UNIVERSITY of VETERINARY MEDICINE

Department of Food Hygiene

Monitoring of heavy metal burden in predatory birds

Written by

Oliver Mackle

Supervised by

József Lehel

Associate professor

Budapest

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Contents

1. INTRODUCTION

In recent years heavy metals have become a great concern for human, animal and environmental welfare. Pollution coming from increased intensity of industrial, commercial and agriculture production all of which are anthropogenic sources has led to increasing levels of heavy metals being deposited in the environment than naturally occur. The health problems associated with the accumulation of heavy metals is partly due to their contamination of the soil and therein also contaminating the plants which serve as a primary source in the food chain either as directly consumed by humans or animals. There is increased uptake of metals in plants situated in soils with a lower pH as the metals become more soluble and mobile in an acidic environment. Progress has been made in recent times to reduce this pollution from industrial sources such as tighter regulation on the use of fossil fuels and better systems for reducing their emissions into the environment which was a major source of increased lead concentrations in urban/industrial areas. The heavy metals most commonly associated with poisoning of humans are lead, mercury, arsenic and cadmium. Heavy metal poisoning may occur from industrial exposure, water or atmosphere pollution, food sources, medicines, improperly coated food containers, or the ingestion of lead-based paints. The largest such incident of heavy metal poisoning occurred in Minamata Bay, Japan, during the mid-20th century. Industrial waste containing methylmercury was dumped into the bay resulting in the deaths of over 1700 people. In 2013 a treaty was signed by 140 countries with the aim of controlling anthropogenic pollution by methylmercury which was named after Minamata. Climate change is also contributing towards increased levels of heavy metals in the environment due to melting of glaciers, heavy metals which have been locked in icecaps are now being released into the environment due to rising global temperatures.

The accumulation of heavy metal in feed stuffs destined for human or animal consumption can easily be checked and monitored for heavy metals whereas the diet of wild animals is almost impossible to regulate. Heavy metals have been shown to accumulate in kidney, liver, blood, feathers, eggs and bones. Due to the accumulation of heavy metals in wild birds tissues they can be used as bioindicators for environmental contamination. Bird feathers have been used in previous studies and have been shown to be appropriate bioindicators of pollution by heavy metals. Feathers of birds of prey have proved to be good indicators of the status of environmental heavy metal pollution because they occupy higher trophic levels within an ecosystem and indicate compounds which bio-accumulate in prey such as methylmercury. Using feathers is a non-invasive way to obtain animal material from birds but most research looking at the metal concentrations has previously been focused on internal organs such as the liver or the excretion of the birds as these two show a much higher uptake of most metals that the bird is exposed too but require harming the animal to obtain these samples.

The aim of this study was to measure and evaluate the arsenic, cadmium, lead and mercury concentration in owl species including Long-eared owl (*Asio otus*), Barn owl (*Tyto alba*), Tawny owl (*Strix aluco*) and Little owl (*Athene noctua*), Buzzard (*Buteo buteo*), Common kestrel (*Falco tinnunculus*) and Eurasian sparrow-hawks (*Accipiter nisus*) collected from around Hungary. For this, feather samples were taken from them and their heavy metal contents were detected by inductively coupled plasma optical emission spectrometry. Statistical analysis was used to evaluate the results and looked to answer some key questions regarding the heavy metal burden accumulating in the birds of prey based on comparisons of 1) difference between male and female birds; 2) difference between adult and juvenile birds; 3) correlation between the four heavy metals that have been tested for accumulation.

2. REVIEW OF LITERATURE

2.1. METALS

Naturally occurring metals in the environment are divided into three different categories according to their use in the body. Metals which are defined as essential are those that are required in the body for optimum health and have a well defined role in biochemical processes. The second group is metals which could be called semi-essential because they do not have a defined physiological or biochemical role but improve growth rates and feed conversion, arsenic belongs in the semi-essential group. The last group of metals is non essential which have no beneficial role in animals and humans. Cadmium, lead and mercury belong to this group. The essential metals have daily requirement amounts and deficiencies will develop if they are not met in the diet. All of the metals can cause toxicosis if the concentration is too high in the diet.

2.1.1. Arsenic

Arsenic is the 20th most common element in the earth crust. The scientific symbol of arsenic is As and an atomic number of 33. It is a solid, brittle metalloid at standard temperature and pressure. The most common stable form of arsenic is metallic or grey arsenic. Arsenic binds covalently with most metals and non metals to form organic compounds. The most common uses for arsenic have been as chromatic copper arsenate which is a preservative for outdoor wood products but this has been phased out. Arsenic is also used as an alloy with lead in car batteries and ammunition. Other uses are in the production of pesticides, herbicides and insecticides. Arsenic is not an essential nutrient but has been shown to cause deficiency if animals are deprived of arsenic in the diet through decreased growth rates. Arsenic has antibacterial and anticoccidial properties and has been used as growth promoters in the swine and poultry industry (Anderson, 1983). Ingested arsenic is sequestered in mucosal tissue and diffuses into the body down the concentration gradient that builds in the mucosa tissue. After inorganic arsenic is absorbed it accumulates in tissues where it is methylated such as in the liver and testes. Arsenic can be excreted in the urine and the most common form is dimethylarsenic acid (Vahter et al., 2000). Arsenic toxicity and deprivation are exacerbated by conditions which limit the availability of mobile methyl groups (Uthus, 2003). There is a metabolic interaction between selenium and arsenic through competition for methyl donors and antioxidants. Arsenic and copper also have interactions. High arsenic concentration induces copper accumulation in the kidney (Uthus, 2001). Oxidative stress is the main mechanism of toxicity. Grains and grasses grown near industrial areas can contain markedly higher levels of arsenic than occur naturally.

2.1.2. Cadmium

Cadmium is the 63rd most common element in the earths crust. The scientific symbol of cadmium is Cd and it has the atomic number 48. Cadmium is a silver-white coloured soft metal which can be cut with a knife at standard temperature and pressure. For a metal it has a low melting pointing of 321°C. It is naturally found in association with zinc, lead and copper. Usually it is extracted as a by-product of mining for zinc. Cadmium has been used in batteries, plastic stabilizers and for metal planting on steel. Solar cells are also a common use for cadmium in recent times. Anthropogenic sources through which cadmium enters the environment at higher levels include zinc refining, coal combustion, mining waste, iron and steel production, pigments, fertilizers and sewage sludge. Cadmium is not considered an essential nutrient. However, it has been shown in low levels to increase weight gain in rodent, chickens and livestock when added to the diets (Bokori and Fekete, 1995). The beneficial mechanism is not understood and might possibly be due to an antibacterial or pharmacologic action. Cadmium can be absorbed in toxicological amounts through oral ingestion and inhalation. Cutaneous exposure requires very long exposure times. Cadmium has a relatively low intestinal absorption compared to similar actions such as zinc and iron. Cadmium absorption occurs mainly in the duodenum and jejunum inadvertently through pathways intended for essential nutrients such as zinc, iron, manganese and cysteine at the brush border. Cadmium bound to peptides may enter enterocytes by endocytosis. Absorption has been shown to decrease with chronic exposure (Muller at al., 1986). The bioavailability of cadmium is links to its solubility in the digestive tract with highly soluble salts being much better absorbed. Cadmium from diets of animal origin is absorbed in lower quantities than the higher soluble salts found in plants. Cadmium is transported in the blood mainly bound to albumin and distributed throughout the body with the highest concentrations appearing in the liver and kidney. Cadmium displaces zinc and copper from metallothionein. Basically, metallothionein is synthesized in the liver. Nephrotoxicity is one of the leading pathological signs of cadmium toxicosis due to the accumulation of it in the kidney. Blood levels of cadmium are the best indication of recent exposure whereas urine levels are more accurate at indicating total body burden (ATSDR, 1999a). Cadmium is excreted very slowly from the body through the urine mainly and also in the faeces. Signs of toxicosis can take over half a year to resolve due to this slow process of excretion. The free cadmium ions are responsible for the toxic effects through disruption of the cellular redox states causing oxidative stress. In the testes cadmium disrupts zinc protein transcription leading to apoptosis (Xu et al., 1999). Sources in the environment of cadmium in high levels can occur due to contamination of water sources for mining. Human activities such as fuel combustion, smelting, mining and incorrect disposal of cadmium products pollute the surrounding environment. Soil levels within a 1km radius of smelters have been shown to have levels of 72 mg/kg compared to the normal concentration of less than 1 mg/kg distributed throughout the earth crust. Dietary levels of 10 mg/kg are tolerated in poultry but this will result in higher levels accumulating in the kidneys and liver.

2.1.3. Lead

Lead has the atomic symbol of Pb and an atomic number 82. It is a bluish silver grey metal. Lead is soft and malleable with a low melting point of 327.46. It has no characteristic smell or taste. Mixed lead and zinc ore accounts for the majority of lead mining sources and is most commonly found in the form of lead sulphide (PbS). The main uses for lead are in acid batteries and ammunition, the use of lead ammunition was banned in Hungary in 2005. Lead was used in ceramics and glass products but is declining for less toxic alternatives. Lead was previously used as an additive to fuels but in the 1990s this use was banned in the European Union due to health and environmental concerns. Lead is one of the most common causes of poisoning through agriculture to companion, wild animals and humans. Lead is not an essential nutrient in the diet of animals and birds meaning it has not known benefit to physiological and biochemical processes in the body of humans and animals. Absorption of lead in the gastrointestinal tract occurs in the duodenum. Absorption most likely occurs through pathways designed for essential nutrients such as non-heme iron. The efficiency of absorption is dependent on the chemical form of lead, other dietary nutrients and physiological state of the animal. Calcium and phosphate are effective at reducing lead absorption (Varnai et al, 2001). Young animals absorb more lead than adults. The absorption of inhaled particles is very efficient and dermal absorption is minimal. 90% of lead entering into the blood is bound to haemoglobin within red blood cells. In peripheral tissue lead is bound to protein. Lead is redistributed from soft tissues to bone and forms a very stable complex with phosphate replacing calcium in hydroxyapatite. Physiological states that are associated with increased rates of bone resorption lead to increased levels of lead in the blood (ATSDR, 1999b). Lead is excreted in the urine and through biliary clearance in the intestines. The mechanism of toxicity is through oxidative stress. The major source of exposure of animals to lead is through soil contamination of feeds stuffs. Plants do not take up lead easily so are not a major concern unless contaminates exogenously. Anthropogenic sources of lead are the major concern to contributing increased levels of lead in the environment such as from smelters and chemical plants. Despite bans on various uses of lead in the 1980s and 1990s those site that were contaminated still contain high levels of lead. The major source of lead to wild birds is spent lead ammunitions. Biomagnification of lead does not typically occur in the higher trophic levels. The highest levels of lead in organisms are associated with proximity to contaminated sites. The predominant clinical signs of lead toxicosis are renal, gastrointestinal, hepatic and immunological signs. Higher doses of lead can be tolerated when consumed through the diet compared to water sources. High levels of cadmium increase lead deposition and toxicity (Phillips et al., 2003). The maximum tolerable levels of lead in chickens diet is 0.5 mg/kg for chronic exposure.

2.1.4. Mercury

Mercury has the scientific symbol of Hg and the atomic number 80. The metal is a dense silvery white and liquid at room temperature. It is also known as quick silver due to these properties. Mercury has uniform expansion over the entire liquid temperature range which made it ideal for use in thermometers. Mercury occurs in the earth crust at an average rate of 80 μg/kg. Naturally occurring mercury is found in cinnabar ore in the form of mercuric sulphide. The biggest producers of mercury worldwide are Algeria, China, Kyrgyzstan and Spain. The main uses of mercury products have been for the production of chlorine-caustic soda, antiseptic, batteries, barometers and dental restoration. Major natural sources of mercury result from volcano eruptions. Anthropogenic release of mercury into the environment results from the burning of fossil fuels, production of steel and phosphate and mining for gold. Mercury has no known benefit to the physiological state of animals and humans, so is considered a non essential nutrient. Absorption of mercury is highly dependent on its chemical form. Metallic mercury is only absorbed in the gastrointestinal tract at about

0.01% of oral dose. Dermal absorption is considered low. Inorganic and organic mercury have better absorption rates with methylmercury having the highest rate of absorption at 90%. Younger animals absorb mercury in higher rates. After absorption mercury is transported in the blood. Methylmercury can bind with cysteine and take advantage of carrier mediated amino acid transport systems to enter cells. Mercury accumulates in the liver, spleen, kidney, brain and muscle. In chickens the concentration of mercury in the feathers is equal to the approximate tissue concentration after consumption of organic or inorganic mercury (March et al., 1983). The mechanism of toxicity through which mercury toxicosis occurs is oxidative stress, lipid perspiration, mitochondrial dysfunction and changes to heme metabolism. Excretion of mercury is mainly via urine and faeces. Animals accumulate mercury at a rate faster than excretion this leads to bioaccumulation in higher trophic levels. Increased levels of mercury in the soil lead to increased levels of mercury in plants grown on those soils. In animal tissues mercury accumulates in the liver, kidneys and muscles. Renal changes and pathological nervous sign are the most sensitive indication of mercury toxicosis. The maximum tolerable level of methylmercury in the diet of birds is 5 mg/kg.

