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ORIGINAL ARTICLE

CONCENTRATIONS OF SOME HEAVY AND TRACE METALS IN SERUM AND BRAIN OF FIVE SPECIES OF WILD VERTEBRATES FROM NIGERIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Environmental pollution from heavy metals is a growing concern, largely driven by increased industrial activity. This study measured the concentrations of seven heavy metals—lead, cadmium, chromium, manganese, cobalt, copper, and iron—and two trace metals—magnesium and nickel—in the serum, cerebrum, cerebellum, and brainstem of the hedgehog (Eulipotyphla), pigeon (Columbiformes), cattle egret (Pelecaniformes), and two species of squirrel (Sciuromorpha), using Atomic Absorption Spectrophotometry. Among the metals tested, magnesium showed the highest concentrations across all sample types and brain regions in all studied animals. Magnesium levels reached as high as 817,598 µg/mL in the serum of female pigeons. Metal levels were generally higher than those reported in previous studies from other regions, except for cobalt, which was undetectable in the tested tissues. The highest concentrations for all metals were found in the serum. No consistent pattern was observed in metal concentrations across the brain regions. These findings reveal complex patterns of metal accumulation and distribution, potentially reflecting interspecies differences in physiology, diet, and environmental exposure. The data offers insights into metal presence in wildlife serum and brains. It will also provide crucial baseline dataset with information that can be invaluable for future ecological and environmental studies.

Keywords: blood; brain regions; heavy metals; wildlife

INTRODUCTION

The increasing human population and accelerating industrialization have contributed to the rising levels of heavy metals in the environment. This is particularly concerning due to the accumulation of these metals in air, water resources, and food consumed by humans and animals. [1, 2]. Heavy metals such as arsenic (As), manganese (Mn), mercury (Hg), lead (Pb), copper (Cu), iron (Fe), cadmium (Cd), nickel (Ni), zinc (Zn), and cobalt (Co) are pollutants that may occur naturally in the environment or result from anthropogenic activities [3]. Metals play principal roles in biological processes such as nucleic acid configurations, serving as transporters or second messengers during neurotransmission as in zinc [4], or vanadium's protective functions on the pyramidal cells of hydrocephalic mice as recently reported [5]. The human body requires essential trace metals like iron, zinc, copper, manganese, cobalt, and molybdenum for proper functioning [6]. In the brain, zinc and copper are represented in ample amounts, while iron has the highest concentration [7, 8]. Previous studies have indicated the role of trace elements in neurodevelopment with early stages of deficiency resulting in foetal anomalies. Iron deficiency during the early stages of foetal development could lead to acute brain dysfunction which is less likely to reverse after iron repletion [9].

Excessive accumulation of metals is known to be neurotoxic and increases the risk for certain neurodegenerative disorders like Parkinson's disease and Alzheimer's disease [10] as well as inducing cytogenotoxicity and hepato-renal toxicity in rats [11, 12]. Although some of these metals are essential due to their biological functions, they are required only in trace amounts and can become toxic when accumulated excessively in the body. These effects could lead to gastrointestinal and kidney dysfunctions, cognitive impairments, vascular damage and immune dysfunctions [13]. Some metals like cadmium, arsenic and chromium exerts disruptive actions on DNA synthesis and repair due to their carcinogenic abilities [14, 15]. Animals get exposed to heavy and trace metals through direct exposure, polluted water, and crops grown on irrigated sewage, industrial effluents as well as through anthropogenic activities [16]. An example of anthropogenic metal exposure was observed in a study carried out in Zamfara State, where indiscriminate small-scale artisanal gold mining led to high levels of lead and cadmium in the liver, kidney, muscle, intestine, cerebrum, cerebellum, and midbrain of goats [17, 18]. It can be quite challenging to get human samples for assessment of metal concentration in serum and tissues especially the brain. Using animals that are readily available as sentinels provides an indicator for the level of metals in the environment. Examples of sentinel indicators of metal exposure are the cases of lead poisoning in horses and cattle living close to a smelter [19] and the ataxic dancing cats of Minamata alerting local residents of methyl mercury poisoning from contaminated sea food [20].

Ibadan, the site for this study, is the capital city of Oyo State in southwestern Nigeria, and it has experienced significant urbanization over the past few decades. Originally established in the early 1800s, Ibadan has grown from a small settlement to one of Nigeria's largest cities [21]. The rapid urbanization of Ibadan has brought about significant environmental pollution challenges. Studies have shown that particulate matter (PM) levels in various locations within Ibadan can vary widely, with some areas experiencing exceptionally high concentrations. This variability is indicative of localized pollution sources, such as traffic emissions, industrial activities, and open burning of waste.

This study investigates the concentrations of heavy metals in the serum and three brain regions (cerebrum, cerebellum, and brainstem) of five sentinel animal species – squirrel (*Heliosciurus gambianus* – Gambian sun squirrel (GSS) and *Funisciurus anerythrus* – Thomas's rope squirrel (TRS)), speckled pigeon (*Columba guinea*), western cattle egret (CE) (*Bubulcus ibis*), and hedgehog (*Atelerix albiventris*) in Ibadan, Oyo State, southwest Nigeria. This study aims to provide insights on the metal presence in serum and brain tissue of specific wildlife, taking into consideration the interspecies differences in the serum, cerebrum, cerebellum, and brainstem. The study will address how the various levels of heavy metal concentrations vary across these different species, thus offering a valuable baseline dataset for future ecological and environmental research.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the National Veterinary Research Institute, Vom, Plateau State (Nigeria). All animals were handled humanely, with the utmost care, and to avoid causing undue pain, according to the NIH guidelines for handling research and experimental animals.

Study Site

All the animals were obtained from Ibadan, a tropical savannah town (7°23'47"N 3°55'0"E). The squirrels and pigeons were captured from within the campus of the University of Ibadan, while the cattle egrets and hedgehogs were captured from the forests in Ibadan with the assistance of local hunters.

Animals

A total of 50 animals were captured from their natural environments by using food baits in metal cage traps and local traps and transported to the laboratory in individual cages. They were certified to be healthy by a qualified veterinarian. The animals (five males and five females for each animal species, 5 species in total) were euthanised with ketamine (100 mg/kg) and xylazine (10 mg/kg) combination through intramuscular injection [22]. Blood (0.5 – 1.5 mL) was collected into plain tubes from all animals via the intracardiac route once there was no breathing and there was cessation of response to tactile stimuli. Blood collected was centrifuged at 10,000 rpm for 10 mins to obtain the sera, which were stored at -20 °C until analysed. These animals were selected because they are quite abundant in Ibadan, which is a suitable habitat for them. The hedgehog and squirrels were sexed by the presence or absence of a scrotal sac, while birds were sexed after sacrifice.

