Long-term monitoring of exposure to toxic and essential metals and metalloids in the tawny owl (*Strix aluco*): temporal trends and influence of spatial patterns

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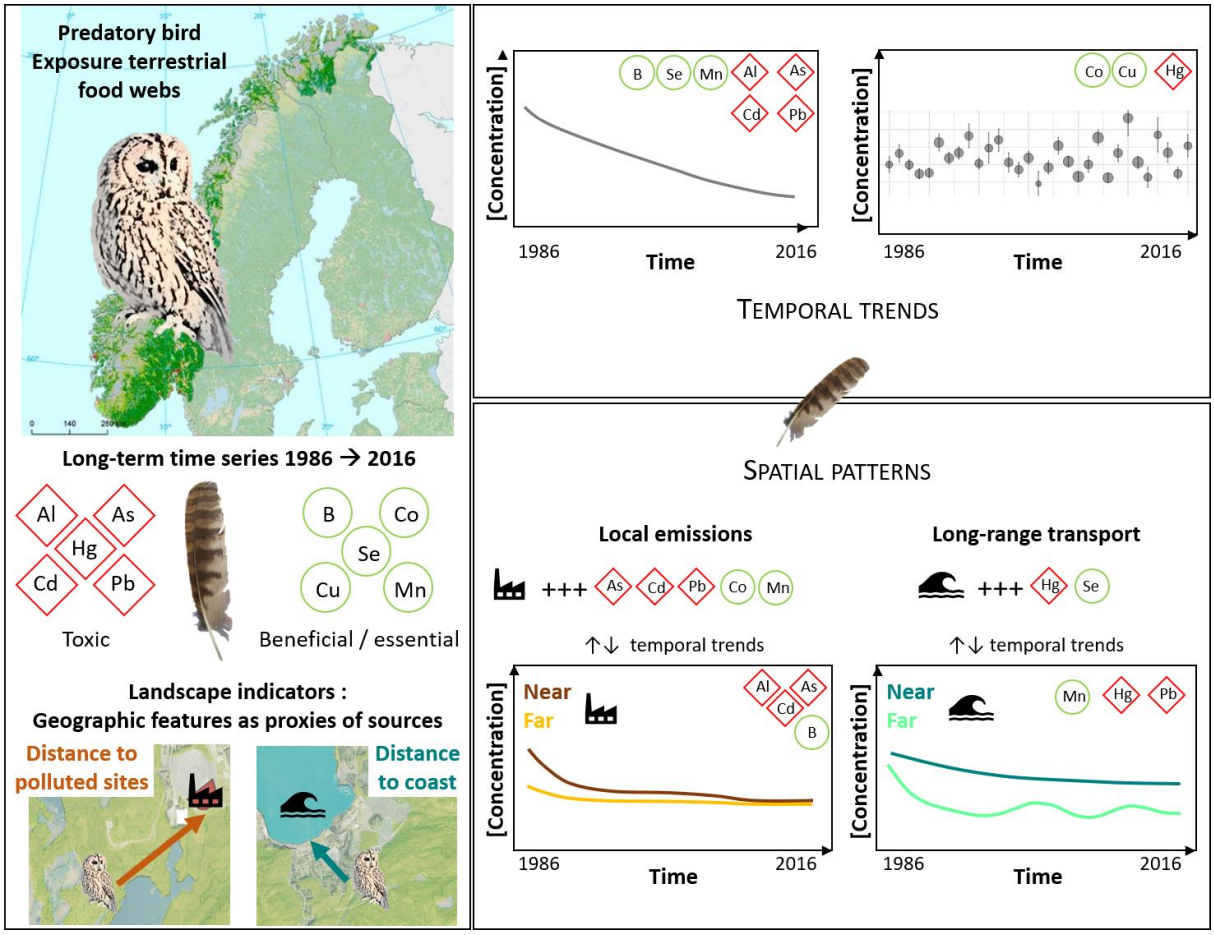
Abstract

As a result of regulatory decisions, atmospheric deposition of most toxic metals and metalloids (MEs) has decreased in Europe over the past few decades. However, little is known about how this reduction translates into exposure at higher trophic levels in the terrestrial environment where temporal trends may be spatially heterogeneous due to local current or legacy sources of emissions (e.g., industry) or long-range transport of elements (e.g., marine transport). The aim of this study was to characterize temporal and spatial trends of exposure to MEs in terrestrial food webs using a predatory bird, the tawny owl *Strix aluco*, as a biomonitor. Toxic (Al, As, Cd, Hg, Pb) and essential/beneficial (B, Co, Cu, Mn, Se) elemental concentrations were measured in feathers of nest-captured females from 1986 to 2016, extending a previous study published over the time-series 1986-2005 (*n*=1051), in a breeding population in Norway. A drastic decline over time was shown for the toxic MEs (-97% for Pb, -89% for Cd, -48% for Al, and -43% for As) except Hg. The beneficial elements B, Mn, and Se showed oscillations but an overall decline (-86%, -34%, and -12%, respectively) whereas the essentials Co and Cu did not exhibit significant trends. The distance to potential sources of contamination influenced both the spatial patterns of concentrations in owl feathers and their temporal trends. The accumulation of As, Cd, Co, Mn and Pb was overall higher in the vicinity of sites recorded as polluted, and a greater temporal decrease of As, B, and Cd concentrations was found in the areas of further distance to polluted sites. The decrease of Pb concentrations was sharper further from the coast during the 1980s than in coastal areas, while the opposite was observed for Mn. The levels of Hg and Se were higher in coastal areas, and Hg temporal trends differed according to the distance to the coast. This study highlights the valuable insights provided by long-term survey of wildlife exposure to pollutants and landscape indicators to reveal regional or local patterns and detect unexpected events, data that are crucial for regulation and conservation of ecosystem health.

Key words: bird of prey, biomonitoring, geographic feature proxy, landscape indicators, landscape ecotoxicology, wildlife toxicology,

Graphical abstract



1. Introduction

Pollution is one of the threats to biodiversity which may occur across multiple spatial scales, from local to global range, and act at different timescales (Bonebrake et al., 2019). Among pollutants, hazardous metallic and metalloid elements (MEs) have been widely emitted by anthropogenic activities (Ali et al., 2019). Trace metals and metalloids naturally occur in the environment at trace levels. Some of them are beneficial, or even essential, to living beings, such as Fe, (Cu, Co, Mn, Mo, Ni, Se, and Zn although being toxic to organisms at high concentrations of exposure (Ali et al., 2019; Steinnes, 2009; Walker et al., 2006). Conversely, several MEs are known as toxic without established biological roles such as Al, As, Cd, Pb, and Hg. Several of the essential or beneficial metals are co-factors for the synthesis of numerous enzymes and participate in antioxidant defences, which are needed to manage reactive oxygen species, thus contributing to the processes against toxic metal-induced oxidative stress. For instance, antioxidant enzymes are metal-dependent, with superoxide dismutase dependent on Cu, Zn or Mn and glutathione peroxidase dependent on Se (Miller et al., 1993).

Over the past decades, consistent efforts in the regulation and industrial practices have led to a drastic decrease in emissions of toxic MEs, especially Cd, Pb and Hg with -66%, -95% and -72% of releases between 1990 and 2019 in Europe (European Environment Agency., 2021). Even if the inputs of MEs in ecosystems have been lowered in recent decades, how this propagates to terrestrial wildlife at higher trophic levels is not well characterized. To tackle this issue, biomonitoring using raptors has proven its effectiveness in assessing pollution levels and impacts on wildlife. Avian top predators are reliable indicators since they are high-order consumers susceptible to secondary poisoning and biomagnification (Bustnes et al., 2013; Gómez-Ramírez et al., 2014). Among European raptors, the common buzzard (*Buteo buteo*) and the tawny owl (*Strix aluco*) have been showed as the most suitable candidates to apply biomonitoring in order to study long-term and large-scale changes in environmental pollution (e.g. toxic metals, anticoagulant rodenticides, pesticides and medicinal products) (Badry et al., 2020). For decades feathers have been used in ecotoxicological studies to assess exposure to contaminants in birds, especially metals, with continuous refinements in both the methods of analytics and interpretation of residue concentrations in this matrix (Borghesi et al., 2017). They are widely used to monitor both bioaccumulation and individual and population health (Espín et al., 2016). Bustnes et al. (2013) studied the trends of essential and non-essential elements in feathers of a population of tawny owlsin Norway from 1986 to 2005. They showed a decrease in exposure to As, B, Cd, Co and Pb over the time period. However, they also evidenced nonlinear shifting trends with increasing concentrations after the 1990s, for Al and iron (Fe) notably, and intra-annual and inter-annual variability can be noticed in As and Co concentrations for example. Conversely, no temporal changes for Hg were detected (Bustnes et al., 2013). These findings highlighted the need for long-term

monitoring in order to confirm the patterns. Further, these results question whether temporal trends are homogeneous over space and may explain the non-decreasing temporal patterns of exposure with opposite trends at local versus regional level.

Although substantially reduced, emissions still occur worldwide. For instance, emissions of Hg to air in Norway were assessed as 267 kg $year^{-1}$ in 2016 versus 1, 007 kg $year^{-1}$ in 1994 (The Norwegian PRTR, 2022). Metal emissions to land surfaces have been calculated as higher in the early 2010s than in the 1980s (1.5 to 3 times higher) despite noticeable declines in hydrologic and atmospheric emissions (Rauch and Pacyna, 2009). This might be explained by growing consumption of metals worldwide which increased stocks in-use and discards in the waste stream (Rauch and Pacyna, 2009).

