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Heavy Metal Bioaccumulation in Rabbit Organs and *Chrysomya albiceps* (Wiedemann, 1819) Larvae: Implications for Forensic Entomology

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Abstract

Background: A closer relationship has been established between entomology and toxicology, giving rise to a new field known as entomotoxicology, which focuses on the bioaccumulation of toxic substances in scavenging insects and other arthropods.

Methods: This study investigates the impact of lethal doses of Mercury Chloride ($HgCl_2$) and Cadmium Chloride ($CdCl_2$) administered to male rabbits (*Oryctolagus cuniculus* Linnaeus) on the distribution of Mercury and Cadmium in rabbit organs and their subsequent accumulation in *Chrysomya albiceps* larvae. The experimental groups were subjected to oral administration of specific concentrations of $HgCl_2$ and $CdCl_2$, while a control group received distilled water. Organ dissection and subsequent larval feeding were followed by acid digestion for sample preparation and inductively coupled plasma torch spectrometry coupled with a mass spectrometer (ICP-MS) was employed for analysis.

Result: Results indicate a significant increase in Mercury concentrations in treated rabbit organs, with the liver exhibiting the highest concentration. Cadmium showed diverse distribution among rabbit organs, with the highest concentrations in the kidneys. *Ch. albiceps* larvae, reared on rabbit organs, exhibited varied concentrations of both Mercury and Cadmium, with significantly higher levels in the treated group. These findings underscore the ecological implications of heavy metal exposure and the potential utility of insect larvae in forensic investigations as bioindicators of environmental contamination.

Key words: Cadmium, *Chrysomia albiceps*, Entomotoxicology, ICP-MS, Mercury, Necrophagous.

Introduction

Forensic entomology is a field of scientific study that involves the intersection of arthropod science with the legal system to address relevant inquiries related to criminal and civil laws (Mahat *et al*., 2019).

Leveraging insects and arthropods in forensic inquiries offers crucial insights into estimating the time of death, tracking the relocation of remains, assessing injuries and detecting the presence of drugs or toxins (Gennard, 2012; Mahat *et al*., 2012). Necrophagous insects also present a viable alternative for obtaining samples for toxicological analysis, particularly in situations where conventional samples are unavailable (Goff and Lord, 2001).

Entomotoxicology, a relatively unexplored subfield within forensic entomology, focuses on two main objectives: (1) identifying drugs and poisons in necrophagous insects and (2) examining how these substances may influence the developmental patterns of these insects (Rivers and Dahlem, 2022). The increasing incidence of deaths linked to drugs and poisons, particularly in remote areas, has led to the discovery of highly decomposed bodies, where organs may be unidentifiable and unsuitable for toxicological sampling (Mahat *et al*., 2012). Simultaneously, necrophagous larvae have the ability to accumulate drugs and poisons during their feeding on decomposing bodies. However, the recoverability of these substances depends on the conditions to which the bodies are exposed and the inherent chemistry of the drugs/poisons (Gennard, 2012).

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The unique ability of insects to accumulate drugs and toxins in their tissues makes them invaluable resources for forensic toxicologists and entomologists (Malejko *et al*., 2020). The use of insects as a reservoir of toxicological evidence in forensic science relies on their tendency to collect pollutants from their feeding sources in their tissues

(Tracqui *et al.*, 2004). Integrating information obtained from insect evidence with other types of evidence allows investigators to enhance their understanding of the circumstances surrounding a death (Pounder, 1991).

Chrysomya albiceps, commonly known as the Oriental latrine fly, holds significant importance in forensic entomology. As an early colonizer of decomposing remains, its presence aids in estimating the time of death by contributing to the determination of the postmortem interval. The larvae of *Ch. albiceps*, found in and around human remains, play a key role in the decomposition process and their analysis assists in establishing a timeline for various decomposition stages. Additionally, *Ch. albiceps* larvae have been utilized in entomotoxicology studies, accumulating drugs and toxins from decomposing substrates. The species' widespread geographical distribution, adaptability to different environments and specific morphological features make it a versatile forensic indicator globally.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was employed to assess the concentrations of Cadmium (Cd) and Mercury (Hg) in third instar larvae of *Chrysomya albiceps*. ICP-MS is widely favored in inorganic testing laboratories due to its numerous advantages, including a broad dynamic range, heightened sensitivity, low limits of detection and the capability to swiftly conduct multi-elemental analysis (Flanagan *et al.*, 2020; Skoog *et al.*, 2013; Wolstenholme *et al.*, 2021).