2.2. Eating habits of investigates birds

Long-eared owl (*Asio otus*)

Long-eared owls eat mostly small mammals, including voles, many kinds of mice, kangaroo rats, shrews, pocket gophers, and young rats or rabbits. They hunt over open ground or below the canopy in sparsely forested areas. Prey items usually weigh up to about 3.5 ounces, often less than 2 ounces. They also sometimes eat small birds, capturing them on the ground or (in the case of roosting birds) from low vegetation (Ivory, 1999).

Little owl (*Athene noctua*)

The Little Owl typically hunts worms, beetles, moths, small mammals and common vole (Schipper et al., 2011).

Buzzard (*Buteo buteo*)

Buzzards’ diet consists of small mammals such as mice, the common vole and rabbits. They also eat reptiles and insects (Csörgő et al., 2012).

Common kestrel (*Falco tinnunculus*)

Common kestrels eat almost exclusively small mammals in Europe. Voles, birds and lizards make up the majority of the diet whilst insects and frogs are rarely consumed. They hunt by soaring in the air and diving quickly onto their prey. When prey is in abundance they have been known to store the prey that they do not eat (Shrubb, 1993; Village, 1990).

Eurasian sparrow-hawks (*Accipiter nisus*)

The Eurasian sparrow-hawk has a diet that can consist of up to 97% small birds such as finches, thrushes and robins. The small mammals that they consume are rabbits, voles, shrews and squirrels. very rarely do they consume insects and carrion (Stevens, 2011).

2.3. USE OF FEATHERS FOR MONITORING OF HEAVY METALS

Feathers have been used in this study for a number of reasons. It has been shown in other studies that heavy metal concentrations in bird feathers are accurate bioindicators of the metal levels in the blood at the time of feathers growth (Burger, 1993; Burger and Gochfeld, 1993). Care should be taken in that feathers as samples will also reflect external contamination from sources such as atmospheric pollution, soil, rain and dust (Jasper at al., 2004). Feathers have also been chosen as the sample for this study as it is non invasive and does not require harming of any birds when compared to studies using tissue samples. Feathers are a good choice of sample for monitoring contaminants also because sample can be taken from an individual animal multiple times, the samples do not quickly degrade and can be stored at room temperature. Studies have shown that cadmium levels in feathers have proven to be accurate indicators of tissue levels (Agusa et al., 2005), while other studies have disputed this (Orlowski et al., 2007). Lead concentrations in feathers have been shown to be representative of tissue levels (Golden et al., 2003) although atmospheric contamination can prove significant over longer periods of time (Franson and Pain, 2011) which could distort comparisons between juvenile and adult birds burden of lead. Mercury has been examined in bird feather in many precious studies and shown to be an accurate representative of blood and tissue mercury levels at the time of feathers growth (Lewis and Furness, 1991, Thompson et al., 1991).

3. MATERIALS AND METHODS

3.1. ANIMALS

The birds were originated from the eastern and north-eastern region of Hungary and the samples were collected in the Hortobágyi Madárpark (Bird Hospital Foundation), Hortobágy, Hungary as shown in Figure 1. The examined area is geographically varied; the northern part mountainous with woody habitats, while heading south it changes to steppe with sand-hills and rivers. Also there are agricultural areas and possible environmental polluting factories, such as oil refinery, drug company, fertilizer warehouse, chemical and incineration plant, from where the heavy metals can get into the food chain.

During the collection, different data were noted as follows: time and location of finding, gender and birds' age. The identification of the age of the birds was carried out by the colour of their plumage, the size of the bird, and for example in the case of the buzzards, the colour of their eyes is different in the first 2 years (in the first year is livid, in the second year is yellow and from the third year is brown).

Samples were collected from predatory birds including 41 Owls – Long-eared owl (*Asio otus*) (3 juveniles and 4 adults), Barn owl (*Tyto alba*) (1 juvenile), Tawny owl (*Strix aluco*) (7 juvenile and 12 adult), Little owls (*Athene noctua*) (8 juveniles and 1 adult), 40 Buzzard (*Buteo buteo*) (18 juveniles and 18 adults, 4 without age), 18 Common kestrels (Falco tinnunculus) (7 juveniles, 10 adults, 1 without age) and 24 Eurasian sparrow-hawks (*Accipiter nisus*) (9 juveniles, 13 adults and 2 unknown).

Figure 1 Collection area of bird species

3.2. ANALYTICAL METHOD

3.2.1. Chemicals used

De-ionized water was produced by a Purite Select Fusion 160 BP water purification system. The conductivity of the ultrapure water was less than 0.1 ΩS/cm. Concentrated nitric acid (69 m/m%) (HNO_3) and hydrogen peroxide (30% m/m%) (H_2O_2) were obtained from Aristar and Normapur. Both of them were for trace analysis quality. For sample digestion, 0.5 g of sample was decomposed by nitric acid and hydrogen peroxide by microwave digestion system. All the laboratory glassware and plastic tools were cleaned by 0.15 M hydrochloric acid (37 m/m%) solution obtained from Aristar then rinsed with de-ionized water.

3.2.2. Analytical standards, ICP measurements

ICP multi- and mono-element standards used for quantitative ICP measurement were obtained from Perkin Elmer and Prolabo. Argon gas of 4.6 purity was from Messer. Quality Control (QC) standards were prepared from standard bovine liver (NIST-1577C).

3.2.3. Sample preparation

Feathers were washed with de-ionized water and acetone, to remove adherent exogenous contamination before the analytical procedure. For sample digestion, 0.5 g from each sample was weighted into a CEM MARS6 MARSXPreSS teflon vessel. Then they were decomposed by 5 ml nitric acid (69 m/m%) and 5 ml hydrogen peroxide (30% m/m%) in a microwave digestion system (Ramp: 35 min; Temperature: 200ºC; hold: 50 min; E: 1700 W). The sample is filled up to 25 ml and analysed by ICP-OES after a double dilution by de-ionized water using 1 mg/l Y solution as internal standard and 0.25 mg/l Au for the stabilization of mercury content. Blank and the QC samples were prepared from NIST-1577C standard by the same method.

3.2.4. Analytical measurements

Determination of heavy metals was carried out by a Perkin Elmer Optima 8300 DV Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) instrument (Figure 2) with the following measurement parameters:

Optical system	Echelle-type, flushed with nitrogen gas
Wavelength	167-782 nm
RF generator	40 MHz solid state, free running, flat plate plasma technology
RF powder	1300 W
Torch	Flat Plate Torch
Detector	solid-state detection circuit, 2 SCD detector (segmented CCD)
Plasma observation	Dual View (DV): axial, radial
Nebuliser type	concentric (BURGENER PEEK MIRA MIST)
Nebuliser gas flow rate	0,7 dm^3/min
Plasma gas flow rate	12 dm^3/min
Auxiliary gas flow rate	0.2 dm^3/min
Resolution of optical system	high
Resolving powder	<6 pm
Observation height	15 mm

Figure 2 ICP-OES instrument

Calibration range was between 0 and 200 mg/kg. The limits of detection (LOD) were the following:

Heavy metals (mg/kg)			
As	Cd	Hg	Pb
0.2	0.02	0.2	0.1

Internal quality control of the measurements was carried out via measuring QC samples of known heavy metal concentration at least 10 times. After discarding the extremes, the standard deviation of data (SD) was established that must have remained within the ±15% of the nominal concentration value in order to accept the QC measurement. The certified Hg content of the QC standard was less than the LOD (0.00536 mg/kg). The Hg content of all the samples was also less than the LOD.

3.3. Statistical analysis

The statistical analysis of the data was performed using the Software: R version 3.4.2. The usual t-test cannot be used because of some unknown values. Wilcoxon rank-sum test was used to compare distributions. Comparisons between age and gender were performed independently. Individuals with unknown age were excluded from the analysis by age, with unknown gender were excluded from the analysis by gender. All data was included in the calculation of correlation between the heavy metals, age and gender are shown on graphs.

Because data was not normally distributed and some numbers were not known exactly, Spearman rank-correlation was calculated and tested. P-value <0.05 was significant. Spearman’s rho and p-value were reported in two different tables. $\rho<0.33$ is a weak correlation, $0.33<\rho<0.67$ is medium and $0.67<\rho$ is strong. Averages and Standard deviations for both sexes and age groups have been calculated separately using Microsoft Excel.

4. RESULTS

The results are shown and represented by Figures 3-58 and Tables 1-24.

4.1. ARSENIC CONTENT

Arsenic was measured in each of the species studied. The average concentrations for Owls are shown in **Table 1**.

Table 1 Heavy Metal Concentration in Owls (mean±SD, mg/kg, n=number of sample)

Metal	Males (n=17)	Females (n=18)	Adult (n=17)	Juveniles (n=18)	Both Sexes (n=41)
Arsenic	0.32±0.38	0.40±0.17	0.36±0.25	0.46±0.39	0.40±0.30
Cadmium	0.08±0.04	0.08±0.05	0.09±0.05	0.12±0.21	0.10±0.14
Mercury	0.68±0.31	0.52±0.35	0.58±0.34	0.60±0.27	0.58±0.31
Lead	2.25±0.80	2.12±1.74	2.10±1.36	2.39±1.77	2.30±1.52

The average for both sexes was 0.40±0.3 mg/kg. The average for males was 0.32±0.38 mg/kg. The average for females was higher (0.4±0.17 mg/kg). The average for adults was 0.36±0.25 mg/kg. The average for juveniles was 0.46±0.39 mg/kg.

Figures 3-4 and **Table 2** shows along with using the Wilcoxon rank sum test there was no significant difference between age and gender.

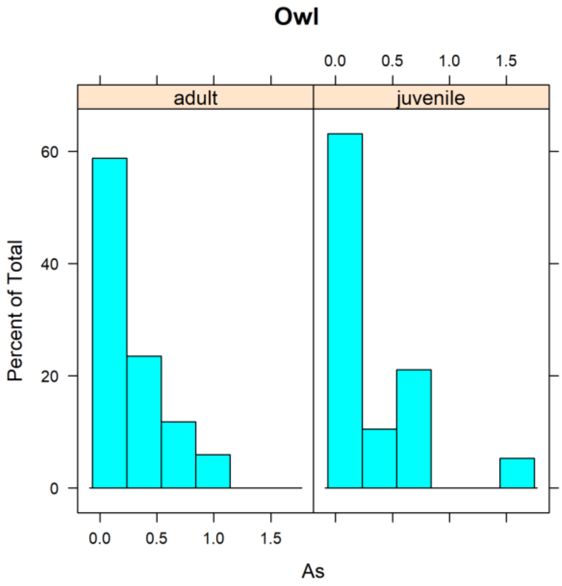

Figure 3 Comparison of As in adult and juvenile Owls

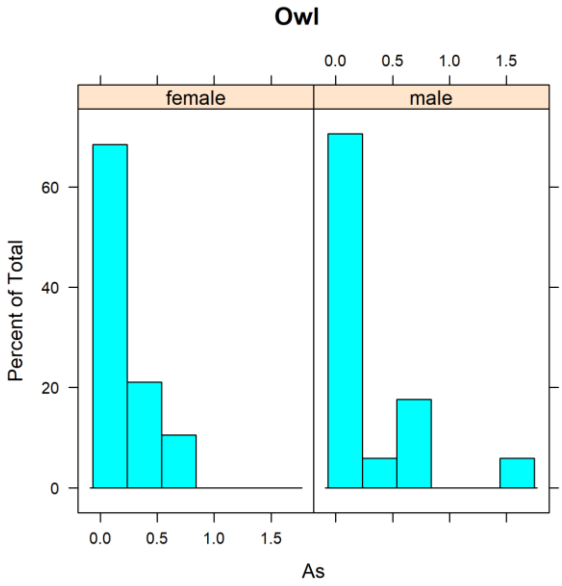

Figure 4 Comparison of As in female and male Owls

Table 2 Arsenic Median in Owls

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0	0.13	0.3	1.11
juvenile	0	0	0.06	0.519	1.68
male	0	0	0	0.24	1.68
female	0	0	0	0.27	0.62

Wilcoxon rank-sum test p-value between age groups: 0.92
Wilcoxon rank-sum test p-value between genders: 0.9307

The average concentration of Arsenic in Buzzards is shown in **Table 3**.

Table 3 Heavy Metal Concentration in Buzzards (mean±SD, mg/kg, n=number of samples)

Metal	Males (n=18)	Females (n=16)	Adult (n=18)	Juveniles (n=18)	Both Sexes (n=40)
Arsenic	0.33±0.17	0.32±0.14	0.4±0.28	0.28±0.13	0.33±0.17
Cadmium	0.08±0.03	0.09±0.03	0.09±0.03	0.78±0.04	0.09±0.03
Mercury	0.63±0.27	0.67±0.43	0.69±0.34	0.63±0.36	0.64±0.35
Lead	0.83±0.53	1.67±2.01	1.16±0.59	1.20±1.99	1.15±1.40

The average for both sexes was 0.33±0.17 mg/kg. The average for males was 0.33±0.17 mg/kg. The average for females was 0.32±0.14 mg/kg. The average for adults was 0.40±0.28 mg/kg. The average for juveniles was 0.28±0.13 mg/kg.

Figures 5-6 and **Table 4** shows along with using the Wilcoxon rank sum test there was no significant difference between age and gender.

