CHAPTER 6 - SPATIAL AND TEMPORAL VARIATION IN BIOMARKERS

6.1 Results

The results from the biomarker studies are summarized in Figure 6.1. All values are represented as mean + standard error and the significant differences were determined using one-way ANOVA and Mann-Whitney U-Test ($p < 0.05$).

6.1.2 DNA Damage

The percentage contribution of each average base pair length (ABPL) class to the overall DNA content is shown in Figure 6.1A. Most of the sites had an even distribution of the different ABPL size classes, with the largest size class having the highest percentage contribution. Olifantsvlei 2002 and Holfontein 2002 did however have larger contributions form the middle size classes than the other sites. Barberspan 2002 and Rietvlei 2002 differed from the rest with having a very high percentage contribution from the largest size class. The average base pair lengths (ABPL) of the DNA fragments are shown in Figure 6.1B. During the 2002 survey the longest ABPL was recorded at Barberspan. Rietvlei had a very similar length. The shortest was at Roodekrans. There was a significant difference ($p < 0.05$) between the APBL recorded at Barberspan and Roodekrans, Olifantsvlei and Holfontein. The same was true for Rietvlei and Roodekrans, Olifantsvlei and Holfontein. During the 2005 survey, the sites had very similar values, but Rietvlei had the longest APBL and Barberspan the shortest. There was no significant difference ($p < 0.05$) between the sites. There was a significant difference ($p < 0.05$) between the ABPL recorded for the two surveys at Barberspan and Rietvlei, but not at Roodekrans.

6.1.2 Catalase Activity

Holfontein had the highest catalase activity during the 2002 survey and Olifantsvlei had the lowest (Fig. 6.2A). There was no significant difference ($p < 0.05$) between the sites. During the 2005 survey Rietvlei had the highest catalase activity and Barberspan the lowest. There was also no significant difference ($p < 0.05$) between

the sites. When comparing the two surveys, there was a significant difference ($p < 0.05$) between the two surveys at Barberspan and Roodekrans. The values at these two sites were much higher in 2002 than in 2005. For Rietvlei the value recorded in 2005 was higher than the value of 2002.

6.1.3 Reduced Glutathione Content

The glutathione content was the highest at Rietvlei during the 2002 survey and the lowest at Olifantsvlei (Fig. 6.2B). There was no significant difference ($p < 0.05$) between the sites. Roodekrans had the highest glutathione content during the 2005 survey and Rietvlei the lowest. There was a significant difference ($p < 0.05$) between Roodekrans and the two other sites. The values recorded for 2005 were higher than the values for 2002. There is also a significant difference ($p < 0.05$) between the values recorded for the two surveys at Barberspan and Roodekrans, but not at Rietvlei.

6.1.4 Multivariate Analysis of the Biomarkers

Multivariate analysis based on Bray-Curtis similarity coefficients and group averaged sorting (Bray and Curtis, 1957) was performed on the data using the PRIMER (Plymouth Routines in Marine Environmental Research) program v4.0, (Plymouth Marine Laboratory). The biomarker data from the different sites and sampling surveys were analysed together. The biomarker data subjected to this analysis consisted of the data of ABPL, catalase activity, reduced glutathione content and all the haematological parameters except the total leucocyte count. Cluster analysis and multi-dimensional scaling (MDS) (Kruskal and Wish, 1978) were performed on the averaged data of each individual biomarker test for every site. For the averaged data the stress value (a measure of the accuracy of the results) of 0.01 was obtained, indicating that the ordination is an accurate description of the temporal bioaccumulation patterns (Clarke and Warwick, 1994). Based on the multivariate analysis, three distinct groupings can be distinguished (Figure 6.3 A and B). Group I consisted of only of Roodekrans 2002. Group II consisted of Barberspan 2005 and Roodekrans 2005. The last group (III) consisted of Rietvlei 2005, Barberspan 2002, Rietvlei 2002, Olifantsvlei 2002 and Holfontein 2002.

