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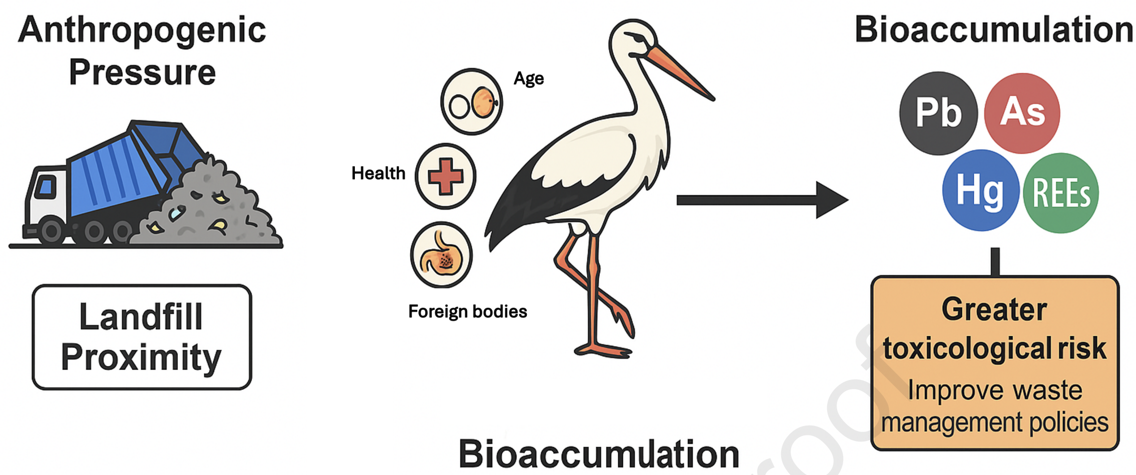
Anthropogenic Pressure
Landfill Proximity
Age
Health
Foreign bodies
Bioaccumulation
Pb
As
Hg
REEs
Greater toxicological risk
Improve waste management policies
Bioaccumulation

Bioaccumulation of Trace Elements in White Storks (Ciconia ciconia): Effects of Age, Health, and Anthropogenic Exposure

Authors

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ABSTRACT

White storks (*Ciconia ciconia*) are recognized as effective bioindicators of environmental contamination due to their wide distribution and trophic flexibility. In this study, we analyzed blood concentrations of 47 essential, toxic, and potentially toxic elements in 189 white storks from central Spain, assessing the influence of age, health status, and anthropogenic pressure on metal accumulation. Birds were grouped into chicks, fledglings, and adults. Statistical comparisons were performed using non-parametric tests and general linear models (GLMs), depending on data distribution. Our findings indicate that age significantly affects metal accumulation, with fledglings exhibiting higher concentrations of lead (Pb, $p = 0.0024$), arsenic (As, $p = 0.0012$), cadmium (Cd, $p = 0.0476$), and manganese (Mn, $p = 0.0467$) compared to adults, suggesting increased exposure through parental feeding and trophic transfer. Health status was also a critical determinant: sick individuals showed significantly elevated levels of Cd ($p < 0.0001$), Pb ($p < 0.0001$), and As ($p = 0.0166$), supporting the role of metal toxicity in avian morbidity. In terms of anthropogenic exposure, storks sampled within 30 km of landfills exhibited significantly higher concentrations of As ($p = 0.0002$), Cd ($p = 0.0118$), and Hg ($p = 0.0412$). Individuals with foreign materials in the digestive tract also showed increased Pb ($p = 0.0007$) and Cd ($p = 0.0008$) levels. Conversely, no significant differences were found between individuals from areas of high versus low human population density. These results highlight the impact of environmental pollution on metal bioaccumulation in white storks and demonstrate the influence of landfill proximity and trophic exposure on contaminant burdens. Given their role as a sentinel species, our findings underscore the need for stricter waste management policies and continued biomonitoring efforts to mitigate toxic metal exposure in wildlife.

1. INTRODUCTION

Trace elements —including heavy metals and other inorganic elements—, are widely distributed in the environment as a result of both natural processes and anthropogenic activities. These elements can be categorized as essential, toxic or potentially toxic, depending on their biological function and effects on organisms. While elements such as iron (Fe), zinc (Zn), and copper (Cu) are fundamental for physiological processes, others —such as Pb, Cd, and Hg— are highly toxic even at low concentrations (Maia et al., 2017; Pérez-López et al., 2016).

The main sources of contamination include industrial activities, mining, agriculture, fossil fuel combustion, and waste disposal (Khademi et al., 2019; Meharg et al., 2002). Industrialization and urbanization have led to a marked increase in environmental concentrations of many trace elements, as demonstrated by biomonitoring studies in both wildlife and human populations (Espín et al., 2020; Gasull et al., 2024; Henríquez-Hernández et al., 2023; Sánchez-Virosta et al., 2021, 2020). Exposure to these elements has been associated with endocrine disruption, immunosuppression, and neurotoxicity (Baos et al., 2012; de la Casa-Resino et al., 2015, 2014). While classical heavy metals such as Pb, Cd, Hg, and As have been extensively studied due to their persistence and toxicity, increasing attention is now being directed toward emerging contaminants, including rare earth elements (REEs), metalloids, and transition metals, due to their rising use in modern technologies and subsequent environmental accumulation (Espín et al., 2020; Tansel, 2017).

Wildlife biomonitoring is essential for detecting bioaccumulation trends and evaluating ecological risks. Sentinel species provide critical insights into the presence, distribution, and biological impact of environmental contaminants (Carneiro et al., 2018, 2015; Espín et al., 2021, 2020; Sánchez-Virosta et al., 2021, 2020). Recent research has documented rising contamination levels across various ecosystems, underscoring the need for effective mitigation strategies (Espín et al., 2020). Among wildlife, birds are considered valuable bioindicators of environmental pollution, due to their ecological diversity and high sensitivity to contaminants (Smits and Fernie, 2013).

The white stork (Ciconia ciconia) has a broad and flexible diet that includes a variety of natural prey, such as insects (particularly orthopterans and coleopterans), earthworms, small mammals, and, in some regions, invasive crayfish. This trophic breadth allows the species to exploit diverse environments and adjust to local prey availability (Antczak et al., 2002; Chenchouni, 2017).

In human-modified landscapes, however, many resident storks have shifted toward anthropogenic food sources, particularly at landfills, which offer predictable feeding grounds year-round. This shift is especially pronounced during the non-breeding season, when natural prey becomes scarce. Some individuals travel over 40 km to access landfills, and those nesting nearby often show strong site fidelity and reduced habitat exploration (Blanco et al., 2023; Gilbert et al., 2015; Jagiello et al., 2018). Adults tend to dominate feeding opportunities at these sites, outcompeting juveniles and affecting their foraging success. The consumption of organic waste and food scraps at landfills has partially replaced wild prey in the diet of some populations, increasing the risk of exposure to toxic elements. Moreover, ingestion of non-digestible debris such as plastics or metal fragments—frequently found in the gastrointestinal tract of affected individuals—may represent an additional route of contamination and a direct health hazard.

(Gilbert et al., 2015)

This trophic plasticity is a key determinant of contaminant exposure in white storks. Foraging behavior plays a critical role in exposure pathways, with landfill reliance notably increasing the risk of contaminant intake. Additionally, the consumption of aquatic and terrestrial prey may facilitate biomagnification of certain elements. Given these factors, assessing trace element concentrations in white storks is essential to understand their contaminant profiles and evaluate potential health impacts (de la Casa-Resino et al., 2014). Moreover, white storks are considered ideal biomonitoring species due to their broad geographic distribution, adaptability, and nesting habits in both urban and agricultural areas. Their tolerance to human activity enables researchers to assess contamination across a variety of environments. Although the white stork is not considered globally endangered (IUCN: Least Concern), it remains a species of conservation interest under Annex I of the EU Birds Directive, owing to its ecological relevance, population fluctuations, and interactions with anthropogenic environments. These characteristics, together with its accessibility for non-lethal sampling, make it a suitable focal species for biomonitoring studies (Baos et al., 2012; de la Casa-Resino et al., 2014).

Trace elements exert a wide range of toxic effects in birds. Neurotoxicants such as Pb and Hg impair cognitive function, motor coordination, and behavior (Baos et al., 2012). Pb exposure has also been associated with skeletal deformities and reduced calcium (Ca) deposition in eggshells (Smits et al., 2007). Cd and As disrupt hormone regulation, stress physiology, and immune function. A major mechanism of trace element toxicity is oxidative stress: elements such as selenium (Se), As, and Cd can induce the formation of reactive oxygen species, leading to cellular damage and DNA alterations (Baos et al., 2006b; de la Casa-Resino et al., 2015; Kamiński et al., 2007). Chronic exposure can negatively impact fitness and longevity, and population dynamics. Among the most critical consequences is reproductive impairment, with Pb and Hg exposure linked to reduced fertility, lower hatching success, and developmental abnormalities in nestlings —factors that may contribute to population declines (Pérez-López et al., 2016).