Utilizing ICP-MS (Inductively Coupled Plasma Mass Spectrometry), isotopes can be distinguished, ensuring the conclusive identification of analytes (Sari *et al.*, 2023). The detection limits for ICP-MS typically range from parts-per-billion (ng/g) to parts-per-quadrillion (fg/g) in a standard solution, depending on the specific element and background levels (Berry, 2015). ICP-MS is applied in various fields, including forensics (Arroyo *et al.*, 2010; Deconinck *et al.*, 2006). This versatility positions ICP-MS as an ideal instrument for quantifying toxic metals like Cd and Hg, frequently implicated in poisoning incidents. The results of the analysis provide valuable insights for toxicological investigations, assisting forensic scientists in reconstructing events and determining the cause of death.

In this study, the objective is to perform a quantitative analysis of $CdCl_2$ and $HgCl_2$ accumulation in *Ch. albiceps* larvae, a species recognized for its significance in forensic investigations, utilizing ICP-MS. The aim is to offer valuable insights into the utilization of entomological evidence in toxicological investigations and contribute to the enhancement of a more robust and effective approach to determining the cause of death.

MATERIALS AND METHODS

For this study, three group of male rabbits, *Oryctolagus cuniculus* Linnaeus, with weights of 2.240 kg, 1.970 kg and 1.970 kg for each rabbit in the group were utilized. The first group rabbits received an oral suspension of a lethal dose of Mercury Chloride ($HgCl_2$) at a concentration of 0.4489 g/kg body weight. The second group rabbits were treated with an oral suspension of a lethal dose of Cadmium Chloride ($CdCl_2$) at a concentration of 0.0348 g/kg (Table 1). Both Mercury and Cadmium Chloride were diluted in 5 ml of distilled water before administration to facilitate absorption into the organism. The administration was carried out orally using syringes with a metal probe, following the method described by Mumtaz *et al.* (2019). The third group rabbit, serving as the control, were treated with 5 ml of distilled water and subsequently euthanized by slaughter.

Table 1: The toxicological treatments of rabbits.

Rabbit	Weight	Substance use	Lethal dose g/kg
R1	2.240	$HgCl_2$	0.448
R2	1.970	$CdCl_2$	0.0348
R3	1.970	-	-

Analysis of toxicity in rabbit carcasses tissues and larva *Ch. albiceps*

We conducted the dissection of rabbit carcasses to obtain various organs, including the lungs, heart, liver, spleen, kidneys, stomach, small intestine, muscle and testicles. Following the dissection, each organ was divided into two parts. The first part was stored in a refrigerator at -25°C for subsequent measurement of mercury and cadmium concentrations in the respective organs of the different rabbits. The second part was used as a nutritious substrate for *Ch. albiceps* larvae, which were bred in the Animal Biology Laboratory of the Biology Department.

In the laboratory, the *Ch. albiceps* females laid their eggs on a prepared substrate, which consisted of cow liver. Approximately 20-25 eggs were placed on each of the internal organs obtained from the dissected rabbits. After six days, third-instar larvae were collected and stored in the refrigerator at -25°C until analysis.

Preparing samples for ICP-MS examination

The mineralization of organic tissues and of larval tissues was carried out through acid digestion. In a crucible, five grams of the sample were combined with a solution comprising nitric acid (HNO3 at 69%), hydrochloric acid (HCl at 37%) and sulfuric acid (H2SO4 at 96%) in proportions of 7:7:1 (v:v:v) for organic tissues and 5:5:0.7 (v:v:v) for larval tissues. The digestion took place by heating the mixture on a hot plate at 150°C for 10 minutes, with the temperature monitored using a thermometer. After digestion, the mixture was placed in a microwave oven for extraction, with the temperature set at 200°C for 30 minutes. The prepared samples were filtered and analysis was conducted using inductively coupled plasma torch spectrometry coupled with a mass spectrometer (ICP-MS) at the National Institute of Forensic Science and Criminology (INCC/GN) in Algiers.

Statistical analysis

Statistical analysis was carried out using SPSS 26 software (SPSS Inc., Chicago, IL) with analysis of variance (ANOVA). Mean separation was accomplished through Tukey's honestly significant difference test. A significance level of P<0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

The presence of mercury was detected in the organs of the control group's rabbits, albeit at very low concentrations. The highest concentration was observed in the stomach 12.18 ppb, while the lowest concentrations were found in both the testicles and muscles at 4.28 ppb. The small intestine exhibited a slightly lowest concentration 4.23 ppb. No significant differences were observed among these three organs. However, statistical analysis revealed significant variations among the remaining organs at a significance level of p≤0.05 (Fig 1A).

