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Occurrence of Rare Earth Elements (REEs) and trace elements in feathers of fledglings of *Ichthyaetus melanocephalus* (Mediterranean gull) and *Chroicocephalus ridibundus* (Black-headed gull) from the Venice Lagoon (Italy)

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To my supervisors

ABSTRACT

Rare earth Elements (REEs) are emerging global pollutants due to their distinctive physical and chemical features that render them vital in a multitude of manufacturing apps. However, few researchers have been able to draw on any systematic study into the presence of REEs in biota, such as birds. So far, no previous study has investigated the total concentration of REEs in gulls using feathers as a non-invasive biomonitoring tool. Feathers are indeed very important tools to monitor exposure to trace elements and REEs, as they are excellent indicators of the concentration of pollutants in the body taken up through diet.

The concentrations of 16 REEs and 5 trace elements (Hg, Pb, Cd, Rb and Se) were analysed in the feathers of the Mediterranean gull (MG) (*Ichthyaetus melanocephalus*) and Black-headed gull (BHG) (*Chroicocephalus ridibundus*). After an acidic digestion with a microwave oven, feathers were analysed with an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) and then the concentrations of the aforementioned elements were evaluated.

The concentrations of REEs and trace elements in MG were higher than those observed in BHG. All the 16 REEs were detected in each feather sample with a strong correlation to each element; only Ce showed concentration below the Limit of Quantification (LoQ). The concentrations of Light rare earth elements (LREEs) were higher than those of heavy rare earth elements (HREEs). In the case of the trace elements, extreme concentrations of Cd and Rb were found in the bird species studied, indicating a greater exposure; while the total concentration of Hg, Pb and Se were found below the LoQ in many of the feathers analysed.

Summary

AIM OF THE STUDY

Increased REE emissions have caused major environmental pollution but there is limited knowledge of their bioavailability, fate and effects on natural ecosystems (MacMillan et al. 2017). Birds of the biosphere are especially susceptible to element pollution, as exposure to non-essential and toxic metals both externally (inhalation or dermal contact) and internally, ingestion of contaminated food (Burger and Gochfeld 2002). In addition, birds are well known as reliable biomonitors of the aquatic environment because they, 1) are relatively easy to study, 2) occupy often high trophic levels in the aquatic food webs, 3) reveal the current environmental exposure, 4) respond relatively rapidly to contamination events, and 5) act as biological vector of contaminants (Burger and Gochfeld 2004; Zhang and Ma 2011; Vizuete et al. 2018).

There is the need to fill the gap in the knowledge of the effects of REEs on birds; most of the available studies dealt primarily with effects caused on poultry, broilers and laying hens by REEs used as feed additives (Abdelnour et al., 2019 and citations herein). As concern bioaccumulation, literature on REEs concentrations in wild bird tissues is scant, and data are available only for a restricted number of species, including common eider (*Somateria mollissima*), ring-billed gull (*Larus delawarensis*), and willow ptarmigan (*Lagopus lagopus*) (MacMillan et al. 2017; Brown et al. 2019).

The studies of MacMillan et al. (2017) and Brown at el. (2019) also recognized the liver as the preferred organ for studying REEs accumulation in vertebrates, including birds. Nevertheless, a recent study performed on Humboldt penguins (*Sphenicus humboldti*) kept in captivity evidenced that feathers can be used as reliable indicator of REEs exposure and accumulation in birds (Squadrone et al. 2019a). The body burden in feathers may be used as a reliable proxy of bioaccumulation; since the blood stream flows through small blood vessels of the feathers only during their development, nutrients, oligo-elements, trace elements and REEs, which were uptaken with food and were bioavailable, reach the feather and accumulate there.

The Lagoon of Venice is one of the major breeding areas in Italy for marine birds (Scarton 2017). Although the level of contamination in the area has been defined to be low to moderate (Picone et al. 2016), recent studies on breeding birds reported that exposure to trace elements (e.g. Hg, Pb, Cd) in the Lagoon is consistent with insurgence of detrimental effects on species conservation (Picone et al. 2019). Thus, to assess whether breeding birds are critically exposed also to REEs, a study was performed using contour feathers collected from fledglings of two species of gulls (*Ichthyaetus melanocephalus* and *Chroicocephalus ridibundus*) nesting in salt marshes of the northern basin of the lagoon. According to Vizuete et al., (2018), gulls were chosen as biomonitors for REEs and trace elements because: 1) they are top predators in the aquatic food web, especially as concern the diet of chicks and fledglings; 2) spend a considerable part of their lives along coastal areas; 3) they have a wide ecological adaptability; 4) they form abundant and easily accessible colonies, allowing for the synoptic sampling of several individuals; 5) developing chicks are fed by parents with prey caught from within a few kilometers of the breeding colony.

This study conducts a field-based analysis of the ecotoxicological impact of REEs and some trace elements with reference to the bioaccumulation in the feathers of the MG and BHG. In this research, we intend to evaluate the presence and concentration of REEs and some trace elements in the marine birds of Venice. Our data will help study REEs and trace element spatial patterns in environments so that the prospective use of REEs and trace elements as ecological tracers in these fields is checked. The trace element and REE levels are comparable with other results around the world and the prospective exposure of humans and animals to these rising contaminants is assessed.

In the Lagoon of Venice, there are no known sources of REEs, i.e. there are no mining activities, REEs processing plants, no known damping sites; hence concentrations were not expected to be high considering these factors, but so many industrial application activities that may contribute to the diffusion of REEs are present. For instance, Gd can be released by hospital effluents or sewage or the glass factories. We hypothesize that REE will be high in concentration as the ceramic and glass industry has historically been present in the field of research.

INTRODUCTION

The Mediterranean gull

The Mediterranean gull (hereafter MG) *Ichthyaetus melanocephalus* is a small gull of family Laridae, originally restricted to Black Sea and East Mediterranean area. With an estimation of 120,000 – 320,000 breeding pairs in Europe, its conservation status is regarded as secured (Birdlife international, 2004). The first colony outside their native distribution area was located in the south of France. In Italy, probably a couple nested in Sardinia later in 1864; Giglioli also noticed without proof of their nesting, the species occurred in Veneto (Canestrini et al. 1872). The first record of nesting of MG in Italy was in the Comacchio Marshes, at the end of June 1978 (Brichetti 1978). Fasola and Brichetti (1993) confirmed their registration in 1978 with a first evidence of nesting in Italy. In 1982 the Mediterranean gull colonised in Valle Bertuzzi, in the Comacchio area, and their population increased up to 1,000 pairs. In the Lagoon of Venice, the first report of adults in reproductive behaviour was in 1995, at Palude Fondello, although neither eggs nor chicks were observed (Scarton and Bon 2009). They were observed in the same colony with the Sandwich tern. The first proof of chicks in the Lagoon of Venice was on 15 June 1996, in the same area, a very recent one (Scarton and Bon 2009). Population size of the MG in the study area during the winter census in the period 2012-2017 (Basso and Bon, 2017) has been estimated in about 2.700 individuals. About 1500 individuals nesting in the Northern Lagoon in 2019 have been estimated (Basso, M., and Picone, M. personal communication).

Figure 1. Mediterranean gull (*I. melanocephalus*). (From: http://www.gull-research.org/melanos/h0f1red.html)

Diet of the Mediterranean gull

There is no or less information concerning the diet of *Ichthyaetus melanocephalus* in Italy but generally gulls have developed different methods of feeding and mastered various feeding strategies for adaptation as opportunistic omnivores (Milchev et al. 2004). They may be classified as mixotrophic, as they feed on variety of diets as available in their habitat. It is reported that mass of gastropods and numbers of insects are the most significant animal prey during the fledging period in chicks as observed in the Macedonian wetland of Greece (Goutner 1994). Wheat, seeds, bivalves, small stones, rubbish dumps, worms and fishes also form part of their diet, as the dietary composition will probably differ substantially between geographical areas (Goutner, 1994). According to Milchev et al. (2004), seeds of cultivated plants (barley, wheat, and sunflower) and of ragwort constituted the staple diet of Mediterranean Gulls during their post-breeding residence at the Atanasovsko Lake Reserve. Also animal remains were found in 27% of the analysed pellets, with terrestrial animals predominating. Invertebrate preys include ground beetles and grasshoppers. Vertebrates consisted mainly of marine and brackish benthic fishes (Milchev et al., 2004).

On site observations in one of the main colonies located within the Lagoon of Venice revealed that chicks unable to fly were fed by parents with: small mullets, small gobies, squids and cuttlefishes. Moreover, they can feed independently on rag worms, small crabs and bivalves. The size of the fish they can eat is no longer than 10 cm as observed also in the sandwich terns. So, although the data are scarce, they corroborate the hypothesis that MG may switch dietary habits during the season, as already observed in other gulls (Isaksson et al. 2016): adults feed mostly on terrestrial habitats, but when chicks require high-energy and digestible food for growth, they switch to marine fish and invertebrates. Since chicks are fed with fishes and/or molluscs, and they cannot find seeds in the small islands where the MG nests in the lagoon, they have a higher trophic relative to the adults (as cited by Isaksson et al. 2015). Food items recovered from the regurgitations of the chicks are reported in Table 1.

Table 1. Food items recovered from Mediterranean gull chick's regurgitations in Valle Sacchetta (2017)

Scientific name	**Common name**	**Length (cm)**	**Sample size**
Sepia officinalis	Cuttlefish	3 - 5	4
Gobius sp.	Goby	4 - 5	4
Liza sp.	Mullet	3 - 5	2
Nereidae ind.	Ragworm	6	1
Coleoptera ind.	Beetle	1	3

Black-headed gull

To make a comparison, we used another species of a gull, a species resident in the Venice Lagoon that has slightly different dietary habit. Though, they do not create larger colonies like the MG yet, the co-evolution history between the two species MG and BHG indicated the option of using them as comparison in order to obtain strong references and contrasting information.

The Black-headed Gull (*Chroicocephalus ridibundus*) is a large migratory species of water bird nesting in various ecosystems of the coastal Eurasia's basins. The first proof of breeding of the BHG in Italy was in 1965 in Sardinia, 120 pairs were discovered in 1980 and 400 pairs in 1981 (Cramp and Simmons 1983). According to Cramp and Simmons (1983), BHG is 10-15% slender than MG; they have a brilliant white edge at the exterior wing and dusky lining for every other primary external wing. Thus, this species have its wings and back pale mostly grey and the remaining plumage white but with some seasonal variation and gender resemblance. The head of the adult breeding BHG is dark chocolate-brown with a white orbital ring in front of their eye but the non-breeding adult retains a little dark brown colour on the head with a grey-black ear spot. In the summer, the red legs and bills of the BHG gets brighter.

The Juvenile BHG are initially richly coloured with boldly variegated wings of grey, black, brown and white cross patterns; yellow or fleshly dull bill, white tail and yellow-flesh dull legs. Their brown and black marks on wings wear or bleach in their first summer but in the first winter, most juvenile BHG assumes a fully dark appearance (Cramp and Simmons 1983).

The flight actions of the BHG vary with buoyancy, agility and wing-beat in summer as compared to winter (Cramp and Simmons 1983). This species abandon the middle Europe's breeding regions between July and November, during their transmigration across Germany and Austria, they distribute themselves throughout the regions of Italy, Croatia and Greece, the Netherlands and Belgium to the western Atlantic shore of Europe and the Mediterranean shore in Southern Europe. The Mediterranean populations are generally resident and did not show seasonal migration to wintering grounds, even if a dispersal of the 1-st year juvenile is possible. As a consequence, in winter the Central European populations mix with the Mediterranean populations and then return to their nesting areas at the beginning of spring.

Cramp and Simmons (1983) gives an account of BHG to prefer habitats of foreshores, lagoons, ponds, lakes, estuaries and other wetter sites. BHG forages on nearby ploughed lands, grasslands and overwater including seacoasts. BHGs use widespread sandy or sandy beaches and even grasslands, playgrounds, sewage farms, urban parks, waste dumping facilities, roads and even reservoirs when they are out of breeding periods. In this case, they tend to feed on a vast range by artificial light from trees, food thrown by people, on buildings, perches, walks or swims in pursuance of a wide range of habitat exploitation.

Food and dietary techniques of BHG differ significantly between the location, season, accessibility of nutrition and individuals and are able to adapt rapidly to altering conditions. Mostly animal product, especially insects and earthworms, but usually complemented by vegetable and domestic or industrial waste. BHG seizes prey (insects, ragwort) on foot or can create a smooth jump. Likewise, bread is taken out of the hand, floor or water. Other feeds for BHG include: mollusc, crustaceans, fish (in particular those in shallow waters or swim just below the surface, also sick and dead individuals), amphibians and plant materials (fruits, seeds and cereals) (Cramp and Simmons 1983). Invertebrates, tiny vertebrates and waste from public deposits or farms form part of the diet of the BHG. During the breeding period, their diet comprises primarily of insects, crop elements and earthworms and sometimes of vertebrates or cherries; within 10 km of the breeding colony birds obtained food (Čížek et al. 2007).

Typically BHGs can be gregarious all year long and their daily distribution habits vary.

The age of flocks represents the distinct dispersion trends of adults and juveniles who move from their natal places. For instance, with the inland range, the proportion of first year birds rises significantly whiles couples or groups may remain together for migration. They may guard feeding territories or resting locations outside breeding season with several variation in distances amid nests. In all, they have the tendency to shift to a more central site. They defend these sites against intruders.

BHGs can eat at night (nocturnals) and roost (in thousands) whenever the tide flows. Flight to roost starts 2-3 hours prior to dusk and peaks in twilight phase. Diurnal roosting and loafing is also communal and it implies preening and bathing or swimming. BHG is less boisterous with the falling of darkness but not absolutely quiet. The evidence of threat in BHGs is when their head and neck extends a little upward, breast and their belly is puffed out. BHGs incubate within 23-26 days and they have 35 days for fledging.

