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## Metals in the Réunion harrier - tissue concentrations and meaning for conservation

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**Abstract** - The Réunion harriers is an endemic raptor on Réunion Island. Several threats endanger its population, poisoning by rodenticides being considered as the main one currently. No information is available on its exposure to other chemicals notably trace metal elements such as lead (Pb), mercury (Hg), and cadmium (Cd). The Réunion harrier is still victim of poaching nowadays. When shooting is not lethal, animals may carry embedded shot in their body and thus be exposed to toxic level of Pb as demonstrated for other raptors. Moreover, recent monitoring suggests a decrease of its breeding success over time. It is known that Hg and Cd could impair reproduction and disturb embryo development in birds. The aim is to measure metal concentrations in the tissues of 30 carcasses of harrier collected from 2016 to 2021. Lead was analysed in liver and humerus while Hg and Cd were measured in livers. Concentrations were compared to toxicological reference values. Overall, the Réunion harrier was not exposed to toxic levels of Pb or Cd. For Hg, 53% of the individuals have residues higher than the threshold compatible with oxidative stress, 13% having liver concentrations above those compatible with reproduction impairment. A positive correlation was found between the proportion of urban habitat in a 55 km<sup>2</sup> area centred on the location where the harrier was found and the concentration of Hg in the liver. We conclude that Hg exposure could be a threat for the Réunion harrier population and recommend monitoring the exposure of the most sensitive stages, i.e., embryos and nestlings, to this metal with non-invasive methods.

**Keywords** : *Circus maillardi*; Réunion harrier; mercury; lead; cadmium; bioaccumulation; island



## 1. Introduction

All over the world, human activities threaten wildlife, which lead to the sixth mass extinction (Ceballos et al. 2017) and make biodiversity conservation one of the main issues of the current century. On islands, the decline of populations and extinction of native species are faster than anywhere else worldwide (Spatz et al. 2017). Several threats impair island biodiversity. Both introduction of invasive species and habitat lost and fragmentation are considered as the main drivers over the recent history, but other contemporary and emerging threats are expected to become of growing importance in the future (Russell and Kueffer 2019). While pollution is the cause of decline for less than 10% of the threatened island species (Russell and Kueffer 2019), it can represent the major threat for some endangered species as reported for the Réunion harrier *Circus maillardi* (Coeurdassier et al. 2019). This raptor is endemic of the Réunion Island, an overseas territory of France located in the Indian Ocean that hosts a breeding population estimated at ~200 pairs. It is classified as endangered by the International Union for the Conservation of Nature (IUCN 2018). Recent findings pointed out that, since 2010, the poisoning by anticoagulant rodenticides (ARs), a family of pesticides applied in the Réunion against rats and mice in agriculture field and urbanized areas, is likely the major driver of its decline (Coeurdassier et al. 2019). In addition, despite protective legislation, poaching and persecution, notably by shooting, are also identified among the most serious current threats (Grondin and Philippe 2011; Augiron 2022). When shooting is not lethal, animals may carry embedded lead (Pb) fragments in their body and (Berny et al. 2017) reported that ammunitions in tissues are frequently noticed on X-rays by veterinarians working on wild birds. Embedded projectiles may release lead (Pb) chronically in the body, and thus, lead to sublethal intoxication and long-term detrimental effects on long-lived animals due to Pb neurotoxicity (Berny et al. 2017). As the Réunion harrier is frequently victim of shot, we can wonder whether injured individuals could suffer Pb intoxication due to bullets potentially embedded in their tissues. Moreover, although the diet of the Réunion harrier is mainly composed of rodents, it is also an opportunistic scavenger (Grondin and Philippe 2011). Thus, another exposure pathway to Pb could occur via the ingestion of gunshots contained in carrions as demonstrated for numerous other scavenging birds (Pain et al. 2019; Monclús et al. 2020). Lead intoxication is reported as a common and increasing threat for raptors worldwide including harriers (Mateo et al. 1999; Komosa and Kitowski 2008; Krone 2018; Pain et al. 2019) and the Réunion harrier exhibits some traits suggesting that it could be exposed to Pb. Additionally, to rodenticides, Pb exposure could therefore be an issue for its conservation.

In raptors, exposure to non-essential metals other than Pb, i.e., mercury (Hg) and more rarely cadmium (Cd), has been shown in different parts of the world (Kim et al. 2009; Ackerman et al. 2016). Access to biological samples of such a rare species is valuable and offers the opportunity for a wider screening of metal exposure to check whether these elements represent or not a conservation issue for this endangered harrier. Since 2016, the monitoring of a local breeding population located in the study area of Bras-Panon, shows very low fecundity and hatching success (Augiron 2022). It is widely reported that exposure of birds to Hg is susceptible to impair their reproduction by disturbing adult physiology (Tartu et al. 2013) and embryo development (Ackerman et al. 2016). Thus, conservationists may wonder whether Hg could be involved in the low breeding success even if no obvious source(s) of exposure has been identified on Réunion island. Considering Cd, its toxicity on wildlife due to nephrotoxicity, carcinogenicity, anaemia, or interaction with the metabolism of calcium and other essential elements is well-documented and may also lead to disturbance in reproduction (Eisler 1985). The main driver of exposure of wildlife to Cd is the degree of contamination of their habitats

(Hurníková et al. 2021). If its breeding habitat is mainly associated to the open woodland, the Réunion harrier forages in diversified habitats including agricultural areas (Bretagnolle et al. 2000) that have been shown to be contaminated by Cd due to the use of mineral fertilizers in the Réunion island (Doelsch et al. 2006b). Altogether, this information makes credible the assumption of an overexposure to Hg and/or Cd and the measurement of their concentrations in harrier's tissues could help to get a first insight for some of the possible causes of disturbance of its reproduction.

