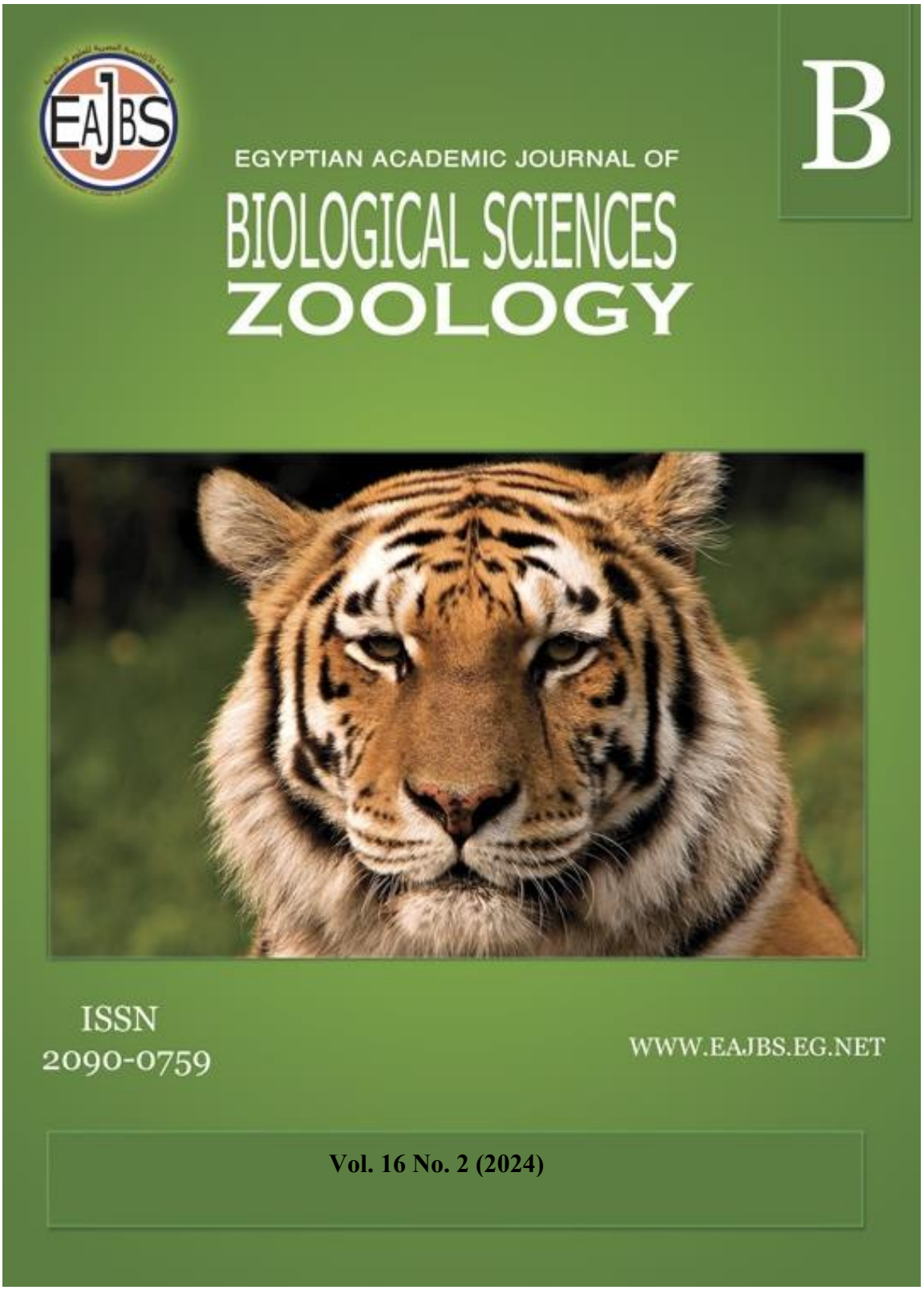

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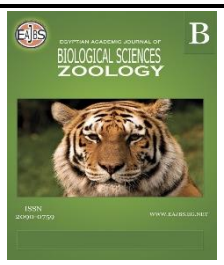

Forensically Importance of Beetle (*Dermestids Maculatus*) on Rat Carcasses ZnCoS Nanoparticles Toxicity at Different Decomposition Stages During Winter Season