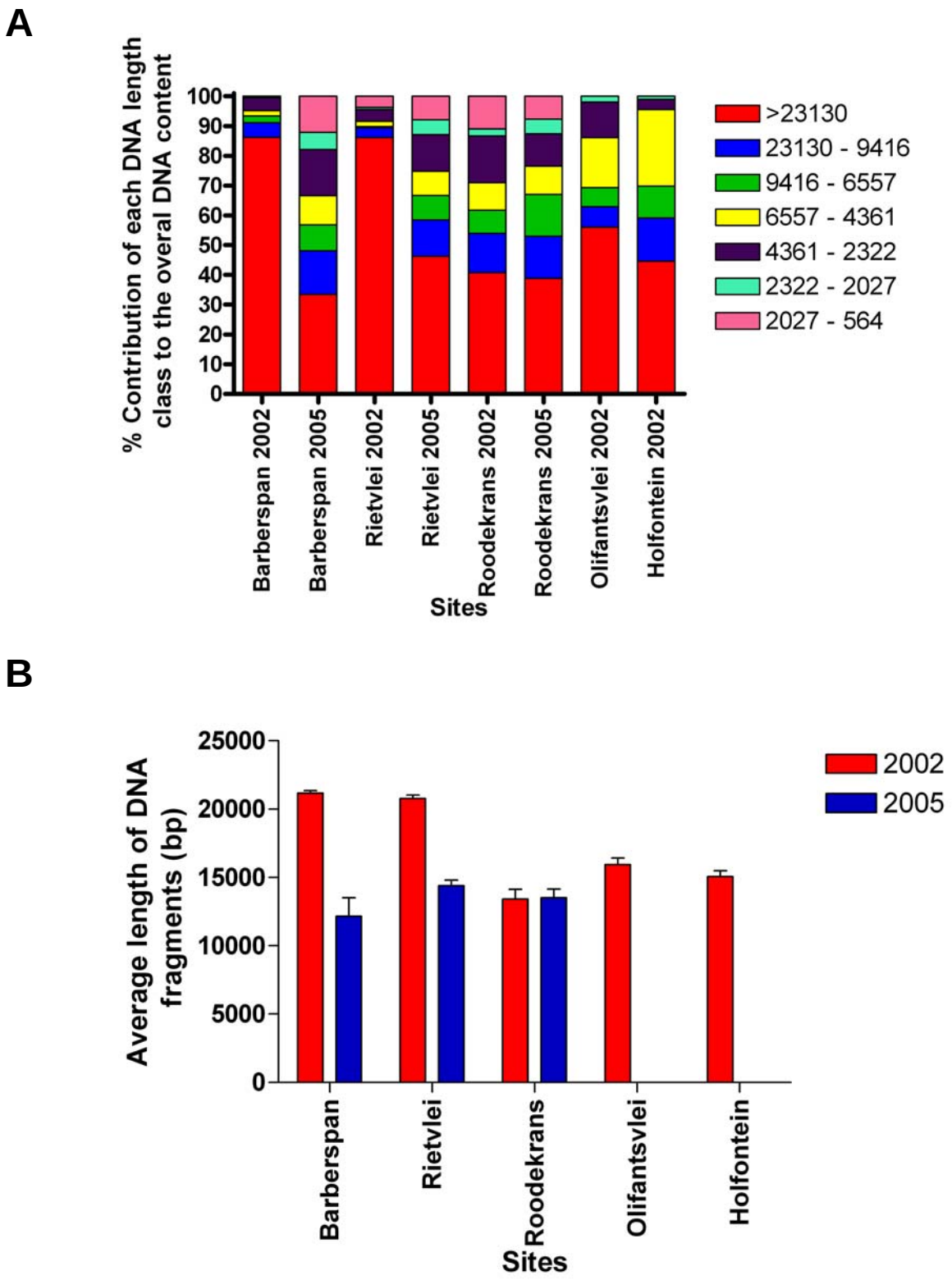

Figure 6.1 The percentage contribution of each DNA length class to the overall DNA content (A) and the average length of DNA fragments (B) at the sites during the two surveys. Table 6.1 provides a matrix of the significant differences between the sites ($p < 0.05$) for the average length of DNA fragments.