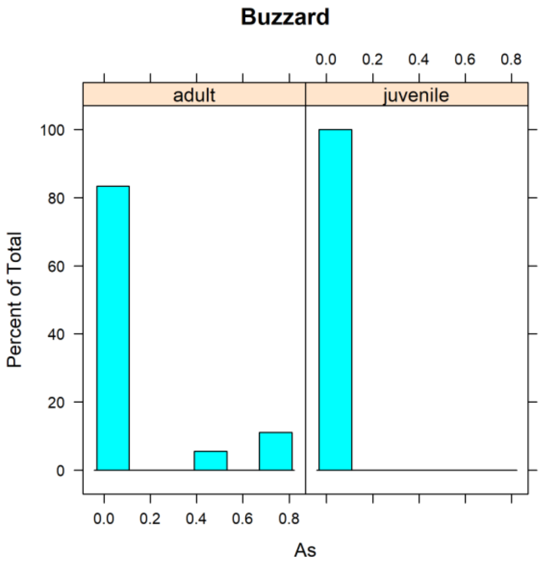

Figure 5 Comparison of As in adult and juvenile Buzzards

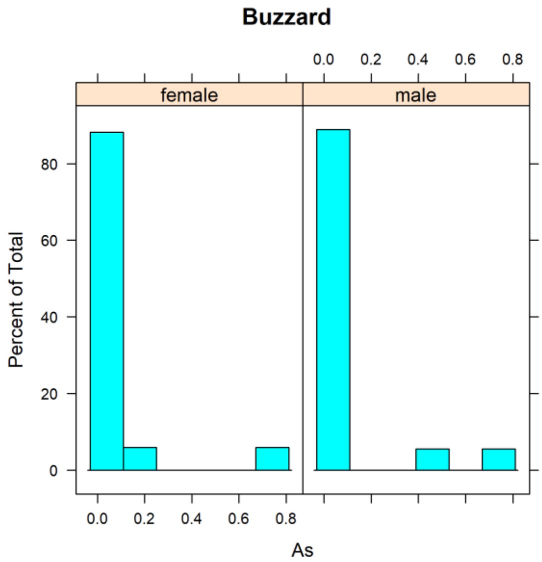

Figure 6 Comparison of As in female and male Buzzards

Table 4 Median Value for Buzzards

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0	0	0	0.78
juvenile	0	0	0	0	0
male	0	0	0	0	0.734
female	0	0	0	0	0.78

Wilcoxon rank-sum test p-value between age groups: 0.0803
Wilcoxon rank-sum test p-value between genders: 0.9762

The average concentration for Arsenic in Common kestrels across both sexes is 0.29±0.24 mg/kg (**Table 5**).

Table 5 **Heavy Metal Concentration in Common kestrels (mean±SD, mg/kg, n=number of samples)**

Metal	Males (n=8)	Females (n=8)	Adult (n=10)	Juveniles (n=7)	Both Sexes (n=18)
Arsenic	0.20±0.00	0.36±0.32	0.30±0.30	0.30±0.16	0.29±0.24
Cadmium	0.13±0.10	0.25±0.20	0.21±0.19	0.20±0.18	0.20±0.18
Mercury	0.64±0.37	0.56±0.37	0.46±0.29	0.75±0.36	0.59±0.36
Lead	2.02±1.30	2.50±1.81	1.85±1.24	2.48±2.02	2.10±1.57

The average concentration for males was 0.20±0.00 mg/kg and in females was 0.36±0.32 mg/kg. The average concentration in adults was 0.30±0.31mg/kg and in juveniles was 0.30±0.16 mg/kg.

Figures 7-8 and **Table 6** have shown using Wilcoxon rank sum that there is no significant difference between ages and genders in the concentration of arsenic in Common kestrels.

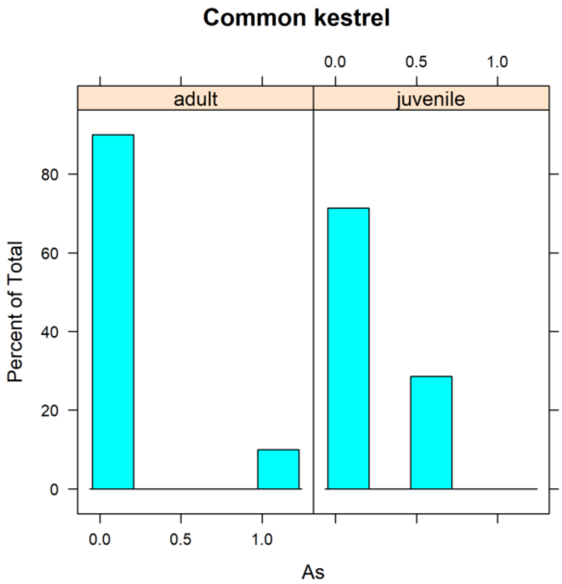

Figure 7 Comparison of As in adult and juvenile Common kestrels

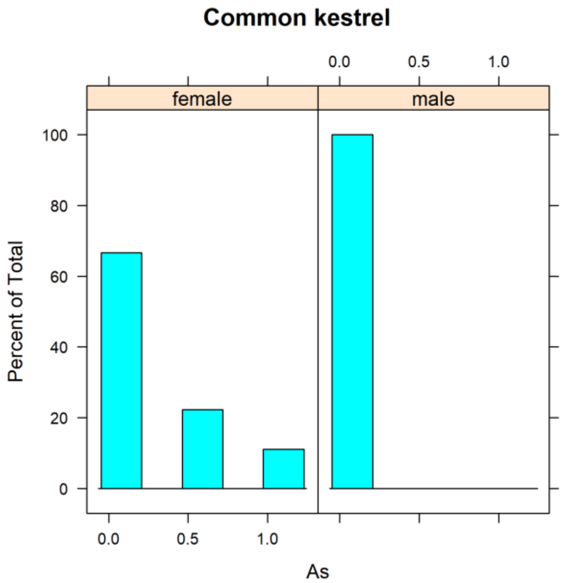

Figure 8 Comparison of As in female and male Common kestrels

Table 6 Median values for Common kestrels

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0	0	0	1.18
juvenile	0	0	0	0.25	0.6
male	0	0	0	0	0
female	0	0	0	0.5	1.18

Wilcoxon rank-sum test p-value between age groups: 0.4632
Wilcoxon rank-sum test p-value between genders: 0.0962

The average concentration of Arsenic in Eurasian sparrow-hawks was 0.32±0.18 mg/kg (**Table 7**).

Table 7 Heavy Metal Concentration in Eurasian sparrow-hawks (mean±SD, mg/kg, n=number of samples)

Metal	Males (n=11)	Females (n=11)	Adult (n=13)	Juveniles (n=9)	Both Sexes (n=24)
Arsenic	0.25±0.12	0.40±0.21	0.37±0.21	0.27±0.12	0.32±0.18
Cadmium	0.12±0.11	0.10±0.05	0.12±0.10	0.09±0.06	0.11±0.09
Mercury	2.09±1.39	2.44±1.10	2.29±1.21	2.30±1.31	2.19±1.26
Lead	2.19±1.10	1.69±0.52	2.06±1.06	1.70±0.64	1.84±0.92

The average concentration in males was 0.25±0.12 mg/kg and in females was 0.40±0.21 mg/kg. The average concentration in adults was 0.37±0.21 mg/kg and in juveniles was 0.27±0.12 mg/kg.

Figures 9-10 and **Table 8** show using Wilcoxon rank sum test that there is no significant difference between genders and age groups.

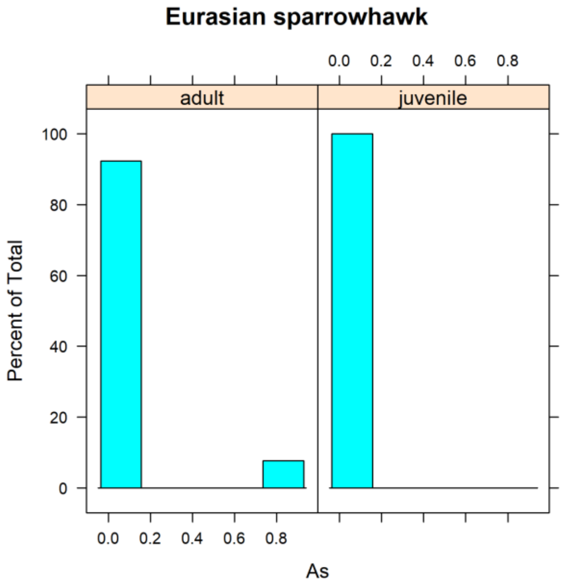

Figure 9 Comparison of As
in adult and juvenile Eurasian sparrow-hawks

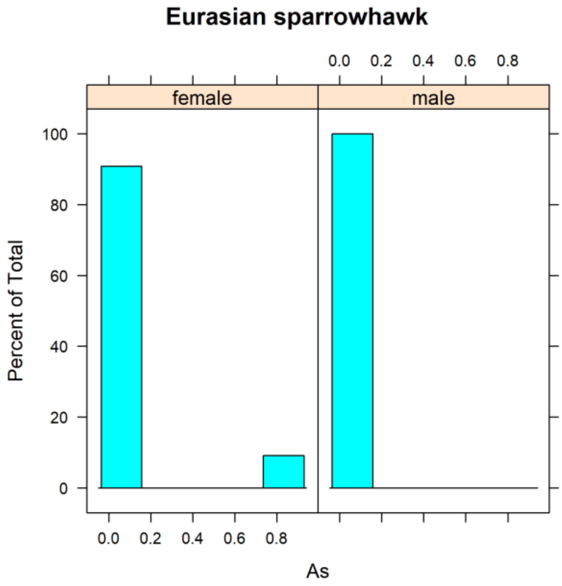

Figure 10 Comparison of As
in female and male Eurasian sparrow-hawks

Table 8 Median value of As in Eurasian sparrow-hawk

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0	0	0	0.893
juvenile	0	0	0	0	0
male	0	0	0	0	0
female	0	0	0	0	0.893

Wilcoxon rank-sum test p-value between age groups: 0.4595
Wilcoxon rank-sum test p-value between genders: 0.3633

4.2. CADMIUM CONTENT

Cadmium was measured in each of the species groups studied. The average concentration in Owls was 0.10±0.14 mg/kg (**Table 1**). The average in males was 0.08±0.04 mg/kg and in females was 0.08±0.05 mg/kg. The average concentration in adults was 0.09±0.05 mg/kg and in juveniles was 0.12±0.20 mg/kg.

Figures 11-12 and **Table 9** shows using the Wilcoxon rank sum test that there was no significant difference between age groups and genders.

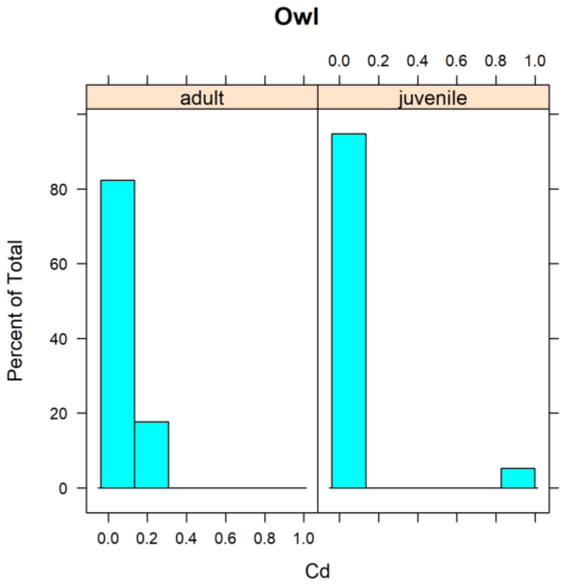

Figure 11 Comparison of Cd in adult and juvenile Owls

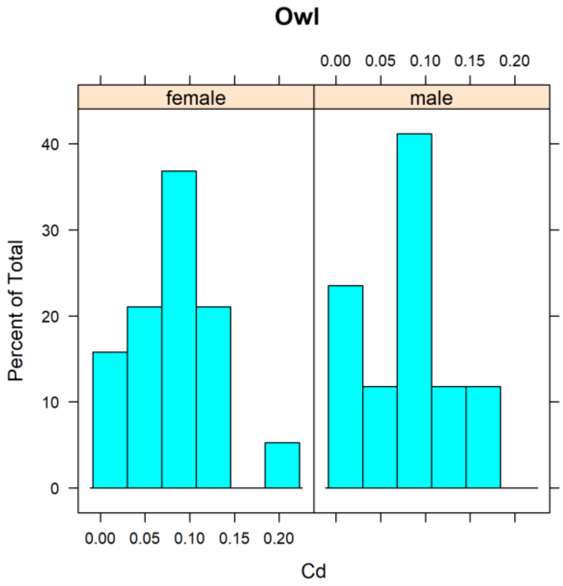

Figure 12 Comparison of Cd in female and male Owls

Table 9 Median value of Cd in Owls

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0.064	0.08	0.122	0.214
juvenile	0	0.065	0.08	0.093	0.96
male	0	0.04	0.08	0.1	0.16
female	0	0.0605	0.07	0.098	0.214

Wilcoxon rank-sum test p-value between age groups: 0.6336
Wilcoxon rank-sum test p-value between genders: 0.7747

The average concentration of cadmium in Buzzards was 0.09±0.03 mg/kg (**Table 3**). The average concentration in males was 0.08±0.03 mg/kg and in females was 0.09±0.03 mg/kg. The average concentration in adults was 0.09±0.03 mg/kg and in juveniles was 0.78±0.04 mg/kg.

Figures 13-14 and **Table 10** shows using Wilcoxon rank sum calculation that there is no significant difference between age groups and genders.