Brain Collection

All the brains (n = 50) were harvested from the skull and subsequently examined *in situ* to rule out any gross morphological pathology. The brains were further carefully dissected to expose the cerebrum, cerebellum, and brainstem as previously described [23]. This was followed by immediate cryopreservation at -20 °C till analysed.

Atomic Absorption Spectrophotometry

Metal concentrations in serum and brain regions were analysed using Atomic Absorption Spectrophotometry (AAS), following the methods described [24, 25, 26]. The concentration of nine (9) metals were analysed – lead (Pb), magnesium (Mg), cadmium (Cd), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), manganese (Mn), and cobalt (Co). For the quality assurance check, deionized water was used to establish a baseline, while calibration standards with known concentrations of the analyte were used to create a calibration curve. Linearity checks were also carried out to ensure the accuracy and reliability of the measurements. This was done using known standard concentrations after every 10 samples and periodically during the analysis to detect drift while all samples were analysed in duplicates to ensure precision. The detection limit for each of the metals are Pb – 0.08 μg/l; Ni – 0.05μg/l; Mg – 0.005μg/l; Fe – 0.05μg/l; Cr – 0.04μg/l; Cd – 0.01μg/l; Mn – 0.03μg/l; Co – 0.05μg/l.

Statistical Analysis

The collected data were analysed using 2-way ANOVA with Tukey post hoc test and descriptive statistics. The data were expressed as means ± SEM and as bar diagrams. The statistical package used was GraphPad Prism version 8.0.0 (GraphPad Software, San Diego).

RESULTS

Values obtained are presented as mean ± standard deviation and presented in μg/mL (sera) or μg/g (tissue) in bar diagrams Figures 1 – 8.

Cadmium

The highest observable concentration was in the serum of the male pigeon (3,015 ± 2,382.95), and there was a statistically significant difference ($p > 0.05$) when compared to the level in male GSS, which had the lowest level (690 ± 500.30) (Figure 1). Non-detectable levels were observed in the cerebrum of the female hedgehog and the cerebellum and brainstem of both male and female pigeons.

Chromium

The sera of the female TRS (1,635 ± 332.34) and female GSS (1,600 ± 1,032.38) had the highest concentration values, while the cerebellum of the male hedgehog had the highest values in the brain region (635.38 ± 31.71), and the cerebrum of the female GSS (0.955 ± 0.26) had the lowest value. However, there was no statistically significant differences (Figure 2).

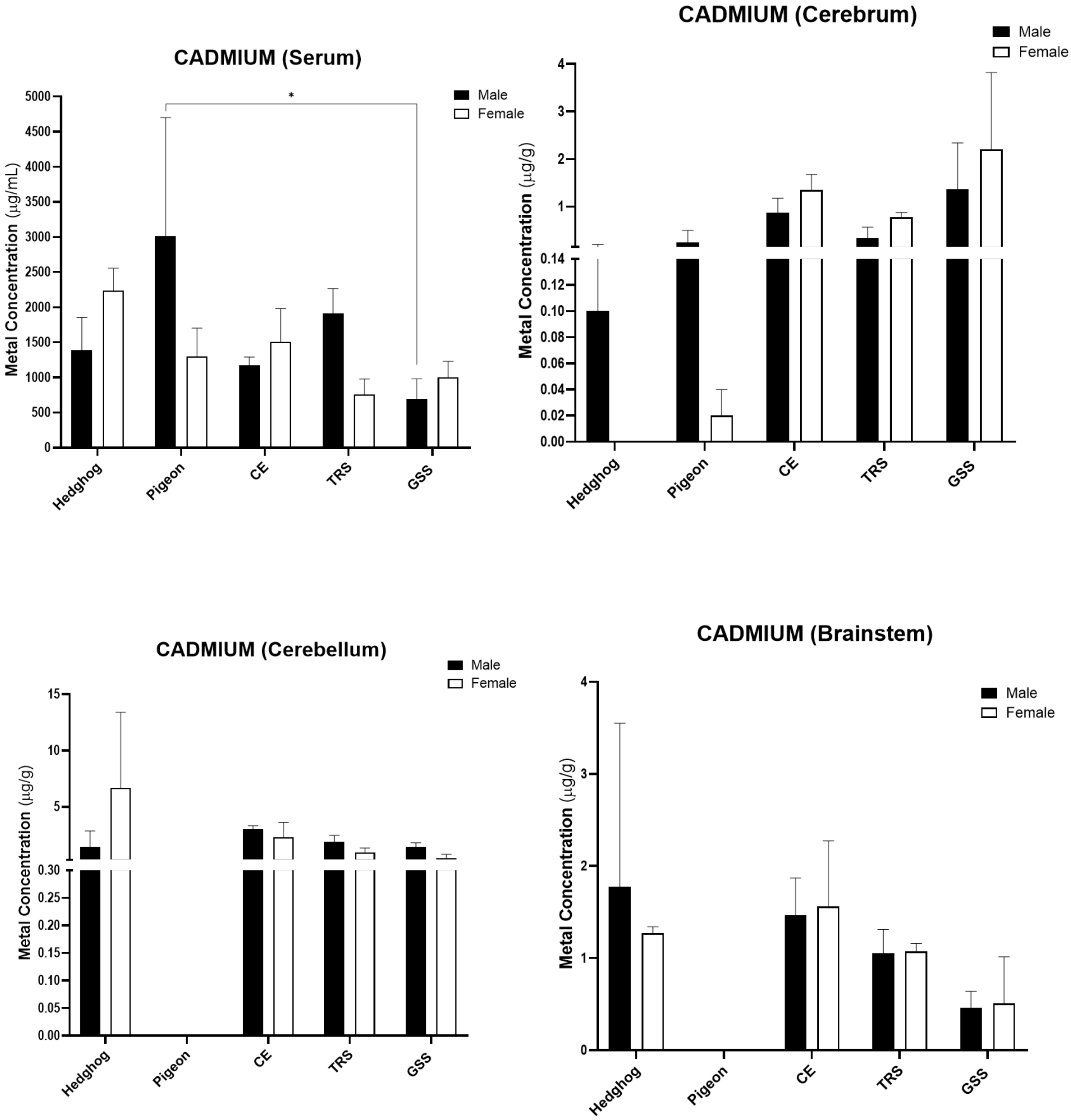

Fig. 1. Bar charts showing concentration of cadmium in serum, cerebrum, cerebellum, and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS). (*- P ≤ 0.05)

Cobalt

There were no detectable levels of cobalt in all the sera and brain regions analysed.

