

The Cattle Egret *Bubulcus ibis* as a near-global indicator of terrestrial pollution

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ABSTRACT

Persistent organic pollutants (POPs) are omnipresent in the environment. Due to their physical and chemical properties, POPs bioaccumulate in higher trophic-level species. Birds inhabit many niches and trophic levels and share physiological characteristics with humans. Wild bird eggs reflect the pollution of the environment and exposure experienced by the female prior to egg formation. Bird eggs are relatively easy to collect, easy to handle and store, and decompose slowly. In addition, bird eggs can also be used as biomarkers by examining the eggshell pigmentation and thickness. Aquatic bird eggs have been used most often with less published on terrestrial birds that also suffer consequences of pollution. Here, we review candidate species whose eggs can be used as near-global indicators. We found the Cattle Egret (*Bubulcus ibis*) as a suitable near-global indicator of terrestrial pollution. It is the most widespread Ardeid with extensive natural expansion, not threatened, occurs in high numbers, breeds in colonies with other Ardeids, has a high trophic status, and eggs are relatively large and easy to collect. Eggs of Cattle Egrets from eight countries and four continents have been analysed for pollutants such as PCBs, DDTs, HCH, HCB, dicofol, aldrin, dieldrin, endrin, heptachlor, mirex, and endosulfan. Multi-country and trans-continental studies using Cattle Egret eggs would therefore be possible, tracking the effectiveness of interventions such as the Stockholm Convention.

1. Introduction

There are many organisms that have been nominated as bioindicator species due to their indicator ability, abundance, and distribution, well-documented taxonomic literature, and importance, both economically and commercially [1]. Birds are taxonomically well described, occupy many niches and trophic levels, and are often associated with humans with whom they share many foods such as grains and fish. Birds are also endothermic, a physiological characteristic only shared with mammals, both therefore facing parallel threats from pollution based on common exposure and endothermic biology [2,3,4,5]. The mobility of birds allows them to discriminate and select between advantageous and non-supporting areas to breed, feed, shelter, and drink [6]. This combination of characteristics is why birds are often used as environmental indicators [1], from small to regional, and global scales [7,8,9].

Due to their mobility, birds encounter a wide range of agricultural and industrial pollutants such as persistent organic pollutants (POPs) and metals via water, air, and food. The differences in the bird's exposure are then due to behaviour, niches, trophic levels, diet, age, sex, and

life histories, further influenced by the wide range of habitats they occupy. It, therefore, stands to reason and has been confirmed, often determined from analyses of their eggs, that bird populations differ in their pollutant profiles because of these factors ([10,11,12]; [13]; [14, 15]).

Wild bird eggs reflect contamination in the environment wherein the female bird feeds prior to and during egg formation [16,17,14]. Eggs of wild birds are effective and useful in measuring POPs concentrations reflecting those of the adult females as well as the exposure experienced by the embryo [18]. Compared with other vertebrates, bird eggs are relatively easy to collect, and the removal of eggs from nests will not have lasting effects except for endangered species. Several authors therefore promote the use of bird eggs as indicators of pollution in the environments in which they live [19,20,21].

Birds experience deleterious effects of pollutants. We list several studies here. Endocrine-disrupting pollutants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) in birds and other animals alter thyroid hormone function and neuro-endocrine systems, alter physiological stress responses [22,23,24], affects gonadal

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steroid hormones [25], delay and impair sexual maturation and mating behaviour [26], cause thinner eggshells, affect embryological development, and may lead to embryo mortality [27,28]. Eggshell pigmentation may be indicators of the female bird's health and habitat condition [29,30]. Therefore, much information can be drawn from eggs about the health of bird populations of the same or similar species when compared across regions.

The use of singular avian species that are located across multiple countries, catchments, and continents is essential to provide highly comparable data to assess terrestrial pollution. This species should meet the recommended bioindicator criteria [1] and should allow researchers to monitor the effectiveness of the Stockholm Convention and other mitigation measures combatting terrestrial pollution.

Most published studies concentrate on aquatic birds and their eggs. This is to be expected as pollutants concentrate through more trophic levels in aquatic than in terrestrial environments [31,32,33]. Osprey eggs (*Pandion haliaetus*) had five to ten times higher *per-* and polyfluorinated alkyl substances (PFAS) concentrations than Tawny Owl (*Strix aluco*) and Common Kestrel (*Falco tinnunculus*) eggs from Sweden [32]. While all three are raptors, the Osprey as aquatic predators had much higher concentrations. Bouwman et al. [31] found concentrations of ΣDDT three orders of magnitude higher in aquatic-feeding heron eggs than in terrestrial-feeding Cattle Egret eggs from the same region. The higher levels in aquatic birds do not mean that terrestrial birds do not suffer consequences. The decline and recovery of the Eurasian Sparrowhawk (*Accipiter nisus*; [34,35]) is a good example. The terrestrial Cattle Egret had significantly thinner eggs with increasing ΣDDT concentrations in their eggs from regions where DDT was used [36,37], and is still used for malaria control [31].

Examples of terrestrial birds as indicators include the Little Owl (*Athene noctua*; [38]), Snow Bunting (*Plectrophenax nivalis*; [39]), Tits (Paridae; [40]) and the Eurasian Sparrowhawk [34,35,21]. Padayachee et al. [21] reviewed POPs in raptors globally. The only raptor used as a terrestrial indicator in both hemispheres was the Peregrine Falcon (*Falco peregrinus*; [21]). However, the Peregrine Falcon is scarce, sensitive to multiple stressors, and nests are widely distributed [41] with much effort needed to collect eggs. There are other terrestrial bird species with a near-global distribution that could serve as a pan-continental indicator of pollution. The Barn Owl (*Tyto alba*) is a candidate [42,43]. Like all owls, they breed solitary and far between. This allows higher spatial resolution of pollutant patterns [42]. However, the number of eggs that can be sampled is limited due to distances between nests and associated logistics. Tit eggs, especially Great Tits (*Parus major*) and Blue Tits (*Parus caeruleus*) eggs are also used as indicators of heavy metals [40] and POPs [44,45]. Tits have a wide northern hemisphere distribution while their range also expands into Southern Africa.

