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The Hooded Crow (*Corvus cornix*) as an Environmental Bioindicator Species of Heavy Metal Contamination

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Abstract This study aims to examine the possible presence of lead and cadmium in the liver and kidneys of hooded crows (*Corvus cornix*). Liver and kidneys of hooded crow carcasses were collected in Province of Cuneo (Piedmont, Italy) in order to detect lead and cadmium content. Significant differences were found in lead and cadmium levels between areas of intensive cultivation versus areas where meadows are prevalent. Moreover, age greatly influenced the burden of heavy metals, while sex did not seem to affect the level of contamination. The source of contamination may be phosphate fertilizers used for intensive cultivation in the study area.

Keywords Heavy metals · *Corvus cornix* · Bioindicator species · Cadmium · Lead

Lead (Pb) and cadmium (Cd) from phosphate fertilizers pose a potentially serious threat to soil quality and, via the food chain, to human health (Alloway and Steinnes 1999; Sheppard et al. 2009). Heavy metals that are present in fertilizers remain in the ground and are absorbed by organic components, thus contaminating surface water. These elements are absorbed by plants (Adams et al. 2004), especially in acidified soils, and are then consumed by the animals, including humans. Once absorbed, cadmium and lead bioaccumulate in high concentrations in liver and kidneys (Godt et al. 2006). This cycle may cause the decline of populations of some avian species in intensively farmed areas (BTO 2012). This may also directly affect humans that consume contaminated water and plants and animals, and secondary contamination of lead and cadmium in edible parts of animals is also of concern (Bilandžić et al. 2010).

It is objectively difficult to define the metal levels related to a state of toxicity or mortality, or to a geochemical background, in the tissues and organs of wild birds. The majority of reports on this subject are on waterfowl (Guitart et al. 1994), seabirds and raptors (Kalisińska et al. 2006), while there are few studies on passerines. Studies have frequently utilized feathers, feces and eggs as organic matrices (Dauwe et al. 2000; Mora 2013; Swaileh and Sansur 2006; Berglund et al. 2011; Markowski et al. 2013). Nonlethal collection methods can be used to detect heavy metal levels and constitute a valid technique to assess environmental contaminants. However, monitoring the concentrations of contaminants within the tissues is an useful way of assessing the potential damage of chemicals to organisms and their predators or scavengers. Liver and kidneys are the most important organs for detoxification processes. The spleen and pancreas are the only possible alternatives to liver and kidneys in lead poisoning diagnostic procedures (Guitart et al. 1994).

Lead poisoning manifests itself as a chronic or acute disease in wild birds. Signs usually include progressive

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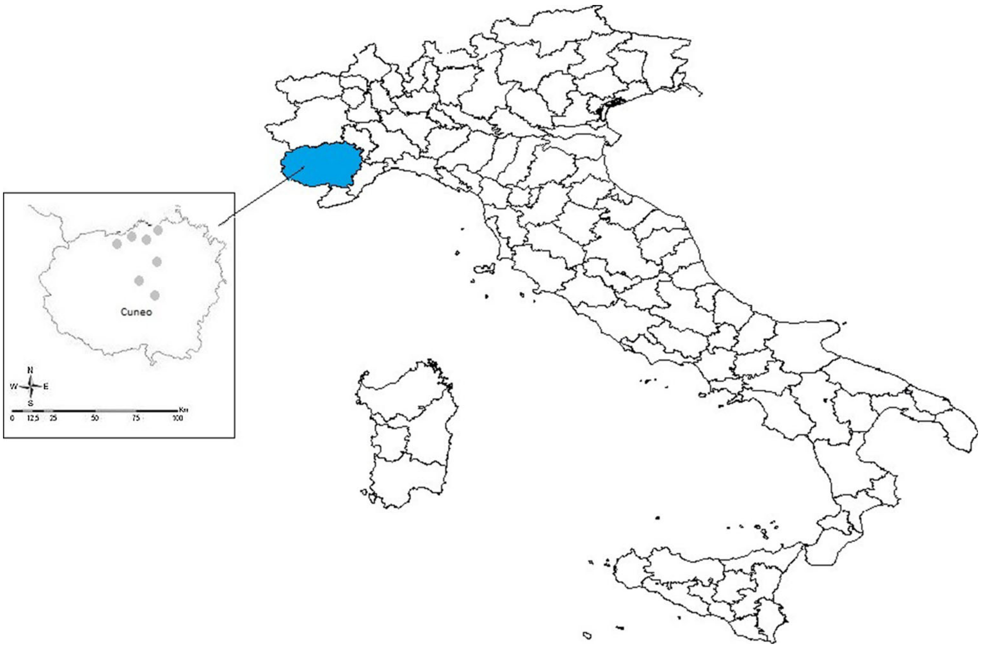