This study aims to assess the internal concentration of Pb in bones and liver of dead Réunion harriers collected by the «Société d'Etudes Ornithologiques de La Réunion» (SEOR) rescue center from 2015 to 2021. These tissues are commonly used to monitor Pb exposure and assess the associated risk for wildlife, bone concentrations reflecting long term accumulation while liver residues help to assess a shorter-term exposure (Espín et al. 2016). Moreover, liver concentrations of Hg and Cd were also measured in the available samples as well as Selenium (Se), an essential element involved in the detoxification of Hg (Cuvin-Aralar and Furness 1991; Yang et al. 2008). For all the non-essential metals analysed, the toxicological meaning of liver and bones concentrations was discussed at the light of threshold values published for wildlife.

## **2. Materials and methods**

### *2.1. Study area and available samples*

The tissue samples came from 30 carcasses of Réunion harrier collected throughout Réunion Island and collected by the SEOR care center between July 2015 and March 2021. Most of the birds were found weakened and/or injured. Some individuals died quickly after being taken in charge by the care center and the others were euthanized because they were too seriously injured. In most cases, the causes of their deaths have been identified by the care center and the veterinary services of Réunion, which included poisoning, collision (electric cables, cars), poaching (shooting), predation, pneumonia, or aspergillosis. The carcasses were stored at -20°C and several information were recorded: age, sex, location and date of discovery. All the carcasses were analysed by X-ray to look for Pb ammunitions in tissues. Then, bones (humerus) and liver were sampled from each of the 30 corpses and stored at -20°C until analysis.

### *2.2. Sample preparation*

Fresh liver samples were freeze-dried and approximately 0.6 g was then digested in 7mL of nitric acid 67-69% (Fisher Scientific, OPTIMA™ quality) in a DigiPrep mineralization system (SCP Sciences, Courtaboeuf, France) for 4h30. After mineralization, the tubes were filled to 50 mL with ultra-pure water (Millipore Synergy system, Molsheim, France). Finally, they were filtered and diluted 10 times with ultra-pure water

The humerus were first cleaned with a stainless-steel blade to remove the remains of tendons and muscles and then cut with a stainless-steel saw. Both extremities of the bone were removed to keep only the diaphysis. Then, they were freeze-dried and manually crushed to retain about 0.4 g for analysis. Digestion was carried out with nitric acid 67-69% (7 mL) and hydrogen peroxide 30-32% (2

mL), both of OPTIMA™ quality (Fisher Scientific), in a DigiPrep for 4h30. After mineralization, the tubes were filled to QSP50 mL with ultra-pure water. Finally, they were filtered and diluted 50 times before analysis due to the high calcium concentrations in these samples.

### 2.3. *Measurement of trace metallic elements (TME) concentrations in tissues*

Lead, Hg, Se and Cd concentrations were measured in Inductively Coupled Plasma Mass Spectrometry (ICP-MS, ThermoFisher Scientific ICAP RQ Model, Thermo Fisher Scientific, Courtaboeuf, France). According to Zhu and Alexandratos (2007), gold(III) chloride was added at a final concentration of 250 µg/L to the rinsing and carrier solutions in order to limit memory effect especially for Hg, the Hg potentially deposited in the ICP-MS being complexed by Au, then released. Repeatability and reproducibility of the measurements were surveyed (checking of relative standard deviation RSD values and use of internal standards, i.e., Ga, Rh and Ir), and quality controls (control solutions and blanks to check for the absence of drift) inserted every 10 samples showed average recovery of 99%, 96%, 96% and 105% for Cd, Hg, Pb and Se, respectively, guaranteeing the absence of drift of the measurements. Accuracy of the analyses was checked using certified reference material (TORT-3 and DOLT-5) with known concentrations of trace elements. Percentage of recovery were 93±4%, 110±4%, 81±4% and 93±1% for Cd, Hg, Pb and Se, respectively. The detection limits were 0.0133 µg Hg/kg, 0.0035 µg Cd/kg and 0.0389 µg Se/kg in liver. The detection limits for Pb were 0.0035 µg Pb/kg in both liver and humerus. Finally, tissue concentrations are expressed in µg/kg dry weight (dw).

### 2.4. *Toxicological interpretation of TME concentration in tissues*

For Pb concentrations, we used the values reported by Taggart et al. (2020). For the liver, Pb exposure was considered abnormal at concentrations > 6 000 µg/kg dw. For concentrations > 20 000 µg/kg dw, the bird was considered to have been acutely exposed, Pb exposure being a likely cause of death. For bones, exposure was considered abnormal at concentrations > 10 000 µg/kg dw and acute and potentially lethal for concentrations > 20 000 µg/kg dw.