Elevated levels of Pb, Cd, and Hg have been reported in white storks inhabiting industrial and agricultural regions (de la Casa-Resino et al., 2014; Maia et al., 2017; Meharg et al., 2002; Pérez-López et al., 2016). However, despite extensive research on trace element contamination in birds, significant knowledge gaps remain concerning the exposure of white storks to both traditional heavy metals and emerging contaminants. Most prior studies have focused primarily on Pb, Cd, and Hg (Baos et al., 2006a, 2006b; de la Casa-Resino et al., 2015, 2014; Kamiński et al., 2009, 2007; Maia et al., 2017; Meharg et al., 2002; Pérez-López et al., 2016), while REEs —alongside metalloids and transition metals— have been largely overlooked in this species.

This study aims to assess the bioaccumulation of 47 essential, toxic, and potentially toxic elements in white storks inhabiting highly anthropized landscapes, characterized by intense urbanization, industrial activity, and environmental pollution. Unlike previous research conducted in rural or natural environments, this study focuses on populations exposed to multiple overlapping sources of contamination, offering a more comprehensive understanding of how human-driven pressures influence trace element exposure in avian species. By analyzing a broad panel of elements, this work provides a nuanced perspective on both classical pollutants and emerging contaminants, contributing to a holistic understanding of environmental metal burdens in complex urban ecosystems.

In doing so, we evaluated the influence of age, health status, proximity to landfills, and local human population density on element accumulation. We hypothesized that fledglings would present higher concentrations of certain metals than adults due to trophic transfer, and that sick individuals would show elevated burdens reflecting impaired detoxification or greater exposure. We also expected higher contamination levels in storks sampled near landfills or in densely populated areas, as proxies for intensified anthropogenic pressure.

2. MATERIAL AND METHODS

2.1. Study Population and Sampling

For this study, we used blood samples collected from white storks (*Ciconia ciconia*) admitted to the GREFA (Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat) Wildlife Hospital in Madrid. All individuals were brought in mainly during the breeding season (May–June), which represented the peak period of admissions, typically after being found injured or grounded by citizens, environmental agents, or local NGOs. Birds were not actively captured for research purposes. When restraint was necessary, it was performed using opaque nets or cloths to minimize stress. These storks originated from free-ranging populations across the Community of Madrid (central Spain). This autonomous region spans approximately 8,030 km^2 and is home to over 6.8 million inhabitants, making it one of the most densely populated areas in Spain. The territory encompasses the vast metropolitan area of Madrid—one of Europe's largest urban agglomerations—as well as small, sparsely populated rural villages. The sampling area thus covered a broad gradient of human influence, ranging from high-density urban and peri-urban zones to extensive agricultural landscapes and natural enclaves. Notably, the region includes major anthropogenic infrastructures such as the Valdemingómez waste treatment complex —one of the largest landfills in Spain— which plays a key role in shaping the foraging ecology of urban-adapted scavenger species. This environmental heterogeneity provided an ideal context for evaluating how varying levels of anthropogenic pressure influence metal accumulation in white storks.

A total of 189 white storks were included in the study, classified as 18 nestlings, 134 fledglings, and 37 adults, based on plumage and biometric parameters. Sampling was conducted between January 2020 and December 2021, ensuring seasonal representation across different life stages.

Upon arrival at the recovery centre, a full clinical assessment was performed on each bird. Clinical parameters included body weight (g), body condition score (BCS), hydration status, temperature and presence of lesions, external parasites or clinical signs indicative of disease Based on the presence or absence of clinical signs requiring medical intervention, individuals were classified as either clinically healthy or sick/injured. The presence of foreign materials in the digestive tract was recorded when ingestion was confirmed by regurgitation of debris (e.g., plastic, rubber bands), radiographic evidence, intraoperative retrieval, or necropsy findings. In one case lacking direct confirmation, the diagnosis was based on characteristic clinical signs of gastrointestinal impaction, including a markedly distended and firm abdomen.

Blood samples were collected from the brachial or caudal tibial vein using 23-gauge needles and sterile syringes. A 1 ml aliquot was placed in heparinized tubes and stored at –20ºC until contaminant analyses.

All samples were transported under controlled temperature conditions and analyzed at the Toxicology Laboratory of the University of Las Palmas de Gran Canaria (Spain), using validated analytical methods for metal and metalloid quantification. All samples were collected as part of routine clinical procedures performed by veterinary staff at GREFA during the admission and health evaluation of wild birds. No specific permits were required for the use of these samples, as they were obtained as residual material during standard diagnostic protocols. No animals were captured, handled, or sampled specifically for this study.

2.2. Standards and Elements

We analyzed the serum concentrations of 47 elements, encompassing five essential elements, the four primary toxic elements —arsenic, lead, mercury, and cadmium— 13 additional elements listed as priority pollutants by the Agency for Toxic Substances and Disease Registry (ATSDR, 2024), and 17 REEs and other minority elements. The REEs are increasingly regarded as emerging environmental contaminants due to their growing use in advanced technological devices (Tansel, 2017). Pure elemental standards dissolved in 5% nitric acid (HNO_3) at a concentration of 100 mg/L were obtained from CPA Chem (Stara Zagora, Bulgaria).

To minimize potential inter-element interferences, two separate 10-point calibration curves (ranging from 0.005 to 20 ng/mL) were prepared: (a) A commercial multi-element standard solution (CPA Chem, catalog number E5B8·K1.5N.L1), containing 21 elements, including essential nutrients and key heavy metals; and (b) A customized multi-element mixture prepared in our laboratory using individual standards from CPA Chem, incorporating REEs and other metallic elements (Sánchez-Virosta et al., 2021, 2020).

2.3. Sample Preparation

Blood samples were digested using a Milestone Ethos Up microwave system (Milestone, Bologna, Italy). Each sample was processed in duplicate by weighing two 250 mg aliquots into individual digestion vessels. To each aliquot, 3.5 mL of Milli-Q water and 1.25 mL of concentrated sub-boiling nitric acid (65%) were added. Microwave-assisted digestion was carried out according to the following program: Step 1: 1800 W – 100°C – 5 min; Step 2: 1800 W – 150°C – 5 min; Step 3: 1800 W – 200°C – 8 min; and Step 4: 1800 W – 200°C – 7 min.

After cooling, the digested samples were transferred into 50 mL polypropylene containers and diluted to a final volume of 7.5 mL with Milli-Q water. Prior to analysis, an aliquot from each sample was spiked with an internal standard solution containing scandium (Sc), germanium (Ge), rhodium (Rh), and iridium (Ir), each at a stock concentration of 20 mg/mL. Blank samples were prepared following the same procedure as the test samples.

2.4. Instrumental Analysis

Trace element quantification was performed using an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan), equipped with standard nickel cones, a MicroMist glass concentric nebulizer, and an Ultra High Matrix Introduction (UHMI) system. The instrument operated in robust mode, with the Integrated Sample Introduction System (ISIS) configured for discrete sampling. To minimize polyatomic interferences, the Octopole Reaction System (ORS4) was used in helium (He) mode. Prior to analysis, the system was optimized using a tuning solution containing cesium (Cs), cobalt (Co), lithium (Li), magnesium (Mg), thallium (Tl), and yttrium (Y) to ensure instrument stability and sensitivity. Element quantification was conducted using MassHunter v.4.2 ICP-MS Data Analysis software (Agilent Technologies).

The analytical method was optimized and validated following previously established protocols (González-Antuña et al., 2017; Henríquez-Hernández et al., 2017). Recovery rates ranged from 89% to 128% for REEs and technologically relevant metals, and from 87% to 118% for toxic heavy metals listed by ATSDR and other trace elements. Calibration curves exhibited excellent linearity for all elements, with regression coefficients (R^2) exceeding 0.998. Limits of quantification (LOQ) were determined by analyzing twenty blank replicates, defining the LOQ as the element concentration yielding a signal three times above the mean blank value. Accuracy and precision were assessed using fortified alkaline solutions at three concentration levels: 0.05, 0.5, and 5 ng/mL). The relative standard deviation (RSD) was generally below 8%, although elements such as Cu, nickel (Ni), Se, Fe, Ba, Zn, and samarium (Sm) exhibited slightly higher RSDs (15–16%) at the lowest concentration. At higher levels, precision improved, with RSDs consistently below 5% for all elements analyzed. Each analytical batch also included calibration blanks, a five-point calibration curve, and certified quality control samples (Seronorm™ Trace Elements Whole Blood L-2, Sero AS, Norway) injected every 20 samples to monitor accuracy and instrumental drift. Internal standard recovery was tracked in each run, and performance criteria followed international QA/QC guidelines for trace element biomonitoring.

2.5. Statistical Analysis

All statistical analyses were conducted using GraphPad Prism v10.0 (GraphPad Software, CA, USA) and Jamovi v2.4 (The Jamovi Project, 2022). Data normality was assessed using the Kolmogorov–Smirnov test. As many of the measured elements exhibited non-normal distributions and heteroscedasticity, non-parametric statistical tests were applied. The Mann–Whitney U test and Kruskal–Wallis test were used to compare non-normally distributed variables. For variables meeting normality assumptions (e.g., essential elements), parametric tests were applied, including the two-tailed Student's t-test or one-way analysis of variance (ANOVA), depending on the number of groups.