Temporal trends may differ between the different elements depending on their potential to contaminate local ecosystems or be transported over long distances (i.e., long-range atmospheric and marine transport). The relative contribution of the different main planetary geographic areas in global ME cycles during the last decades have changed, the levels in Europe being more and more impacted by emissions from other parts of the world such as Asia (Rauch and Pacyna, 2009). This trend may enhance the role of long-range transport versus local or regional emissions in environmental contamination. Based on a national scale long-term monitoring of metals in terrestrial moss samples in Norway since 1977, Steinnes et al. (2011) emphasized the role of European transboundary pollution in the atmospheric deposition of As, Cd, Mo, and Pb but with a temporal decrease of such a contribution of long-range transport over the last decades. Conversely, the deposition of Fe, Ni, Cu, Cr, and Co was mostly influenced by local sources, with a decreasing time trend as well, except in some areas subjected to deposition of metals from smelters located in neighboring countries (Steinnes et al., 2011). Some elements such as B, Se, and Sr mainly originate from the marine environment (Steinnes, 2009, 1995). Marine environment could also be an important source for Hg, since this element is particularly prone to long-range airborne and marine transport, and because more than half of the annual emissions to air originate from ‘re-emissions’ of previously released mercury that has accumulated in surface soils and oceans (United Nations Environment Programme, 2013). Further, non-precipitation Hg wet deposition has been identified as contributing by 5 –10 % to Hg wet deposition over the land surfaces, being therefore an important process in regions considered a hotspot for cloud, fog, dew, and frost that participate to Hg non-precipitation deposition such as coastal areas (Zhang et al., 2019).

Metals and metalloids being persistent, the legacy of contamination remains in soils and sediments, with MEs accumulating both in the environment and living organisms and being transferred through food webs (Ali et al., 2019). Current levels of wildlife exposure could thus be related to historical inputs from industry, mining and transport for instance, with higher concentrations in individuals living in the surroundings of polluted sites where hot-spots of ME loads remain in the environment. Studying

bioaccumulation of Cd and Pb in European blackbirds (*Turdus merula*) around a former Pb- and Zn-smelter in Northern France, Fritsch et al. (2012) found a decrease of exposure with increasing distance to the smelter. They highlighted that on a broad scale, local contamination resulting from industrial inputs was the prominent factor influencing exposure of blackbirds, although the smelter closed down several years before the sampling (Fritsch et al., 2012). The proximity to local past or present sources of metal contamination, such as industrial facilities, and the proximity to the coast which undergoes marine inputs may shape the spatial exposure patterns of wildlife to MEs. The geographical proxies describing the distribution of owls towards such potential sources of inputs acting at local to regional spatial scale may be considered as "landscape indicators" to study the spatial patterns of owl exposure to metals and metalloids (Cairns Jr and Nlederlehner, 1996).

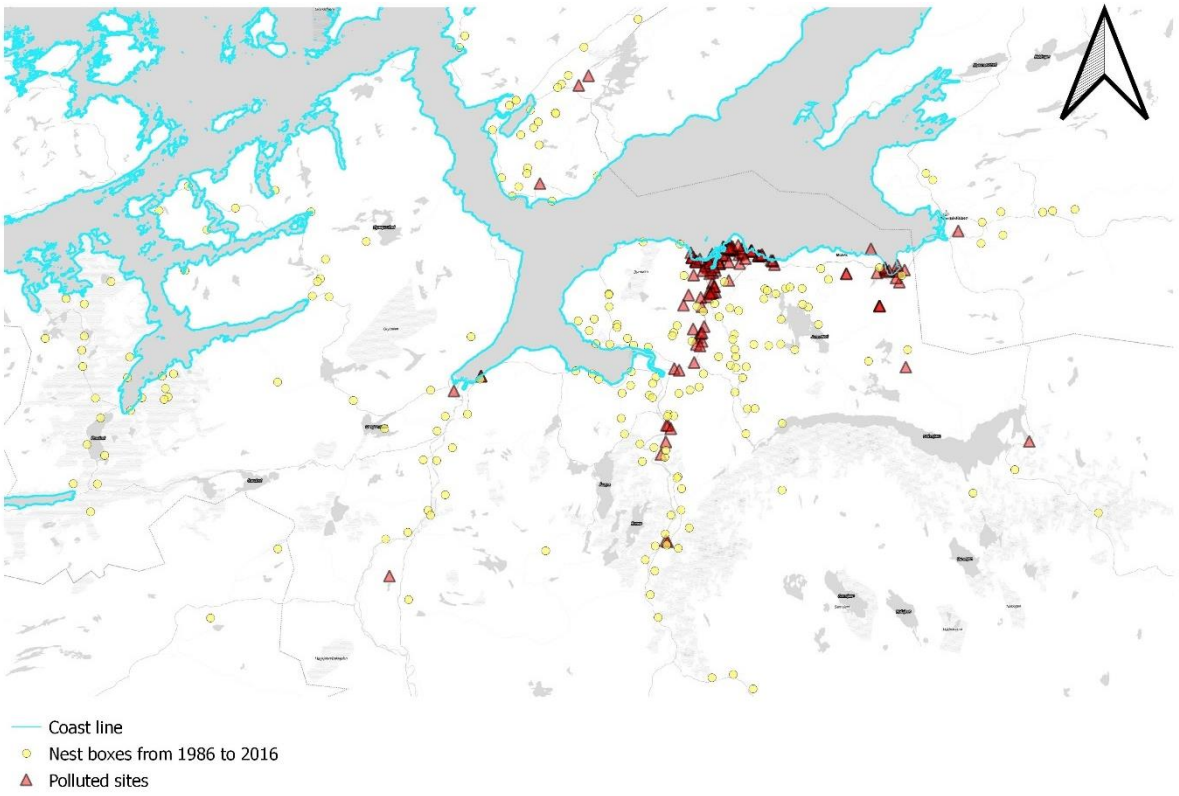
We aimed to explore the temporal and spatial dynamics of exposure to metals and metalloids in terrestrial food webs in Central Norway studying a predatory bird, the tawny owl, as a biomonitoring model , . Our first objective was to further characterize long-term trends of wildlife exposure to MEs, expanding the time series analysis of element loads presented in Bustnes et al. (2013) from 1986 to 2005 until 2016, with a focus on comparing toxic versus beneficial elements. Due to national and international legislative measures with subsequent decrease of emissions, a decrease of exposure to toxic elements is awaited. For the essential and beneficial elements, we expect a two-step pattern. First, a decrease over the time sequence due to decrease of emissions or reduction of need in detoxification processes against toxic MEs. Second, we expected steady or even increasing concentrations if deficiencies occurred when exposure to toxic MEs was the highest. Our second objective was to investigate spatial patterns and how they interact with temporal trends, using "landscape indicators". Exposure may exhibit spatial heterogeneity according to the geographical location towards sources of concurrent inputs or legacy contamination. Since the origin of contamination differ depending on the element of concern, the spatial patterns should vary according to the main sources of environmental input such as local anthropogenic contamination or long-range marine transport. A higher exposure of owls to MEs typically emitted by industrial activities, namely Al, As, Cd, Co, Cu, Mn and Pb is expected nearby local sources recorded as "polluted sites". An increase of owl exposure to MEs that may originate from marine environment inputs such as B, Hg and Se is predicted in coastal areas. Due to environmental contamination and persistent nature of MEs, a weaker decrease or even an absence of decrease in exposure over time in the vicinity of such landscape indicators of sources" is hypothesized.

2. Materials and methods

2.1 Study area

This work is part of the ENVISTRESS project which aims to document how pollution may modulate stress in avian top predatory species from central Norway. The study area is located in Norway in the region of the Trøndelag County (formerly Sør-Trøndelag County) (63°25N, 10°23E) and includes the municipalities of Trondheim (~180 000 inhabitants) (Fig. 1). Fjords and boreal forests area describe a typical coastal landscape of Norway. The oceanic climate is predominant. The area surface is around 7752 km² with a distance of 114 km from West to East and 68 km from South to North.

Figure 1. Map of the study site with the location of nest boxes, coastline and polluted sites.



2.2 Data collection

The tawny owl is a long-lived, nonmigratory territorial bird living in woodland, forest, parkland, urban areas and churchyards with large trees. The tawny owl is a resident territorial bird, making this species a relevant sentinel for the monitoring of contaminants in local terrestrial ecosystems. In the population living in the surroundings of Trondheim where our study was conducted, the home-range size was observed to vary from 91 to 1780 hectares with a median of 286 hectares (Sunde et al., 2001). The tawny owl is a bird of prey mostly feeding on small mammals, that constitute the major part of its diet, as well as passerine birds and amphibians (Capizzi, 2000; Petty, 1999; Sunde et al., 2001).

About 200 tawny owls nest boxes were set up in mixed forest, and each nest box was annually visited. The data collection provides a long-term retrospective from 1986 to 2016. Female owls were caught

in the nest boxes in early May before the beginning of the moulting period (Hirons et al., 1984) as part of a private driven monitoring / ringing program, running since the 1980s, and one of the two mid-positioned tail feathers were sampled during each breeding season. Pulling a feather can be considered as minimal invasive sampling since feathers are rapidly regrown, i.e. the owls show no altered behavior (i.e. they survive, stay in their territory and continue to reproduce for years) and we have no records of owls where feathers have not regrown when recaptured. The moult of wing and tail in tawny owls is indeed documented as starting in early June and being completed by the end of September in most of birds (Hirons et al., 1984). Time lags may occur in some birds depending on breeding success and breeding timing, a few birds can start moulting before the end of May or still in active moult beyond September (Hirons et al., 1984). The standardization of the type of feather studied and the choice of flight or tail feathers rather that contour feathers are essential to ensure reliable comparisons especially when studying temporal trends of contaminants such as metals, as shown in the tawny owl (Debén et al., 2012; Varela et al., 2016). A total of 409 female tawny owls were monitored, resulting in a total of 1051 feathers sampled since some individuals were monitored for several years. Moulting commonly starts with the central pair of rectrices in the second prebasic moult, and Strix owls continue the molt the year after during the third prebasic moult where the previous moult finished, and such schedule then follows consistently (Zuberogoitia et al., 2018). The moulting pattern can be asymmetrical or incomplete, especially in starving or diseased birds (Hirons et al., 1984; Zuberogoitia et al., 2018). Therefore, feathers moulted in the previous moult season can be found instead of feathers moulted in the current year. This means that some feathers sampled may have been grown two years before, inducing a bias in the correspondence of the year in the time sequence. Owing to the huge sample size in this study (more than 1000 feathers) and the substantial length of the survey which extends over 3 decades, such a one- or two-year lag for some feathers is not likely to induce bias in the significance and interpretation of temporal trends.