For liver Hg concentrations, we considered that concentrations >1 600 µg/kg dw were consistent with induction of oxidative stress while 6 900 µg/kg dw (calculated from the value of 2000 µg/kg ww, Shore et al. 2011; Ackerman et al. 2016) was retained as an indicative value for impaired reproduction. Liver concentrations above 76 000 µg/kg dw (calculated from the value of 22 000 µg/kg ww, Shore et al. 2011 in Ackerman et al. 2016) were retained as indicative concentration for death. The critical concentrations provided on a wet weight basis were converted in dry weight assuming a water content of 71% for the liver of a bird (Hughes 1974). We also calculated the molar ratio Se:Hg as a proxy of Hg detoxification by Se. For a given individual, the Se:Hg molar ratio was calculated by dividing the liver concentration of each element by its molar mass, i.e., 78.96 and 200.59 g/mole for Se and Hg, respectively. A molar ratio Se:Hg <1 means that Hg is in excess compared to Se and thus, may more likely induce toxic effect while a ratio above 1 is considered as protective for adverse mercury affects (Eisler 1985; Burger et al. 2012). As Se can also be toxic for wildlife at high concentration, 10 000 µg/kg dw was retained as a toxicity threshold concentration for Se in the liver (Ohlendorf and Heinz 2011).

For Cd hepatic concentrations, we referred to the value proposed by Kitowski et al. (2020) and considered the Réunion harrier has been abnormally exposed for concentrations > 3 000 µg/kg dw.

## 2.5. Statistical analyses

Since the measured tissue concentrations for the three TMEs (Pb, Hg, Cd) follow a log-normal distribution, the data were log<sub>10</sub>-transformed for all parametric analyses presented below. We used general linear models (LM) to check the existence of correlations between log-transformed concentrations of TMEs in the different tissues analysed. For an individual, when the concentration was below the detection limit, we assigned it the detection limit value of the TME considered, this change involved only one individual for Pb. Possible differences in log-transformed concentrations of TMEs between sex or age classes (juvenile, immature, adult determined by plumage colour) were investigated using LM. Temporal trends were checked for the concentrations (log-transformed) of the three TMEs with generalized additive model GAM (Gaussian family with identity link). Given the relatively limited sample size (n = 30), we restricted the maximum number of degrees of freedom of smoothing terms to k = 5 to avoid overfitting in the GAM. For each test performed, the threshold of significance was 0.05. Normality of residues and homoscedasticity of variances were checked in all cases. Finally, we conducted Spearman rank correlations between the concentrations of the different elements to look for a possible multi-exposure of the Réunion harrier to TMEs.

For TMEs exhibiting liver concentration above the toxicity threshold for more than 10% of the individuals analysed, we checked whether landscape composition influences harrier exposure. In this case, we assumed correlations between liver concentration and landscape variables considering the main sources of wildlife exposure known for the different TMEs. For Hg, the sources may be industrial and urban areas or wetlands (Jackson 1998; Chalmers et al. 2014) while they were industrial activities and mineral fertilizers used in agriculture for Cd (Burger 2008). As previously reported, exposure of wildlife to Pb is due to embedded or ingested lead shot, mining and smelting activities could be secondary sources (Buekers et al. 2009). If needed, the urban, agricultural and/or wetland areas within the discovery area of an individual were extracted from a habitat shapefile (CIRAD 2018). For each harrier, we defined two buffers centred on the location where the individual was found of 1.2 km radius and 4.2 km radius, corresponding to the mean (4.5 km<sup>2</sup>) and to the maximum value (55.5 km<sup>2</sup>) of the harrier's home range (Chiron and Augiron 2019). Moreover, the potential presence of industrial sites polluted by TMEs within harrier's discovery area was checked in the GEORISQUES database (Ministère en charge de l'Environnement 2022). Thus, relationships between liver TME concentrations (log-transformed) and the urban, agricultural and/or wetland areas or industrial site within the surrounding landscape of an individual have been tested with LM or generalized additive model GAM (Gaussian family with identity link). Given the relatively limited sample size (n = 30), we restricted the maximum number of degrees of freedom of smoothing terms to k = 5 to avoid overfitting in the GAM.

All the statistical analyses were computed with R 4.1.1 (R Development Core Team 2022) and four libraries were used: *ggplot2*, *mgcv*, *SCALE*, *tidyverse*.

### 3. Results

In total, 15 of the 30 individuals studied were females, 13 were males and the gender was not reported for 2. Concerning the age class, 3 harriers were juveniles, 21 immatures, 5 were adults, and it was not reported for one of them. No temporal trend was found for TMEs concentrations from 2015 to 2021 (Pb: GAM, edf = 1,  $p = 0.28$ ; Hg: GAM, edf = 1,  $p = 0.93$ ; Cd: GAM, edf = 1,  $p = 0.39$ ).