Although the House Sparrow (*Passer domesticus*) is common where they occur, breeding pairs occupy small ranges, and the eggs are small (ca. 0.6–2.9 g depending on locality; [46,47]) requiring pooling for analyses. Probably because of these factors, little work on pollutants in House Sparrow eggs has been published [31,46], indicating its lack of monitoring utility.

The Rock (Feral) Pigeon (Dove) *Columba livia* has a near-global distribution (All about Birds, [48]) with very large numbers (260 million globally; [48]). It is granivorous but also feeds on berries, shoots, green leaves, soil, and food scraps in cities and surrounding farmlands [49,50,51,52]. This bird breeds solitary, mostly on buildings and rocky ledges. Obtaining enough eggs though, would be problematic given the difficulty of reaching solitary nests.

Terrestrial, trans-equatorial migrants breed mostly in the Northern Hemisphere; only some in the Southern Hemisphere [53,54,55], so hardly any corresponding conspecific eggs from the south or north, reducing the utility of migratory bird eggs as global indicators of terrestrial pollution. It may be argued that eggs from hemispheric migratory birds should indicate pollution from the non-breeding hemisphere, and up to a point they do. However, they encounter pollutants

during both legs of migration, at the non-breeding sites, and after their return before breeding, confounding geographic assessments [56]. There are very few terrestrial bird species that migrate across all continents. There are migratory subspecies of the Peregrine Falcon (*F. peregrinus calidus* between Eurasia and Africa, and *F. peregrinus tundrius* in the America's), all of whom also breed in the Northern Hemisphere. Birds such as Barn Swallows (*Hirundo rustica*), House Martins (*Delichon urbicum*), European Bee-eaters (*Merops apiaster*), and White Storks (*Ciconia ciconia*) breed in the Southern Hemisphere, but in small numbers in small areas. Therefore, these birds have limited utility as a global indicator.

There is, however, one terrestrial bird that is predatorial, with a near-global breeding distribution that would be especially useful, the Cattle Egret (*Bubulcus ibis*, with three subspecies *B. ibis ibis*; *B. ibis coromandus*, and *B. ibis seychellarum*) (Fig. 1). Cattle Egrets are the most abundant and omnipresent heron species globally [57] (Fig. 2), with an estimated global population of 4 to 9.85 million birds [58]. It feeds almost exclusively on terrestrial prey, therefore at a higher trophic level than the Rock Pigeon *C. livia*. The aim of this article is to review and assess the eggs of the Cattle Egret as a near-global indicator of terrestrial pollution. We review the biology of the bird, distribution, and concentrations of organochlorine in eggs worldwide. We discuss some of the implications such pollutants may have on birds and assess the utility and advantages and disadvantages of their eggs as a near-global indicator of terrestrial pollution.

2. Materials and methods

2.1. Literature

A literature search was conducted to find studies that measured the concentrations of POPs in eggs of all *Bubulcus ibis* subspecies. Google Scholar was used as the primary search engine. Additional studies were traced using the references of already identified publications. The cut-off date was August 2023.

Key words used: Cattle egret, *Bubulcus ibis*, cattle egrets and DDT, cattle egrets and organochlorines, cattle egrets and POPs, *Bubulcus ibis* and DDT, *Bubulcus ibis* and organochlorines, *Bubulcus ibis* and POPs, cattle egret distribution, cattle egret range expansion, heron eggs and DDT, DDT, and bird eggs, heron eggs and organochlorines. The cutoff date was august 2023

2.2. Data selection criteria

The literature used was restricted to publications in peer-reviewed journals. Only concentrations presented in table format with clear concentrations and units were considered; data represented only graphically were excluded. Concentration data in Cattle Egret eggshells, feathers, carcasses, tissues, and droppings were excluded from this review, only data on egg contents were used.

2.3. Measuring unit conversions and groupings

Values reported in parts per million (ppm), parts per billion (ppb), milligram per kilogram (mg/kg), and nano-gram per gram (ng/g) were compiled in a table and converted to microgram per kilogram (µg/kg). Concentrations were converted from dry mass (dm) to wet mass (wm) [76].

2.4. Statistics

As far as possible, we report summed (Σ) concentrations with the number of congeners analysed in subscript, such as ΣPCB₆. In cases where this information was not apparent, we report it as ΣPCB₇. In studies where more than one set of samples were reported from a country, the arithmetic mean was calculated from the reported means as

