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Lead (Pb) and Copper (Cu) Concentration in the Eggshells of Audouin's Gulls (*Larus audouinii*) in Turkey

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Abstract: Audouin's gull (*Larus audouinii*) eggshells from 2 archipelagos (Karaburun-İzmir and Aydıncık-Mersin) in Turkey were collected between 2002 and 2003, and analyzed for lead (Pb) and copper (Cu) content. According to the results, mean Cu level of the eggshells was 1.855 ppm for the Aydıncık colony and 10.202 ppm for the Karaburun colony. Mean Pb level of the eggshells was 0.954 ppm for the Aydıncık colony and 4.601 ppm for the Karaburun colony. The detected metal levels did not seem to have any toxic effects on the Audouin's gull populations. It is suggested that eggshells from abandoned nests of Audouin's gulls may be useful as bio-indicators for monitoring heavy metal contamination.

Key Words: Audouin's gull, eggshells, Cu, Pb, Turkey

Türkiye'deki Ada Martılarının (*Larus audouinii*) Yumurta Kabuklarındaki Kurşun (Pb) ve Bakır (Cu) Konsantrasyonu

Özet: Ada martısı yumurta kabukları iki adacıktan (Karaburun - İzmir ve Aydıncık - Mersin) 2002-2003'de toplanmış ve kurşun (Pb) ve bakır (Cu) analizleri yapılmıştır. Yumurta kabuklarındaki ortalama Cu düzeyi, Aydıncık kolonisi için 1,855 ppm ve Karaburun kolonisi için ise 10,202 ppm saptanmıştır. Yumurta kabuklarındaki ortalama Pb düzeyi ise Aydıncık kolonisinde 0,954 ppm ve Karaburun kolonisinde 4,601 ppm saptanmıştır. Saptanan metal düzeyleri ada martısı populasyonlarında herhangi bir toksik etki oluşturacak düzeyde değildir. Bu çalışma, terkedilmiş yuvalardan toplanan ada martısı yumurta kabuklarının, ağır metal kontaminasyonunun izlenmesi için biyo-indikatör olarak kullanılabileceği fikrini vermiştir.

Anahtar Sözcükler: Ada martısı, yumurta kabuğu, Cu, Pb, Türkiye

Introduction

The number of Audouin's gulls (*Larus audouinii*) in the Mediterranean is estimated to be 18,600 pairs. Approximately 70% of the Audouin's gull population breeds at the Ebro Delta in Spain. The remaining part of the population is distributed among the Chafarinas islands (Spain) and Aegean islands. The Hellenic Ornithology Association reported 20 colonies consisting of 530 pairs on the Greek islands and islets in the study they conducted in 1997 (Oro, 1998). Additionally, there were 4 colonies reported in Algiers, 1 colony on Cyprus, 2 colonies in France, 10 colonies in Italy, 4 colonies in Morocco, and 1 colony in Turkey. The most northern breeding site of the bird is Gorgona Island, Tuscany, Italy (Int. Act. Plan for A., 1996).

Audouin's gull colonies were previously studied by Çağlayan (2003) in Turkey (Aydıncık-Mersin and Karaburun-İzmir). The literature concerning population densities and breeding sites of Audouin's gulls is clearly insufficient, and the related data are quite varied. Until recently, Audouin's gulls were known to breed only on Aydıncık Island near the coast of Mersin province. The Aydıncık colony was monitored by Witt in 1973-74 and he also monitored the colony during his visit to the site in 1995-1997. According to his observations, there were 28 pairs breeding on Aydıncık Island in 1974, 30 pairs in 1987, and 6 pairs in 1996. Apart from that, 10 pairs were observed exhibiting flirting behavior in 1998 (Witt, 1997).

In 1998, Audouin's gulls were also observed on Karaburun Island in the Aegean Sea by amateur bird

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watchers. There were 5 adult pairs and 1 young gull recorded; however, a detailed investigation of the pairs, which bred after this observation at the end of April (Çağlayan, 2003), were not made. With the presence of the Audouin's gull, Aydıncık Island was declared an important bird site (IBS) (Kılıç and Eken, 2004). Moreover, Aydıncık and Karaburun islands were declared natural sites of first degree importance. The first detailed investigation of Audouin's gulls (*Larus audouinii*) in Turkey was carried out between 2001 and 2003. According to this there were 17 pairs observed on Aydıncık Island in 2001 and 8 pairs in 2002, and there were 18 pairs of Audouin's gulls (*Larus audouinii*) recorded on Karaburun Island.