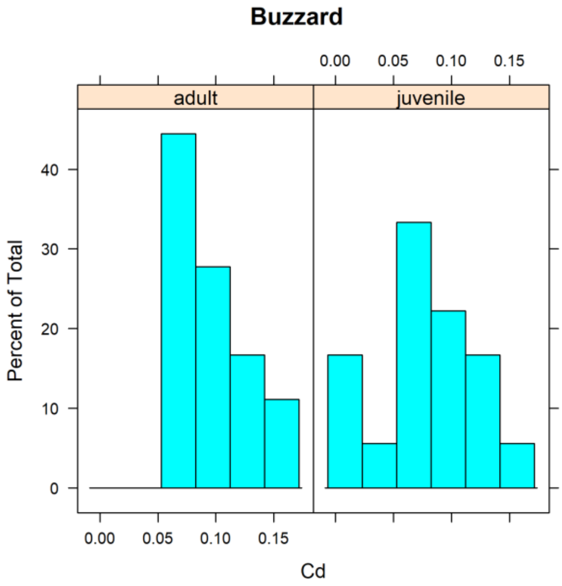

Figure 13 Comparison of Cd in adult and juvenile Buzzards

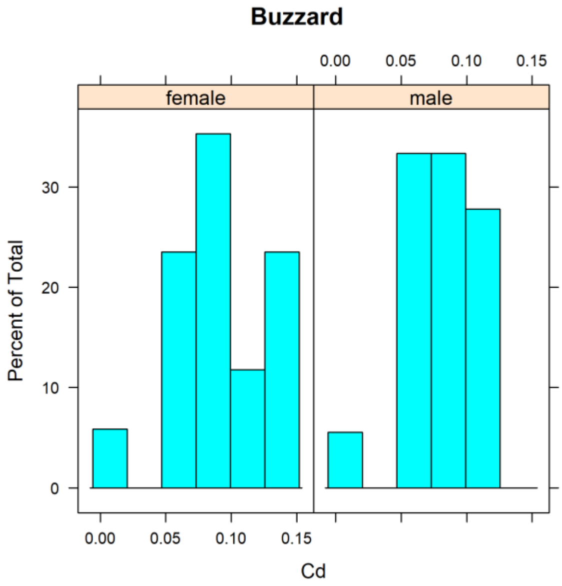

Figure 14 Comparison of Cd in female and male Buzzards

Table 10 Median value of Cd in Buzzards

	minimum	lower quartile	median	upper quartile	maximum
adult	0.058	0.074	0.0855	0.116	0.165
juvenile	0	0.055	0.074	0.11	0.146
male	0	0.063	0.077	0.101	0.118
female	0	0.072	0.086	0.117	0.146

Wilcoxon rank-sum test p-value between age groups: 0.2749
Wilcoxon rank-sum test p-value between genders: 0.2218

The average concentration of cadmium in the Common kestrels studied was 0.20±0.18 mg/kg (**Table 5**). The average concentration in the males was 0.13±0.10 mg/kg and in females was 0.25±0.20 mg/kg. The average concentration in the adults was 0.21±0.19 mg/kg and in juveniles was 0.20±0.18 mg/kg.

Figures 15-16 and **Table 11** shows using the Wilcoxon rank sum test that there was no significant difference in concentration of cadmium between age groups and genders.

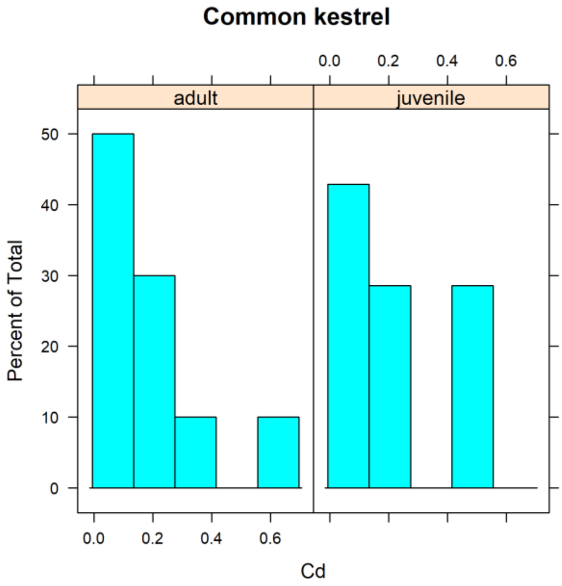

Figure 15 Comparison of Cd in adult and juvenile Common kestrels

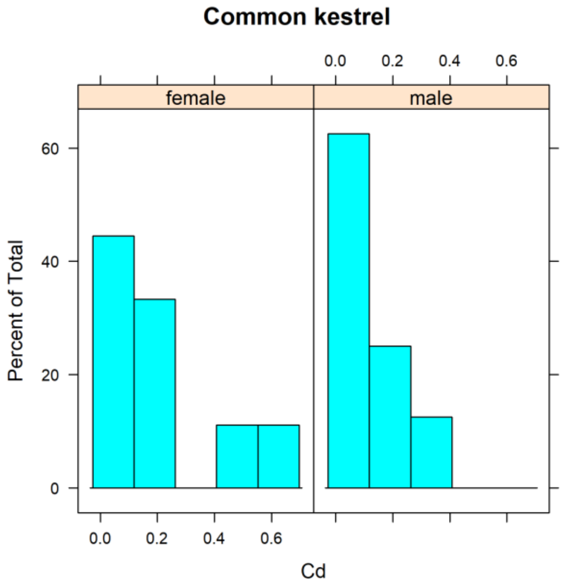

Figure 16 Comparison of Cd in female and male Common kestrels

Table 11 Median value of Cd in Common kestrels

	minimum	lower quartile	median	upper quartile	maximum
adult	0.06	0.086	0.122	0.262	0.67
juvenile	0.02	0.061	0.155	0.3	0.5
male	0	0.068	0.0905	0.1525	0.36
female	0.02	0.082	0.18	0.262	0.67

Wilcoxon rank-sum test p-value between age groups: 0.8125
Wilcoxon rank-sum test p-value between genders: 0.4234

The average concentration of cadmium in Eurasian sparrow-hawks was 0.11±0.09 mg/kg (**Table 7**). The average concentration in males was 0.12±0.11 mg/kg and in females was 0.10±0.05 mg/kg. The average concentration in adults was 0.12±0.10 mg/kg and in juveniles was 0.09±0.06 mg/kg.

Figures 17-18 and **Table 12** shows using the Wilcoxon rank sum test that there is no significant difference in the concentration of cadmium between the age groups and genders studied.

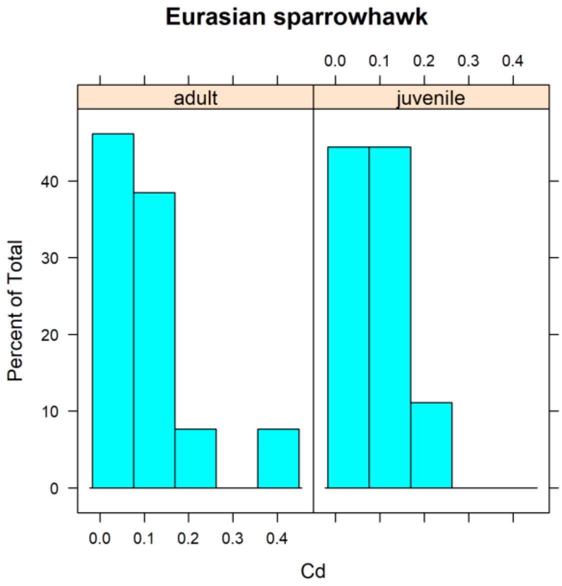

Figure 17 Comparison of Cd in adult and juvenile Eurasian sparrow-hawks

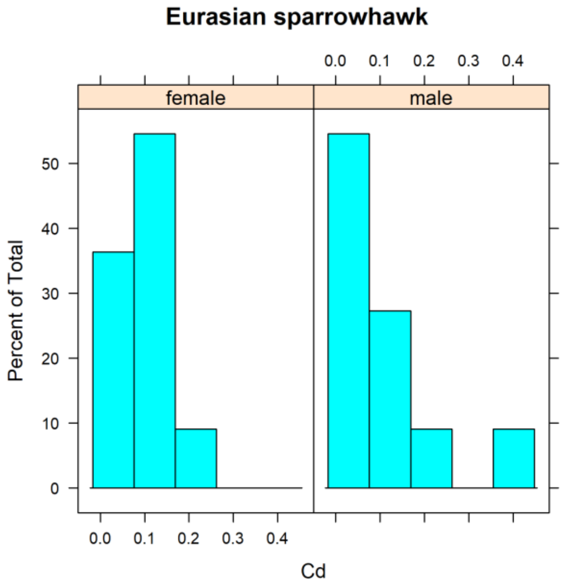

Figure 18 Comparison of Cd in female and Male Eurasian sparrow-hawks

Table 12 Median value of Cd in Eurasian sparrow-hawks

	minimum	lower quartile	median	upper quartile	maximum
adult	0.048	0.072	0.085	0.147	0.431
juvenile	0	0.055	0.092	0.108	0.196
male	0	0.0545	0.075	0.132	0.431
female	0	0.0725	0.086	0.126	0.196

Wilcoxon rank-sum test p-value between age groups: 0.8412
Wilcoxon rank-sum test p-value between genders: 0.8182

4.3. MERCURY CONTENT

Mercury concentration was measured in each of the species groups studied. The average concentration of mercury in Owls was 0.58±0.31 mg/kg (**Table 1**). The average concentration in males was 0.68±0.30 mg/kg and in females was 0.52±0.37 mg/kg. The average concentration in adults was 0.58±0.34 mg/kg and in juveniles was 0.6±0.27 mg/kg.

Figures 19-20 and **Table 13** shows along with using Wilcoxon rank sum test that there is no significant difference in the concentrations of mercury between the age groups and genders studied.

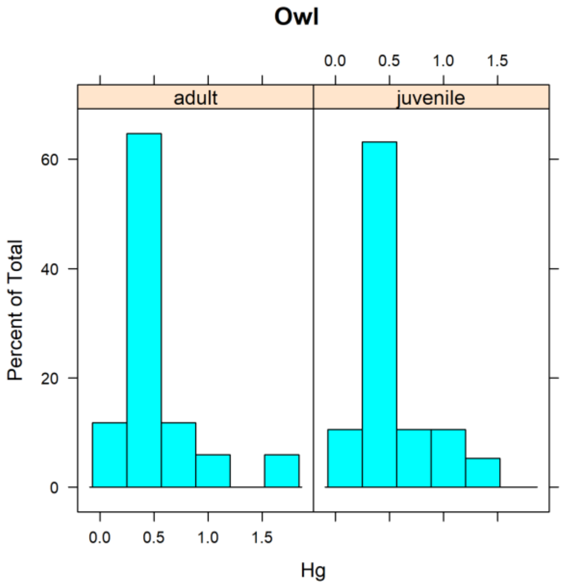

Figure 19 Comparison of Hg in adult and juvenile Owls

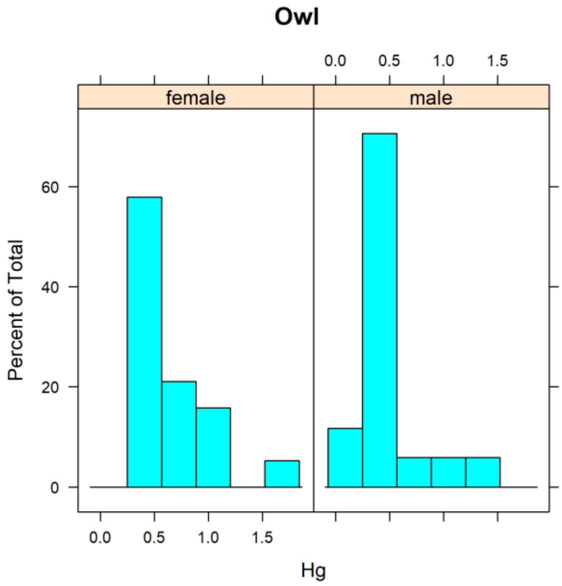

Figure 20 Comparison of Hg in female and male Owls

Table 13 Median value of Hg in Owls

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0.34	0.48	0.52	1.77
juvenile	0	0.415	0.47	0.55	1.44
male	0	0.34	0.47	0.51	1.44
female	0.25	0.45	0.51	0.79	1.77

Wilcoxon rank-sum test p-value between age groups: 0.9873
Wilcoxon rank-sum test p-value between genders: 0.132

The average concentration of mercury in Buzzards studied was 0.64±0.35 mg/kg (**Table 3**). The average concentration in the males was 0.63±0.27 mg/mg and in the females was 0.67±0.43 mg/kg. The average concentration in the adult buzzards was 0.69±0.34 mg/kg and in the juveniles was 0.65±0.34 mg/kg.

Figures 21-22 and **Table 14** shows along with using Wilcoxon rank sum test that there was no significant difference between the age groups and genders studied.