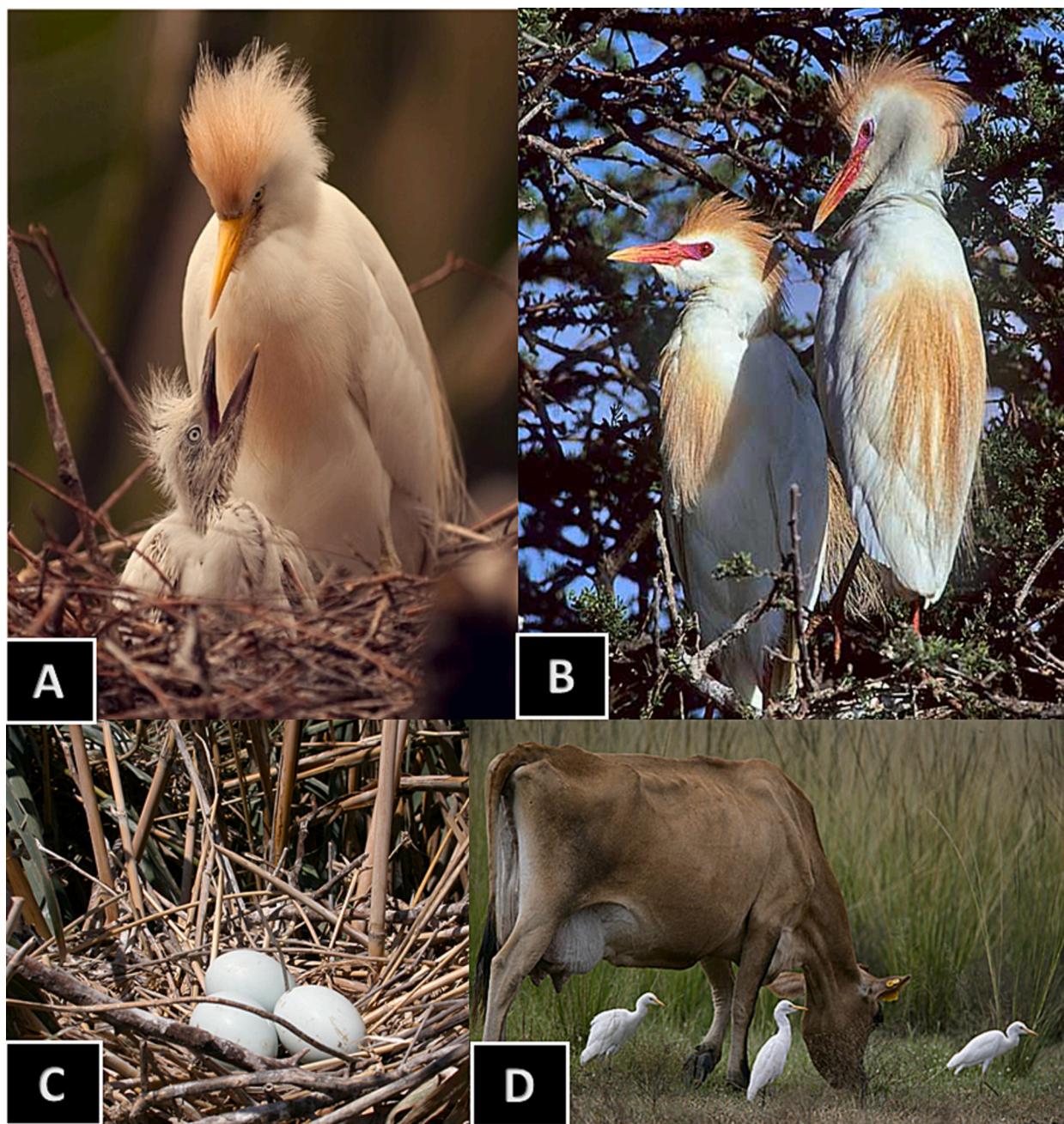


Fig. 1. A) Adult Cattle Egret along with a nestling. B) Cattle Egrets in breeding plumage. C) Cattle Egret nest with eggs. D) Cattle Egrets foraging alongside cattle. Images with permission from Warwick Tarboton.

individual data were normally not supplied. Descriptive statistics for the residue concentrations of the organochlorines in eggs are listed in Tables S1–10 in the supplementary material.

3. Cattle Egret biography

3.1. Description

The Cattle Egret is a medium-sized heron (Ardeidae) with white feathers and a sharp yellow bill [57]. They measure on average 51 cm in length with a wingspan between 24 and 27 cm for males and 24 to 26 cm for females. Their mass can vary between 270 and 510 g, depending on sex and feeding status [57]. During the breeding season, adults develop orange/gold breeding plumage (Fig. 1) that differs slightly between subspecies. Cattle Egrets are provisionally divided into three subspecies;

Bubulcus ibis ibis, the nominate subspecies, which refers to Cattle Egrets originating from Africa and spread to western Asia, southern Europe, and the Americas; *B. ibis coromandus*, the eastern subspecies, which refers to Cattle Egrets located in the remaining regions of Asia, Philippines, Australia, New Zealand, and Oceania; and *B. ibis seychellarum*, that are Cattle Egrets endemic to the Seychelles in the Indian Ocean (Fig. 2). However, the status of *B. ibis seychellarum* as a subspecies is questioned [77,78] and we will not consider this subspecies further.

3.2. Reproduction

Cattle Egrets breed during various times of the year depending on local climate, location, and availability of food [57]. However, they do not breed everywhere where they occur, especially in the colder regions. The nests are constructed over a period of four to five days, using reeds,