It was observed that these small colonies chose Aydıncık and Karaburun islands as their breeding grounds; however they left the islands during certain years due to excessive fishing, animal grazing, egg collection, and competition with herring gulls, *Larus argentatus* (Çağlayan, 2003). Previously, no studies have been conducted in Turkey on the deposition of contaminants in their breeding grounds and habitats, or on the passage of these contaminants to their eggs.

Studies related to metal contamination in the Audouin's gull (Goutner et al., 2000) were carried out in the western Mediterranean Basin, mainly in Italy (Leonzio et al., 1989) and at the Ebro Delta and on the Chafarinas islands of Spain (Morera et al., 1997). There are no studies related to metal levels in the colonies of Turkey, which is the most eastern breeding ground of the population.

The aim of the present study was to report reference values for continuous monitoring in the future and to compare colonies under stress from different pollutants. It is hoped that this study will be a suitable reference for the determination of regional contamination and realization of a protection plan for this top predator species.

Material and Methods

Collection Sites and Sampling

In this study, 2 known archipelagos in Turkey (Aydıncık and Karaburun) with Audouin's gull colonies were selected for eggshell collection. Aydıncık consists of 1 big and 1 small islet about 2 km off the southern Mediterranean coast of Turkey, 5 km southeast of Aydıncık Township in Mersin Province, located at lat 36°08′N, long 33°20′ E. Karaburun Island is within the boundaries of Karaburun Township in İzmir Province, on Turkey's Aegean coast. It is approximately 1.5 km from the port of Karaburun. Its southern slope is covered with dense bushes. There are steep rocks coming from the sea on the northern and northwestern parts of the Island.

The eggshells were collected from deserted nests on both islands at the end of the breeding period. There were 8 eggshells collected from Aydıncık Island and 10 eggshells from Karaburun Island, corresponding to different nests in order to avoid pseudoreplication. Collected eggshells were labeled according to nest number, placed in chemically cleaned glass jars, and transported to the laboratory.

Chemical Analysis

Square wave stripping voltammetry (SWASV) is much more suitable to our purposes than linear wave stripping voltammetry due to its much shorter deposition time and lower detection limit (Somer and Ülkü, 2004); therefore, SWASV (Locatelli and Torsi, 2004) was used to measure Cu and Pb in the eggshells of Audouin's gulls. Analyses were carried out by the standard addition method using the increase in the magnitude of the stripping peak.

Eggshells were not cleaned before metal analysis. Samples (0.2 g) of each eggshell batch were placed in beakers and dried at 100 °C until they reached constant weight. The egg samples were digested in nitric acid and then 1.5 ml of ultra pure HNO_3 (Merck) was added to each sample. The temperature was gradually increased until the sample was completely dissolved (without complete dryness) and 10 ml of de-ionized water was added to each of them. All the reagents were of analytical grade and all solutions were prepared with de-ionized water.

The heavy metals in the dissolved samples were first determined using a Perkin-Elmer Optima 4300 DV ICP-OES spectrophotometer; however, metal content was too low to give meaningful results; therefore the determinations were carried out with the SWASV method using a CHI 660 B electrochemical analyzer equipped with a BAS cell stand. The working and auxiliary electrodes included a hanging mercury drop electrode (HMDE) and Pt wire, and the potentials were measured against a Ag/AgCl (3 M KCl) reference electrode. To the electrochemical cell, 1.5 ml of the dissolved sample was

transferred using perchloric acid (70%, Merck) as a supporting electrolyte and to acidify the working medium. Then, 0.1 M of stock solutions prepared from Cu $(NO_3)_2$ (Merck) and Pb $(NO_3)_2$ (Merck) were used for the preparation of standard addition solutions of 10^{-3}, 10^{-4}, and 10^{-5} M by daily dilution. The increase in the magnitude of the peak after standard additions of the stock solutions was recorded to determine the heavy metal content. Two blank solutions were prepared using equivalent amounts of acid, and Pb and Cu were determined in these solutions and extracted to calculate the actual amounts present in the samples. The detection limit was 0.1 ppm for each metal.

Statistical Analysis

Pb and Cu concentrations in the eggshells collected from the Aydıncık and Karaburun colonies were compared. Descriptive statistics are shown in the Table. Since the sample size was very small, and Cu and Pb data were not normally distributed, we used nonparametric Kruskal-Wallis statistical procedures. Correlations were analyzed between Pb and Cu values for the 2 islands using Spearman's rank correlation coefficients. Statistical analyses were performed using SPSS v.13.0.