Fatma El-Zahraa A. Abd El-Aziz[1]*, Asmaa F.A. Dawood[2&3] , Shahina Khan[3], Shereen M. Refaie[3], Aliya E. M. Elbadwi[3], Mariem A. Rabab[1], Mostafa A. Asmaey[4] , Dalia A. Ahmed[5]

[1]Zoology and Entomology Department, Faculty of Science, Assiut University, Assiut, Egypt.
[2]Department of Histology, faculty of Medicine, Assiut University, Assiut, Egypt.
[3]Department of Biomedical Sciences, College of Medicine, King Faisal University, Alhsa, Saudi Arabia.
[4]Department of Chemistry, Faculty of Science, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt.
[5]Physics Department, Faculty of Science, Assiut University, Assiut 71516, Egypt.
***Email:** fatma.abdelgalil1@science.aun.edu.eg, F_abdelhameed@yahoo.com

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Dermestids maculatus.

ABSTRACT

Background and Objective: Forensic entomotoxicology examines how toxins affect the development of arthropods that feed on decaying bodies, which can influence post-mortem interval (PMI) calculations. This research focuses on the impact of ZnCoS nanoparticles (NPs) on the decomposition stages of rat carcasses and their effects on *Dermestes maculatus*; a species of forensic relevance. **Materials and Methods**: Thirty albino rats were assigned to control and treatment groups, receiving different doses of ZnCoS NPs. The decomposition was observed daily for a month, with arthropods collecting regularly. The development rates and structural changes in *Dermestes maculatus* were examined using light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). **Results**: Exposure to ZnCoS NPs resulted in a 22–33 hour delay in the PMI for specific insect species. Structural damage, especially to the wings of *Dermestes maculatus*, was evident, showing signs of apoptosis. These findings indicate that ZnCoS NPs alter both insect growth and the rate of decomposition. **Conclusion**: ZnCoS NPs have a notable impact on decomposition and PMI estimation, underscoring the importance of further forensic investigation into nanoparticle toxicity. SEM and TEM proved an efficiency in conducting postmortem toxicological analyses.

INTRODUCTION

Forensic entomotoxicology focuses on understanding how drugs and toxins influence the developmental rates of insects that feed on decomposing remains (Kim, 2002). Evidence derived from forensic entomology is increasingly valuable in criminal investigations, as it can provide insights into the cause of death, estimate the postmortem interval (PMI), determine the location of death, indicate whether remains were moved postmortem, and assist

in diagnosing antemortem injuries (Campobasso *et al.*, 2004; Tracqui *et al*., 2004; Amendt *et al.,* 2007;). During postmortem crime investigations, Diptera and other arthropods can serve as dependable toxicological specimens for analysis, replacing the typical tissues and fluids specimens usually collected for this purpose (Introna *et al.,* 2001). Arthropodology is a novel discipline within a criminal investigation that analyzes arthropod information to determine if medications or poisons were administered before death (Samnol *et al.*, 2020). Throughout the decomposition process, the decaying carcass becomes a magnet for a different type of arthropod known as a sarcosaprophagous insect. Some insects are attracted to the remains as a suitable place to lay their eggs or feed, while others are attracted to the groups of other arthropods that provide a food source (Payne, 1965).

Changes in agricultural land use are one of the most significant human impacts on climate. Croplands, pastures, and farms have increased globally in recent decades, resulting in large amounts of energy, water, and pesticide consumption ~~increases~~ and severe biodiversity losses. Soil animals mediate several fundamental ecological processes critical to the entire ecosystem, including organic matter decomposition, nutrient cycling and carbon sequestration, in addition to the establishment and maintenance of soil structure. All of which affect gas and water movement by manually breaking down leftovers and distributing microbial reproduction (Power, 2010).

One of the main goals of forensic toxicology is to discover the presence of toxins and determine their type: whether they are drugs, high-grade medicine, or pesticide poisons. Careful exploration of entomological findings affecting arthropods, including insects that live on and in carcasses, can help uncover a lot of information important to investigations into the death of an individual that would otherwise be lost in the absence of awareness on the part of forensic science personnel (Sardar *et al.,* 2021; Tabor *et al.,* 2004). The process of decomposition resulting from insect activity on and within a body occurs progressively and can be measured, enabling precise estimations of the PMI even months after death depending on the circumstances. Numerous studies have indicated that the presence of drugs and toxins can influence the developmental rates of insects feeding on decaying remains (Alan and Sarah ,2012; Tabor *et al*., 2004).