Descriptive statistics were reported as means ± standard deviations (SD) for normally distributed variables, and as medians with interquartile ranges (IQR) for non-normally distributed data. Categorical variables were summarized as proportions. For concentrations below the limit of quantification (LOQ), a single imputation approach was applied, assigning values between zero and the LOQ threshold. A two-tailed *p*-value <0.05 was considered statistically significant.

A principal component analysis (PCA) was performed to explore multivariate structure in the elemental concentration profiles. The analysis included all 47 quantified elements and employed Promax rotation. Sampling adequacy and factorability were assessed using the Kaiser–Meyer–Olkin (KMO) test and Bartlett's test of sphericity, respectively. The number of components retained was based on the scree plot and eigenvalues >1. Factor loadings ≥0.5 were considered indicative of meaningful contribution to each component. The results were used to support interpretation of co-occurring elemental patterns, particularly among rare earth elements (REEs).

To assess the combined influence of individual and environmental factors on metal accumulation, General Linear Models (GLMs) were constructed for five representative trace elements: lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and the sum of rare earth elements (REEs). The dependent variable in each model was the blood concentration of a given element, and the fixed factors included age group (chick, fledgling, adult), health status (healthy vs. sick), presence of traumatic injuries (yes/no), presence of foreign materials in the digestive tract (yes/no), human population density (high/low), and proximity to landfills (≤30 km / >30 km). Proximity to landfill was defined as the straight-line distance (km) between the nesting site and the nearest active landfill. Following Gilbert et al. (2016), who reported maximum landfill visitation distances of 28.1 km during the breeding season, we adopted a 30 km threshold to distinguish colonies considered proximal (≤30 km) or distant (>30 km) to landfills. This threshold reflects a biologically plausible movement range for foraging white storks during the reproductive period. All predictors were treated as categorical variables, and model significance was evaluated via ANOVA at a threshold of $p < 0.05$. All models were implemented as GLMs assuming normal error distribution and identity link function. Given the study design and data structure—based on independent individuals without repeated measures or clear hierarchical grouping—Linear Mixed Models (LMMs) were not appropriate.

We also explored the effect of log-transformation on highly skewed variables, such as mercury (Hg), and reanalyzed the data using parametric tests (t-test and ANOVA). As the statistical significance of the results remained unchanged, we retained the non-parametric approach for consistency across all elements and to ensure robustness in cases with small or unbalanced sample sizes.

All statistical decisions, including the choice of parametric or non-parametric methods and the structure of GLMs, were based on data distribution and study design. Full datasets, detailed statistical outputs, and metadata are publicly available through

Mendeley Data (https://doi.org/10.17632/j7tz8fd6tc.1), ensuring complete transparency and reproducibility.

3. RESULTS AND DISCUSSION

3.1. Background Levels of Essential, Toxic, and Emerging Trace Elements in White Storks.

The blood concentrations of essential, toxic, and potentially toxic elements in clinically healthy white storks with normal body condition (n = 130 out of 189; 68.8%) from central Spain provide valuable baseline data for assessing trace element exposure in wild populations inhabiting highly anthropized environments. Table 1 presents these concentrations, stratified by age group, offering a reference framework for numerous elements, including several for which no prior data exist in this species. Establishing such baseline levels is essential for future ecotoxicological research, enabling more accurate assessments of environmental contamination and its potential health impacts on avian populations.

Measured concentrations were generally consistent with previously published baseline values for white storks in other regions of Spain. For instance, blood concentrations of Fe, Cu, Zn, and Se in chicks and fledglings closely matched those reported in central and northwestern Spain (Maia et al., 2017; Pérez-López et al., 2016), supporting the reliability of these reference values across geographically distinct populations. Specifically, Fe and Cu levels aligned with those reported by Maia et al. (2017), who also documented age-dependent differences, emphasizing the physiological relevance of these elements during development. Similarly, concentrations of As, Cd, Pb, and Hg fell within previously documented ranges for both nestlings and adults in Spain (Maia et al., 2017; Pérez-López et al., 2016). Notably, Pb and Hg levels were slightly lower than those reported by Pérez-López et al. (2016), suggesting potential regional differences in environmental exposure.

Significant age-related differences in trace element concentrations were detected, with fledglings generally exhibiting levels more comparable to adults than to chicks. Kruskal-Wallis tests revealed statistically significant differences across age groups for several elements. Among essential elements, Fe and Cu concentrations were significantly lower in chicks relative to fledglings and adults, likely reflecting physiological demands and dietary changes during early development. This trend is consistent with findings in white storks and other avian species, which show distinct patterns of metal assimilation between younger and older individuals (Baos et al., 2006a; Pineda-Pampliega et al., 2021; Scheuhammer, 1987).

Among toxic elements, As concentrations were significantly lower in adults compared to chicks and fledglings, potentially indicating reduced exposure over time or more efficient detoxification in older birds. Conversely, Pb levels peaked in fledglings, exceeding those found in both chicks and adults. This may reflect elevated environmental exposure during the post-fledging period when birds shift to independent foraging, —a stage in which oportunistic behavior and consumption of contaminated prey may increase metal accumulation (Meharg et al., 2002). The elevated Pb levels in fledglings are consistent with prior findings highlighting the susceptibility of juvenile white storks in urbanized environments to metal accumulation due to their exploratory behavior and reliance on human-modified food sources (de la Casa-Resino et al., 2014).

This study also reports, for the first time, baseline values of REEs in white storks. While classical toxic metals such as Pb and Cd have been extensively monitored in avian species, much less is known about the accumulation and biological effects of REEs. In our dataset, the sum of REEs was highest in chicks and declined significantly in fledglings and adults. This may be linked to differences in dietary sources or age-related variations in metal metabolism. Given the rising environmental presence of REEs resulting from their extensive use in modern technological processes, elucidating their bioaccumulation patterns in wildlife has emerged as a growing research priority (Picone et al., 2024, 2022; Sánchez-Virosta et al., 2020). Previous research has documented REE accumulation in avian species such as the eagle owl (*Bubo bubo*), Kentish plover

(*Charadrius alexandrinus*), Sandwich tern (*Thalasseus sandvicensis*), Humboldt penguin (*Spheniscus humboldti*), particularly among urban-adapted omnivorous birds (Brown et al., 2019; Picone et al., 2024, 2022; Sánchez-Virosta et al., 2020; Squadrone et al., 2019). These findings underscore the need for continued monitoring and investigation into the potential toxic effects of REE exposure in birds.

The data presented in Table 1 highlight the importance of age group differentiation when assessing metal exposure in avian species. While essential elements such as Se and Zn remained relatively stable, several toxic elements exhibited distinct age-related patterns. Notably, fledglings showed elevated levels of Pb and Cd, which may reflect recent shifts toward more opportunistic foraging behaviors combined with an immature detoxification capacity, rather than cumulative exposure. These findings emphasize the dynamic nature of metal accumulation throughout development (Baos et al., 2006a, 2006b; Blanco et al., 2023; de la Casa-Resino et al., 2014; Pérez-López et al., 2016).

In summary, this study provides foundational reference values for a broad range of trace elements in white storks, enhancing our understanding of exposure patterns and physiological regulation. The age-related differences observed reinforce the importance of considering developmental stages when assessing contaminant burdens in avian species, particularly those inhabiting human-modified environments.

To further explore the structure of elemental co-occurrence patterns, a principal component analysis (PCA) was performed using the complete set of 47 trace elements (Supplementary Figure 1). The analysis yielded five components with eigenvalues greater than one, collectively explaining 51.0% of the total variance. The first component, which alone accounted for 25.1% of the variance, grouped nearly all rare earth elements (REEs), suggesting a shared environmental origin and supporting their treatment as a unified group in subsequent analyses. In contrast, the remaining components lacked a clear toxicological or ecological interpretation, as they clustered both essential and toxic elements without a consistent pattern. This result highlights the complexity of environmental exposure in anthropized landscapes and underscores the importance of combining univariate and multivariate approaches when evaluating contaminant profiles in wildlife.

3.2. Influence of Health Status on Blood Elements Levels in White Storks.

Health status had a significant impact on the blood concentrations of several essential and toxic elements in white storks (see Table 2, Table 3, and Figure 1). Individuals classified as sick or injured at the time of sampling exhibited elevated concentrations of multiple toxic elements, supporting a potential association between contaminant exposure and adverse health outcomes in white storks.