#### 3.1. Pb concentrations in liver and bones

Median Pb concentrations were 29.6  $\mu\text{g}/\text{kg dw}$  in liver and 204.9  $\mu\text{g}/\text{kg dw}$  in humerus (Tab. 1). For all individuals, both liver and bone concentrations were below the toxicity thresholds defined for these tissues. The highest concentration measured in liver was 609.6  $\mu\text{g}/\text{kg dw}$ , which is 10 times lower than the liver toxicity threshold of 6 000  $\mu\text{g}/\text{kg dw}$  for Pb. In humerus, the highest measured concentration, 3 767  $\mu\text{g}/\text{kg dw}$ , was 5 times lower than the threshold in bones. The lowest concentrations measured were 5.7  $\mu\text{g}/\text{kg dw}$  in liver and 28.9  $\mu\text{g}/\text{kg dw}$  in humerus.

**Table 1.** Concentrations ( $\mu\text{g}/\text{kg dw}$ ) of the studied metallic elements in Reunion harrier ( $n = 30$ ) and toxicity threshold ( $\mu\text{g}/\text{kg dw}$ ) for Pb, Cd, Hg and Se in avian livers. The last column n+/% indicates the number and the percentage of individuals with a liver concentration above the threshold.

		Min	Median	Max	Toxicity threshold	n+/%
<b>Pb</b>	Liver	5.7	29.6	609.6	6 000 <sup>1</sup>	0/0
	Humerus	28.9	204.9	3 767	20 000 <sup>1</sup>	0/0
<b>Hg</b>	Liver	177.6	2 022	22 656	1 600 <sup>2</sup>	16/53
					6 900 <sup>3</sup>	4/13
					76 000 (lethal) <sup>2</sup>	0/0
<b>Cd</b>	Liver	95.3	359.9	9 229	3 000 <sup>4</sup>	1/3
<b>Se</b>	Liver	2 409	3 855	8 616	10 000 <sup>5</sup>	0/0

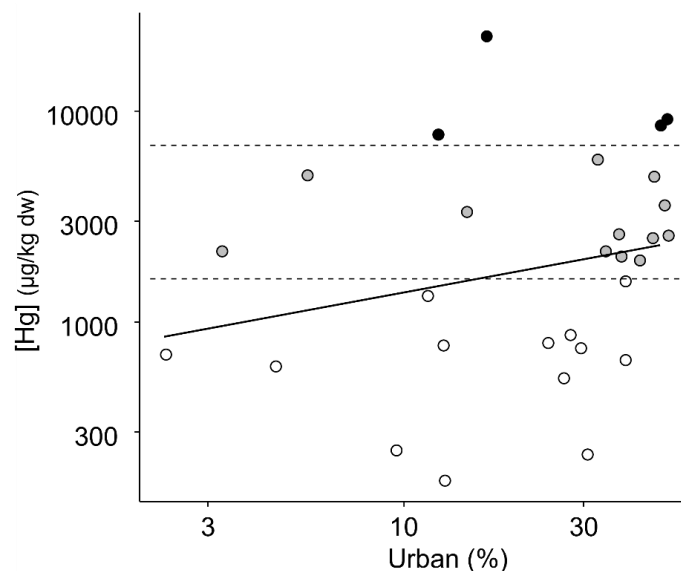
<sup>1</sup> Taggart et al. (2020); <sup>2</sup> Shore et al. (2011); <sup>3</sup> Ackerman et al. (2016); <sup>4</sup> Kitowski et al. (2020); <sup>5</sup> Ohlendorf and Heinz (2011)

Radiographs of the 30 harriers revealed that 2 individuals contained lead shot. One individual had one shot in the left leg and another in the head, it was euthanized at the care center. The other individual had one shot in the head and died because of shooting. The Pb concentrations in the livers of these two individuals were the highest compared to the other harriers, with values of 370.2 and 609.6  $\mu\text{g}/\text{kg dw}$ , respectively. In humerus, the concentrations measured, 125.5 and 179.2  $\mu\text{g}/\text{kg dw}$ , respectively were quite low compared to those measured in other Réunion harriers.

No correlation was observed between liver and bone concentrations (LM,  $p = 0.27$ ). In addition, no differences in Pb concentrations were detected between the sexes (LM,  $p = 0.85$ ) or age classes (LM,  $p = 0.59$ ). As the Pb concentrations were all below the toxicity threshold, no relationship was checked with landscape composition.