Some distinguishing features of a MG as compared with BHG include: a lot denser blood red bill; thicker red legs; white ring around its eye; no black tips on ends of its wings; a much whiter and somewhat stocky creature (bird) than BHG. Clearly different are also the calls: MG makes a "catty-calls" like "meeeows" but BHG's make vibrato calls, "kreeo-calls" when screaming, "kek- calls" in flight and the adults make a "kraaahh-krraaahhh-krah-gra" call sometimes. BHG's are used to occupying the marginal areas in small groups of the colony. They are not so gregarious during breeding and nesting. As concern population size in the study area, about 18.000 individuals were estimated in winter (Basso and Bon 2017); some hundreds estimated in northern lagoon over breeding season (Bon et al. 2014).

Figure 2. : Black-headed Gull (*Chroicocephalus ridibundus*)

Figure 3. Black-headed Gull (*Chroicocephalus ridibundus*)

Birds as environmental monitors

The insatiable demand for resources and technological growth leaves a massive increase of anthropological impact on the environment. As a result, the need to monitor the environment has never been this crucial and the idea of using birds is not new. Birds have been essential tools for the monitoring of the environment. For instance, (Furness and Camphuysen 1997) reveals historical remarks which indicate certain elements of bird behaviour, which are now being substituted with sophisticated weather prediction techniques. Furness and Greenwood (1993) also confirms the presence of seabirds used as a significant indication of approaching landfall or fishing banks even in eastern Canada around 1700. Hence the use of biological indices as a precautionary system for environmental damage and as an assessment instrument is the most vital instrument in order to access exposure to contaminants (Abdullah et al. 2015). Many ecological experiments are needed to monitor all forms of pollution, in this case, the detrimental impacts of metals on the environment.

The accumulation of information is a significant component in the interpretation of tracking outcomes both in biological and non-biological monitoring, making this data increasingly valuable with a continuation of the surveillance system (Furness and Greenwood 1993). Since birds can be subjected both internally and externally to trace components by consuming contaminated food (Markowski et al. 2013), they were identified as possible environmental pollution bioindicators and for multiple pollutants, including trace elements, since the 1960s (Borghesi et al. 2017). In addition, the trophic range of certain seabirds such as gulls permits them to feed on anthropogenic food resources that make them especially exposed to pollutant accumulation (Signa et al. 2013). The dietary structure of seabirds is thus useful in supplying the abundance of poisonous components in the sea.

Birds are easy to monitor, classify and identify; the risk of misinterpretation is decreased because birds are well established ecologically and have been studied extensively. They are high in the food chain and their long lifetime ensures that environmental stress effects are incorporated over time (Furness and Greenwood 1993). For this reason, a local species that represents the study area to a feasible extent is normally chosen for this form of surveillance, taking all possible exposure paths into account. Unlike few studies that examined the impacts of heavy metals on passerine birds (Dauwe et al. 2002; Tsipoura et al. 2008; Hofer et al. 2010; Markowski et al. 2013) birds at the top of the food chain such as raptors or birds that are piscivorous were intensively used in various biomonitoring research (Burger 1993; Muralidharan et al. 2004; Hosseini Alhashemi et al. 2011; Signa et al. 2013; Ullah et al. 2014; Abdullah et al. 2015; Kushwaha 2016).

Feather as tissue for analysis

The use of feathers for biomonitoring began long time ago with most of the studies focusing on metallurgy (García-Fernández et al. 2013). The concentration of trace elements can be evaluated in different organs (liver, kidney, brain), tissues (bones, fat, muscles) and eggs; nevertheless, feathers are alternatives to tissue samples for ethical, realistic and conservatory purposes (Dauwe et al. 2000; Markowski et al. 2013; Zheng et al. 2018; Picone et al. 2019).

In birds, feather deposition and egg sequestration are common ways of eliminating food-borne pollutants that reach the bloodstream and are absorbed by the guts and intestines (Signa et al. 2013; Kaur and Khera 2018). Trace elements may be concentrated in feathers more than in other tissues at least for some contaminant for the 3-4 week period of feather development (Borghesi et al. 2017). According to Burger (1993) and Furness and Greenwood (1993), sulphydryl-rich keratin and melanin-pigment affinity of trace elements generally lead them to bind to feather protein during the brief span of feather development, when plume is linked to the bloodstream by means of tiny shrub blood vessels. In this case, feathers act as register of the bloodstream metal concentrations (Hofer et al. 2010). After the feathers are fully matured, they are not linked to the bloodstream and then the concentrations of structural elements and non-structural elements (i.e. hormones, trace elements and REEs) are a reflection of the blood concentrations throughout the period of feather growth (Bortolotti 2010).

Contamination by xenobiotics in feathers can be endogenous or exogenous (Jaspers et al.,2011) (Fig. 2). When the level of xenobiotics in the feathers increases, it reflects the amount of contaminants accrued in the inner organs of a bird since the last moult cycle and the amount contaminants ingested with the food during the moulting process. In case of exogenous contamination, feathers reach a minimum level soon after the moult is completed and then increase when the bird becomes vulnerable to long-term xenobiotic pollution (Jaspers et al. 2011). The exogenous contamination is defined by Borghesi et al., (2017) as external contamination, which is usually ascribed to atmospheric dust, water or deposition of contaminants on feathers (Dmowski 1999; Dauwe et al. 2002; Borghesi et al. 2017). External contamination can be removed by an efficient washing (Picone et al., 2019).

The use of feathers in biomonitoring studies is gradually becoming common and provides many benefits, as well as precious data regarding the quality of the setting by determining the impurities deposited in bird feathers during their lifetime (Rutkowska et al. 2018). Some advantages of feather assessment are: it is harmless and ethical since feathers can be readily obtained even if it requires sampling another time and again to study trace element accumulation, without affecting the individual's well-being or continued existence (Adout et al., 2007; Picone et al., 2019). Table 2 shows other advantages and disadvantages of using feathers for biomonitoring.

Table 2. Advantages and disadvantages of feather assessment in biomonitoring (after Rutkowska et al., 2018).

ADVANTAGES		DISADVANTAGES
harmless sampling	Direct connection to environmental pollution	Internal and external pollution distinguishing challenge
easy collection, transport and storage	Could be stored in nature history museums	Missing accredited material of reference
non-invasive, significant especially in handling protected species	may be sampled irrespective of the season, age or gender	There is no "ideal feather"

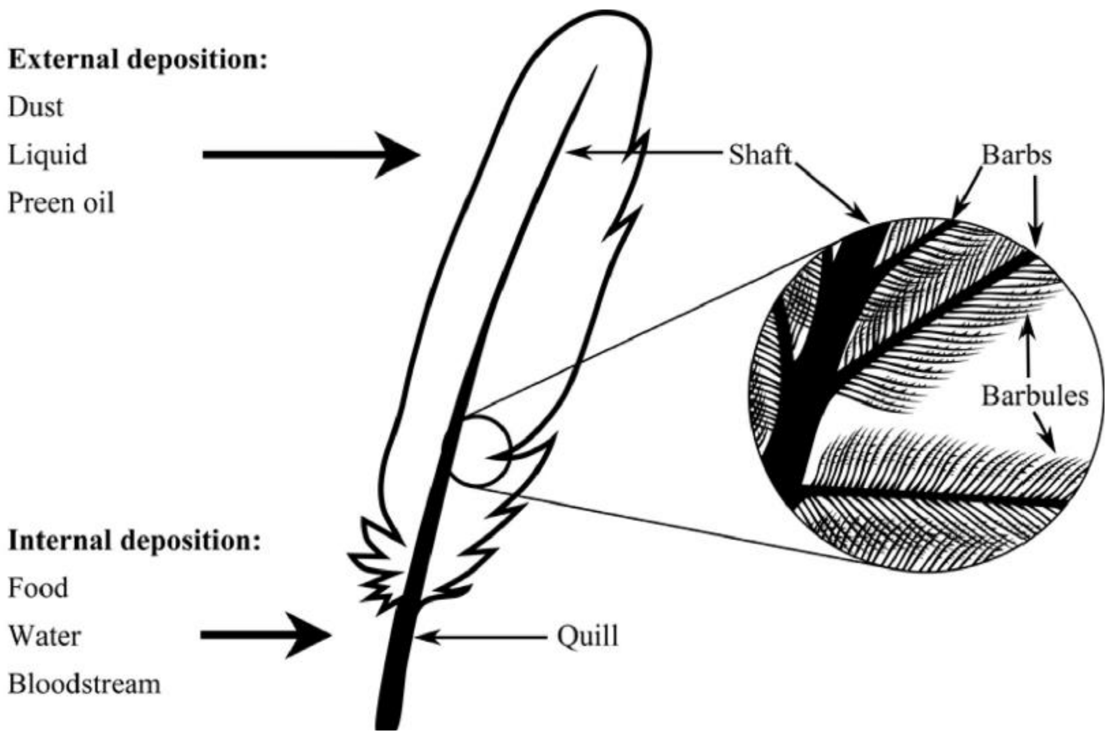

Figure 4. Structure of feather illustrating the possible contamination pathways. Source: Jaspers et al. (2011)

The use of chicks

The outcomes of feather assessment should take into consideration variables like the moulting period, collection time, feather type, internal surface contamination, and age, gender and dietary status of birds that may interfere with data interpretation (García-Fernández et al., 2013, Espin et al., 2012; Jasper et al., 2011). To overcome these restrictions, this study considers the use of chicks rather than adult birds. Using flightless chicks eliminates the problem of where burdens were accumulated. For instance, during an environmental pollution assessment in birds, Borghesi et al., (2017) used a non-invasive sampling of chicks to prevent the confounding variable factors (age, environmental heterogeneity), which gives access to the demonstrated sedimentary particles that is embedded in their plumage.

Young birds have limited mobility and obtain their nutrients from their parents, who in turn gather them from near the nest or colony site, with feeding range varying by species up to few kilometres. Thus, element concentrations in feathers of chicks at fledging time reflect local exposure (Burger and Gochfeld 1997). Some may point out that hatchlings acquire part of their body burdens from parents via the egg; nevertheless this is a negligible part of their burdens, due to the growth-dilution occurring in post-hatch and fledging periods.

The outcome may not be significantly different from that of the adult birds, just as Dauwe et al. (2005) noted that PCB and DDT feather levels were not very different between the young and the old feathers of Great Tit. Chicks are prone to bioaccumulation and potentially biomagnification of toxic substances because of their nutritional habit. After trace elements enter birds, they are directly removed by excretion or absorbed into their feathers. Footprint accumulation can be linked to a certain time period, corresponding to the time necessary to fully grow the feather varying from species to species (Grubb 2006; García-Fernández et al. 2013; Picone et al. 2019).

Trace elements

Trace elements can be non-essentials (e.g. As, Cd, Pb, Hg, Rb, Sr,) and essentials (e.g. Co, Cr, Cu, Mn, Se, Zn) (Jenkins 1969**).** The trace elements in biota may be essential for performing metabolic functions, or they may be nonessential, reminders of our geochemical origins and indicators of environmental exposure, which can be also due to anthropogenic activities. Non essential trace elements may exert toxic effects on biota, but some of them may be beneficial to health through pharmacological action (Nielsen 2003). However, even essential trace elements may exert toxic effects at concentrations beyond those necessary for their biological functions (Fraga 2005).

In view of the anthropogenic impacts throughout the years, environmental concentrations of trace elements increased, altering the balance of their biogeochemical cycles and in some cases becoming a threat for biota. The chemical speciation of trace elements in the environment, namely the form in which they are present in the environment, describes their bioaccessibility, bioavailability and mobility, which affect their fate and their effects on various environmental receptors, including human beings (Corami et al. 2019, submitted). In their biogeochemical cycle, trace elements have sinks, where they are accumulate over years, and sources, from which they can spread back into the environment. Thus, the bioaccessible and bioavailable forms of trace elements may be assimilated by organisms, becoming part of the cell metabolic activities or exerting toxic effects.

Environmental monitoring of trace elements is necessary to investigate their effects on biota. Developing methods to investigate metal exposure, intake and bioaccumulation is essential (Borghesi et al. 2017). Biological indicators of exposure as early warning systems can be successfully employed for environmental worsening and as an evaluation tool for trace element pollution (Abdullah et al. 2015). Birds are environmentally sensitive models for testing pollutant concentrations in broad fields as they often occupy elevated positions on the food web, enabling bioaccumulation and detection of adverse concentrations for many years (Burger 1993; Walsh 2018). Trace elements may affect the survival of chicks and adult life in several ways (e.g. behaviour modification, reproduction and growth reduction); on the other hand deficiency of essential trace elements may cause decreased antioxidant potential, accelerated ageing, embryo and chick developmental delay, and an increased incidence of abnormal pregnancies, immunological abnormalities and lifestyle conditions in organisms (Wada 2004). The trace elements selected for this study include: Hg, Se, Rb, Pb, and Cd.

Mercury

Mercury (Hg) is a extremely poisonous, non-essential metal that is discharged into the environment from natural occurring sources such as volcanic emissions, continental and volatile particulates and sea fluxes (Arcos et al. 2002). According to Tchounwou et al. (2012). Hg is a transitional metal element in nature with a specific level of toxicity in each of its three forms (elemental, inorganic and organic). Also the most common and more toxic form in the environment is methylmercury ($MeHg^+$) which is higher in aquatic environments as compared to terrestrial environments. Forms of Hg with comparatively low toxicity (e.g. elemental Hg) can be converted to very toxic forms by biological procedures (Eisler 2000).

Neurotoxicity is the most likely acute response to nutritional methylmercury in wild adult fishes, despite the fact that further laboratory tests have shown also other effects. A 7 μg g^{-1} wet weight or higher levels in the brain may trigger serious, and possibly lethal effects. Methylmercury is neurotoxic also in birds and mammals. Adverse reproductive impacts have been correlated with 3 mg kg^{-1} of Hg in the diet for some bird species, such as mallards. In specific, in water birds Hg has demonstrated to cause reduced development in chicks, malformations of embryos, reduced hatching capacity, reduced survival of the young, reproductive modifications, and endocrine disorder (Burger and Gochfeld 1997; Jayasena et al. 2011).