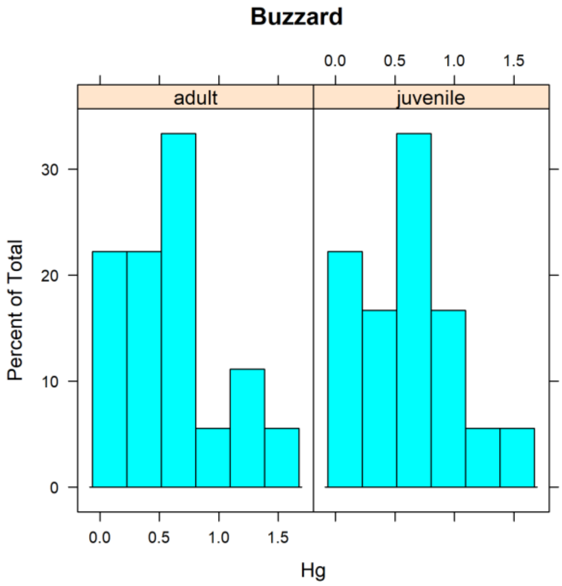

Figure 21 Comparison of Hg in adult and juvenile Buzzards

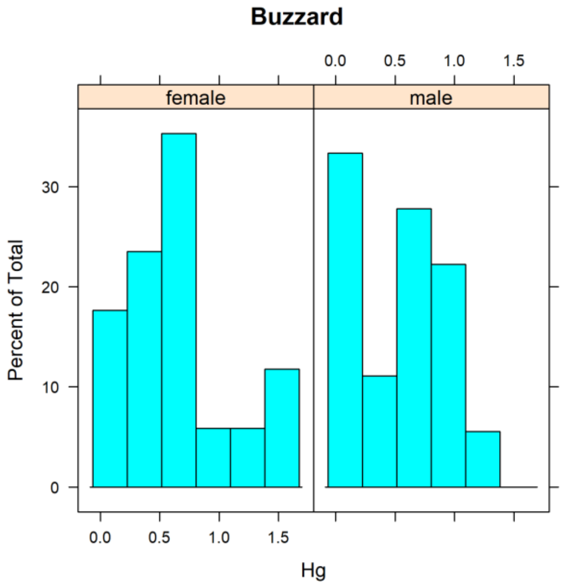

Figure 22 Comparison of Hg in female and male Buzzards

Table 14 Median value of Hg in Buzzards

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0.44	0.53	0.77	1.57
juvenile	0	0.29	0.57	0.89	1.61
male	0	0	0.55	0.82	1.34
female	0	0.32	0.56	0.77	1.61

Wilcoxon rank-sum test p-value between age groups: 0.9367
Wilcoxon rank-sum test p-value between genders: 0.466

The average concentration of mercury in the Common kestrels studied was 0.59±0.36 mg/kg (**Table 5**). The concentration in the Males was 0.64±0.37 mg/kg and in females was 0.56±0.38 mg/kg. The concentration in adults was 0.46±0.29 mg/kg and in juveniles was 0.75±0.36 mg/kg.

Figure 23-24 and **Table 15** shows along with using Wilcoxon rank sum test that there is no significant difference in the concentration of mercury in the common kestrels between the age groups and genders studied.

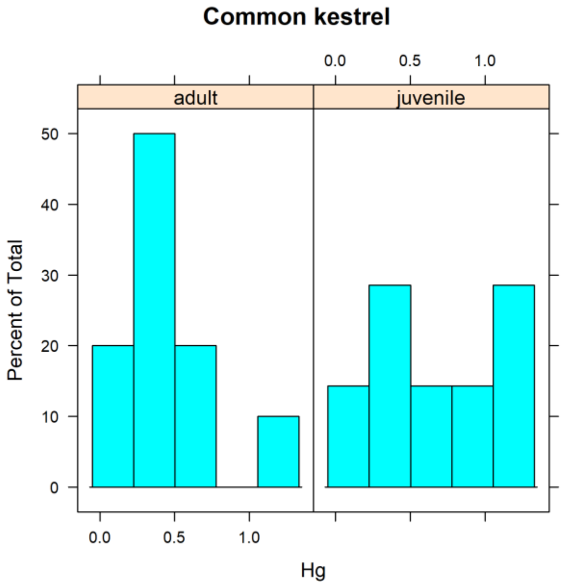

Figure 23 Comparison of Hg in adult and juvenile Common kestrels

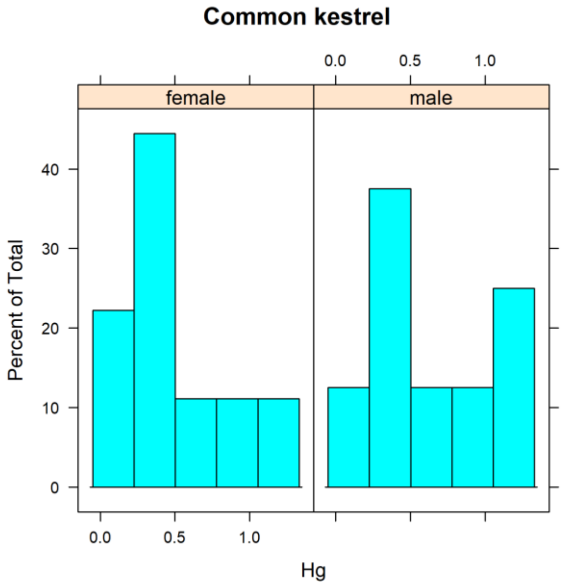

Figure 24 Comparison of Hg in female and male Common kestrels

Table 15 Median value of Hg in Common kestrels

	minimum	lower quartile	median	upper quartile	maximum
adult	0	0.24	0.3805	0.58	1.13
juvenile	0	0.425	0.68	1.115	1.28
male	0	0.2655	0.6135	0.975	1.2
female	0	0.248	0.46	0.58	1.28

Wilcoxon rank-sum test p-value between age groups: 0.2413
Wilcoxon rank-sum test p-value between genders: 0.7001

The average concentration of mercury in the Eurasian sparrow-hawks studied was 2.19±1.26 mg/kg (**Table 7**). The average concentration in the males was 2.09±1.39 mg/kg and in the females was 2.44±1.10 mg/kg. The average concentration in the adults was 2.29±1.21 mg/kg and in the juveniles was 2.30±1.31 mg/kg.

Figures 25-26 and **Table 16** shows along with using Wilcoxon rank sum test that there is no significant difference in concentration of mercury due to gender or age in the Eurasian sparrow-hawks studied.

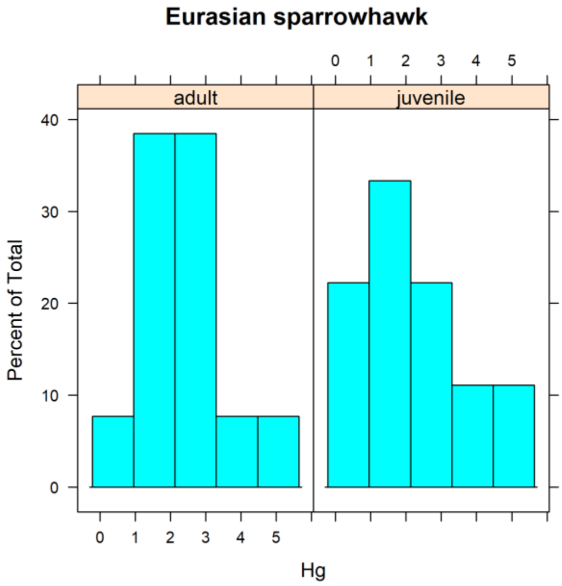

Figure 25 Comparison of Hg in adult and juvenile Eurasian sparrow-hawks

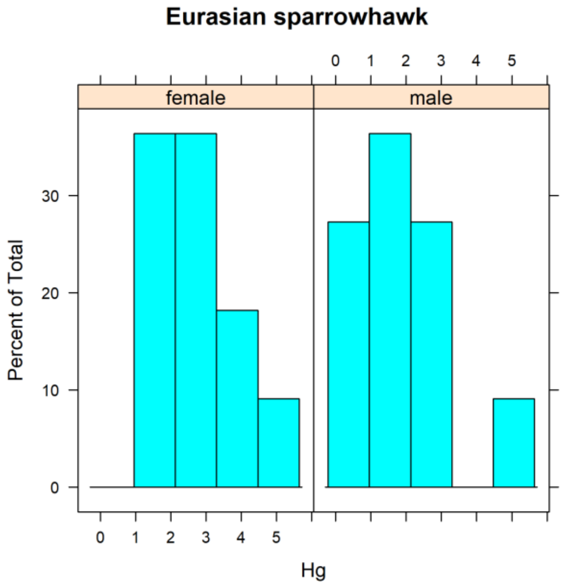

Figure 26 Comparison of Hg in female and male Eurasian sparrow-hawks

Table 16 Median value of Hg in Eurasian sparrow-hawks

	minimum	lower quartile	median	upper quartile	maximum
adult	0.487	1.53	2.22	2.54	5.43
juvenile	0	0.975	2.11	3.16	4.53
male	0	1.115	1.76	2.895	5.43
female	0.975	1.66	2.38	3.035	4.53

Wilcoxon rank-sum test p-value between age groups: 1
Wilcoxon rank-sum test p-value between genders: 0.4009

4.4. LEAD CONTENT

Lead concentration was measured in each of the species studied. The average concentration of lead in the Owls studied was 2.30±1.52 mg/kg (**Table 1**). The concentration in males was 2.25±0.80 mg/kg and in females was 2.12±1.74 mg/kg. The average concentration in the adult owls was 2.10±1.36 mg/kg and in the juvenile group was 2.39±1.77 mg/kg.

Figure 27-28 and **Table 17** show along with using the Wilcoxon rank sum test that there is no significant difference in the lead concentrations between genders or age groups of the Owls studied.

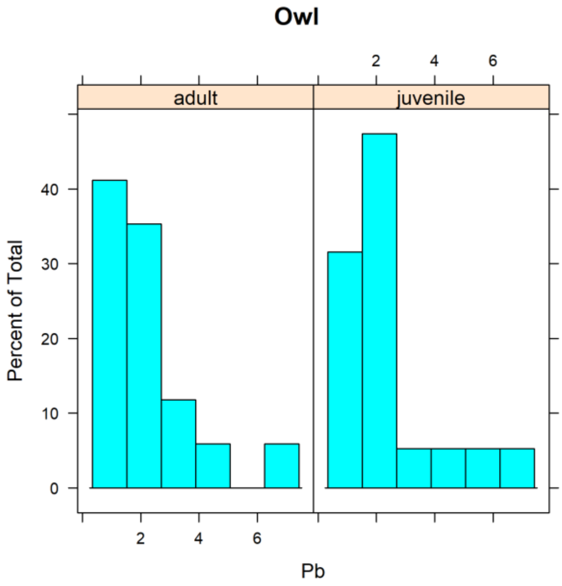

Figure 27 Comparison of Pb in adult and juvenile Owls

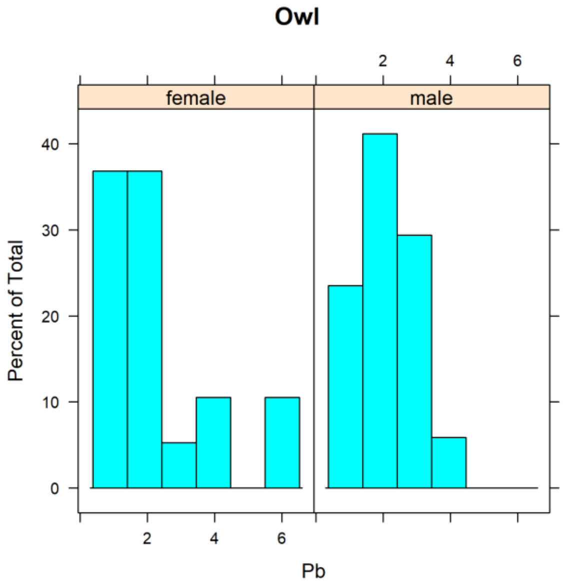

Figure 28 Comparison of Pb in female and male Owls

Table 17 Median value of Pb in Owls

	minimum	lower quartile	median	upper quartile	maximum
adult	0.67	1.16	1.76	2.18	6.29
juvenile	0.6	1.335	1.74	2.38	7.16
male	1.04	1.47	1.92	2.77	4.07
female	0.6	0.925	1.71	2.44	6.29

Wilcoxon rank-sum test p-value between age groups: 0.7634
Wilcoxon rank-sum test p-value between genders: 0.3498

The average concentration of lead in the Buzzards analysed is 1.15±1.40 mg/kg (**Table 3**). The average concentration in the male group was 0.83±0.53 mg/kg and in the females was 1.69±2.01 mg/kg. The concentration of lead in the adult Buzzards was 1.16±0.59 mg/kg and in the juveniles was 1.2±1.99 mg/kg.

Figures 29-30 and **Table 18** shows along with using Wilcoxon rank sum test that there was no significant difference between genders or age groups of the Buzzards studied.