This study examines the toxicity of ZnCoS NPs on arthropods, specifically targeting both adults and larvae of the hide beetle (*Dermestes maculatus*), a species commonly found on exposed decomposing rats carcasses. The findings could have significant forensic implications.

MATERIALS AND METHODS

Study Design:

A total of 30 male albino rats (8 weeks, 250–300 g) were acquired from the animal house at the Faculty of Medicine, Assiut University, Egypt. The rats were kept in ideal environmental conditions, with temperature~~s~~ ranging from ~~+~~11 ºC to ~~+~~26 ºC in Assiut, December 2023, in accordance with the standards outlined in the Care and Use of Laboratory Animals (Sikes et al., 2011). Every rat was positioned on a plate and surrounded by a perforated plastic cage to prevent the entry of birds and other animal patrons. The site consisted predominantly of sand and dust, devoid of any flora. After decomposition, arthropods were gathered daily during the initial week and then once daily for a minimum duration of one month (Fig. 1).

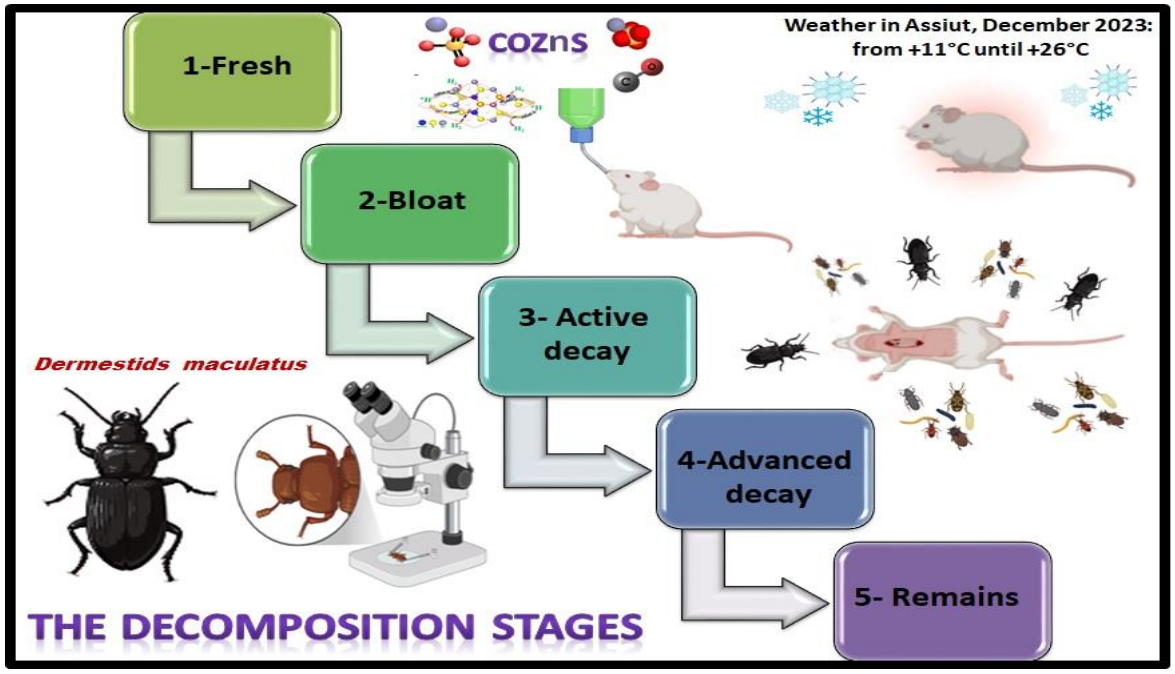

Fig. 1: Illustration displays the investigation plan.

Synthesis and Characterization of ZnCoS Nanoparticles:

$Zn_{0.5}Co_{0.5}S$ nanoparticles were synthesized by a hydrothermal method. The following ingredients were required for the manufacture of $Zn_{0.5}Co_{0.5}S$ nanomaterials: $ZnCl_2$, cobalt chloride ($CoCl_2.6H_2O$), sodium sulphide non-hydrate ($Na_2S.9H_2O$) (assay 99.5%) from Alpha Chemical Co., and ethanol (C_2H_5OH). Water was distilled and de-ionized (D.I.) with Milli-Q water purification equipment. All of the aforementioned chemical precursors were employed in this study without additional purification. In a typical procedure, a solution of $ZnCl_2$ (3.69 g) and the required amount of $CoCl_2.6H_2O$ (6.43 g) were mixed with 20 ml of deionized water and stirred for 30 min. A separately prepared $Na_2S.9H_2O$ (1 M) solution was added dropwise to the above mixture. The stirring was continued for a specific time in order to facilitate complete nanoparticle precipitation. The obtained product was moved to a stainless-steel autoclave lined with Teflon. The autoclave was heated in a furnace to 180 ºC for 12 hours. After the furnace is turned, the autoclave is kept in it for 12 hours to gradually drop to room temperature (25 ºC). To eliminate the NaCl, the suspension was washed three times with double distilled water and twice with pure ethyl alcohol. Finally, the produced samples of the nanocomposite were dried at room temperature while the vacuum was kept constant at $\approx 5.2 \times 10^{-5}$ torr for 10 h.

The X-ray powder diffraction patterns (XRD) data was collected using a Philips PW 1700 diffractometer with Cu Kα radiation at $\lambda = 0.154056$ nm wavelength. The sample was scanned from 4º to 90º of 2θ at a rate of 0.06° per second. A JEOL scanning electron microscope (model JSM-5400 LV) was used for characterisation. The charge was removed from the samples by applying a thin layer of gold (Fig. 2).

The XRD pattern of the produced ZnCoS sample was obtained and displayed in Figure 2. The figure depicts six diffraction peaks at values 28.5, 32.9, 47.6, 56.4, 69.6 and 76.9. The peaks are caused by reflection from the (111), (200), (220), (311), (400) and (311) planes of the cubic phase of ZnS. The XRD pattern of the prepared samples is well matched with the standard cubic ZnS (JCPDS Card file 00-005-0566, a = b = c = 5.406 Å). When compared to the standard sample (JCPDS card No. 00-005-0566), the X-ray diffractogram and 2Θ values of ZnS showed good agreement, confirming the zinc mix crystal structure. No diffraction peaks corresponding to Co precipitates or Co-related impurity phase were

detected, which further confirmed the formation of ZnS:Co^{2+} Solid solution instead of Co-precipitation or second phase. The average crystallite size of **ZnCoS** nanoparticles was estimated using Debye-Scherrer, $D=K\lambda/(\beta \cos\theta)$, where K is the shape factor equal to 0.9, λ the X-ray wavelength for Cu kα radiation (λ = 1.5418 Å), θ the Bragg diffraction angle (in radian), and β the full width at half maximum of the most intense XRD peak. The **ZnCoS NPs** exhibited an average crystallite size of about 17.6 nm, which corresponded to the most intense diffraction lines (111), (220) and (311).

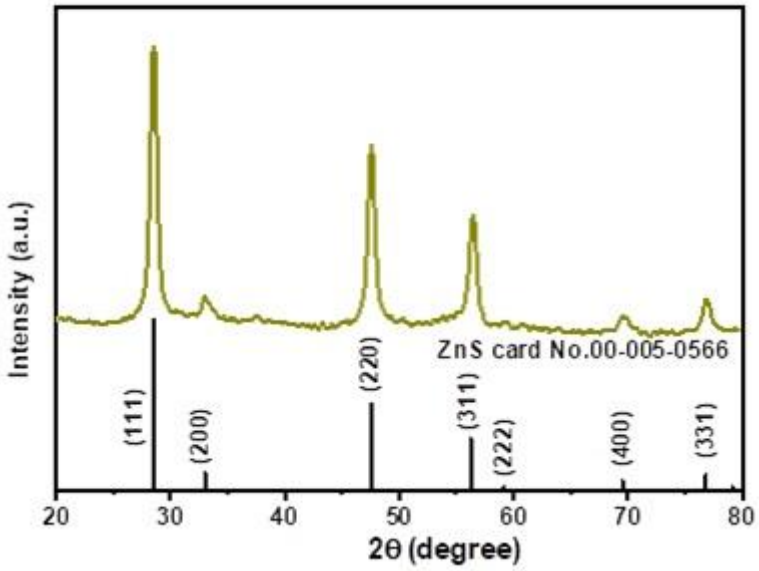

Fig. 2: XRD pattern of ZnCoS nanoparticles.

SEM micrographs were used to assess the surface appearance and distribution of particle sizes in all samples. Figure 3 shows the typical SEM picture of ZnCoS nanoparticles. The result shows that the as-synthesized ZnCoS NPs are in narrow size distribution, and the shape of the particles is approximately cube. The morphology of samples is composed of agglomerated particles.

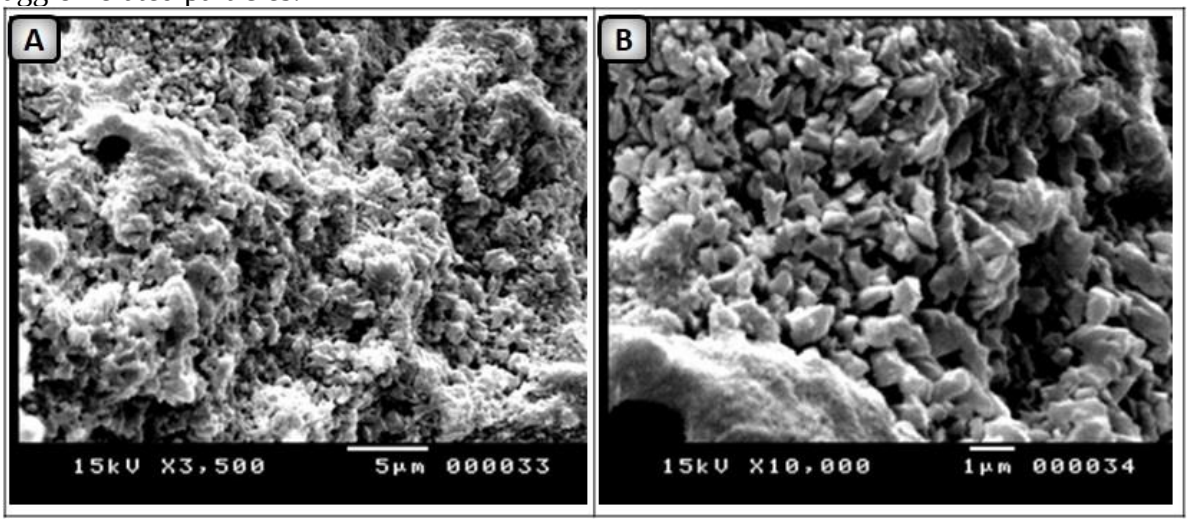

Fig. 3: (A & B) SEM image of ZnCoS nanoparticles.

ZnCoS NPs Toxicity Dosage and Administration:

Rats were classified equally randomly into uncovered two groups (n = 10): I (control) and treated group II (ZnCoS NPs toxicity) . Rats in group I received neither saline

nor drugs and anaesthetized with 50 mg/kg intraperitoneal thiopental then euthanized by cervical dislocation, following by left uncovered in direct sunlight and plate covered completely beneath sand, respectively. Rats in group II were euthanized by receiving 500 mg/kg of ZnCoS NPs one shot orally by gastric gavage, representing, single and double the lethal dose (LD_{50}) modified (Rohn & Bond, 1953), and were left uncovered in direct sunlight with up plate beneath sand from all sides, respectively.

Arthropods Identification:

Herein, we identified insect larvae and nymphs, separated and identified the collected arthropod specimens as larvae, spiders, isopods, and nymphs, and identified mites and bugs according to Tiemeyer *et al.*, (2009); Whitworth, (2019); Yassin, (2015). The arthropod specimens were preserved in plastic jars containing a 7% neutral buffered formalin solution for 5 days. The samples were kept in 72% ethyl alcohol for long-term preservation.

Scanning Electron Microscope (SEM):

Adult and larvae hide beetle (*Dermestids maculatus*) from two different groups (control and treated) were placed on ~~cover~~ glass slides and fixed in a solution of 5% glutaraldehyde in sodium cacodylate buffer for 1.5 h. After that, they were rinsed with distilled water and dehydrated using ethanol. The critical point drying process was performed, where the samples were placed on stubs, covered with a layer of either carbon or gold and examined using a Joel JSM 35 scanning electron microscope at 20 kV.

Histological Investigations:

Adult hide beetles (*Dermestids maculatus*) from all groups were sectioned (5 µm), placed on slides, and left to dry at 37 ºC overnight. The sections underwent de-waxing in xylene and hydration in a graded series of alcohols before staining with hematoxylin and eosin (Odendaal & Reinecke, 2003).

Ultrastructural Study:

Specimen Preparation for Semithin Sections:

Adult hide beetles (*Dermestids maculatus*) were immersed in a 4% cold glutaraldehyde solution, followed by three rinses in phosphate buffer (pH 7.2) for 20 min each. The specimens were postfixed in a 1% osmium tetroxide (OsO_4) solution for 2 h and then subjected to four more washes in the same buffer. The specimens were dehydrated using increasing concentrations of ethyl alcohol and soaked in propylene oxide for 30 min to remove any remaining alcohol residue. Subsequently, they were soaked in a mixture of propylene oxide and Epon 812 (1:1, v/v) for an additional 30 min before finally being soaked in Epon 812 for 4 h. The tissue blocks were immersed in capsules filled with the embedding mixture and then subjected to polymerization in an oven at 60 ºC for two days. Sections of a thickness of 0.5 µm were produced using an LKB ultramicrotome and then stained with toluidine blue (Ayub *et al.*, 2017).

Specimen Preparation for Transmission Electron Microscopy (TEM):

Ultrathin sections were produced after examining semithin sections to determine the specific location of the tissues. The process involved the utilization of a Leica AG ultramicrotome to cut ultra-thin slices measuring 50–80 nm. These slices were then treated with uranyl acetate and lead citrate for staining. The sections were examined using a TEM (Jeol 100 CXII) operating at 80 kV. Electron micrographs were obtained for semithin sections, processed, and edited using Photoshop for sample analysis. Micrographs were employed to illustrate the findings.

Statistical Analysis:

The analysis of statistical data was conducted using Prism 8. Data were obtainable as frequencies and proportions. By using T-test and ANOVA for comparing diverse groups. $P < 0.05$ indicated statistically significant.

RESULTS

The Decomposition Stages of PMI of Rat Carrions in Different Groups:

The decomposition of rat carcasses in all groups progressed through five distinct stages: fresh, bloated, active decay, advanced decay, and dry remains (Fig. 4). During the fresh stage (0–1 days postmortem), there were no statistically significant differences across the groups in terms of the presence of eggs, adults, larvae, or pupae. In the bloated stage (2–4 days postmortem), both Groups II and III displayed a marked decrease in the number of eggs, larvae at various instars, and adults compared to Group I. During the active decay stage (5–7 days postmortem), Group III showed a pronounced decline in eggs, larvae across all instar stages, pupae, and adults relative to Group I. Nevertheless, Group III exhibited a higher number of eggs, second- and third-instar larvae, and pupae compared to Groups I and II in the latter part of the active decay phase. By the advanced decay stage (8–9 days postmortem), Group III had notably fewer eggs, larvae, pupae, and adults than Groups I and II. In the final dry remains stage (16–31 days postmortem), Group III demonstrated a significant reduction in the presence of eggs and adults, with no observable second- or third-instar larvae or pupae in comparison to Groups I and II.

Furthermore, 1st larval stage did not appear in all three groups. The comparison of species growth at the end of the study showed that some species had different distributions across the studied groups. There were birds, including *Passer passer domesticus, Corvus corone, Upupa epops, Egretta ibis,* cats, and reptiles (*Mabuya quinquetaeniata).* Regarding the PMI, there was a delay in the development of *Musca domestica* (house flies) by 31 h in Group III and 22 h in Group II. Additionally, there was a delay in the development of *Sarcophaga sp*. (flesh flies) by 33 h in Group III and 26 h in Group II.

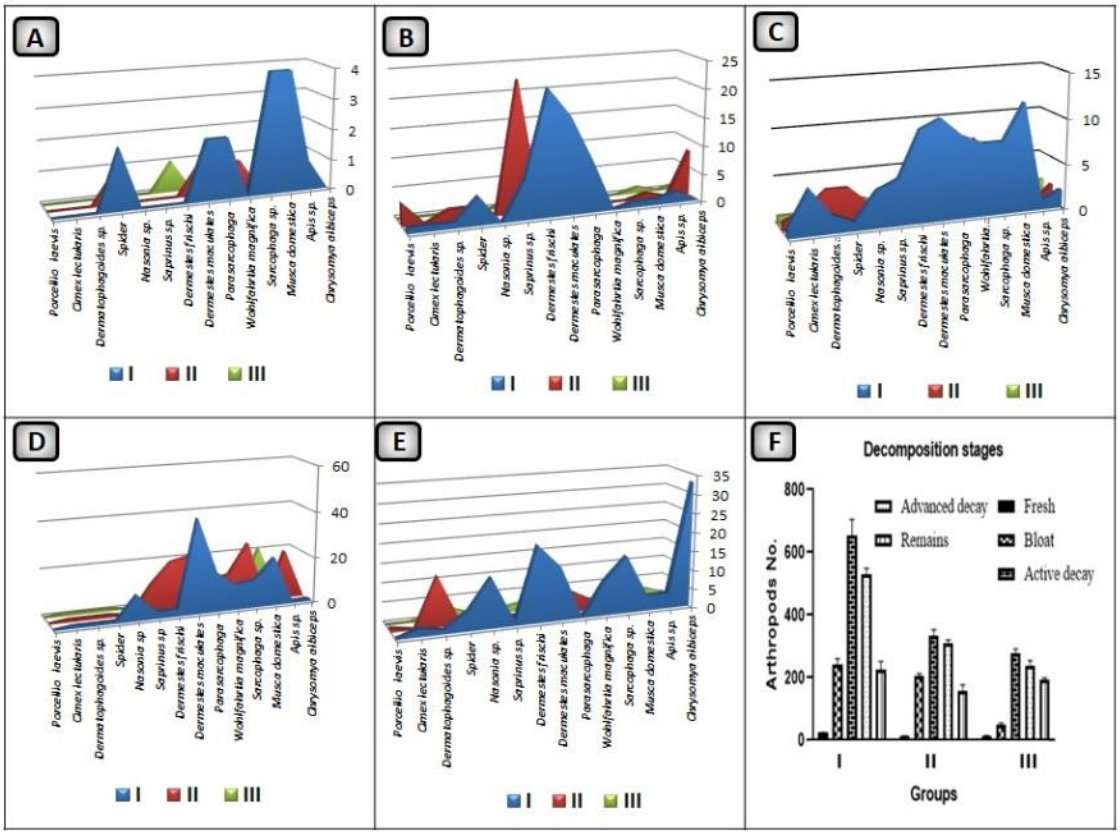

Fig. 4: The decomposition Stages, A: Fresh stage, B: Bloat stage ,C: Active decay stage, D: Advanced decay stage , E: Dry or remains stage and F: Stages of decomposition from fresh to dry stages in the all groups.

Macroscopic and SEM Observations:

The SEM results of Group I showed that in normal adult and larva of hide beetle (*Dermestids maculatus*), the adult body size from 5.5: 10.0 mm, it was separated into three different regions: head, thorax, and abdomen (Fig. 5). The SEM photomicrograph of adult and larva of hide beetle (*Dermestids maculatus*) in Group II and III showed severe damage in the head accompanied by complete loss of some legs, with an ulcer in the body, and thickening of the epidermis with hyperplasia of the epithelial layer, and in all the body (Fig. 6).