In the rabbits treated with the lethal dose, the concentration of mercury increased significantly compared to the control group. Notably, significant differences were observed among the various organs. The liver exhibited the highest concentration 16348.24 ppb, while the testicles showed the lowest concentration 8623.13 ppb (Fig 1B).

Entomotoxicology not only examines the impact of drugs on insects but also employs insects as an alternative matrix (Goff and Lord, 1994; Introna *et al*., 2001). Analyzing toxic substances in highly decomposed bodies is more straightforward with insects compared to traditional matrices such as blood and urine, as there are fewer disruptions caused by decomposition (Kharbouche *et al*., 2008; Nolte *et al*., 1992). The current study showed that *Ch. albiceps* larvae reared on the various organs of rabbits for six days contained different concentrations of all mercury chloride and this concentration varied between the organs in the control group. The highest concentration reached 1.99 ppb in larvae reared on the small intestine and the lowest concentration was 0. 03 ppb in muscle-reared larvae (Fig 2A). Mercury accumulated at a greater concentration in the group treated with the Lethal dose, the highest concentration reached 71.71 ppb in the stomach-reared larvae and the lowest concentration in the lung-reared larvae was 10.24 ppb (Fig 2B).

Nuorteva and Nuorteva, (1982) pioneered the analysis of mercury (Hg) in entomological specimens to determine the geographic origin of an unidentified body discovered in Inkoo, Finland. The low Hg concentrations (0.12-0.15 ppm) led to the inference that the victim likely hailed from a region with minimal Hg pollution, later confirmed as Turku-a city free of Hg pollution. This successful application of Hg analysis in entomological specimens guided the police investigation accurately. In a recent study, El-Ashram *et al.* (2022) Extracted aluminum phosphide (AlP) (is a low-cost insecticide, rodenticide and fumigant) from a pooled sample of the 3rd instar larvae of *Ch. albiceps*.

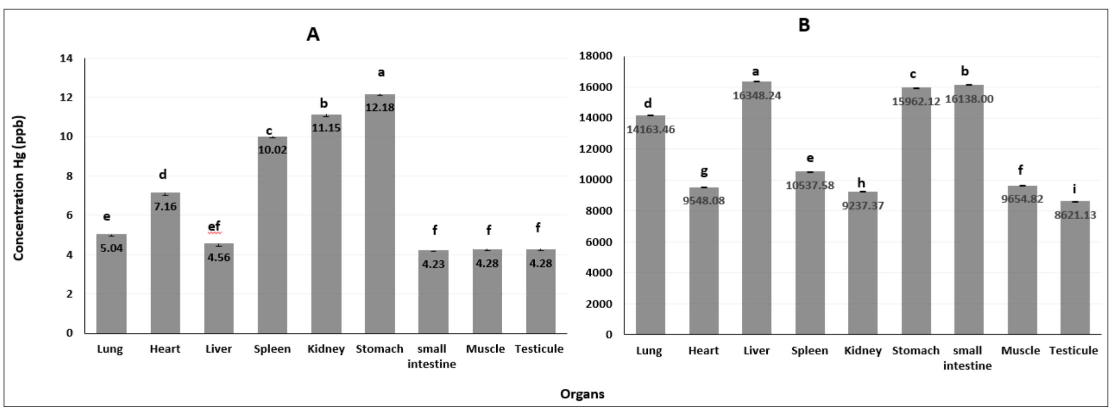

Fig 1: Concentrations of Hg (ppb) in rabbit organs: (A) Non-treatment (control), (B) Treatment.