### 3.2. Hg concentrations in liver and relationship with Se

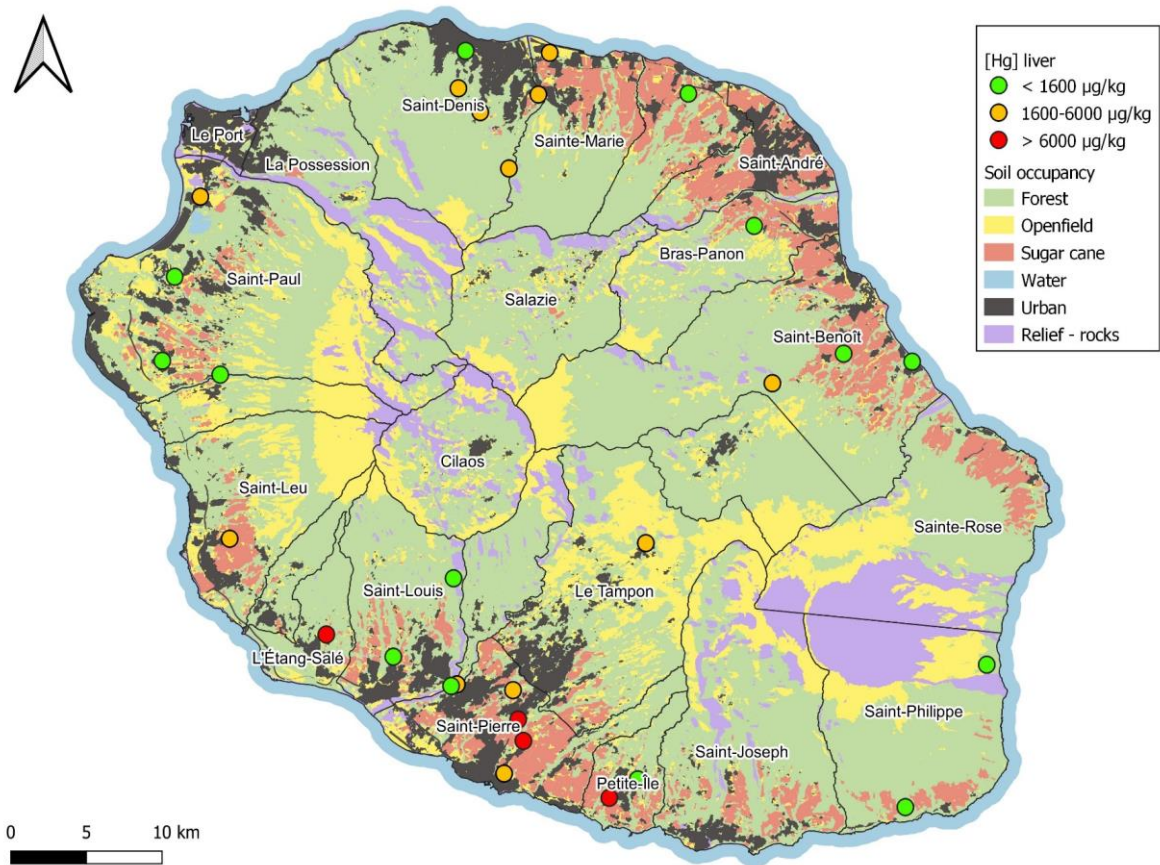
The median Hg concentrations measured in liver was 2 022  $\mu\text{g}/\text{kg dw}$  ( $n = 30$ ) and is above the first threshold of toxicity, 1 600  $\mu\text{g}/\text{kg dw}$ . The maximum liver Hg concentration was 22 656  $\mu\text{g}/\text{kg dw}$  and the minimum Hg concentration was 177.6  $\mu\text{g}/\text{kg dw}$ . Among the 30 livers analysed, 16 exceeded the first toxicity threshold of 1 600  $\mu\text{g}/\text{kg dw}$  and four were above the upper threshold of 6 900  $\mu\text{g}/\text{kg dw}$  (Fig. 1). None of them exceeded the lethal threshold of 76 000  $\mu\text{g}/\text{kg dw}$ .



**Fig. 1** Correlation between Hg liver concentrations ( $\mu\text{g}/\text{kg dw}$ ) and the percentage of urban area within 55  $\text{km}^2$  around the place where the harrier was found. The first and second dashed lines indicate the toxicity thresholds of 1 600  $\mu\text{g}/\text{kg dw}$  and 6 900  $\mu\text{g}/\text{kg dw}$ , respectively (Shore et al. 2011; Ackerman et al. 2016) and the grey and black dots correspond to the individuals with a liver concentration above these thresholds. Both axes are on a logarithmic scale

The spatial distribution of harriers according to their liver Hg concentration is shown in Fig. 2. The four harriers with the highest Hg concentrations ( $> 6 900 \mu\text{g}/\text{kg dw}$ ) were all found south-west of the island, between the municipalities of Etang Salé and Petite île. Birds with liver concentrations above the first toxicity threshold of 1 600  $\mu\text{g}/\text{kg dw}$  tended to be aggregated in the Saint-Denis and Saint-Pierre areas, while birds below that threshold were distributed more evenly across the island. No difference in Hg concentrations were detected between the sexes (LM,  $p = 0.86$ ) or between age groups (LM,  $p = 0.45$ ). Concentrations of Hg in liver were not correlated to the urban area proportion within 4.5  $\text{km}^2$  around the place where the harrier was found (LM,  $p = 0.59$ ; GAM, edf = 1,  $p = 0.59$ ) or with the proportion of wetlands (LM,  $p = 0.60$ ; GAM, edf = 1,  $p = 0.60$ ). Within the area of 55.5  $\text{km}^2$  around each harrier, we found a positive linear correlation between Hg liver concentrations and urban area proportion (LM,  $p = 0.046$ ,  $R^2 = 0.13$ ; GAM, edf = 1.44,  $p = 0.094$ ; Fig. 1) while wetland area proportion were still not correlated with Hg concentrations (LM,  $p = 0.64$ ; GAM, edf = 1,  $p = 0.64$ ). No site polluted with Hg was referenced in the GEORISQUE database (Ministère en charge de l'Environnement 2022) in the Réunion Island.