2.3 Metal and metalloid measurements

To avoid analysis of the external deposit elements, only the lower shaft of the feather (not the vane) was used in the metal analyses. It has been shown for metals such as As, Cd and Pb in a study on the tawny owl that shaft analysis is one of the best option to study these elements avoiding the bias related to atmospheric deposition and to apply harmonized protocols for pollutant biomonitoring, although the concentrations of metals were found lower in feather shafts than in barbs (García Seoane et al., 2018). The rest of the feather samples were scraped with a scalpel to remove residuals of external contamination. The lower shaft was acid digested in concentrated HNO3 (ultra-pure grade) and maintain in a microwave oven (Milestone Mls Mega) at 180 °C during 45 min (from 20°C to 180°C to 20°C). MQ-water was used for the dilution of the HNO3 acid solution (q.s. 0.6M). Chemical analyses

were carried out using HR-ICP-MS Element 2 (Thermo Electronics) at the heavy metal laboratory at the Norwegian Institute for Nature Research in Trondheim. Measurement accuracy, uncertainty and reproducibility were checked to ensure quality control (details in Appendix Table A1). Simultaneous analysis has been run for campaign of 1986 to 2004 and then separate analyses were run for 2005, and then for 2006-2016. Among the 39 metallic elements which have been measured, the present study focuses on 10 elements chosen based on their potential for toxicity or beneficial role in birds (cf Introduction) and considering their potential sources of contamination (i.e., mostly local or long-range transport). Besides local soil and vegetation factors (e.g., geological background), Al, As, Cd, Co, Cu, Mn and Pb may mostly originate from urban and industrial local to regional emissions whereas B, Hg and Se may furthermore originate from marine transported emissions (Ali et al., 2019; European Environment Agency, 2021; Rauch and Pacyna, 2009; Steinnes, 1995). All elements could not be analysed in all feather samples. Data were available for 1015 samples for Al, As, Cd, Co, Cu, Hg, Mn, Pb and Se while data were available for 946 samples for B.

2.4 Landscape indicators: computation of geographical features

Two geographical factors considered as proxy for potential input sources of MEs in the environment were computed: the nearest distance to "polluted sites" and the nearest distance to the coast. Polluted site location and surfaces were downloaded from the Norwegian Environmental Agency website (www.miljodirektoratet.no). This official national database maps contaminated land as well as municipal, private and industrial landfills based on information about sites with contaminated soil or with suspicion of contaminated soil due to various anthropogenic activities. In this study, the sites contaminated by metals and metalloids due to anthropogenic pollution releases caused by industry and factory, residential and urban facilities, smelting, or mining were considered. The maps used to locate coast boundaries were accessed through the Norwegian online mapping resource repository "geonorge" (www.geonorge.no). The coastline map was used (Fig. 1). The proximity to potential sources of contamination was calculated with the NNJOIN QGIS® extension as the minimum Euclidean distance (in meters) between the nest boxes (n=201) and the proximal polluted sites ("DistPollut") or coastland borders ("DistCoast"). The discretization package "arules" was used to split the distance to polluted sites or to the coast in 2 classes each (referred to as class 1 "close" and class 2 "far") based on the frequencies (i.e. obtaining balanced sample sizes in each class). The distance to polluted sites was split as class 1 "close": 104 - 2114 m (n=529) and class 2 "far": 2114 - 16596 m (n=522). The distance to the coast classes were class 1 "close": 20 - 4046 m (n=528) and class 2 "far": 4046 - 41202 m (n=523). The geographical distribution between the two sets of distances was checked to assess their spatial independence. The spatial distribution of the nest boxes was unbalanced towards a negative association between distances to the coast and to polluted sites (X^2 p-value = 0.002, Appendix Table

A3), meaning that more nest boxes classified as “close” to polluted sites were in the class “far” from the coast and that more nest boxes classified as “far” from polluted sites were in the class “close” to the coast. This will drive the cautious interpretation of the results to avoid any bias related to potential lack of independence (see Fig. 1 and below in statistical analyses and results). However, it is worth noting that even if the geographical distributions regarding the classes of distances were not statistically balanced, the sample size in each category reached between 235 to 288 (Appendix Table A3). Moreover, the minimum distance to polluted sites and the minimum distance to the coast in the full dataset were not significantly correlated (Spearman’s rank correlation, *p-value*=0.12; Fig. 2 and Appendix Fig. A1). Therefore, the dataset allowed testing the role of each category of distance and allow performing robust statistics.

2.5 Statistical analyses

Statistics were performed using the software “*R”* version 3.6.1 (R Core Team, 2020). The statistical distribution of the metal concentrations in feathers were cheked using the test of Shapiro, and were found to be skewed (log-normal distributions) for all elements but Se (normal distribution).

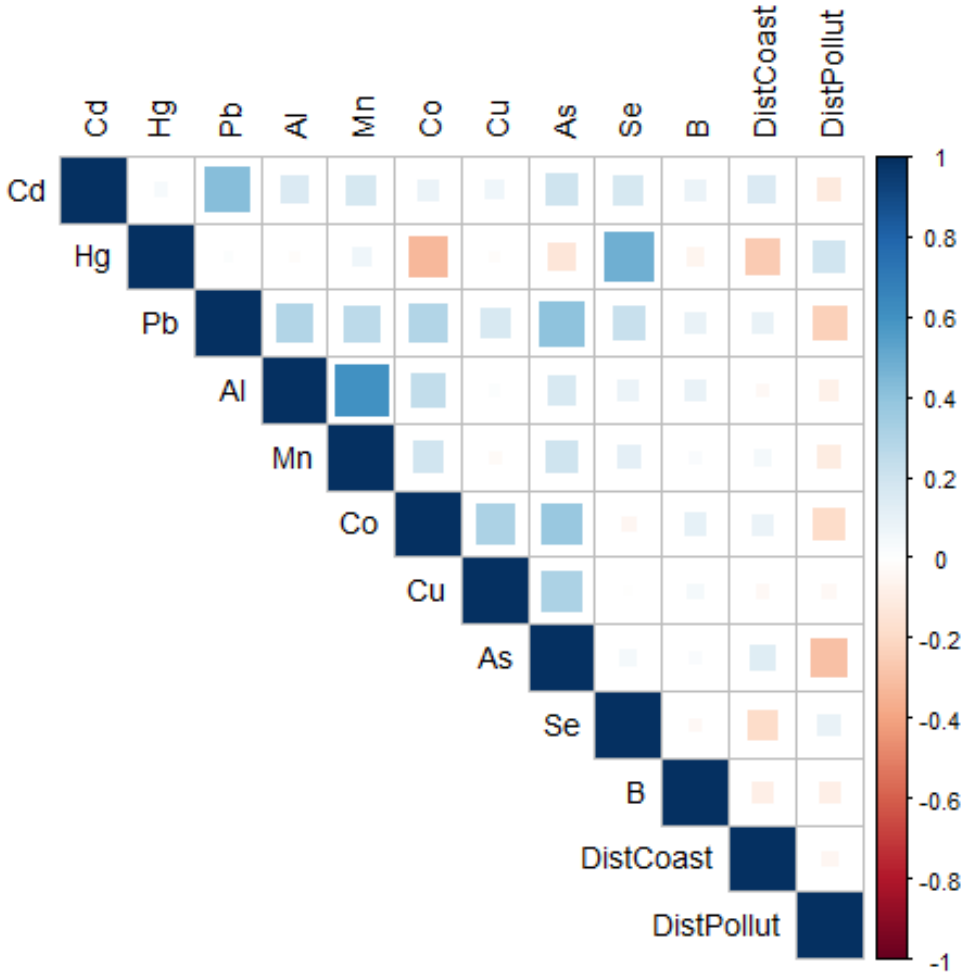
As a first exploratory analysis in order to get a global picture about correlations between the concentrations of the MEs and their association with geographical features, Spearman’s rank tests were performed on raw data, and displayed graphically together with statistical outputs in Figure 1 (package “corrplot”).

The percentages of concentration variation for each ME were calculated based on differences between average concentrations of the three first years of the sequence (1986, 1987, 1988) and average concentrations of the last three years (2014, 2015, 2016) to avoid an overweight of punctual fluctuation of the values on general trends if considering only the first and last year. General Additive Models (GAMs) were fitted on the year-specific average concentration of MEs (μ_{year}) in response to the year of sampling, log-transformed as $\log_e(x+0.1)$. The fitted GAM parameters used for cubic regression splines (bs=”cr”, *k*=4; γ=1.4) and the weights $\log_e(n_{year})$, corresponding to the annual size of samples, are detailed in Bustnes *et al.* (2013). The value of *k* was verified using knots criteria from 1 to 5 to 10 to 20 (Wood, 2017). GAMs were fitted using the package “mgcv” (Wood, 2017).

