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THE ROLE OF NECROPHAGOUS INSECTS IN DEATH INVESTIGATIONS: INTEGRATING FORENSIC ENTOMOTOXICOLOGY AND TOXICOLOGY WITHIN CULTURAL AND MEDICOLEGAL CONTEXTS

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ABSTRACT

This study addresses the limitations of conventional forensic toxicology in advanced decomposition, where biological samples are often unavailable. It aims to examine the role of necrophagous insects as alternative biological matrices in death investigations, while integrating forensic entomotoxicology and toxicology within broader medicolegal and cultural contexts. An experimental laboratory approach was conducted using beef and pork tissues exposed to methamphetamine (3 µg/mL), followed by the cultivation and collection of fly larvae (maggots) at different developmental stages (days 3–7). The samples were analyzed using qualitative (Marquis reagent), instrumental (FTIR and GC-MS), and validation methods. The results demonstrated that methamphetamine was successfully detected in maggot tissues, with concentrations increasing from day 3 and peaking on day 6 (49.57 µg/mL in beef and 34.55 µg/mL in pork), before declining as larvae transitioned to later stages. FTIR analysis confirmed the presence of characteristic functional groups, while method validation showed satisfactory performance (recovery 101.90%, RSD 1.19%, R = 0.991). These findings highlight that necrophagous insects not only function as biological indicators of decomposition but also act as carriers of toxicological evidence through bioaccumulation processes. The integration of entomotoxicological data with conventional toxicology and medicolegal interpretation provides a more comprehensive framework for determining cause and manner of death, particularly in complex cases. Furthermore, incorporating cultural and legal perspectives strengthens the evidentiary value and contextual interpretation of insect-based findings in forensic practice. This study demonstrates that forensic entomotoxicology can serve as a reliable and integrative approach, bridging biological, chemical, and socio-legal dimensions in modern death investigations.

KEYWORDS: Forensic Entomotoxicology; Necrophagous Insects; Methamphetamine; Medicolegal Context; Cultural Context.

1. INTRODUCTION

In death investigations, particularly under advanced decomposition conditions, the availability of conventional biological matrices such as blood, urine, and soft tissues is often severely limited or completely absent due to progressive degradation processes [1]. This condition poses a critical challenge for forensic toxicology, as the identification and quantification of toxic substances become increasingly unreliable when standard biological samples are no longer viable [2]. Consequently, alternative biological matrices are required to preserve and recover toxicological information in such complex forensic scenarios [3].

Necrophagous insects, especially fly larvae (maggots), have emerged as promising alternative matrices in forensic investigations because of their ecological role in consuming decomposing tissues [4]. During this process, larvae are capable of bioaccumulating xenobiotic substances, including drugs and toxic compounds, from the tissues they ingest [5]. Previous studies have demonstrated that substances such as methamphetamine can be detected within larval tissues and that their concentrations may vary depending on developmental stages [6]. These findings indicate that necrophagous insects not only contribute to postmortem interval (PMI) estimation but also hold significant potential as carriers of toxicological evidence [7].

Despite these advances, a critical gap remains in the integration between forensic entomology and forensic toxicology [3]. Classical entomological approaches primarily focus on insect succession patterns for PMI estimation, while forensic toxicology traditionally relies on human biological samples and the principle that "the dose makes the poison" [8]. The interaction between toxic substances and insect development, including its impact on both toxicological interpretation and PMI estimation, has not been fully incorporated into existing forensic frameworks [9]. Moreover, most previous studies have emphasized either detection capability or biological effects in isolation, without sufficiently linking experimental findings to medicolegal interpretation [10].

In addition, limited experimental studies have quantitatively examined the dynamics of drug accumulation in necrophagous insects across developmental stages using validated analytical techniques [4]. The absence of such data restricts the reliability and applicability of entomotoxicological evidence in real forensic cases [2]. Therefore, there is a need for an integrative approach that combines experimental toxicological analysis, insect biology, and medicolegal interpretation within a unified framework [11].

To address this gap, the present study employs an experimental laboratory design to investigate the role of necrophagous insects as alternative biological matrices in detecting methamphetamine under decomposition conditions [6][12]. Fly larvae were cultivated on beef and pork tissues exposed to methamphetamine, and samples were collected across developmental stages to evaluate bioaccumulation patterns [5]. The detection and characterization of methamphetamine were conducted using qualitative (Marquis reagent) and instrumental techniques, including Fourier Transform Infrared (FTIR) and Gas Chromatography–Mass Spectrometry (GC-MS) [10]. Experimental findings demonstrate that methamphetamine can be detected in larval tissues with increasing concentrations during active feeding stages and declining during later developmental transitions, reflecting bioaccumulation and metabolic processes [6].