3.2.1. Health Status and Elemental Concentrations

Table 2 compares elemental concentrations between clinically healthy and sick white storks. Concentrations of Pb, Cd, and As were significantly higher in sick individuals, suggesting that chronic exposure to these toxic metals may contribute to health deterioration. These findings are consistent with previous research in white storks, where Pb and Cd exposure has been linked to oxidative stress, immune suppression, and metabolic dysfunction (Baos et al., 2006a; de la Casa-Resino et al., 2015). Pb toxicity in birds is well-documented, and has been linked to neurological impairment, immunosuppresion, and reduced reproductive success (Maruyama et al., 2024; Pain et al., 2019; Plaza et al., 2018). Similarly, Cd is known to bioaccumulate in soft tissues, —particularly the kidneys—, where it may induce renal damage and metabolic disruption (Baos et al., 2006a; Rahman et al., 2017).

Figure 1A further supports this pattern, showing that the cumulative burden of the four most toxic elements (As, Hg, Cd, Pb) was significantly higher in sick individuals (p = 0.0007). This finding suggests a strong association between contaminant exposure and compromised health status, reinforcing the potential role of these elements as drivers of disease susceptibility in white storks. Previous studies have consistently linked elevated Pb and Cd levels with reduced immune competence, renal impairment, and reproductive dysfunction in birds inhabiting contaminated environments

(Scheuhammer, 1987). Increased concentrations of Pb and Cd in avian tissues have also been associated with oxidative stress, as evidenced by elevated biomarkers such as malondialdehyde (MDA) and altered antioxidant enzyme activities, which compromise overall health (de la Casa-Resino et al., 2015). Similar interactive effects between metal exposure and oxidative stress biomarkers have also been reported in plants, supporting the notion that trace element toxicity often manifests through disruption of antioxidant defenses (Saeed et al., 2024). Additionally, elevated leukocyte counts —indicative of chronic inflammation and immunological stress— have been reported in birds exposed to high levels of Pb and Cd, suggesting a systemic impact of these contaminants on avian health (Bauerová et al., 2020). Furthermore, tissue accumulation patterns in multiple bird species identify the kidneys and liver as primary targets of heavy metal toxicity, supporting the hypothesis that these elements may act synergistically to compromise the health of exposed individuals (Khwankitrittikul et al., 2024).

Interestingly, Se and Fe levels were significantly lower in sick birds, potentially indicating impaired antioxidant defenses or disruptions in iron metabolism associated with illness. Se is a key micronutrient involved in antioxidant protection and plays a vital role in mitigating oxidative damage induced by heavy metal toxicity; its depletion may thus exacerbate the physiological consequences derived from heavy metal exposure (Pineda-Pampliega et al., 2021). Similarly, reduced Fe concentrations may indicate dysregulation of iron metabolism, commonly associated with chronic diseases and inflammatory conditions (Pineda-Pampliega et al., 2021; Sánchez-Virosta et al., 2020). In addition, elevated manganese (Mn) levels observed in sick individuals raise concerns about its potential neurotoxic effects. Excess Mn has been associated with neurological impairment and inflammatory responses in avian species, primarily through Th1/Th2 immune imbalance and the activation of pro-inflammatory pathways (Miao et al., 2021). The elevated concentrations of REEs, observed in sick individuals further highlight the potential health risks posed by these emerging contaminants. The environmental prevalence of REEs has increased markedly due to their widespread industrial applications, particularly those associated with electronic waste (Sánchez-Virosta et al., 2020; Tansel, 2017).

Supplementary Table 1 presents elemental concentrations in relation to body condition (normal vs. underweight/emaciated). Although no consistent trend emerged, certain essential elements —notably Fe and Se— were more abundant in birds with normal body condition, supporting their role in maintaining physiological balance and metabolic resilience. Conversely, higher concentrations of gold (Au) and vanadium (V) were detected in underweight individuals —an unexpected finding that warrants further investigation. Surprisingly, As levels were significantly higher in birds with good body condition, a result that is difficult to interpret and may reflect differences in diet or environmental exposure.

3.2.2. Presence of Foreign Materials in the Digestive Tract and Metal Accumulation

Table 3 and Figure 1 illustrate the effect of foreign material ingestion on elemental concentrations. Birds with detectable non-food materials in their digestive tracts exhibited significantly higher concentrations of Pb and Cd compared to individuals without such materials. These findings suggest that the ingestion of contaminated items —such as metallic debris or polluted prey— may directly contribute to metal bioaccumulation. The presence of foreign objects in the digestive tract has previously been associated with elevated heavy metal burdens in scavengers and waterbirds, likely due to the ingestion of anthropogenic materials (Gilbert et al., 2015). In particular, landfills and urban environments represent key sources of metal exposure for white storks, as contaminated waste can provide a direct pathway for the intake of toxic elements (Bjedov et al., 2024).

Figure 1B further supports this association, showing that the cumulative burden of the four most toxic elements (As, Hg, Cd, Pb) was significantly higher in birds with foreign materials present in their digestive tract ($p = 0.0021$). This finding is consistent with previous research showing that foraging behavior, particularly in anthropogenic environments, plays a key role in trace element exposure. Ingestion of non-food or contaminated materials—common among birds foraging at landfills—has been associated with increased accumulation of toxic metals, either directly or through trophic pathways (Abbasi et al., 2015; Kar et al., 2018; Loera et al., 2024; Xia et al., 2021).

These findings underscore the dual impact of direct environmental exposure and the ingestion of anthropogenic materials on heavy metal accumulation in white storks. The strong association between metal burden and dietary habits highlights the need for improved waste management and stricter environmental regulations. Continued monitoring of contaminant levels in wildlife is essential for assessing ecosystem health and mitigating potential ecological risks.

3.3. Influence of Anthropogenic Pressure on Elemental Levels in White Storks.

Environmental exposure to trace elements is strongly influenced by human activities, particularly in urbanized areas and near waste disposal sites. To assess the impact of anthropogenic pressure, we analyzed the blood concentrations of essential and toxic elements in white storks based on two parameters: human population density (Table 4, Figure 2A) and proximity to landfills (Table 5, Figure 2B). The results revealed significant associations between metal accumulation and both of these environmental variables.

3.3.1. Effect of Urbanization

Table 4 compares elemental concentrations in white storks from municipalities with high versus low human population densities, using the median population density (1500 inhabitants/km^2) of the sampled areas as the cut-off. The results show that As and Cd concentrations were significantly higher in storks from more densely populated areas (p = 0.043 and p = 0.044, respectively), suggesting increased exposure to anthropogenic sources such as industrial emissions, traffic-related pollution, and contaminated food sources. These findings are consistent with previous research on metal accumulation in urban-dwelling birds, where heavy traffic and industrial activity have been identified as major contributors to both airborne contaminants and dietary contaminants (Kar et al., 2018).

Additionally, V concentrations were significantly higher in storks from high-density urban areas (p = 0.020), aligning with known primary sources, including fossil fuel combustion and industrial processes (Khademi et al., 2019). Despite these individual differences, Figure 2A shows that the cumulative burden of the four most toxic elements (As, Hg, Cd, Pb) did not differ significantly between high- and low-density areas. This suggests that urbanization alone may not be the primary driver of heavy metal exposure in white storks and instead highlights the role of dietary habits and habitat use in shaping exposure patterns (Bauerová et al., 2020).

3.3.2. Effect of Proximity to Landfills

Table 5 presents a comparative analysis of elemental concentrations in storks sampled at distances <30 km versus >30 km from an urban solid waste landfill. In contrast to population density, proximity to landfills had a much stronger influence on metal accumulation, with significant increases observed for multiple toxic elements in birds foraging near these waste sites.

Storks living closer to landfills exhibited significantly elevated concentrations of Hg, As, Cd, and Pb ($p < 0.05$ for all comparisons), reinforcing the notion that landfills are major sources of environmental contamination for foraging birds. The accumulation of these elements has also been documented in other avian species that regularly exploit landfills —particularly scavengers that consume organic waste contaminated with industrial and domestic pollutants (Bjedov et al., 2024; Gilbert et al., 2015).

In addition to the classical toxic metals, storks foraging near landfills exhibited significantly higher levels of aluminum (Al), uranium (U), and REEs ($p < 0.05$ for all comparisons), none of which were associated with urban population density. The presence of these contaminants suggests exposure to industrial residues, electronic waste, and other anthropogenic pollutants commonly found in landfill environments. This pattern is further supported by Figure 2B which shows that the cumulative burden of highly toxic elements was significantly greater in storks residing near landfills ($p = 0.045$).

These results highlight the ecological risks associated with landfill foraging behavior in white storks and other urban-adapted bird species. Given the widespread reliance of storks on waste sites —particularly in regions where landfill food availability influences migratory behavior— there is an urgent need to implement targeted measures to reduce metal contamination in waste streams. Policy interventions, such as enhanced landfill waste treatment, stricter regulation of industrial metal emissions, and efforts to reduce the heavy metal content of consumer products could play a critical role in mitigating these ecological risks.

3.3.3. Combined Influence of Multiple Predictors on Metal Accumulation

To better capture the complexity of environmental exposure, General Linear Models (GLMs) were applied to assess the combined effects of biological and environmental predictors on blood concentrations of five key trace elements: Pb, Cd, As, Hg, and REEs. Model outputs are summarized in Supplementary Table S2.