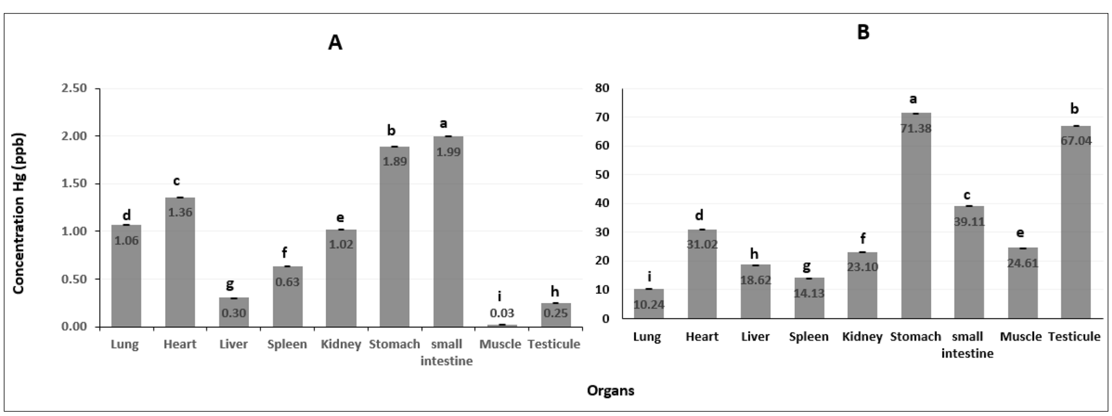

Fig 2: Concentrations of Hg (ppb)In *Ch. albiceps* fly larvae reared on rabbit organs: (A) Non-treatment (control), (B) Treatment.

The outcomes of the experiments involving fly rearing demonstrated a distinct bioaccumulation pattern of mercury (Hg) in blowfly larvae. When the larvae were raised on carcasses of vertebrates known to harbor methyl mercury, such as Fennoscandian fish, the average mercury content in the larvae was 4.3 times greater than that in the provided food (Nuorteva and Nuorteva, 1982).

The rabbit's organs differed in cadmium concentrations in both the control group and the treatment group, as the highest concentration in the stomach reached 18.364 parts per billion, while the lowest concentration in the lungs reached 3.896 parts per billion in the control group (Fig 3A). As for the treatment group, the highest concentration was in the kidneys, 24577.87 parts per billion, followed by the small intestine and stomach, with concentrations of 22743.44 and 21327.25, respectively and the lowest concentration was in the heart, 10231.24 parts per billion (Fig 3B).

Typically, the highest concentrations of cadmium (Cd) are found in the kidneys, followed by the liver. However, in cases of low intake initially, the liver may contain more Cd than the kidneys. Due to its larger size, the liver harbors a greater total Cd content. Substantial amounts of Cd are present in tissues and contents of the gastrointestinal tract. Despite representing a significant proportion of body mass, muscles contain a relatively small portion of the total body Cd (Gašparík *et al.*, 2017; McAuley *et al.*, 2018; Barrasso *et al.*, 2018).

Cadmium chloride, a highly toxic compound among heavy metals, negatively impacts living organisms and can accumulate in the food chain, leading to severe consequences such as increased mortality, reduced longevity, decreased fecundity and lower hatching ability in insects and other arthropods (Postma *et al.*, 1994; Postma and Davids, 1995; Schmidt *et al.*, 1991; Williams *et al.*, 1987). In criminal investigations, the presence of toxins and drugs in a corpse can be determined through insect evidence. These studies primarily focus on the quantitative and qualitative analysis of toxicants, without emphasizing their effects on insect growth, survival and development (Malejko *et al.*, 2020).

This study revealed the presence of cadmium chloride in *Ch. albiceps* larvae raised on various organs, including the lungs, heart, liver, spleen, kidneys, stomach, small intestine, muscle and testicles. In the control group, it reached 1.32, 1.03 and 1. 0.004, 0.226, 0.036, 1.105, 1.632, 0.365, 0.543 ppb respectively (Fig 4A), while in the treated group it reached 83.47, 97.014, 1, 117.721, 91.014, 65.275, 54.75, 87.734, 105.24, 245.7 ppb respectively (Fig 4B).

Larvae of four fly species, worldwide, were analysed for the presence of 48 heavy metals and trace elements (Charlton *et al.*, 2015). Cadmium was found to be present-

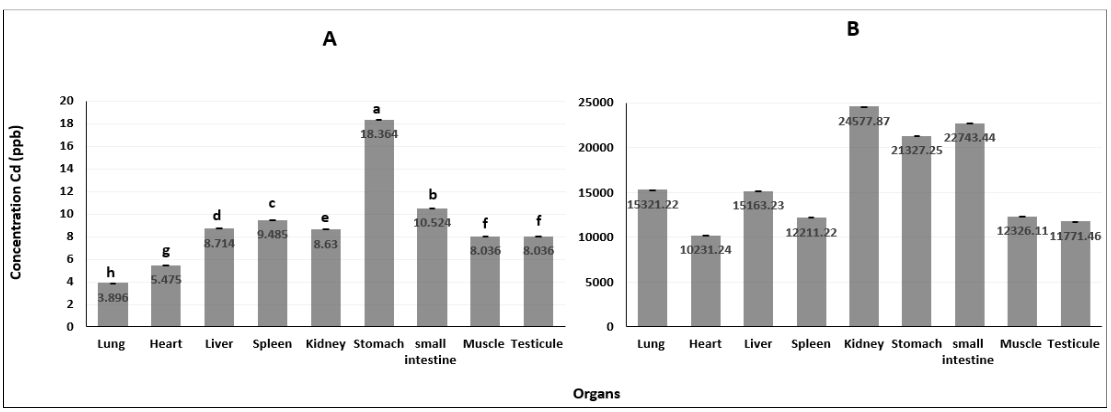

Fig 3: Concentrations of Cd (ppb) in rabbit organs: (A) Non-treatment (control), (B) Treatment.