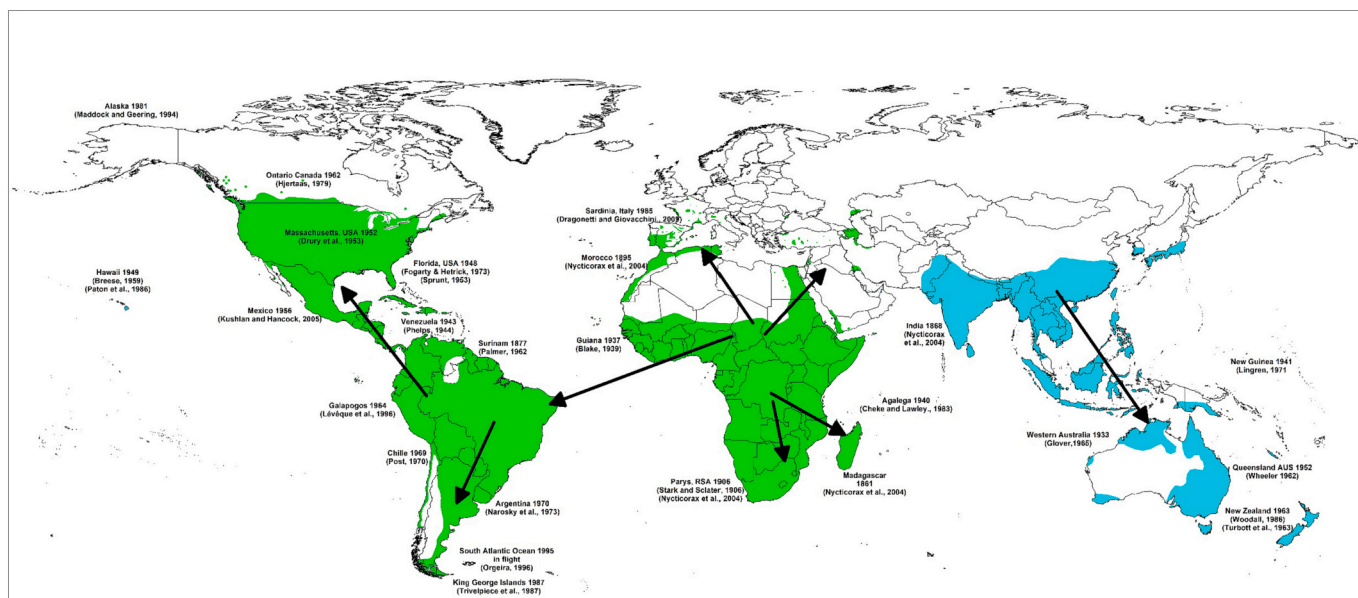


Fig. 2. Range expansion of the Cattle Egret and the first confirmed sighting at new locations [59]. This map is not up to date. For instance, Cattle Egrets are now breeding in the UK in small numbers. Two additional distribution maps are available at Birdlife International, and All About Birds. [60–75]

twigs, and branches. They build nests in reed beds, bushes, shrubs, trees, or on the ground near water [57,79]. Cattle Egrets roost and breed in small to large colonies, in many cases alongside other egret, heron, and similar colonially breeding water bird species [77]. Males perform courting rituals and may display aggressive and territorial behaviour towards rival males. Females pair with different males each season and extra-pair copulations occur [57]. Once paired, the females lay two to five medium oval eggs that have a pale blue tint. Eggs become faded with time [80], and eggshells shift to whitish-green when dried [81]. Both parents incubate and feed the chicks [57]. Siblicide is common in this species, especially under restricted food availability [82].

3.3. Cattle egret range expansion

Since the early 1900s, the Cattle Egret has undergone a rapid natural and human-facilitated near-global range expansion (Fig. 2). It is one of the fastest range-expanding bird species [83]. It occurs on all continents and on many large and small islands. Cattle Egrets can fly long distances, even crossing the Atlantic Ocean ([57]; Kylin, Pers. Obs. 2007 and 2008 in the Atlantic, flying westwards). The range expansion of both the nominate and the eastern subspecies are summarised by Maddock and Geering [84] and elaborated in Fig. 2.

Not only is the Cattle Egret now a cosmopolitan species, but the number of breeding colonies has also increased dramatically [77]. For instance, breeding colonies in Algeria increased from 51 to 87 between 1999 and 2007 [85], while approximately 22 countries in Europe have reported breeding colonies of Cattle Egrets [86]. Most of this expansion was unaided by humans. However, Cattle Egrets were intentionally released on the Hawaiian Islands in 1959 [87] and in Australia [88] as a biological control agent of pests. Although perceived to be benign [79] as they infiltrate largely vacant terrestrial niches, they may impact their new prey populations [89], and have been mentioned as a possible vector of Japanese encephalitis when flying between Australia and New Zealand [90]. In addition, humans have aided in creating favourable habitats for these birds by habitat alteration, farming, deforestation, and redirecting of waterways [91,57,89]. The current global population is estimated at 4 to 9.85 million birds [58].

3.3.1. *Bubulcus ibis ibis*

The nominate subspecies has its historic range in tropical central

Africa, whereafter it quickly spread northwards towards Spain and Portugal (Fig. 1). The first record we could find for Europe was an [92] bird list [92]. Since then, it has spread across Europe and Great Britain. The first breeding pair of Cattle Egrets in Great Britain was reported in 2008 and sightings of Cattle Egrets have expanded as far north as the Scottish highlands (BTO, 2023). Cattle Egrets gradually expanded southwards, with breeding in South Africa recorded by the 1920s [57,93]. Today, this species occupies most of the African continent except some deserts. Cattle Egret also expanded westwards, towards South America and later to North America [94] spreading as far north as Alaska by 1981 [84] and as far South as King George Islands in the Antarctic [95]. Since their invasion of North America, they have spread over most of the southern half of the continent, breeding in all but four contiguous USA states, and in Manitoba, Ontario, and Saskatchewan in Canada [96,97]. All About Birds [98] has an observation map online, although many historical records are not shown.

3.3.2. *Bubulcus ibis coromandus*

The distribution and range expansion of the eastern subspecies of the *B. ibis coromandus* is less well documented than the nominate. The eastern subspecies have a historical range in southern Asia, extending from India all the way east towards southern Japan and the Philippines (Fig. 2). It expanded to Australia [99] and New Zealand [84,100]. The range expansions summarised here can be attributed to the bird's ability to utilise rice fields and nest in cities [77].

3.4. Habitat and diet

Cattle Egrets occur in a variety of habitats, including grasslands, wetlands, pastures, waste and sewage dumps, and in agricultural landscapes [57]. Cattle Egrets occupy a high trophic level as they primarily prey on insects such as grasshoppers, crickets, flies and their maggots, beetles, moths, spiders, and wasps. They occasionally also catch and eat frogs, fish, crayfish, small snakes, small mammals, lizards, and earthworms [57,101,89].