The GLMs confirmed and refined the patterns observed in univariate analyses. Proximity to landfills emerged as a significant predictor of elevated concentrations for As and REEs ($p = 0.0026$ and $p = 0.0223$, respectively), underscoring the relevance of landfill foraging as an exposure route. Age group was also a key determinant, with fledglings and chicks exhibiting higher concentrations of As and REEs, respectively. In the case of Pb, the presence of foreign materials in the digestive tract was the only significant predictor ($p = 0.040$), supporting the hypothesis that ingestion of anthropogenic debris increases contaminant burdens. By contrast, health status, trauma, and human population density did not significantly influence element concentrations in any of the models. These findings highlight landfill exposure and foraging behavior as the primary drivers of trace element accumulation in white storks, with age-related factors modulating susceptibility to specific contaminants.

3.4. Ecological and Conservation Implications

Our findings underscore the role of white storks as effective bioindicators of environmental pollution, given their sensitivity to heavy metal exposure and their strong association with human-modified habitats. Elevated concentrations of toxic elements — such as Pb, Cd, As, Hg, Al, U, and REEs— in individuals foraging near landfills clearly demonstrate that anthropogenic feeding grounds significantly amplify contaminant burdens in avian populations. Notably, the detection of emerging contaminants such as REEs —previously associated with oxidative stress, DNA damage, and cytotoxicity effects (Brouziotis et al., 2022; Pagano et al., 2015)— raises additional ecological concerns due to their increasing environmental prevalence and potential impacts on wildlife health.

These results highlight the urgent need for targeted conservation and environmental management interventions. Stricter waste management policies, —including improved processing at landfill sites and enhanced recycling practices— could substantially reduce wildlife exposure to toxic contaminants. In parallel, policies aimed at reducing the use of heavy metals in consumer products, enforcing tighter control on industrial emissions, and regulating electronic waste disposal form core components of comprehensive environmental protection strategies.

Establishing ongoing biomonitoring programs focused on sentinel species such as the white stork can provide early detection of ecological risks, and support timely, evidence-based interventions. Future research should further investigate the sublethal and chronic effects of trace element exposure, with particular emphasis on reproductive outcomes, behavioral changes, and long-term population-level impacts. Collaborative efforts involving conservationists, policymakers, waste management authorities, and the scientific community are essential for developing integrated strategies to mitigate environmental risks and safeguard avian populations in increasingly anthropized landscapes. These findings support specific policy recommendations, including the improvement of landfill waste treatment processes, regulation of emerging contaminants such as REEs, and the implementation of long-term wildlife monitoring programs to track exposure trends and guide adaptive conservation strategies.

4. CONCLUSIONS

This study provides critical baseline data on the concentrations of essential, toxic, and emerging elements in white storks (*Ciconia ciconia*), revealing significant variation associated with age, health status, ingestion of anthropogenic materials, and proximity to landfills. Our results confirm the heightened vulnerability in fledglings —likely linked to dietary transitions— and underscore critical windows for contaminant exposure during development. Moreover, the clear associations between poor health status, presence of foreign materials in the digestive tract, and elevated concentrations of toxic metals —including Pb, Cd, As, Hg, Al, U, and REEs— highlight the direct health risks posed by environmental pollution.

The strong influence of landfill proximity on contaminant burdens reinforces the urgent need for targeted policy measures to reduce wildlife exposure. Improved waste management practices, stricter environmental regulations, and reduced use of heavy metals in consumer products are essential to minimize contamination sources affecting storks and other avian sentinel species.

Continued biomonitoring of white stork populations is vital to support early-warning systems for ecosystem health and to inform evidence-based conservation strategies. Future research should further investigate the long-term physiological, reproductive, and demographic impacts of chronic exposure to both traditional and emerging pollutants, particularly in highly anthropized environments. Ultimately, integrating robust scientific evidence into proactive environmental management and conservation frameworks is crucial to safeguard biodiversity and ecosystem integrity in the face of escalating anthropogenic pressures.

5. FIGURE CAPTIONS

Figure 1. Total concentration (ng/mL) of highly toxic elements (Pb, As, Cd, Hg, and Tl) in the blood of white storks (Ciconia ciconia), categorized by **(A)** health status (n = 149 healthy, n = 34 sick) and **(B)** presence of foreign materials in the gastrointestinal tract (n

= 121 with absence, n = 57 with presence). Differences were analyzed using the Mann–Whitney U test. Asterisks denote statistically significant differences: *$p < 0.05$; ***$p < 0.001$..

Figure 2. Total concentration (ng/mL) of highly toxic elements (Pb, As, Cd, Hg, and Tl) in the blood of white storks (Ciconia ciconia), categorized by **(A)** human population density in the nesting area (<1500 vs >1500 inhabitants/km^2; n = 138 and n = 45, respectively), and **(B)** distance to the nearest landfill (>30 km vs <30 km; n = 102 and n = 81, respectively). Differences were analyzed using the Mann–Whitney U test. Asterisks denote statistically significant differences: *$p < 0.05$; n.s., not significant.

Supplementary Figure 1. Scree plot showing eigenvalues obtained from a principal component analysis (PCA) based on 47 trace elements measured in the blood of white storks (Ciconia ciconia). The analysis was performed using Promax rotation and included all individuals with complete elemental data. The first five components exceeded the eigenvalue threshold of 1 and together explained 51.0% of the total variance. The steep decline in eigenvalues after the first component supports its dominant contribution, primarily driven by rare earth elements (REEs).

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Table 1. Concentration of essential and toxic elements in whole blood of chicks, fledglings, and adults of white storks (*Ciconia ciconia*) from central Spain. Results are expressed in ng/g of blood (fresh weight).

	Chicks (n = 10)			Fledglings (n = 93)			Adults (n = 27)			Significance			
Essential element	**Mean ± SD**	**Median**	**p25 –p75**	**Mean ± SD**	**Median**	**p25 –p75**	**Mean ± SD**	**Median**	**p25 –p75**	**P ***	**P #**	**P †**	**P ††**
Essential elements													
Fe	282458 ± 113888	229555	201739 - 385421	308784 ± 46239	306475	280274 - 336070	304417 ± 57198	302885	270286 - 338103	n.s.	0.0167	0.0432	n.s.
Cu	330.2 ± 169.8	256.7	210.4 – 385.2	380.5 ± 107.8	358.3	314.9 – 436.9	385.9 ± 96.7	398.0	305.7 – 447.4	0.0462	0.0247	0.0138	n.s.
Zn	4017 ± 1865	3539	3083 - 4130	3867 ± 998	3816	3305 - 4324	4027 ± 1449	3874	2983 - 5304	n.s.	n.s.	n.s.	n.s.
Se	477.4 ± 174.9	388.9	350.5 – 602.8	503.1 ± 136.3	485.0	400.0 – 594.8	551.7 ± 183.0	537.5	427.2 – 649.5	n.s.	n.s.	n.s.	n.s.
Mn	50.6 ± 31.6	44.1	27.9 – 57.3	34.4 ± 19.3	30.8	24.6 – 38.6	33.7 ± 13.7	33.4	22.9 – 43.3	0.0467	0.0132	0.0389	n.s.
Major toxic elements													
As	74.2 ± 64.1	62.5	36.9 – 100.5	71.2 ± 50.2	58.6	32.7 – 95.3	41.0 ± 43.4	34.9	1.9 – 152.0	0.0012	n.s.	0.0411	0.0002
Cd	0.81 ± 0.62	0.73	0.34 – 1.07	1.04 ± 1.33	0.74	0.43 – 1.15	0.81 ± 1.01	0.38	0.11 – 1.06	0.0476	n.s.	n.s.	0.0189
Hg	78.2 ± 85.4	48.8	32.9 – 104.9	77.2 ± 58.6	63.0	41.0 – 94.4	92.5 ± 128.6	67.7	4.3 – 108.1	n.s.	n.s.	n.s.	n.s.
Pb	106.4 ± 59.3	94.5	58.8 – 94.5	155.7 ± 100.6	135.8	94.0 – 194.2	116.6 ± 90.6	95.8	58.7 – 146.9	0.0024	0.0186	n.s.	0.0034
Other toxic or potentially elements													
Al	48.2 ± 148.0	10.1	8.8 – 17.6	33.9 ± 125.0	9.5	5.2 – 12.4	21.5 ± 48.6	9.5	8.5 – 13.8	n.s.	n.s.	n.s.	n.s.
Au	0.13 ± 0.07	0.14	0.11 – 0.18	0.06 ± 0.16	0.01	0.0 – 0.01	0.09 ± 0.13	0.01	0.0 – 0.18	< 0.0001	< 00001	n.s.	0.0028
Ba	16.3 ± 33.7	8.3	0.31 – 14.6	5.3 ± 15.7	0.4	0.21 – 0.62	3.4 ± 5.2	0.5	0.18 – 5.74	0.0366	0.0147	0.0081	n.s.
Co	2.02 ± 1.82	1.38	1.03 – 2.25	0.92 ± 0.43	0.83	0.65 – 1.12	1.65 ± 2.92	0.66	0.47 – 1.67	< 0.0001	< 0.0001	0.0011	n.s.
Cr	0.75 ± 0.96	0.42	0.0 – 1.31	1.08 ± 3.55	0.58	0.02 – 1.15	0.81 ± 0.86	0.59	0.02 – 2.71	n.s.	n.s.	n.s.	n.s.
Ni	1.28 ± 0.93	0.91	0.42 – 2.31	8.78 ± 33.2	0.96	0.47 – 2.21	9.32 ± 45.38	0.75	0.46 – 1.33	n.s.	n.s.	n.s.	n.s.
Mo	18.56 ± 5.77	18.91	14.84 – 23.61	17.15 ± 4.84	17.02	13.41 – 19.86	14.54 ± 5.11	13.15	11.37 – 17.37	0.0024	n.s.	0.0192	0.0013
Sb	0.15 ± 0.26	0.0	0.0 – 0.26	0.93 ± 5.33	0.08	0.0 – 0.54	1.16 ± 3.04	0.13	0.03 – 0.89	0.0208	0.0387	0.0084	n.s.
Sn	15.94 ± 12.81	14.49	5.51 – 22.37	6.02 ± 10.81	3.12	0.08 – 8.62	7.11 ± 10.6	4.06	0.09 – 8.71	0.0014	0.0002	0.0052	n.s.
Sr	63.29 ± 21.81	62.54	50.51 – 72.72	58.59 ± 26.08	55.72	39.75 – 71.68	38.37 ± 21.05	34.57	19.68 – 47.87	< 0.0001	n.s.	0.0002	< 0.0001
Tl	0.15 ± 0.08	0.18	0.08 – 0.21	0.04 ± 0.08	0.0	0.0 – 0.01	0.06 ± 0.09	0.01	0.0 – 0.13	< 0.0001	< 0.0001	0.0031	0.0068
U	0.78 ± 2.21	0.0	0.0 – 0.19	2.63 ± 3.98	0.07	0.0 – 4.52	3.06 ± 3.53	2.28	0.04 – 4.72	0.0329	n.s.	0.0031	n.s.
V	6.26 ± 5.77	5.11	1.85 – 9.02	3.86 ± 4.53	3.18	0.0 – 6.65	2.01 ± 3.09	0.05	0.0 – 4.91	0.0076	n.s.	0.0042	0.0132
Sum REE [a]	0.69 ± 3.30	0.75	0.39 – 1.69	0.38 ± 0.48	0.16	0.05 – 0.56	0.33 ± 0.38	0.20	0.06 – 0.46	0.0012	0.0002	0.0007	n.s.

