ELSEVIER

The Science of the Total Environment 267 (2001) 169–176

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

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Lead and cadmium in eggs of colonially nesting waterbirds of different position in the food chain of Greek wetlands of international importance

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Received 1 April 2000; accepted 24 August 2000

Abstract

Lead and cadmium concentrations were measured in eggs of collonially nesting waterbirds with different position in the food chains of Greek wetlands of international importance. Differences were found between species in the levels of both lead and cadmium in the Evros and Axios Deltas attributable to their different diets. Nevertheless, the concentration in eggs was unrelated to the position of each species studied in its food chain. There was no significant difference in lead levels among four wetlands sampled for the cormorant and in Cd levels among three wetlands sampled for the Mediterranean gull, probably implying species-specific accumulation patterns. A higher lead pollution of the Axios Delta area was only reflected in the eggs of the Mediterranean gull. The very low concentrations of both metals found in the eggs may either suggest low environmental inputs or lack of sensitivity in using eggs as lead and cadmium biomonitors, thus a more sensitive bioindicator still remains to be found. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ecotoxicology; Lead; Cadmium; Waterbird eggs; Wetlands

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PII: S0048-9697(00)00808-1

1. Introduction

Lead and cadmium, both non-essential heavy metals, occur widely in the terrestrial and aquatic environment mainly due to inputs from human activities. In biota, these metals enter the food chains and find their way through mechanisms, that have been partly understood (Bryan and Langston, 1992; Schuhmacher et al., 1995; Furness, 1996a,b; Stewart et al., 1996). Both metals are very toxic to humans and animals (Goyer et al., 1995). Long-term field and laboratory research of the effects of lead on the behavioural development of gulls indicated lower survival rates, significant lead-induced impairments in righting response, locomotion, thermo-regulatory behaviour, begging and feeding behaviour and disrupted sibling recognition of chicks (Burger and Gochfeld, 1994, 1995a, 1996a, 1997, 1998). Cadmium also results in a variety of physiological disturbances in birds. Effects may include altered behaviour, disturbance in the metabolism of some essential elements, suppression of egg production, kidney damage, testicular damage and other impairments (Furness, 1996a).

Mediterranean wetlands are ecosystems of great importance as human resources, in regard to fisheries, aquaculture and grazing that have developed long ago in wetlands. Besides, Mediterranean wetlands are valuable for providing suitable habitat for numerous species of wildlife, especially birds, of both economic and conservation importance (Goutner, 1994b; Pearce and Crivelli, 1994; Zalidis and Mantzavelas, 1994).

Use of bird organs (liver, kidney, etc.) and tissues (eggs and feathers) have extensively been used to monitor metals in the environment. Transfer of lead and cadmium in bird eggs has been demonstrated in various cases (Burger and Gochfeld, 1991, 1993, 1995b, 1996b). As eggs at least partly reflect metal uptake from local foraging, waterbird eggs have been used in monitoring pollution by these metals, among others (Furness, 1993; Burger and Gochfeld, 1995b). Such studies are scarce in the eastern Mediterranean.

The purposes of this study are: (a) to investigate the levels of lead and cadmium in the eggs of a number of colonial waterbird species from Greek wetlands of international importance where such information was lacking; (b) to compare lead and cadmium levels in some waterbird species' eggs in relation to their position in the food chain of two coastal wetlands; and (c) to decide whether these waterbirds' eggs could be useful for biomonitoring of these metals in the wetlands studied.

2. Materials and methods

2.1. Study areas

Five northern Greek wetlands were investigated in this study. The Evros Delta, at the Greek–Turkish border (Fig. 1), is the eastern-most Greek wetland (40°47′N, 26°05′E) extending over 11 000 ha, including a variety of habitats such as temporary and permanent fresh water marshes, saltmarshes, lagoons, brakish lakes, coastal sandy islets and beaches with ammophilus and nitrophilous vegetation, bush and forest stripes along the river and cultivated land (Britton and Hafner, 1978; Babalonas, 1980). The river Evros, originating from Bulgaria, receives considerable transboundary pollution from Bulgaria and Turkey as of its total drainage area (52 500 km^2) only 6.5% belong to Greece.