Cattle Egrets display highly opportunistic feeding behaviours [102] and have symbiotic relationships with large herbivorous mammals (Fig. 1) such as cattle (Family: Bovidae) and rhinoceroses (Family: Rhinocerotidae). Evidence supports that this symbiotic relationship developed in Africa when the birds followed slow-moving African

Buffalo herds (Genus: *Syncerus*) during grazing ([103]; [57]). Cattle Egrets are not only twice as successful in obtaining prey when they feed in close association with large mammals, but this strategy also requires two-thirds less energy expenditure [104,105]. As a result of the high trophic level, it is possible that Cattle Egrets bio-accumulate contaminants [106]. Breeding Cattle Egrets feed within 15 km (maximum 28 km) of their breeding colonies, restricting the area where they can take up pollutants [103].

3.5. Cattle Egrets as bio-indicators of water quality

Although the Cattle Egret is essentially a terrestrial bird based on its diet, they breed and roost near water. The presence or absence of these birds near aquatic roosting and breeding locations can be used as a bio-indicator of the associated water and environmental quality [77]. During the 1970s, Cattle Egrets, among other species, started to disappear as breeding birds along parts of the Nile Delta in Egypt. One of the possible explanations was the use of DDT on cotton plantations which may have caused unfavourable conditions and a decline in insect populations. However, due to a lack of chemical analyses at the time, this was never proven. Only in the 1980s when organochlorine use was restricted, did some of the bird populations recover [107]. This shows that the Cattle Egret responds according to environmental stressors and will recover once the stressor is reduced [1].

Cattle Egrets, among other egrets and herons, also bring in and continuously enrich wetlands where they roost and breed with nutrients, influencing the associated ecologies [108]. It is conceivable that pollutants from elsewhere are also translocated here.

4. Results and discussion

We report on arithmetic concentrations as published by various authors or calculated by ourselves (Figs. 3 and 4). It should be kept in mind though, that different extraction and analytical methods were used. We also had to apply conversion factors to obtain ng/g wet-mass-based concentrations. Therefore, comparisons should be read in context of factor- or order-of-magnitude patterns, not small-scale differences. The data are available in Table S1 in Supplementary Materials.

4.1. Compounds

The literature review shows that quantifiable residues of POPs occur in Cattle Egret eggs when measured, except in eggs from Florida, USA [109]. PCBs were detected in samples from five countries of which samples from Spain had the highest mean Σ PCB concentration (Fig. 3A). The eggs used in that particular study [110] were collected in 1979 from Ebro Delta; a wetland in the north-eastern part of Spain that houses over 60,000 bird breeding pairs and hosts an additional 250,000 birds during winter months [111]. This area is used for agriculture, specifically rice cultivation which may make use of pesticides [111,112]. Lower mean Σ PCB concentrations were detected in eggs collected almost 30 years later in Aiguabarreig Spain; an ecologically important wetland that eventually flows via the Ebro River into the Ebro Delta.

All reported mean Σ PCB concentrations from the USA, Egypt, and Mexico were within the same order of magnitude [113,37,107,114]. These reports date back to the 1970s and 80s when PCBs were still used extensively. No recent reports are available from those countries. The mean Σ PCB concentrations reported from countries in the Northern Hemisphere were higher than those from the Southern Hemisphere (Fig. 3A).

The same pattern can be observed for compounds such as DDT, hexachlorobenzene (HCB), and hexachlorocyclohexane (HCH), with some exceptions (Fig. 3C–D). The highest Σ DDT concentrations were detected in Mexicali Valley, Mexico [37] where bird carcasses had Σ DDE concentrations of up to 20,000 ng/g wm [37]. The reported Σ DDT concentrations from the USA (Cali), Spain, Egypt, and Israel were of the

same order of magnitude as those from Mexico. The USA banned the use of DDT in 1972 [115], while Mexico still made use of DDT until 2001 ([116]; Stockholm [117]).

Eggs from the Southern hemisphere had lower Σ DDT concentrations than those of the Northern hemisphere except for sites from Limpopo province in South Africa (Fig. 4B). Eggs collected from heronries at Tshakhuma Dam and Xikundu Dam had Σ DDT concentrations in the same order of magnitude as those from China and Pakistan. This was to be expected since parts of Limpopo (a malaria-endemic region) are sprayed with DDT to control malaria, to this day [118]. It should be noted that DDT is also a by-product of dicofol production and a formulation contaminant [119,120] and was only reported in studies from Pakistan (Fig. 4A). Dicofol has a half-life of about two years in the environment and only a few studies report dicofol concentrations.

Aside from dicofol, other compounds such as HCH, aldrin, dieldrin, DDT, endrin, and heptachlor were also quantified in eggs from Pakistan (Figs. 3 and 4). Malik et al. [36] not only investigated the egg contents of Cattle Egrets for POP residues but also in their prey and sediments associated with their heronries. They found biomagnification of POPs through the food web as higher levels of pollutants were found in the eggs. This claim was later strengthened by Khan et al. [121] with comparable results. Both studies attributed the organochlorine residues detected to the current and historical use of such compounds in agriculture and to current industrial practices.

Carcasses of adult herons from the USA were found with lethal concentrations of organochlorines, especially dieldrin, PCB, and DDE, in their brain tissues [122]. However, samples collected in Louisiana USA, prior to those collected in Mexico, had the lowest dieldrin concentration. It should be kept in mind that aldrin can break down to dieldrin, both being used as pesticides [123].

HCH concentrations from Pakistan were higher than concentrations reported from other countries (Fig. 4D). The report found β -HCH and γ -HCH (lindane) in greater quantities than the other isomers, most likely from use in agricultural pest control [121]. Eggs collected from Tai Lake, China, had the second highest HCH concentration, followed by Egypt. Only samples from Spain had HCH concentrations of the same order of magnitude as those reported from the Southern hemisphere. Endrin and endosulfan concentrations were the highest in samples from Pakistan with the lowest concentrations of both compounds found in eggs from China (Figs. 4 D and F).