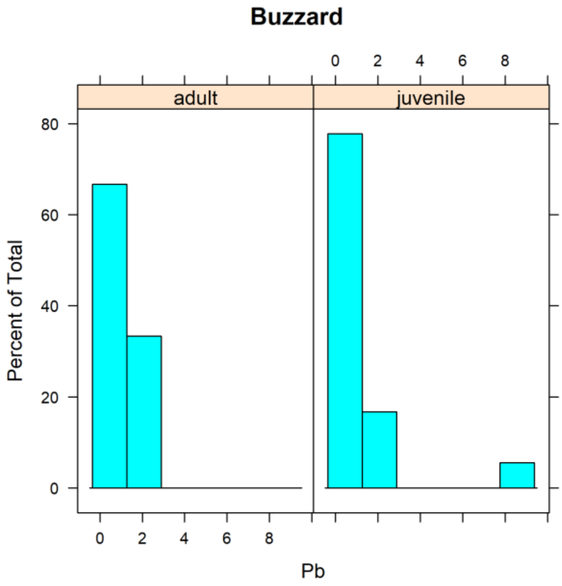

Figure 29 Comparison of Pb in adult and juvenile Buzzards

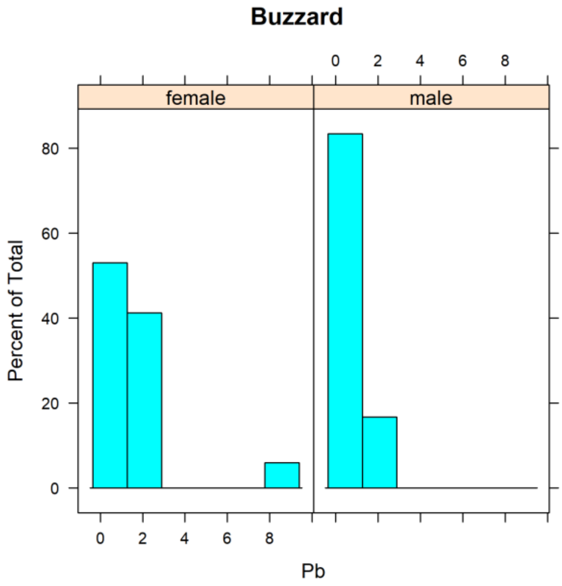

Figure 30 Comparison of Pb in female and male Buzzards

Table 18 Median value of Pb in Buzzards

	minimum	lower quartile	median	upper quartile	maximum
adult	0.24	0.56	1.16	1.4	2.61
juvenile	0	0.47	0.58	1.26	9.03
male	0.07	0.47	0.635	1.24	1.89
female	0	0.56	1.18	1.69	9.03

Wilcoxon rank-sum test p-value between age groups: 0.1209
Wilcoxon rank-sum test p-value between genders: 0.0988

The average concentration of lead in the Common kestrels analysed was 2.10±1.57 mg/kg (**Table 5**). The average concentration of lead in the male kestrels was 2.02±1.30 mg/kg and in the females was 2.50±1.82 mg/kg. The average concentration in the adult kestrels was 1.85±1.24 mg/kg and in juveniles was 2.48±2.02 mg/kg.

Figures 31-32 and **Table 19** shows along with using Wilcoxon rank sum test that there is no significant difference between in the age groups or genders studied.

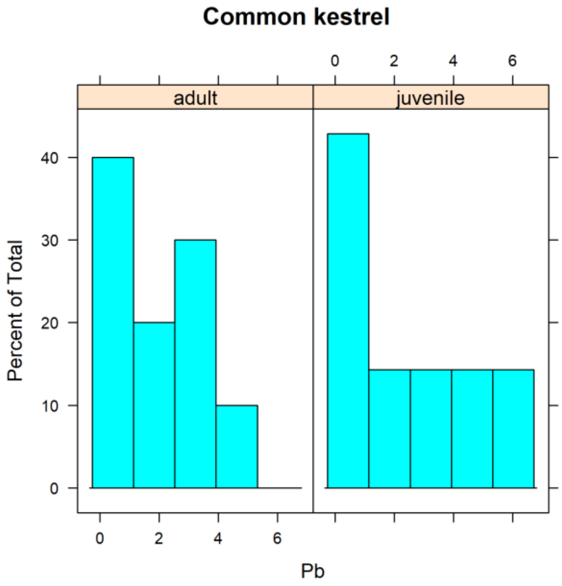

Figure 31 Comparison of Pb in adult and juvenile Common kestrels

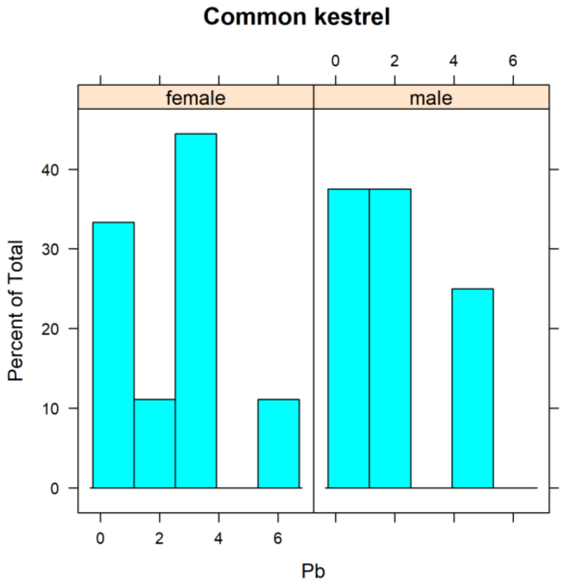

Figure 32 Comparison of Pb in female and male Common kestrels

Table 19 Median value of Pb in Common kestrels.

	minimum	lower quartile	median	upper quartile	maximum
adult	0	1.06	1.495	2.74	4.28
juvenile	0.63	0.965	1.16	3.6	6.46
male	0.75	1.09	1.495	2.975	4.28
female	0	1	2.54	2.93	6.46

Wilcoxon rank-sum test p-value between age groups: 0.8868
Wilcoxon rank-sum test p-value between genders: 0.9626

The average concentration of lead in the Eurasian sparrow-hawks studied was 1.84±0.92 mg/kg (**Table 7**). The average concentration in the males was 2.19±1.10 mg/kg and in females was 1.69±0.52 mg/kg. The average concentration in the adults was 2.06±1.06 mg/kg and in juveniles was 1.7±0.64 mg/kg.

Figures 33-34 and **Table 20** shows along with using Wilcoxon rank sum test that there is no significant difference in the concentration of lead in Eurasian sparrow-hawks between genders or the age groups studied.

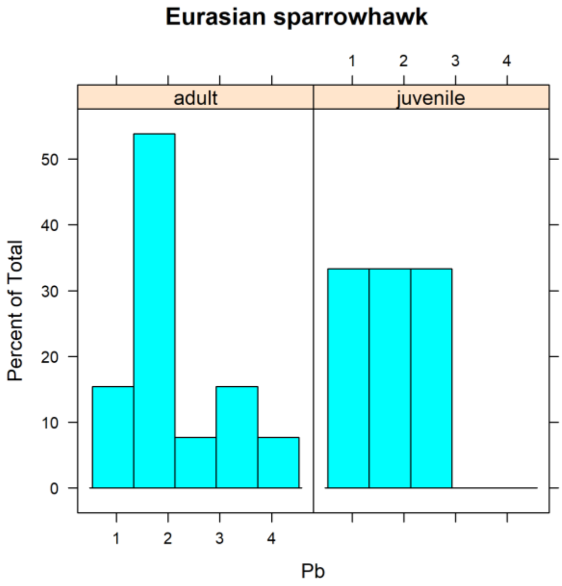

Figure 33 Comparison of Pb in adult and juvenile Eurasian sparrow-hawks

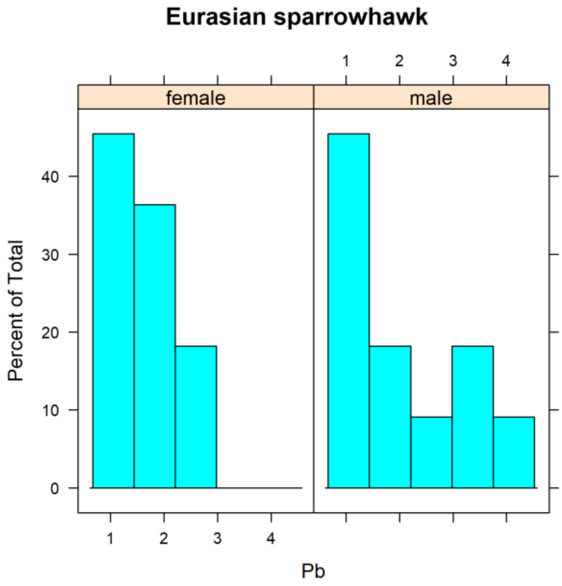

Figure 34 Comparison of Pb in female and male Eurasian sparrow-hawks

Table 20 Median Value of Pb in Eurasian sparrow-hawks

	minimum	lower quartile	median	upper quartile	maximum
adult	1.04	1.36	1.4	2.65	4.38
juvenile	0.68	1.05	2.02	2.2	2.27
male	1.04	1.285	2.13	2.86	4.38
female	0.81	1.365	1.87	1.975	2.65

Wilcoxon rank-sum test p-value between age groups: 0.8151
Wilcoxon rank-sum test p-value between genders: 0.5112

4.5. CORRELATION AMONG THE INVESTIGATED HEAVY METALS

The results regarding the correlation of heavy metal concentration in the predatory bird's feathers studied had some positive correlations. Because low concentrations were not known exactly and were substituted by zero, Spearman rank-correlation has been used.

In the Owls studied Cadmium was significantly correlated with Arsenic (**Figure 35**) and lead (**Figure 39**), the Spearman rank correlation coefficient in was 0.43 and 0.38 respectively, the connection in both cases was medium.

In the Buzzards studied a significant correlation was found between Cadmium and lead (**Figure 45**). The Spearman rank coefficient is 0.51 and the connection is medium. There was no significant correlation between any of the heavy metals examined in the case of Common kestrels.

In the Eurasian sparrow-hawks samples analysed cadmium was significantly correlated with lead (**Figure 57**) having a Spearman rank-correlation coefficient of 0.49, the connection was medium.

