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Mercury concentrations in eggshells of the Southern Ground-Hornbill (*Bucorvus leadbeateri*) and Wattled Crane (*Bugeranus carunculatus*) in South Africa

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ABSTRACT

In this study, wild hatched eggshells were collected from the nests of threatened Wattled Crane and South Ground-Hornbill in an attempt to determine their total Hg concentrations. A total of fourteen eggshell samples from both bird species were collected from different study areas in the Mpumlanga and KwaZulu-Natal Provinces of South Africa. The eggshells were acid digested under reflux and their total Hg concentrations were determined using cold-vapour atomic absorption spectrophotometry (CV-AAS). The observed total Hg levels for the South Ground-Hornbill samples ranged from 1.31 to 8.88 μg g^{-1} dry weight (dw), except for one outlier which had an elevated 75.0 μg g^{-1} dw. The levels obtained for the Wattled Crane samples were relatively high and these ranged from 14.84 to 36.37 μg g^{-1} dw. Generally, all the measured total Hg concentrations for the Wattled Crane samples exceeded the estimated total Hg levels derived for eggshell which were known to cause adverse reproductive effects in avian species from previous studies. Based on these findings, it is, therefore, possible that the exposure of these birds to elevated Hg may have contributed to their present population decline.

1. Introduction

Mercury (Hg), in its different forms, has become an important environmental contaminant of global concern where several natural and anthropogenic sources continue to contribute to its growing release into the environment. Notably, emissions from coal-fired power stations and artisanal gold mining practices were identified as the major anthropogenic Hg sources (Williams et al., 2010; Walters et al., 2011). In South Africa, where there is unprecedented dependence on coal for energy generation, coal combustion has remained a leading source of Hg pollution. Presently, South Africa is ranked amongst the highest emitters of Hg contributing more than 10% to the global Hg emissions (Pacyna et al., 2010).

Both the South Ground-Hornbill (SGH) (*Bucorvus leadbeateri*) and Wattled Crane (WC) (*Bugeranus carunculatus*) populations have been declared to be under significant threat (Burke and Rodwell, 2000). Presently, these two large avian species are among the South African wild birds listed on the International Union for the Conservation of Nature (IUCN) red list of globally threatened birds as vulnerable and critically endangered, respectively (Burke and Rodwell, 2000). These birds are widely distributed in the southern Africa region, particularly in Angola, Botswana, Democratic Republic of Congo, Malawi, Mozambique, Namibia, Zambia and South Africa.

Regardless of their geographical location, habitat destruction which is mostly linked to increased agricultural, industrial and other human-related activities has been implicated as possible factors contributing to their rapid population decline. Furthermore, increased livestock grazing and anthropogenic disturbances in close proximity to their breeding sites are also responsible for their declining breeding success. It has also been reported that accidental and purposeful poisoning of both species do occur, particularly where poisoned baits set out for problematic animals or baits laid out specifically to kill birds for the traditional medicine market have been ingested (Burke and Rodwell, 2000; Engelbrecht et al., 2007). While the various physical threats and their

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impacts on the continued survival of these threatened birds have been documented, the potential deleterious effects as a result of their exposure to elevated levels of harmful environmental pollutants, particularly within their habitat, have not been properly considered. The present study, therefore, aims to evaluate the total mercury concentrations in eggshells of these threatened birds whether they exceed the effect thresholds previously established for other species in the literature.

2. Materials and method

2.1. Sampling sites, sample collection and treatment and instrumental analysis

In this study, the total Hg concentrations of fourteen (14) hatched eggshell samples were determined. Of these, eight (8) eggshell samples were obtained from the Ground-Hornbill, which were collected from different nest sites within the Kruger National Park during 2012 and 2013 breeding seasons. The remaining six (6) were crane eggshells, which were also collected from different nest sites within the KwaZulu-Natal midlands in 2011. The limited sample size employed in this study was due to the present conservation status of both bird species as well as their unique reproductive behaviours where fully matured female birds only lay a maximum of two eggs. For clarity, individual eggshells from both species were collected from different nests. The detailed description of the crane samples is provided in the Supplementary Table 1.

With respect to their reproductive and breeding behaviours, these birds are known to lay a maximum of two eggs but only rear one chick. Usually, the first chick hatched by the Ground-Hornbill, for instance, outcompetes its younger and weaker sibling, thus only one chick usually survives (Kemp and Kemp, 1991). Incidentally, the only surviving chick could also be lost via other forms of man-made and natural threats, including forest fires, poaching or even as prey to some carnivorous animals. As a result of their population decline in South Africa, several conservation efforts, including the Wattled Crane Recovery Programme (WCRP) and Mabula Ground-Hornbill Conservation Project were initiated to mitigate these identified physical threats (Barnes, 2000; Theron et al., 2008).

