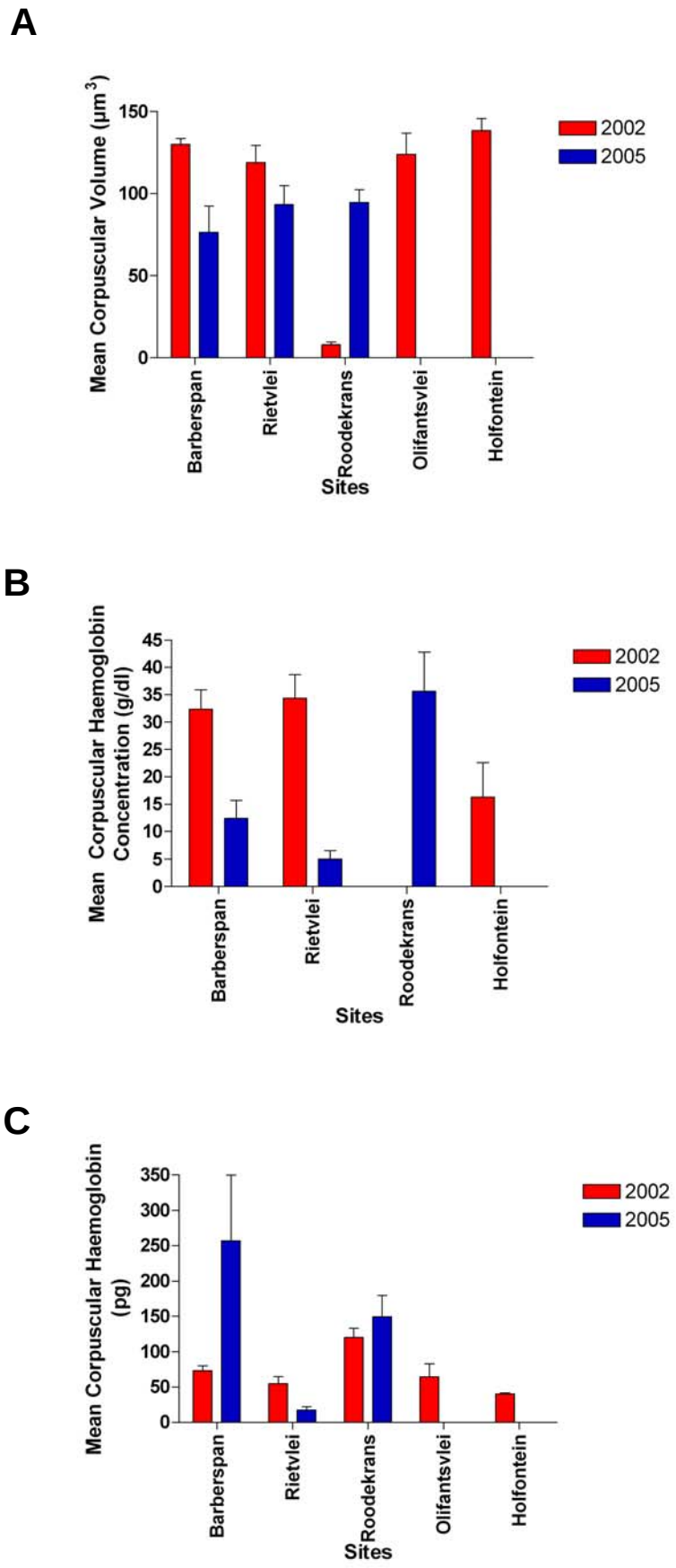


Figure 5.2 The mean corpuscular volume (A), Mean Corpuscular Haemoglobin concentration (B) and Mean Corpuscular Haemoglobin (C) for all the sites from the different surveys.

### 5.1.6. Mean Corpuscular Haemoglobin Concentration

The MCHC was the highest at Rietvlei, followed by Barberspan and then Holfontein in 2002 (Fig. 5.2B). There was no significant difference ($p < 0.05$) between the values of the different sites. During the 2005 survey the highest value was at Roodekrans and the lowest at Rietvlei. There was a significant difference ($p < 0.05$) between the values obtained at Barberspan and Roodekrans and Roodekrans and Rietvlei. The MCHC values were significantly higher ($p < 0.05$) in 2002 than in 2005 at both Barberspan and Rietvlei, with both sites having a significant difference ($p < 0.05$) between the two surveys.