Only studies from South Africa analysed Cattle Egret eggs for mirex (Fig. 4G). The highest mean mirex concentrations were found in eggs collected along the Vaal River, specifically near Parys, with slightly lower concentrations found upstream in Gauteng. Mirex has not been registered in South Africa as a pesticide. The authors attributed the presence of Mirex to background contamination, possibly from its use as a flame retardant [124].

Although not the main aim of this assessment, Cattle Egret eggs, feathers, and organs have been used to monitor metals such as lead, copper, mercury, and cadmium [17,125,126,127,107,128,129,130]. Perry et al. [131] reported DDE residues in organs such as liver, heart, brain, muscle, skin, and feathers. DDE residues were higher in the skin and feathers compared with other organs, while these concentrations were all lower than in eggs. In addition, HCH, HCB, and DDTs were detected in Cattle Egret carcasses in Mexico [132].

4.2. Implications of exposure

4.2.1. Monitoring populations

DDT exposure can lead to breeding impairment, embryo defects, and mortality [16], causing population declines in some Ardeid populations [119,31,133,134,135]. One of the best ways to assess the impact pollutants have on Cattle Egret is to monitor breeding, breeding success, hatching success, population size, and growth. The Cattle Egret's population growth shows that this species can colonise new habitats and that their numbers grow rapidly once established. Furthermore, Cattle

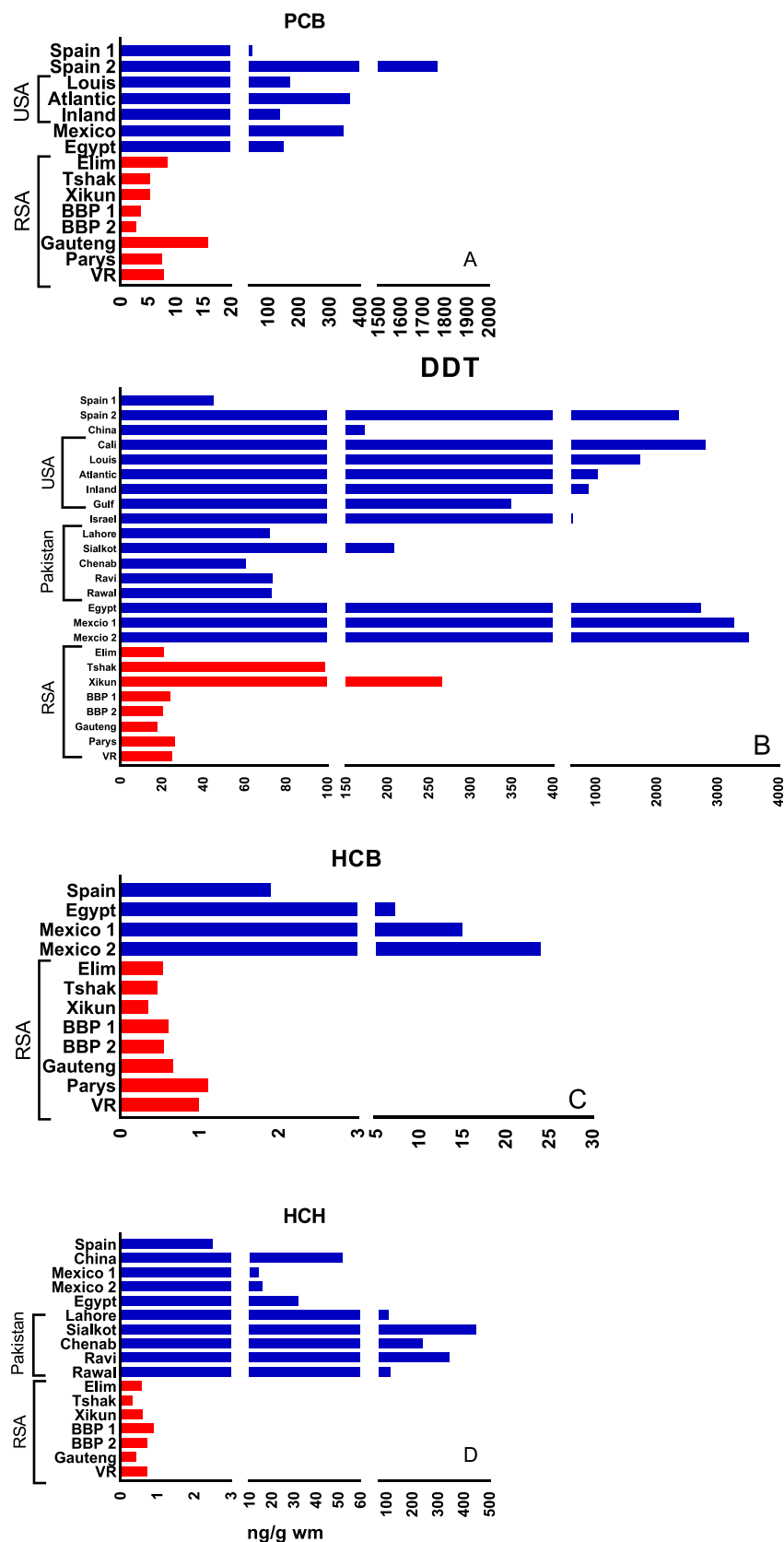


Fig. 3. Bar graphs representing A) PCB, B) DDT, C) HCB, and D) HCH concentrations in Cattle Egret eggs reported by various authors (Table S1). Blue bars are from the Northern Hemisphere, and red from the Southern Hemisphere. RSA – Republic of South Africa. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