To study the influence of the distance to potential sources of contamination, GAMs were fitted using the classes of distances in interaction with the predictor “year” to fit the concentration of MEs using log-transformed concentrations as detailed above. The value of parameter *k* was set to -1. The structure of GAMs with interactions did not allow testing full models including the two geographic proxies as factors with several splines in interaction with the continuous predictor “year”. In order to avoid bias due to multicollinearity in alternative models, i.e. GAM gathering the two geographic proxies but with only one interaction spline, GAMs including one or the other geographic proxies were run

independently and then compared (see below). GAMs including each landscape indicator (i.e. distance to polluted site or distance to the coast) were compared, for each ME, by performing hypothesis tests on the two fitted GAM objects using the function “anova.gam” in “mgcv” with the setting test=chisq. Further, the models differing according to the previous test were compared based on their adjusted determination coefficient (R^2-adj), the deviance explained (*D*), Akaïke criterion (AIC) and Generalized Cross Validation (GCV) criterions.

Figure 2. Correlation matrix (Spearman’s rank correlation test) on the dataset gathering metal and metalloid element concentrations (µg.g-1) in owl feathers from 1986 to 2016 and the distance of the nest box where feathers were collected to the coast (“DistCoast”) and to polluted sites (“DistPollut”).



Squares coloured in red (negative correlation) to blue (positive correlation) are plotted in the matrix when the correlation is significant (p-value < 0.05) with their size being inversely proportional to the level of significance (i.e. increased size with lower p-value). The association strength assessed by Spearman’s rho value is figured by the colour of the squares according to the legend at the right side of the graph.

3. Results and Discussion

3.1 Correlations between ME concentrations and association with geographical features

Many correlations were detected between toxic MEs with the concentrations of As, Al, Cd, and Pb being all positively correlated (Fig. 2). The strongest correlation coefficients and levels of significance, were found between Cd and Pb, Al and Mn, and As and Pb (Fig. 2). The pattern for Hg was comparatively different since no positive association with any other toxic ME was found. Still, a negative correlation with the concentrations of As and Co was observed. Co and Mn concentrations were positively correlated with each other and with As, Al, Cd, and Pb. The concentrations of Cu were positively correlated with the levels of a few other elements, namely As, Co, and Pb. The concentrations of Se were positively correlated to Hg with a high coefficient and a high significance, to Pb also and to a lower extent to Cd. No correlation with any other element was detected for B (Fig. 2). The concentrations of Cd were positively correlated with the distance to the coast, indicating an increase of Cd in sites further from the shore, whereas the concentrations of both Hg and Se were higher in coastal areas (Fig. 2). The concentrations of several elements were negatively correlated with the distance to polluted sites, showing a decrease in concentrations of As, Cd, Co, Mn and Pb with increasing distance to polluted sites. Conversely, a positive correlation was evidenced for Hg, depicting lower concentrations near polluted sites. No correlation with any of the geographical features was detected concerning Al, Cu and B (Fig. 2).

Table 1. Temporal trend predictions of metal(loid) element concentrations (µg.g-1) in tawny owl tail feathers.

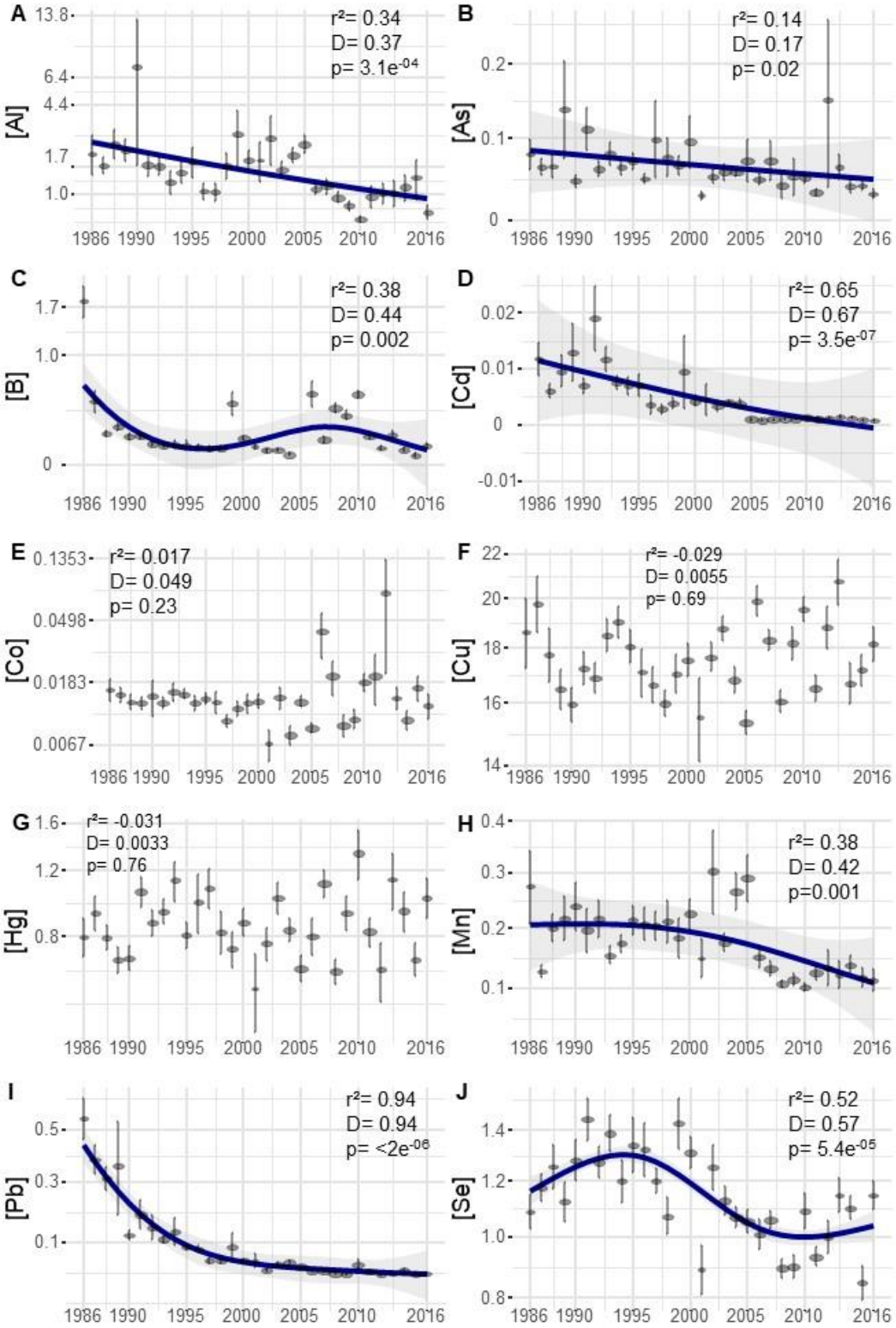
Trace elements	*n*	Variations (µg.g^{-1})				Model output parameters			
		Mean 1986-1988	Mean 2014-2016	Var(%)	Abs.Var	R^2-adj	*D*	edf	
Al	1015	2.10	1.10	-48%	-1.02	0.345	0.367	1.0	***
As	1015	0.067	0.039	-43%	-0.029	0.142	0.170	1.0	*
B	946	0.690	0.100	-86%	-0.590	0.379	0.440	2.9	**
Cd	1014	0.008	0.001	-89%	-0.008	0.653	0.670	1.4	***
Co	1015	0.015	0.013	-15%	-0.002	0.029	0.006	1.0	ns
Cu	1015	18.6	17.2	-7.8%	-1.45	0.017	0.049	1.0	ns
Hg	1015	0.830	0.890	6.6%	0.055	0.031	0.003	1.0	ns
Mn	1015	0.190	0.130	-34%	-0.064	0.379	0.416	1.8	***
Pb	1015	0.380	0.013	-97%	-0.370	0.938	0.944	2.8	***
Se	1015	1.20	1.00	-12%	-0.140	0.525	0.569	2.8	***

*The variations in the concentrations (Var.%: Variation in percent; Var.Abs. absolute variation) along time were computed comparing the dataset 1986-1988 (mean using the three years) and 2014-2016 (mean using the three years). GAM models were fitted using year as predictor, with output parameters: adjusted coefficient of correlation ("R2-Adj"), effective degree of freedom ("edf"), explained deviance ("D"), and levels of significance (ns: non-significant p-value > 0.05; * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001)*

3.2 Temporal trends

A significant continuous decline over time of the concentrations was found for toxic MEs, except Hg (Table 1, Fig. 3). The average variation percentages between 1986 and 2016 showed indeed a drastic decrease of the concentrations in feathers of Cd (-89%) and Pb (-97%) and, to a lower extent, of Al (-48%) and As (-43%) (Table 1). Decreasing linear trends over the thirty years were found for Al (edf = 1, R^2-adj=0.345, *p-value* < 0.001), As (edf = 1, R^2-adj =0.142, *p-value* < 0.05) though outlying concentrations of As in 2012 were detected around the general trends, and Cd (edf = 1.4, R^2-adj =0.653, *p-value* < 0.001) which exhibited a stationary state around 0 by 2005 (Fig. 3). The concentrations of Pb displayed a highly significant exponential decrease shape (edf = 2.8, R^2-adj =938, *p-value* < 0.001), with a sharp decline during the first decade when levels in 1996 ([Pb] ~ 0.1µg.g^{-1}) dropped to around one fifth of the levels measured in 1986 ([Pb] ~ 0.5 µg.g^{-1}), and then a steady slow decrease (Fig. 3I). The temporal variation found for the toxic elements As, Cd and Pb confirmed the continuous decreasing trends presented in Bustnes *et al.* (2013) between 1986 and 2005. Such a decrease in the meso-predator exposure corresponded to the changes in emissions and atmospheric deposition of these elements across Europe and especially in Norway (Pacyna et al., 2007, 2009; Steinnes et al., 2011), highlighting the efficiency of regulation to reduce the risk for environmental health. Comparing the declines of concentrations in owl feathers with the declines of emissions in the EU between 1990 and 2019 (European Environment Agency, 2021), interestingly, the decrease reached the same order of magnitude for Pb (dropped by 97% and 95 %, respectively) while it was stronger in owls for Cd (dropped by 89% versus 66 %) and it was slower in owls for As (dropped by 43% compared to 88 %). Pacyna et al. (2009) showed for Cd and Pb in Europe that the reduction of air concentrations followed similar trends as the reduction of anthropogenic emissions over the 1980s to the 2000s, while different patterns were found for concentrations in precipitation. For instance, a 45% decrease of Cd and Pb was found in precipitation, whereas the emissions dropped by 60% and 70%, respectively. However, the decrease of Cd and Pb in precipitation falling in Norway since the 1980s was larger and reached more than 90% (Pacyna et al., 2009). Except for the unexpected As peak around 2012, As and Cd concentrations exhibited similar patterns of regular decrease in owl feathers, with the lowest exposure levels reached over the last decade. Such a pattern matched the decline in As and Cd emissions in Europe, which occurred over two periods: in the mid-1970s and the mid-1980s, followed by a massive reduction in the 1990s (Pacyna et al., 2007).