Results

Pb and Cu concentrations in the eggshells belonging to the Aydıncık and Karaburun colonies are shown in Table, with mean, median, standard deviation, and variance values. There was a significant correlation between Pb and Cu in the Karaburun colony ($r = 0.671$, $P < 0.05$). On the other hand, there was a strong correlation between the 2 metals in the Aydıncık colony ($r = 0.972$, $P < 0.001$). Pb concentration of 5 of the 8 samples collected from the Aydıncık colony ranged between 0.59 and 2.62 ppm. Pb content in 9 of the 10 samples collected from the Karaburun colony ranged between 0.98 and 18.42 ppm. Mean eggshell Pb concentration in the Karaburun colony (4.601 ± 5.804 ppm) was much higher than in the Aydıncık colony (0.954 ± 1.005 ppm). The difference in Pb levels was statistically significant ($H = 3.04$; $P > 0.05$).

Cu in 5 of the 8 Aydıncık samples ranged from 0.44 to 5.86 ppm, while Cu in 9 of the 10 Karaburun samples ranged between 0.56 and 49.953 ppm. Mean Cu level in the Karaburun eggshells (10.202 ± 16.040 ppm) was much higher than that of the Aydıncık eggshells (1.855 ± 2.570 ppm). Cu content in the Aydıncık samples was

Table. Mean levels and variance values for Pb and Cu (ppm: calculated in dry weight samples) in each sample from the Karaburun and Aydıncık colonies (nd: not determined).

Eggshell number	Pb		Cu	
	Karaburun	Aydıncık	Karaburun	Aydıncık
1	8.89	1.25	20.39	1.17
2	18.42	2.62	1.69	5.86
3	1.34	0.59	0.56	0.44
4	1.85	nd	4.18	nd
5	nd	nd	nd	nd
6	2.42	nd	2.52	nd
7	1.17	2.11	1.13	6
8	0.98	1.06	1.21	1.37
9	2.05		49.95	
10	8.89		20.39	
n	10	8	10	8
Mean	4.601	0.954	10.202	1.855
Median	1,95	2,11	0,295	0,220
Min	0	0	0	0
Max	18.42	2.62	49.95	6
Std Dev	5.80493	1.005243	16.03965	2.570459

homogenous; however, Cu content in the Karaburun samples was heterogeneous, with high variance (Table). Although Cu content in the eggshells collected from Karaburun seemed to be significantly higher than that of the eggshells collected from Aydıncık, the difference in Cu levels was not statistically significant ($H = 2.17$; $P > 0.05$).

Discussion

Due to the limited number of eggs found in the nests, the studies were condcuted with broken eggshells rather than egg contents in order to avoid inducing any adverse effect on the breeding activities of the birds. Materials used to determine metal accumulation in birds are internal organs and egg content (Stronkhorst et al., 1993), eggshells (Burger, 1994; Morera et al., 1997; Ayas, 2007), feathers (Burger, 1993; Goutner et al., 2001), and feces (Dauwe et al., 2000).

It was estimated that the number of gulls in the studied colonies would increase in the future, provided that anthropogenic factors, such as fishing and grazing, are appropriately controlled. The limited number of collected samples represents a problem that hampered statistical analysis. The metal values found in eggshells were highly variable, with high intra group variances (Table). For instance, Pb levels observed in the Karaburun Island eggshells were very different from each other and significantly higher than those in the Aydıncık samples; however, the difference in Pb levels in the eggshells obtained from the 2 islands were not statistically significant due to high variation in the Karaburun values.

The fact that some of the Karaburun samples had Cu and Pb levels higher than those of the Aydıncık samples was probably due to the close proximity of Karaburun to the industrial regions and high pollution levels of İzmir Bay. Previous studies reported that the rivers and streams flowing into the Aegean Sea are the main sources of heavy metal pollution in İzmir Bay (TÇSV, 1991; Tekin, 1992; Balcı and Turkoglu, 1993).

It is known that heavy metals in the environment can be accumulated by gulls and pass into their eggs (Furness, 1993). Arınç and Şen (1999) determined that heavy metals are deposited in sediments and in pelagic fish, which are the main food source of gulls. Burger (1994) found that egg laying was one of the methods of removing heavy metals from the organs of herring gulls (*Larus argentatus)*. Scheuhammer (1987) stated that the passage of heavy metals to gull eggs could only take place when there is an excessive accumulation of heavy metals in the organs of the adult birds.