### 5.1.7 Mean Corpuscular Haemoglobin

The MCH was the highest at Roodekrans in 2002 and the lowest at Holfontein (Fig. 5.2C). There was a significant difference ($p < 0.05$) between the values of Rietvlei and Roodekrans, Roodekrans and Olifantsvlei and Roodekrans and Holfontein. In 2005 the highest MCH value was at Barberspan and the lowest at Rietvlei. The only significant difference ($p < 0.05$) was between Roodekrans and Rietvlei. The MCH values were higher in 2005 than in 2002 at Barberspan and Roodekrans, but lower at Rietvlei. There was a significant difference ($p < 0.05$) between the two surveys at Rietvlei.



## 5.2 Discussion

### 5.2.1 Leucocyte Count

The common range for the number of leucocytes assumed for the majority of bird species is 15 to 30 x $10^3/mm^3$ (Sturkie, 1976; Van Wyk *et al*., 1998). Little haematological data is available for passerines, especially for small seed-eating weavers and sparrows. Mandal *et al.* (1986) found a normal leucocyte count of 15.68 x $10^3/mm^3$ for Baya Weavers (*Ploceus philippinus*) and 14.25 x $10^3/mm^3$ for House Sparrows. Birds treated with an organic pollutant had higher leucocyte counts (18 x $10^3/mm^3$ and 16.7 x $10^3/mm^3$ respectively) (Mandal *et al.*, 1986). Common Mynas (*Acridotheres tristis*) showed an even bigger difference between normal (13.33 x $10^3/mm^3$) and treated (24 x $10^3/mm^3$) values. Puerta *et al.* (1995) found higher values

for House Sparrows, 21.8 x $10^3$/mm$^3$ for early summer and 16 x $10^3$/mm$^3$ for late summer. Birds treated with testosterone had slightly higher values, 25 x $10^3$/mm$^3$. The values obtained during the 2005 survey falls within the common range of leucocytes for birds, except for Barberspan, which is significantly lower. The values for the 2002 are very high and fall outside the common range. The highest values measured by Manta et al. (1986) were for domesticated ducks, which was 22.9 x $10^3$/mm$^3$ normal and 23.15 x $10^3$/mm$^3$ for treated birds. Values as high as 51.2 x $10^3$/mm$^3$ have been reported for domesticated ducks (Hemm and Carlton, 1967). The leucocyte count measured by Alonso *et al.* (1991) for juvenile White Storks was 62.1 x $10^3$/mm$^3$. Although stress causes the number of leucocytes to increase, the abnormally high 2002 values may be due to instrumental error. For this reason the total leucocyte count was not used in the multivariate analysis of the different biomarkers (see Chapter 6).

### 5.2.2 Erythrocyte Count

The values for the total erythrocyte count were close to the values recorded for Baya Weavers (Mandal et al., 1986), House Sparrow (Mandal *et al.*, 1986, Puerta *et al.*, 1995) and various Neotropical passerines (Booth and Elliot, 2003). The total erythrocyte count decreases with an increase in stress to the bird (Mandal *et al.*, 1986). Smaller types of birds also tend to have a higher number of erythrocytes (Balasch *et al.*, 1974). There are many factors that influence the amount of erythrocytes, including, sex, age, time of day (Campbell and Dein, 1984). This could explain the differences between the sites and between the two sampling surveys. There were in general higher metal levels recorded in the 2005 survey (see Chapter 4), which could account for the lower erythrocyte counts.

### 5.2.3 Haematocrit

Only one value was below the range of normal values for birds (35 to 55%) used in avian veterinary science (Campbell and Dein, 1984). The values were also similar to those recorded for Baya Weavers (Mandal *et al.*, 1986), House Sparrows (Puerta *et al.*, 1995), Great Tits (*Parus major*) (Ots *et al.*, 1998) and various Neotropical passerines (Both and Ellot, 2003). According to Campbell and Dein (1984) haematocrit is the quickest and most practical method for evaluating the erythrocyte status of a bird. The haematocrit indicates the level of anaemia or dehydration, which

are both difficult to evaluate by physical examination alone. A haematocrit value below the normal range suggests anaemia and one above dehydration. There were very high levels of Al, Cd, Co, Cu and Pb at Roodekrans during the 2005 survey which might have lead to the low haematocrit (See Chapter 4).