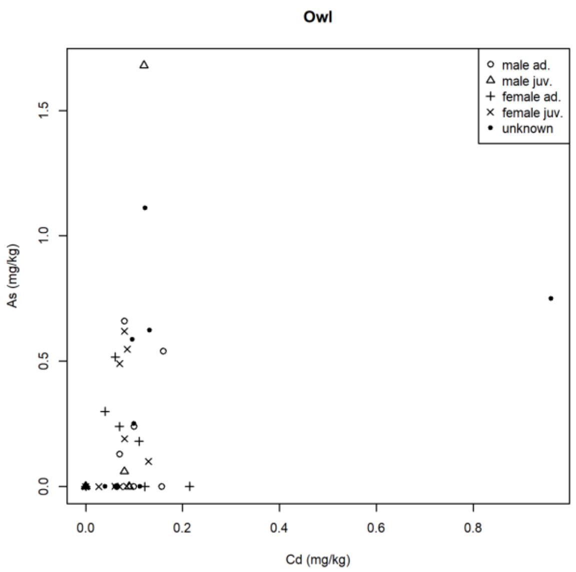

Figure 35 Correlation of As and Cd in Owls

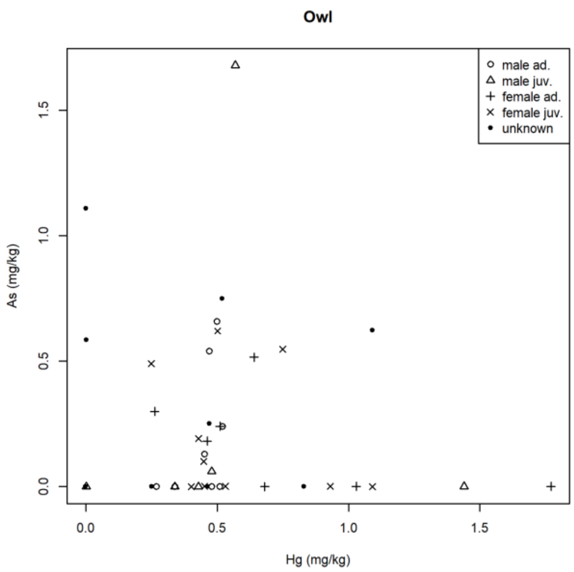

Figure 36 Correlation of As and Hg in Owls

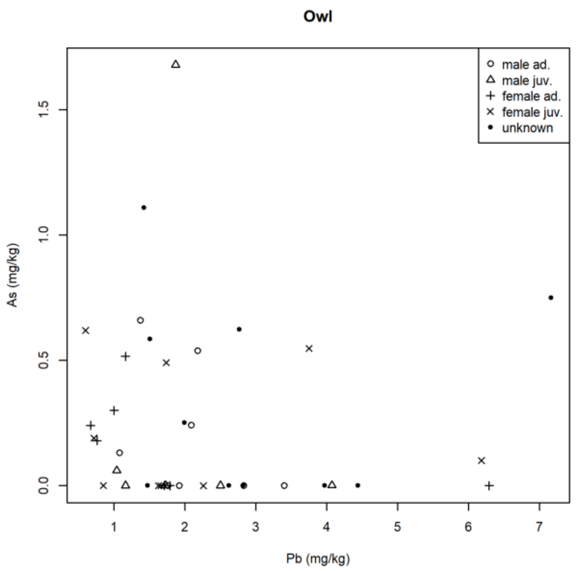

Figure 37 Correlation of As and Pb in Owls

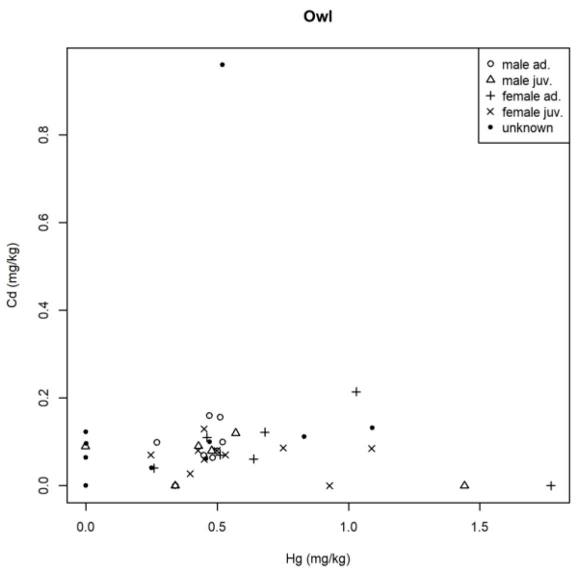

Figure 38 Correlation of Cd and Hg in Owls

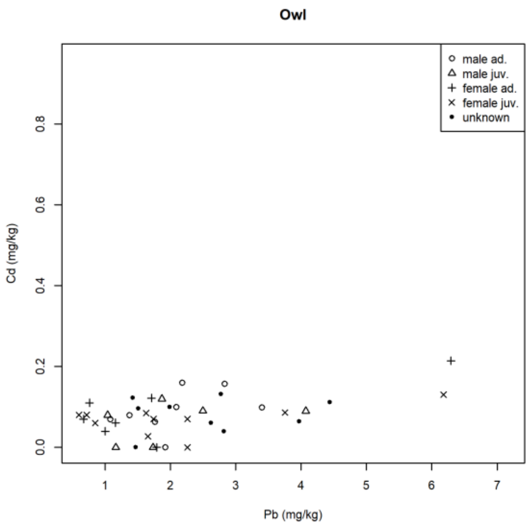

Figure 39 Correlation of Cd and Pb in Owls

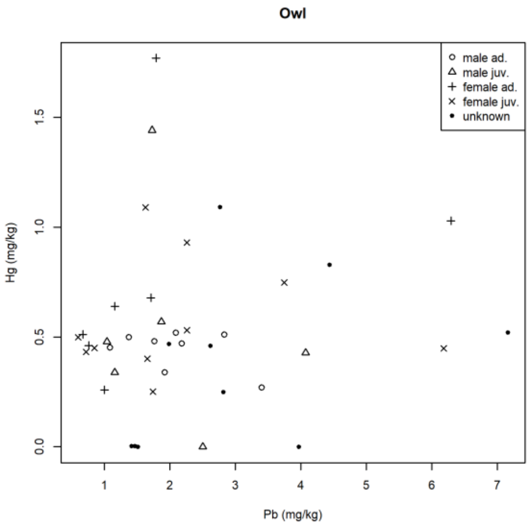

Figure 40 Correlation of Hg and Pb in Owls

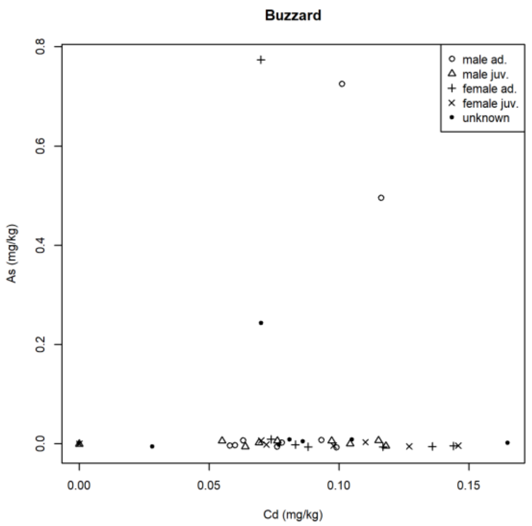

Figure 41 Correlation of As and Cd in Buzzards

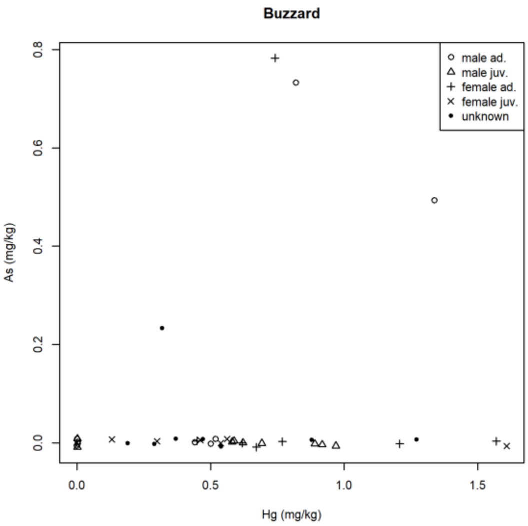

Figure 42 Correlation of As and Hg in Buzzards

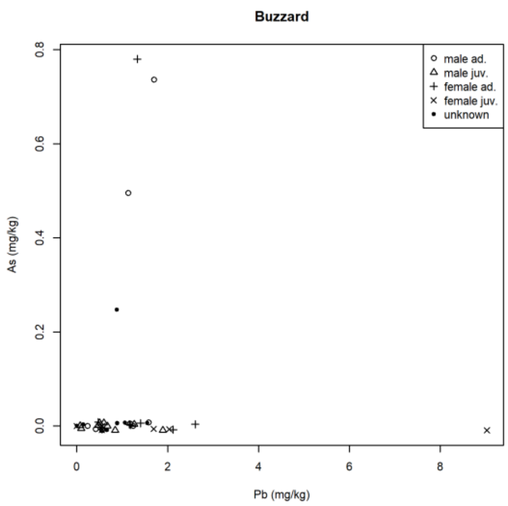

Figure 43 Correlation of As and Pb in Buzzards

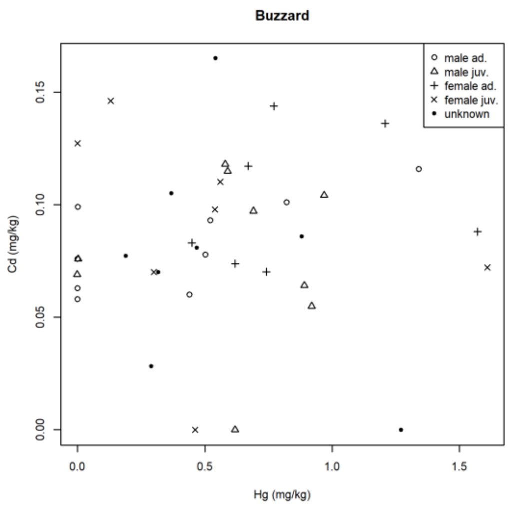

Figure 44 Correlation of Cd and Hg in Buzzards

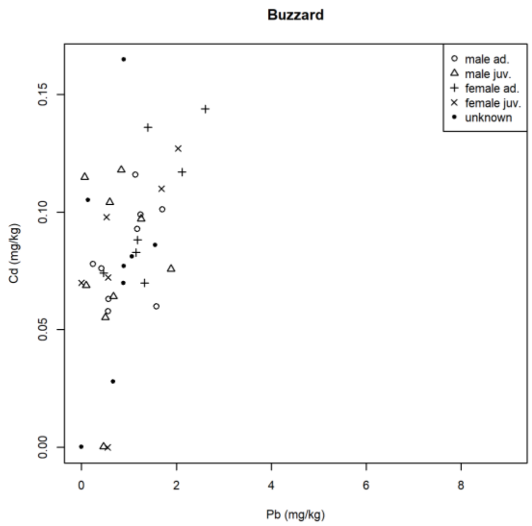

Figure 45 Correlation of Cd and Pb in Buzzards

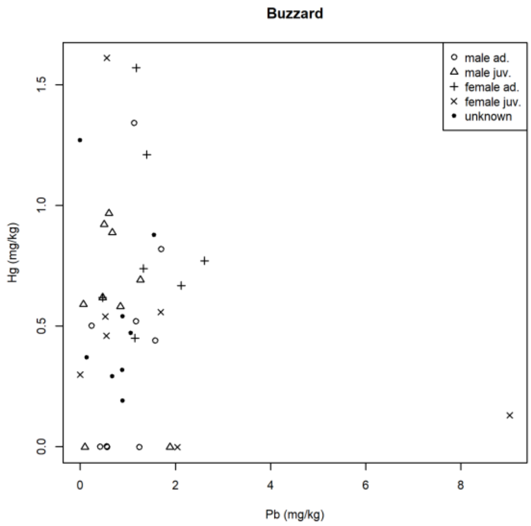

Figure 46 Correlation of Hg and Pb in Buzzards

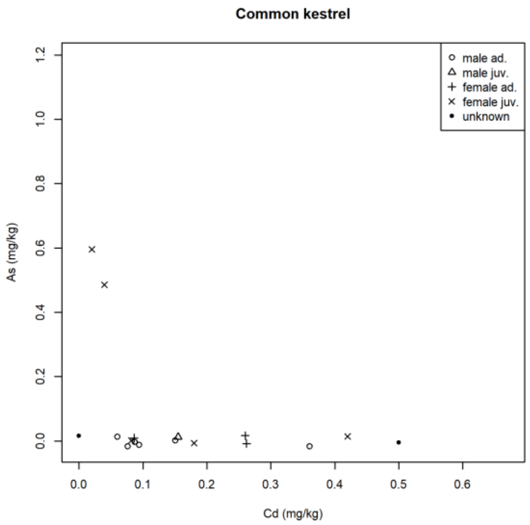

Figure 47 Correlation of As and Cd in Common Kestrels

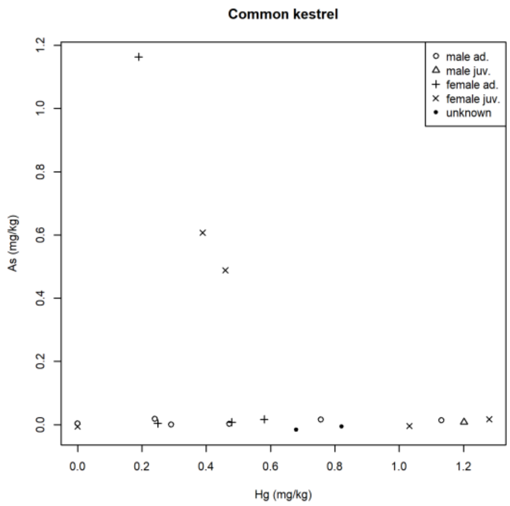

Figure 48 Correlation of As and Hg in Common Kestrels

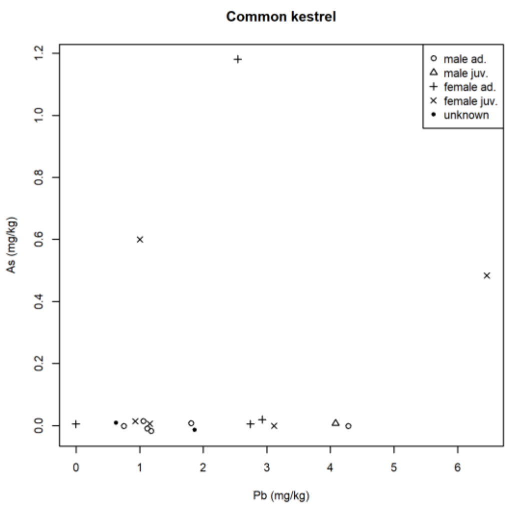

Figure 49 Correlation of As and Pb in Common Kestrels

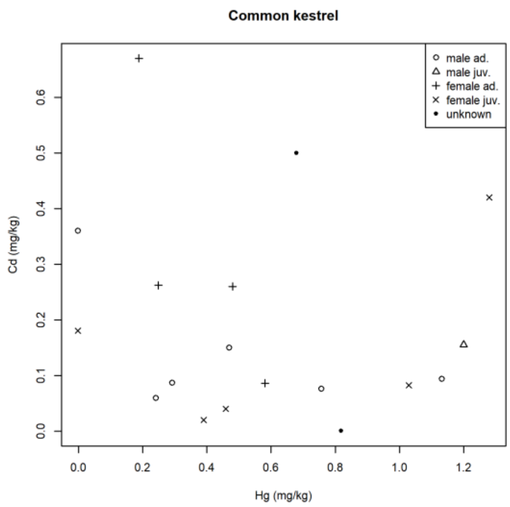

Figure 50 Correlation of Cd and Hg in Common Kestrels

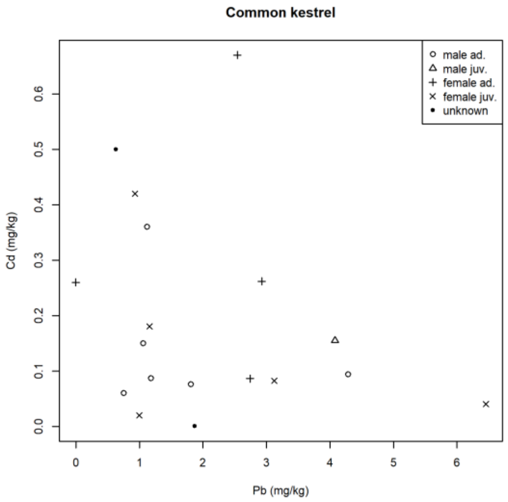

Figure 51 Correlation of Cd and Pb in Common Kestrels

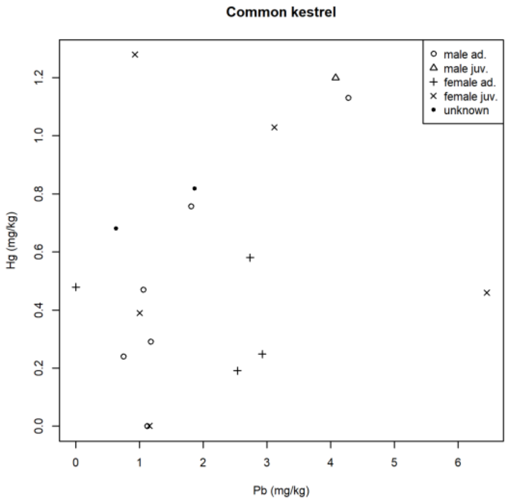

Figure 52 Correlation of Hg and Pb in Common Kestrels

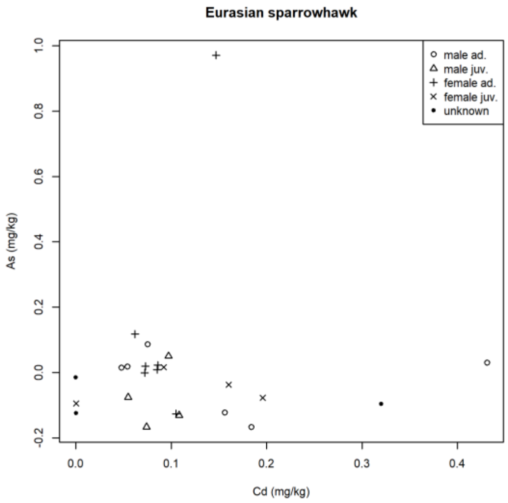

Figure 53 Correlation of As and Cd in Eurasian sparrow-hawk

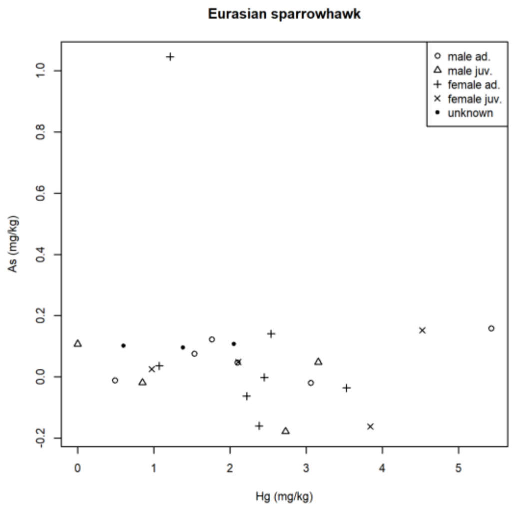

Figure 54 Correlation of Hg and As in Eurasian sparrow-hawk

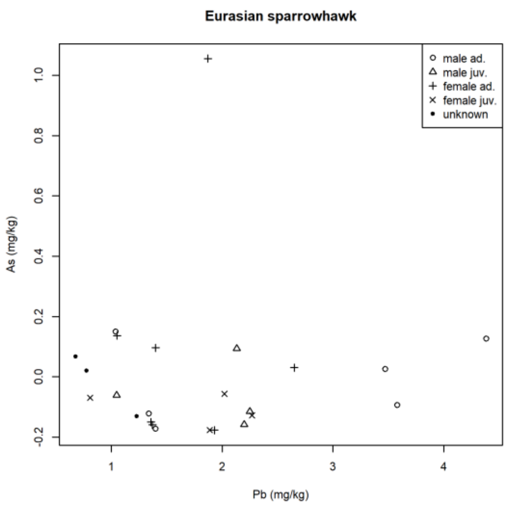

Figure 55 Correlation of As and Pb in Eurasian sparrow-hawk

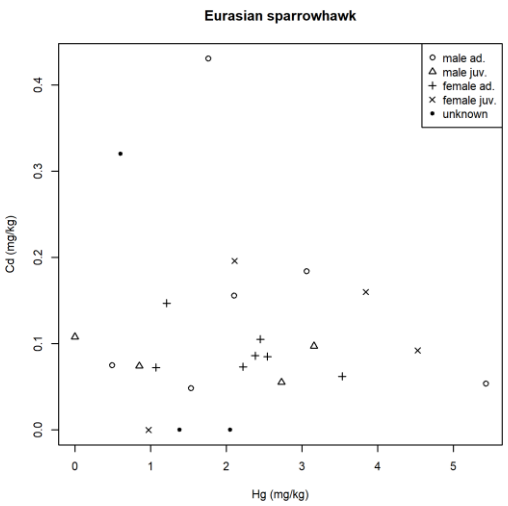

Figure 56 Correlation of Cd and Hg in Eurasian sparrow-hawk

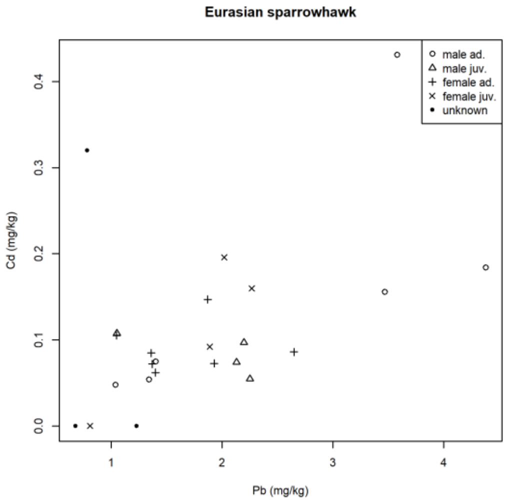

Figure 57 Correlation of Cd and Pb in Eurasian sparrow-hawk

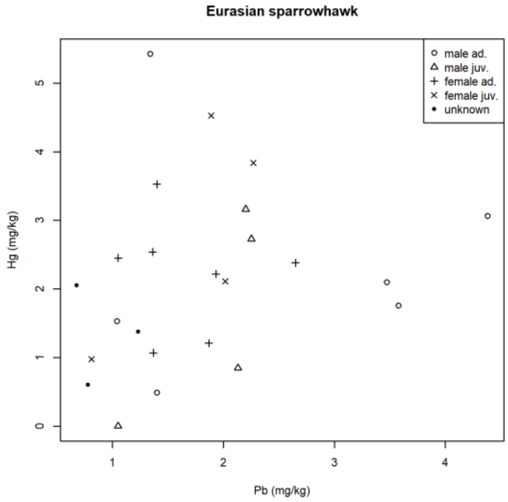

Figure 58 Correlation of Hg and Pb in Eurasian sparrow-hawk

5. DISCUSSION

The average concentration of Arsenic in the four species groups studied was 0.40±0.30 mg/kg in Owls, 0.33±0.17 mg/kg in Buzzards, 0.29±0.24 mg/kg in Common kestrels and 0.32±0.18 mg/kg in Eurasian sparrow-hawk which are significantly lower than the concentration of 5 mg/kg found in the USA in an area of possible arsenic pollution (Wiemeyer et al., 1980), There are few relatable studies that use feathers to analyse the quantity of Arsenic which makes it difficult in establishing whether the concentration of Arsenic recorded in this study is significant or not (Burger et al., 2015). The threshold value for Cadmium in birds is accepted as 3 mg/kg (Nighat et al., 2013), our concentrations measured are much lower than this value at 0.10±0.14 mg/kg in Owls, 0.09±0.03 mg/kg in Buzzards, 0.20±0.18 mg/kg in the Common kestrels and 0.11±0.09 mg/kg in Eurasian sparrow-hawks. In accordance with data from other studies analysing heavy metals in predatory birds (Naccari et al., 2009), adverse effects have been found at lower levels ranging from 0.1 mg/kg to 2 mg/kg (Burger, 1993), such as reduced growth rates (Spahn et al., 1999). So it is possible that the birds analysed in this study are experiencing mild adverse effects due to the cadmium concentrations in the environment. The threshold for toxicity of Mercury in bird species is highly variable amongst difference species. Sublethal levels from 2.4 mg/kg have been shown to cause impairments in the reproductive processes (Scheuhammer et al., 2007; Jackson et al., 2011). Our values recorded are much lower at 0.58±0.31 mg/kg in Owls, 0.64±0.35 mg/kg in Buzzards and 0.59±0.36 mg/kg in the Common kestrels studied. The highest concentration of Mercury was recorded in the sample of Eurasian sparrow-hawks, the average was 2.19±1.25 mg/kg and the highest individual concentration was 5.43 mg/kg which is a level that could be considered to impact on the reproductive performance of that individual. Mercury accumulates in the higher trophic levels of the food chain and the Eurasian sparrow-hawk' diet may explain the higher concentrations recorded compared to the three other species groups studied. Adverse effects of Lead concentration can be observed above 4 mg/kg (Burger and Gochfield, 2000). The average value that was recorded in this study was between 1.15 mg/kg and 2.3 mg/kg which is low enough that adverse effects should not be observed in these birds. Although there were 5 Owls, 1 Buzzard, 3 Common kestrels and 1 Eurasian sparrow-hawk sample over the threshold value which could be arising from spent ammunition or other common sources of Lead pollution.

In the four species groups that have been analysed in our study there was no significant difference in the concentration of the heavy metals due to gender. Several other studies that have addressed gender difference have also concluded that there is little difference in the concentration of heavy metals between the males and the females (Esselink et al,. 1995; Movalli, 2000; Zaccaroni et al., 2003) and any difference found was attributed to physiological and ecological differences. Naccari et al. (2009) found lower levels of metals in female buzzards that they attributed to the excretion of heavy metals in the eggs of those birds but we found no such difference in this study.