Selenium is known to detoxify the reproductive impairments of Hg in vertebrates, including birds (Ohlendorf and Heinz 2011), however a significant exception to the suspected protective intervention of Se against methylmercury was observed as mallards were supplied with a mixture of selenomethionine and methylmercuric chloride. The toxic effects on bird embryo development were far larger than when methylmercury without selenium was added. The same experiment also showed a reduced the toxicity of methylmercury in adult mallards. So, even if Se is reported to mitigate the effects due to Hg in birds (Burger et al., 1993; Ohlendorf and Heinz, 2011), under certain circumstances the reproductive impact of methylmercury cannot be mitigated by Se (Wiener et al. 2003).

Cadmium

Cadmium (Cd) is a metal that is available in little quantities in zinc ores of the earth crust (Tchounwou et al. 2012). Cd can be naturally released by volcanic activities and forest fires but main sources for the environment are several manufacturing processes (e.g. cement, bricks, fertilizers, alloys, batteries) (PO 2017). Birds are generally exposed to Cd by food intake or inhalation from Cd contaminated sources (soil, water and atmosphere).

Uptake through the food intake is a major route of exposure and increasing. Cd enters in the blood circulation and reach target organs such as liver and kidneys, where it can be complexed by metallothioneins, low molecular weight and sulphydryl-rich proteins acting as detoxification agent (Scheuhammer 1987). Unlike adult birds, juveniles are more vulnerable to the poisonous impacts of Cd, which may include delayed development, anaemia, kidney and testicular damage. Cd may also cause harm to the kidney, hyperplasia of the marrow, egg suppression (Furness 1996; Spahn and Sherry 1999). At levels varying from 0.1 to 2 μg g^{-1} in plumage have been noted toxic impacts of Cd towards seabirds (Burger and Gochfeld 2000a).

Lead

Lead (Pb) is a non essential element and has been acknowledged as highly toxic for biota. It primarily exists as Pb(II), but can be oxidized to Pb(IV). Pb is comparatively stable and usually occurs in soluble salts, $PbSO_4$ or $Pb_3(PO_4)_2$, after release to soil as halide, hydroxides, oxides, carbonates and sulphates. Pb forms organic and clay complexes, fixes and limits its transfer to aquatic systems. The leaching of Pb from highly contaminated sites may however be quick for soil (EPA, 2005d).

According to Tchounwou et al. (2012), Pb is primarily introduced into the atmosphere through smelters, emissions of vehicles and anthropogenic activities such as burning fossil fuels, mining and production. It is used in the manufacturing of batteries, ammunitions, metal products and X-rays protection systems, hence Pb has many distinct industrial, environmental and domestic relevance (Tchounwou et al. 2012). The majority of the Pb in air is eventually laid on certain surface areas, including plants, soil, water bodies, man-made surfaces, by dry or wet deposition procedures (Wiener et al. 2003).

The ingestion of lead gunshots and bullets are considered a direct cause for millions of the deaths of birds, i.e. those that feed on substrates in shot-over fields, such as wetlands, clay-pigeon shoots, firearms training sites, and upland game regions (Wiener et al. 2003).

Lead is a metabolic poison that has an impact on a variety of biological traits in birds: breeding, growth, development, comportment and survival (Henny et al. 1991; Gonzalez et al. 2014). Series of effects of Pb poisoning after constant exposure include: its interference with their biological routes and also result in death (Blus et al. 1995) according to the amount of lead concentration in the body of the organism. Pb can cause impairment of reproduction and thyroid dysfunction. It alters the function and composition and generates unfavourable biochemicals, neurotoxic, teratogenic and replicative impacts in kidneys, bones, core nervous systems and hematopoietic system (Eisler 2000). Clinical evidence of Pb toxicity in birds includes depression, emaciation, anaemia, vomiting, ataxia, diarrhoea, blindness, and convulsions (Gonzalez et al. 2014).

Encephalopathy and gastrointestinal dysfunction are clinical indications of toxicity observed in domestic animals. Anxiety, hyper-excitability and potential brutal behavior are all compelling indications of toxicity. Furthermore, Pb can interfere with heme synthesis, changing urine and blood enzyme concentration. Locomotive changes vary from ataxia and coordination deficiency to rigidity across the back muscles to compulsive hypermotility. Fatigue, weight loss, reduced manufacturing, paraplegia, death and opposition to infectious diseases are other indications of toxicity (EPA, 2005d).

Burger and Gochfeld (2000b) argue that Pb levels can be measured in feathers, but it is difficult to interpret as they can be affected by external contamination and environmental exposure. However, the results of Scheuhammer (1987) disclosed that, laying female birds usually collected 4-5 times more Pb in bones than non-laying females. This higher deposition can be linked to an enhanced skeletal Ca turnover caused during reproduction by the creation of egg shells. Thus, for Pb in feathers, the avian toxicity thresholds is 4 µg g^{-1} (Burger and Gochfeld 2000b; Hargreaves et al. 2010). Anaemia was revealed to Bald eagles when the levels of blood Pb are greater than 0.6µg ml^{-1} (Hoffman et al. 1981; Redig et al. 1991; Gonzalez et al. 2014) however, the levels of blood lead as small as 0.42 µg ml^{-1} were also linked with a haematocrit reduction (Miller et al. 2001).

Selenium

Selenium (Se) is a naturally occurring metalloid which is required in ultra-trace concentrations by birds and other animals; however, when it exceeds the physiological requirements, it is harmful. High Se levels in natural vegetation and foodstuffs of wildlife are not limited to areas in which soils are naturally high in Se but can also be the result of wastewater sludge or fly ash waste, mining and metal smelters emissions (Spallholz and Hoffman 2002). Selenium deficiency can severely affect some physiological aspects of birds.

Evaluation of poisoning of Se is complex due to its occurrence in a wide range of chemical types and forms which differ significantly in bird poisoning. The four prevalent oxidation conditions are selenide (-2), elemental Se (0), selenite (+ 4) and selenate (+ 6). Selenite and selenate are both bird-toxic, but organic selenides present the biggest risk. Selenomethionine has shown to be extremely toxic to animals among organic selenides, and seems to be the most susceptible to damage wild animals, as it leads to a elevated bioaccumulation of Se in their eggs (Spallholz and Hoffman 2002). In badly aerated soils, Se(-2) and Se(0) prevail, which are virtually water insoluble, because Se (+4) is soluble but can be heavily adsorbed onto soil and soil-based organic matter, while the Se (+6) has a large level of water-solubility and is not adsorbed by soil particles (Eisler 2000).

Naturally, Se occurs in sulphide ores and metals, limestone, clay, carbonates, metals, charcoal, and mineral water. Se is also introduced into the environment by other natural processes like volcanic eruptions, leaching, rock weathering and biomethylation (EPA, 2007). Industrial sources such as the combustion of coal and oil, nonferrous metal production (mainly zinc, copper, nickel and lead and cadmium), the production of steel and iron, the incineration of municipal and waste-water systems, and manufacturing phosphate fertilizer introduce significantly more Se into the environment than natural resources like volcanic activity or the weathering of seleniferous rocks (Wiener et al. 2003).

The design and manufacturing of glasses, pigments, rubber, metallic alloys, textile products, oil, and medical treatments are the main anthropogenic sources; anti-dandruff shampoos, veterinary pharmaceutical products, fungicides, cosmetic and gaseous insulators contributes significantly also (EPA, 2007).

The most common impacts of Se toxicity are unusual posture and motion, diarrhoea, difficulty in breathing, abdominal discomfort, prostate, and mortality in livestock who feed on seleniferous forage (EPA, 2007). Se in birds hinder significant proteins and enzymes and its concentrations (selenomethionine) over 3 ppm on a wet weight basis in egg lead to decreased hatchability and embryos deformity (Spallholz and Hoffman 2002). In birds Se induces abnormalities in eye, feet or leg, beak, brain and stomach, hepatic pathology and alters metabolism of glutathione. Bilateral alopecia may be noted as a marker of chronic selenosis when nutritional levels are high. Se causes a lower hatchability of eggs and a high incidence of embryo deformations. In the diet of laying females, an excess of Se may affects egg fertility (Ohlendorf and Heinz 2011).

Birds are deemed under good health and reproduction range for food concentrations of less than 0,3mg kg^{-1} whereas those of 3,0 to 5,0 mg kg^{-1} and more than 5,0 mg kg^{-1} are toxic (Ohlendorf and Heinz 2011). All the same, Se concentrations in birds recognized to have poisonous impacts vary, based on species, from 1.8 μg g^{-1} to 26 μg g^{-1} (Ohlendorf and Heinz 2011). Depending on the individual chronic exposure, levels up to 26 μg g^{-1} may cause severe effects, but a Se level of 5 μg g^{-1} is regarded target for feathers (St. Clair et al. 2015; Ashbaugh et al. 2018).

Selenium is also important for the detoxification and protection of vertebrate from methylmercury toxicity (Wiener et al. 2003); often birds with higher Hg levels may have higher Se concentrations also (Burger et al. 2008; Ashbaugh et al. 2018), due to interactions between these two elements (Wiener et al. 2003).

Rubidium

Rubidium (Rb) is a trace element with two natural isotopes: ^{85}Rb (72.15 percent) and ^{87}Rb (27.85 percent). Rb is a major alkaline component that has biochemical qualities comparable to potassium (K). Small quantities of Rb are found in the rock-forming silicate minerals, like potassium feldspars and micas that are naturally found inside the earth's crust. Its radioisotope, ^{85}Rb, is often employed to trace the metabolism of K. Rubidium in some way is a replacement for K, but has some growth and longevity impacts that prevent complete convertibility (Lombeck et al. 1980).

Water-soluble are Rb_2O, RbOH and RbI. There are a number of medicinal uses of these compounds. As an antidepressant, for example, RbCl was used. Since salt (RbCl, RbI) and alkali (RbOH) will exist as ions in the environment, no water or soil volatilization is expected. However, they are expected to leach quickly from soil to groundwater due to their high solubility in water (O' Neill 2006). They are used in glasses and ceramics; also they are used to produce the purple colours in fireworks. Potential applications are as working liquid in vapour turbines and as getter in vacuum tubes, in space vehicle ion engines.

There is little information available for Rb toxicity as compared with other trace elements. The data concerning Rb toxicity threshold in rats is 500 mg kg^{-1} d^{-1} but there are no threshold values for Rb toxicity in birds. Rb is now of ecotoxicological interest due to the evidence that it may biomagnify in arctic food webs (Campbell et al. 2005).

Rare earth elements (REE's)

The rare earth elements (REEs) are a group of elements that comprises the 15 lanthanide elements in group III A of the periodic table, together with yttrium (Y) and scandium (Sc) which share a comparable or common property with lanthanides. The lanthanides (which means: "remain hidden" from Greek) include: Lanthanum (La), Cerium (Ce), Praseodymium (Pr), Neodymium(Nd), Promethium (Pm), Samarium (Sm), Europium (Eu), Gadolinium (Gd), Terbium (Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium (Tm), Ytterbium (Yb), and Lutetium (Lu). Despite their name these elements are not rare (other than the radioactive promethium); for instance, Ce is as abundant as Cu on the Earth's crust. However, they are not found in high concentrations, but are evenly scattered about the Earth crust. Lanthanides are divided into two groups: light REEs (La – Eu) and heavy REEs (Gh – Lu) depending on the electron arrangement for each component (as shown in Table 3), which determines the way in which they interact with other compounds and components. Light rare earth elements (LREE) are regarded as soluble rather than the heavy rare earth elements (HREE) (Sneller et al. 2000). The lanthanides in nature occur in more than 200 minerals (Squadrone et al., 2019; Hu et al., 2004). For instance, it has been discovered that uranium ores contain significant quantities of REEs (Khan et al. 2017).

The chemical and physical characteristics of REEs are very comparable. The distinctive oxidation state is +3, with only a few exceptions. REE's have magnetic and optical properties, high electrical conductivity and high luster, but they tarnish readily in air. Most REE compounds are strongly paramagnetic; many REE fluoresce strongly under UV light and have high melting and boiling points (Zohravi 2007).

REEs are widely used in sophisticated technologies as shown in Table 3. They are very important for a wide range of technology globally (Squadrone et al. 2019b).Their use is becoming progressive in electronics, petro-chemistry, metallurgical, mechanical, domestic defense, power, lighting, agriculture, medical and environmental safety industries (Migaszewski and Gałuszka 2015; Wang et al. 2017). Increasing emissions of the REEs have caused significant environmental pollution but there is limited knowledge of their destiny and impact on natural ecosystems (MacMillan et al. 2017).

Several toxicological studies indicate that REEs cause human multifunctional and multi-systematic harm, particularly among individuals with kidney disease, smokers, pregnant females and infants who are breastfed (Pałasz and Czekaj 2000; Redling 2006; Pagano et al. 2015; Wang et al. 2017). Extreme pain, ataxia, dyspnoea and depression are the clinical signs (Wang et al. 2017). According to Wang et al. (2017), Grobner and Prischl (2007), renal fibrosis, spread peritonitis, peritoneal adhesion, ascites, opacification of blood and hepatic inflation are all pathological phenomena. Several anthropogenic routes for REE pollution could be attributed to electronics, ceramics and glass production industries.

REEs are introduced into surface water systems through e-waste, surface runoffs, atmospheric deposition and recycling activities. The increase in gadolinium concentration by more than 2 orders of magnitude in Berlin, Germany, was from a waste water treatment plant (Bau and Dulski 1996). As with other metals, pH and the occurrence of some cations in the environment heavily influence the accessibility of lanthanum (Herrmann et al. 2016).

Little is known about the link between accumulation and toxicity of REEs in birds (Brown et al. 2019). Hence, exposure rates for individuals have not been fully evaluated, nor have been the food and feed maximums set (Squadrone et al. 2019a). Good bioindicators of REEs contamination are lichen and moss, marine and freshwater animals at the lower levels of the food web (MacMillan et al. 2017; Squadrone et al. 2019b).

Among the uses of REEs is their use as food supplements or amendments for poultry. REEs in China have been used as improvements in agricultural production for over 40 years and amazing results from China's agricultural operations have been stated (Zohravi 2007). There has been reported cases where 250, 500 and 750 mg kg^{-1} probiotic supplements recorded in the layer-hens diet boosted the output of eggs but reduced the egg proportion to damage, the egg yolk and blood cholesterol concentrations (Kurtoglu et al. 2004; Zohravi 2007).