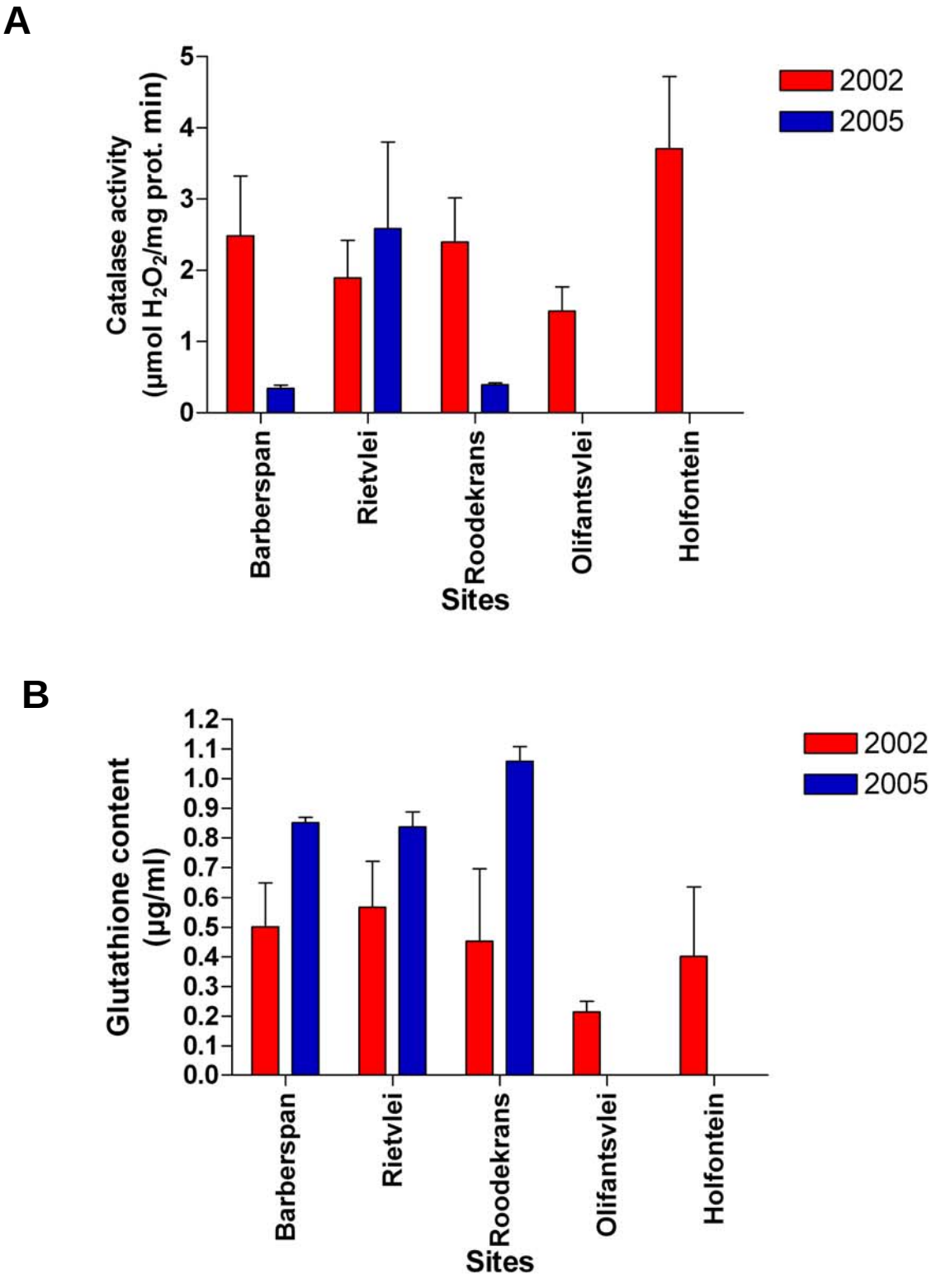

Figure 6.2 The catalase activity (A) and reduced glutathione content (B) at the sites during the two surveys. Table 6.1 provides a matrix of the significant differences between the sites ($p < 0.05$).