Porto Lagos (41°01′N, 25°08′E) (Fig. 1), is a multifarious coastal area south of the Lake Vistonis within a wide wetland complex of eastern Macedonia (Zalidis and Mantzavelas, 1994). The complex includes a lagoon of 560 ha, peripherally fringed with saltmarshes also occurring on its numerous islands. Low sand dunes and sandy beaches with ammophilus vegetation occur seawards. The area is extensively used for fisheries (fish ponds).

Kerkini Lake (41°22′N, 23°13′E) (Fig. 1), is an artificial lake (7300 ha) made as a water reservoir in the thirties by damming the river Strymon originating from Bulgaria. Vegetation of the lake includes reedbeds and aquatic species. Wet fields and an extensive forest occur in the mouth of the river to the lake (Zalidis and Mantzavelas, 1994).

The Axios Delta (40°30′N, 22°53′E) (Fig. 1), is a part of an extensive wetland complex situated at

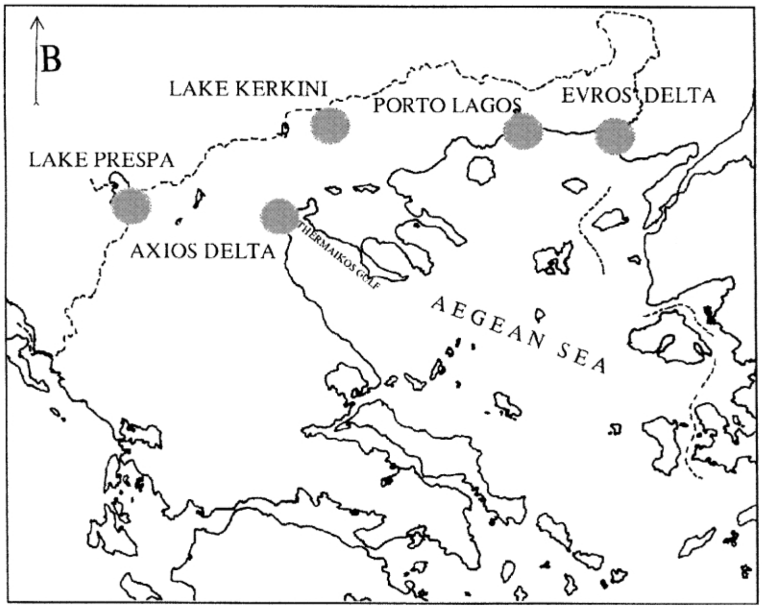

Fig. 1. Geographic overview of northern Greece showing five sampling areas.

the west coast of Thermaikos Gulf. Habitats include salt and fresh water marshes, ricefields, lagoons, vegetated coastal islets, few sandy shores, forested river banks and tamarisk bushland (Kazantzidis et al., 1997). This river originates from the former Yugoslavia and bears considerable pollution, probably being the mostly polluted water body of the north-eastern Greece (Fytianos et al., 1986).

Lake Mikri Prespa (40°76′N, 21°09′E) extends over 4800 ha being situated at 853 m above sea level. The lake lies at the north-western edge of Greece bordering Albania and the former Yugoslavia. Habitats include aquatic vegetation, wet meadows, reedbeds, cultivations and sparse forestland.

2.2. Methods

Eggs of colonially nesting waterbirds were collected from colonies between the end of March to the beginning of June, depending on species different timing of laying. In the Evros Delta we sampled cormorants (*Phalacrocorax carbo*), avocets (*Recurvirostra avosetta*), yellow-legged gulls (*Larus cachinnans michahellis*) and Mediterranean gulls (*Larus melanocephalus*). In the Axios Delta we sampled cormorants, common terns (*Sterna hirundo*) and Mediterranean gulls. For comparisons, eggs of cormorants from Lakes Kerkini and Prespa and of Mediterranean gulls from Porto Lagos were also collected. All eggs were freshly laid, either abandoned among nests or deserted in flooded nests excepting cormorant eggs collected from active nests, under licence. All eggs were collected in 1997 excepting Prespa and a part of Evros Delta cormorant egg-samples. In the latter area due to the similarity of pollutant levels in both years samples were compiled in one (1997/98). In the field, eggs were placed in cotton-spread cartons and were then brought to the laboratory where they were opened in the same day, their contents poured in chemically cleaned containers and were subsequently deep frozen to −20°C.