A wide range of toxic impacts of REEs have been investigated on humans, but their toxic impacts and binding objectives are not well understood in cells (Wang et al. 2017). However, REEs may affect the embryogenesis of sea urchins and may also affect risk assessment of human health (Oral et al. 2010; Li et al. 2010; Wei et al. 2013; Gonzalez et al. 2014). A number of endpoints such as inhibition of growth, cytogenesis and organ specific toxicity are negative outcomes for exposures to REEs. Various research have shown that REEs follows hormetic concentration-related patterns, like a variety of other xenobiotics, which imply stimulating or protective impacts at a small level then negative impacts at greater concentrations.

Lanthanides are poorly soluble, so once released in the environment, they can readily plug or attach to complex ions, such as hydroxides, carbonates, fluorides, phosphates or organic ligands (Sneller et al. 2000; Herrmann et al. 2016). They may be in colloidal, suspended particles or soluble forms (Herrmann et al. 2016). REEs can accumulate in organisms; interfere with cells and adsorbed particles.

However, the majority of bioaccumulation studies of REEs included only three or four elements, usually LREE, which do not enable for a definite pattern of bioaccumulation within either species or distinct species through the whole range to be established (Gonzalez et al. 2014).

Dose-response studies of animals have shown that REEs concentrations in the biota accumulate preferentially in the liver, and that concentrations tend to follow the gradient liver > renal > bone > muscular tissue (Schwabe et al. 2012; Squadrone et al. 2019b).

Squadrone et al. (2019a) reported a strong linkage between the levels of REEs in feathers and the diets of a colony of Humboldt penguins located in the Acquario di Cattolica (Italy). The use of feathers as a bioindicator tissue for REEs is thus recommended.

Recent studies on trophic webs suggested that in the biota there is a restricted biomagnification hazard due to REEs. MacMillan et al. (2017) discovered that REEs bioaccumulate more at the base of the food web. To date, the trophic webs taken into consideration are few (Artic and temperate food webs in Canada, freshwater food web in the Gret lakes in USA, terrestrial and freshwater food web in Western Italy), so more date are needed to verify if the biomagnifications risk is low in all the food webs.

Table 3. Main industrial applications of REEs (after Naumov, 2008; Gonzalez et al., 2014; Snow, 2012)

Heavy Rare earth element	**Possible application(s)**
(Pr) Praseodymium	Hybrid Electric Motor and Generator, Ceramics, glasses, pigments
(Tb) Terbium	Hybrid Electric Motor and Generator; Phosphors for lighting and exhibition
(Dy) Dysprosium	Hybrid Electric Motor and Generator; High-power magnets, lasers
(Ho) Holmium	Ceramics, lasers, nuclear industry; premier power magnets well-known
(Er) Erbium	Ceramics, dyes or colorant for glass, optical fibers, lasers, nuclear industry
(Tm) Thulium	Electron bean tubes, visualization of images in medicine; High-voltage magnets
(Yb) Ytterbium	Metallurgy, chemical industry; Fiber-optic technology; solar panels; alloys (stainless steel); lasers; radiation supply for transferable X-ray units
(Lu) Lutetium	Single crystal scintillators; X-ray phosphors
(Y) Yttrium*	Carbon nanotubes, LCD screen, Component Sensors, Capacitors, phosphors, microwave filters, glasses, oxygen sensors, radars, lasers, superconductors

(Gd) Gadolinium	Visualization of images in medicine, optical and magnetic detection (used in a reactor to control rods to control fission reaction and as a contrast agent to improve magnetic resonance imaging (MRI)), ceramics, glasses, crystal scintillators; Magnetic quality imaging disparity agent; additives for glass
(Pm) Promethium	Sources for measuring devices, miniature nuclear batteries, phosphorus, phosphorescent pigment, starter switch for fluorescent lamps; beta radiation source; fluid-cracking catalysts
Light Rare earth element	
(La) Lanthanum	Glasses (high-end optical lenses), ceramics, car catalysts, phosphors, pigments, accumulators, in lanthanum nickel-metal hydride (NiMH) rechargeable batteries (electric cars, laptops); petroleum refining catalysts; high-tech digital cameras; X-ray films; lasers; communication devices; medical use (Gonzalez et al., 2014; Bernard et al., 2005; Hutchison et al., 2004).
(Ce) Cerium	LCD screen, UV cut glass, Glass and Mirrors Polishing Powder, Catalytic Converter, Diesel Fuel Additive, Polishing powders, ceramics, phosphors, glasses, catalysts, pigments, misch metal, UV filters, used in catalytic converters and the oxide as a polishing powder; Catalysts; metal alloys; lens polishers (for glass, television faceplates, optical glass, silicon microprocessors, and disk drives); medical use (Monafo et al., 1976)
(Pr) Praseodymium	Hybrid Electric Motor and Generator, Ceramics, glasses, pigments, oxide is used as a catalyst in plastic manufacturing and is combined with zirconium oxide to produce a vivid yellow pigment used in ceramics; Improved magnet corrosion resistance; pigment; searchlights; airport signal lenses; photographic filters
(Nd) Neodymium	Hybrid Electric Motor and Generator, Headlight, Permanent magnets, catalysts, IR filters, pigments for glass, lasers, super-strong magnets; neodymium-iron-boron (NeFeB) magnets which are used to make cell phones vibrate; Magnets for laptops; lasers; fluid-fracking catalysts; electric motors and communication devices
(Sm) Samarium	Permanent and high strength magnets, microwave filters, nuclear industry, servo-motors; High-temperature magnets; reactor control rods; electric motors
(Eu) Europium	LCD screen, phosphors, notably the reddish-orange colour of screens and monitors; Liquid crystal displays (LCDs); fluorescent lighting; glass additives; communication devices
(Sc) Scandium	High-strength Al-Sc alloys, electron beam tubes

MATERIALS AND METHODS

Study area

Feathers of the MG were sampled in Valle Sacchetta and Valle Paleazza, which faces the Venice Lagoon on the northern edge. BHG feathers were sampled in Valli Treportine. A presentation of the sampling or study area is shown in Figure 4.

In these areas a population of about 1.000 pairs were observed in the study period of 2017-2018. Their nests were found in islands closer to the study area. The Mediterranean gull in this area fed the chicks with cuttlefish, ragworm, goby, mullet and beetle.

Concentrations of As, Cd, Cu, Hg, Pb and V in shallow sediments of the Venice Lagoon are well known (Picone et al. 2018), but for many other trace elements such as Se and REEs, information is inadequate. The industrial districts and urban areas typically have higher metal concentration level as compared to the shoals and mudflats of the northern basin (Picone et al. 2018). As and Hg are only elements occurring above the effect range low (ERL) and effect range median (ERM) (as cited by Picone et al., 2019a). Recent studies highlighted that trace elements may biomagnify (Dominik et al. 2014) and exert toxic effects toward aquatic invertebrates (Picone et al., 2016, 2018) also in shallows far from the urban centres and industrial districts, corresponding to the areas were the gull prey upon fishes and invertebrates. Even though there is little knowledge about the fate and transportation of REEs (MacMillan et al. 2017), it cannot be excluded that they could enter the food web sustaining the water birds.

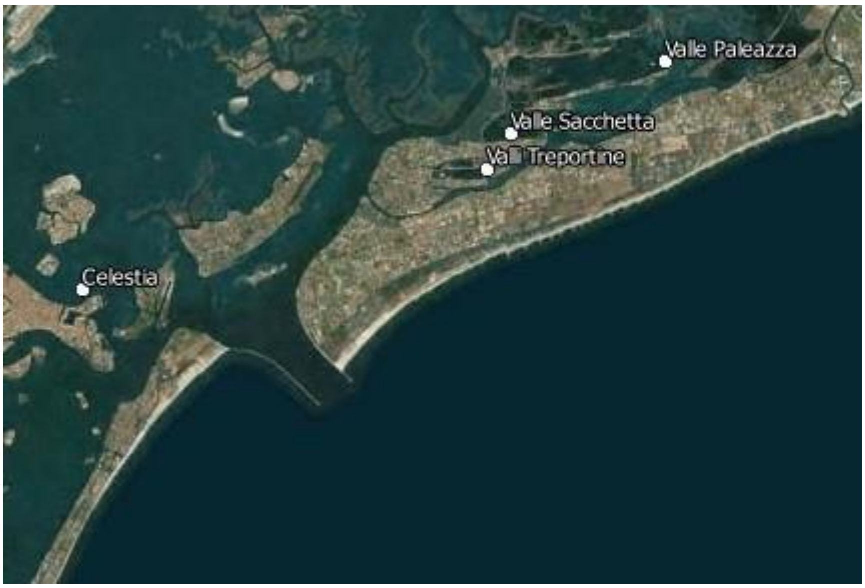

Figure 5. The locality of the sampling area for MG and BHG within the Venice Lagoon.

Sampling

Feather samples from the Mediterranean gull (MG) and Black-headed gull (BHG) were collected during late spring (June 2018) in Valle Paleazza and Valle Sacchetta.

Flightless chicks were hand-collected in the colonies by authorized operators during periodical ringing and monitoring campaigns. The chicks were then deployed into large plastic tanks covered with jute sheets to minimize the stress and the excessive sunlight on them. Chicks were kept for no longer than 90 minutes in the tanks.

A metal ring and a colour ring (blue for MG, and yellow for BHG) with 4-digit alphanumeric code were also applied on the left and right tarsus, respectively, to univocally identify the birds and avoid duplicate sampling. A bunch of contour feathers was retrieved from the rump of each individual chick (n=15 for MG; n = 12 for BHG). Contour feathers were chosen as matrix since they are not necessary for flight, so their collection does not affect the fledging of the chicks.

After the sampling, feathers collected from different specimens were separately preserved in paper envelopes, labelled with date, species, ring number and weight in grams. The samples were then stored at room temperature until washing and subsequent chemical analyses were carried out. Feather removal did not cause birds to experience noticeable trauma and did not influence their ability to fly.

Sample Pre-treatment

Feathers were vigorously washed with acetone (2% solution in ultrapure water) and deionized water alternatively (for at least 3 times), left overnight to dry in a decontaminated fume hood and placed back into their respective decontaminated plastics bags. This washing was performed to eliminate adhesive external contaminants.

Sample mineralization

In order to be analyzed through ICP-MS, feathers were mineralized through wet acid digestion. Acid digestion or mineralization is the complete decomposition of the solid matrix (i.e. feathers) to completely transfer the analytes (i.e. trace elements and REEs) into solution, while avoiding loss or contamination of the analyte.

The vessel-inside-vessel technology (Milestone, Ethos 1), as seen in Figure 6, was used for the mineralisation of the feathers. This method is preferred because it is able to digest the sample faster and allows a high control on the reaction. The samples were placed inside quartz vessels, which were then place inside TFM vessels. TFM is a microwave-transparent fluorinated material which allows direct heating of the sample.. The containers have a relief valve, which prevents the internal pressure from becoming too high. A probe inside one of the vessels ensures a constant internal temperature monitoring.

The feathers together with nitric acid (69% Plasma Pure Plus) and hydrogen peroxide H_2O_2 (30-32% Ultrapure Romil, ratio 4:1) were put into the quartz vessel. On the other side, the primary vessel (TFM vessel) contained the solution necessary to perform precise control of temperature i.e. Ultrapure water, Elga, and hydrogen peroxide H_2O_2 (Ultrapure Romil, ratio 4.5:1). Samples were excluded in blank reagents and were randomly placed in location to check for any matrix effects. All the digested samples were recovered according to their corresponding identifications, weighed, and suitably diluted (with ultrapure water, ratio 1:4) after the mineralisation was done and cooled. The mineralised samples were stored frozen at -20ºC till the analysis with ICP-MS was done.

The primary and secondary vessels were cleaned after every digestion to prevent any interference with the next set of samples to be mineralised. Thus, vessels were rinsed with ultrapure water alternatively. Afterwards, the primary vessel was filled with 10 ml of $HNO_{3,}$ the secondary vessel also with Ultrapure water, Elga and H_2O_2 (4.5:1) All measures and steps were taken under a decontaminated fume hood, in order to diminish any form of contamination.

Figure 6. Microwave digester (Milestone, Ethos 1)

Source of image: http://www.speciation.net/Database/Instruments/Milestone-Srl/ETHOS-1--Advanced-Microwave-Digestion-Labstation-;i2574

Instrumentation

The Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique was used for the analysis of the elements. Analyses of trace elements and REEs were performed using the Thermo Scientific iCAP TQ ICP-MS. The plasma (ICP-MS) is used as ionization system. Compared to other techniques of elementary determination, this allows the inclusion of more elements; it has a very wide linear dynamic range, a shorter analysis time and superior performances with regard to the limits of detection. In inorganic mass spectrometry, samples are nebulized, ionized in argon plasma at high temperatures and then analyzed on the basis of their mass/charge ratio.

The parts that make up this mass spectrometer with this inductively coupled plasma source are:

- *Spray chamber:* a peltier cooled high purity PFA, low-volume, baffled cyclonic spray chambers that efficiently filter out larger aerosol droplets for improved plasma stability.
- *Nebulizer:* is a high performance, concentric nebulizers for optimal sample consumption.
- *Torch:* is a plasma formation structure. It consists of concentrated quartz pipes at the bottom, where there is an in-tension copper spiral that leads to a generator generating a magnetic oscillating current of 27.4 MHz. The electrical shock is introduced to gas (usually argon) to begin ionization which leads to temperatures of between six thousand and 10.000 K. The fluid is kept by the magnetic field generating a constant motion of loaded electrons that collide with other polar ionizing electrons.
- *Peristaltic Pump*
- *RF Generator:* Digital, solid state RF generator, Argon ICP ion source. Cold plasma operation highly stable and reliable.
- *Load Coil:* Water cooled PTFE coated load coil for enhanced service life and secure plasma inflammation.
- *Inert Tubing Cones:* Ni optimized cones (orifice with a diameter of 1.1 mm) and skimmer (orifice with a diameter of 0.5 mm) for reduction and servicing of matrix deposition.
- *Extraction Lens*
- *Cold Plasma Interface*
- *Ion Optics:* the unique 90° cylindrical ion lens, the RAPID Lens, provides a strong ion flow throughout the full mass spectrum.
- *Q1 Quadrupole:* is the pre- and post-filter, high-frequency quadrupole mass analyser for ion isolation. In all TQ methods, it is user-defined resolution. Switch to high resolution (< 1u) for optimum results in any matrix, between intelligent mass selection (iMS). Mass calibration is evaluated and updated automatically. The electrons are separated by the mass /

load proportion and transferred to the sensor. The stream of ions is carried through electromagnetic lenses inside four conductor bars and is affected by the oscillating magnetic field applied to the opposite sides of bars. This domain causes the ions to travel on a certain sinusoidal trajectory that only crosses the quadrouple with some mass ions. Based on the mass / load ratio the frequency of the oscillating field is varied to determine the different ions.

