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Associations between DDT and egg parameters of the House Sparrow *Passer domesticus* from the Thohoyandou area of South Africa

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HIGHLIGHTS

- DDT influences the eggshell thickness of House Sparrow eggs.
- Pore numbers and - densities of eggshells are not significantly influenced by DDTs.
- Pore shape and volume density of pores is not affected by DDT.

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ABSTRACT

This study investigated whether the pesticide DDT (Dichlorodiphenyltrichloroethane) and its metabolites, DDE (Dichlorodiphenyldichloroethylene) and DDD (Dichlorobischlorophenylethane) were associated with adverse effects on multiple endpoints of the eggs of House Sparrows from the Thohoyandou area in South Africa, where DDT is used for malaria control. Eggshell thickness, pore numbers, pore shapes, and volume densities of the pores were measured to test possible adverse effects. Analysis was done using a scanning electron microscope and the concentrations of the pesticides were determined with the aid of gas chromatography-mass spectrometry. The highest concentrations recorded was *p,p′*-DDE at 0.84 μg/g wm (wet mass) in the eggs collected from Mangondi (a site last sprayed five years before sampling). Overall, the concentrations of total DDT recorded in this study were lower than reported by most other studies conducted in the same area. The association between DDT concentrations and House Sparrows eggshells were noticeable in the eggshell thicknesses, with significant differences between the eggs collected from Muledane (a site last sprayed 30 years before sampling) and Makula (a site sprayed both years of sampling) ($P < 0.0022$). Limited differences were found between the pore numbers and pore density of eggshells from the various sites. It may be that the limited effect on the pore numbers and volume densities of the pores are associated with low concentrations of DDT in the House Sparrow eggs.

1. Introduction

Malaria is a deadly disease caused by the protozoan parasite (*Plasmodium* sp.) that is transmitted from human to human by the *Anopheles* mosquito (Roberts and Janovy, 2009). The pesticide DDT (Dichlorodiphenyltrichloroethane) can be used under the Annex A restrictions of the Stockholm Convention (Parts I and II) for the purpose of malaria vector control (Stockholm Convention on POPs, 2010). DDT and its metabolites are toxic in nature, have a long half-life of ~5–15 years, are transported over great distances in water, wind and biota, and have the ability to bio-accumulate in the food web (Wells and Leonard, 2006). The effect of pesticides and especially DDT on bird eggs is a topical and environmentally relevant research area (De Kock and Randall, 1984; WHO, 2002; Bustnes et al., 2006; Vasseur and Cossu-Leguile, 2006; Polder et al., 2008; Bouwman et al., 2007, 2013, 2015). DDT and its metabolites, DDE (Dichlorodiphenyldichloroethylene) and DDD (Dichlorobischlorophenylethane) are known to have both lethal and sub-lethal effects on birds (Fry, 1995). According to Zimmermann et al. (1997), *p,p′*-DDE is responsible for delayed sexual maturity and altering mating behaviour which includes the ability of birds to successfully

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incubate as well as attend to the eggs and feeding the chicks (Bustnes et al., 2001). *p,p′*-DDE leads to thinning of the eggshell (Brunstrom et al., 2002). This isomer acts as an androgen receptor antagonist and is responsible for the inhibition of testosterone secretion (Carlsen et al., 1992; Kelce et al., 1995; Danzo, 1997). Organochlorine pesticides inhibit the carbonic anhydrase production by the shell gland that results in softer shells as well as shell-less eggs (Lundholm, 1990). Japanese Quails (*Coturnix japonica*) experienced 10% decrease in their eggshell calcium being fed DDT in their diet (Bitman et al., 1969). In Peregrine Falcons (*Falco peregrinus*) DDE caused eggshell thinning in 1945–1960, concentrations of 0.6–9.3 μg/g wm showed a 1.83 to 1.36 μm variation in the eggshells (Peakall et al., 1976). In addition to its effect on eggs, DDT and its metabolites also affects the hatching success and survival rate of the birds (Aurigi et al., 2000).

The House Sparrow (*Passer domesticus*) is a passerine bird that often lives in close proximity to humans (Vincent, 2005). Globally, this birds' numbers has declined (Summers-Smith, 1999; Crick et al., 2002; Dandapat et al., 2010). In India a decline of 70% has been noticed in certain areas over a five year period (Dandapat et al., 2010), while in Great Britain their population numbers have declined by 60% over a thirty year period (Summers-Smith and Thomas, 2002). A number of reasons for the global decline have been suggested, including pesticides, lack of nest sites and changes in breeding behaviour (Summers-Smith, 1999; Crick et al., 2002; Giesy et al., 2003). In Thohoyandou, the study area, House Sparrows build their nests under the roofs or in the thatch of the roofs that are in close proximity to the indoor residual spraying (IRS) performed for malaria control. The nests and eggs in particular are directly exposed to the pesticides (Bouwman et al., 2013). Environmental studies on the effect of DDT and its metabolites on passerine birds, and specifically on House Sparrows, are lacking (Douthwaite, 1995). The aim of the study was to assess the association between DDT spraying and the structural properties of the eggs (shell thickness, pore numbers, and pore shapes) of the House Sparrow in an area that is regularly being sprayed for malaria control.