[a] Sum of individual concentrations of Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, and Yb.

* Statistical significance based on the Kruskal-Wallis test.

Mann-Whitney U test significance: chicks vs.fledglings.

† Mann-Whitney U test significance: chicks vs. adults.

†† Mann-Whitney U test significance: fledglings vs. adults.

Table 2. Concentration of essential and toxic elements in whole blood of white storks (*Ciconia ciconia*) from central Spain, according to health status at the time of sampling. Results are expressed in ng/g of blood (fresh weight).

	Sick (n = 46)				Healthy (n = 144)				
Essential element	**Mean ± SD**	**Median**	**p25 –p75**		**Mean ± SD**	**Median**	**p25 –p75**		**P ***
Essential elements									
Fe	307088 ± 58753	302726	280550 - 335923		305462 ± 56967	305718	265227 - 336734		n.s.
Cu	365.4 ± 101.9	336.4	311.6 – 436.6		378.9 ± 113.9	365.7	305.7 – 439.6		n.s.
Zn	3723 ± 830.1	3587	3211 - 4142		3938 ± 1127	3841	3245 - 4405		n.s.
Se	465.7 ± 161.1	425.2	344.5 – 591.7		517.0 ± 148.6	490.7	403.5 – 606.4		0.0454
Mn	45.8 ± 32.0	35.3	27.7 – 48.7		34.2 ± 17.4	30.8	23.5 – 41.9		0.0016
Major toxic elements									
As	79.6 ± 49.4	75.3	36.9 – 105.3		63.4 ± 51.6	50.5	28.3 – 89.2		0.0166
Cd	1.8 ± 2.4	1.1	0.7 – 1.9		0.9 ± 0.9	0.6	0.3 – 1.1		< 0.0001
Hg	81.5 ± 62.5	69.0	53.6 – 88.8		80.2 ± 81.8	61.3	37.9 – 96.3		n.s.
Pb	197.7 ± 92.3	187.8	134.9 – 240.8		136.1 ± 95.8	116.2	81.7 – 172.6		< 0.0001
Other toxic or potentially toxic elements									
Al	73.6 ± 234.5	10.3	7.4 – 15.9		26.9 ± 86.3	9.5	6.7 – 12.7		n.s.
Au	0.06 ± 0.18	0.0	0.0 – 0.01		0.12 ± 0.29	0.01	0.0 – 0.12		0.0382
Ba	3.0 ± 6.2	0.3	0.1 – 8.3		6.1 ± 17.8	0.4	0.2 – 7.3		n.s.
Co	1.2 ± 0.6	1.1	0.7 – 1.3		1.2 ± 1.6	0.8	0.6 – 1.2		0.0047
Cr	1.2 ± 0.9	1.1	0.4 – 1.9		1.0 ± 3.2	0.5	0.0 – 1.2		0.0015
Ni	5.7 ± 8.4	2.1	0.7 – 2.8		8.6 ± 36.8	0.7	0.5 – 1.8		0.0013
Mo	18.1 ± 6.8	17.5	13.3 – 21.0		16.6 ± 4.8	16.4	13.0 – 19.7		n.s.
Sb	3.0 ± 12.7	0.0	0.0 – 0.4		0.7 ± 1.6	0.1	0.0 – 0.4		n.s.
Sn	5.9 ± 6.2	4.9	2.8 – 6.6		7.3 ± 11.8	3.4	0.0 – 9.7		n.s.
Sr	60.3 ± 25.1	56.1	45.1 – 69.8		54.1 ± 26.2	52.0	36.0 – 69.1		n.s.
Tl	0.06 ± 0.09	0.0	0.0 – 0.2		0.05 ± 0.08	0.0	0.0 – 0.09		n.s.
U	1.4 ± 2.7	0.0	0.0 – 0.4		2.6 ± 3.9	0.3	0.0 – 4.4		n.s.
V	6.7 ± 5.2	6.7	3.3 – 8.5		3.3 ± 4.2	0.2	0.0 – 5.9		< 0.0001
Sum REE [a]	0.8 ± 1.4	0.6	0.0 – 0.7		0.5 ± 1.1	0.2	0.0 – 0.5		0.0455

[a] Sum of individual concentrations of Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, and Yb.

* Statistical significance based on the Mann-Whitney U test.

Table 3. Concentration of essential and toxic elements in whole blood of white Storks (*Ciconia ciconia*) from central Spain, according to the presence of foreign materials in the digestive tract. Results are expressed in ng/g of blood (fresh weight).

	Presence (n = 41)				Absence (n = 149)				
Essential element	**Mean ± SD**	**Median**	**p25 –p75**		**Mean ± SD**	**Median**	**p25 –p75**		**P ***
Essential elements									
Fe	293475 ± 50203	300175	278663 - 323838		307111 ± 57777	306288	269297 - 337774		n.s.
Cu	355.3 ± 99.5	332.4	298.2 – 435.3		379.9 ± 113.7	365.8	306.7 – 439.9		n.s.
Zn	3671 ± 1001	3481	3041 - 4089		3941 ± 1205	3857	3257 - 4311		0.0428
Se	443.1 ± 144.8	416.7	343.2 – 576.4		518.8 ± 149.8	491.7	403.4 – 608.6		0.0114
Mn	40.4 ± 21.1	34.6	28.1 – 47.6		35.1 ± 19.9	30.9	23.7 – 42.4		0.0117
Major toxic elements									
As	66.3 ± 42.5	65.4	32.5 – 92.3		65.2 ± 52.4	55.0	28.6 – 90.2		n.s.
Cd	1.3 ± 1.0	1.0	0.7 – 1.8		0.9 ± 1.2	0.6	0.3 – 1.1		0.0008
Hg	70.5 ± 66.6	62.5	40.1 – 78.3		81.6 ± 81.0	63.0	38.3 – 98.9		n.s.
Pb	188.9 ± 104.1	164.6	128.6 – 234.5		138.2 ± 95.3	117.4	87.8 – 177.9		0.0007
Other toxic or potentially toxic elements									
Al	29.0 ± 80.9	9.9	8.0 – 15.6		33.1 ± 119.3	9.5	6.4 – 12.8		n.s.
Au	0.03 ± 0.05	0.0	0.0 – 0.07		0.08 ± 0.16	0.01	0.0 – 0.11		n.s.
Ba	4.4 ± 7.0	0.3	0.1 – 8.9		6.0 ± 17.6	0.4	0.2 – 7.4		n.s.
Co	1.2 ± 0.6	1.0	0.7 – 1.3		1.2 ± 1.6	0.8	0.6 – 1.2		n.s.
Cr	1.3 ± 1.0	1.2	0.3 – 1.9		1.0 ± 3.2	0.5	0.0 – 1.1		0.0022
Ni	5.5 ± 8.5	2.1	0.8 – 2.6		8.6 ± 36.5	0.8	0.5 – 1.8		0.0012
Mo	17.9 ± 6.8	17.2	13.3 – 20.9		16.6 ± 4.8	16.6	12.9 – 19.8		n.s.
Sb	3.5 ± 13.7	0.0	0.0 – 0.4		0.6 ± 1.6	0.1	0.0 – 0.5		n.s.
Sn	8.5 ± 11.0	5.6	3.0 – 9.5		6.9 ± 11.2	3.3	0.1 – 9.5		n.s.
Sr	55.1 ± 23.8	49.9	38.9 – 62.2		54.9 ± 26.4	53.2	36.3 – 69.6		n.s.
Tl	0.05 ± 0.08	0.0	0.0 – 0.13		0.05 ± 0.08	0.0	0.0 – 0.1		n.s.
U	1.2 ± 2.3	0.1	0.0 – 0.7		2.7 ± 3.9	0.4	0.0 - 4		n.s.
V	6.1 ± 5.1	6.2	0.9 –8.2		3.4 ± 4.4	1.2	0.0 – 6.1		0.0042
Sum REE [a]	0.4 ± 0.3	0.4	0.0 – 0.7		0.5 ± 1.2	0.2	0.0 – 0.6		n.s.