Figure 3. Temporal trends (1986-2016) of toxic elements (Al, As, Cd, Hg, Pb) and essential or beneficial elements (B, Co, Cu, Mn, Se) concentrations in tawny owl feathers (µg.g-1).



(grey points: Average concentrations (µyear) are plotted as dots where point size corresponds to the weights of the model (log(nyear)) with standard error bars (±SE). Model parameters: adjusted r² (r2), deviance explained (D) and p-value (p). The splines are not displayed when the trend was not statistically significant.

The lengthening of the temporal monitoring allowed to identify a decrease of exposure to Al that was not detected until 2005. In the first investigated time series lasting until 2005, Al concentrations showed a U-shape with a decline around 1995, reaching 30–40%, but concentrations in the first and last years were almost identical (less than 1% change) (Bustnes et al., 2013). In the present work, the decrease was linear, reaching 48% change, and the highest concentrations measured around 1990 appeared as outliers. Aluminium is rarely considered in studies and monitoring of atmospheric emissions and deposition, rendering difficult the interpretation of long-term trends of exposure in predators. For this element, contrary to Pb for instance, total natural flows (i.e., mass movements in the natural system: crustal production and subduction, volcanic emissions, sediment erosion and denudation, and ocean deposition) have been calculated to largely exceed the anthropogenic flows related to natural biomass combustion, mining extraction, metal production, and metal consumption (Rauch and Pacyna, 2009). Natural emissions may thus be responsible for punctual increases within the global decline. However, other environmental processes such as storms (inputs through sea salt deposition on land) and precipitation or snow melt (modification in soils pH which can affect environmental availability of MEs) might also cause intermittent releases of Al (Bustnes et al., 2013).

The concentrations of Hg did not exhibit any significant trend but oscillating values showing peaks in some years during all the decades, and with the lowest concentrations in 2001 and the highest values in 2010 (Table 1, Fig. 3). The concentrations over the last decade were of the same order of magnitude or slightly higher than in the 1980s, showing no decline and even a trend for increase (Table 1). Similarly, the previous study in owl feathers did not show significant temporal variations in Hg concentrations between 1986 and 2005 (Bustnes et al., 2013). Regarding temporal trends of Hg in Europe, surveys in Sweden showed a decrease of 70% of gaseous elemental mercury between 1980 and the 2000s whereas concentrations in precipitation did not exhibit any significant trend (Pacyna et al., 2009). Other studies highlighted decreasing trends of Hg deposition which was quantified at 10% and 30% over the periods 1995–1998 and 1999–2002, respectively (cited in Pacyna et al., 2009). Pacyna et al. (2009) mentioned that both the emissions in Europe and the concentrations of Hg in air and precipitation dropped by a factor three over the period 1980-2000s. However, during the most recent period of the surveys from 2003 to 2005, an increase in Hg concentrations is highlighted especially in precipitation. The authors suggest that the impact of the European emissions was stronger than the global background during the first part of the time series, which translated in a decrease of environmental contamination when the emissions were reduced in Europe. However, over the most recent decades the emissions outside Europe may be contributing the most and may explain the reversal of the downward trend observed in air and precipitation concentrations. Such changes in sources, global cycle and deposition of Hg over time may explain the lack of clear trend in our owl dataset. Interestingly, focusing on the owl data from 1991 to 2001, a downward trend could be seen

graphically, which matched the trend in European emissions and air or precipitation concentrations measured in Sweden. Accordingly, in a Swedish population of white-tailed eagles (*Haliaeetus albicilla*), a decline of 70% between 1967 and 2011 was found for Hg in feathers (Sun et al., 2019). In eagles from Norway, Hg in feathers of white-tailed eagles showed a significant humpbacked exposure trend with a 60% increase between 1866 and 1957 and then a 40% decline until 2015 (Sun et al., 2019). Such clear declining patterns of Hg concentrations in eagles over the most recent decades were not observed in owls. Moreover, Sun et al. (2019) showed a relationship between the isotopic signatures of carbon and nitrogen and the decreasing trend of Hg in the Norwegian population, which may indicate that diet could modulate exposure to Hg in this top-predator. Conversely, no significant temporal trend was detected in the West Greenland population of white-tailed eagles between 1970 and 2013, showing overall oscillations and large variations within the population (Sun et al., 2019). Similarly, measurements of Hg concentrations in feathers of tawny owls in Spain over the period 1997 to 2014 did not reveal any temporal trends but a high intraspecific variability and maximum concentrations in 2001 and between 2011 and 2012 (Varela et al., 2016). The latter results suggested a fluctuating exposure around a steady average with a tendency to increase during the survey's last decade. This matches our results on owls and seems in accordance with the role of global background and emissions in Hg environmental concentrations in some geographical areas, as mentioned in Pacyna et al. (2019). In accordance with our predictions, the essential elements Co and Cu showed oscillations (Table 1, Fig. 3). The present results suggested that quite steady exposure levels around background values were reached for the essentials Co and Cu, the variations between the 1980s and 2016 being -15% for Co and -7.8% for Cu. A significant weak Co decline of 30% in owl feathers has been shown for the time sequence until 2005, which was in line with the reduction of atmospheric deposition in Norway. Indeed, in this monitoring of atmospheric deposition for three decades until 2005, a decreasing trend of moss concentration was shown overall for both Co and Cu with relatively low changes in the south of Norway (35% for Co and 50% for Cu) (Steinnes et al., 2011). A continued increase of deposition close to the Norwegian border was likely due to emissions of Cu–Ni smelters in Russia, but such an influence was limited to areas within 150–200 km from the sources (Steinnes et al., 2011). These two elements were more affected by local sources than As, Cd and Pb, which mostly originated from transboundary pollution . Thus, the input levels were likely low during the last decades to be biologically regulated by vertebrates.

The beneficial elements B, Mn and Se showed fluctuations yet overall declines, with varying percentages of -86% for B, -34% for Mn, and -12% for Se. Boron decreased significantly but also showed a fluctuating pattern (edf = 2.9, R^2-adj=0.379, *p-value* < 0.01). It showed declining phases between 1986 ([B] >0.5 $\mu g.g^{-1}$) and the late 1990s ([B] < 0.25 $\mu g.g^{-1}$). The latter was followed by a period of increase from 2005 to 2010, and a subsequent reduction during the last period (Fig. 3). These results

provided new insights with regards to the interpretation of B temporal trends, which were characterized by a decrease in the previous study until 2005 but with a deviating pattern showing high values in the late 1999s (Bustnes et al., 2013). Since several phases of fluctuations were exhibited over a longer time sequence, it might suggest that the highest B concentration measured in 1986 was an outlier. The main sources of B are marine salts, volcanic activity and industrial pollution (Steinnes, 2009). The first phase of strong decline might be related to the decrease of industrial atmospheric emissions in the 1980s-90s, while further oscillations may be due to natural flows.

Manganese levels appeared constant around 0.2 $\mu g.g^{-1}$ from 1986 to 2000 and then decreased to 0.1 $\mu g.g^{-1}$ within the last 10 years (Fig. 3), thus showing a weak decreasing non-linear stepping pattern (edf = 1.8, R^2-adj=0.379, *p-value* = 0.001). However, it is worth notifying that outlying concentrations of Mn in feather were identified around the general trends in 2002 and 2004-05 (Fig. 3). Variations of Se showed a sinusoidal shape (edf = 2.8, R^2-adj=0.525, *p-value* < 0.001) (Fig. 3). The concentrations of Se presented a slight increase to approximately 1.3 $\mu g.g^{-1}$ between 1986 and 1996. A decreasing period occurred until 2010 when the concentration averaged around 1 $\mu g.g^{-1}$. A final tendency to slightly increase over the last years was observed. The temporal trends for these two essential elements were insignificant within the time series 1986-2005, whereas the present longer trends shed light on lowered concentrations during the last decade. Since Mn and Se are micronutrients involved in biochemical processes in animals, occurring in metalloenzymes and participating to anti-oxidative system for instance (Steinnes, 2009), lower loads in owls might be related to reduced needs because of the temporal decrease in exposure to toxic MEs. However, since deficiencies can have severe consequences on health, the continuation of a decline in concentrations toward levels close to zero should be regarded as alarming, rendering necessary further survey of these elements in wildlife in the future.

3.3 Spatial variability in temporal trends

Differences in effective degrees of freedom (indicating differences in the shape of the temporal trend between the sets of distances “close” and “far”) and significant coefficients (indicating differences in concentrations between the sets of distances “close” and “far”) were found for both distance to the coast and distance to polluted sites for most elements (Table 2). Model comparisons showed signicant differences between the influence of the distance to the coast *versus* the distance to polluted sites in the case of As, B, Cd, Cu, Hg, Mn and Se (Table 2).