2.3. Analytical procedures

One gram from each defrosted and whirlmixed egg sample was transferred into a Teflon bomb and 3 ml conc. HNO_3 (65%, Suprapur) was added. The bomb was placed then in a microwave oven for 5 min at 200 W and 5 min at 350 W. After cooling the content was transferred into a 15-ml polypropylene volumetric flask and rinsed with double distilled water to volume. Blank solutions were prepared similarly.

Cd and Pb concentrations in egg samples were determined by means of a Perkin-Elmer 560 atomic absorption spectrophotometer with a 400 graphite furnace (GF-AAS) and background correction. All reagents were Merck-Suprapur and the standards were BDH-Spectrosol solutions.

2.4. Statistical procedures

The Kruskal–Wallis^{-2} test was used for statistical comparison of Cd and Pb egg levels, between nesting areas and between species within a nesting area. In Evros and Axios Deltas differences between species were assessed by Mann–Whitney *U*-tests using Bonferroni corrections for multiple comparisons. Spearman rank correlations were estimated between Pb and Cd concentrations for each species in each area studied. Metal concentrations were expressed as ppb wet weight.

3. Results

Lead levels in waterbird eggs from the study area varied from below detection limits to 372 ppb in Evros Delta avocets (Table 1). In the Evros Delta the lowest median levels were found in the cormorant (7 ppb) and maximum in the yellow-legged gull (47 ppb). Lead levels were significantly different between species (Kruskal–Wallis^{-2} = 19.326, d.f. = 3, $P = 0.0002$) and more specifically were significantly higher in yellow-legged gull and in avocet than in cormorant ($P = 0.0019$ and $P < 0.0001$ respectively, Mann–Whitney *U*-tests. In these comparisons the P level was 0.0083).

In the Axios Delta, the lowest median lead levels were also found in the cormorant (8 ppb) and maximum in the common tern (50 ppb), the overall difference being highly significant (Kruskal–Wallis^{-2} = 19.819, d.f. = 2, $P < 0.0001$). This was due to significantly lower median lead levels in the cormorant eggs in comparison with both common tern and Mediterranean gull lead levels ($P = 0.0002$ and $P < 0.0001$ respectively, Mann–Whitney *U*-tests. In these comparisons the P level was 0.017). Median Pb concentrations in cormorants were not significantly different among four wetland areas (Kruskal–Wallis^{-2} = 5.548, d.f. = 3, $P = 0.136$). In contrast, the median lead levels of Mediterranean gulls were significantly different in the three sampled coastal wetlands (Kruskal–Wallis^{-2} = 15.225, $P = 0.0005$), due to significantly higher lead levels of Axios compared to both Evros Delta and Porto Lagos ($P = 0.0014$ and $P = 0.0113$ respectively, Mann–Whitney *U*-tests. In these comparisons the P level was 0.017).

Cadmium levels were much lower than Pb in all areas and in all species studied. They varied from below detection limits in some of Evros Delta Mediterranean gull and cormorant eggs to 24 ppb in Mediterranean gulls in the Axios Delta. In the Evros Delta, Cd levels among species were significantly different (Kruskal–Wallis^{-2} = 29.795, d.f. = 3, $P < 0.0001$). Pairwise, significantly higher levels were found in the avocet eggs than in Mediterranean gulls and cormorant eggs (both $P < 0.0001$, Mann–Whitney *U*-tests.). In the Axios Delta, lowest median cadmium levels were found in the cormorant (4 ppb) and maximum in the common tern (7 ppb). The overall difference was significant (Kruskal–Wallis^{-2} = 7.585, d.f. = 2, $P = 0.022$). In pairwise comparisons the median levels in the cormorant eggs were marginally significantly lower than in common tern ($P = 0.011$, Mann–Whitney *U*-test). Median concentrations of Cd in cormorants were significantly different among the four wetland areas (Kruskal–Wallis^{-2} = 16.580, d.f. = 3, $P = 0.0009$). This was due to significantly higher median levels in Kerkini than in both Evros and Prespa ($P = 0.0003$ and $P = 0.0054$ respectively, Mann–Whitney *U*-tests). Median Cd levels in Mediterranean gull eggs were not significantly different in the three sampled coastal wetlands (Kruskal–Wallis^{-2} = 5.529, d.f. = 2, $P = 0.063$).