- *Vacuum System*
- *Turbo pump*
- *Standard Interface pump*
- *Dry Interface pump*
- *Detector:* This comprises of an electromultiplier that amplifies the production of the electrodes and offers a measurable electrical signal in which collide ions (emission of the ratio of electrons) and electromultipliers. The current intensity is proportional to the amount of ions reaching the detector.

The instrument was calibrated for quantitative analysis by means of a calibration curve, used to correlate the concentration of analytes in solution (mapped on the x-axis) with the instrument's response signal (mapped at the y-axis). Multielement standard solutions with different concentrations are used as the most common calibration method for ICP-MS to create a calibration curve covering a range of concentrations which include the concentration in the sample.

A single element standard was employed for Hg, as it has a low deterioration time. Variables such as changes in the effectiveness of plasma ionisation, the probable clogging or erosion of cone apertures and the distinct matrix quantity within the samples, may affect the instrument sensitivity. To restrict the effect of these factors, internal standard (Pt 10 μg L^{-1}) is added to all the samples and standards.

As the analytes have a comparable behavior, nuclear mass and ionisation potential, it is vital to check whether inner signal drifts have occurred by using an internal standards. Internal standards are elements that are not present in samples and follow the same sample analysis process to provide a signal distinguishable from the analyte signal. Every factor affecting the analyte signal will also affect the internal standard signal in the same degree. Thus there will be less variability in the ratio of the two signals.

It is recommended to use elements Y, Ge, Rh or In and Re or Pt as internal standards. Relative standard deviation Percentage (RSD %) was <10% for each element analysed.

Referenced certified material, human hair ERM DB001, was also used to test for the accuracy of the analysis (quality assurance of the data). The recovery was higher than 80% for all the elements.

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 10.0 for Windows (SPSS Inc., USA). A one-way Analysis of Variance (ANOVA) has been carried out for checking for differences in feather's concentrations between MG and BHG.

Variance is an arithmetic mean dispersion measure (how statistics are far from arithmetic mean) i.e. data dispersion:

$$var(y_1,\ldots,y_n)=1n\Sigma(y_i-\bar{y})2ni=1$$

This assessment is intended to determine if (for a particular variable) there is a statistically distinct arithmetic mean of sample groups or not within a particular confidence level (H_0 hypothesis). The relationship between the sum of squares among the groups (SSB) and sum of squares within the groups (SSW) in line with the following equation can confirm or reject this hypothesis.

An F distribution table with critical values and a relation between the degree of freedom of the numerator (p-1) and the degree of the denominator (n-p) can be used to obtain a number (F_{crit}) below the significant value of the hypothesis H_o. If F > Fcrit then H_o, otherwise it will be dismissed, will be verified.

Kolmogorov-Smirnov test and Levene's test have been performed to verify data normality and variance homogeneity, respectively.

The Kolmogorov-Smirnov statistic test is the maximum absolute difference of the two observed distribution functions. It compares the empirical distribution function of the data (F_{obs}) and the cummulative distribution function associated with the hypothesis (F_{exp}).

$D_n = \max_x |F_{exp}(x) - F_{obs}(x)|$.

Levene's Test is an homogeneity-of-variance test that is less dependent on the assumption of normality than most tests. For each case, it computes the absolute difference between the value of that case and its cell mean and performs a one-way analysis of variance on those differences. Assumptions:1. The samples from the populations under consideration are independent. 2. The populations under consideration are approximately normally distributed.

When data normality and variance homogeneity conditions were not met, a non parametric Mann-Whitney U-test has been performed. The Spearman's correlation was used to check whether the REEs were correlated to each-other or not.

RESULTS

Analysis of Trace elements

Cadmium

The range of the concentration of Cd in MG was 1 µg kg^{-1} to 528 µg kg^{-1} and 1 µg kg^{-1} to 88 µg kg^{-1} for BHG, as shown in Figure 7. An individual feather of the MG (IMVS183) contained extremely large concentration of Cd of 528 µg kg^{-1} which was classified as an outlier according to Tukey's fence of detection of outliers[1]. The arithmetic mean values of concentration varied between bird species group, their concentrations were 80 ± 4 µg kg^{-1} for MG and 50 ± 3µg kg^{-1} for BHG. It is an obvious trend in the graph that, the Cd concentration in MG increased sharply above that of the BHG during the period of feather formation.

Mann-Whitney U test was selected for statistical analysis because the intercept of the data was not significant (p-value = 0, 07), thus, ANOVA was not the best option to analyse the data. The results from Mann-Whitney U test for Cd (Z = 3, 04; p-value = 0,002) revealed that, there are differences between the 2 species. It can therefore be emphasized that, MG accumulated more Cd than BHG.

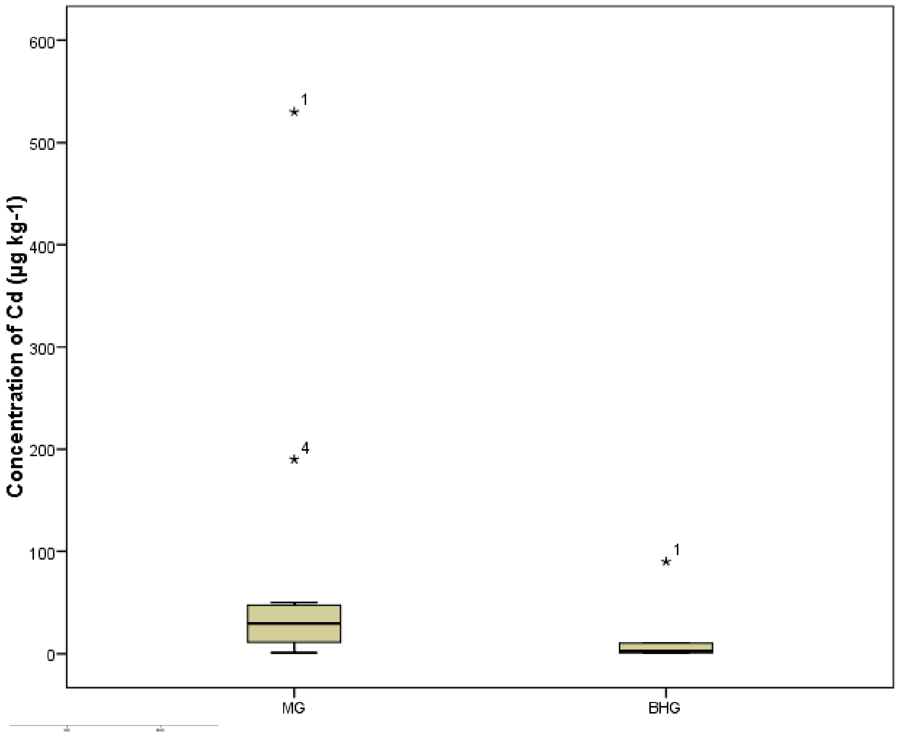

Figure 7. Concentration of Cd in the feathers of MG and BHG. The values were expressed in µg kg^{-1}.

[1] Tukey's rule says that the outliers are values more than 1.5 times the interquartile range (IQR = Q3-Q1) from the quartiles — either below Q1 − 1.5IQR, or above Q3 + 1.5IQR

Table 4. Summary of concentration of Cd in the feathers of MG and BHG

Trace metal	Minimum	Median	Geometric mean	Arithmetic mean	Maximum
MG	1	15	17	50	528
BHG	1	3	3	10	88

Rubidium

The range of the concentration of Rb in MG was 4 µg kg^{-1} to 697 µg kg^{-1} and 4 µg kg^{-1} to 65 µg kg^{-1} for BHG, as shown in Figure 8. As in the case of Cd, the same individual feather of MG (IMVS183) contained extremely large concentration of Rb of 697 µg kg^{-1} which was considered as an outlier according to Tukey's fence of detection of outliers. The arithmetic mean values of concentration varied between bird species group, their concentrations were 80 ± 2 µg kg^{-1} for MG and 20 ± 0.5 µg kg^{-1} for BHG. As shown in the graph, MG tends to accumulate more Rb than BHG.

Mann-Whitney U test was selected for statistical analysis because both normality and variance homogeneity conditions were not met hence ANOVA was not the best option to analyse the data. The results from Mann-Whitney U test for Rb (Z = 1, 56; p-value = 0,12) revealed that, there are no differences between the mean concentrations of the 2 species. Statistical analysis (Mann-Whitney) showed that the results are not statistically different, so even if Rb concentrations may reach higher concentration in MG than in BHG, the interspecific variation is so high that the difference between the mean values cannot be reported as significant.

Table 5. Summary of concentration of Rb in the feathers of MG and BHG

	Minimum	Median	Geometric mean	Arithmetic mean	Maximum
MG	4	33	35	80	697
BHG	4	20	19	20	65

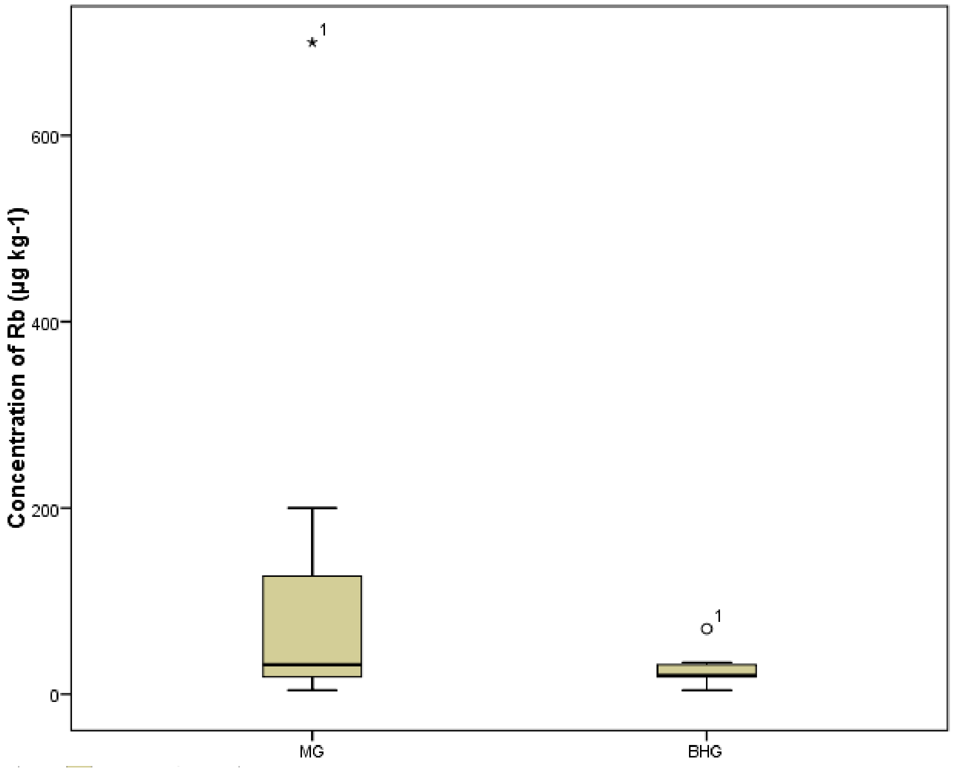

Figure 8. Concentration of Rb in the feathers of MG and BHG. The values were expressed in µg kg^{-1}.

Mercury

The range of the concentration of Hg in MG was 2 µg kg^{-1} to 313 µg kg^{-1} and 2 µg kg^{-1} to 293 µg kg^{-1} for BHG, as shown in Table 6. Some individual feathers contained extremely large concentration of Hg such as 313 µg kg^{-1} in the MG and 293 µg kg^{-1} in BHG. The arithmetic mean values of concentration varied between bird species group, their concentrations were 37 ± 4 µg kg^{-1} for MG and 116 ± 12 µg kg^{-1} for BHG.

Statistical analysis of Hg data was not performed, due to the large number of data below the limit of quantification for MG (see Table 5).

Table 6. Summary of Hg concentrations in feather of MG and BHG (data reported as µg kg^{-1})

	Median	Geometric Mean	Arith. Mean	minimum	maximum	valid data
MG	17	21	37	8	313	10 (50%)
BHG	116	102	116	8	293	11 (92%)

Selenium

The range of the concentration of Se in MG was 80 µg kg^{-1} to 900 µg kg^{-1}**,** for BHG only 2 data were above the LoQ (104 µg kg^{-1} and 165 µg kg^{-1}), as shown in Table 7. An individual feather of the MG (IMVS183) contained extremely large concentration of Se of 14.557 µg kg^{-1} which is an outlier according to the Tukey's fences test. The arithmetic mean values of concentration varied between bird species, their concentrations were 1.050 ± 32 µg kg^{-1} for MG and 73 ± 2 µg kg^{-1} for BHG. Values below LOQ were substituted by half of the LOD value and taken into account when calculating the average value.

Statistical analysis of Se data was not performed; due to the large number of data below the limit of quantification. The available data are not conclusive as concern a comparison in the accumulation potential of Se between the 2 species.

Table 7. Summary of Se concentrations in feather of MG and BHG (data reported as µg kg^{-1})

	Median	Geometric Mean	Arith. Mean	minimum	maximum	valid data
MG	61	141	1.050	8	14.557	11 (55%)
BHG	61	69	73	61	165	2 (16%)

Lead

In the case of Pb, there was only 1 data above the LoQ for MG and no data was above the LoQ for BHG. No further analyses were performed on Pb data.

Analysis of Rare Earth Elements

All the 16 REEs were detected in each feather sample; only in the case of Ce, there were data (1 for MG and 7 for BHG) below the Limit of Quantification (LoQ).

The Spearman's R correlation analysis proved that REE's are positively correlated both in BHG and MG. A stronger positive correlation was observed in the BHG (lowest R = 0, 69 and highest R = 1). In MG the correlation was significant and positive as well, although less than in BHG. The only exception to this trend is Ce in MG. The lack of correlation of Ce with Sc, Eu, Gd, Tb, Ho, Er, Tm and Lu in MG might be related to the fact that feathers of MG were collected in 2 different areas of the Lagoon, where local levels of Ce concentration might be different. Reference tables with Spearman's R and p-values are provided in the Appendix (from Appendix Table 1 to Appendix Table 4).

The profiles of the graphs for each REE were similar for almost all of the elements (from Appendix Figure 1 to Appendix Figure 16). This similarity trend is also reflected in the descriptive statistics reported Appendix Table 5 Appendix Table 6. Due to the strong correlation and similarity of the graph profiles, statistical analysis was performed on the sum of REEs rather than for the single REE, even as was also performed by Macmillan et al. (2017).

Verification for normality and variance homogeneity by Kolmogorov-Smirnov test and Levene's were not met, even also after a log transformation of data. So, the Mann-Whitney U test was used to compare the sum of REEs in MG and BHG feathers. The test showed that there are no significant differences between the sums of the REE concentration of the two species ($Z= -1{,}60$ and p-value = 0,11), probably due to the strong interspecies variability observed. Even if there is slightly a higher sum of REE in the MG as compared with the BHG, there is so great variation within single species that the two mean values cannot be considered as different.

There is no consistence for the LREE/HREE ratio in the two species as indicated in Appendix Table 7 and Appendix Table 8. Few individuals have a higher concentration of HREE as compared with LREE, whilst for most of the analysed specimens LREE occurred at higher concentrations than HREE.

DISCUSSION

The results were evaluated by statistical analysis and certain key issues in connection with the accumulation of toxic metal load in MG and BHG were answered comparatively.

The sample concentrations in this study are expected to reflect about 3 weeks of dietary exposure, corresponding to the approximate age of the chicks. During the few weeks of feather formation, trace elements in feathers constitute circulating blood concentrations that reflect both local exposure and mobility from inner tissues (Lewis and Furness 1991; Monteiro et al. 1995). The MG and BHG sample outcomes were compared to those of other species analyzed in the same study area (Kentish plover) and in other seabird research projects.

However, it should be taken into consideration that most of the research concerning birds focused mainly on concentration measured in adults; contaminant concentrations in adults are generally greater than in chicks, mainly because, adults have metabolized and bioaccumulated contaminants longer, according to Burger (1993).

Squadrone et al., (2017) and other scientists pointed out that the liver provides the highest measure of exposure to the REEs; however it is not feasible to kill the bird, hence feathers are the best ethical option. There are differences in the information provided by feathers (short-term accumulation in a definite area) and liver or other organs (integration for a longer exposure time). The longer the period of accumulation, the more the different areas the birds have stayed. The feathers accumulate and integrate concentration for a short period of time unlike that of the tissues and organs of the birds. The feathers have a specific or definite time and space contest as concerned the life cycle of the birds whilst the organs integrate all the exposure of a life span.

Comparison among trace elements.

<u>Mercury</u>

In marine ecosystems, Hg is the main element of concern (Thompson et al. 1998). Maximum concentration of Hg in MG and BHG were 313 µg kg^{-1} and 293 µg kg^{-1} respectively. Nevertheless, both data for Hg were below the toxicity threshold of 5.000 µg kg^{-1} reported in the literature (Burger and Gochfeld 2004; Hargreaves et al. 2010; St. Clair et al. 2015). Hence, chicks of MG and BHG seem to be not exposed to Hg through their diet. This is in contrast to the Hg body burden observed for Kentish plover (KP), *Charadrius alexandrinus*, in the Venice Lagoon, whose average Hg level was 8.710 ± 976 µg kg^{-1} in its tail feathers (Picone et al. 2019).

Hg levels below the adverse effect threshold were observed for several different bird species. For instance, about 4.100 μg kg^{-1} of Hg have been observed in feathers of black-tailed gulls of Rishiri Island in Japan (Asunaga et al. 2005); also many birds from the Midway Atoll in the northern pacific Ocean, including the Red-footed booby (3790 μg kg^{-1}) or the Great frigate bird, (1640 μg kg^{-1} of Hg, Burger and Gochfeld 2000c) or the black legged kittiwakes from Prince William Sound, Alaska, (average concentration of 2910 μg kg^{-1}, (Burger et al. 2008) showed Hg concentrations below the adverse effect threshold. Also, the levels of Hg recorded in breast feathers in chicks of the glaucous-winged gull from an isolated oceanic Island in Aleutians was 2830 μg kg^{-1} (Burger et al. 2009). The concentrations of Hg observed in chicks of MG and BHG are in agreement with the data reported in the aforementioned bird species.

The difference between chicks of gulls and other birds of the Venice Lagoon (e.g. KP) may be related to the different feeding habits: the KP and other waders feed on benthic crustaceans, ragworms and bivalves in the intertidal area, whilst chick of gulls are fed by parents with fishes and molluscs less dependent from the benthic habitats, and thus, less exposed to the sediment Hg contamination.

Thus, exposure to Hg is not a factor of concern for the chicks of the gulls in the Venice Lagoon.

Cadmium

In the case of Cd, although median and geometric mean are below the toxicity threshold of 0.1 mg kg^{-1}, there were 2 individuals of MG with Cd body burdens higher than this threshold, suggesting that some individuals may have been exposed to Cd to an extent that may lead to sub-lethal effects, as reported by Burger and Gochfeld (2000a, 2000b) and (Burger et al. 2008). Moreover, Cd distribution and accumulation presented patterns consistent with the previous observation obtained for KP in the Venice Lagoon (Picone et al. 2019).

The present research work also is in agreement with the earlier observations of (Burger et al. 2008) who recorded a lower Cd concentration than the toxicity threshold in the black-legged kittiwake and black oystercatcher from Prince William Sound of Alaska, which had a dry weight of 78.5 ng g^{-1} and 16.0 ng g^{-1} respectively. In feathers of juvenile *Larus dominicanus*, a Cd concentration of 0.021 μg g^{-1} was observed in the Florianópolis, SC, Brazilian coast (Barbieri et al. 2010). The results obtained in the present work also agree with the findings of other studies by Kim and Oh (2014) in which chicks of black-tailed gull, heron and egret showed Cd accumulations of 0.05–0.03 μg g^{-1}, 0.07 μg g^{-1}, 0.48–0.88 μg g^{-1}, respectively.

Lead

The results showed that exposure to Pb are negligible, which again corroborate the findings of the previous work on KP by (Picone et al. 2019). Lead levels from MG and BHG are lower than the values found in literature (Orłowski 2007). The mean value observed is also lower than the concentration observed in *P. apricaria* (2700 µg kg^{-1}) by Lucia et al. (2010) which is in turn lower than the approximate threshold level for toxic effects (4000 µg kg^{-1}).

The levels of Pb and Cd in seabirds according to Burger and Gochfeld, (2009) vary greatly across research due to factors of feeding, intensity and exposure periods in foraging regions, physiology of birds as well as the biochemical features of Cd and Pb. Consequently, further similar researches on Pb pollution in other water birds are therefore necessary in the area of study.

Selenium

In the present study median and geometric mean for Se are below the toxicity threshold of 5 mg kg^{-1} reported by Ashbaugh et al. (2018); nevertheless, a single individual of MG has feather concentrations above this threshold, suggesting a possible exposure to Se. These results were consistent with those of the studies of Picone et al. (2019) in the KP.

Selenium concentrations observed in other studies concerning waders, according to Lucia et al. (2014), were generally below the toxicity threshold. Also, Ashbaugh et al. (2018) observed a value of 2700 µg kg^{-1} for *Charadrius nivosus* in Oklahoma. Another observation by Lucia et al. (2010) for *P. squatarola* was 1900 µg kg^{-1}. As concern Gulls, the black-legged kittiwake from Prince William Sound of Alaska also registered Se concentrations at a mean of 2110 ng g^{-1} (Burger et al. 2008). In the research concerning seabirds from the midway Atoll in the northern pacific Ocean, low levels of Se were again recorded in the Red-footed booby (2310 ng g^{-1}) and the Great frigatebird (3850 ng g^{-1}, Burger and Gochfeld 2000c).

According to the literature, waders tend to have higher Se levels than gulls and other waterbirds (Burger et al. 1993; Lucia et al. 2010, 2014; Picone et al. 2019). A possible explanation to the variation in the observed accumulation of Se in birds may include difference in diet, different foraging locations or variation in metal kinetics.

Rubidium

The distribution and concentration of Rb in the marine and freshwater webs have been given far too little attention. Thus, there are no toxicity thresholds available in literature so it is not possible to foresee any toxic effect due to exposure to Rb. However, low-K and high-Rb diets associated with physiological interference with K^+ and sodium (Na^+) were observed in mammals (Kosla et al. 2002; Campbell et al. 2005).

In any case, the fact that Rb was detectable in most of the specimen suggests that this element in the Lagoon of Venice may be bioavailable, also for gulls. This hypothesis is corroborated by the fact that also in *C. alexandrinus* Rb was detected in all the analysed specimens, with a mean concentration of 490 ± 250 μg kg^{-1} (Picone et al., unpublished data). On the other hand, the higher concentration observed in KP than in MG and BHG suggest once again that the benthic food web may be a preferential way of accumulation for trace element within the Lagoon.

Similar outcomes of about 0.034 ± 0.028 ng g^{-1} level of Rb have been observed in the feathers of the black-tailed gulls of Rishiri Island in Japan (Asunaga et al. 2005). However, since Rb concentration in vertebrates has been shown to depend both on the quantity of ambient Rb and the proportions of Rb to other alkali metals, further studies are needed to elucidate the possible correlation effects between Rb and alkali metals (Campbell et. al, 2005).

Future work is recommended also to establish toxicity threshold in literature and toxic effects of Rb exposure.

Evaluation Rare Earth Elements (REEs)

There is a general lack of information concerning REEs accumulation on birds in the literature, despite the recognized need to collect data for these elements in birds colonizing exposed regions, densely-populated areas and suburban areas (Brown et al. 2019).

To our knowledge, Squadrone et al., (2019) who found a mean concentration of 160 μg kg^{-1} in Humboldt penguins (*Spheniscus humboldti*) and Borghesi et al. (2017) who observed the ΣREEs 120 μg kg^{-1} in Greater flamingos housed in a zoo are the only research that has evaluated REE in feathers. Other studies focused only on internal organs (Macmillan et al. 2017; Brown et al. 2019).

Contrary to the previous findings on penguins and flamingoes, much higher concentration of REEs were observed in MG and BHG, with an average of 6.546 μg kg^{-1} and 9.838 μg kg^{-1} respectively. Higher concentration in wild gulls as compared with penguins kept in an Aquarium and flamingoes housed in a zoo were expected, due to the much higher complexity of the exposure in brackish water lagoons as compared with artificial housings, where birds are fed with a single type of food. In natural environments trace elements may be taken up not only through food ingestion, but also by sediment/soil particles co-ingestion or inhalation of dust (Brown et al. 2019). Different prey-items may contain different concentrations of REE. Moreover, as noted by Squadrone et al. (2019) and Borghesi et al. (2017), there is probably a strict link between geochemistry of the environment and accumulation in feathers.

The data collected for this thesis underline that REEs are bioaccessible and bioavailable to birds. Nevertheless, it is not possible to quantify the risk due to the exposure to REEs, because there is a lack of benchmark data relating feather concentrations (but also for other organs) with toxic effects.

The accumulation of REEs, however, is of concern, because some of these elements may interfere with Ca metabolism at various levels, due to similarity in size, bonding properties, coordination geometry and donor atom preferences (Redling 2006). For instance, Jakubek et al. (2009) reported that Y may compete with Ca both for Ca-ion channels (inhibiting them significantly at concentration as low as 0,07 ppm) and with Ca-binding proteins. Brown et al. (2019) during the nesting period observed different accumulation pattern between females and males in Ring-billed gulls (*Larus delawerensis*) for some REEs, including Y, and related this difference to potential sex-difference in Ca-binding proteins. In facts, according to these authors, Y could bind to albumin and other Ca-binding proteins such as globulins in the plasma of female gulls, which could explain the greater concentrations of Y in female liver relative to males during the incubation period.

Squadrone et al. (2019) suggested also that deposition of Y in lieu of Ca in the eggshells may be a relevant route of exposure for the developing embryo, since Ca may be mobilized from the eggshell for the calcification of the skeleton, though the mediation of calcium-binding proteins (Chien et al. 2009; Kaweewong et al. 2013).

Correlation of REE

In the case of BHG, the correlation values are very high (lowest r = 0,69; see in appendix table 1 to appendix table 4), this depicts a strong positive correlation of all the REEs in the BHG which confirms the findings of Amyot et. al., (2017) and Macmillan et. al., (2017). On the contrary, MG's correlation is not so strong as compared to BHG because, Ce does not correlate with most elements, including Sc, Eu, Tb and most HREEs. This difference in correlation can be related to the fact that, there is more variability in the diet of MGs than in BHGs. It is well known that REEs act as a homogenous set of elements in non-biological arrays, but this strong correspondence between REEs has not been well researched or correctly explored in biological fields.

LREE and HREE ratio

Based on the ratio of LREE and HREE, the concentrations of LREEs are higher than in HREEs which confirms the study of Squadrone et al., (2019) and Li et al., (2016) that, there is often an excess of LREE than in HREE. Higher concentration of LREE have also been observed in previous studies on birds (even if different tissues were considered), whilst the prevalence of HREE was not reported before in studies on birds.

CONCLUSION

One of the more significant findings emerged from this study is that MG tends to accumulate more trace elements (e.g. Cd, Rb) and REEs than BHG. However, there is little risk of Pb, Hg, Se and Ce in the study area to the gulls since most of their bioconcentration was below the limit of quantification.

The second major finding was that, calculated apparent correlation coefficients between the REEs in MG and BHG clearly highlights a greater variability in the diet of MG and consistence in the diet of BHG. This evidence of homology provides a theoretical and technical enhancement for the research, bioavailability and efficacy of the REE migration and transformation in the marine environment. Even though, a big discrepancy exist between the correlation of Ce with Sc, Eu, Gd, Tb, Ho, Er, Tm and Lu in MG as evidenced by concentrations below the LoQ.

Our study demonstrates consistency in the fact that, most birds have a higher concentration of LREE as compared to HREE evidenced by the ratio >1 for most LREEs. This suggests a useful trace of LREE in extreme levels in the lagoon of Venice.

In addition, our findings provide estimates to the baseline for lanthanides concentration in the feathers of the MG and BHG. Since these baselines are useful to other disciplines of medicine, advanced tech or agriculture, utilization of feathers must be promoted for further surveillance especially in other areas where augmentation of REEs occur due to anthropogenic activities.

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APPENDIX

Appendix Table 1. Spearman's R correlation for BHG

	39-Y	57-La	58-Ce	59-Pr	60-Nd	62-Sm	63-Eu	64-Gd	65-Tb	66-Dy	67-Ho	68-Er	69-Tm	70-Yb	71-Lu
21-Sc	0,80	0,90	0,69	0,92	0,90	0,92	0,87	0,90	0,87	0,93	0,87	0,89	0,87	0,89	0,83
39-Y		0,97	0,84	0,95	0,97	0,95	0,87	0,97	0,87	0,93	0,87	0,91	0,87	0,91	0,85
57-La			0,83	0,99	1,00	0,99	0,92	1,00	0,92	0,98	0,92	0,95	0,92	0,94	0,89
58-Ce				0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83
59-Pr					0,99	1,00	0,94	0,99	0,94	0,99	0,94	0,96	0,94	0,97	0,91
60-Nd						0,99	0,92	1,00	0,92	0,98	0,92	0,95	0,92	0,94	0,89
62-Sm							0,94	0,99	0,94	0,99	0,94	0,96	0,94	0,97	0,91
63-Eu								0,92	1,00	0,97	1,00	0,99	1,00	0,99	0,99
64-Gd									0,92	0,98	0,92	0,95	0,92	0,94	0,89
65-Tb										0,97	1,00	0,99	1,00	0,99	0,99
66-Dy											0,97	0,98	0,97	0,98	0,94
67-Ho												0,99	1,00	0,99	0,99
68-Er													0,99	0,98	0,97
69-Tm														0,99	0,99
70-Yb															0,97

Appendix Table 2. P-value results for the correlation analysis of BHG

	39-Y	57-La	58-Ce	59-Pr	60-Nd	62-Sm	63-Eu	64-Gd	65-Tb	66-Dy	67-Ho	68-Er	69-Tm	70-Yb	71-Lu
21-Sc	$p < 0.01$	$p < 0.01$	$p = 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
39-Y		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
57-La			$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
58-Ce				$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
59-Pr					$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
60-Nd						$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
62-Sm							$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
63-Eu								$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
64-Gd									$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
65-Tb										$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
66-Dy											$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
67-Ho												$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
68-Er													$p < 0.01$	$p < 0.01$	$p < 0.01$
69-Tm														$p < 0.01$	$p < 0.01$
70-Yb															$p < 0.01$

Appendix Table 3. Spearman's R correlation for MG

	39-Y	**57-La**	**58-Ce**	**59-Pr**	**60-Nd**	**62-Sm**	**63-Eu**	**64-Gd**	**65-Tb**	**66-Dy**	**67-Ho**	**68-Er**	**69-Tm**	**70-Yb**	**71-Lu**
21-Sc	0,67	0,74	**0,37**	0,67	0,72	0,65	0,65	0,67	0,61	0,68	0,61	0,67	0,56	0,63	0,59
39-Y		0,97	0,83	0,93	0,98	0,90	0,74	0,88	0,67	0,88	0,67	0,77	0,60	0,76	0,61
57-La			0,86	0,84	0,99	0,80	0,64	0,79	0,56	0,79	0,56	0,66	0,48	0,65	0,50
58-Ce				0,62	0,86	0,58	**0,34**	**0,56**	**0,26**	**0,54**	**0,26**	**0,37**	**0,18**	**0,38**	**0,18**
59-Pr					0,87	0,99	0,92	0,99	0,87	0,98	0,87	0,93	0,81	0,92	0,82
60-Nd						0,83	0,66	0,82	0,58	0,82	0,58	0,69	0,51	0,68	0,52
62-Sm							0,94	0,99	0,89	0,99	0,89	0,95	0,83	0,95	0,84
63-Eu								0,95	0,99	0,95	0,99	0,99	0,96	0,99	0,96
64-Gd									0,90	0,99	0,90	0,96	0,84	0,95	0,85
65-Tb										0,90	0,99	0,98	0,99	0,97	0,97
66-Dy											0,90	0,96	0,84	0,96	0,86
67-Ho												0,97	0,99	0,97	0,99
68-Er													0,94	0,99	0,95
69-Tm														0,94	0,99
70-Yb															0,94

Appendix Table 4. P-value results for the correlation analysis of MG

	39-Y	57-La	58-Ce	59-Pr	60-Nd	62-Sm	63-Eu	64-Gd	65-Tb	66-Dy	67-Ho	68-Er	69-Tm	70-Yb	71-Lu
21-Sc	$p < 0.01$	$p < 0.01$	**$p = 0.11$**	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
39-Y		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
57-La			$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.01$	$p < 0.01$	$p = 0.01$	$p < 0.01$	$p = 0.03$	$p < 0.01$	$p = 0.03$
58-Ce				$p < 0.01$	$p < 0.01$	$p < 0.01$	**$p = 0.14$**	$p = 0.01$	**$p = 0.27$**	**$p = 0.01$**	**$p = 0.27$**	**$p = 0.11$**	**$p = 0.43$**	**$p = 0.10$**	**$p = 0.43$**
59-Pr					$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
60-Nd						$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.02$	$p < 0.01$	$p = 0.02$
62-Sm							$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
63-Eu								$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
64-Gd									$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
65-Tb										$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
66-Dy											$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
67-Ho												$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
68-Er													$p < 0.01$	$p < 0.01$	$p < 0.01$
69-Tm														$p < 0.01$	$p < 0.01$
70-Yb															$p < 0.01$

Appendix Table 5. Summary of REE concentrations in the feathers of MG

REE	Minimum	Median	Geometric mean	Maximum
Sc	288	733	702	1.844
La	460	376	369	1.537
Ce	4	319	270	1.539
Pr	16	117	151	1.496
Nd	40	368	358	1.629
Sm	13	104	136	1.469
Eu	7	37	63	1.421
Gd	9	99	125	1.495
Tb	6	27	52	1.609
Dy	10	91	113	1.701
Ho	5	28	52	1.676
Er	7	56	80	1.651
Tm	3	20	38	1.652
Yb	6	43	71	1.625
Lu	3	19	38	1.616
Y	32	293	305	1.552

Appendix Table 6. Summary of REE concentrations in the feathers of BHG

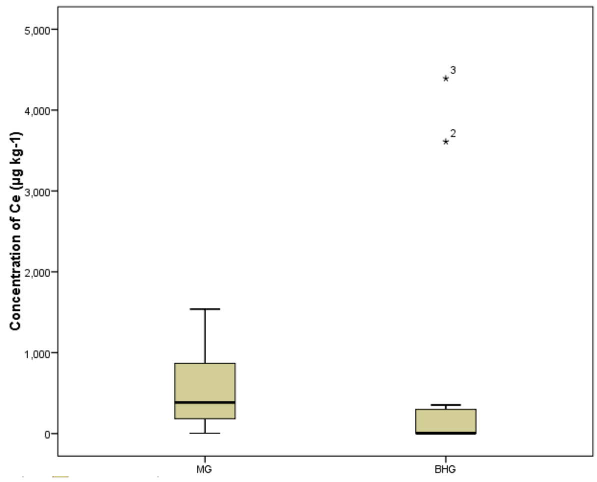
REE	Minimum	Median	Geometric mean	Maximum
Sc	250	547	724	4.811
La	260	950	162	4.463
Ce	4	4	24	4.392
Pr	7	28	70	4.557
Nd	13	83	134	4.551
Sm	6	24	66	4.584
Eu	4	12	45	4.596
Gd	5	20	55	4.574
Tb	3	10	41	4.794
Dy	6	22	62	4.886
Ho	3	10	42	4.800
Er	5	15	51	4.896
Tm	3	9	40	4.931
Yb	5	13	47	4.831
Lu	2	9	39	4.927
Y	14	78	116	4.600

Appendix Table 7. Ratio of the sum of HREE and LREE of MG (µg kg^{-1})

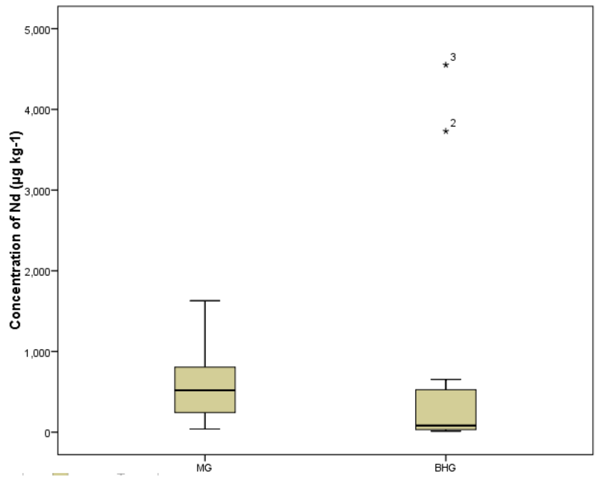
MG	ΣHREE's	ΣLREE's	ΣLREE's/ΣHREE's
IMVS183	8540	6063	0,71
IMVS65	5476	4191	0,77
IMVS97	14577	10935	0,75
IMVS279	4108	3939	0,96
IMVS511	2045	1629	0,80
IMVP123	2783	3486	1,25
IMVP325	834	1586	1,90
IMVP2927	574	2297	4,00
IMVP529	1187	4526	3,81
IMVP2631	979	3766	3,85
IMVP2733	85	487	5,72
IMVP2835	370	1325	3,58
IMVP437	354	918	2,59
IMVP239	770	1740	2,26
IMVP2549	693	2305	3,33
IMVP2451	410	1818	4,43
IMVP2353	850	3344	3,93
IMVP2255	372	1401	3,77
IMVP2157	276	1027	3,72
IMVP2059	552	2118	3,84

Appendix Table 8. Ratio of the sum of HREE and LREE of BHG (µg kg^{-1})

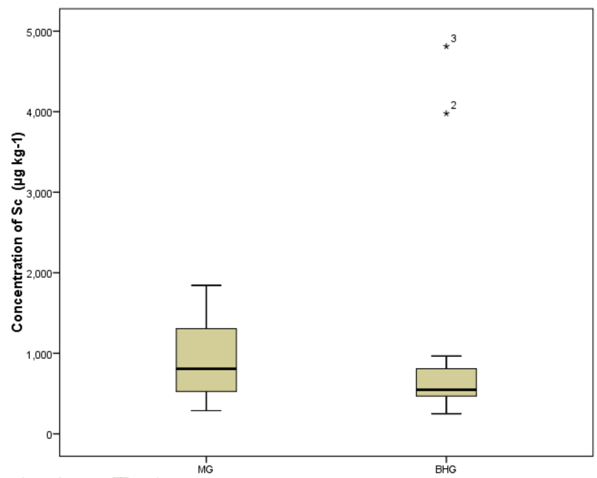
BHG	ΣHREE's	ΣLREE's	ΣLREE's/ΣHREE's
CRVT813	5408	4197	0,78
CRVT315	33736	26022	0,77
CRVT617	43239	31954	0,74
CRVT519	3847	2853	0,74
CRVT1043	214	863	4,03
CRVT1145	92	707	7,68
CRVT947	222	997	4,49
CRVT763	75	513	6,83
CRVT465	170	711	4,18
CRVT267	74	464	6,26
CRVT169	52	311	5,97
CRVT71	79	619	7,83



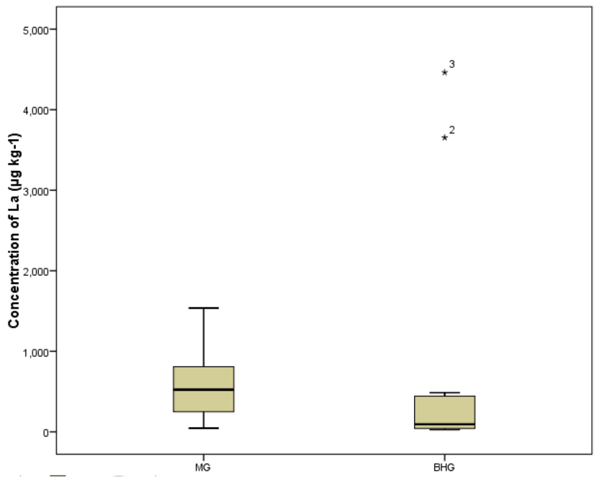
Appendix Figure 1. Concentrations of Ce in feathers



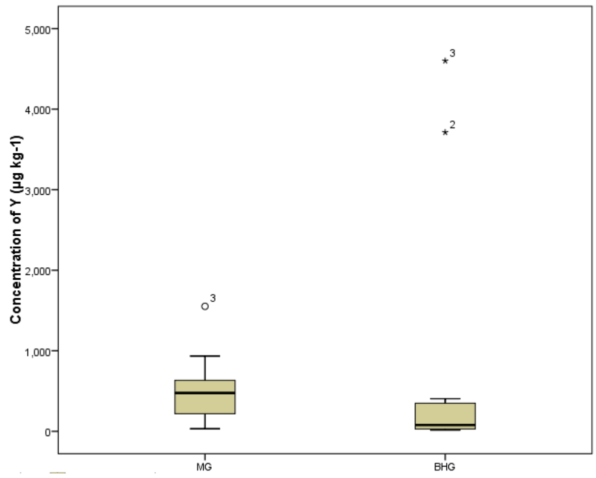
Appendix Figure 2. Concentrations of Nd in feathers