Copper

The sera, cerebrum and brainstem of all 5 species analysed, as well as the cerebellum of female CE and TRS, had no detectable level of copper. The detectable levels observed in the cerebellum ranged from 0.18 ± 0.31 (male GSS) to 5.29 ± 2.23 (male pigeon) (Figure 3).

Iron

The highest values were observed in sera of male hedgehog (14,988.3 ± 8,302.57) and female CE (11,668 ± 7,819.30), while the lowest value was observed in the cerebellum of the female GSS (23.48 ± 29.30). Iron concentration in the brainstem as well as the cerebrum showed statistically significant differences between either males or females across species (Figure 4).

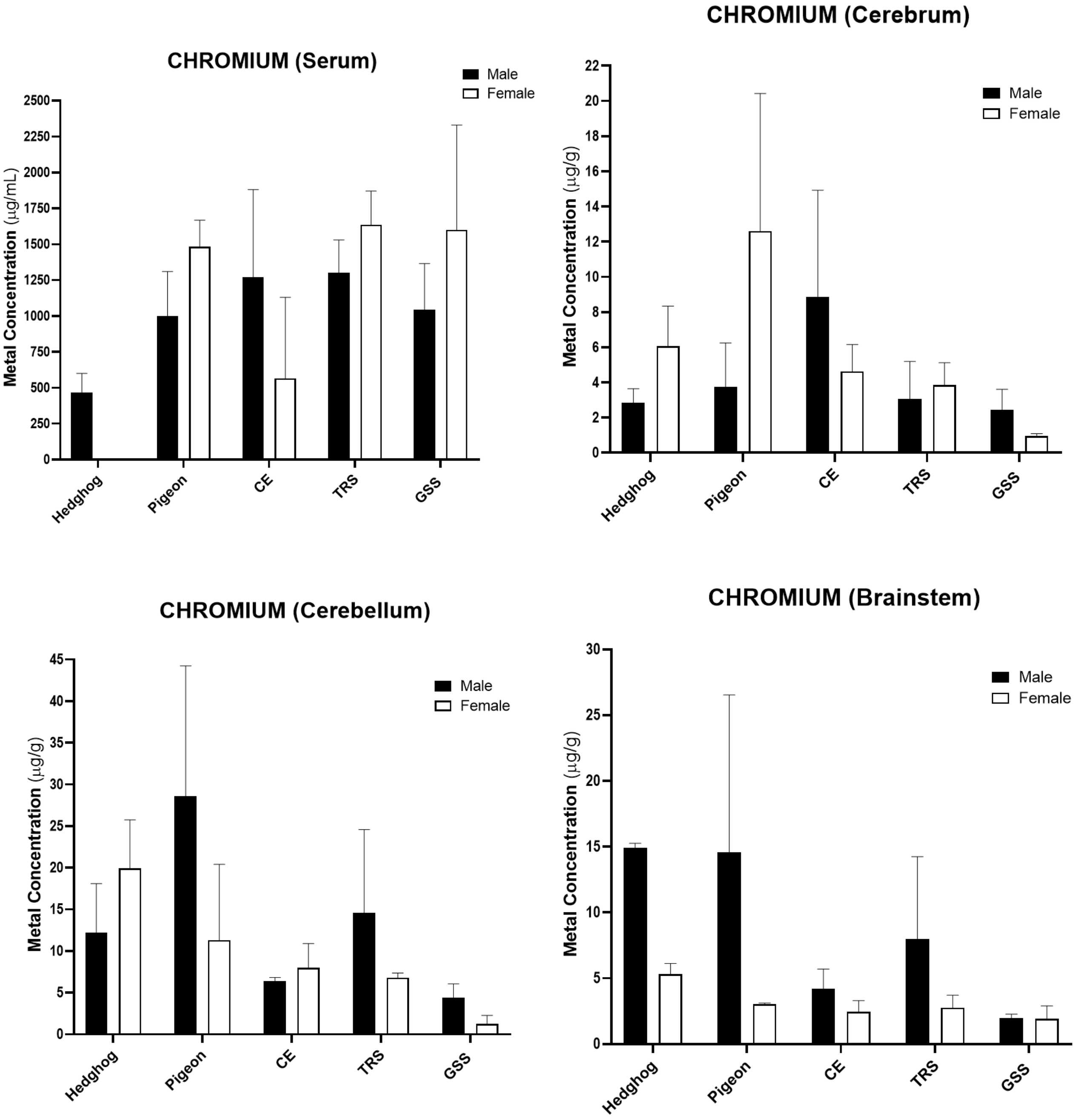

Fig. 2. Bar charts showing concentration of chromium in serum, cerebrum, cerebellum, and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS).

Lead

The highest values were observed in the serum of both male and female of all the species. CE (male 15,330 ± 2,828.43; female 5,000 ± 1,414.21) and TSS (male 5,110 ± 2,33.77; female 14,000 ± 1,880.90) showed the highest variations between genders in serum concentrations as seen in Figure 5. Some regions of the brain however had values below detection limit.

Magnesium

The highest values were observed in the serum of the male hedgehog (617,540 ± 77,5407.4) and female TRS (736,668 ± 409,859.8). Of all the metals assessed, Mg showed the highest values across all the brain regions and in serum (Figure 6). The cerebrum of the pigeon had the highest concentration of Mg across all the brain regions and was statistically significantly different from the levels in the cerebrum of the other males.