The primary objective of this study is to analyze the role of necrophagous insects as alternative matrices in forensic toxicology and to evaluate their reliability in detecting methamphetamine under advanced decomposition conditions [1]. Furthermore, this study aims to integrate entomotoxicological findings with forensic toxicology and medicolegal interpretation, including cultural considerations that influence the understanding and evaluation of death [8].

The working hypothesis of this study is that fly larvae developing on tissues containing methamphetamine can serve as valid and reliable biological indicators for toxicological analysis when conventional matrices are unavailable [1]. Additionally, it is hypothesized that the integration of entomotoxicological data with medicolegal and cultural perspectives will enhance the accuracy, contextual relevance, and evidentiary value of forensic conclusions [11]. By combining experimental data with multidisciplinary interpretation, this study seeks to contribute to the development of a more comprehensive forensic framework that bridges biological, chemical, and socio-legal dimensions in modern death investigations [2].

2. METHOD

2.1 Research Design

This study employed a controlled experimental laboratory design to investigate the role of necrophagous insects as alternative biological matrices for detecting methamphetamine under decomposition conditions [6] [13]. The experimental

approach was selected to provide empirical evidence addressing the limitations of conventional biological matrices in forensic toxicology, particularly in advanced decomposition cases [1]. This design enables direct observation of bioaccumulation processes of toxic substances in insect larvae under controlled conditions [2].

2.2 Materials and Sample Preparation

The experimental materials consisted of fresh beef and pork tissues used as decomposition media for maggot cultivation [5]. Each sample (250 g) was treated with methamphetamine solution at a concentration of 3 μg/mL to simulate drug exposure in decomposing tissues [6]. A total of five experimental units were prepared for each treatment group (n = 5), including one control and four treated samples, to ensure reproducibility and statistical validity [10] The treated tissues were placed in open containers covered with perforated lids to allow insect colonization while preventing contamination.

2.3 Environmental Conditions

All experimental setups were maintained under controlled environmental conditions, with temperatures ranging between 25–30°C and relative humidity of approximately 60–80%, simulating natural tropical decomposition environments [2]. The samples were placed in shaded outdoor conditions protected from direct sunlight and rainfall to allow natural insect colonization while minimizing environmental variability [14]. Environmental factors such as temperature and humidity were considered critical variables influencing larval development and bioaccumulation processes [7].

2.4 Maggot Cultivation and Sampling Procedure

Necrophagous insect larvae were allowed to develop naturally on treated tissues over an eight-day observation period [14]. Sampling was conducted on days 3 to 7, corresponding to larval developmental stages, with emphasis on instar III larvae due to their high feeding activity and bioaccumulation potential [15]. At each sampling point, larvae were collected using sterile forceps, with five replicates per sampling day (n = 5) to ensure data reliability [16]. The collected larvae were immediately inactivated using hot water (>80°C) and stored under refrigeration to prevent chemical degradation [3].

2.5 Extraction of Methamphetamine from Maggot Samples

Maggot samples were homogenized and subjected to sonication extraction using dichloromethane followed by methanol–chloroform (1:3) solvent systems to optimize analyte recovery[17]. Sonication was performed for 10 minutes at room temperature to enhance solvent penetration and analyte release [18]. The extract was filtered and purified using solid-phase extraction (SPE) to remove interfering substances and concentrate the analyte prior to analysis [10].

2.6 Qualitative Analysis

Preliminary identification of methamphetamine was performed using the Marquis reagent test, which produces a characteristic color change (orange to brown) indicating the presence of aromatic amine compounds. The observed color change was used as an initial confirmation of methamphetamine presence in maggot samples. This qualitative screening step is commonly used in forensic toxicology prior to instrumental confirmation.

2.7 Instrumental Analysis

Quantitative and confirmatory analysis was conducted using Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS) (Groth et al., 2025). FTIR analysis was used to identify characteristic functional groups such as N–H, C–H, and aromatic C=C bonds [3]. GC-MS analysis was performed to determine methamphetamine concentration based on retention time and mass spectral fragmentation patterns [13]. These techniques provide high sensitivity and specificity for forensic toxicological analysis [2].