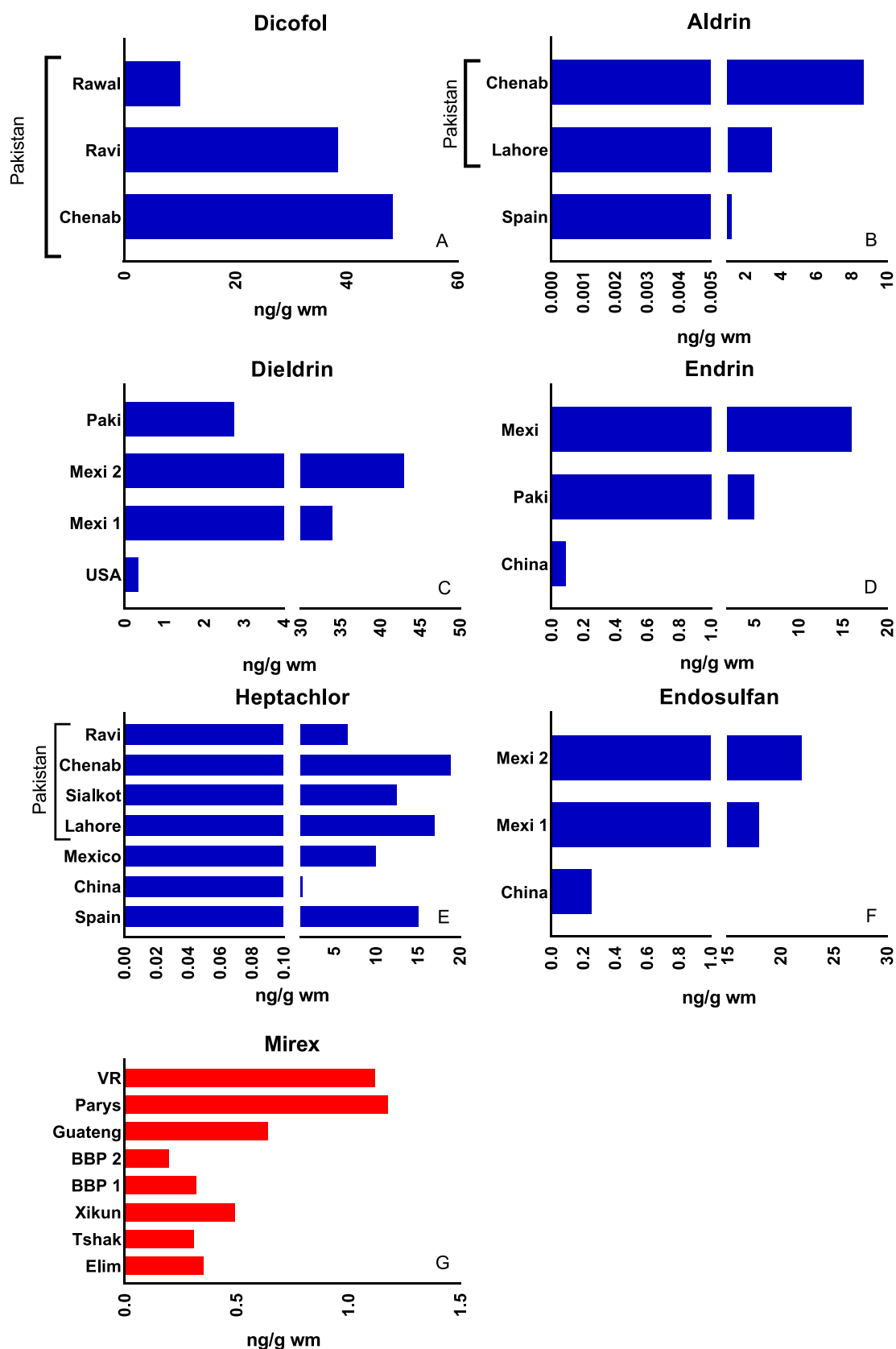


Fig. 4. Bar graphs representing A) Dicofol, B) Aldrin, C) Dieldrin, D) Endrin, E) Heptachlor, F) Endosulfan and G) Mirex concentrations in Cattle Egret eggs reported by various authors (Table S1). The blue and red bars are from the Northern and Southern hemispheres, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Egrets demonstrated rapid recovery after being faced with declining numbers due to biological control measures imposed by some countries [136]. Moreover, Cattle Egrets produce larger clutch sizes when occupying new areas [137], suggesting faster population growth compared with natal African colonies. This change in clutch size is temporary as the population over time stabilizes.

4.2.2. Eggshell thinning

Eggshell thinning is the process by which the eggshell does not develop to the thickness it should be during egg formation. Thinned eggshells are a principal cause of reproductive failure, explaining the decline in some bird populations [138]. Eggshell thinning during embryo development does occur; calcium and other minerals are mobilised from the shell to subsidize embryo development ([139]: [140]). The thinning of the eggshell induced by the developing embryo is referred to as embryo-induced eggshell thinning, or EET. Orlowski and Hałupka [141] found that altricial bird species, which include Cattle Egrets, displayed the highest thinning (12%) EET. Several factors can influence the eggshell thickness other than EET, including the size, colour, and maculation of the eggs. Furthermore, the genetics, health, egg incubation time, and the diet of the female bird may also influence the eggshell thickness [139,142,143].