The location toward polluted sites better explained the variations of As, B, Cd and Se than the location towards the coast (Table 2). Conversely, the geographical situation in relation to the coast better explained the variations of Cu, Hg, and Mn.

Table 2. Summary of general additive models (GAMs) relating concentrations of metallic and metalloid elements (µg.g-1) in tawny owl feathers over time (year used as predictor) according to the distance to polluted sites (class 1: 104-2114 m, class 2: 2114-16 596 m) or to the distance to the coast (class 1: 20-4046 m, class 2: 4046-41 201 m).

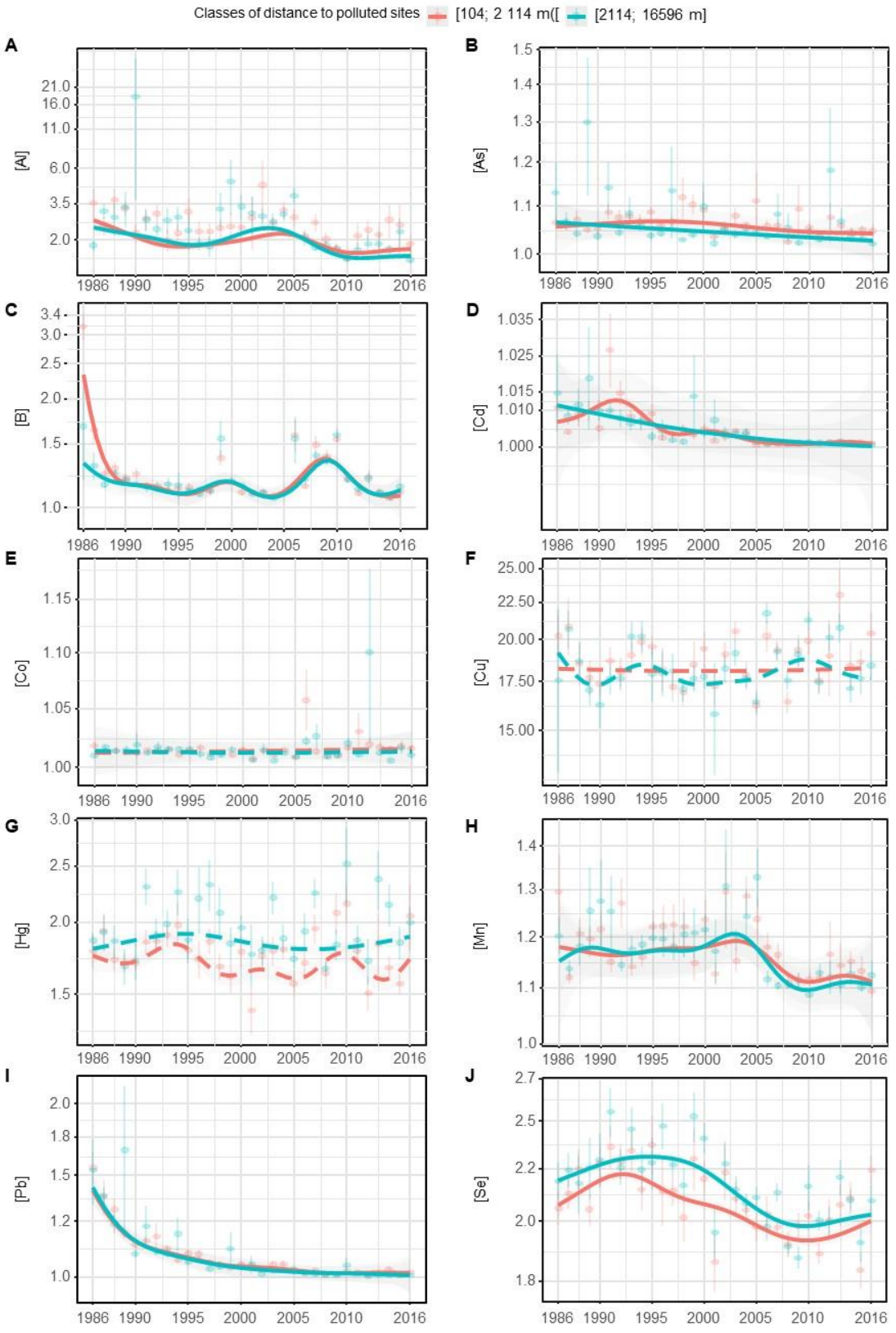
Trace elements	*n*	Models with distance to polluted sites					Models with distance to the coast					Comparison between models
		R^2-adj	Parametric coefficients		Smooth terms		R^2-adj	Parametric coefficients		Smooth terms		
			intercept	β class 2	edf class 1	edf class 2		intercept	β class 2	edf class 1	edf class 2	Pr(>Chisq)
Al	1015	0.076	0.017	-0.013 ^{ns}	**5.7 *****	**5.7 *****	0.077	0.012	0.003 ^{ns}	**5.9 *****	**6.8 *****	0.128
As	1015	0.047	-1.86	**-0.078 *****	**2.9 ****	**1.0 *****	0.031	-1.90	0.000 ^{ns}	**1.0 *****	**1.0 ****	**< 0.001**
B	946	0.343	-1.29	-0.063 ^{ns}	**8.7 *****	**8.6 *****	0.328	-1.31	-0.005 ^{ns}	**8.8 *****	**8.5 *****	**< 0.001**
Cd	1014	0.163	-2.26	-0.003 ^{ns}	**7.6 *****	**2.1 *****	0.157	-2.27	0.007 ^{ns}	**1.0 *****	**5.6 *****	**0.021**
Co	1015	0.001	-2.17	-0.011 ^{ns}	1.1 ^{ns}	1.5 ^{ns}	0.006	-2.18	0.009 ^{ns}	5.4 ^{ns}	1.0 ^{ns}	0.066
Cu	1015	0.005	2.85	-0.011 ^{ns}	1.2 ^{ns}	7.0 ^{ns}	0.021	2.86	**-0.027 ***	1.0 ^{ns}	**8.4 ****	**< 0.001**
Hg	1015	0.043	-0.242	**0.177 *****	8.1 ‘	3.2 ^{ns}	0.049	-0.056	**-0.200 *****	1.0 ^{ns}	**6.4 ***	**0.038**
Mn	1015	0.097	-1.37	-0.021 ^{ns}	**6.6 *****	**7.0 *****	0.110	-1.40	0.036 ^{ns}	**6.2 *****	**8.0 *****	**< 0.001**
Pb	1015	0.584	-1.88	-0.035 ^{ns}	**6.8 *****	**6.5 *****	0.587	-1.91	0.005 ^{ns}	**3.8 *****	**7.6 *****	0.546
Se	1015	0.121	0.127	**0.075 *****	**4.9 *****	**4.1 *****	0.117	0.199	**-0.068*****	**5.4 *****	**3.7 *****	**0.046**

*Abbreviations: R^2-adj = adjusted R^2 of the model; β = estimate deviation from the intercept for the class 2 distance, edf = effective degree of freedom; n = number of individuals used for the model. Statistical significance: ns for p > 0.06, ‘ for 0.06 > p > 0.05 * for p < 0.05, ** for p < 0.01, *** for p < 0.001. Significant values are highlighted in bold.*

Negative coefficients for the distance class further from polluted sites were found for all elements (i.e. Al, As, B, Cd, Co, Cu, Mn, and Pb) except Hg and Se, indicating a trend for a decrease in contamination of owl feathers with increasing distance to polluted sites (Table 2, Fig. 4). However, this negative association was significant only in the case of As, underlying an important role of polluted sites as a local source of As contamination (Table 2). The majority of the elements, except Hg, Se and Cu, did not show any significant coefficient in the models testing the distance to the coast, showing a slight or even no influence of the distance to the shore in owl exposure (Table 2, Fig. 5). Together with the previous insights from raw correlations, the results showed that accumulation of ME in owls was overall higher in the vicinity of sites recorded as polluted for As, Cd, Co, Mn and Pb. The levels of Hg and Se in feathers were significantly higher in birds nesting close from the coasts, and (Table 2, Fig. 4 & 5). The opposite was found far away from the polluted sites (Table 2, Fig. 4 & 5). Interestingly, the concentration of Hg and Hg/Se molar ratio in Bonelli's eagles' feathers was positively associated with more considerable distances to active mines in southern Portugal (Badry et al., 2019). In the present study, mapping sites recorded as “polluted” include various types of contamination sources such as industry and mining. Most areas considered “polluted” in our work may not participate in Hg emissions, such as mines.