Spearman rank correlations between the two metals were insignificant for all species in all areas excepting Mediterranean gull in Porto Lagos (Table 2).

Table 1
Concentrations of lead and cadmium (ppb wet weight) of colonially nesting waterbirds from Greek wetlands[a]

	Mean	Median	Minimum	Maximum	No. of eggs
Lead					
Evros Delta					
P. carbo	8	7	nd	22	38
L. cachinnans	68	47	nd	177	7
L. melanocephalus	20	12	nd	100	15
R. avosetta	70	32	3	372	20
Axios Delta					
P. carbo	10	8	nd	30	13
S. hirundo	83	50	4	313	13
L. melanocephalus	61	44	nd	165	15
P. Lagos					
L. melanocephalus	29	16	nd	142	13
Kerkini					
P. carbo	28	22	nd	127	15
Prespa					
P. carbo	7	7	nd	15	12
Cadmium					
Evros Delta					
P. carbo	3	3	nd	6	38
L. cachinnans	5	4	1	8	7
L. melanocephalus	3	4	nd	5	15
R. avosetta	6	6	3	9	20
Axios Delta					
P. carbo	4	4	2	8	13
S. hirundo	7	7	1	12	13
L. melanocephalus	7	5	3	24	15
P. Lagos					
L. melanocephalus	4	4	1	12	13
Kerkini					
P. carbo	6	6	3	10	15
Prespa					
P. carbo	4	3	2	5	12

[a]Mean values are given for comparisons with the literature. nd, Not detected.

4. Discussion

The differences found between species in the levels of both lead and cadmium in the Evros and Axios Deltas are attributable to their different diets. Dietary data are lacking for the study years but have been available for most species and areas through previous studies. In the Evros Delta the avocet feeds upon nereid worms (*Hediste diversicolor*), amphipods such as *Gamarus aequicauda* and *Corophium orientale* and aquatic insects (Goutner, 1985). The yellow-legged gull is an omnivorous species, whereas the Mediterranean gull has a very variable diet: in the Evros Delta prey composition has varied greatly from almost exclusively fish in one year to almost exclusively insects in another (Goutner, 1986). In the Thermaikos Gulf area these birds feed greatly on fish from trawler discards during the pre-laying period, whereas during the chick rearing period their diet is mainly composed by wheat seeds and insects (Goutner, 1986, 1994a). In the Axios Delta, the common terns feed on surface-living, small-sized fish such as *Atherina* sp., *Spratus* sp. and *Aphanius fasciatus* (V. Goutner, unpublished data). Cormorants are exclusively fish feeders,

Table 2
Spearman rank correlation coefficients and probability levels between lead and cadmium concentrations from eggs of colonially breeding waterbirds from Greek wetlands

Species	r_s	P	N^a
P. carbo (Evros)	−0.172	0.302	38
P. carbo (Axios)	0.473	0.103	13
P. carbo (Kerkini)	0.194	0.488	15
P. carbo (Prespa)	0.228	0.476	12
L. melanocephalus (Evros)	0.333	0.224	15
L. melanocephalus (P. Lagos)	0.718	0.006	13
L. melanocephalus	0.343	0.211	15
R. avosetta (Evros)	−0.014	0.953	20
L. cachinnans (Evros)	0.667	0.102	7
S. hirundo (Axios)	−0.008	0.978	13

[a] *N*, number of eggs analysed.

therefore top carnivores in the food chain in wetlands. Nevertheless their dietary composition differs among the wetlands studied: in the Axios Delta it is mainly constituted by gobies (*Gobius jozo*) and mugilids (Goutner et al., 1997), at Kerkini Lake mainly by *Alburnus alburnus* (T. Nazirides, personal communication) whereas at Prespa Lake by *Chalcalburnus belvica* (Catsadorakis, 1997).

Based on the above-mentioned diet information, the Evros Delta avocet, with the lowest position in the food chain considered, had higher egg Pb levels than cormorants (being in the highest trophic level) and higher levels of Cd than both cormorants and Mediterranean gulls. Also, the yellow-legged gull, being at a lower trophic level than cormorant had higher egg Pb levels; and in the Axios Delta common terns and Mediterranean gulls had higher egg Pb levels than cormorants, and common terns higher levels of Cd than cormorants. Consequently Pb and Cd concentration in eggs was unrelated to the position of each species studied in its food chain. Similarly, Burger and Gochfeld (1996b), found no clear food chain pattern for these two metals in five waterbird species they studied.