2.8 Method Validation

The analytical method was validated using parameters including accuracy, precision, linearity, limit of detection (LoD), and limit of quantification (LoQ) [19]. Accuracy was evaluated using recovery tests, while precision was assessed through repeated measurements expressed as relative standard deviation (RSD). Linearity was determined through calibration curves of methamphetamine standards, with correlation coefficients (R) used to evaluate the strength of the analytical response. These validation procedures ensure the reliability and reproducibility of the analytical method in forensic applications.

2.9 Statistical Analysis

Statistical analysis was conducted to evaluate differences in methamphetamine concentrations across sampling days and between treatment groups [10]. One-way analysis of variance (ANOVA) was used to determine significant differences in concentration levels among different larval

developmental stages [6]. Additionally, linear regression analysis was applied to assess the relationship between sampling time and methamphetamine concentration in maggot samples. A significance level of $p < 0.05$ was used to determine statistical significance. Statistical analysis was performed using SPSS software (IBM Corp., Armonk, NY, USA) to ensure accuracy and reliability of the results [3].

2.10 Data Interpretation and Medicolegal Context

The analytical results were interpreted within a forensic toxicological framework to assess the reliability of maggots as alternative biological matrices [1]. Furthermore, the findings were integrated into medicolegal and cultural contexts to evaluate their relevance in determining cause and manner of death [8]. This integrative approach recognizes that forensic evidence must be interpreted not only scientifically but also within legal and socio-cultural frameworks.

3. RESULTS AND DISCUSSION

3.1 Growth and Development of Maggots

The growth of maggots followed a typical developmental pattern, with rapid increases in larval length observed between days 3 and 7, corresponding to instar I–III stages [14]. This trend is clearly illustrated in **Figure 1**, which shows the progressive increase in larval size prior to pupation.

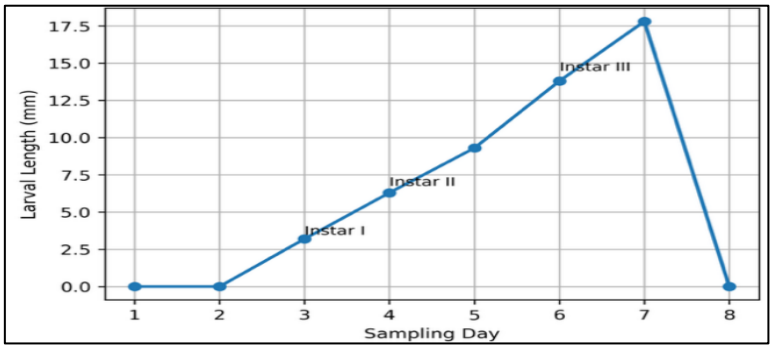

Figure 1. Maggot growth Curve Across Developmental Stages

Figure 1. Growth curve of necrophagous insect larvae under controlled conditions. No larvae were observed on days 1–2, followed by the emergence of instar I on day 3 (~3.2 mm), progression to instar II on day 4 (~6.3 mm), and rapid development to instar III on days 5–7 (~17.8 mm) [20] [14]. The absence of larvae on day 8 indicates transition to the pupal stage. The observed growth dynamics align with established developmental ranges and reflect active feeding phases critical for bioaccumulation processes [7]. These results underscore the importance of larval stage in postmortem interval estimation and entomotoxicological interpretation [2].

3.2 Qualitative Detection of Methamphetamine

The qualitative detection of methamphetamine using the Marquis reagent revealed a progressive increase in color intensity corresponding to larval developmental stages, indicating enhanced bioaccumulation during active feeding phases. This trend is desceribed in **Figure 2**, which presents the semi-quantitative variation in reaction intensity across sampling days.

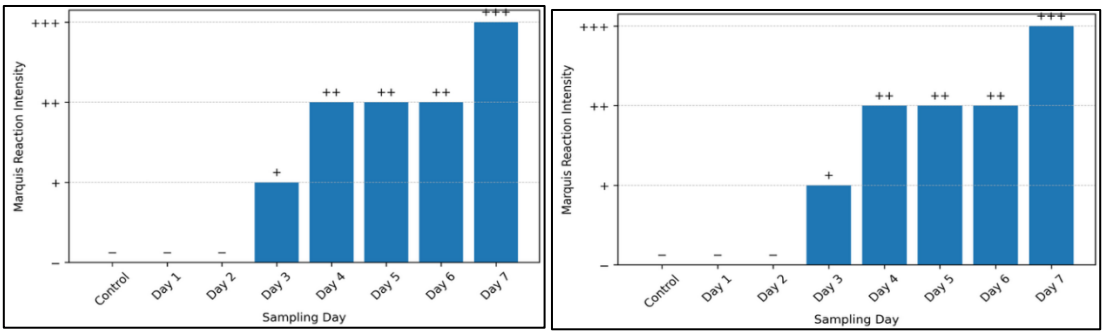

Figure 2. Semi-quantitative detection of Methamphetamine in Maggot Samples.