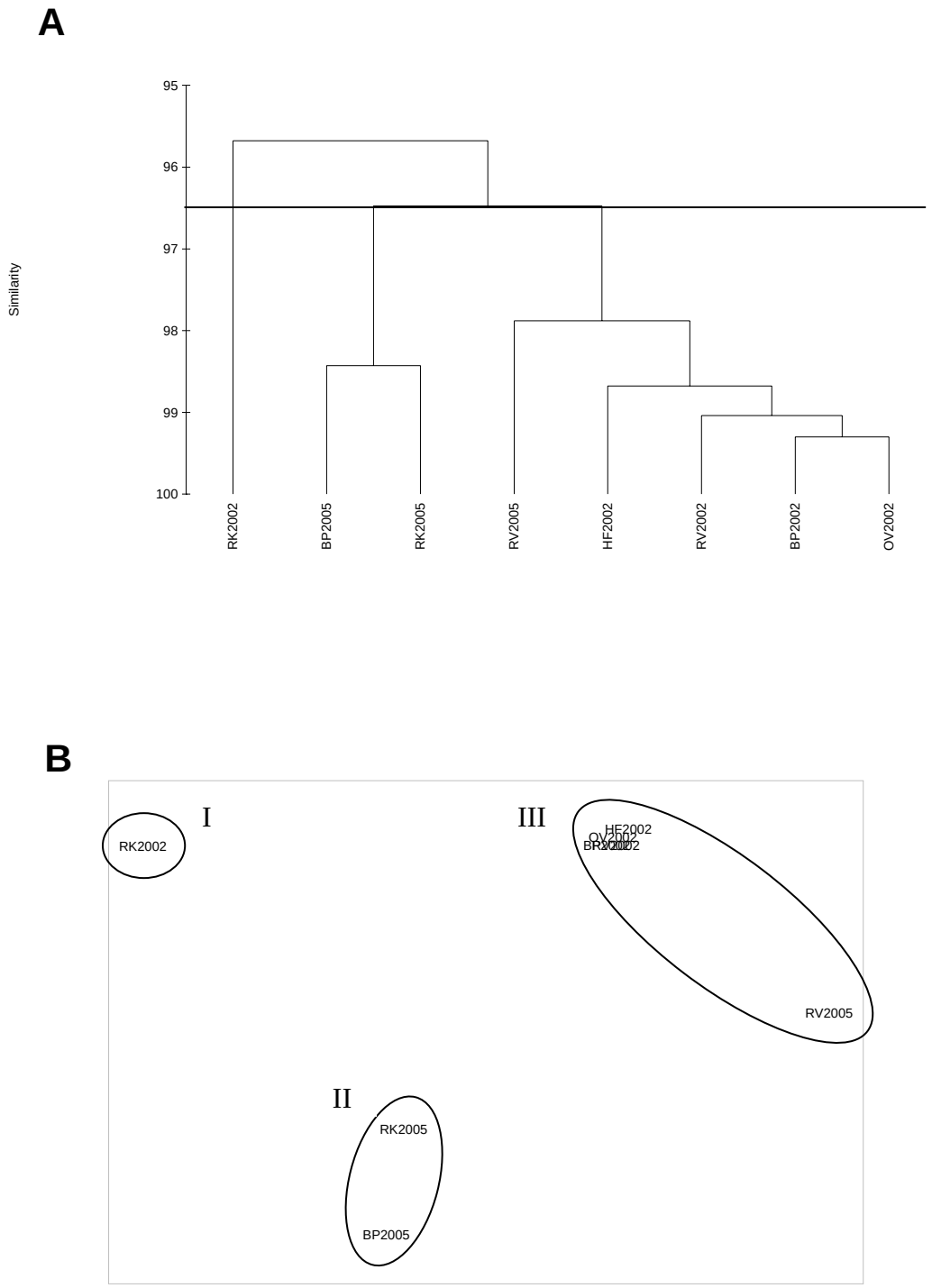

Figure 6.3 Dendogram (A) and MDS plot (B) constructed from multivariate analysis of the biomarker data at each site based on the similarities between the sites. Groupings were made at a similarity level of 96.5.

Table 6.1 The significant differences ($p < 0.05$) of the various biomarkers between the surveys. The sites, sampling periods and biomarkers are represented by the following abbreviations: BP02 = Barberspan 2002; BP05 = Barberspan 2005; RV02 = Rietvlei 2002; RV05 = Rietvlei 2005; RK02 = Roodekrans 2002; RK05 = Roodekrans 2005; OV02 = Olifantsvlei 2002; HF02 = Holfontein 2002; CAT = catalase activity; DNA = DNA damage; GLU = glutathione content.

	BP02	BP05	RV02	RV05	RK02	RK05	OV02
BP05	CAT; DNA; GLU						
RV02	-	-					
RV05	-	-	DNA				
RK02	DNA	-	DNA	-			
RK05	-	GLU	-	GLU	CAT; GLU		
OV02	DNA	-	DNA	-	-	-	
HF02	DNA	-	DNA	-	-	-	-

6.2 Discussion

The three biomarkers used in this study are biomarkers of genotoxicity and has been used in various exotocicological studies (Hoff *et al*., 2003; Li *et al.*, 2005; Pandey *et al.*, 2003; Sanchez *et al.*, 2005; Wepener *et al.*, 2005). These biomarkers test for the effects of pollutants, in this case metals, at both genetic and cellular level. When organisms are exposed to a pollutant, some or all these biomarkers will theoretically react in relationship to the severity of the pollution.