Fig. 1 Province of Cuneo and traps location

weakness, green-stained feces and vent, deposition of eggs with thin shells, disorders of the central nervous system (Burger 1995), weight loss and emaciation, as well as making no attempt to escape in the presence of humans. Lesions consist of severe wasting of breast muscles, impaction of esophagus or proventriculus, enlarged gall-bladder containing dark green bile, and hemorrhagic enteritis. High levels of lead exposure decreases resistance to infectious disease (Gainer 1974) and can result in nestling and adult death (Clark and Wilson 1981).

Cadmium is an extremely toxic element; it is able to cause both acute and chronic tissue lesions and has been found to have teratogenic, mutagenic and carcinogenic effects (Krajnc et al. 1987).

The aim of this study is to determine the prevalence of lead and cadmium levels in hooded crows (*Corvus cornix*) collected from province of Cuneo (Piedmont, Italy) and to compare the levels recorded in hooded crows that reside in intensely cultivated territory where chemical fertilizers are used, with those of hooded crows that reside in regions used for pasture where no chemical fertilizers are used. Cuneo is a province in the southwest of the Piedmont region of Italy (Fig. 1). The municipalities included in the monitoring plan are located in the Po valley.

Hooded crows are quite sedentary, never venturing far from their nests and can therefore be considered as residing in this contained area. In addition, their propensity to scavenge, their trophic level, and the fact that they are abundant, makes them candidates for bioindicators of environmental contamination by lead and cadmium (Spellerberg 1991). The crow may be contaminated either by consuming water and forage directly or by eating animal carcasses with the pollutants. We have found no data in the literature regarding the hooded crow, nor any data relating to other passerines in this study area.

The present paper reports on lead and cadmium pollution in kidney and liver of hooded crown (*C. cornix*). The possible role as bioindicator species of the environmental contamination of heavy metal is investigated. This species is considered damaging for the area considered and it is under a plan of control. Then liver and kidney from of hooded crown are easily available and constitute useful specimen to assess the heavy metals contamination of rural territories. We hypothesize that the habitat of hooded crows heavily influences the levels of heavy metals in its organs. In particular the specimens living in highly cultivated areas where fertilizers are extensively used can adequately reflect the level of environmental pollution. In addition we want to verify if *C. cornix* age or gender can affect the levels of cadmium and lead in the tissues examined, in order to identify the parameters that make this species a good environmental indicator.

Materials and Methods

Hooded crows were caught with Larsen traps placed in five different localities in the Cuneo plain (district of Caramagna Piemonte 44°47′N 7°44′E, Savigliano 44°39′N 7°38′E, Murello 44°45′N 7°36′E, Sant'Antonio Baligio 44°33′N 7°39′E, Racconigi 44°46′N 7°41′E, Moretta 44°45′N 7°32′E, Cavallermaggiore 44°43′N 7°41′E) (Fig. 1). In the areas considered there are no landfills or industries of ceramics or paints, being the economy mainly agricultural and pastoral. The municipality of Caramagna Piemonte is mostly composed of polyphite meadows, with limited areas used for intensive cultivation. The territory of the other municipalities considered in this study is used almost exclusively for intensive cultivation of corn, wheat and barley.

Crows were euthanized using cervical dislocation, a method permitted by EU Regulation 1099/2009 (Protection of Animals at Time of Killing) during a management program of pest containment. A total of 127 birds were examined; 84 from Caramagna Piemonte, and the remaining birds from the other areas. In these areas we estimated crow density to be 20.3 individuals per km^2 (with 95 % CI [16.3–25.3]) using the line-transect method (Buckland et al 1993) and DISTANCE software. Individuals were examined during the period of August 2005–June 2006. Carcasses were classified by sex and age (Svensson 1992). For age classification, crows were grouped according to the following Euring codes: 3 (born in the year in which they were caught, or young), 5 (born in the year preceding that in which they were captured, or sub-adult), 6 (born before the year preceding that in which they were captured, or adults). In order to avoid possible sources of contamination, liver and kidneys were collected from animal carcasses by using single-use instruments. Immediately after sampling, materials were frozen and sent to the laboratory for analysis.

