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Heavy metal accumulation as a threat to the endangered Egyptian vulture (*Neophron percnopterus* L.) in Turkey

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Abstract: Due to anthropogenic activity, most vulture species are threatened. *Neophron percnopterus*, the Egyptian vulture, is a vulture species with a declining population. One of the most important factors threatening the species is exposure to chemicals. The second largest population of this bird species is found in Turkey. Accumulation of heavy metals for this species has not been investigated. The aim of this study was to detect and measure As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn values in the feathers of *Neophron percnopterus*. Calamis and vane, which are different part of the feathers, were analyzed separately with ICP-OES. According to the results, the mean value of heavy metal accumulation is below the threshold levels, except for Pb (mean ± SD = 32.26 ± 74.71 µg/g d.w.) and Cr (mean ± SD = 17.82 ± 33.74 µg/g d.w.) in vanes. On the other hand, all heavy metal values other than Hg and Zn had critical levels, which may result in lethal and sublethal effects on individuals.

Key words: Anatolia, birds of prey, ecotoxicology, feather, noninvasive methods, scavenger

1. Introduction

Heavy metals are metallic elements with atomic density greater than that of water (Fergusson, 1990). The natural origins of heavy metals in the environment are volcanic eruptions and natural crust erosion (Mandon et al., 2019), while anthropogenic activities such as mining are the primary source of metals throughout the world (Ma et al., 2015; Zhu et al., 2017).

Many heavy metals, such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), nickel (Ni), and cobalt (Co), are essential trace nutrients for plants and animals (Rengel, 2004). On the other hand, some metals are highly toxic even at low concentrations (Zahir et al., 2005). Heavy metal pollution is one of the most important threats to marine (Ling et al., 2018), freshwater (Abdelhady et al., 2019), and terrestrial (Belskii and Mikryukov, 2018) biodiversity. Exposure to heavy metals has resulted in growth and developmental abnormalities, detrimental effects on productivity, and death in animals (Migliarini et al., 2005; Burki, 2012; Hargitai et al., 2016; Pavlaki et al., 2016; Zeng et al., 2019).

Vultures are among the most affected birds because of negative anthropogenic factors (Ogada et al., 2012). Due to dramatic decreases arising from stress factors in regions where vulture species are found, many vulture populations have become almost extinct, which is seen as a real vulture crisis throughout the world (Green et al., 2004; Virani et al., 2011; Ogada et al., 2016). Heavy metals are one of the major threats for vultures (Gangoso et al., 2009, Bounas et al., 2016). Like many other birds of prey, vultures have much more exposure to chemicals through bioaccumulation and biomagnification because they are at the top of the food chain (Miller et al., 2002). Furthermore, feeding on contaminated carcasses makes scavenger vultures more vulnerable to chemical exposure than other predators (Ogada et al., 2012). It is known that metals, depending on their concentrations, have sublethal (Espín et al., 2014) or lethal effects (Espín et al., 2014) on vulture species. As a result of all of these threat factors, developing conservation strategies and action plans for vulture species has become a serious issue worldwide (Bowden, 2017).

The Egyptian vulture is listed as an endangered vulture species on the IUCN Red List (BirdLife International, 2017). Poisoning, poaching, and destruction of natural habitats are the main threats to the species' populations (Angelov et al., 2013; Grubač et al., 2014; Sanz-Aguilar et al., 2015; Velevski et al., 2015). Turkey is estimated to have the second largest Egyptian vulture population

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after Spain in the Western Palearctic region (BirdLife International, 2017). The protection of Egyptian vulture populations in Turkey is very important in terms of increasing the numbers of the species around the world. Effective conservation studies may be carried out only if the negative factors affecting populations are established.

It has been reported that heavy metals have adverse effects on Egyptian vultures (Donázar et al., 2002; Pikula et al., 2013; Berny et al., 2015; Bounas et al., 2016). In Turkey, there have been no studies on heavy metal accumulation in the Egyptian vulture. Thus, heavy metal contamination has not been revealed as a possible threat factor for the species in Turkey. From this point of view, it is essential to analyze to what extent the Egyptian vulture is exposed to heavy metals, and also to determine whether these metals are a risk factor for the species.

Using feathers in heavy metal assessment is a common practice, especially for endangered species, as a noninvasive method (Abdullah et al., 2015; Theuerkauf et al., 2015; Verma et al., 2017; Yamac et al., 2019). The amount of heavy metal accumulation may vary in different parts of a feather (Dauwe et al., 2003); therefore, both the vane and calamus of the collected feathers were analyzed separately. This study was conducted in order to obtain data concerning heavy metal accumulation in feathers of the endangered Egyptian vulture in Turkey.

2. Materials and methods

2.1. Study area and sampling

The study was performed in Northwest Anatolia (Figure 1). Detailed information regarding the study area was previously presented by Balaban and Yamaç (2018). Feathers were used for heavy metal analysis, being a noninvasive method. Primary feather samples were collected from the base of 10 different Egyptian vulture nests (Figure 2). The distance of the nearest neighbor from each sampled nest ranged between 5.5 and 41.2 km. In total, 26 feathers approximately 26.3 ± 3.2 cm in length were collected from 10 occupied nests in the latter half of September after adults and juveniles had left the breeding area. To avoid collecting samples belonging to the same individual, only a single feather from each nest was used for heavy metal analysis.

2.2. Metal analysis

Initially, feather samples were washed with tap water for rough cleaning. After soaking the feathers in pure acetone for 10 min, they were then washed using ultrapure water (0.055 µS/cm) and air-dried. Following this, the feathers were dried at 80 °C for 24 h until they reached dry weight. A sample of 250 mg of each feather was added to a mixture of 4 mL of nitric acid (HNO_3, 65%) and 1 mL of hydrogen peroxide (H_2O_2, 30%) in PFA plates and left until the acid discharge was complete. The covered PFA plates were processed for 30 min at 200 °C in a CEM Mars Express sample burning unit (CEM Corporation, Matthews, NC, USA). After cooling, the samples were filtered (Macherey-Nagel MN640; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and bathed in distilled water up to 50 mL. The entire process performed on the feather samples was implemented in order to prepare blank samples for acid samples. This process was repeated 3 times (US EPA, 1998).

An ICP-OES device (inductively coupled plasma optical emission spectrometer, Varian 720-ES; Agilent, Santa Clara, CA, USA) was used for the analysis of 8 metals (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn). The detection limits of the metals were 1 µg/L for arsenic, 0.05 µg/L for cadmium, 0.15 µg/L for chromium, 0.3 µg/L for copper, 0.8 µg/L for mercury, 0.3 µg/L for nickel, 0.8 µg/L for lead, and 0.2 µg/L for zinc. In addition to the metals, standard reference material (human hair, CRM, ERM-DB001) was also analyzed.

2.3. Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data statistically. A Shapiro–Wilk test was conducted to assess the normality distribution of the data. Normally and nonnormally distributed data were evaluated with Student's t-test and the Mann–Whitney U test, respectively, to identify whether there was any significant difference in heavy metal accumulation between different parts of the feathers. A significance level of 0.05 was accepted for all of the tests. Data are reported as mean ± standard deviation (SD).

3. Results and discussion

Data on heavy metal accumulation for the calamus and vane parts of each of the sampled feathers are presented in the Table. According to our results, the As concentration in the vane of the feathers of 2 individuals and the As concentration in both the calamus and vane of feathers of 1 individual were higher than the threshold value, which is 1 µg/g (Sánchez-Virosta et al., 2015) (Table). Exceeding this limit mostly results in poor reproductive success among bird populations (Eeva et al., 2009; Berglund and Nyholm, 2011). To the best of our knowledge, data for the As accumulation in feather samples from the Egyptian vulture have not been reported previously. Nevertheless, it has been reported that the As value reached 19.61 µg/g in contaminated feathers of individuals in the family Falconidae and 11.04 µg/g in the family Accipitridae (Nighat et al., 2013). In addition, the As rate was 0.158 (0.015–0.885) µg/g in an owl species, *Tyto capensis* (Ansara-Ross et al., 2013), whereas it was 0.22 µg g^{-1} in the vane part of a vulture species, *Cathartes aura* (Haskins et al., 2013). Even though the As concentration in the majority of the evaluated samples was beneath the threshold level, arsenic

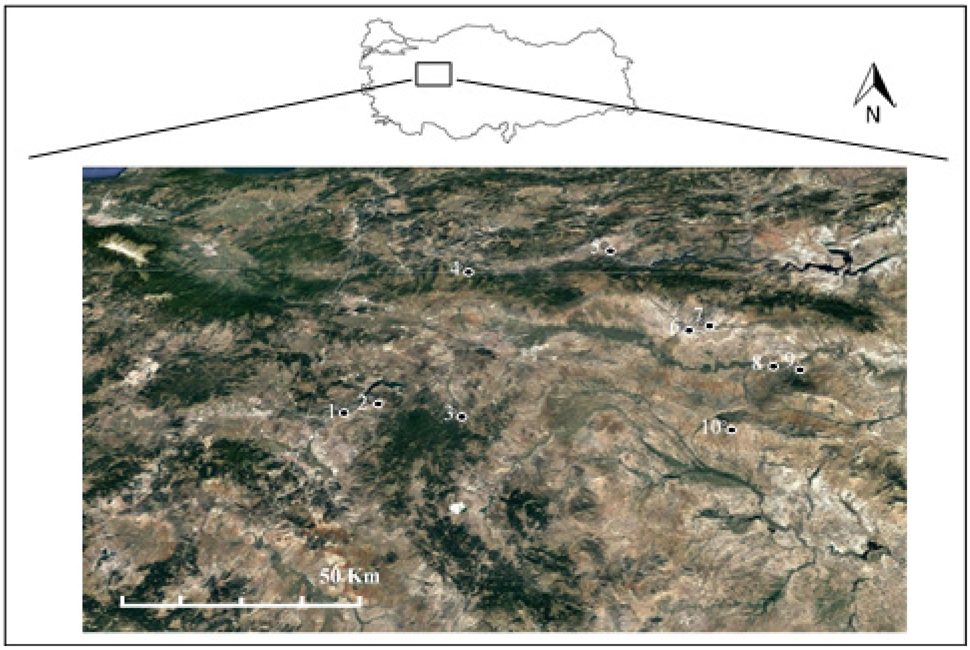

Figure 1. Study area and the sampled nests.

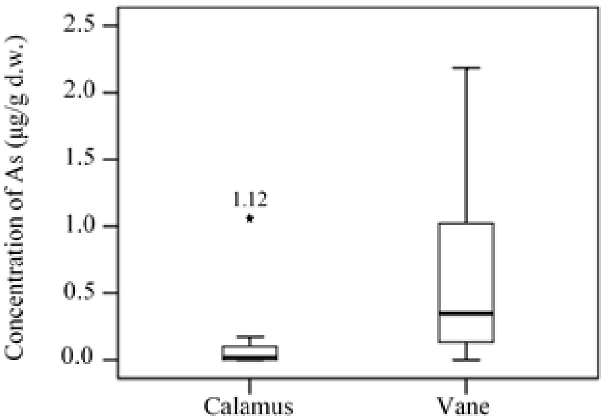

Figure 2. Distribution of As values for calamus and vane (Z= −2.49, P = 0.01).

may still be an important risk factor when considering individuals with extreme As levels with regard to the findings of this study.

Individuals numbered 3 and 10 had slightly higher Cd concentrations than the threshold level, which is 0.1 µg/g according to Burger (1993). On the other hand, these Cd values are relatively low in comparison to the Cd value (1.42 µg g^{-1}) detected in Egyptian vulture feathers in a study conducted by Abbasi et al. (2015).

The amount of Cr in the samples was very high for 5 individuals in our study (Table). Individual number 8, in particular, had a concentration 38.5 times the threshold value of 2.8 µg/g (Burger and Gochfeld, 2000). Cr values were declared to range between 2.02 and 66.71 µg/g in studies on different species from both Falconiformes and Strigiformes (Ansara-Ross et al., 2013; Manjula et al., 2015; Yamac et al., 2019). High concentrations of Cr might be explained by Cr deposits in the region. Cr deposits are found all over Turkey, but they are mainly concentrated in 6 regions (MTA, 2018). One of these regions includes the study area. It is known that different forms of Cr are toxic and carcinogenic (Gomez and Callao, 2006). It has been reported that Cr causes embryonic mortality and abnormalities in birds (Kertész and Fáncsi, 2003; Butkauskas and Sruoga, 2004). Thus, the effect of Cr on reproduction can be suspected as an important threat to the Egyptian vulture population in the area, which is a threatened species. On the other hand, because of the fact that the Egyptian vulture is a migratory species, it is necessary to have information about conditions in their wintering area and along their migration route in order to obtain exact data for Cr exposure.

Cu is an essential metal for all living organisms. However, high amounts of Cu in the body may lead to maldevelopment of fetuses and problems in the gastrointestinal and immune systems (ATSDR, 2004). Data regarding Cu accumulation in Egyptian vulture feathers are lacking. However, there are limited data from different raptor species. The mean Cu value of this study is similar to the available data from Kushwaha (2016) (*Gyps indicus*, 2.90–8.11 µg/g) and Naccari et al. (2009) (*Buteo buteo*, 9.24 µg/g). However, when considering an

Table. Heavy metal concentrations (µg/g d.w.) in feathers of *Neophron percnopterus*. Values higher than threshold level are indicated in bold (* V = vane, C = calamus; ND = nondetectable).

Metals	Feather*	Nest numbers										Mean ± SD	Median	Threshold
		1	2	4	4	5	6	7	8	9	10			
As	V	0.46	0.07	0.13	0.21	ND	0.34	**1.26**	**2.19**	0.36	**1.02**	0.60 ± 0.69	0.34	1.00
	C	0.17	ND	ND	0.05	ND	0.04	**1.12**	ND	ND	0.10	0.15 ± 0.35	ND	
Cd	V	0.02	0.06	**0.13**	0.10	0.01	0.01	0.08	0.33	0.07	**0.16**	0.10 ± 0.10	0.07	0.10
	C	0.01	0.03	0.03	0.00	0.05	0.03	0.05	0.02	0.03	0.07	0.03 ± 0.02	0.03	
Cr	V	ND	ND	ND	**24.35**	**32.70**	1.36	**12.04**	**107.752**	ND	ND	**17.82 ± 33.748**	0.68	2.80
	C	ND	ND	ND	ND	ND	ND	ND	ND	ND	**16.04**	1.60 ± 5.07	ND	
Cu	V	12.53	4.09	8.11	3.91	0.28	10.26	9.58	**32.13**	14.81	4.58	10.03 ± 8.94	8.85	Undetermined
	C	3.00	12.99	11.24	8.11	12.83	7.77	12.45	12.60	9.72	19.21	10.99 ± 4.27	11.85	
Hg	V	0.35	0.54	ND	1.371	ND	ND	ND	ND	ND	ND	0.23 ± 0.45	ND	5.00
	C	0.49	0.39	ND	0.78	ND	ND	ND	ND	ND	ND	0.17 ± 0.29	ND	
Ni	V	ND	ND	ND	ND	ND	ND	2.20	**36.77**	4.32	ND	4.33 ± 11.49	ND	5.00
	C	ND	ND	ND	ND	ND	ND	ND	ND	ND	**5.72**	0.57 ± 1.81	ND	
Pb	V	ND	ND	ND	ND	ND	ND	**15.96**	ND	**71.53**	**235.083**	**32.26 ± 74.71**	ND	4.00
	C	ND	0.770	ND	1.342	ND	ND	ND	ND	ND	ND	0.21 ± 0.47	ND	
Zn	V	114.075	195.426	184.724	126.561	33.75	185.988	130.394	87.91	189.338	128.130	137.63 ± 52.34	129.263	Undetermined
	C	104.197	119.483	121.837	99.61	150.262	126.774	163.651	113.487	158.919	136.366	129.46 ± 22.28	124.306	

individual assessment, the outcome of the vane analysis of individual number 8 is similar to the mean Cu value (32.17 µg/g) of Falconidae species from regions contaminated by heavy metals.

The mean Hg levels of vane and calamus were determined as 0.23 ± 0.45 µg/g and 0.17 ± 0.29 µg/g, respectively. These values are below the threat level (Table). It has been reported that the sublethal effects of Hg on the breeding success and flight efficiency of birds occur at 5–40 µg g^{-1} d.w. (Burger and Gochfeld, 1997; Carlson et al., 2014; Varian-Ramos et al., 2014).

The mean values of nickel in this study are below the threshold level (5 µg/g) (Table). Nevertheless, when individuals are separately analyzed, the Ni value in the calamus of individual number 10 is slightly higher than the threshold (5.72 µg/g). Moreover, the value in the vane of individual number 8 is 7.5 times the threshold value (36.77 µg/g) (Table). These findings emphasize that nickel pollution has reached a critical level, which could pose a problem for fauna and flora in the area. Although Ni is an essential metal, it may affect animal species negatively at high levels (Cain and Pafford, 1981; Harasim and Filipek, 2015). Its range is 0.1–5.0 µg/g in mammal and bird tissue in noncontaminated regions, whereas it reaches 80 µg/g in birds in contaminated regions (Outridge and Scheuhammer, 1993). Nighat et al. (2013) stated that the mean Ni value is 3.2 µg/g for an Accipitridae species (*Sarcogyps calvus*) and 500 µg/g for a Falconidae species (*Falco tinnunculus*) in contaminated areas.

All Pb values in the calamus parts of our samples had noncritical rates based on a threshold of 4.0 µg/g (Burger et al., 2009). Apart from 3 individuals, the Pb values in the examined vane parts were determined as "nondetected" (Table). However, these 3 contaminated samples were found to be over 6.40 µg/g, which is the highest rate of Pb accumulation found to date for vulture species. Furthermore, individuals 9 and 10 had extremely high rates, with 17.88 and 58.77 times the threshold equivalent, respectively. According to studies on lead accumulation in vulture species, the mean Pb value range is 0.09–6.40 µg/g (Kavun, 2004; Cardiel et al., 2011; Kushwaha, 2016; Yamac et al., 2019). This rate was stated as 4.01 µg/g in Egyptian vulture samples by Abbasi et al. (2015).

This heavy metal release into nature is from several sources, such as mining and other industrial processes (ATSDR, 2007). In addition, bullets used for hunting are a significant source of lead (Church et al., 2006; Fisher et al., 2006; Berny et al., 2015). Scavengers feeding on hunted animals are being negatively affected by lead contamination in carcasses (Gangoso et al., 2009; Margalida et al., 2013; Berny et al., 2015; Bounas et al., 2016).

Large amounts of lead intake into the body have lethal and sublethal effects on living organisms (Pikula et al., 2013; Ferreyra et al., 2015; Ecke et al., 2017). Donázar et al. (2002) reported that lead contamination due to ammunition could be a potentially limiting factor for the Egyptian vulture population. In addition, the negative effect of lead on reproductive success in birds has been shown to increase with the Cr level (Butkauskas and Sruoga, 2004). High concentrations of Cr were determined in this study. Thus, lead accumulation should be particularly taken into consideration.

It was reported that 90 µg/g Pb was detected in the vane of a developing feather of a California condor that had died due to lead poisoning (Church et al., 2006). On the other hand, a higher Pb concentration in the vane than in the calamus of fully developed feathers can indicate exogenous contamination because of the vane's position (Cardiel et al., 2011). Our finding of high Pb levels in the vane was supported by the results of a study on cinereous vultures' feathers in the region (Yamac et al., 2019).

Zinc is another important metal, but exposure to high concentrations of Zn may cause poisoning (Beyer et al., 2004). The mean Zn concentration for this study was 137.63 µg/g for the vane and 129.46 µg/g for the calamus (Table). The highest rates found were 195.43 µg/g in the vane of individual 2 and 158.92 µg/g in the calamus of individual 9. Our results are supported by the findings of Kavun (2004), Nighat et al. (2013), and Yamac et al. (2019). Data for the Zn threshold value in feathers are nonexistent (Clair et al., 2015). The mean Zn values for different raptor species have been reported as 119.14 µg/g for the family Accipitridae (Nighat et al., 2013), 60.01 µg/g for *Buteo buteo* (Naccari et al., 2009), and 140 µg/g (Kavun, 2004) and 121.84 µg/g (Yamac et al., 2019) for *Aegypius monachus*.

3.1. Vane and calamus comparison

Heavy metal accumulation in feathers occurs both endogenously and exogenously (Dauwe et al., 2003). In endogenous contamination, the feather position becomes important; it is known that areas outside of the feathers (outside of the primaries) and the feather parts (vane rather than calamus) contain higher metal concentrations (Dauwe et al., 2003; Cardiel et al., 2011). In this study, there was no significant difference between the vane and calamus in terms of metal accumulation, except for As ($Z = -2.49$, $P = 0.01$) (Figure 2). Arsenic contamination rates were higher in the vane than those in the calamus. When considered on an individual basis, certain feather parts contain heavy metal accumulations (Cr, Ni, and Pb) over the threshold value, whereas other parts were determined as "nondetectable" (Table). It has been reported that the potential for heavy metal accumulation in the calamus is lower than that for the vane because of the structure of the calamus (Roque et al., 2016). Cr values for 4 individuals, Ni values for 1 individual, and Pb values for 3 individuals in the vane exceeded the threshold limit, whereas these metal values were nondetectable in the calamus. This situation could be explained by exogenous contamination in this case. Egyptian vultures muddy their feathers up (Overveld et al., 2017), and thus external contamination may originate not only from the air but also from the soil for this species.

On the other hand, Ni and Cr values for individual number 10 show a reverse situation (nondetectable for the vane and above the threshold value for the calamus). Therefore, internal contamination could be mentioned in this case. The Cr concentration, in particular, was determined as being extremely high. A study on turkey vultures conducted by Haskins et al. (2013) showed that Cr could accumulate at different levels in separate feathers and even in different parts of the same feather sample. The authors emphasized that a single feather sample is insufficient for this assessment. In addition, the data for the Cr element in feathers (Haskins et al., 2013) were much higher than the values found in our study. Consequently, individual number 10 is thought to have been exposed to endogenous contamination of both Cr and Ni.

3.2. Conclusions

In this study 8 different heavy metals were determined in the calamus and vane parts of feathers of the Egyptian vulture. When evaluated on an individual basis, all heavy metal values other than Hg and Zn showed critical levels that may result in lethal and sublethal effects.

When different feather parts were analyzed with respect to accumulation, it was found that heavy metals other than Cu had accumulated in the vane. This indicates exogenous contamination rather than nutritional intake.

In conclusion, this study clearly finds that the endangered Egyptian vulture has a high level of heavy metal accumulation in its feather samples. Future studies need to focus on the effects of heavy metal accumulation both regionally and nationwide, determination of the sources of metal contamination, and actions that need to be taken.

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