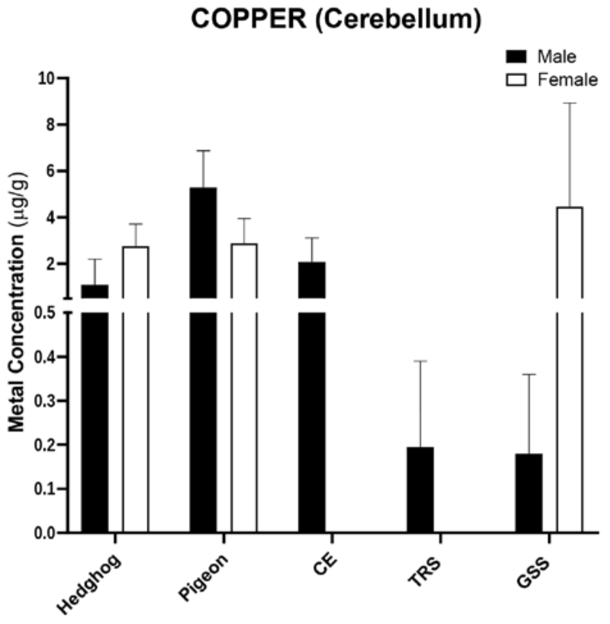

Fig. 3. Bar charts showing concentration of copper in the cerebellum of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS).

Manganese

The only detectable levels were observed in the sera of the female CE (266 ± 594.79) and that of the male TRS (168 ± 335) (Figure 7).

Nickel

The highest values were observed in the serum of the male CE (3,235 ± 3,061.77) and female TRS (3,300 ± 3,776.32). (Figure 8). There was no nickel detected in the cerebrum of all the animals.

DISCUSSION

Environmental pollution by metals is becoming a global health concern as it threatens the ecosystem due to bioaccumulation. These metals last a long time in the environment and cause various health effects when there is an exposure to them. Heavy and trace metals are important environmental pollutants, with their toxicity being an increasingly significant challenge for ecological, nutritional, environmental and evolutionary reasons [18]. Entry of these metals into the environment is notably through natural means (like soil erosion and natural weathering of earth's crust) and human activities (like industrial effluents, mining, sewage discharge, use of insecticides and use of disease control agents on crops) [27]. Metal pollution is a universal environmental problem affecting all living organisms through their bioaccumulation and biomagnification along the food chain [28]. Bioaccumulation of heavy metals in the brain has been documented in wildlife like the polar bears [29–31], beluga whales [31, 32], bottlenose dolphins [33], river otters [34], and rodents [35]. Using animals as bioindicators helps to determine the extent of environmental contamination and also the exposure to certain compounds [33].

Blood serum serves as a short-term indicator of the extent of exposure to certain elements, thereby enabling their current intake to be evaluated [33]. Accumulations of heavy metals in different body tissues have been documented, but the blood-brain barrier serves as a semi-permeable membrane to limit the entrance of noxious substances into the brain [36]. This could be the reason why the highest concentrations of metals in this study were found in the serum.

Cadmium

Reports have shown that exposure to cadmium in the human population primarily occurs through the use of rechargeable nickel-cadmium batteries and cigarettes, while exposure through food is the main route in non-smokers. Cadmium affects the nervous system by the inhibition of acetylcholinesterase activity [37]. Brain concentration levels obtained from this report were considerably higher than those reported in mallards, 0.006 ± 0.012 µg/g wet weight (adults) and 0.013 ± 0.012 µg/g wet weight (immature) [38] and slightly higher than those reported in various carnivorous mammals from northwest Poland [39]. A study from Argentina, in three different species of birds (Great Kiskadee – *Pitangus sulphuratus*; Neotropic Cormorant – *Nannopterum brasilianum*; Great Grebe – *Podiceps major*) reportedly had varied values of cadmium in the whole brain [40]. In a similar study in Nigeria, in the African giant rat from three ecological zones, brain cadmium levels ranged from 0.03 to 0.05 mg/L [22], values that are generally lower than that obtained in different brain regions in animals in this current study.

Cobalt

Cobalt is categorised as one of the less common heavy metals and is essential for the synthesis of vitamin B_{12} [41]. Cobalt serum levels obtained from grazing cattle from different reserves in Kenya were on the average 0.017 and