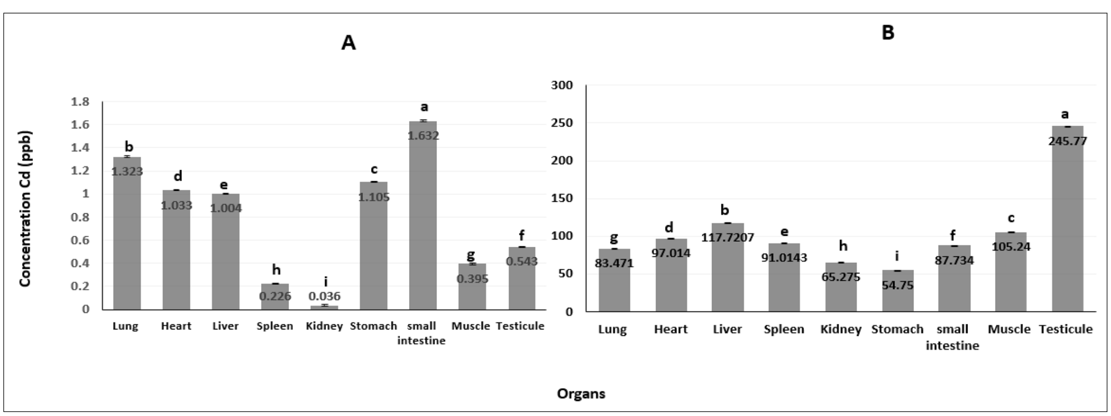

Fig 4: Concentrations of Cd (ppb) in *Ch. albiceps* fly larvae reared on rabbit organs: (A) Non-treatment (control), (B) Treatment.

above the limit of detection of the analytical method used-in all samples. In all samples from Musca domestica, the cadmium concentration was above the EC maximum limit (ML). Different accumulation patterns of Cd and Pb, were observed for the yellow mealworms and black soldier flies (Van der Fels-Klerx *et al*., 2016).

In a study where Dipteran larvae were supplied with Cd-contaminated material and subsequently used as prey for a predatory Coleopteran, the findings revealed Cd accumulation in the Dipteran. However, the Coleopteran exhibited lower Cd concentrations, providing additional evidence for categorizing Coleoptera as Cd deconcentrators and Diptera as Cd macroconcentrators (Dallinger and Rainbow, 1993; Maryanski *et al*., 2002).

In general, the effects of drugs and/or poisons are not only dependent on their concentrations but also vary among different necrophagous insects. For instance, Mahat *et al.* (2009) observed a delayed oviposition and extended pupation period in *Ch. megacephala* on malathion-treated carcasses, with the impacts showing a dose-dependent relationship. In contrast, *Boettcherisca peregrina* exhibited accelerated development (shorter larval stages) when exposed to tissues containing cocaine compared to control conditions (Gennard, 2012).

CONCLUSION

In conclusion, the administration of lethal doses of Mercury Chloride and Cadmium Chloride to male rabbits significantly influenced the distribution of Mercury and Cadmium in the rabbit organs and their accumulation in *Ch. albiceps* larvae. Markedly increased concentrations of Mercury were observed in the treated rabbit organs, particularly in the liver, indicating varied distribution among organs. For Cadmium, there was diverse accumulation in rabbit organs, with the highest concentrations found in the kidneys. Additionally, *Ch. albiceps* larvae demonstrated significant accumulation of both Mercury and Cadmium, with varying concentrations among organs and notably higher concentrations in the treatment group. These findings shed light on the environmental impact and biological accumulation of heavy metals, emphasizing the role of insects as environmental indicators and in forensic investigation techniques.

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Authors' contribution

F. Boulkenafet and S. Bouhayene designed the study, F.A. Al-Mekhlafi and S. Lambiase conducted data analyses and wote the manuscript. S. Benzazia and M. Toumi conducting experiments and ICP-MS analysis. M.S. Al-Khalifa, F. Boulkenafet and F.A. Al-Mekhlafi helped in writing the manuscript and conducted data analysis.

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Data availability statement

All the data is available within the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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