Elevated Pb levels in yellow-legged gull eggs may be understandable due to its omnivore, wide-range feeding habits implying that these birds may partially feed over lead-polluted areas. Nevertheless, similarly high Pb levels in the avocet should probably be searched in the mechanisms its invertebrate prey incorporates lead. In lead-contaminated areas the sediment dwelling *Nereis diversicolor* (former name of *Hediste diversicolor*) and other worms may accumulate high amounts of lead being a source of contamination for birds (Bryan and Langston, 1992). Cadmium is taken up by nereid worms rather from interstitial water than from sediments (Bryan and Langston, 1992; Ferns and Anderson, 1994). Certain amphipods accumulate considerable concentrations of cadmium and may represent significant sources of this metal in the diet of certain seabirds (Rainbow, 1989).

Despite considerable differences in diet, there was a lack of significant difference in Pb levels among areas sampled for the cormorant and in Cd levels among those sampled for Mediterranean gull, probably implying species-specific accumulation patterns. A significant Spearman rank correlation between the two metals in Mediterranean gull eggs in Porto Lagos probably denote a common pollution source in this particular area. Insignificant correlations between the two metals are also known from studies in the common tern (Burger and Gochfeld, 1991, 1993) and the herring gull (Burger and Gochfeld, 1996b, Gochfeld, 1997) although, in another study, a significantly positive correlation was detected in the latter species (Burger and Gochfeld, 1995b).

Levels of both metals were not considerably higher in the Axios Delta, despite our predictions based on wetlands' bordering to a highly industrialized area and also being in the vicinity of Thessaloniki the second highly populated city of Greece. Nonetheless, highest lead levels in Mediterranean gull eggs from Axios may indicate a higher lead pollution of this area reflected in the egg due to the feeding habits of this bird greatly using terrestrial habitats surrounding its colonies (Goutner, 1994a).

In this and in other studies on waterbird eggs, Pb and/or Cd levels were either below detection limits (Cheney et al., 1981; Fossi et al., 1984;

Ohlendorf and Harrison, 1986; Becker and Sperveslage, 1989; Crivelli et al., 1989; Mora, 1996; Morera et al., 1997), or in very low concentrations comparable to our findings (Cheney et al., 1981; Focardi et al., 1988; Burger and Gochfeld, 1991; Weseloh et al., 1997). However, considerably higher levels of Cd and especially Pb have been detected in eggs of waterbirds in other parts of the world such as in New York Bight and Captree herring gull colonies (Burger and Gochfeld, 1993; Gochfeld, 1997). Also, in the Agassiz National Wildlife Reserve (ANWR), Minnesota, concentrations of Cd in double-crested cormorant (*Phalacrocorax auritus*) and in black-crowned night-heron (*Nycticorax nycticorax*) as well as Pb levels in Franklin's gull (*Larus pipixcan*) eggs were even higher (Burger and Gochfeld, 1996c). These examples indicate that where high concentrations are reflected in birds due to environmental exposure, eggs can be useful tools for environmental biomonitoring. The very low concentrations of Pb and Cd found in the eggs from the Greek wetlands may either suggest low environmental inputs or lack of sensitivity in using eggs of these species as metal biomonitors in our areas. Therefore, a more sensitive metal bioindicator still remains to be found.

5. Conclusions

1. Birds such as the avocet and yellow-legged gull are positioned at a lower trophic level than the cormorant, and exhibit higher egg Pb levels.
2. Prey sources are probably determinative for Cd and Pb egg burden in birds since water-based bird food is less polluted than the sediment-based type.
3. Lack of significant differences in Pb levels among areas sampled for cormorant and in Cd levels sampled for Mediterranean gull, imply species-specific accumulation patterns.
4. A significant correlation between Pb and Cd in Mediterranean gull eggs in Porto Lagos is probably due to a common pollution source in this area.

Acknowledgements

We are indebted to Dr S. Kazantzidis, S. Goutner, D. Skartsi, T. Nazirides and M. Malakou, for aid in the field. Chemical analyses were supported by the University of Ioannina.

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