As shown in **Figure 2**, no detectable reaction was observed in control and early-stage samples (days 1–2), confirming the absence of methamphetamine in initial conditions. The emergence of a weak positive

response (+) on day 3 indicates the onset of compound accumulation, which increased to moderate levels (++) during days 4–6 and reached maximum intensity (+++) on day 7. This pattern reflects the relationship between larval feeding activity and the accumulation of toxic substances, where instar III larvae exhibit the highest metabolic and ingestion rates. These findings support previous studies demonstrating that necrophagous insects can serve as effective bioindicators of toxic compounds, particularly under advanced decomposition conditions where conventional matrices are unavailable.

3.3 FTIR Analysis

FTIR analysis revealed consistent spectral features corresponding to methamphetamine functional groups across maggot samples derived from different decomposition media. Characteristic absorption peaks and their variations across sampling days are clearly illustrated in Figure 3, which presents the annotated FTIR spectra for both beef (a) and pork (b) substrates.

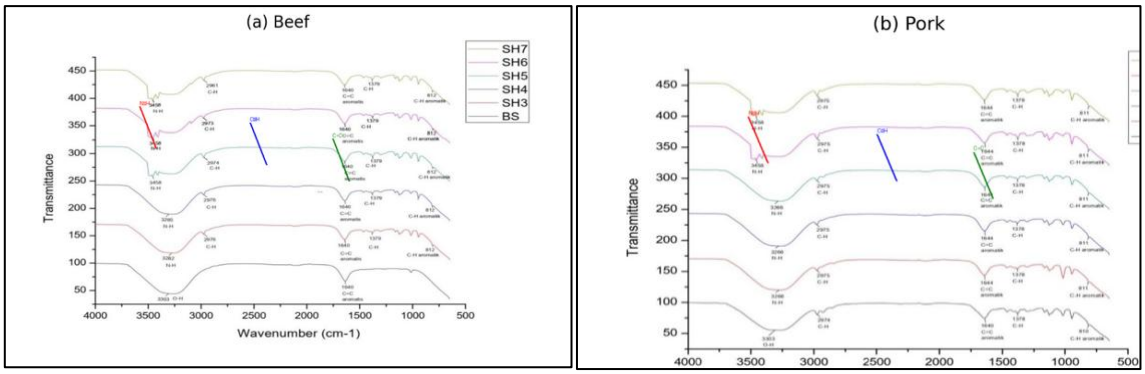

Figure 3. FTIR spectra of maggot samples from beef and pork decomposition media with annotated characteristic peaks of methamphetamine

Figure 3. Annotated FTIR spectra of maggot samples derived from beef (a) and pork (b) decomposition media across sampling days (H3–H7) compared with control samples. Both spectra exhibit consistent characteristic absorption bands associated with methamphetamine, including N–H stretching (~3260–3458 cm^{-1}), C–H stretching (~2960–2975 cm^{-1}), and aromatic C=C vibrations (~1640–1644 cm^{-1}). Additional bands at ~1378–1379 cm^{-1} and ~810–812 cm^{-1} correspond to C–H bending and aromatic out-of-plane vibrations. The persistence and increasing clarity of these peaks across developmental stages indicate progressive bioaccumulation in larval tissues. Minor peak shifts and band broadening, particularly in the N–H/O–H region, reflect matrix interactions during decomposition. Overall, the spectral consistency across both substrates confirms the presence of methamphetamine and demonstrates the reliability of FTIR as a qualitative tool in forensic entomotoxicological analysis.

3.4 Quantitative Analysis Using GC-MS

Quantitative GC-MS analysis revealed a clear temporal pattern of methamphetamine accumulation in maggot samples, with increasing concentrations observed during active larval feeding stages followed by a slight decline prior to pupation. This trend is illustrated in **Figure 4,** which compares methamphetamine concentrations across sampling days in both beef and pork substrates.