**Fig. 2** Spatial distribution of the Réunion harriers according to the Hg concentration in liver (in  $\mu\text{g}/\text{kg}$  dw,  $n = 30$ ).

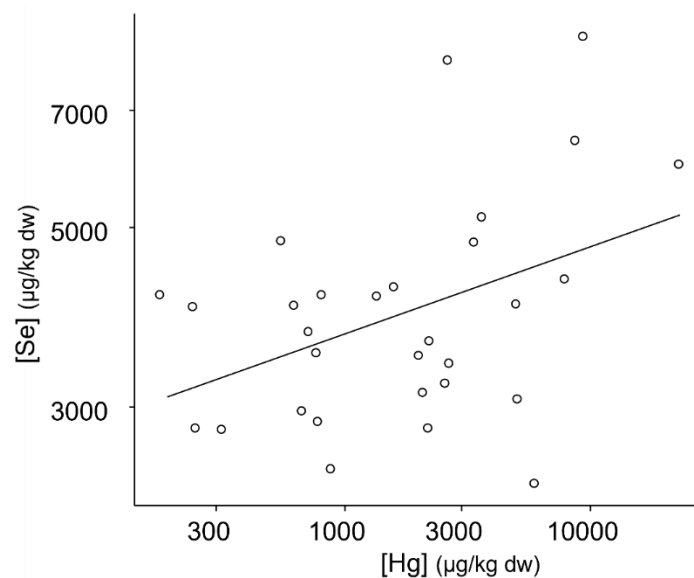
The median for Se concentrations in livers was  $3\,855\ \mu\text{g}/\text{kg}$  dw ( $n = 30$ ) with a maximum concentration of  $8\,616\ \mu\text{g}/\text{kg}$  dw and a minimum one of  $2\,409\ \mu\text{g}/\text{kg}$  dw. For all individuals, liver concentrations were below the toxicity threshold defined for this tissue (i.e.,  $10\,000\ \mu\text{g}/\text{kg}$  dw). A positive relationship was found between the log-transformed Hg and Se concentrations in liver (LM,  $p = 0.023$ ,  $R^2 = 0.17$ , Fig. 3).

Then, we computed the Se:Hg molar ratio in the liver of the harriers which allow to potentially evaluate the quantity of Se allocated to Hg detoxification. The median of the ratio was 5.6, the maximum being 59.1 and the minimum 0.67. This ratio was below 1 only for the individual with a Hg liver concentration of  $22\,656\ \mu\text{g}/\text{kg}$  dw. In the studied population, half of the individuals had a Se:Hg ratio between 2 and 10 ( $n = 15$ ) and the rest had a ratio higher than 10.

### 3.3. Cd concentrations in liver

The median concentration of Cd was  $359.9\ \mu\text{g}/\text{kg}$  dw. Only one individual with the highest concentration measured,  $9\,229\ \mu\text{g}/\text{kg}$  dw, exceeded the abnormal exposure threshold, i.e.,  $3\,000\ \mu\text{g}/\text{kg}$  dw. A significant difference was found between Cd concentrations between sexes (LM,  $p = 0.011$ ). The

median concentrations of Cd in females were 710.4  $\mu\text{g}/\text{kg dw}$  and 199.2  $\mu\text{g}/\text{kg dw}$  in males. There was no difference in Cd concentrations between age classes (LM,  $p = 0.17$ ). No relationship was checked between Cd concentrations and landscape composition.



**Fig. 3** Correlation between Hg concentrations ( $\mu\text{g}/\text{kg dw}$ ) and Se concentrations ( $\mu\text{g}/\text{kg dw}$ ) in Réunion harrier's livers ( $n = 30$ , LM,  $p = 0.023$ ,  $R^2 = 0.17$ ). Both axes are on a logarithmic scale.

### 3.4. Multi-exposure to TME

Hepatic Hg and Cd concentrations were positively correlated (Spearman rank test,  $\rho = 0.47$ ,  $p < 0.01$ ), indicating that Réunion harriers with a high Hg concentration had also a relatively high Cd concentration. Correlations between liver Pb and Hg or Cd concentrations were not significant (Spearman rank test,  $p = 0.79$  and  $p = 0.62$ , respectively).