### 5.2.4 Haemoglobin Content

Birds exposed to organic pollutants showed a decrease in the haemoglobin content (Mandal *et al.,* 1986). Results for Baya Weavers (Mandal *et al.,* 1986), House Sparrows (Mandal *et al.,* 1986; Puerta *et al.,* 1995), Neotropical Passerines (Booth and Elliot, 2003), Great tits and Pied Flycatchers (*Ficedula hypoleuca*) (Eeva *et al.,* 2000) were all similar to the range of values obtained for both the surveys. As with the total erythrocyte count, the metals levels were generally higher in the 2005 survey, which could lead to the lower levels of haemoglobin content (See Chapter 4).

### 5.2.5 Mean Corpuscular Volume

The values obtained during the 2005 survey were lower than the values reported for Baya Weavers (Mandal *et al.,* 1986) and House Sparrows (Mandal *et al.,* 1986; Puerta *et al.,* 1995). The values from the 2002 survey were however closer to these reported values, except for Roodekrans, which was a lot lower than the rest of the values. There were very high levels of Ni, Sr and Zn at this site in 2002, which might be the cause (See Chapter 4). This might be due to an increase in the number of new blood cells. This can be seen in the higher MCHC values at these sites (See Section 5.2.3 below). There was a decrease in the MCV of several bird species after exposure to a pesticide (Mandal *et al.,* 1986). The MCV also increases if the birds are stressed (Bearhop *et al.,* 1999).

### 5.2.6. Mean Corpuscular Haemoglobin Concentration

A decrease in the MCHC indicates a swelling of the erythrocytes (Pickering, 1981). The MCHC decreased in some bird species exposed to a pesticide, but increased in other species. It increased in the Baya Weaver and domestic duck, but decreased in the House Sparrow and Common Myna (Mandal *et al.,* 1986). Most of the values obtained in the study are lower than the values obtained for Baya Weaver (Mandal *et al.,* 1986) and House Sparrow (Mandal *et al.,* 1986; Puerta *et al.,* 1995). There is a variation in the MCHC values available for birds, with the highest value reported

being 58.25 g/dl (Rose-ringed Parakeet, *Psittacula krameri*, exposed to a pesticide) (Mandal *et al.*, 1986) and the lowest 28.7 g/dl (juvenile Great Bustard) (Alonso *et al.*, 1990). The values reported during this study are all lower than the highest value reported, thus maybe indicating that the values are normal.

### 5.2.7 Mean Corpuscular Haemoglobin

The MCH decreased in some bird species exposed to a pesticide, but increased in other species. It increased in the Baya Weaver and domestic duck, but decreased in the House Sparrow and Common Myna (Mandal *et al.*, 1986). The MCH values reported show a variation among species. Values as low as 34.6 pg (Kelp Gull, *Larus dominicanus*) and as high as 92.5 pg (Gentoo Penguin, *Pygoscelis papua*) have been reported (Melrose and Nicol, 1992). High values however indicate an adaptation to conditions where more oxygen is needed for increased metabolic processes. There is such a huge difference between the various values obtained during the study that no good comparison can be made with other species. Some values were however very similar to the values obtained for small passerines (Mandal *et al.*, 1986; Puerta *et al.*, 1995).

## 5.3 Conclusion



Although some studies have shown that certain blood parameters can be used as an indicator of the general health of the birds, they are too general to indicate stress due to specific metals. There are too many variables that influence the haematological parameters, including sex, age, season, diet, etc. (Campbell and Dein, 1984). But they can still be used to indicate the general state of health of the birds. After statistical analysis, no correlation was found between the level of the various metals and the values of the haematological parameters recorded. Thus the haematological parameters can be used as indicators of general health for the birds, but they are not sensitive enough and react to too many variables to be good bioindicators for metal pollution. They can however be used in conjunction with other biomarkers during multivariate analysis to show trends rather than specific values. This was done and the results can be seen in Chapter 6.