Increased concentrations of organochlorine compounds may lead to reproductive failure and mortality for many bird species, especially due to eggshell thinning associated with *p,p'*-DDE but also PCBs [27,37,144,145]. Aurigi et al. [16] reported that DDE can thin eggshells by as much as 20% and can lead to embryo mortality and defects. A study conducted in 1964 in the USA found DDE concentrations of 202,000 ng/g wm along with an 18% decline in eggshell thickness to cause embryonic mortality and lead to a population decline in gull colonies. A reduction in eggshell thickness of 11% was associated with shell flaking and egg breakage [146]. Concentrations as low as 2 ng/g DDT wm have been reported to cause a reduction in eggshell thickness in predatory birds [147]. Cattle Egret eggs, collected in 1953, had a 0.2 mm thicker mean eggshell thickness than eggs laid in 1986 [37]. Cattle Egret eggshells from an area in South Africa where DDT is still used for malaria control were up to 33% thinner associated with increased DDT concentrations [31]. Studies examining DDT concentrations in eggshells pre-, during-, and after DDT exposure reveal the same pattern [148]. Eggshell thickness drastically declined during times of DDT exposure and eggshells in general were thicker pre-exposure [148].

4.2.3. Cattle Egret eggs as terrestrial indicator of POPs

The wide distribution, colonial breeding, high and expanding numbers, well-known biology, trophic position, and previous use of the eggs suggest that the Cattle Egret is the best candidate for a near-global bird species to research and monitor terrestrial pollution when weighed against other candidates, notably the omnivorous Rock Pigeon *C. livia*. Holt and Miller [1] list characteristics of indicators. These include good indicator ability (provide a measurable response, the response reflects the whole population, and the response is in proportion to the treat), the species should be classified as none threatened, abundant and often encountered, well studied, and hold economical and or commercial importance. We list the reasons and advantages of using the Cattle Egret that also include the criteria by Holt and Miller [1].

Biology:

- Cosmopolitan, near-global bird distribution.
- Not threatened and often encountered in large numbers.
- Taxonomically well-documented and stable.
- With climate change, further range expansion may be expected.
- Easy to identify and well-studied.
- Cattle Egrets live up to 23 years [149].
- Mostly insectivorous but also small vertebrates.
- Frequently forage in agricultural areas.

Indicator utility:

- Residues of organochlorines as well as metals have been quantified in Cattle Egret eggs and organs (Supplementary material Table S2 and S3)
- Cattle egret eggs can also be used to analyse other persistent compounds such as organophosphate flame retardants [150].
- Cattle Egret eggs are large enough for individual analyses of contents and eggshells.
- Thinner Cattle Egret eggshells have been associated with DDT (measurable response)
- Much data exists on other Ardeids that are aquatic, allowing comparisons between aquatic and terrestrial pollution in closely related bird species.
- They are terrestrial feeders that are likely to accumulate POPs and other pollutants from terrestrial environments.
- Cattle Egrets feed within 15 km of their breeding colonies, restricting the areas where they are exposed to pollutants in their food during breeding.
- Cattle Egret eggs are consumed by humans in some parts of the world [150].

Egg collection:

- Breeding colonies are easy to locate from the ground and aerial reconnaissance.
- Colonial nesting, also with other Ardeids and aquatic birds.
- Cattle Egret eggs are relatively easy to collect in sufficient numbers from breeding colonies and do not require specialised and/or expensive collection equipment.
- Because of colonial breeding, single eggs can be collected from multiple nests, leaving eggs behind in each nest for incubation.
- Unhatched eggs can also be used.
- Collection of eggs has no long-term effects on population growth and can be done with minimal stress and disturbance.
- With care, minimal disturbance during collection can be achieved, and displaced chicks find their way back to their nests (personal observations, H Bouwman).
- Because multiple Ardeids breed colonially together, eggs from both terrestrial and aquatic birds can be collected from the same locations and at the same time.
- Eggs can be stored frozen for very long periods to investigate legacy pollution or trends at a later stage.
- Other sample matrixes include adult and juvenile feathers, whole carcasses, organs, blood, and droppings.

Considerations:

- There are ethical implications and permits required for the collection, transporting, and storage of Cattle Egret eggs. However, this is the case for all birds in most countries.
- Although we list the collection of multiple Ardeid species eggs from the same colony at the same time as an advantage, breeding may not always be synchronous.
- Cattle Egrets are normally not considered as threatened, presenting a lower ethical threshold. However, this may not be the case for other species breeding alongside the Cattle Egrets.
- Collection at colonies must be done carefully so as not to disturb other breeding colonies. Mora [37] observed nestling mortality and destroyed nests after repeated visits.
- The Cattle Egret is a species that is dramatically increasing its distribution range and establishing new breeding grounds. However, as of 2023, there are some regions that have not yet reported the breeding of Cattle Egrets (even though they are observed in those regions).

- The concentrations quantified in the eggs reflect the pollution load of the female bird only and do not reflect the response of the whole population.
- Cattle Egrets eggs are consumed in certain parts of the world but the organisms themselves are not harvested for any commercial purposes.

Cattle Egrets are well suited for the role of indicator species and comply with most of the criteria listed by Holt and Miller [1]. Their suitability will increase with time as they continue to expand their distribution range.

5. Conclusions

We conclude that organochlorine residues were detected in various concentrations in Cattle Egret eggs even long after the application of such pollutants had terminated in the particular area. Cattle Egrets have undergone one of the most impressive natural expansions of the twentieth century and continue to spread and populate newly cultivated areas. They comply with most of the criteria associated with an indicator species. Cattle Egrets are one of the few terrestrial-feeding heron species. The use of this species as an indicator of terrestrial pollution may aid in understanding why populations of other bird species are declining due to reproductive failure because of increasing environmental pollution. Using the same species across multiple countries, catchments, and continents will provide highly comparable data to assess terrestrial pollution, allowing us to monitor the effectiveness of the Stockholm Convention and other mitigation measures combatting terrestrial pollution.

CRediT authorship contribution statement

Veselia Lesch: Writing – original draft. **Henrik Kylin:** Writing – review & editing. **Hindrik Bouwman:** Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.encco.2023.11.003>.

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