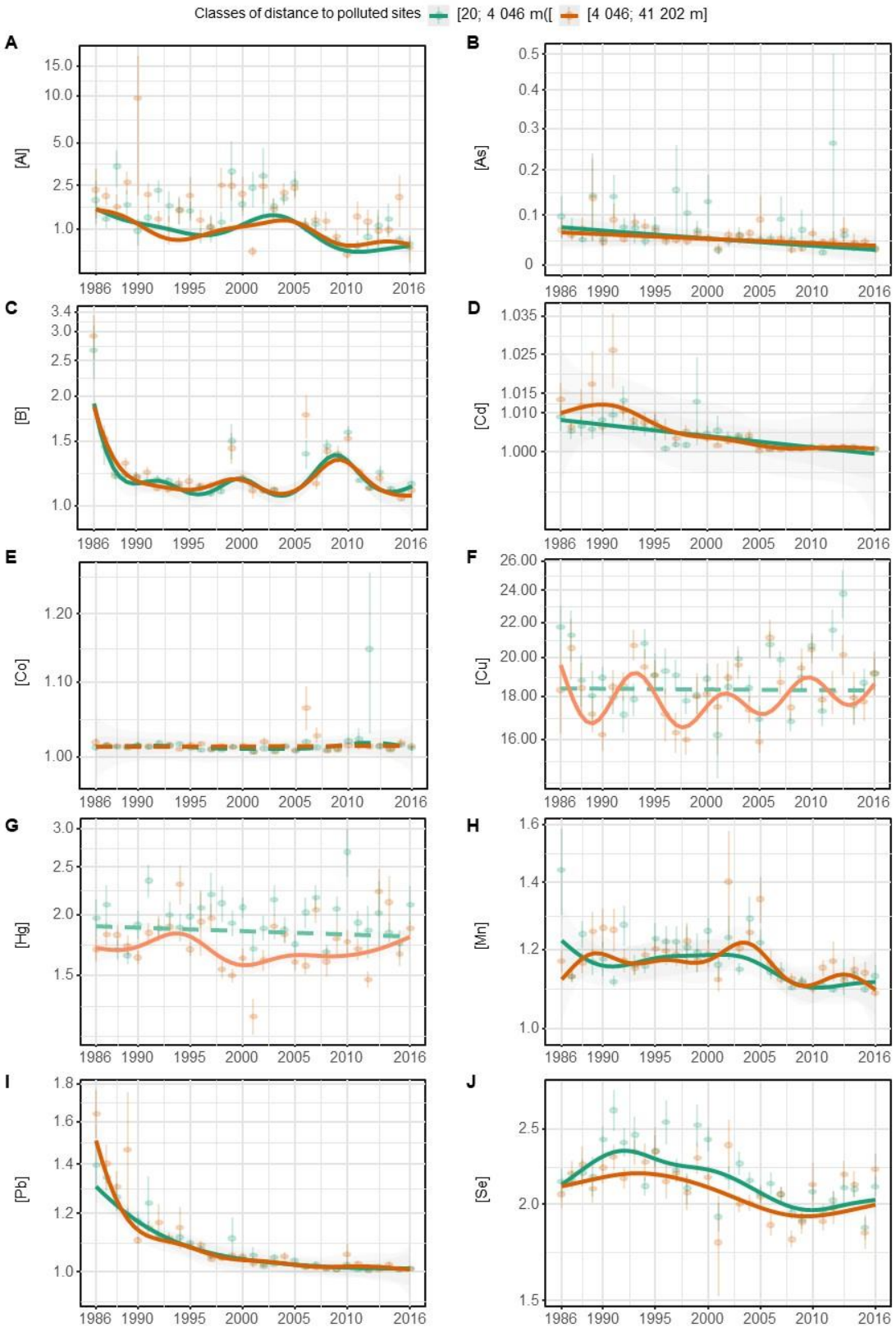
No specific time-dependent association to geographical features were detected in the case of Al and Co, while temporal trends were found to vary depending on the distance to polluted sites or to the coast for the other elements (Table 2). Interestingly, the results showed a faster, sharper, or greater decrease over time of the concentrations of As, B, and Cd in the areas further from polluted sites (Table 2, Fig. 4). This pattern suggests that local environmental contamination was of primary importance in owl exposure to these MEs especially before the decrease of emissions that prominently occurred in Europe from 1980s to 2000s. Such a pattern of prominent influence of local hot-spots of contamination has been also shown in the literature for predatory and passerine birds (Erry et al., 1999; Fritsch et al., 2012; Janssens et al., 2001), even though exposure of owls due to long-distance transport likely have co-occurred and may have represented a significant contribution in some periods. For instance, Cd concentrations in birds nesting close to the coast stabilized and decreased earlier than in birds nesting inland (Fig. 5).

Figure 4. Temporal trends (1986-2016) in the concentrations of toxic elements (Al, As, Cd, Hg, Pb) and essential or beneficial elements (B, Co, Cu, Mn, Se) in tawny owl feathers (in µg.g-1 - average ± standard error) by class of distance to polluted sites.



Straight lines correspond to statistically significant trends and dotted lines correspond to non-significant trends (Table 2)

Figure 5. Temporal trends (1986-2016) in the concentrations of toxic elements (Al, As, Cd, Hg, Pb) and essential or beneficial elements (B, Co, Cu, Mn, Se) in tawny owl feathers (in µg.g-1 - average ± standard error) by class of distance to the coast.



Straight lines correspond to statistically significant trends and dotted lines correspond to non-significant trends (Table 2).

The concentrations of Mn in feathers decreased since the beginning of the survey in areas closer to the coast, while were stable or even slightly increasing for birds nesting inland until 2005 (Fig. 5).The temporal trends of Cu in birds nesting closer to the coast exhibited a steady but a non-significant temporal trend compared to the fluctuating sinusoidal pattern in birds nesting far from the coast (Table 2, Fig. 5). These results suggest that accumulation of Cu was biologically regulated by owls, without signs of intoxication or deficiency.

The decrease of Pb concentrations was sharper in birds nesting further from the coast during the late 1980s than in birds nesting closer to the coast (Fig. 5). The shape of the decrease rapidly overlapped regardless of the distance to the shore. In contrast, the trends coincided independently of the distance from the polluted sites (Fig. 4). This pattern was surprising since a slighter decrease in sites of the shorter distance to polluted sites was expected, which was observed for As and Cd. Considering emissions, Pacyna et al (2009) highlighted different spatial distribution patterns between Cd and Pb at the European scale, Cd being mainly emitted from point sources and showing a “hot spot” distribution map while Pb being mostly emitted from area sources (e.g. traffic) and exhibiting a more homogeneous spatial distribution. Moreover, the monitoring of atmospheric deposition in Norway showed that the contribution of trans-boundary contamination from other European countries decreased significantly between the 1970-1980s and the 2000s (Steinnes et al., 2011). To gain more insights about the origin of Pb would be helpful to identify the relative contribution of the various sources of inputs, as done for instance in a study using Pb isotopic signature in blood of griffon vulture *Gyps fulvus* which showed the influence of both environmental lead of geological sources and point sources such as lead-based ammunition in exposure (Mateo-Tomás et al., 2016).

The temporal trends of Hg concentrations in owl feathers differed according to the distance to the coast, showing an absence of variation in owls nesting close to the coast. In contrast, the concentrations in owls breeding further from the coast exhibited a significant downward trend over the late 1990s and early 2000s, followed by an increase (Table 2, Fig. 5). Altogether, these results suggested a prominent role of the spatial situation in relation to the shore on exposure of the owls to Hg, with a higher level of exposure in coastal areas throughout the time over the 30-years. In their study dealing with exposure of subarctic top predator birds to persistent organic pollutants (POPs), Eulaers et al. (2013) showed that levels of POPs decreased following a spatial pattern coast > fjord > inland. They underlined that exposure depended on regional nest location, reflecting historical POP environmental contamination (Eulaers et al., 2013)**.** Similarly, our results showed that exposure to Hg was dependent on spatial location with a decrease of exposure from coast to inland which may reflect the sources of Hg inputs. However, this may be caused by current long-range transport of Hg instead of legacy local contamination contrary to the study of POPs (Pacyna et al., 2009; United Nations Environment Programme, 2013). Studying elemental concentrations of feathers in the Bonelli's eagle

(*Aquila fasciata*) in southern Portugal, Badry et al. (2019) showed a decrease of Hg with increasing distance to a coal-fired power plant known as a major regional source of Hg contamination. As mentioned above, they also showed a decrease in Hg with shorter distances to active mines. We support this by detecting decreasing Hg in owls breeding closer to polluted sites (Table 2, Fig. 3), which might be considered as a confirmation of prominent regional or global sources of Hg contamination rather than local sources. Additional information about the inorganic or organic (methylmercury) forms of Hg accumulated in owl tissues may be helpful in gaining further insights about the sources and pathways of exposure of the birds and investigating the role of food web transfer and biomagnification in the temporal and spatial trends of owl exposure.

The concentrations of Se varied with geographical features. Still, the GAM suggest that distance to the polluted sites was the most important predictor. The temporal changes of Se concentrations in owl feathers showed similar patterns when comparing the distances to polluted sites or the coast (Tables 2, Fig. 4 & 5). Parallel sinusoidal patterns were observed for Se for each class of distance, demonstrating an influence of the nest box's location on Se levels but not on their temporal variations (Fig. 4 & 5). Based on evidence from the literature, for instance, through monitoring Se in moss in Norway, a marine origin of this ME was expected (Steinnes et al., 2011). Our results are partly in accordance with this since higher concentrations closer to the coast were found. However, statistical parameters suggested strongest associations to the distance to polluted sites. Such complex results suggest time-dependent associations between Se concentrations and geographical features, the relative contribution of the proximity to polluted sites or the coast varying over the time sequence. The element Se, like some other essentials such as Zn, can be toxic at high doses and accumulation in wildlife can be related to environmental contamination (Burger et al., 2015). Besides, Se is involved in the detoxification mechanisms of other trace elements, especially Hg and Pb (Fu et al., 2019; Khan and Wang, 2009; Yang et al., 2008). The variations in Se concentrations may be attributed to Se environmental contamination and the large panel of selenium-mediated (i.e. HgSe complex with the selonoprotein P, glutathione peroxidase activity) detoxification mechanisms leading to accumulation of this element. In Bonelli's eagles, higher Se concentrations close to a power plant were also associated with an increase of both Hg and the Hg/Se molar ratio at shorter distance to the power plant (Badry et al., 2019). The authors hypothesized that such an increase of Se concentrations in feathers may be due to enhanced binding of Se to Hg. In our study, Se concentrations were found to be correlated to both Hg and Pb concentrations, and to Cd concentrations to a lesser extent (Fig. 2). Such associations between Se concentrations and Hg or Pb tissue burdens in birds have sometimes been found in the literature, but not systematically (Burger et al., 2015; Janssens et al., 2001). The various mixtures of trace elements in polluted sites and temporal variations in ME inputs hamper disentangling the causal role of Se, Hg or Pb environmental contamination in the exposure of wildlife

in Se trends. One might hypothesize that Se variations were driven partly by Se environmental contamination itself, partly by Hg contamination, and partly by Pb contamination with varying relative contribution over the time sequence.