6.2.1 DNA Damage

DNA damage has been a useful biomarker in fish and freshwater molluscs (Hoff *et al*., 2003; Wepener *et al.*, 2005). DNA is still a relatively new technique and needs to be further developed, especially for birds. DNA damage has been proven to increase in White Storks and Black Kites (*Milvus migrans*) after massive exposure to heavy metal pollution (Pastor *et al*, 2001a; Pastor *et al*, 2001b). Rietvlei 2002 and Barberspan 2002 had significantly ($p < 0.05$) longer DNA fragment lengths than the other surveys. Rietvlei 2002 however had very high metal levels. Hoff (2003) found that perfluorooctane sulfonic acid and other organic pollutants may induce DNA repair and/or effect DNA breakdown. The self-repairing capability of DNA might affect the interpretation of genotoxic induced stress results (Connell *et al.*, 1999).

Wepener *et al.* (2005) also found that freshwater mussels from polluted sites had longer average DNA fragments than control sites, which indicate that the DNA repair system was induced. This might also be the case when there are very high metal levels, as with Rietvlei 2002. Barberspan is located in an area of intensive agricultural and other contaminants might also have contributed to the development of genotoxic stress. The fact that the other sites had very similar results for DNA damage, but different levels of metals, might show that the biomarker is not sensitive enough for bird blood and needs to be further investigated.

6.2.2 Catalase Activity

The level of catalase is usually higher under stressful conditions (Sanchez *et al.*, 2005). A threshold value may however be reached where the levels actually decrease when the stress is too high. Various studies found that high metal levels actually inhibited the catalase activity in gastropods (Moolman, 2004) and fish (Pandey *et al.*, 2003). This may be the case for Roodekrans 2005, which had very little catalase activity but very high metal levels. The level of catalase activity at Barberspan in 2005 was very low, although the metal levels were not that high. However, the metal levels were higher than those from sites with higher catalase activity. Other contaminants might have played a role. There was no correlation at the other sites regarding their metal levels and the level of catalase activity.

6.2.3 Reduced Glutathione Content

Various studies have shown an increase in the reduced glutathione content in biota exposed to pollutants (Li *et al.*, 2005; Pandey *et al.*, 2003). Hoffman (2002) showed that there is a positive correlation between selenium levels and the levels of reduced glutathione in birds. In this study, sites with high metal levels also had higher levels of reduced glutathione. The surveys from 2005 generally had higher metal levels in 2002. They also had higher levels of reduced glutathione. The survey at Rietvlei in 2002 also had higher levels than the other surveys in 2002, but it was not significantly higher. It did have higher metals in general as well. Thus glutathione seems to be a good bioindicator of metal pollution in this study. Isaksson (2005) however suggests that the ratio between oxidised and reduced glutathione should be used, to overcome the possibility of physiological adaptation.

6.2.4 Multivariate Analysis of the Biomarkers

There was not that much differences between the different sites and surveys regarding the biomarker responses. The similarity level between the sites for the biomarkers was very high (96.5%), especially when compared to the metals levels (80% - see Chapter 4). This shows that there was not that much spatial and temporal difference in the response of the biomarkers. In general it can be concluded that three groupings based on the magnitude of the collective biomarker and haematological responses could be distinguished, with group II having the highest level of stress, followed by group I and then group III with the lowest. This follows the pattern of metal levels, as the sites with the highest level of metals falls into group I and II, except for Rietvlei 2005. Thus although the individual biomarkers gave varying results, when using multi-variate analysis there seems to be a correspondence to the level of organism stress and the metals levels in the feathers.

6.3. Conclusion

Not all the biomarkers gave clear dose-response results. Thus other biomarkers also need to be investigated for their use in birds. Some biomarkers need to be expanded, for example using the ratio between oxidised and reduced glutathione instead of just the reduced glutathione content. Laboratory studies on birds are also needed to test whether there are clear dose-response relationships between the various biomarkers and the various metals. The three biomarkers used in this study are biomarkers of genotoxicity. Looking at the multi-variate analysis of both the biomarkers and the metals, it can be seen that the sites with the higher metal levels mostly have corresponding results in the biomarkers. Thus the metals induced the appropriate biomarker action. Investigation into the levels of other contaminants present, especially organic contaminants, should also be taken at the study sites, as these might influence the biomarker results. However, as there seem to be similar groupings after multivariate analysis for both metal levels and biomarker responses, feathers seem to be a good indicator of metal contamination.