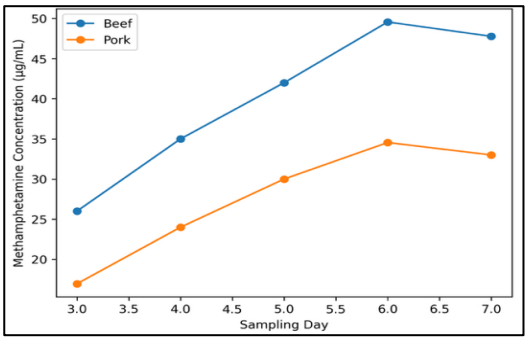

Figure 4. GC-MS quantification of methamphetamine in maggot samples

As shown in **Figure 4,** methamphetamine concentrations increased progressively from day 3 to day 6 in both substrates, reaching peak levels before slightly declining on day 7. This pattern reflects the bioaccumulation dynamics associated with larval development, where instar III larvae exhibit maximum feeding activity. The higher concentrations observed in beef compared to pork suggest a potential matrix effect influencing drug absorption and retention. Statistical analysis confirmed significant differences across sampling days ($p < 0.05$), while regression analysis demonstrated a strong positive

correlation ($R \approx 0.991$), indicating a consistent accumulation trend during active feeding phases. The subsequent decrease in concentration corresponds to the transition toward the pupal stage, where feeding activity ceases, reinforcing the importance of developmental stage in interpreting entomotoxicological data.

3.5 Method Validation Results

Method validation demonstrated satisfactory analytical performance, as indicated by a recovery value of 101.90%, confirming the accuracy of the method [19]. Precision analysis yielded a relative standard deviation (RSD) of 1.19%, indicating good repeatability. The linearity of the method was confirmed by a correlation coefficient (R) of 0.991, reflecting a strong relationship between analyte concentration and detector response. Additionally, the limit of detection (LoD) and limit of quantification (LoQ) were determined to be 6.62×10^{-6} µg/mL and 1.25×10^{-4} µg/mL, respectively, demonstrating high sensitivity of the GC-MS method. Overall, these results confirm that the analytical method is reliable and suitable for detecting methamphetamine in maggot samples within forensic entomotoxicological applications.

3.6 Mechanism of Bioaccumulation and Interpretation

The observed increase in methamphetamine concentration during larval development can be explained by bioaccumulation processes, where larvae continuously ingest contaminated tissues [21]. During instar III, larvae exhibit maximum feeding activity, resulting in peak accumulation levels [15]. The subsequent decrease in concentration during later stages is associated with metabolic transformation and reduced feeding activity as larvae transition to pupae [10]. This phenomenon highlights the importance of considering developmental stages in interpreting entomotoxicological data [9].

3.7 Integration with Forensic, Medicolegal, and Cultural Contexts

The findings demonstrate that necrophagous insects can serve as reliable alternative matrices for toxicological analysis when conventional biological samples are unavailable [1]. This has significant implications in forensic investigations, particularly in cases involving advanced decomposition [2]. From a medicolegal perspective, the ability to detect drugs in insect tissues strengthens evidentiary reliability and supports legal decision-making processes [8]. Furthermore, integrating cultural perspectives in death investigations is essential, as interpretations of death may be influenced by societal and legal frameworks [22]. This study demonstrates that forensic entomotoxicology provides a multidisciplinary approach that bridges biological, chemical, and legal dimensions, thereby enhancing the accuracy and contextual relevance of forensic interpretations [11].

4. CONCLUSION

This study demonstrates that necrophagous insects, particularly fly larvae, play a significant role in death investigations by serving as alternative biological matrices for detecting methamphetamine under advanced decomposition conditions. Experimental results confirmed the presence of methamphetamine in maggot tissues through qualitative (Marquis reagent) and instrumental analyses (FTIR and GC-MS), with increasing concentrations observed from day 3 to day 6, followed by a slight decline on day 7, reflecting bioaccumulation dynamics during larval development. Statistical analysis further supported significant differences across developmental stages ($p < 0.05$) and a strong correlation between time and concentration ($R \approx 0.991$). These findings highlight that necrophagous insects extend beyond their conventional role in postmortem interval estimation to function as carriers of toxicological evidence. The integration of forensic entomotoxicology with toxicological analysis and medicolegal interpretation provides a more comprehensive and reliable framework for complex death investigations.

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Author Contributions

All the authors contributed significantly to this manuscript, participated in reviewing and editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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