Goutner et al. (2000) explained that the high level of mercury accumulation observed in the feathers of Audouin's gulls was due to the elevated concentration of mercury in the Aegean Sea, resulting from the high pollution load of the rivers and streams flowing into it. Aydıncık is very far from any industrial region, and the rivers flowing near it pass through agricultural areas rather than industrial. The largest source of water close to Aydıncık Island is the Göksu River. Previous studies revealed that the pollution load of this river consists mainly of agricultural contaminants, such as organochlorine pesticides and fungicides containing mercury, zinc, and manganese (Ayas and Kolankaya, 1996; Ayas et al., 1997).

The Cu level in seabirds is general fairly small (0.15-1.8 ppm/fresh-weight) (Walsh, 1990; Stronkhost et al., 1993). Morera et al. (1997) reported that the level of Cu in egg contents and eggshells of Audouin's gulls was 2.58 and 2.14 ppm, respectively. Leonzio et al. (1989) found that the eggs of Audouin's gulls were heavily contaminated with mercury and chloride-containing hydrocarbons, and that the level of Pb was > 0.1 ppm; however, in the present study the mean Pb level in Audouin's gull eggshells from the eastern Mediterranean were much higher (from Karaburun: 4.601 ppm; from Aydıncık: 0.954 ppm).

The metal content of eggshells can provide a good idea about the metal content of egg contents. Morera et al. (1997) found that the Cu level in egg contents and eggshells were very close to each other (2.58 and 2.14 ppm, respectively). In some studies the content of certain metals in egg contents were 2-35 times higher than the corresponding concentrations in eggshells (Mora, 2003); therefore, it can be concluded that the Cu and Pb levels in egg contents of the Audouin's gull colonies on Karaburun and Aydıncık islands must be at least equal to or higher than the respective concentrations determined in their eggshells.

As seen from the Table, the levels of Cu and Pb in each egg sample were quite different. This difference is more apparent in the Karaburun samples. The main reason for this difference is the restricted number of samples; however, this may also be due to other

environmental and physiological factors, such as environmental pollution load, age of the egg laying female, and inter- and intra-clutch variability (Becker, 1989; Sanpera et al., 1997).

In seabirds, Becker (1992) found inter-clutch differences in Hg concentration, both in *Larus argentatus* and *Sterna hirundo*, as well as decreasing Hg levels according to the laying sequence (a-, b-, c- eggs). Additionally, Morera et al. (1997) detected a significant female effect on Hg concentrations in *Larus audouinii*. Morera et al. (1997) found that concentrations of elements such as Zn, Cu (which was also determined in our study), Mn, and Hg were 80%-99% greater in the egg contents of Audouin's gulls than in eggshells (*Larus audouinii*).

In conclusion, the realization of population and pollution studies related to Audouin's gull will be useful to future planning and programs on behalf of its protection. Moreover, it is suggested that Audouin's gull can be an appropriate bio-indicator organism for monitoring environmental pollution.

References

Arınç, E. and Şen, A. 1999. Hepatic cytochrome P4501A and 7-ethoxyresorufin-O-deethylase induction in mullet and common sole as an indicator of toxic organic pollution in İzmir Bay, Turkey. Marine Environmental Research. 48: 147-160.

Ayaş, Z. and Kolankaya, D. 1996. Accumulation of some heavy metals in various environments and organisms at Göksu Delta, Turkey, 1991-1993. Bulletin Environmental Contamination and Toxicology. 56: 65-72

Ayaş, Z., Barlas, N. and Kolankaya, D. 1997. Determination of organochlorine pesticide residues in various environments and organisms in Göksu Delta, Turkey. Aquatic Toxicology. 39:171-181.

Ayaş, Z. 2007. Trace element residues in eggshells of grey heron (*Ardea cinerea*) and black-crowned night heron (*Nycticorax nycticorax*) from Nallihan Bird Paradise, Ankara-Turkey. Ecotoxicology. 16: 347–352

Balcı, A. and Turkoglu, M. 1993. Heavy metals in sediments from İzmir Bay, Turkey. Marine Pollution Bulletin. 26: 106-107.

Becker, P.H. 1989. Seabirds as monitor organisms of contaminants along the German North Sea coast. Helgol Meeresunter. 43: 395-403.

Becker, P.H. 1992. Egg mercury levels decline with the laying sequence in Charadriiformes. Bulletin Environmental Contamination and Toxicology. 48: 762-767.