The crane eggshells were collected from each nest once the eggs had hatched and the chicks had moved off with the parents. Similarly, hatched eggshells of the Ground-Hornbill were removed from each nest by staff of the Mabula Ground Hornbill Project at the time the second chick was collected for hand-rearing purposes. Incidentally, these sampling locations are situated in close proximity to important hotspots for Hg in their respective localities.

During the sample preparation, the dried membrane in the crane samples were carefully separated from the eggshells. The cleaned and dried eggshell were then ground and stored in well labelled self-sealing polyethylene bags. In contrast, due to the fragile nature of the Ground-Hornbill samples which prevents the separation of eggshell from the dried membrane, each sample was treated as one ground mass.

The sampling locations as well as the associated activities related to Hg releases into the environment are shown in Fig. 1. It is equally important to point out that a large open cast copper mine with smelter and a phosphate mine is located south of the sampling site, the Kruger National Park, and the Umgeni River catchment traverses the crane sampling location. In the 1990s, Hg-containing effluents were reportedly discharged into the Umgeni River catchment and consequently, this area is now considered as “historically contaminated with Hg” within the KwaZulu-Natal Province. Several years following this Hg spill, monitoring studies conducted indicated total Hg levels exceeding the global average of 5 ng L^{-1} for surface waters within the Umgeni River catchment (Williams et al., 2011).

The procedures for the determination of total Hg in eggshell samples in this study were derived from the established cold vapour atomic absorption spectrometric (CV-AAS) technique (USEPA Method 245.1, revision 3), previously developed for the determination of Hg in water samples, with some modifications. Briefly, approximately 1.0 g of dried eggshell sample was weighed and transferred into clean round bottom flask. The eggshell sample was suspended in 100 mL of ultrapure water (Siemens LaboStar™

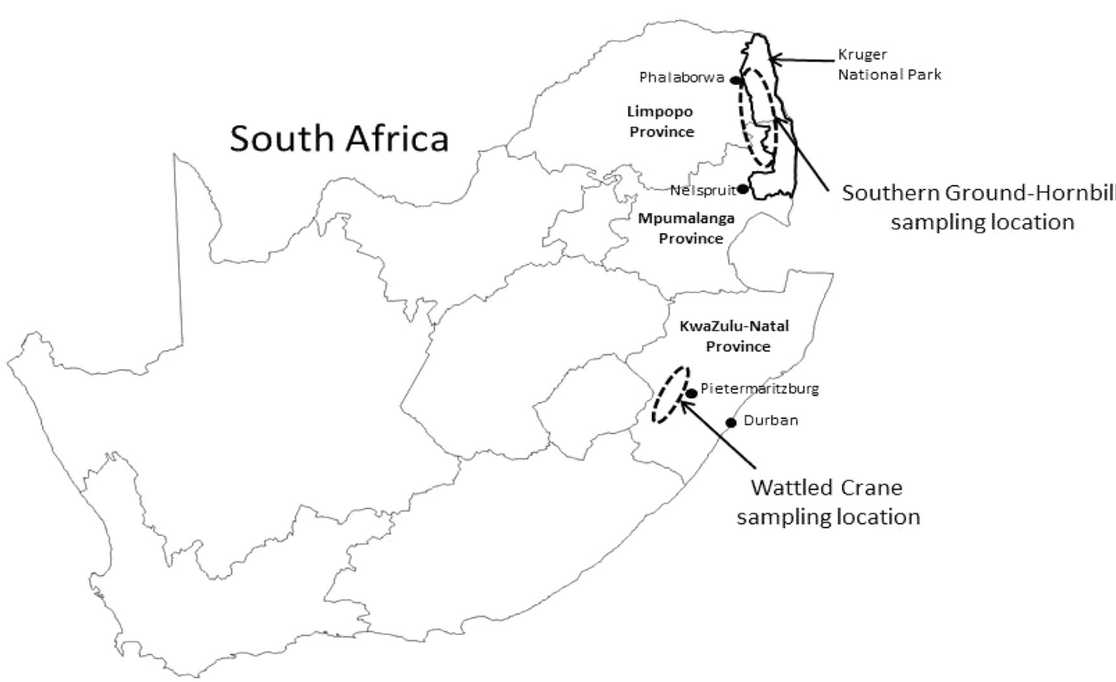

Fig. 1. Map of the study areas showing the sampling locations within the three South African Provinces.