Studying intra- and interspecific variation in exposure of nestlings of the northern goshawk (*Accipiter gentilis*), the white-tailed eagle (*Haliaeetus albicilla*) and the golden eagle (*Aquila chrysaetos*) to POPs, Eulaers et al. (2013) showed that spatial factors were important drivers of exposure but ecological factors also played a crucial role in shaping exposure patterns. Across-species differences in exposure was determined by both trophic level and dietary carbon sources that featured biomagnification and food chain discrimination between terrestrial and marine food chains (Eulaers et al., 2013). Trophic ecology may also be an important feature in determining the levels of exposure of the tawny owls and their temporal trends. Diet shifts and changes in food web structure involving preys of marine, freshwater and terrestrial origin have been showed to be responsible for temporal and spatial trends in exposure to contaminants such as Hg and POPs in gulls and dippers, for instance (Hebert et al., 2009, 2000; Morrissey et al., 2010a, 2010b). Moreover, the influence of geographical features may not be due only to their role as contamination proxies, but also their relevance as proxies of the owls' feeding behaviour. The diet of the Tawny owl is composed of small mammals, notably voles, birds and amphibians (Petty, 1999; Sunde et al., 2001), and different preys can differ in their abilities to accumulate contaminants and their potential to participate in food web biomagnification (Baudrot et al., 2018). The relative contribution of the various preys in the diet of owls is likely to depend on the prey item availability, which differs according to landscape features and over time (Capizzi, 2000; Petty, 1999; Sunde et al., 2001). Availability of voles has been found to influence the accumulation and its temporal trends of POPs in tawny owls, and to be related to clutch size in the population studied here (Bustnes et al., 2011; Yoccoz et al., 2009). Such issues of diet shifts and vole availability deserve further attention and should be addressed in future studies to gain better insights into understanding spatio-temporal trends in wildlife exposure. Further investigation are required to explore the toxicological meaning of co-exposure of owls to several toxic metals and metalloids and its temporal trends.

4. Conclusion

Showing declining trends of exposure of a predatory bird to several toxic metals over three decades, but a lack of decrease of Hg accumulation, this study underlines the critical insights provided by long-term biomonitoring to survey the efficiency of regulation as well as to detect rare events, unexpected variations, and potential threats to ecosystems. . Using “landscape indicators” to investigate how spatial exposure patterns may be shaped by local or global inputs of metals and metalloids, the study showed that the concentrations of MEs in predatory bird feathers were related to geographical proxies of potential legacy and current sources of ME. Especially, toxic metal(loid)s whose environmental

release originated mainly from industrial activities such as As, Cd, and Pb were found at higher concentrations in owls nesting nearby polluted sites, while Hg was found at higher concentrations in owls nesting in coastal areas. Interestingly, the landscape patterns also influenced the temporal trends of exposure to metals, exhibiting a greater temporal decrease of Al, As, and Cd in the areas further away from polluted sites, while the temporal trends of Hg differed according to the distance to the coast. The accumulation of essential and beneficial elements was also dependent on time and spatial features in some cases, overall in a lesser extent than toxic elements. Their variations might be related to both anthropogenic emissions and their biological roles, especially towards the antioxidant and detoxification systems involved in fighting against toxic metal-induced stress. These results raise issues about the relative importance of emission from local to global sources in wildlife exposure, and how such relative contribution shifted over time during the last decades, especially for Hg. Such insights are crucial to inform regulation and provide basis for mitigation levers. Exposure being dependent on complex interplays between environmental and ecological factors, further studies are needed to investigate the contribution of trophic and behavioural ecology of owls to both temporal and spatial patterns of exposure to toxicants.

CRediT authorship contribution statement

Quentin Devalloir: Data curation, statistical analyses, writing
Clementine Fritsch: Conceptualization, supervision, writing, review & editing
Bård-Jørgen Bårdsen: Conceptualization, review & editing.
Georg Bangjord: Methodology, data collection
Sophie Bourgeon: Conceptualization, review & editing
Igor Eulaers: Conceptualization, review & editing
Jan Ove Bustnes: Funding acquisition, project administration, conceptualization, review & editing

Acknowledgements

The project has been funded by the Norwegian Research Council Project EnviStress (No. 268482) and by the FRAM Centre Flagship Program “Hazardous substances – effects on ecosystems and human health” (project: Impacts of environmental contaminants and natural stressors on northern raptors: RAPTOR). We thank Syverin Lierhagen for carrying out the element analyses and Venke Ivarrud for help with preparation of the samples. We thank the anonymous reviewers who provided comments that greatly helped in improving the manuscript.

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Appendix:

Table A1. Detailed quality control for trace metals and metalloids measurements in tawny owl feathers.

Trace elements	1986-2005*						2005-2016*					
	Detection limit		Blank	CRM : Tea, GWD fra China		Samples RSD	Detection limit		Blank	CRM : Polish Virginia Tobacco Leaves (INCT-PVTL-6)		Samples RSD
	µg/l	µg/g	µg/l	CRM recovery (%)	95% confidence uncertainty certified values (%)	%	µg/l	µg/g	µg/l	CRM recovery (%)	95% confidence uncertainty certified values (%)	%
Al	3.000	4.14	0.69780	75.3 ± 9.2	not provided	5.14	0.200	0.0480	0.42999	99.0 ± 2.6	19.4	2.32
As	0.014	0.019	0.00056	98.5 ± 14.7	10.7	15.83	0.025	0.0060	0.00078	61.4 ± 10.0	7.2	37.93
B	0.230	0.317	0.06346	104.1 ± 12.7	20.0	10.48	0.050	0.0120	0.00000	77.1 ± 8.8	5.7	2.89
Cd	0.009	0.012	0.00036	127.7 ± 14.2	14.0	13.29	0.002	0.0005	0.00008	76.9 ± 4.1	5.4	34.14
Co	0.004	0.006	0.00088	107.9 ± 12.8	11.1	13.82	0.004	0.0010	0.00022	65.9 ± 4.1	4.5	13.80
Cu	0.030	0.041	0.13757	107.4 ± 14.4	5.8	2.96	0.020	0.0048	0.00366	62.8 ± 4.2	3.9	2.14
Hg	0.010	0.014	0.00072	73.3 ± 12.3	not provided	3.84	0.001	0.0002	0.00000	90.5 ± 4.0	6.9	3.53
Mn	0.013	0.018	0.01369	107.2 ± 15.2	3.2	5.79	0.006	0.0014	0.00024	74.4 ± 7.3	3.7	4.23
Pb	0.004	0.006	0.02304	94.6 ± 10.6	4.5	2.38	0.002	0.0005	0.00053	58.1 ± 5.6	15.1	6.00
Se	0.040	0.055	0.02693	95.3 ± 38.8	not provided	12.67	0.050	0.0120	0.00656	No certified value		5.48

**General information: HR-ICP-MS, certified multi element calibration solutions, matrix matched concerning ion strength and acid, verified against certified reference material in solution (SPS-SW2). Measurement accuracy and uncertainty verified against certified reference material (CRM). Contamination verified with at least 3 blanks. Repeatability assessed through RSD computed from values of repeated measurements*

Table A2. Average (mean ± sd), median (Med[1st Quartile-3rd Quartile]) and effective size of the data about ME concentrations (µg.g-1 dry weighted) of toxic (Al, As, Cd, Pb and Hg) and beneficial or essential (B, Cu, Co, Mn and Se) elements in tawny owl tail feathers from 1986 to 2016 and the euclidian distance of the nest box where feathers were collected to the nearest polluted sites ("DistPollut") or to the coast ("DistCoast") in meters.

	Mean ± sd	**Median [Q1-Q3]**	***n***
Al **(µg.g^{-1})**	1.71 ± 5.118	0.86 [0.44 – 1.8]	1015
As **(µg.g^{-1})**	0.066 ± 0.151	0.04 [0.02 -0.061]	1015
B **(µg.g^{-1})**	0.24 ± 0.348	0.15 [0.081 – 0.27]	946
Cd **(µg.g^{-1})**	0.0044 ± 0.012	0.001 [0.0005 – 0.004]	1014
Co **(µg.g^{-1})**	0.016 ± 0.046	0.011 [0.006 – 0.017]	1015
Cu **(µg.g^{-1})**	17.4 ± 3.708	17.2 [14.8 – 19.8]	1015
Hg **(µg.g^{-1})**	0.88 ± 0.588	0.73 [0.53 -1.02]	1015
Mn **(µg.g^{-1})**	0.18 ± 0.194	0.12 [0.09 - 0.2]	1015
Pb **(µg.g^{-1})**	0.77 ± 0.214	0.024 [0.007 – 0.08]	1015
Se **(µg.g^{-1})**	1.13 ± 0.381	1.07 [0.87 – 1.3]	1015
Dist. Pollut (m)	2946 ± 3223	2114 [1099 - 3567]	201*
Dist. Coast (m)	6892 ± 7678	4046 [1093 - 9094]	201*

** number of nest boxes*

Table A3. Distribution of the nest boxes (number of data) depending on their classes of distance to polluted sites and distance to the coast and results of the statistics.

Distance classes	**Close to polluted sites [104, 2 114 m[**	**Far from polluted sites [2 114, 16 596 m]**
Close to the coast [20, 4 046 m[	**241**	**287**
Far from the coast [4 046, 41 202 m]	**288**	**235**
Result of the Chi ² test of Homogeneity: Pearson's Chi-squared test X-squared = 9.3323, df = 1, p-value = 0.002		

Figure A1. Relation between the minimum distance to polluted sites and the minimum distance to the coast in the full dataset (ρ: Spearman's coefficient of correlation)