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. Review of Environmental Toxicology. 5: 203-311

Burger, J. 1994. Heavy metals in avian eggshells: Another excretion method. Journal of Toxicology and Environmental Health. 41: 207-220.

Çağlayan, E. 2003. Population Studies on Audouin's gull (*Larus audouinii*) in the Karaburun and Aydıncık Islands, Turke. MSc Thesis. Hacettepe University, Institute for Graduate Studies in Pure and Applied Sciences.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R. and Ens, M. 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Archive of Environmental Contamination and Toxicology. 39: 541-546.

Furness, R.W. 1993. Birds as monitors of pollutants. In: Furness RW Greenwood JJD (Eds.). Birds as monitors of environmental change. Champan and Hall, London, pp 86.

Goutner, V., Furness, R.W. and Papakonstantinou, K. 2000. Mercury in Feathers of Audouin's Gull (*Larus audouinii*) Chicks from Northeastern Colonies. Archive of Environmental Contamination and. Toxicology. 39: 200-204.

Goutner, V., Albanis, T., Konstantineu, I. and Papakonstantinou, K. 2001. PCB and organochlorine residues in eggs of Audouin's Gull (*Larus audouinii*) in the north-eastern Mediterrenean. Marine Pollution Bulletin. 42: 377-388 pp

International Action Plan for Audouin's Gull. 1996. Bird Life International on behalf of the European Commission, http://www.europa.eu.int/ comm/environment/nature/directive/birdactionplan/larusaudouinii.htm

Kılıç, D.T. and Eken, G. 2004. Türkiyenin Önemli Kuş Alanları, 2004 Güncellemesi. Doğa Derneği, Ankara, Türkiye, s. 74-100.

Leonzio, C., Lambertini, M., Massi, A., Focardi, S. and Fossi, C. 1989. An assessment of Pollutants in Eggs of Audouin's Gull (*Larus audouinii*) A Rare Species of the Mediterranean Sea. The Science of the Total Environment. 78: 13-22.

Locatelli, C. and Torsi, G. 2004 Simultaneous square wave anodic stripping voltammetric determination of Cr, Pb, Sn, Sb, Cu, Zn in presence of reciprocal interference: application to meal matrices, Microchemical Journal. 78: 175–180.

Mora, M.A. 2003. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. Environmental. Pollution. 125: 393-400.

Morera, M., Sanpera, C., Crespo, S., Jover, L. and Ruiz, X. 1997. Inter and Intraclutch Variability in Heavy Metals and Selenium Levels in Audouin's Gull Eggs from Ebro Delta, Spain. Archive of Environmental Contamination and Toxicology. 33: 71-75.

Oro, D. 1998. Audouin's Gull account. The Birds of Western Palearctic In: Ogilvie, M.A., (Eds.) Vol 2. Oxford University Press, Oxford, UK.

Sanpera, C., Morera, M., Crespo, S., Ruiz, X. and Jover, L. 1997. Trace elements in clutches of yellow-legged gulls, *Larus cachinnans*, from the Medes Islands, Spain. Bulletin Environmental Contamination and Toxicology. 59: 757-762.

Scheuhammer, A.M. 1987. The chronic toxicity of aluminum, cadmium, mercury and lead in bird: A Review. Environmental Pollution. 46: 263-295.

Somer, G. and Ülkü, U. 2004. A new and direct method for the trace element determination in cauliflower by differential pulse polarography. Talanta. 62: 323-328.

Stronkhorst. J., Ysebaert. T.J., Smedes, F., Meininger, P.L., Dirksen, S. and Boudewijn, T.J. 1993. Contaminants in eggs of some waterbird species from Scheldt Estuary, SW Netherlands. Marine Pollution Bulletin. 26: 572-578.

TÇSV (Environmental Problems Foundation of Turkey) 1991. Aegean Sea. In: Environmental Profile of Turkey, pp. 127-132. Önder Matbaası, Ankara.

Tekin, H. 1992. Environmental Recovery of the İzmir Inner Bay Determination of Effects of Sediment Load on Bay Water Quality. MSc Thesis, Middle East Technical University (METU). Graduate school of Natural and Applied Sciences, Ankara-Turkey.

Walsh, P.M. 1990. The use of seabirds as monitors of heavy metals in the marine environment. In: Furness RW, Rainbow PS (Eds.) Heavy metals in the marine environment, CRC Press Inc., Boca Raton, FL, pp. 183.

Witt, H.H. 1997. Poster, International Seminar on Audouin's Gull, 9-12 June 1997 Melilla, Spain.