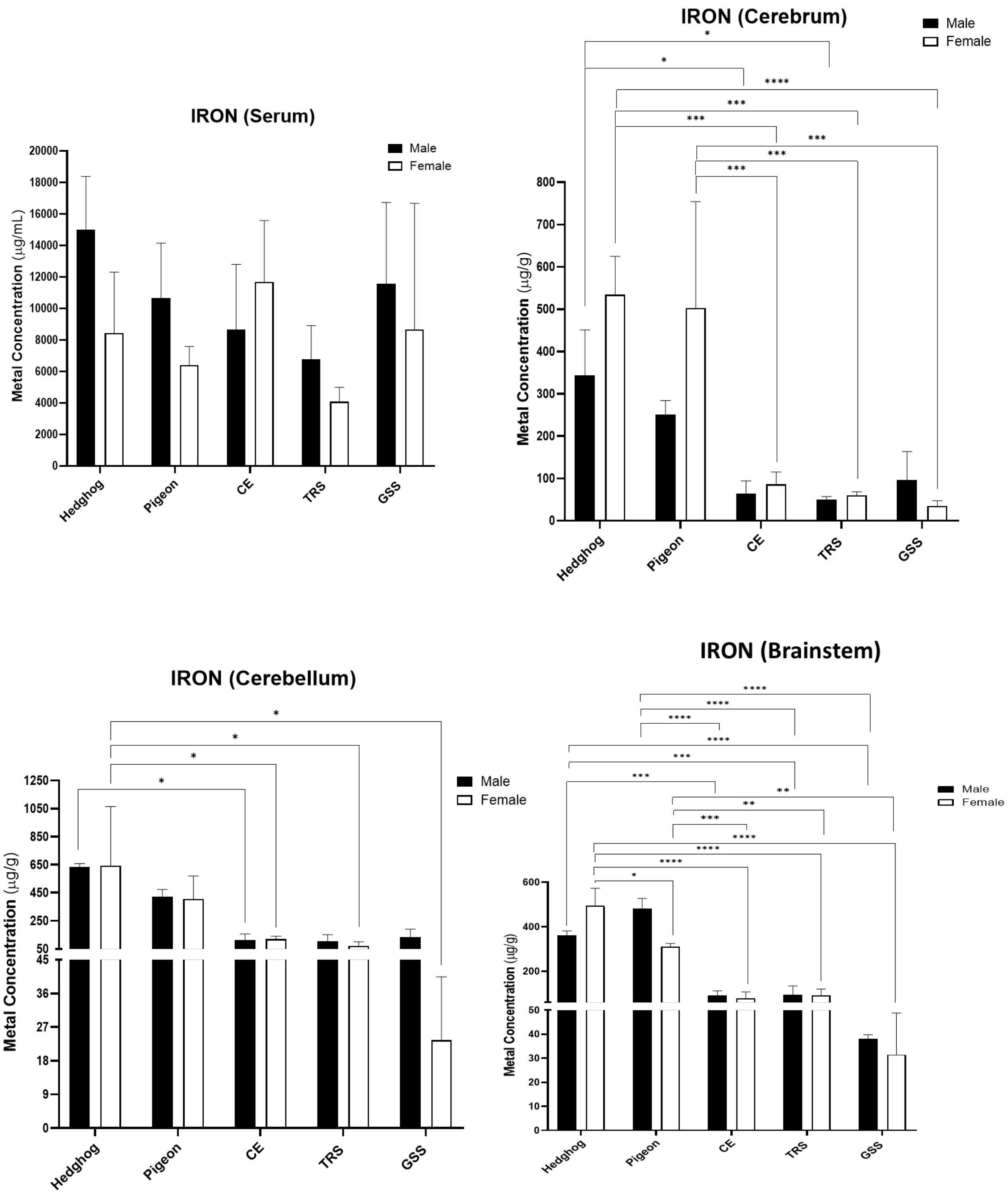

Fig. 4. Bar charts showing concentration of iron in the sera, cerebrum, cerebellum, and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS). (*- P ≤ 0.05; **-P ≤ 0.01; *- P ≤ 0.001; ****-P ≤ 0.0001)**

0.018 mg/L [42], unlike the non-detectable levels in this study.

Chromium

Chromium is a heavy metal found in plants, rock and soil. Chromium exposure is usually by consuming contaminated food and water and breathing contaminated air. Theses can cause ulcer formation which may take months to heal, lead to cancer, anaemia and also cause damage to the stomach and intestines [43, 44]. Along with arsenic, cadmium, mercury, copper, and lead, chromium has been listed by the United States Environmental Protection

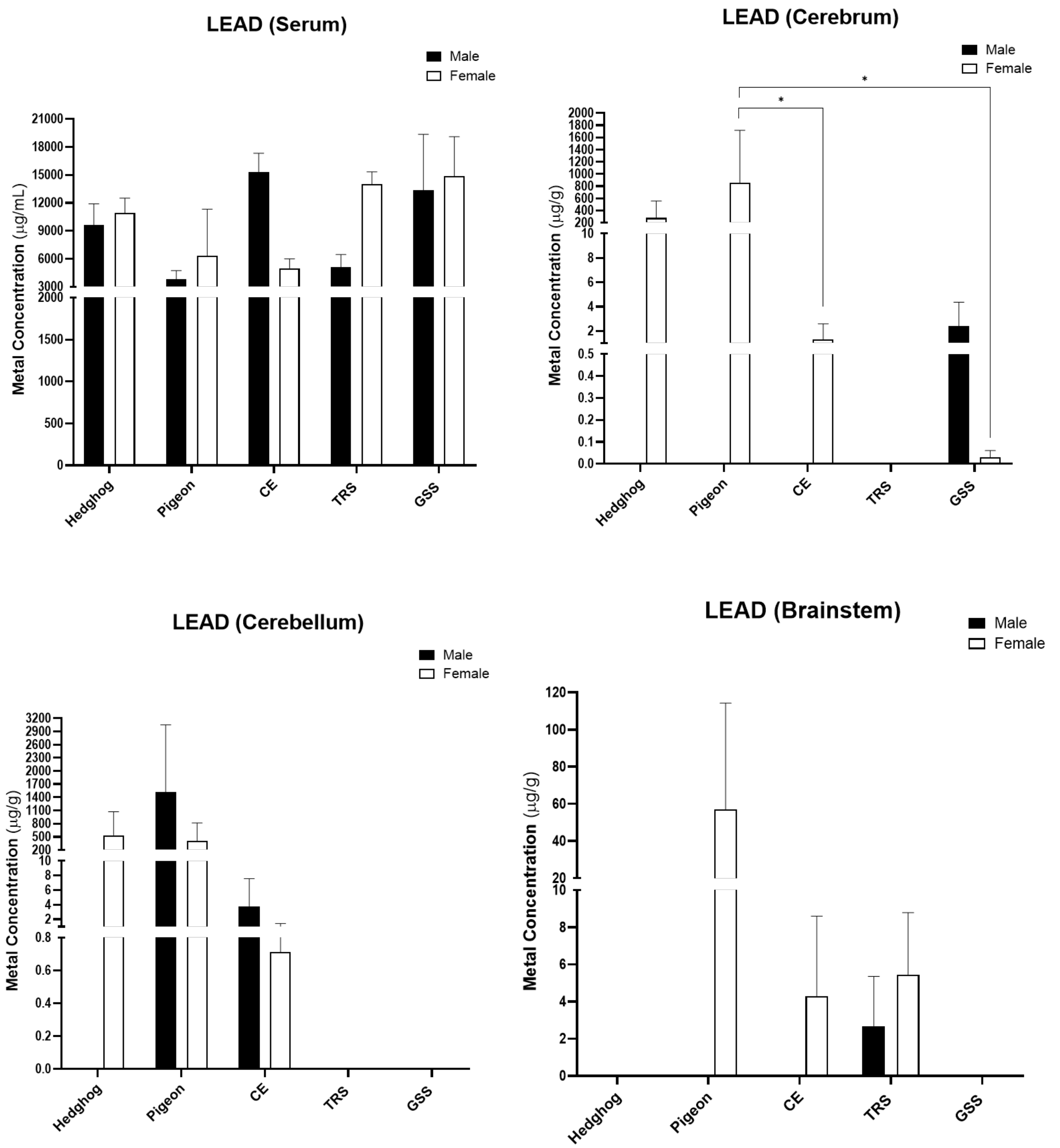

Fig. 5: Bar charts showing concentration of lead in the sera, cerebrum, cerebellum, and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS). (*- P≤0.05)

Agency (USEPA) as priority control pollutant because of its persistence and its irreversible toxic characteristics [44]. Concentration of this metal in tissues from wildlife from two regions in Slovakia were reported not to be dangerous [45], unlike reports from this current study. Concentrations levels observed in the brains of different waterfowls from Evros Delta, Greece, were all below 0.5 mg/kg of metal/tissue [46], which is considerably lower than results obtained from this study. Maximum concentration in drinking water should not exceed 0.1 mg/L [44].