2. Study sites

The study sites were in the Limpopo Province of South Africa, in the northern regions of the malaria DDT spraying area. More specifically, in the Thohoyandou area, six sites were selected:

- two sites were sprayed thirty years before sampling (Tshakuma and Muledane)
- two sites were last sprayed five years before sampling (Makonde and Mangondi)
- two sites that were sprayed both years (2014–2015) of sampling (Makula and Lefule) (Fig. 1).

DDT spraying is conducted yearly in areas that are being controlled, during the Austral summer months from October to March so that the pesticide is in place during the malaria transmission season (Kruger, 2014). However, the spraying period also coincides with the main breeding season of the birds in South Africa (Ryan, 2004). The pesticides are applied on the inner walls and thatch, as well as the outside rafters – in the case of DDT, at 2 g/m^2. This means that sparrows, that often make their nests in the thatch, come in very close proximity of the applied DDT (Bouwman et al., 2013). The House Sparrow can live up to nine years (Jensen et al., 2013), which allows for a comparison with the spray histories. Spray schedules were obtained from the Malaria Control Programme of the Department of Health of the Limpopo Province, and confirmed by interviewing the residents of the various villages.

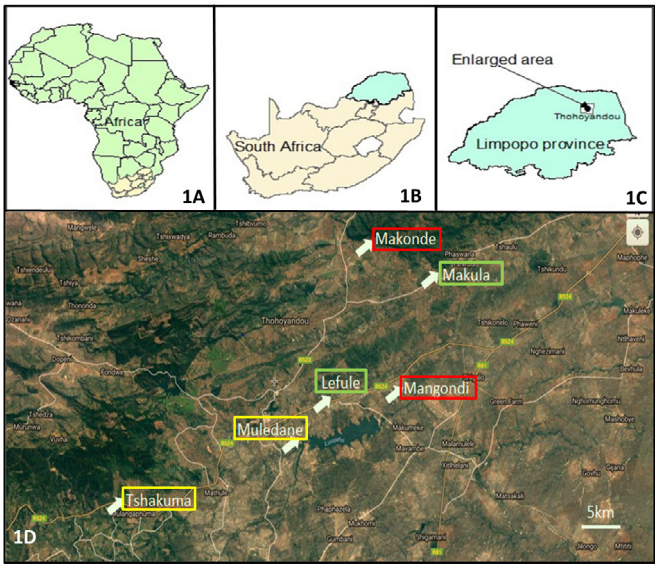

Fig. 1. A. Map of Africa with the location of South Africa highlighted. B. Location of Limpopo Province in South Africa. C. Location of the town of Thohoyandou in the Limpopo Province. D. The locations of all the study sites; Sites last sprayed thirty years before sampling were Tshakuma and Muledane (yellow); Sites last sprayed five years before sampling were Mangondi and Makonde (red); Sites sprayed both years of sampling were Lefule and Makula (green) (mapdata© 2017 AfriGIS (Pty)Ltd, Google).

3. Materials and methods

Permission to conduct research was given by the Animal Ethics Committee of the University of Johannesburg (LSteyn10Sept 2015) and permits (0112-mkt001-00004) were obtained from the Department of Economic Development, Environment and Tourism in the Limpopo Province. Hundred and twenty eggs were collected between September 2014 and October 2015 from about 40 nests. Eggs were collected from nests the sparrows built under the rafters. Nest selection was random for those nests that could be reached safely with a 5 m ladder (including presence of snakes). The ages of the eggs could not be determined. Sixty eggs, randomly chosen per village, were used for eggshell analysis, while the remaining sixty (as well as one eggs content from the eggs used for the eggshell measurements) was used for the determination of DDT concentrations.

3.1. Determination of shell thickness by scanning electron microscope (SEM)

The five eggs per site per year were weighed and the shell and contents separated. The shells were dried in a desiccator for three weeks before analysis. The contents were individually stored at −20 °C in amber polytope containers until chemical analysis. Three pieces of ~5 mm by 5 mm size were cut from each eggshell from the sharp, blunt, and equatorial regions of the egg. The pieces were sputter-coated with gold (the deposition process where the shells are covered with a thin layer of gold functioning as a conductive material) for 2 min at 40 mA in an Emscope SC500 corning OVF sputter coater, and viewed on a TESCAN 3 LMH nanospace scanning electron microscope (SEM) with a Vega 3 operating system. The shells were placed perpendicularly on the stage before photomicrographs were taken on the SEM at 500 times magnification. Measurements were done using the imaging analyser program ImageJ (Public Domain, Java).