## 4. Discussion

### 4.1. Exposure to Pb: a threat for the Réunion harrier?

Considering the relatively low concentrations measured in both livers and bones compared to the toxicity thresholds, we show that the Réunion harrier is not abnormally exposed to Pb. Lead is an element occurring naturally in the environment, so it is often detected at varying concentrations in biological tissues even in uncontaminated areas. Lead concentrations above toxic thresholds have been found in several species of raptors in different continents (Pain et al. 2019; Taggart et al. 2020) and are commonly related to hunting or poaching activities (Taggart et al. 2020; Green et al. 2022). In the case of the Réunion harrier, the measured concentrations never exceed the threshold values defined for different tissues (Taggart et al. 2020). This finding suggests that the Réunion harrier is not threatened currently by an overexposure to Pb due to embedded shot or Pb ingestion from prey. Lead may be accumulated non-homogeneously in the different organs of vertebrates and the information

given by the measured Pb concentrations in different tissues of the same individual is therefore complementary. Bones provide information on the long-term exposure of individuals because Pb can be accumulated in this tissue for several years as it is not remobilized, except in females for the formation of eggshells (Finley and Dieter 1978). Thus, bone concentrations represent relevant indicators for studying Pb exposure of animals over their entire lifespan (Krone 2018). In our study, no individual had concentration in humerus above the toxicity threshold (10 000 µg/kg dw) and we can conclude that we got no evidence that the studied individuals experienced excessive exposure to Pb during their lifetime. However, among the 30 individuals analysed, two harriers had Pb shots in the body, these shots being considered as the cause of death. This shows the current reality of poaching that still represents a threat for the conservation of this species due to its very small population size. In these 2 harriers, no abnormal contamination was detected, probably because the Pb contained in these munitions did not have time to be spread into the body.

#### 4.2. *Mercury and Se concentrations in the Réunion harrier*

The Réunion harrier exhibit relatively high concentrations of Hg in liver, more than 50% of the individuals analysed having levels over 1 600 µg/kg dw. Above this threshold, Hg is susceptible to cause deleterious effects in the Réunion harrier at the cellular level, particularly through the induction of oxidative stress (Ackerman et al. 2016). In addition, 13% of individuals have liver residues greater than 6 900 µg Hg/kg dw, which has been related to reproductive disturbance in birds such as decrease in egg weight, hatching rates and/or an increase in unfertilized eggs (Shore et al. 2011). The Réunion harrier population could suffer troubles in breeding success (Augiron 2022). Nowadays, about 200 pairs live on the island and represent the entire breeding population in the world. Current knowledges suggest that both the number of fledging per pair and the hatching success would have decrease since the 1970's. In 1975-76, the annual fecundity was estimated at 1.4 young per pair (Clouet 1978), it was 1.2 in 2000 (Grondin and Philippe 2011) while, between 2013 and 2019, it declined to 1 young (Augiron 2022). The hatching rate of eggs measured in the period 2013-2019 was 42% (Augiron 2022), which is low compared to other harrier species (Corbacho et al. 1997; Curtis et al. 2004). Moreover, the number of fledging per egg was 0.68 in 1975-1976 and decreased to 0.28 for the period 2013-2019 but the low sample size in 1975-76 and the absence of intermediate points prevent drawing robust conclusion (Augiron 2022). Several studies reported the impact of Hg on the embryonic development of birds. Moreover, (Heinz et al. 2009) showed experimentally that raptors are among the most sensitive group to Hg that at the embryonic stage. Thus, it is not excluded that the Hg exposure was responsible or at least contribute to the low reproductive success since the transfer to the egg usually occurred from the mother (Scheuhammer 1987; Ackerman et al. 2011). The origin of Hg measured in the harriers remains undetermined. Liver concentrations were correlated to urban areas within the area of 55.5 km<sup>2</sup> around the individuals, which suggests possible urban and/or industrial origin of Hg present in the Réunion harrier. A major part of individuals with high residues levels have been found in the vicinity of agglomerations, such as Saint-Pierre and Saint-Denis, but to our knowledge, there is no known Hg contamination in these areas. Moreover, any site polluted by Hg was listed in the Réunion Island in the GEORISQUES database (Ministère en charge de l'Environnement 2022). In birds, Hg contamination levels are mostly related to their diet, particularly when they are carnivorous and related to aquatic environments, even indirectly (Ackerman et al. 2016). In aquatic ecosystems under anaerobic conditions, Hg may be methylated as methylmercury, which is highly lipophilic. It then has increased

transfer capacity in food chains according to a biomagnification process. The Réunion harrier's diet is mainly composed of terrestrial animals notably small mammals (35%), small birds (29%), or reptiles (22%) and it rarely feeds in aquatic environments, fish representing 5% of the diet (Probst 2008; Chiron and Augiron 2019; Augiron 2022). In addition, occasional opportunistic scavenging behaviours have been reported (Probst 2008). Moreover, Bretagnolle et al. (2000) observed that the Réunion harrier forages only rarely in wetlands. Added to the fact that Hg concentrations in harriers were not correlated to the wetland area within the areas considered, these trophic traits do not support the hypothesis of a transfer from aquatic ecosystems with the current data accessible. Soils in Réunion island have moderately high concentrations of Hg, due to the volcanic origin of the island (Døelsch et al. 2006a). The median is 0.16 mg Hg/kg (min-max: 0.03-0.81 mg/kg, (Døelsch et al. 2006a) while it is 0.041 mg Hg/kg (min-max: 0.005-1.370 mg/kg, (Marchant et al. 2017), 0.10 mg Hg/kg (min-max: 0.07-1.22) and 0.23 mg Hg/kg (min-max: 0.07-1.53) in rural and urban soils, respectively, in the United Kingdom (Ross et al. 2007). Some of the Réunion soils have Hg contents above the threshold value of 0.5 mg/kg proposed by the Finnish Ministry of the Environment for contaminated soils. However, all the concentrations reported in (Døelsch et al. 2006a) are below the guide value corresponding to an environmental or health risk, i.e., 2 mg/kg (Marchant et al. 2017). We cannot exclude that the relatively high exposure of the Réunion harrier we have highlighted has as main origin the Hg occurring naturally in the Réunion Island soils. In this case, this would mean that the harrier population is naturally exposed to Hg since a very long time and thus, it would not explain the recent decrease of breeding success suggested over the last 45 years. Investigations remain to be carried out to better understand the origin and pathways of Hg transfer to Réunion harrier and whether this toxic metal may threaten its population or not.