[a] Sum of individual concentrations of Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, and Yb.

* Statistical significance based on the Mann-Whitney U test.

Table 4. Concentration of essential and toxic elements in whole blood of white storks (*Ciconia ciconia*) from central Spain, according to human population density in the municipality where they were found. Results are expressed in ng/g of blood (fresh weight).

	High density (n = 111)			**Low density (n = 79)**			
Essential element	**Mean ± SD**	**Median**	**p25 –p75**	**Mean ± SD**	**Median**	**p25 –p75**	**P ***
Essential elements							
Fe	304146 ±55471	302146	272138 - 333079	306820 ± 60203	310027	261529 - 346058	n.s.
Cu	368.7 ± 115.1	359.9	299.1 – 422.1	390.4 ± 107.7	362.9	310.6 – 485.7	n.s.
Zn	3948 ± 1269	3839	3245 - 4372	3841 ± 1045	3757	3135 - 4372	n.s.
Se	508.2 ± 139.8	481.6	401.8 – 587.9	517.9 ± 168.0	522.0	381.9 – 630.1	n.s.
Mn	30.6 ± 14.4	26.7	22.8 – 35.9	43.6 ± 25.1	37.3	29.0 – 48.7	< 0.0001
Major toxic elements							
As	70.2 ± 54.1	59.1	32.4 – 97.9	58.1 ± 45.9	44.5	26.2 – 74.5	0.0432
Cd	1.3 ± 1.7	0.8	0.4 – 1.2	0.8 ± 0.7	0.6	0.3 – 1.0	0.0441
Hg	74.4 ± 60.4	62.7	40.9 – 90.7	91.3 ± 103.9	62.9	36.9 – 109.4	n.s.
Pb	139.7 ± 99.2	121.3	84.8 – 165.2	151.5 ± 94.5	137.9	85.3 – 192.7	n.s.
Other toxic or potentially toxic elements							
Al	25.8 ± 84.4	9.5	5.6 – 11.8	44.3 ± 155.3	10.0	8.5 – 15.4	n.s.
Au	0.08 ± 0.17	0.01	0.0 – 0.12	0.06 ±0.12	0.01	0.0 – 0.09	n.s.
Ba	6.5 ± 20.4	0.4	0.2 – 7.1	4.5 ± 7.6	0.3	0.2 – 8.7	n.s.
Co	1.3 ± 1.8	0.9	0.6 – 1.2	1.0 ± 0.5	0.8	0.6 – 1.2	n.s.
Cr	1.1 ± 3.8	0.5	0.02 – 1.2	0.9 ± 0.8	0.7	0.2 – 1.4	n.s.
Ni	10.0 ± 42.2	0.8	0.5 – 2.1	5.4 ± 15.4	1.0	0.5 – 2.4	n.s
Mo	16.4 ± 4.9	15.9	12.8 – 19.3	17.2 ± 5.4	17.5	13.1 – 20.7	n.s.
Sb	1.1 ± 5.8	0.13	0.0 – 0.6	0.6 ± 1.6	0.03	0.0 – 0.5	n.s.
Sn	7.7 ± 12.3	3.8	0.1 – 10.5	6.2 ± 9.1	3.6	0.1 – 7.3	n.s.
Sr	53.3 ± 25.6	50.0	34.1 – 69.6	57.8 ± 26.9	56.6	38.0 – 67.7	n.s.
Tl	0.05 ± 0.09	0.0	0.0 – 0.1	0.05 ± 0.08	0.0	0.0 – 0.1	n.s.
U	2.8 ± 3.7	0.9	0.0 – 4.8	2.2 ± 4.0	0.1	0.0 – 3.5	n.s.
V	4.9 ± 5.6	4.3	0.0 – 7.7	2.9 ± 3.5	0.1	0.0 – 5.7	0.0203
Sum REE [a]	0.5 ± 1.3	0.2	0.0 – 0.6	0.4 ± 0.5	0.1	0.0 – 0.6	n.s.

[a] Sum of individual concentrations of Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, and Yb.

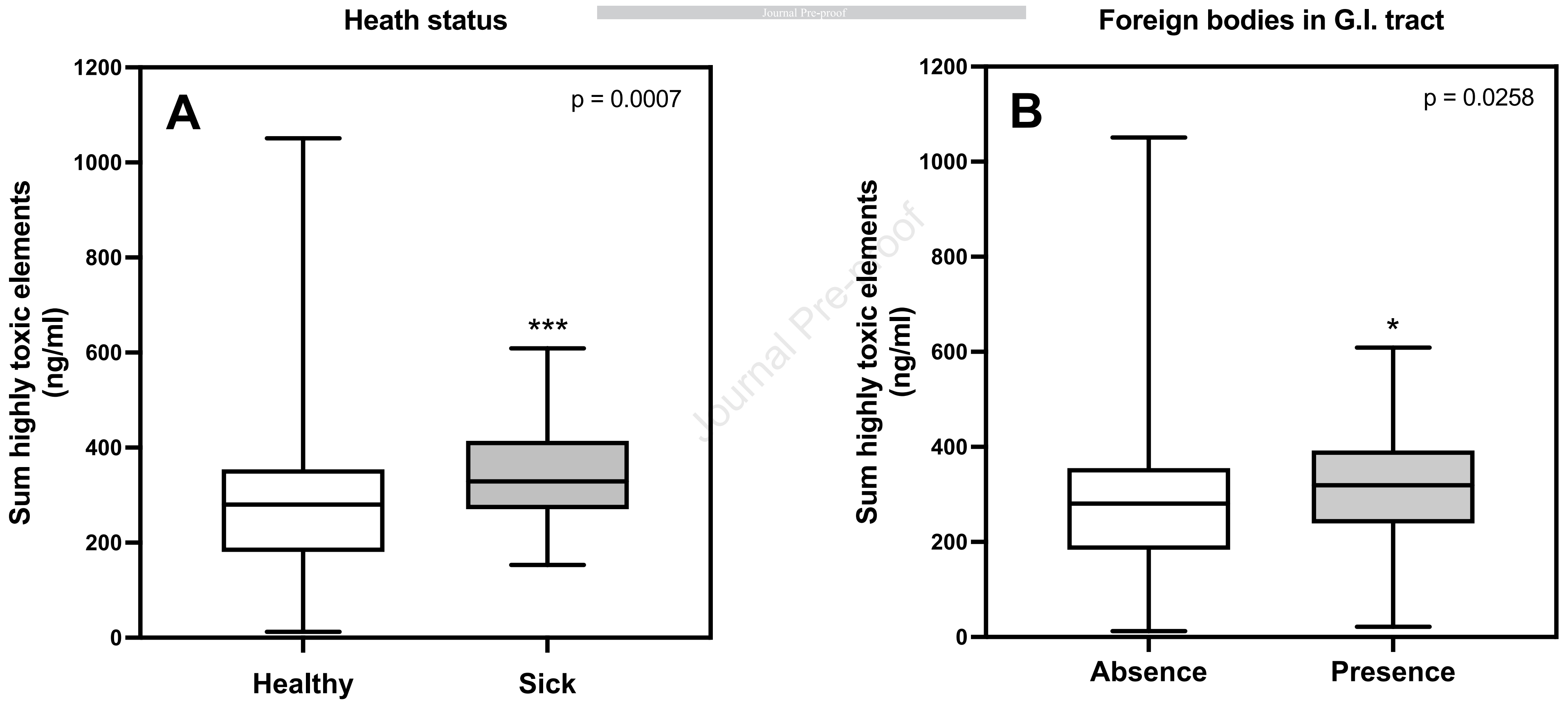
* Statistical significance based on the Mann-Whitney U test.