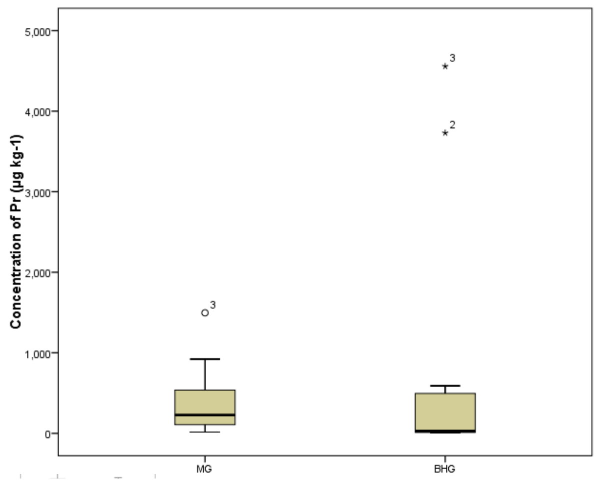
Appendix Figure 3. Concentrations of Sc in feathers



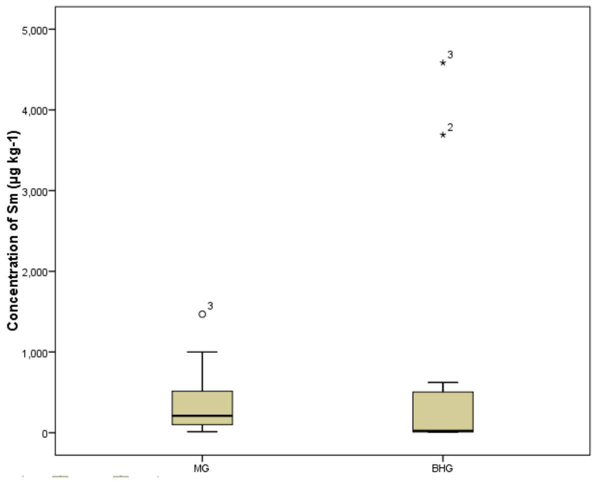
Appendix Figure 4. Concentrations of La in feathers



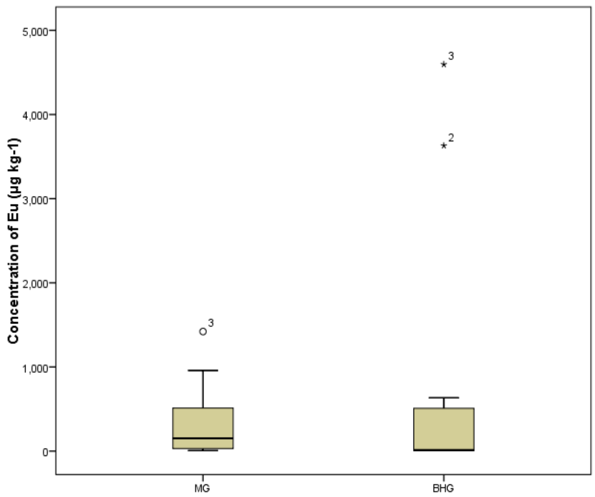
Appendix Figure 5. Concentrations of Y in feathers



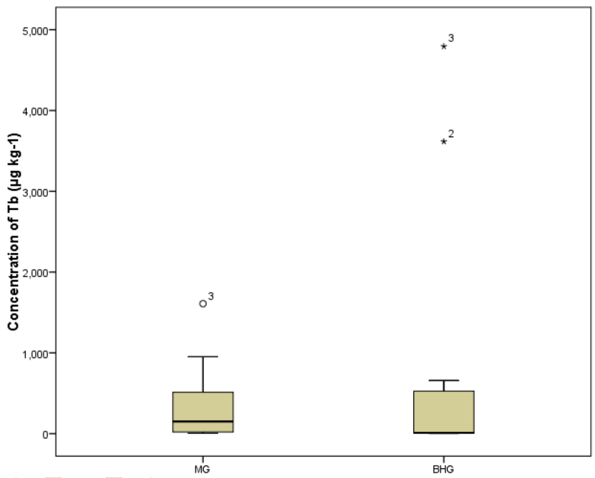
Appendix Figure 6. Concentrations of Y in feathers



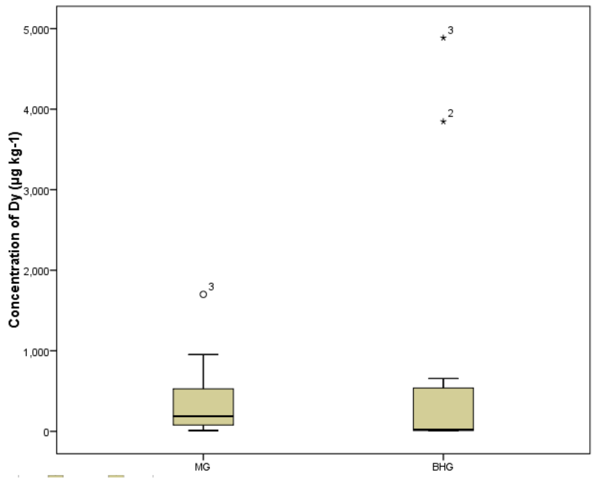
Appendix Figure 7. Concentrations of Sm in feathers



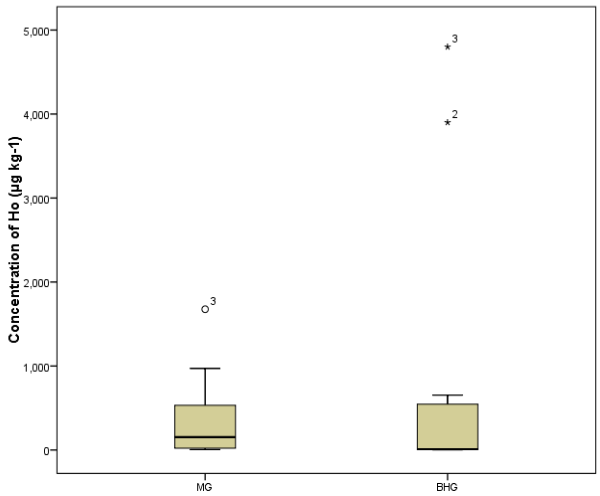
Appendix Figure 8. Concentrations of Eu in feathers



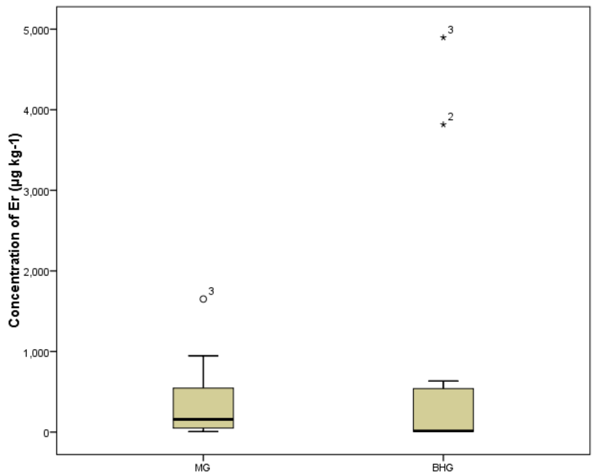
Appendix Figure 9. Concentrations of Tb in feathers



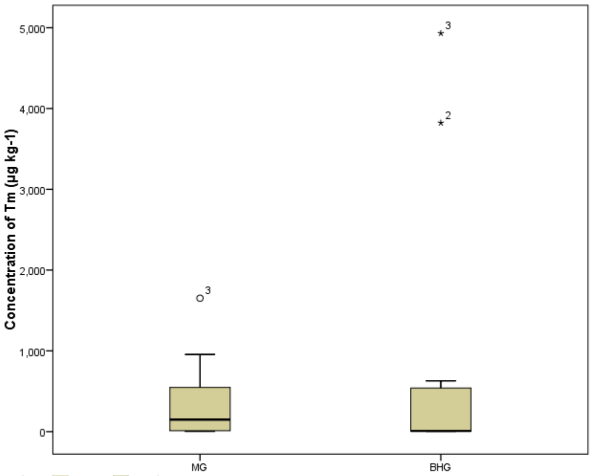
Appendix Figure 10. Concentrations of Dy in feathers



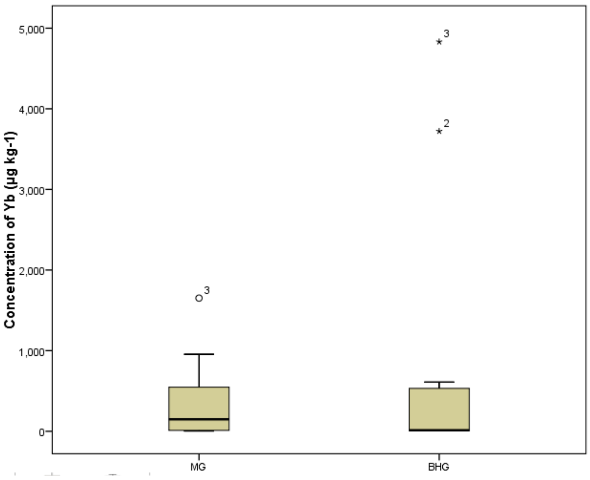
Appendix Figure 11. Concentrations of Ho in feathers



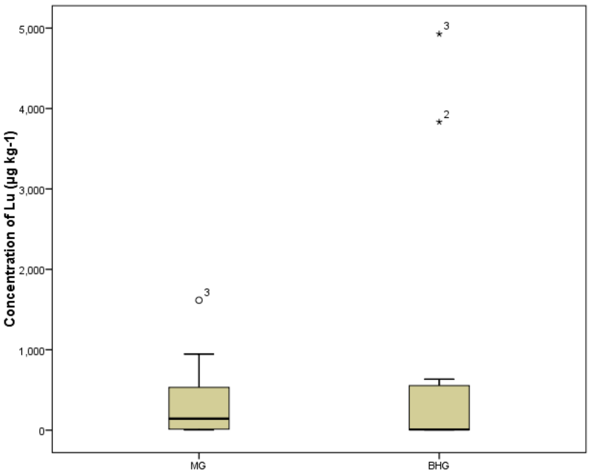
Appendix Figure 12. Concentrations of Er in feathers



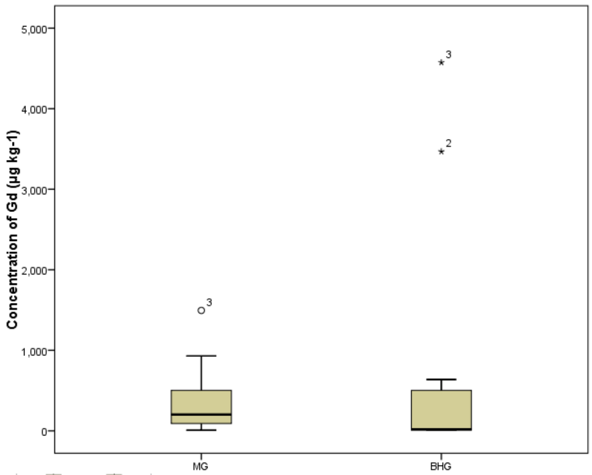
Appendix Figure 13. Concentrations of Tm in feathers



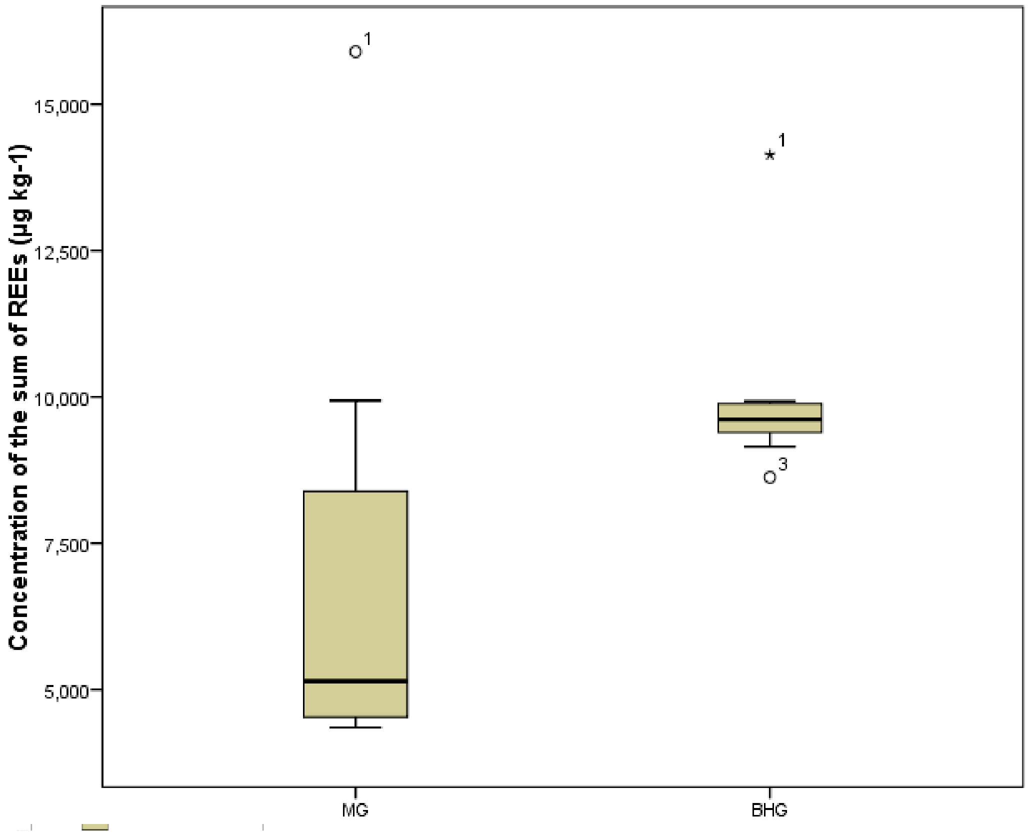
Appendix Figure 14. Concentrations of Yb in feathers



Appendix Figure 15. Concentrations of Lu in feathers



Appendix Figure 16. Concentrations of Gd in feathers



Appendix Figure 17. Concentration of the sum of REEs in the feathers of MG and BHG