If Se is an essential element for animals that is known for its role in Hg detoxification, it may become toxic for birds in case of overexposure (Ohlendorf and Heinz 2011). Reproductive impairment is more sensitive to Se overexposure than are the health and survival of young and adult birds (Ohlendorf and Heinz 2011). Reproductive problems, primarily deformities of embryos and hatchling failure, may occur from liver concentration of about 10 000 µg/kg dw (3 000 µg/kg wet weight) in egg-laying females. The two highest Se concentrations measured in the Réunion harrier, 8 616 and 8 069 µg/kg dw, are closed but below this threshold. They were measured in two adult females found in May and July, so, during the breeding period. Sublethal effects other than reproductive disturbance may occur for liver concentration over 30 000 µg/kg dw (10 000 µg/kg wet weight) in nestlings and adults (Ohlendorf and Heinz 2011). This suggests that Réunion harrier is likely not exposed to toxic level of Se. Considering the protective role of Se against Hg toxicity, the molar ratio Se:Hg revealed a deficiency in Se only in the individual exposed to the highest Hg concentration. For all the others, molar ratios were higher than one, which lead to assume that the Réunion harrier is able to detoxify efficiently methylmercury and limits effects. However, we have to keep in mind that in some animals such as fish, the role of Se in Hg detoxification is still a controversial issue, notably the actual Se:Hg ratio protecting against Hg toxicity that remains unclear (Burger et al. 2012).

#### 4.3. Cadmium concentrations and multi-exposure

The Réunion harrier is not severely exposed to Cd, as shown by the relatively low concentrations measured in livers which are below the threshold of 3 000 µg/kg dw except for one individual. We show that Cd concentrations in liver increased with those of Hg. This evidence a

concomitant exposure within the surrounding landscape of an individual but, to our knowledge, the potential mixture effects of these two toxic metals have never been investigated in birds. If Cd concentrations remain below toxic level, such threshold is not defined by considering exposure to other chemicals that can modulate the overall response of organisms. From a toxicological point of view, a synergistic interaction between Hg and Cd, i.e., an increased effect of the mixture compared to equitoxic exposure to single metal, was reported in invertebrates (Mohan et al. 1986; Frías-Espericueta et al. 2009). In humans, exposure to a mixture of Hg and Cd significantly reduced their concentrations in the blood, liver, kidneys and brain compared to those measured when each element is administrated alone at the same dose (Orr et al. 2018). Overall, mixture toxicity remains poorly understood in the field of toxicology and ecotoxicology and more studies are needed to conclude whether co-exposure to Cd and Hg may impact or not the Réunion harrier into the wild.

## 5. Conclusion

Currently, anticoagulant rodenticides represent a major threat for the conservation of the Réunion harrier (Coeurdassier et al. 2019). The results we obtained on harrier exposure to TMEs show no or a low exposure to Pb and Cd while Hg concentrations in liver are compatible with toxic effects at the cellular level for 53% of the individuals analysed and on reproduction for 13% of them. Mercury is known to be highly toxic for embryo and nestlings through transfer to eggs from mother and thus, could contribute to impair breeding success but no data are currently available on Hg residues in the eggs of Réunion harrier. A huge field of research has been dedicated to the impact of Hg on eggs and guidelines for the interpretation of concentrations are available for birds (Shore et al. 2011). In the coming years, we recommend monitoring of Hg concentrations in both unhatched and hatched eggs using content (i.e., mixed of vitellus and albumen) for unhatched eggs and/or eggshell (for both hatched and unhatched) as proposed by Peterson et al. (2017). Moreover, both nestling and adult exposure to Hg should be monitored in alive individuals using none or minimally invasive samples such as blood or down and feathers. The use of down and feathers will need to follow the methodological recommendations of Peterson et al. (2019). Such assessment of exposure could be complemented by monitoring (1) individual traits that could be related to exposure, such as breeding success for adults, hatching success for eggs or growth and fledging success for nestlings; (2) intra-individual responses related to Hg toxicity like oxidative stress (Balali-Mood et al. 2021) or endocrine disruption (Tartu et al. 2013). If exposure to toxic level of Pb was not evidenced in this study, the threat of shooting persists and must be the subject of special attention for the conservation of the Réunion harrier.

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### **Author contribution**

SA got the funding and managed the project. MC and SA conceptualized the experiment; SA, SR, and JT collected the samples; NC, CA, VD and CH prepared and analysed the samples; CH and MC analysed the data; CH and MC supervised the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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### **Declarations**

Ethics approval: Not applicable.

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