D1 4 system, Germany) and 5 mL of 0.5 N H_2SO_4 and 2.5 mL of HNO_3 (1+1) (Sigma-Aldrich, South Africa) added. Thereafter, 15 mL of freshly prepared $KMnO_4$ (50 g in 1000 mL) (Sigma-Aldrich, South Africa) was added followed by the addition of 8 mL of $K_2S_2O_8$ (25 g in 500 mL) (Sigma-Aldrich, South Africa). Finally, five acid pre-washed boiling chips were added to prevent bumping during heating. The samples were then heated under reflux for 2 h on a heating block and, thereafter allowed to cool down at room temperature. The samples were then filtered and 6 mL of sodium chloride-hydroxylamine hydrochloride solution was added to reduce the excess permanganate. The samples were then made up to the 250 mL mark and kept in the refrigerator until analysis.

The total Hg concentrations of the eggshell samples was determined with a Shimadzu AA-6300 Atomic Absorption Spectrometer (Tokyo, Japan) equipped with a mercury vapour unit. The optimum conditions employed for the Hg analysis are presented in Supplementary Table 2. The analysis was performed in a close-circuit system and the Hg vapours leaving the absorption cell were trapped in $KMnO_4$ solution contained in the waste bottle.

2.2. Quality control and quality assurance

To avoid possible contamination, glassware was soaked in dilute nitric acid for at least 24 h. Similarly, the pestles and mortars employed for grinding were thoroughly washed and dried overnight in the oven. Laboratory reagent blanks (LRBs) as well as laboratory fortified blanks (LFBs) were prepared to assess potential contamination within the laboratory and to ascertain the laboratory performance, respectively. The mean Hg levels in the LRBs were deducted from all the results obtained.

Furthermore, matrix spike experiments were performed where a laboratory matrix (domestic chicken eggshell) was employed. In this case, each of the three replicates was spiked with 0.5 μg of Hg in order to determine the accuracy of the method. Recoveries ranging from 66.4% to 97% were obtained which were consistent with the USEPA recommendations for total Hg determination, the results obtained in this study were not recovery corrected. However, due to the compositional differences between the laboratory matrix and the real eggshell samples, the possibility of inconsistency in the overall accuracy of the method cannot be completely ruled out. As a result, the reported Hg levels in the analysed samples may have been underestimated.

3. Results and discussion

3.1. Inter-species variations in total Hg concentrations of eggshell samples

The results obtained for the total Hg determination in hatched eggshells of the investigated birds are presented in Table 1. Due to the limited sample quantities, only single measurements were performed for all the Ground-Hornbill samples. However, duplicate analyses were performed for most of the crane eggshell samples except for samples I and J, where single measurements were performed. Generally, the total Hg concentrations were relatively higher in the crane samples than those found in the Ground-Hornbill samples. Nevertheless, the highest total Hg concentration (75 $\mu g\ g^{-1}$, dw) was obtained in a Ground-Hornbill sample which was several orders of magnitude higher than those found in other eggshells from the same bird species in this study. With the exclusion of this outlier, the overall mean total Hg concentration for the cranes (23.45 $\mu g\ g^{-1}$, dw) was about five-fold higher than that obtained for the Ground-Hornbill samples.

Generally, the total Hg variations between these two species can be due to a number of factors or combinations of exposure variations, trophic level considerations, locational or geographical differences in Hg concentrations in soil, water and air and species-specific absorption rates (Burger and Gochfeld, 1997). It is equally necessary to take into account that the total Hg concentrations in avian eggs are mostly a reflection of the recent dietary uptake rather than via contributions from accumulated tissue stores (Wolfe et al., 1998). Therefore, the measured total Hg concentrations in the eggshells of both species could serve as a reliable indicator of exposure to Hg in the vicinity of their breeding site prior to their laying season.

The Ground-Hornbill is a predatory species in terrestrial grasslands, savannah and woodlands where its diet ranges from a variety of invertebrates, reptiles, amphibians, tortoises to small mammals; whereas the crane forages predominantly in wetlands for tubers and grass seeds but they have also been known to feed on small aquatic snails, fish and frogs and sometimes, their young chicks also feed on insects (Hancock, 2003). These factors could further explain some of the observed differences in the total Hg concentrations measured in their eggshells.