For digestion, 1 g of liver or kidneys was placed into a Teflon pressure vessel, and 5 mL of HNO_3 (65 %) and 1.5 mL of H_2O_2 (30 %) were then added. After sealing, vessels were introduced into a microwave digestion rotor and the oven was programmed to the following settings: step 1:8 at 110°C; step 2:2 min at 130°C; step 3:13 min at 200°C. After cooling, digested samples were transferred into 25 mL measuring tubes. Inner walls of Teflon pressure vessels were rinsed with ultrapure distilled water and the rinse water was transferred into the sample-containing tubes. Ultrapure distilled water, to a final volume of 25 mL, was then added to the tubes.

Accuracy of analysis was tested using certified reference material NRCC-DORM-2 Dogfish muscle (National Research Council of Canada, Ottawa, Canada) Concentrations measured in reference material fulfil the range of the certified values.

Quantitative determination of lead and cadmium was performed using a Perkin-Elmer AAnalyst 800 Atomic Absorption Spectrophotometer, equipped with a transversely-heated graphite atomizer (THGA) system, with Zeeman-effect for background correction and integrated auto sampler. End cap graphite tubes with a pyrolytic graphite coating and platforms made of pyrolytic graphite were used throughout the study. Argon (99.998 % purity) was used as an inert gas. Cadmium and lead determinations were carried out by the standard additions method, and the standard concentrations were 25–50 ng mL^{-1} for lead and 1–2 ng mL^{-1} for cadmium. The correlation coefficient (R) of the calibration curves was 0.99. The detection limit for Cd was 0.01 and 0.04 mg kg^{-1} for Pb. All samples were analyzed in batches and compared with blanks and samples with known metal content (certified reference material from the National Bureau of Standards). Recovered concentrations of certified samples were within 10 % of the certified values. We applied the Shapiro-Wilk test of normality, the Wilcoxon test for comparing two samples, the Kruskal-Wallis test for more than two samples and the Bonferroni procedure for multiple comparisons (Lehmann and Romano 2005). Thus, we compared the p values with the conventional level of significance (e.g. 0.05) divided by the number of simultaneous tests. Statistical analyses were conducted using R software (version 2.11). Analysis of the data (and their logarithmic transformation of the data) with the Shapiro-Wilk test did not allow the use of parametric tests due to the non-normal distribution of the data itself, so therefore we adopted a nonparametric approach.

Results and Discussion

Results of metal analysis, calculated in mg kg^{-1} ww, are shown in Table 1. We compared lead and cadmium levels recorded in liver and kidneys from hooded crows, and we found that lead is stored in a non-significantly different proportion in the two tissues ($W = 7{,}803$, $p = 0.987$), while cadmium tends to accumulate more in kidneys than in the liver ($W = 5{,}206.5$, $p = 0.000$). Lead and cadmium levels among the three age groups are shown in Table 2, and results of the statistical analyses are shown in Table 3. Both in kidneys and liver, the differences of lead and cadmium levels between the three age categories are highly significant ($p < 0.001$), with the exception of the case of lead for categories 5 and 6 (in liver: $W = 196.5$, $p = 0.925$; in kidney: $W = 174.5$, $p = 0.0289$). Lead and cadmium levels recorded from category 5 are significantly higher than those from category 3: for lead in liver $W = 108$, $p = 0.0003$; for lead in kidney $W = 610.5$, $p = 0.0004$; for cadmium in liver $W = 463.5$, $p = 0.0000$; for cadmium in kidney $W = 443.5$, $p = 0.0000$. Lead and cadmium levels recorded from category 6 are significantly higher than those from category 3: for lead in liver $W = 66.5$, $p = 0.0009$; for lead in kidney $W = 190.5$, $p = 0.0000$; for cadmium in liver $W = 82$, $p = 0.0000$; for cadmium in kidney $W = 29$, $p = 0.0000$. Cadmium levels recorded from category 6 are significantly higher than those from category 5: in liver $W = 120.5$, $p = 0.0014$; in kidney $W = 69.5$, $p = 0.0000$. Moreover, according to the Bonferroni procedure (Lehmann and