Copper

Copper remains one of the most commonly found heavy metals in wastewater [47]. Its pollution is caused majorly by manufacturing operations, farming, mining, industrial and municipal wastewater. Signs of toxicity in-

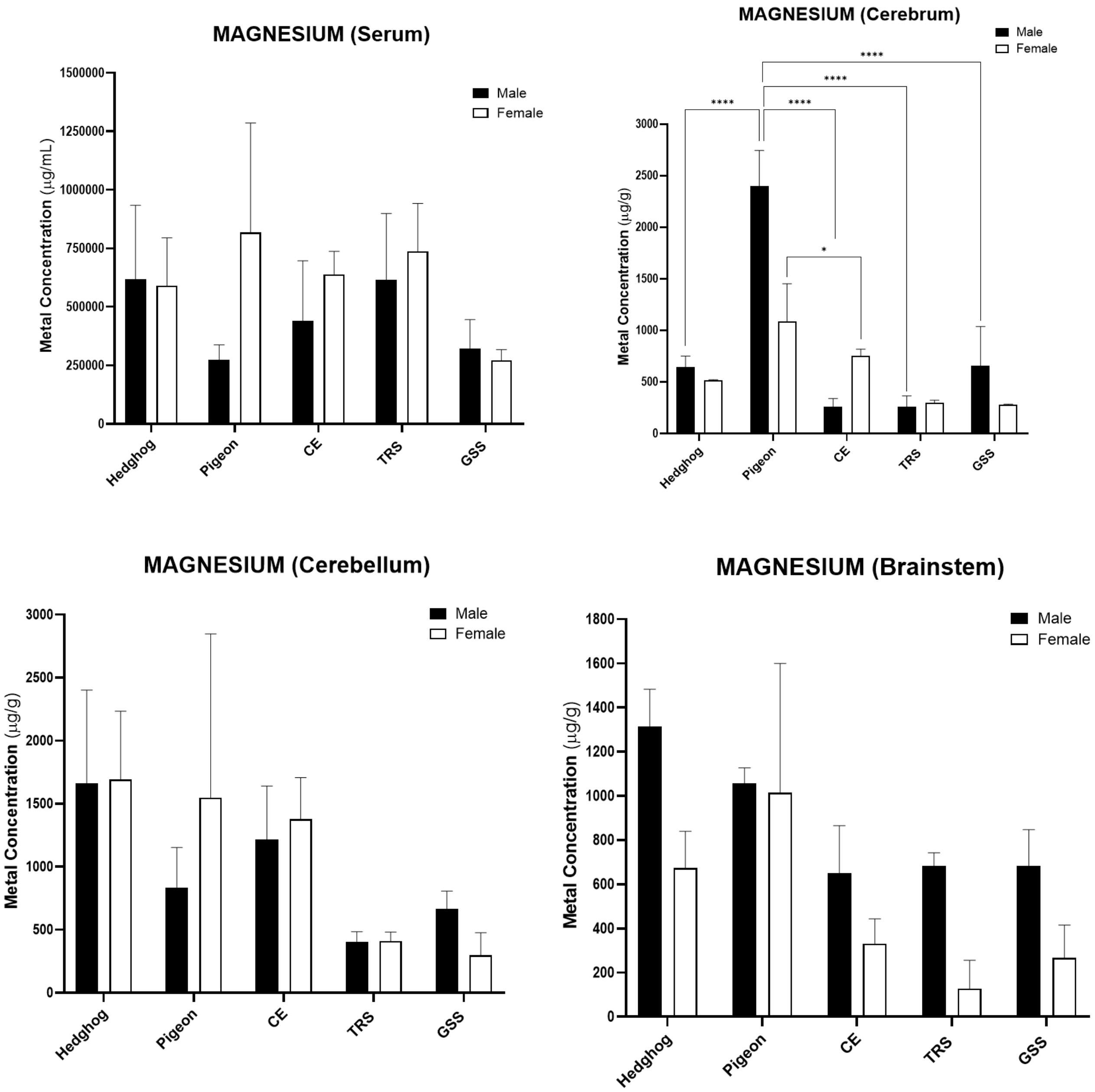

Fig. 6. Bar charts showing concentration of magnesium in the sera, cerebrum, cerebellum and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS) and Gambian sun squirrel (GSS). (*- P ≤ 0.05; **-P ≤ 0.0001)**

clude vomiting, jaundice, abdominal pain, gastrointestinal distress and hypotension. Long-term exposure results in damage to the brain, liver, and kidney [48, 49]. Unlike the report in goats [50], where there were detectable levels in all regions analysed, only the cerebellum had detectable levels. However, concentration levels from the cerebellum in this study were similar to that obtained from the brains of mallards [38]. Copper serum levels from cattle in three game reserves in Kenya were 0.652, 0.659, and 0.785 mg/L [42], which were all considerably higher than the non-detectable levels observed in this study.

Iron

This is the second most abundant metal on the earth's crust. It is a very important element for the survival and growth of practically all living organisms, being a very important component of enzymes and also found in organisms [43]. Iron is one of the heavy metals required for proper function of various enzymes and proteins [37]. In a study in goats, the olfactory bulb was seen to have the highest concentration (98.66 ± 23.02 ppm) relative to other regions tested (cerebral cortex, cerebellum, thalamus, and hippocampus) [50]. The increase in mining activities has

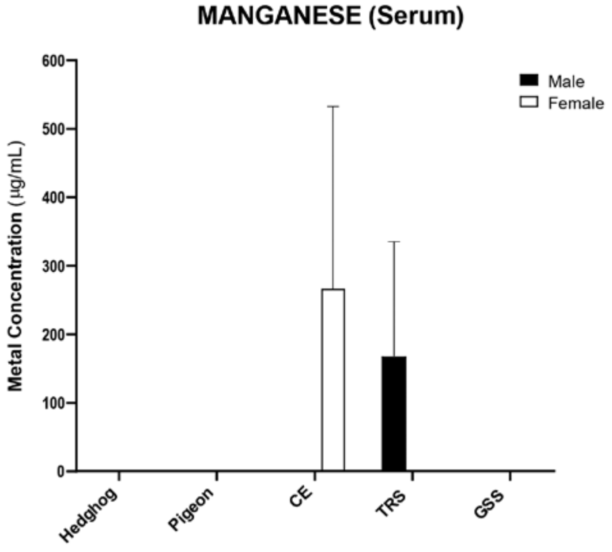

Fig. 7. Bar charts showing concentration of manganese in the serum of female cattle egret (CE) and male Thomas's rope squirrel (TRS).

been reported to increase the incidence or presence of iron in surface water [51], resulting in human exposure to iron by drinking water [43]. Values obtained from the brains of mallards in Poland, without anatomical region specificity, ranged from 20.6 to 25.5 µg/g [38], values far lower than what was obtained from any animal in this current study, which may be because the location is heavily urbanized and industrialized.