Recent findings suggest that mining activities could significantly contribute to excessive accumulation of selected heavy metals in certain plant species (Kendall, 2014). Given the dietary preferences of these birds, the uptake of Hg via the consumption of aquatic flora and fauna might represent important pathway for Hg accumulation contributing to the relatively high total Hg levels observed amongst the crane species. Other possible sources of Hg within the study areas include the prolong use of mercury-based pesticides and through the unpopular artisanal gold mining practices. Furthermore, the sampling sites for cranes are situated within the same water management area (WMA) known to have been historically contaminated with Hg.

A major disadvantage with respect to the use of birds as bio-indicators of environmental pollutants is their mobility. Consequently, the observed pollutant levels in any part of a bird might not necessarily reflect its immediate environmental conditions. However, the use of avian eggshells as indicators of environmental contaminants is rather advantageous as it often reflects the exposure of female birds to harmful contaminants within their nesting sites prior to egg laying. Generally, there was less disparity in the mean total Hg concentrations in crane eggshells, which ranged between 14.84 and 28.42 $\mu g\ g^{-1}$, dw when compared with those of the Ground-Hornbills (1.31–75.0 $\mu g\ g^{-1}$, dw). With the

Table 1
Concentrations of the total Hg ($\mu g\ g^{-1}$, dw) in eggshell samples of Southern Ground-Hornbill and Wattled Crane.

Species (SGH)								
Sample code	A	B	C	D	E	F	G	H
Concentrations	5.10	6.37	2.01	75.0	8.88	1.31	2.73	6.04
Species (WC)								
Sample code	I	J	K	L	M	N		
Mean	16.29	14.84	21.26	28.42	25.40	26.59		
Range	–	–	17.42 – 25.10	20.48–36.37	24.88–25.91	22.23–30.95		

SGH—South Ground-Hornbill; WC—Wattled Crane; dw—dry weight.

Table 2
Estimated total mercury levels in avian eggshells (ppm) associated with adverse effects in birds derived from total mercury levels established for egg content.

Species	Reported level (ppm) for egg content	[a]Estimated equivalent level (ppm, dry weight) for eggshell	Observed effects	References
Pheasant (*Phasianus colchicus*)	0.05–1.5 (ww)	0.0015–0.045	Reduced hatchability Reduced chick survival Reduced egg weight Shell-less eggs	Fimreite (1971)
Pheasant	0.9–3.1 (ww)	0.027–0.093	Reduced hatch rate (by 50–80%) Reduced chick survival	Spann et al. (1972)
Black duck (*Anas rubripes*)	5.53 (ww)	0.166	Lower hatchability	Finley and Stendell (1978)
Mallard (*Anas platyrynnchos*)	0.53 (ww)	0.016	Increased mortality Decreased embryonic growth 58% abnormal chicks	Hoffman and Moore (1979)
	0.7 (ww)	0.021	Embryo malformation 27% abnormal chicks	Heinz (1975)
	0.5–0.9 (ww)	0.015–0.027	Lower hatching rate	Heinz (1974), Heinz (1976a), Heinz (1976b)
			Decreased embryonic growth 58% abnormal chicks	
	5.46 (dw)	0.049	Reduced survival rate	
	1.0 (ww)	0.030	Chicks hyper-responsive to frightening stimuli	Heinz (1976b)
	5.46 (dw)	0.049	Eggs of chicks from eggs with these levels had thinner shells	Heinz (1979)
Common tern (*Sterna hirundo*)	3.65 (ww)	0.110	Reproductive failures	Fimreite (1974)

(Adapted from Burger and Gochfeld, 1997)

[a] Estimated based on the assumption that the moisture content of fresh egg is 70% and 0.9% total Hg allocated to the eggshell; ww—wet weight; dw—dry weight; ppm—parts per million.

exclusion of considerably high total Hg from one particular Ground-Hornbill sample, there was no apparently large difference in the total Hg concentrations amongst the Ground-Hornbill samples. This elevated sample may be in close proximity to a potential Hg source within the study areas. It is, however, difficult to associate the outlier with any possible sources of Hg within the study area due to their mobility. For example, it has been reported that the Ground-Hornbills within the Kruger National Park, may have clearly defined home ranges between 10 and 20 ha (Theron et al., 2013). On the other hand, the cranes are largely non-migratory and will only travel outside their foraging sites if there is severe seasonal water shortage (Ndirima, 2007).

4. Assessing ecological risks associated with Hg levels

Owing to the present status of these threatened bird species, the analyses of fresh eggs, which is conventional to assessing pollutant's levels in avian eggs, is highly discouraged. Since it is crucial to understand the underlying factors contributing to their declining population trends, the use of hatched eggshells is an attempt to evaluate the extent of their exposure to environmental Hg.

For avian species, the most important toxicological endpoints associated with exposure to Hg are those related to reproduction. These could produce effects such as reduced hatchability, eggshell thinning, reduced clutch size, and increased numbers of eggs laid outside the nest, aberrant behaviours of juveniles, amongst others. Therefore, the relationship between the observed total Hg concentrations in avian eggs or eggshells and their associated adverse effects are useful indicators to evaluate their potential reproduction risks.

Laboratory studies indicate that mercury levels of 1.5 $\mu g\ g^{-1}$ in avian species are associated with adverse effects, including impaired reproduction (Aliakbari et al., 2011). Unfortunately, it is difficult to make a good extrapolation employing this benchmark which is established for the whole egg samples since the total Hg concentrations were only obtained for the eggshell samples in the present study. It is, therefore, needful to rely on the available information in the literature to predict Hg distribution in the different components of the egg of avian species. One such study recently reported for Gentoo Penguins showed that up to 92% of the total Hg in their eggs were concentrated in the albumen, 6.7% in the yolk, 0.4% in the membrane and 0.9% in the eggshell (Brasso et al., 2012).

To derive an appropriate adverse effect estimate using the 0.9% composition of total Hg in an eggshell, a concentration of 0.0135 $\mu g\ g^{-1}$ wet-weight is obtained as a suitable cut-off for assessing the potential reproductive risks via exposure of these birds to environmental Hg. For better interpretation of the obtained results, it will be necessary to convert the predicted adverse effect estimate on a dry-weight basis. Assuming the average moisture content of these eggs is 70%, then the estimated adverse effect level of total Hg on dry-weight basis will be equivalent to 0.4054 $\mu g\ g^{-1}$. A comparison of the total Hg concentrations obtained for the eggshell samples for both bird species in this study and by using this benchmark, the total Hg levels in all our samples exceeded the estimated adverse effect benchmark. Based on these calculations and other extrapolations made from previous studies (presented in Table 2), we conclude that the exposure of the investigated birds to elevated environmental Hg may be contributing negatively to the reproductive success of these species and subsequently contributing towards their rapidly declining populations.

There are a number of other important factors that need to be considered while evaluating the ecological risks to avian populations. Some of these include: the age of the bird, longevity, maximum body weight, feeding and excretion rates, fledging age, reproductive maturity, length of stay at breeding sites prior to egg laying, type of habitat (protected area or wild), amongst others. For instance, these crane and Ground-Hornbill species are relatively large birds with a maximum body weight of up to 7.9 kg and 4.6 kg, respectively. Their average lifespan is also estimated to be 20–30 years and 30–40 years, respectively. So far, there are no laboratory reports on the total Hg levels that can produce reproductive impairment for these species. Therefore, it would be difficult to associate the total Hg levels in the eggshells of these birds with the adverse effects estimated for other avian species without taking the aforementioned factors into consideration.

High levels of ingested contaminants are often found and reflected in the eggs of birds. Sometimes, up to 5–20% of the contaminants present in their diet could be eliminated via egg laying (Falkowska et al., 2013). It remains unclear whether the elimination of harmful contaminants via egg laying is an important adaptation strategy often adopted by exposed female birds. We suggest that future studies should be focused on this possibility. Ultimately, there is a need to also look into the possible synergistic interactions of different pollutants and how these interactions contribute to the declining population of these threatened bird species.

5. Conclusions

In this study, the possible contributory roles of Hg to the declining population of both the Wattled Crane and South Ground-Hornbill were evaluated and the observed total Hg levels in their eggshells were considerably higher than those reported for other avian species investigated thus far. An assessment of the potential ecological risks that could be posed by the observed levels was performed and this revealed that these species may be at risk and susceptible to adverse effects of high Hg concentrations which may include lower hatchability (impaired embryonic growth), reduced clutch size and reproductive failures, amongst others. Because of the species-specific differences amongst the avian species, we suggest that other factors such as the age, longevity, body weight, feeding and excretion rates, fledging age, and length of stay at the breeding sites prior to egg laying, amongst others, be considered, if the potential ecological risks that could be posed by a pollutant is to be evaluated for avian species with no established dose-response data. Finally, this study demonstrates that hatched eggshells of avian species, particularly those that are considered threatened, may be used non-invasively to evaluate their extent of exposure to environmental Hg.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2015.01.010.

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