Burger et al. (1994) examined metal concentration in breast feathers of Common terns and found no age related difference in the levels of Mercury, Lead and Cadmium which supports the results that have been found in the study relating to the comparison of age groups.

Similarly, there was no correlation between adults and juveniles of investigated bird species in our study. This may be due to the difficulty in the precise determination of the age of the birds that have been sampled from. Other literature had found that there was age related differences when examining cadmium and lead (Zaccorini et al., 2003) which found increasing levels with age. It would be expected that the concentration of heavy metals would increase with age as juvenile birds would have had less exposure time to pollution of the environment but the older birds have also had seasons to excrete heavy metals during their moulting of feathers (Burger, 1993).

Regarding the correlation of heavy metals, a significant correlation between the concentration of Cadmium and Lead was recorded in the Owls, Buzzards and Eurasian sparrow-hawks analysed. This may be due to the area from which the birds inhabited. Due to the pollution from agricultural or industrial activities, Lead is especially associated with spent Lead ammunition and as it was only banned as recently as 2005, it is very possible that there is Lead ammunition in the environment of these birds. Radiographs of the birds used in this study would have been useful to establish if lead ammunition was in fact in the digestive system of these birds and contributing towards their feather concentrations of lead. There is sparse literature available on the study of metals in birds within the region that has been sampled from which would have been useful to establish trends over time in the concentration of the heavy metals analysed. It would be expected that the levels of lead and cadmium are in decline as their use in anthropogenic source such as cadmium batteries and leaded fuels have also declined (ATSDR, 1999a).

In conclusion the use of feathers for monitoring heavy metals in these species has been a useful and non-invasive sample type that revealed there to be no difference between genders and age groups in heavy metal accumulation. Although feathers are susceptible to external contamination affecting results this sampling method would be recommended especially in the case of endangered species.

7. SUMMARY

The aim of this study was to investigate the concentrations of heavy metals such as Arsenic, Cadmium, Lead and Mercury in the feathers of predatory birds including Owl species [Long-eared owl (*Asio otus*), Barn owl (*Tyto alba*), Tawny owl (*Strix aluco*), Little owls (*Athene noctua*)], Buzzard (*Buteo buteo*), Common kestrels (Falco tinnunculus) and Eurasian sparrow-hawks (*Accipiter nisus*). The birds were originated from the eastern and north-eastern region of Hungary and the samples were collected in the Hortobágyi Madárpark (Bird Hospital Foundation). During the samplings, different data were noted as follows: time and location of finding, gender and birds' age. Feather samples have been collected as a non-invasive method of sampling and have been shown to be representative of heavy metal burden in the body, with the added benefit of feathers being moulted yearly allowing these samples to represent the exposure of these birds in the previous year. The samples have been analysed using inductively coupled plasma mass spectrometry to determine the concentration of the heavy metals mentioned previously. The statistical analysis of the measured concentrations was performed using the Software: R version 3.4.2. Wilcoxon rank-sum test was used to compare distributions and Spearman rank-correlation was calculated and tested.

The mean values for the heavy metals varied between species groups, Arsenic values were from 0.29±0.24 mg/kg to 0.4±0.3 mg/kg, Cadmium concentrations averaged from 0.09±0.03 mg/kg to 0.2±0.18 mg/kg, Lead concentrations averaged from 1.15±1.40 mg/kg to 2.30±1.52 mg/kg and Mercury concentrations averaged from 0.58±0.31 mg/kg to 2.19±1.25 mg/kg. Based on these values recorded the heavy metal burden is not over what is considered the threshold values for these heavy metals and in accordance with similar concentrations of heavy metals that have been recorded in similar species within Europe. The individual samples that had increased concentrations of heavy metals may have come from the diet of the bird or due to external contamination of the feather stemming from pollution of the habitat. There were no significant differences in concentration between genders or age groups for the heavy metals analysed.

There was medium significant correlation between Cadmium and Arsenic (0.43), and lead (0.38) in Owls, between cadmium and Lead (0.51) in Buzzards, and between Cadmium and Lead (0.49) in Eurasian sparrow-hawks based on the Spearman rank-correlation test. There was no significant correlation among the investigated metals in Common kestrels.

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