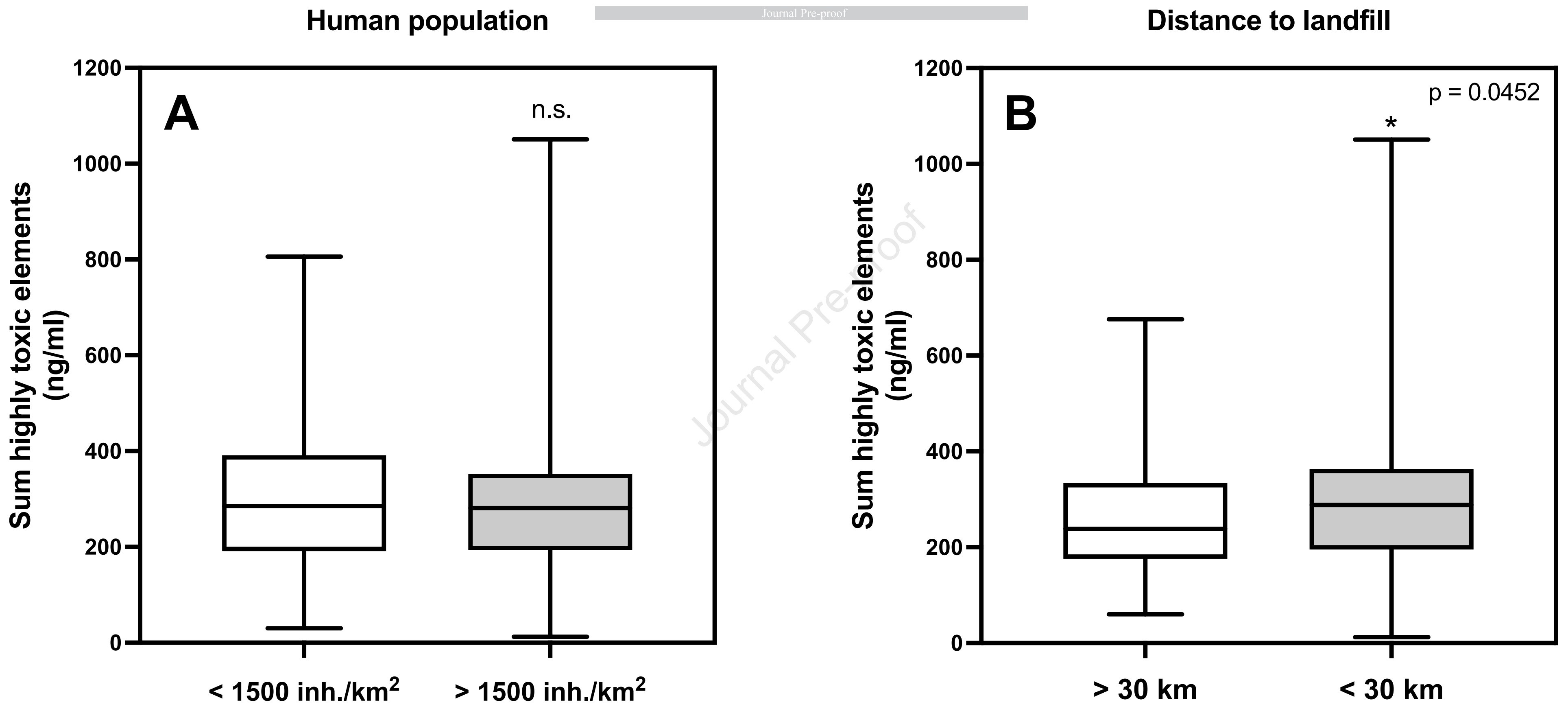
Table 5. Concentration of essential and toxic elements in whole blood of white storks (*Ciconia ciconia*) from central Spain, according to the distance between the sampling point and an urban solid waste landfill. Results are expressed in ng/g of blood (fresh weight).

	< 30 km (n = 41)				> 30 km (n = 149)				
Essential element	**Mean ± SD**	**Median**	**p25 –p75**		**Mean ± SD**	**Median**	**p25 –p75**		**P ***
Essential elements									
Fe	304370 ± 50376	303268	276778 - 334717		310113 ± 81915	310429	238271 - 359189		n.s.
Cu	377.0 ± 105.1	365.0	307.7 – 438.1		376.2 ± 143.4	356.5	264.0 – 435.1		n.s.
Zn	3869 ± 1151	3768	3179 - 9210		4087 ± 1343	3841	3391 - 4374		n.s.
Se	513.8 ± 139.3	494.2	406.1 – 596.2		502.4 ± 195.9	425.9	375.7 – 620.8		n.s.
Mn	47.1 ± 25.7	42.1	30.5 – 52.4		33.0 ± 17.7	29.9	23.6 – 38.5		< 0.0001
Major toxic elements									
As	69.8 ± 51.8	61.3	31.6 – 95.3		46.6 ± 44.6	36.2	22.1 – 51.7		0.0002
Cd	1.3 ± 1.2	0.9	0.5 – 1.5		0.9 ± 1.2	0.6	0.3 – 1.1		0.0118
Hg	82.7 ± 81.5	68.4	44.9 – 99.5		67.4 ± 60.2	52.6	33.9 – 80.1		0.0412
Pb	145.1 ± 100.9	138.3	85.3 – 196.1		139.4 ± 79.1	123.3	83.6 – 177.4		0.0468
Other toxic or potentially toxic elements									
Al	35.1 ± 106.5	11.3	9.2 – 18.2		32.2 ± 118.1	9.5	5.2 – 11.7		0.0006
Au	0.07 ± 0.15	0.01	0.0 – 0.1		0.09 ± 0.14	0.01	0.0 – 0.14		n.s.
Ba	5.0 ± 14.8	0.5	0.2 – 4.8		9.2 ± 24.1	0.5	0.2 – 11.5		n.s.
Co	1.1 ± 1.5	0.8	0.6 – 1.2		1.4 ± 1.4	1.0	0.7 – 1.5		n.s.
Cr	1.0 ± 3.3	0.5	0.0 – 1.2		1.0 ± 0.9	0.7	0.3 – 1.7		n.s.
Ni	8.9 ± 37.9	0.8	0.5 – 2.1		5.0 ± 10.7	1.6	0.6 – 2.5		0.0298
Mo	19.3 ± 5.8	19.1	14.5 – 24.1		16.2 ± 4.7	16.0	12.7 – 19.3		0.0003
Sb	1.0 ± 5.2	0.1	0.0 – 0.5		0.5 ± 0.9	0.1	0.0 – 0.5		n.s.
Sn	6.9 ± 11.4	3.3	0.1 – 9.5		7.9 ± 10.5	4.9	0.1 – 4.9		n.s.
Sr	55.9 ± 27.4	53.3	37.2 – 71.1		50.1 ± 18.2	51.1	36.0 – 60.3		n.s.
Tl	0.05 ± 0.09	0.0	0.0 – 0.1		0.08 ± 0.09	0.02	0.0 – 0.2		n.s.
U	2.8 ± 3.9	0.6	0.0 – 4.7		1.5 ± 3.1	0.2	0.0 – 1.3		0.0426
V	5.8 ± 6.2	4.5	0.0 – 8.5		3.2 ± 3.9	0.8	0.0 – 6.2		0.0035
Sum REE [a]	0.5 ± 1.2	0.5	0.06 – 09		0.4 ± 0.5	0.2	0.05 – 0.6		0.0411

[a] Sum of individual concentrations of Ce, Dy, Er, Eu, Ga, Gd, Ho, In, La, Lu, Nb, Nd, Pr, Sm, Ta, Tb, Tm, Y, and Yb.

* Statistical significance based on the Mann-Whitney U test.


Heath status
Foreign bodies in G.I. tract
A
p = 0.0007
B
p = 0.0258
Sum highly toxic elements (ng/ml)
1200
1000
800
600
400
200
0
***
*
Healthy
Sick
Absence
Presence


Human population
Distance to landfill
A
B
n.s.
*
p = 0.0452
Sum highly toxic elements (ng/ml)
0
200
400
600
800
1000
1200
< 1500 inh./km2
> 1500 inh./km2
> 30 km
< 30 km
Journal Pre-proof

HIGHLIGHTS

- Blood levels of 41 elements were assessed in 189 free-living white storks from Spain.
- Fledglings showed higher concentrations of Pb, As, Cd, and Mn than other age groups.
- Sick individuals had elevated levels of Pb, Cd, As, and several rare earth elements.
- Proximity to landfills increased exposure to Hg, As, Cd, Pb, Al, U, and REEs.
- Ingested foreign materials were linked to higher blood levels of Pb and Cd.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: