UNIVERSITI PUTRA MALAYSIA

HEAVY METALS ACCUMULATION IN HOUSE CROW (Corvus splendens Vieillot) IN KLANG, SELANGOR, MALAYSIA

MOHAMMED ALI AHMED AL-JANAYDEH

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By

MOHAMMED ALI AHMED AL-JANAYDEH

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Chairman : Professor Ahmad Ismail, PhD
Faculty : Science

Heavy metals have become a major concern to the world due to its transferability through the food chain, and eventual bioaccumulation in animals and human beings. There are indications that exposure to these heavy metal pollutants could cause death and other serious health risks in men and women such as in study areas. In particular, kidney infections, urinary tract, uterus, diabetes and rental infection in men and women are common cases, which can be directly linked with excessive toxicity of high concentration from heavy metals contamination. This study explores the potential of Caspase-3 and antioxidant enzymes as biomarkers for assessing heavy metals in house crows (*Corvus splendens*). Specifically, the objectives of this study were: 1) to determine the accumulation of heavy metals in house crow in order to evaluate the status of heavy metal pollution in the Klang area. 2) to evaluate the relationship between heavy metals accumulation and antioxidant status of SOD activity, Cp ferroxidase activity and GSH concentration in house crow from Klang area. 3) to determine the relationship between heavy metals accumulation and apoptosis marker and HSP70 overexpression in house crow tissues especially in liver and kidney tissues. House crow samples were collected from the Klang area through the Department of Public Health, Klang Municipal Council. Quantitative determination of heavy metals (Cu, Zn, Ni, Fe, Cd and Pb) was carried out using atomic absorption spectrophotometer (AAS). In addition, standard procedures were for evaluating the liver and kidney function profile, antioxidant enzyme activities in plasma blood and tissues have been determined using colorimetric kits. Immunohistochemistry protocol for liver and kidney tissues were performed by using Caspase-3 and HSP70 monoclonal antibodies.

Results show that heavy metals were accumulated in all body parts and organs of house crows tested. Fe concentrations were the highest, followed by those of Zn, Cu, Pb, Ni and Cd. Feathers, bone and kidney accumulated high concentrations of Pb and Cd whereas liver accumulated high concentrations of essential heavy metals (Fe>Zn>Cu>Ni). This study revealed positive and significant correlation *($r= 0.405$, $P<0.05$)* between gizzard content (food) metals accumulation and internal organ's

heavy metals of house crow for heavy metals such as Pb, Cd, Ni, and Fe. Significant variations in the SOD activities among adult and juvenile and/or male and female crow samples were also detected in liver and kidney tissues. SOD activity was observed to be age and/or sex-dependent in liver and kidney organs while Cp ferroxidase activity was also age and/or sex-dependent in plasma of the house crows. In addition, Caspase-3 and HSP70 marker results were age and/or sex-dependent in liver tissues while Caspase-3 results was age and/or sex-dependent only in kidney tissues of the house crows. Statistically, the adult female house crow had the highest concentration of heavy metals in the liver than the male and juvenile house crows. Furthermore, the adult male house crows had the highest concentrations of heavy metals in the kidney than the female and juvenile house crows.

The findings of the present study indicated that non-essential heavy metal was bioaccumulated at a significantly lower rate in the liver compared to bioaccumulation in other body organs as a result of liver detoxifications. Analysis of non-essential and essential heavy metals in house crows' body tissues in this study allows assessing environmental pollution of heavy metals in the bird and their environment. Bioaccumulation of metals in internal body organs is reflection of chronic exposure, which is dependent on contaminant level in food ingested, because liver and kidney are sites of detoxification while muscle, brain, bone and heart are sites of accumulation. Moreover, lung tissues and feathers are sites of exposure and excretion. Accumulation of heavy metals in breast feathers and lungs reflect the presence of pollutants in the environment. The breast feathers could be utilized as a detector of heavy metals in house crows, to evaluate quantitatively the concentrations of heavy metals in breast feathers related to the internal organs such as liver and kidney. More importantly, this study reveals the suitability of breast feathers, as an alternative sample to internal organs, in studying heavy metals accumulation in house crow.

The results from this study show that, contamination of heavy metals contamination has various effects on the antioxidant biomarker levels in the blood and tissues (liver and kidney) of house crow in peninsular Malaysia. Adult and female house crows showed reduced Cp ferroxidase activity, which can be associated with increased heavy metal concentration (Pb, Cd, Zn and Ni). However, the Pb exposed house crows showed decreased Cp ferroxidase activity with increasing Pb concentration while the Pb and Cd exposed house crows showed decreased SOD activity with increased Pb and Cd concentration in liver and kidney tissues. Additionally, Caspase-3 immunoreactivity in this study was concentration dependent; the higher Pb and Cd concentration in the liver or kidney, the higher the reactivity of Caspase-3. The present study clearly shows the utility of Caspase-3, SOD activity and Cp ferroxidase activity, especially in the house crow as suitable biomarkers for oxidative stress of Cd and Pb metals in the Klang area.

The implication of this study on the Malaysian environment policy is the need for a review as to mitigate the presence of heavy metals in the environment, which are capable to cause severe harm to human population. Again, there is a need to strengthen the policy and it is compliance in order to reduce activities that cause high level of heavy metal pollutions in the environment such as highly usage of chemicals in agriculture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGUMPULAN LOGAM BERAT DALAM GAGAK RUMAH (*Corvus splendens* Vieillot) DI KLANG, SELANGOR, MALAYSIA

Oleh

MOHAMMED ALI AHMED AL-JANAYDEH

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Pengerusi : Profesor Ahmad Ismail, PhD
Fakulti : Sains

Logam berat telah menjadi satu masalah yang besar kepada dunia kerana keupayaannya dipindahkan melalui rantai makanan, dan seterusnya mengakibatkan biopengumpulan dalam haiwan dan manusia. Terdapat tanda pendedahan kepada pencemaran logam berat boleh menyebabkan kematian dan risiko kesihatan yang serius dalam kalangan lelaki dan wanita khususnya di kawasan kajian ini. Sebagai contoh, jangkitan pada buah pinggang, saluran kencing dan rahim dan juga penyakit diabetes pada lelaki dan wanita adalah kes biasa, yang boleh dikaitkan secara langsung dengan pendedahan ketoksikan yang berlebihan daripada pencemaran logam berat. Kajian ini melihat keberkesanan atau potensi enzim Caspase-3 dan antioksidan sebagai *biomarker* untuk menilai logam berat dalam burung gagak. Secara khususnya, objektif kajian ini adalah untuk: 1) mengkaji pengumpulan logam berat dalam burung gagak *Corvus spledens* untuk menentukan status pencemaran logam berat di kawasan Klang. 2) menilai hubungan diantara pengumpulan logam berat dan status aktiviti antioksidan (SOD), aktiviti Cp dan kepekatan GSH dalam burung gagak dari kawasan Klang. 3) untuk menentukan hubungan diantara pengumpulan logam berat dengan paras apoptosis dan HSP70 dalam tisu burung gagak khususnya dalam tisu hati dan ginjal. Sampel burung gagak telah diperolehi daripada Jabatan Kesihatan Awam, Majlis Perbandaran Klang. Penentuan kuantitatif logam berat (Cu, Zn, Ni, Fe, Cd dan Pb) dari kawasan Klang dibuat menggunakan spektrofotometer serapan atom (AAS). Prosedur piawai juga dilakukan untuk menilai profil fungsi hati dan ginjal, aktiviti enzim antioksida dalam darah dan tisu plasma menggunakan kit kolorimetri. Protokol Immunohistokimia juga dilakukan untuk tisu hati dan ginjal menggunakan Caspase-3 dan HSP70 antibodi monoklon.

Kajian menunjukkan logam berat mengumpul di semua bahagian badan dan organ burung gagak yang diuji. Kepekatan Fe adalah tertinggi, diikuti oleh Zn, Cu, Pb, Ni dan Cd. Bulu, tulang dan ginjal mempunyai kepekatan Pb dan Cd yang tinggi manakala turutan kepekatan logam berat dalam hati ialah Fe > Zn > Zn > Ni. Kajian ini

menunjukkan korelasi yang positif dan signifikan ($P < 0.05$) antara kandungan makanan dalam hempedal dengan pengumpulan logam berat dalam organ dalaman burung gagak untuk logam berat seperti Pb, Cd, Ni, Zn, Fe dan Cu. Terdapat variasi yang signifikan untuk aktiviti SOD pada burung dewasa dan burung muda dan/atau sampel jantan dan betina yang juga dikesan dalam tisu hati dan buah pinggang. Aktiviti SOD dilihat berkait dengan umur dan/atau bergantung pada jantina- dalam organ hati dan ginjal manakala aktiviti Cp juga bergantung pada umur dan/atau jantina di dalam plasma burung gagak. Di samping itu, penanda Caspase-3 dan HSP70 juga bergantung kepada umur dan/atau jantina- dalam tisu hati manakala keputusan Caspase-3 hanya ditunjukkan dalam tisu ginjal burung gagak. Secara statistik, burung gagak betina dewasa mempunyai kepekatan logam berat yang paling tinggi dalam hati dan, gagak rumah jantan dewasa mempunyai kepekatan logam berat yang paling tinggi dalam buah pinggang.

Dapatan kajian ini menunjukkan bahawa kadar bio-pengumpulan logam berat tidak perlu adalah jauh lebih rendah di dalam hati berbanding biopengumpulan dalam organ lain disebabkan detoksifikasi dalam hati. Analisis logam tidak perlu dan logam berat penting dalam tisu burung gagak membolehkan penilaian pencemaran alam sekitar untuk logam berat dalam burung dan persekitaran habitat mereka. Biopengumpulan logam dalam organ dalaman menunjukkan pendedahan yang kronik, yang berkait rapat dengan tahap pencemaran dalam makanan yang diambil, kerana hati dan ginjal adalah tapak detoksifikasi manakala otot, otak, tulang dan jantung adalah tapak pengumpulan. Tisu paru-paru dan bulu juga adalah tapak pendedahan dan perkumuhan. Pengumpulan logam berat dalam paru-paru dan bulu mencerminkan kehadiran agen pencemar dalam alam sekitar. Bulu di bahagian dada boleh digunakan untuk mengesan logam berat dalam burung gagak, untuk menilai secara kuantitatif kepekatan logam berat dalam bulu di bahagian dada yang berkaitan dengan organ dalaman seperti hati dan ginjal. Lebih penting lagi, kajian ini menunjukkan kesesuaian bulu di bahagian dada sebagai sampel alternatif kepada organ dalaman, dalam mengkaji pengumpulan logam berat dalam burung gagak.

Hasil kajian ini juga menunjukkan pencemaran logam berat memberikan pelbagai kesan ke atas paras *biomarker* antioksidan dalam darah dan tisu (hati dan ginjal) pada burung gagak di Semenanjung Malaysia. Burung gagak dewasa dan betina menunjukkan aktiviti Cp feroksidase yang berkurangan dan berhubung terus dengan peningkatan kepekatan logam berat (Pb, Cd, Zn dan Ni). Burung gagak yang terdedah kepada Pb mempamerkan pengurangan aktiviti Cp feroksidase dengan peningkatan kepekatan Pb manakala burung gagak yang terdedah kepada Pb dan Cd menunjukkan pengurangan aktiviti SOD dengan peningkatan kepekatan Pb dan Cd dalam tisu hati dan ginjal. Tambahan pula, Reaktiviti imuno Caspase-3 dalam kajian ini juga bergantung kepada kepekatan; semakin tinggi kepekatan Pb dan Cd dalam hati atau ginjal, semakin tinggi aktiviti Caspase-3. Kajian ini dengan jelas menunjukkan penggunaan Caspase-3, aktiviti SOD dan aktiviti Cp feroksidase, khususnya dalam burung gagak merupakan calon penunjuk biologi yang sesuai kepada tekanan oksidatif logam Cd dan Pb di kawasan Klang.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Ahmad bin Ismail, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohd Hair bin Bejo, PhD
Professor Dato'
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Nor Azwady bin Abd. Aziz, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Syaizwan Zahmir bin Zulkifli, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____

Name of Chairman of Supervisory Committee: Professor Dr. Ahmad bin Ismail

Signature: _____

Name of Member of Supervisory Committee: Professor Dato' Dr. Mohd Hair bin Bejo

Signature: _____

Name of Member of Supervisory Committee: Associate Professor Dr. Nor Azwady bin Abd. Aziz

Signature: _____

Name of Member of Supervisory Committee: Associate Professor Dr. Syaizwan Zahmir bin Zulkifli

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ALT	Lactate Dehydrogenase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BSA	Bovin serum albumin
CAT	Catalase
Cd	Cadmium
cm	Centimeter
Cp	Ceruloplasmin
CPK	Creatine phosphokinase
CREA	Creatinine
CRM	Certified material
Cu	Copper
DDW	Double Distilled Water
DNA	Deoxyribonucleic Acid
dw	Dry Weight
EDTA	Ethylenediaminetetraacetic Acid
Fe	Iron
fw	Fresh weight
g	Gram
GGT	Gamma-Glutamyl transferase
GSH	Glutathione

H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric Acid
$HClO_4$	Perchloric Acid
H&E	Hematoxylin and Eosin
Hg	Mercury
HNO_3	Nitric Acid
HSE	Heat Shock Element
HSF	Heat Shock Factor
HSP	Heat Shock Proteins
HSR	Heat Shock Responses
IPS	Immunoperoxidase Staining
LDH	Lactate Dehydrogenase
M	Molar Volume
mg/L	Milligram Per liter
ml	Milliliter
mm	Millimeter
Ni	Nickle
^0C	Degree Celsius
PBS	Phosphate Buffered Saline
pH	Potential of Hydrogen
ppm	Part per Million
RBCs	Red Blood Cells
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
rpm	Rotation Per Minute

SOD	Superoxide Dismutase
UREA	Urea
URIC	Uric Acid
UV	Altera violate
WHO	World Health Organization
WWTP	Wastwater treatment Plant
δ-ALAD	δ-aminolevulinic acid dehydratase
µg/g	Microgram per gram
µm	Micrometer
*	Significant at 0.05 probability level
**	Significant at 0.01 probability level
***	Significant at 0.001 probability level
%	Percentage

LIST OF CHEMICAL SYMBOLS

Ag	Silver
Zn	Zink
Pb	Lead
Cu	Copper
Ni	Nikel
Fe	Iron
Cd	Cadmium
Ar	Argon
Cr	Chromium
Hg	Mercury
Cl	Chlorine
O_2	Oxygen
Se	Selenium
Sn	Tin
As	Arsenic
Co	Cobalt
Mn	Manganese
$NICl_2$	Nickel Chloride
$CdCl_2$	Cadmium Chloride

LIST OF GLOSSARY

1- Anthropogenic activities: It is of, relating to, or resulting from the influence of human beings on nature.

2- Heavy metals: Heavy metals are defined as those elements having an atomic number greater than 20 and atomic density above 5 g cm− 3 and must exhibit the properties of metal.

3- Food Chain: The sequence of transfers of matter and energy in the form of food from organism to organism.

4- Bioaccumulation: Is the gradual accumulation of substances, such as pesticides or other chemicals, in an organism.

5- Pollution: Is the introduction of contaminants into the natural environment that cause adverse change.

6- Infection: The invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body.

7- Biomarker: It is biological marker is a measurable indicator of some biological state or condition.

8- Bio indicators: Species or ecological community that is so closely associated with particular environmental conditions that its presence is indicative of these conditions in a particular environment Bats are what naturalists call bio-indicators.

9- Biomonitoring: It refers to the measurement of chemicals in human body fluids and tissues, such as blood, urine, breast milk, saliva, and hair.

10- Exposure: It refers to contact of an organism with a harmful agent, such as chemicals

11- Contamination: The presence of a constituent, impurity, or some other undesirable element that spoils, corrupts, infects, makes unfit, or makes inferior a material, physical body, natural environment, workplace, etc.

12- Oxidative stress: This is caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.

13- Oxidative damage: It is formed as a consequence of exposure to ionizing radiation and a variety of chemical agents and as byproducts of normal cellular metabolism.

14- Omnivorous: Animals feeding on a variety of food of both plant and animal origin.

15- Synanthropic Organism: It is used rarely in relation to pathogenic microorganisms especially in wildlife.

16- Toxicity: The quality of being very harmful or unpleasant in a pervasive or insidious way

17- Caspase: It is a family of protease enzymes playing essential roles in programmed cell death.

18- Apoptosis: It is the process of programmed cell death during early development to eliminate unwanted cell.

19- Antioxidant enzymes: proteins involved in the catalytic transformation of reactive oxygen species and their by-products into stable nontoxic molecules, which are capable of defending against oxidative stress-induced cell damage.

20- Bioavailability: This refers to the extent and rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby accessing the site of action.

21- Cp enzymes: It is an enzyme (EC 1.16. 3.1) synthesized in the liver containing 6 atoms of copper in its structure. Ceruloplasmin carries more than 95% of the total copper in healthy human plasma

22- SOD: Superoxide dismutases constitute a very important antioxidant defense against oxidative stress in the body

23- CAT: Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals) which catalyzes the decomposition of hydrogen peroxide to water and oxygen.

24- GSH: Glutathione is known as the body's master antioxidant, which is composed of three amino acids - cysteine, glycine, and glutamate - glutathione can be found in virtually every cell of the organisms' body.

25- Monoclonal antibodies

26- Immunohistochemistry: It is a method for detecting antigens or haptens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

27- Detoxification: It typically implies following a specific diet or using special products that claim to rid your body of toxins, thereby improving health and promoting weight loss.

28- Redox-active metals: These have the ability to occupy multiple valence states in proteins and most notably activate oxygen used by various enzymes involved in cellular respiration

29- Redox-inactive metals: These are critical components in many biological electron transfer reactions.

30- Reactive Oxygen Species: It is highly reactive chemicals formed from O_2 including: peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen.

31- Heat shock protein: It is a protein induced in a living cell in response to a rise in temperature above the normal level.

32- Necrosis: It is the death of body tissue. It occurs when too little blood flows to the tissue. This can be from injury, radiation, or chemicals.

33- DNA: Deoxyribonucleic acid is the chemical name for the molecule that carries genetic instructions in all living things. DNA is the hereditary material in humans and almost all other organisms.

34- RNA: Ribonucleic acid is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.

35- Lymphocytic infiltration: It is an uncommon skin condition that presents as non-scaly red patches and lumps on the face, neck and upper back.

36- Tubular degeneration: It is a medical condition involving the death of *tubular* epithelial cells that form the renal tubules of the kidney.

37- Chronic Toxicity Vacuolization: It is the formation of vacuoles in the cytoplasm of neutrophils in response to severe infections or inflammatory conditions

38- Severe necrosis: It is a form of cell injury, which results in the premature death of cells in living tissue by autolysis.

39- House crow: It is the Indian, greynecked, Ceylon or Colombo crow, is a common bird of the crow family that is of Asian origin.

40- Haemoglobin: A protein found in the red blood cells carries oxygen in your body and gives blood its red colour.

41- Feathers: These provide a light but tough covering, and keep birds warm in cold conditions. They also help the birds to fly.

42- Metalloid: An element has properties that are intermediate between those of metals and nonmetals. It is also called semimetals.

43- Mortality: The state or condition of being subject to death; mortal character, nature, or existence.

44- Concentrations: It is typically expressed as molarities and can be prepared by dissolving a known mass of solute in a solvent.

45- Ecosystem: It is a geographic area where plants, animals, and other organisms, as well as weather and landscape, work together to form a bubble of life.

46- Biotic: It describe living or once living components of a community such as organisms - animals and plant.

47- Immunoperoxidase staining: This procedure target tissue sections, either frozen or formalin-fixed and paraffin-embedded.

48- Environmental habitats: It describes geographic habitat types including polar, temperate, subtropical and tropical regions.

49- Wildlife: These are living things and especially mammals, birds, and fishes that are neither human nor domesticated.

50- Sentinel animals: These are purposefully placed into an animal colony to aid in the detection of disease.

CHAPTER 1

INTRODUCTION

1.1 General overview

Environmental pollution has been a worldwide problem in human habitats (Biswasf et al., 2020; Aazami and KianiMehr, 2018). Indiscriminate or improper waste disposal into the environment and water bodies have resulted in deleterious effects on the environment. Contaminated water bodies pose a significant threat to aquatic animals, while land contamination poses threat to humans and animals (Ahmad et al., 2021). Similarly, the pollution of aquatic and terrestrial environments has resulted in a major ecological problem, which corresponds to rapid domestication and industrialization of cities (Borghesi et al., 2017) such as Selangor, Malaysia. Out of the major environmental pollutants reported, heavy metals have received considerable attention over the years (Ayangbenro and Babalola, 2017).

Heavy metal pollution from anthropogenic activities poses a potential threat to human health through bioaccumulation of these metals in the food chain (Rai, 2018; Tóth et al., 2016). Through atmospheric deposition, heavy metals find their way to the soil, eventually contaminating water sources and plants. As the heavy metals accumulate in the water and plants, it results in high levels in humans through the food chain (Nkosi et al., 2021; Sonone et al., 2020; Alihttps et al., 2019). According to Kar and Patra (2021), heavy metals are usually bioaccumulated in the liver and kidneys, which are main biotransformation organs in the body, which affected are directly proportional to the mortality rate of animal species in the regions where pollution is high. This is consistent with the report by Tayebi and Jahangiri (2020).

Heavy metals induce different cellular damages in the organs of animals depending on the level of toxicity. Since most heavy metals' bioaccumulate in the liver and kidney of humans and animals. In wild birds species such as house crows, heavy metals acquired from contaminated environments through contaminated feed and water are bioaccumulated in these organs (Janaydeh et al., 2018; Kaur, at al., 2018; Sharma and Vashishat, 2017). Lead is a very common heavy metal that is found in polluted areas, and has been shown to cause hepatic damage typified by moderate to severe hepatocyte necrosis with marked diffuse vascular dystrophy and perivascular infiltration in the liver of Japanese quails exposed to experimental heavy metal toxicity (He et al., 2020). Cd has also been reported to induce interstitial nephritis and tubular degeneration in the kidneys of Magpie (*Pica pica*) (Iemmi et al., 2021).

Wild bird species within the vicinity of human habitats, also called Synanthropic organisms are at a higher risk of being affected by pollutants due to their size and feeding habits, hence the choice of utilizing them as bio-indicator organisms in eco-

toxicological studies (Joshua et al., 2021; Nkosi et al., 2021). Among wild bird species living within the vicinity of human habitats, the house crow is the most commonly encountered, living close to human settlement and feeding from the environment. Hence, these bird species can be used as sentinel animals to monitor heavy metal pollution in human populations (Aziz et al., 2021; Kalisińska, 2019).

Several investigators from various countries such as Korea (Nam and Lee, 2006); Finland (Kekkonen at al., 2012); Spain (Tejedor & Gonzalez, 1992), France (Scheifler et al., 2006; Jenkins, 1975), and Brazil (Brait and Filho, 2011) have assessed the levels of heavy metal accumulation in synanthrophic organisms.

Omnivorous birds such as *corvids* have been investigated for bioaccumulation of non-essential heavy metals. The use of *corvids* was reported by Horai et al. (2007) in Japan where concentrations of Pb and Cd in hepatic tissues of Jungle Crow (*Corvus macrorhynchos)* were analyzed. Similarly, a study conducted by Komosa et al. (2012), reported accumulations of Cd and Pb in the liver of the rook (*Corvus frugilegus*). In addition, the hooded crow (*Corvus cornix*) was used as a bio-indicator in a study conducted by Giammarino et al. (2014) to assess heavy metal contamination in environmental matrices in Italy.

A study noted that the deleterious effects of non-essential heavy metals are particularly due to metal-induced oxidative stress (Ercal at al., 2005). Oxidative damage is usually related to the production of reactive oxygen species (ROS) by exposure to metals. Therefore, antioxidant defense has an important role in the protection of organisms against metal-induced oxidative stress (Koivula and Eeva, 2010). Many types of compounds, including metals, can induce oxidative stress. Redox-active metals, such as Iron (Fe), Copper (Cu), and Zink (Zn) induce ROS production through the redox cycling processes (Das and Roychoudhury, 2014; Koivula and Eeva, 2010). Redox-inactive metals such as Lead (Pb), Cadmium (Cd), and Mercury (Hg) can also increase ROS formation by affecting mitochondria function (Chang et al., 2013), or by depleting antioxidant levels of glutathione and other thiol-containing antioxidants in the cell (Hoffman et al., 1998). Hence, the use of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), Ceruloplasmin (Cp) activity and Glutathione (GSH) have been found valuable in evaluation of oxidative stress in birds (Guo et al., 2020; Kurhaluk et al., 2021).

Surface soil acts as sinks for heavy metals and other pollutants (Adimalla, 2020), which affects soil habitat and to human health through food chains (Alihttps et al., 2019). Accumulation of metals especially in liver and kidney (Behrooz and Burger, 2021; Tayebi and Jahangiri, 2020), which may increase the mortality rate amongst species of animals in urban areas (Adimalla et al., 2020). These heavy metals are known Contamination of environmental habitats by heavy metals as a result of the high human activities (Gupta et al., 2021). Heavy metals can accumulate in main organs of the Synanthropic organisms and causes a damage through oxidative stress (Misztal-Szkudlińska et al., 2018). ROS which inhibits antioxidant enzymes and activates

Caspase-3 and HSP70 markers (Koizumi et al., 2013). More so, there is the persistence of lead in the soils of regions of Malaysia, Spain, South Korea, and France causing high anthropogenic activities and their transfer across the food chains could have necessitated the assumptions about presence of large toxic heavy metals in the environment. This raises concerns for safety of birds (such as house crows), which possibly ingest high amounts of heavy metals through feeds. Heavy metals including: Ni, Pb, Zn, Cu, and Cd are considered to be lethal to bird species and adversely affects the respiratory system, endocrine system, stomach, breeding, kidneys, behavioural response, migration, shedding, catalysts needed in the formation and development speeds of haemoglobin (Kaur and Khera, 2018).

In Malaysia, the studies concerning heavy metal contamination among bird species, especially house crow, are still evolving. Thus, this study was designed to assess heavy metal pollution statuses in the surface soil, street dust, food and house crow as the biomonitor organisms. It is expected that the data gnerated from this study would explain whether certain anthropogenic activities in the surrounding Klang areas are responsible for heavy metal pollution in the area.

Oxidative stress is a condition where there is an imbalance between antioxidant defense and the production of reactive oxygen species (ROS), resulting in cellular damage to cells by ROS (Power et al., 2011). Oxidative damage is usually related to the production of ROS by exposure to metals, therefore antioxidant defense has an important role in the protection of organisms against metal-induced oxidative stress (Ercal et al., 2005). Many different types of compounds, including metals, can induce oxidative stress. Redox-active metals, such as Fe and Cu induce ROS production through the redox cycling processes (Das and Roychoudhury, 2014; Harris and Shi, 2003). Caspase-3 as a death protease catalyzes the cleavage of many key proteins by interacting with Caspase-8. Activation of caspases involved in inflammation results in the production of pro-inflammatory cytokines like interleukins, which promote immune response in animals and man (McIlwain at al., 2013). In response to cell death, Caspase-3 is activated to aid in cleaning up dead cells through apoptosis (McIlwain et al., 2013; Porter, 1999). Heavy metal toxicity has been shown to increase the production of ROS in cells. The ROS produced cause destruction of the cell membrane through lipid and protein peroxidation and eventually results in cell death (Ercal et al., 2005). Heat shock proteins (HSP) are produced by cells in response to cell damage known as heat shock responses (HSR). The response triggers the production of HSP in order to maintain normal physiologic state (Steurer et al., 2018). In heavy metal toxicity, the activation of heat shock element (HSE) is important for recognition by heat shock factor 1 (HSF-1). Thus, it has been reported that the absence of HSE leads to failure of HSP response in hosts subjected to metal induced toxicity (Koizumi et al., 2013). These changes in expression of HSP in the literature may be attributed to the absence of heat shock element (HSE) in some of the models or due to species variation.

Specifically, this study intends to evaluate the level of heavy metals (such as Zn, Fe, Ni, Cd, and Pb) pollution in Klang, Selangor, Malaysia for the purpose of ascertaining the risks and problems heavy metals pollution on the environment and accumulation in

animals such house crows which could cause possible human mortality in nearest future. The biological samples made up of muscle heart, bone brain, liver, kidney, lung, feathers, boold and faeces from 42 individuals of house crows, and 42 samples of Food and Gizzard content were collected; while abiotic samples comprised 40 samples of surface soil and street dust were obtained through the Department of Public Health, Majis Perbandaran Klang in December, 2014.

1.2 Objectives of the study

The objectives of the study are:

1. To assess heavy metals concentration in the habitats and different body parts of house crow (*Corvus splendens*) in Klang area, Malaysia.
2. To determine antioxidant status and serum biochemistry (liver and kidney function test) as potential biomarkers for metal stress in the blood and tissues of house crow (*Corvus splendens*).
3. To evaluate the effect of age, sex and metal concentrations on tissue lesions severities in the liver and kidney of house crows (*Corvus splendens*) using H&E, Caspase-3 and HSP70 markers.

1.3 Hypothesis of the study

The following hypotheses are constructed for investigation in this study including:

H1: There are no significant accumulation of heavy metals concentration in the habitats and different body parts of house crow in the Klang area, Malaysia.

H2: There are no significant antioxidant status and plasma biochemistry (liver function and kidney function test) as potential biomarkers for metal (Pb, Cd, Cu, Zn, Ni and Fe) stress in the blood and tissues of house crow.

H3: There are no significant effects of age, sex and metal concentrations of Pb, Cd, Cu, Zn, Ni and Fe on tissue lesions severities in the liver and kidney of house crows.

1.4 Theoretical framework of the study

Bioaccumulation of heavy metals (Cd, Pb, Cu, Zn, Fe, and Ni) was investigated in various tissues including kidney, muscle, bones, liver, brain, bone and feathers in many bioaccumulation studies. Consequently, the hypothesis was formulated that, heavy metal accumulations have a significant impact on the morphometry of birds (such as house sparrow *Passer domesticus*). Then, it was found that the morphological variations in close areas can be accounted for the accumulation of various heavy metals of birds' tissues in the selected areas of study (Albayrak and Pekgoz, 2021).

Another perspective on bioaccummulation of heavy metals in invertebrates's food chain has significant impact on ecotoxicological properties. Invertebrates (such as insects) are a good bioindicator species useful for heavy metal contamination in terrestrial and aquatic ecosystems biomonitoring activities (Ahmad et al., 2021). Therefore, the potential risks posed by contaminated invertebrates significantly impact on the health of humans whenever heavy metals are consumed in food chains of the former in the wild (Goto et al., 2018). Hypothetically, heavy metals are taken up by the living organisms from the abiotic environment, which amassed in biota at diverse trophic levels, and consequently pollute the food chains. Trophic transfer, biomagnification, and bioaccumulation of harmful heavy metals in food chains have significant effects on the health of wildlife and humans (Alihttps at al., 2019).

The concentrations of the environmental trace elements have significant effects on ecosystem, individual wellbeing and health of the population. The assessment of the bioaccumulation of trace elements is fundamental in attempts to understand the ecological effect of pollution. The birds are common bioindicators because the non-invasive sampling can be readily obtainable using sampling of feathers that controls certain factors of variability (such as age and environmental heterogeneity). There are diverse methods being developed to reliably identify and bioaccumulation of common trace elements (such as As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sn and Zn) in feathers of birds including: the Greater Flamingo *Phoenicopterus roseus* (Borghesi et al., 2017).

The birds accumulate heavy metals by means of respiration and the ingestion of insects, plants, and earthworms. The feeding patterns of birds are a major source of heavy metals transfer in birds, which cause several health problems especially breeding inabilities. However, research efforts continue to favour use of bird feathers as effective bioindicators of heavy metal concentrations due to their relative ease of gathering, and processing (Lin et al., 2021).

The atomic weight of heavy metals is more than the molecular weight of water. Aside this, certain heavy metals (such as Fe, Zn, Cu, Se, Co, Mb) contribute in metabolic activities such as in coenzymes or functional macromolecules, which are referred to as microelements needed by living organisms at micro concentrations. But, high concentrations of these microelements could pose serious harm to ecosystems. Toxic heavy metals are persistent pollutants that influence biomagnifications, as they could accumulate with time in the body of animals based on their food chains. The toxic action of heavy metals happens at different trophic chains, usually at the microbiota of the soil, across plants, up towards the largely complex organisms within the echelon of the food chain (Iemmi et al., 2021).

This research work adopted the theoretical framework underlisted in order in developing the following research hypotheses to guide the investigation into level of contamination of heavy metals in Klang area of Malaysia similar to the studies in other regions of Malaysia, Korea, Spain and France on the persistence of heavy metals in the

environment, and possible hazards due to the levels of contaminations (Kaur and Khera, 2018; Albayrak and Pekgoz, 2021; Fernando et al., 2020).

The following hypotheses are constructed for proper investigation in this study including:

H1: There are no significant accumulation of heavy metals concentration in the habitats and different body parts of house crow in the Klang area, Malaysia.

H2: There are no significant antioxidant status and plasma biochemistry (liver function and kidney function test) as potential biomarkers for metal (Pb, Cd, Cu, Zn, Ni and Fe) stress in the blood and tissues of house crow.

H3: There are no significant effects of age, sex and metal concentrations of Pb, Cd, Cu, Zn, Ni and Fe on tissue lesions severities in the liver and kidney of house crows.

H31: There are no significant effects of age and metal concentrations of Pb, Cd, Cu, Zn, Ni and Fe on tissue lesions severities in the liver and kidney of house crows.

H32: There are no significant effects of sex and metal concentrations of Pb, Cd, Cu, Zn, Ni and Fe on tissue lesions severities in the liver and kidney of house crows.

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