Lead

Bioaccumulation of lead in the striatum and nucleus accumbens of the Mexican spiny pocket mouse (*Liomys irroratus*) has been documented to result in variations in the dopaminergic neurons in those areas and cause changes in behavioural tests, showing modifications in learning and memory [26]. In a previous study on mallards, concentrations of Pb, expressed as µg/g, in the whole brain from two different locations in Poland were 0.294 ± 0.603 and 0.672 ± 1.128 (adults) and 0.564 ± 0.600 and 0.401 ± 0.425 (immature subjects) [38]. No distinction was made as to gender in the Polish study. These values are generally lower than the values obtained in the brain regions of the pigeon and cattle egret and even the hedgehog and squirrels obtained in the current study, except for the values obtained in the cerebrums of the male pigeon and cattle egret and the cerebellum of the female cattle egret, which were higher. In a similar study in goats from Nigeria, Pb levels across five brain regions were observed to range from 0.19 to 0.22 ppm, the lowest levels being in the hippocampus and thalamus and the highest levels being in the cerebellum [50]. A study in 3 different species of birds (Great Kiskadee, Neotropic Cormorant, and Great Grebe) from the Argentina had varied values in the whole brain: 5.16 ± 2.44, 0.44 ± 0.05, and 1.27 ± 0.43 ppm (wet weight) and 11.15 ± 5.04, 1.76 ± 0.17, and 5.34 ± 1.84 ppm (dry weight), respectively [40]. A similar study in wild-captured rodents (African giant rat) from Nigeria, across three ecological zones, showed non-detectable levels of Pb in the serum and brain [22]. These varied results buttress the fact that some animals may have a greater tendency to accumulate some particular metals relative to other animals which may be due to variations in their habitats. Levels of Pb in the brain above 5 µg/g are believed to be indicative of poisoning in birds [52], while levels above 16 µg/g are believed to show an advanced level of exposure [53]. Lead affects every organ in the body, with the nervous system being the primary target. Long-term exposure can result in cognitive deficits, weakness in the extremities, anaemia and kidney damage [44]. Lead values obtained from this study were in some cases far above these values, probably indicating an advanced state of exposure from and in the environment since the location is heavily industrialized metropolitan areas (which could be from the soil, water, or even plants eaten), and posing a significant health hazard to humans to eat these animals as a source of protein and even other predatory animals.

Magnesium

This is an essential nutrient required for various biochemical and physiological functions [3]. Values obtained for magnesium in serum and other regions of the brain remained considerably higher than those obtained for other metals. Literature search revealed that magnesium is usually tested to ascertain or monitor the health status of animals, not commonly for toxicology studies. Magnesium serum concentration in wild northern goshawks (*Accipiter gentilis*) in Pennsylvania was 1.9 ± 0.17 mg/L [54], while a study to establish reference values for Mg in plasma in African grey parrots and Hispaniolan parrots gave results as 2.10 – 3.40 and 1.80 – 3.10 mg/dL, respectively [55]. These values in both these studies were far lower than that obtained from this current study. This result raises a concern on the effect of Mg toxicity, not only in the serum, plasma, or brain, but also in other organs that are generally not tested or seen to be of any concern.

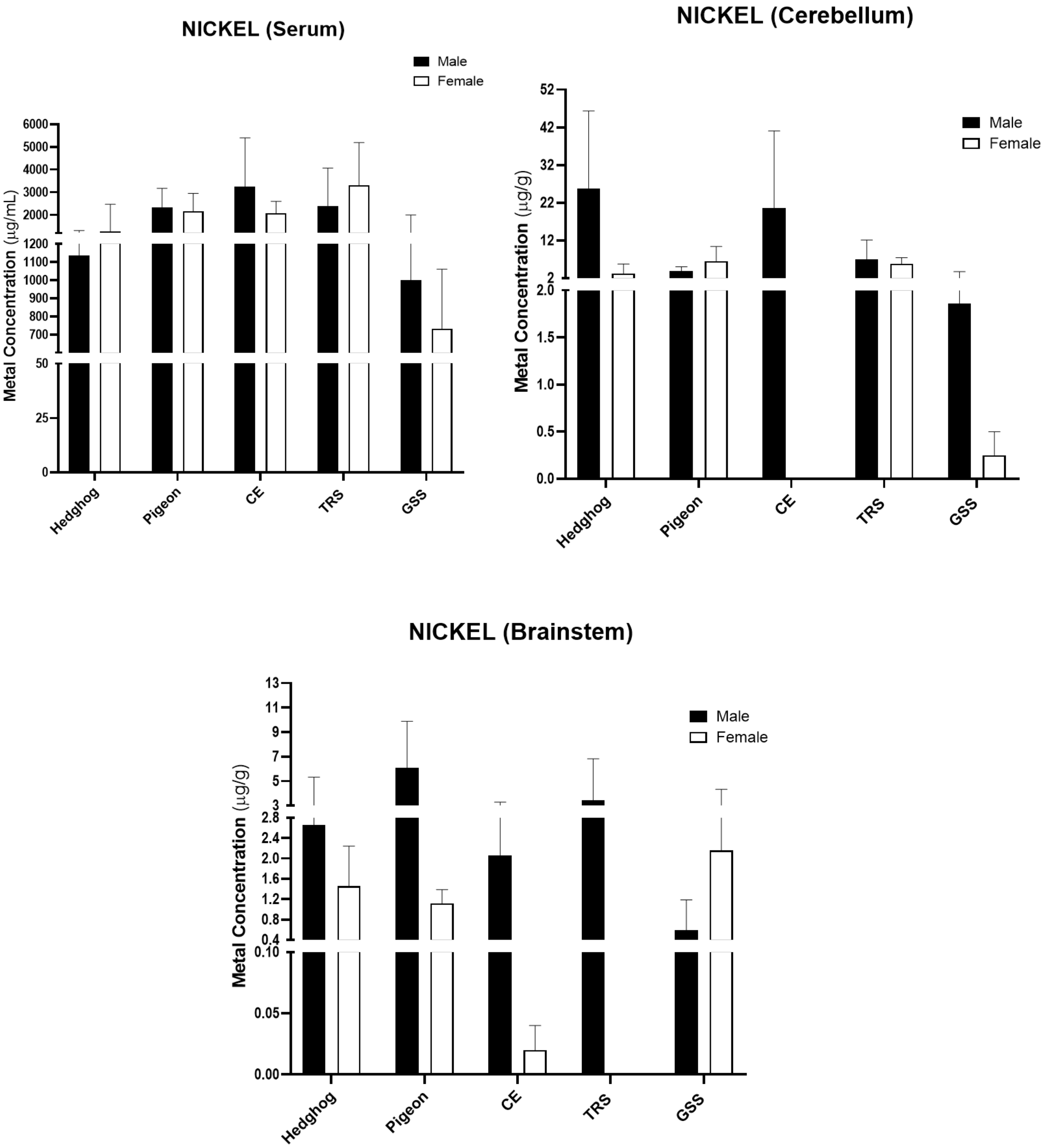

Fig. 8. Bar charts showing concentration of nickel in the sera, cerebellum, and brainstem of male and female hedgehog, pigeon, cattle egret (CE), Thomas's rope squirrel (TRS), and Gambian sun squirrel (GSS).

Manganese

Manganese is involved in the metabolism of proteins, carbohydrates and lipids, while also being a co-factor in many antioxidant enzymes like glutamine synthase. Neurotoxicity of manganese occurs when there is imbalance between antioxidant systems and reactive species. In addition, it can modify epigenetic mechanisms like DNA methylation, altering gene expression and resulting in neurotoxicity [56]. Concentration levels reported in the brains of mallards ranged from 0.28 to 0.40 µg/g [56], while that from different brain regions of the *Liomys irroratus* rodent, obtained from mining areas in Mexico, ranged from 400.1 to 858 µg/kg [34]. These two reports differ greatly from observations in this study, where detectable levels were only observed in the serum of the male *F. anerythrus* and female cattle egret, and no levels in the brain.

Nickel

This is one of the most commonly found heavy metals in wastewater, posing risks to the environment and humans [47]. Bioaccumulation of Ni in brain regions has been reported to result in changes in dopaminergic function and activities like exploratory and memory activities [57]. With values expressed as µg/kg, reports of Ni concentration in rodent brains from a mining area varied from 165.1 ± 89.8 to 572 ± 122.3, with the highest concentration in the nucleus accumbens and the lowest in the striatum [26]. Results from our current study were varied, with the cerebrum having non-detectable levels across all animals, the cerebellum of the female cattle egret and the brainstem of the hedgehog, Gambian sun squirrel, male pigeon, and male cattle egret (see Table 2). Reports from concentration in the mallard brain (where there were detectable levels) – 0.012 ± 0.022 (adults) and 0.020 ± 0.022 (immature birds) [38] showed values lower than those obtained in the current study.

The major means by which living organisms are exposed to heavy metals is by food intake. Grazing animals are exposed by contaminated soil, and the absorbed metal binds to blood cells or blood plasma components [50]. The study of heavy metal accumulation in wildlife is important for ecological and conservation reasons, as exposure to these substances have been known to cause alterations in behaviour, breeding, and survival, resulting in long-term effects that may have deleterious effects on the population of affected animals [26, 31, 33, 42, 58].

Where values were available, metal concentration observed from other results seemed to be generally higher in this study than other reported studies, except in cobalt, where there were no detectable values. This result is alarming and should be a call to increase the awareness of heavy metal pollution and the hazard not only to the animals tested and those that prey on them but also to humans living in these environments and who possibly feed on these.

The reasons for the non-detectable levels in some brain regions in spite of high levels in some other regions in the same animals may possibly be an indicator for the differences in predilection sites for various metals in each animal and differences in environmental factors. But it should be noted that non-detectable levels of some metals in tissue samples have been reported by other authors as well. The irregular pattern and level of bioaccumulation is similar to reports obtained in ducks in Greece [46].

CONCLUSION

The information obtained from this study has provided valuable insights and crucial baseline data into the level of metals in wildlife, which can be useful for future ecological and environmental studies involving the need for conservation of wildlife and flora. The high level of metal concentrations observed generally in this study is a cause for concern, as prolonged exposure to some of these metals (lead, magnesium) has been reported to cause neurodegenerative conditions that may affect behavioural patterns and ultimately alter breeding and survival and subsequently the population and the ecosystem. [30, 32, 33, 34, 55]. Brain regions have been reported to give varied responses when exposed to insults; however, the reason for the sometimes-wide differences in metal concentration across regions in the same species and differences between the sexes still needs investigation. This study has also highlighted the need for surveillance of metal concentration in the environment using animals and wildlife as sentinels since rapid urbanization is increasing the contact of humans with wildlife, meaning that humans are increasingly cohabiting with wildlife, and metal levels in these animals can be essential indicators of metal toxicity in human population.

Ethical Approval and Consent to Participate

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was granted by the Animal Care Committee, National Veterinary Research Institute, Vom, Plateau State (Nigeria), ethical code NVRI/AEC/03/116/22.

Clinical Trial Number

Not applicable

Consent to Publication

All authors approved the final version and consented to the publication.

Data Availability Statement

Data are available on reasonable request from corresponding author.

Conflict of Interest

The authors declare there are no conflicts of interest.

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Competing Interests

All the authors hereby declare that there are no financial or non-financial interests directly or indirectly related to this work.

Generative AI Statement

Authors declare that no generative AI and AI-assisted technologies were used in writing the manuscript.

Authors' Contributions

Conceptualisation – JOO; experimental design – JOO, OOI, FEO; sample collection and analysis – TTG, AAO, ICO, OOI, FEO, OIF, IKP, KIA; manuscript draft – AAO, TTG, ICO and OOI; manuscript editing – JOO and OOI. All authors approved of the final version of the manuscript.

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