Table 1 Levels of lead and cadmium in liver and kidneys of hooded crows in mg kg^{-1} wet weight

Organ	Metal	n	Min–max (mg kg^{-1})	Mean (mg kg^{-1})	SD	Median (mg kg^{-1})
Liver	Lead	124	0–2.93	0.09	0.27	0.04
	Cadmium	124	0–1.75	0.15	0.26	0.06
Kidney	Lead	126	0–0.61	0.07	0.10	0.04
	Cadmium	126	0.01–9.42	0.39	0.95	0.13

Table 2 Lead and cadmium levels among the three age groups

Organ	n				Min–max (mg kg^{-1})			Mean (mg kg^{-1})			SD			Median (mg kg^{-1})		
	Age	3	5	6	3	5	6	3	5	6	3	5	6	3	5	6
Liver	Pb	66	30	18	0–0.45	0–0.27	0–2.93	0.06	0.08	0.24	0.10	0.07	0.67	0	0.08*	0.10*
	Cd	66	30	18	0–0.25	0.01–0.55	0.02–1.75	0.05	0.16	0.50	0.05	0.17	0.51	0.03	0.09	0.32
Kidney	Pb	67	31	18	0–0.40	0–0.27	0–0.61	0.04	0.08	0.17	0.08	0.07	0.14	0	0.08	0.15
	Cd	67	31	18	0.01–0.47	0.02–1.02	0.1–9.42	0.11	0.34	1.57	0.01	0.29	2.10	0.08	0.28	1.34

Categories not sharing a common symbol * are significantly different

Table 3 Comparison by age

Age code	Pb		Cd	
	Liver	Kidney	Liver	Kidney
3 versus 5 versus 6	Kruskal ($\chi^2 = 15.4289$; $p = 0.0004463$)	Kruskal ($\chi^2 = 27.9929$; $p = 8.345\text{e}{-07}$)	Kruskal ($\chi^2 = 41.9726$; $p = 7.687\text{e}{-10}$)	Kruskal ($\chi^2 = 52.5052$; $p = 3.969\text{e}{-12}$)
3 versus 5	Wilcoxon (W = 108; $p = 0.0003377$)	Wilcoxon (W = 610.5; $p = 0.0004422$)	Wilcoxon (W= 463.5; $p = 2.744\text{e}{-05}$)	Wilcoxon (W = 443.5; $p = 5.338\text{e}{-06}$)
3 versus 6	Wilcoxon (W = 66.5; $p = 0.000923$)	Wilcoxon (W = 190.5; $p = 1.943\text{e}{-06}$)	Wilcoxon (W = 82; $p = 1.775\text{e}{-08}$)	Wilcoxon (W = 29; $p = 6.353\text{e}{-10}$)
5 versus 6	Wilcoxon (W = 196.5; $p = 0.925$)	Wilcoxon (W = 174.5; $p = 0.02893$)	Wilcoxon (W = 120.5; $p = 0.001443$)	Wilcoxon (W = 69.5; $p = 1.388\text{e}{-05}$)

Romano 2005), we compared the Wilcoxon p values reported in Table 3 with the significance level (i.e., $p < 0.05$) divided by the number of simultaneous tests (i.e., 3). We also compared levels obtained in the municipality of Caramagna Piemonte with those of the other municipalities considered in this study, and the observed differences turned out to be highly significant ($p < 0.0001$): median for lead in liver: 0.00 versus 0.08 mg kg^{-1}; median for cadmium in liver: 0.04 versus 0.19 mg kg^{-1}; median for lead in kidneys: 0.00 versus 0.11 mg kg^{-1}; median for cadmium in kidneys: 0.09 versus 0.42 mg kg^{-1} (as shown in Table 4). Lead and cadmium levels recorded from males and females were not found to be significantly different (Table 5); for lead in liver: W = 481.5, $p = 0.904$; for lead in kidneys: W = 472, $p = 0.498$; for cadmium in liver: W = 370, $p = 0.096$; for cadmium in kidneys: W = 420, $p = 0.178$. When analysis was limited to age categories 5 and 6, results still demonstrated no differences between males and females: for lead in liver: W = 138.5, $p = 0.056$; for lead in kidneys: W = 144.5, $p = 0.056$; for cadmium in liver: W = 170.5, $p = 0.281$; for cadmium in kidneys: W = 144, $p = 0.079$). Age category 6 alone (adults): for lead in liver: W = 15, $p = 0.079$; for lead in kidneys: W = 23, $p = 0.366$; for cadmium in liver: W = 32, $p = 1.000$; for cadmium in kidneys: W = 30, $p = 0.874$).

Hooded crows, when subjected to population control, could be used as valid bioindicators of environmental contamination by heavy metals due to their large geographical distribution, feeding habits and easy sample collection (Chambers 2008). No heavy metal values obtained from the liver and kidneys of the target species,

Table 4 Levels of lead and cadmium in liver and kidneys by type of land

Municipality		N		Min-max (mg/kg)		Mean (mg/kg)		SD		Median (mg/kg)	
		M	Int.C.	M	Int.C.	M	Int.C.	M	Int.C.	M	Int.C.
Liver	Pb	83	41	0–0.45	0–2.93	0.05	0.17	0.09	0.45	0	0.08
	Cd	83	41	0–0.51	0.02–1.75	0.06	0.31	0.08	0.39	0.04	0.19
Kidney	Pb	83	43	0–0.4	0–0.61	0.05	0.12	0.07	0.12	0	0.11
	Cd	83	43	0.01–1.02	0.02–9.42	0.13	0.89	0.15	1.49	0.09	0.42

M meadows, *Int.C.* intensive cultivation

All differences are highly significant ($p < 0.0001$, Wilcoxon)

Table 5 Levels of lead and cadmium in liver and kidneys by sex

Organ		n		Min–max (mg kg^{-1})		Mean (mg kg^{-1})		SD		Median (mg kg^{-1})	
		M	F	M	F	M	F	M	F	M	F
Liver	Pb	35	28	0–0.34	0–2.93	0.07	0.17	0.09	0.55	0.05	0.05
	Cd	35	28	0–1.75	0.02–1.33	0.22	0.25	0.38	0.29	0.07	0.11
Kidney	Pb	36	29	0–0.33	0–0.61	0.08	0.10	0.10	0.12	0.04	0.07
	Cd	36	29	0.01–9.42	0.03–3.13	0.67	0.55	1.59	0.66	0.18	0.28

M males, *F* females

comparable with our results, are available from the literature. Other species of passerines can be considered, but results for these species concern non-European locations (Lee et al. 1989; Deng et al. 2007).

The acute lethal dose for lead (LD50), determined by injection in rats, was found to be 70 mg kg^{-1} body weight (Kotsonis and Klaassen 1977), while the dose in wild passerines is unknown. In Falconiformes, lead content in the liver and kidneys exceeding 3 mg kg^{-1} wet weight (ww) is considered a toxic concentration (Pain et al. 2009). In Galliformes, levels exceeding 6 mg kg^{-1} ww in liver and 15 mg kg^{-1} in kidneys are considered toxic (Pain et al. 2009). A level as low as 0.16 mg kg^{-1} in waterfowl can be toxic (Wachnik 1984). For raptors, a liver content of 7.5 mg kg^{-1} ww and kidney content of 5 mg kg^{-1} ww are suggested as levels of acute lead intoxication with symptoms; a liver content that exceeds 1.5 mg kg^{-1} ww and a kidney content of 2 mg kg^{-1} ww is considered a sublethal level. Lower levels than those mentioned above are considered deriving from natural exposure to the normal geochemical background concentrations (Mateo et al. 2003; Kalisinska et al. 2006). Regarding Passeriformes, in livers of *Turdus pallidus*, *Turdus hortulorum* and *Turdus dauma*, Lee et al. (1989) was able to detect lead in concentrations ranging from 0.81 ± 0.17 mg kg^{-1} (max 1 mg kg^{-1}) ww. In Chinese *Parus major*, Deng et al. (2007) recorded lead concentrations of 1.21 ± 0.37 mg kg^{-1} dw (dry weight) in kidneys and 0.64 ± 0.15 mg kg^{-1} dw in liver, while in *Carduelis sinica*: 0.68 ± 0.08 and 2.59 ± 0.41 mg kg^{-1} dw of renal lead and 0.45 ± 0.06 mg kg^{-1} dw of hepatic lead.

Cadmium in birds is responsible for anorexia, growth dysfunction, and liver and kidney lesions, as well as having a negative impact on reproduction and survival. In rats, the acute lethal dose of cadmium (LD50), administered orally, was found to be 225 mg kg^{-1} of body weight. Certain studies have demonstrated that background cadmium levels are below 0.9 mg kg^{-1} ww for freshwater waterfowl (Giulio and Scanlon 1984; Scheuhammer 1987), whereas concentrations greater than 1.5 mg kg^{-1} ww are considered to be in excess (Guitart et al. 1994). Regarding the study by Lee et al. (1989), the amount of cadmium found in Korean *Turdus* sp. is 0.81 ± 0.47 (max 1.16) mg kg^{-1} ww in liver and 2.45 ± 1.37 (max 4.5) mg kg^{-1} ww in kidneys. In Chinese *P. major*, cadmium concentrations were 1.32 ± 0.25 were dw in kidneys and 0.68 ± 0.1 were dw in liver. In *C. sinica*, cadmium levels were 2.59 ± 0.41 were dw in kidneys and 0.56 ± 0.09 were dw in liver (Deng et al. 2007).

In our study, levels of contamination by lead and cadmium showed mean values in liver and kidneys that are consistent with those recorded in *C. sinica*, *P. major*, and *Turdus* sp. The high tolerance of the hooded crow to heavy metal, allowed us however to determine exceptional extreme values of contamination. In particular, we highlight the value of 9.42 were of cadmium in the kidneys of an adult male from Murello and to the value of 2.93 were of lead in the liver of an adult female, also from Murello. These are extreme values, previously unreported in passerine birds similar to those of carnivorous birds (Hontelez et al. 1992; Lee et al. 1989; Kalisinska et al. 2006), perhaps due to their propensity to scavenge.

While lead content is similar in both liver and kidneys, cadmium tends to accumulate in kidneys, rather than in the liver, as already observed in other species. The trend to bioaccumulate in different tissues is evidenced by the statistically significant differences of the median values, considering the three age categories: young, sub-adult and adult. Adults and sub-adults have, on average, higher levels of contamination. Results obtained are in accordance with previous reports (Kuiters 1996; Falandysz et al. 2005; Srebocan et al. 2011) for other species. The same trend was also observed in lead distribution, in contrast with data reported in other studies (Wolkers et al. 1994; Kuiters 1996) on other species. It is likely that saturation levels of heavy metals in the involved tissues are reached in the second year of life.

Male crows, being more dedicated to the pursuit of food, could be assumed to be more easily exposed to pollution sources, during the time in which females brood. Our study, however, suggests that contamination levels are not affected by sex.

The principal difference between the two habitats compared in this study is in the practices of agricultural production: in the municipality of Caramagna Piemonte there are pastures and meadows dedicated to the production of hay. The others municipalities are devoted to intensive cultivation for which inorganic fertilizers are essential to obtain high productivity. Among the inorganic fertilizers, phosphorus fertilizers in the Province of Cuneo are still widely used: 1.6 kg per hectare of area in 2004 (Province of Cuneo—Settore Agricoltura 2006). However, they contain heavy metals that can contaminate the soil and threat the health of animals and humans (Mendes et al. 2006) and European Commission (2014) is aware of the need to take action in accordance with its risk reduction strategy. The main source of lead and cadmium contamination, in this study, could be identified as being phosphate fertilizers. In fact, there is a highly significant difference in heavy metal contamination between the area dedicated to meadows and the areas principally devoted to intensive cultivation. We can consider that the same sources of contamination may have an important effect on other bird species whose populations are declining (BTO 2012) and, in general, on hunted species. The latter could also represent a risk for public health, if the tissues containing heavy metals constitute edible parts. Future studies could be focused on establishing the levels of heavy metals in a population of hooded crows residing in areas with high levels of contamination, with particular consideration of colonies living in urbanized or industrialized areas, in order to determine tolerance levels and toxicity thresholds of lead and cadmium for the species.

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