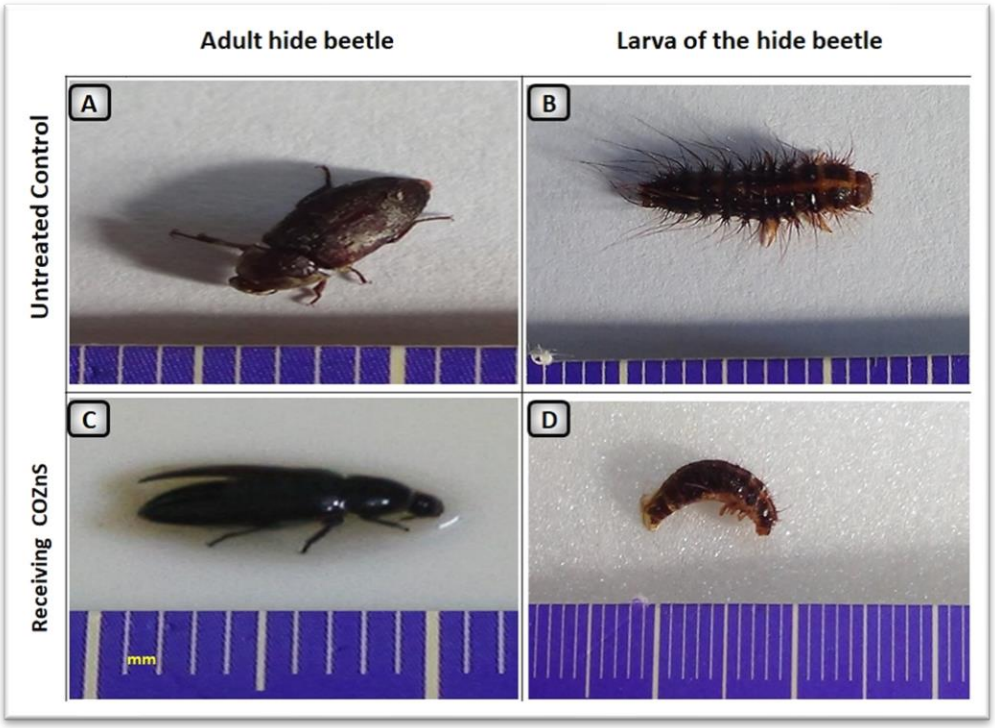

Fig. 5: Macroscopic observation of the different groups of beetle (*Dermestids maculatus*) ,(A),(B) normal group,(C),(D) treated group.

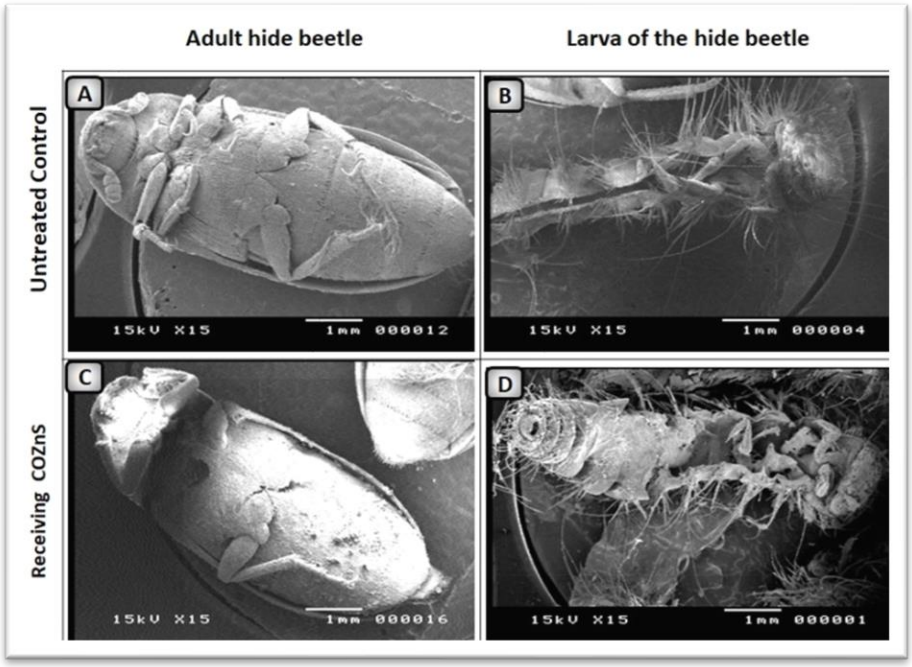

Fig. 6: Scanning electron photomicrograph of the different groups of beetle (*Dermestids maculatus*) ,(A),(B) normal group,(C),(D) treated group.

Histological and Ultrastructural Observations:

For light microscopy investigations, a section from the control group of adult hide beetles (*Dermestes maculatus*) showed that the stomach was divided into distinct dorsal, lateral, and ventral chambers. Additionally, the filter region was clearly identifiable. The typhlosole in the hindgut exhibited that the gut tube was extensive and filled with diverse food particles. The pairs of laterally aligned midgut gland diverticula showed the anterior dorsal caecum and four endodermal hepatopancreatic tubules. Histopathological changes in beetle (*Dermestids maculatus*) of treated groups showed a breakdown of typhlosole, necrotic epidermis, circular muscle, and longitudinal muscle, destroyed epidermis and circular muscle, and only the longitudinal muscle is present. Moreover, the results showed severe damage in the stomach and endodermal hepatopancreatic (Fig. 7 A&B).

A photomicrograph of a semithin section of the wing from *Dermestes maculatus* in the control group revealed the typical structural features. These included the exocuticle, endocuticle, haemolymph space, and microtrichia. Significant alterations were observed in the various layers of the wing structure, with the most pronounced effects seen in the groups treated with ZnCoS nanoparticles. These groups exhibited complete destruction of the layers, along with fragmentation of the wing, which ultimately resulted in the death of all beetles (Fig. 7 C&D). Ultrastructural evidence from the treated groups of *Dermestes maculatus* beetles provides strong support for the initiation of apoptosis or other forms of cell death in body cells. This toxicity-induced cell death was more pronounced in comparison to group I (Fig. 7 E&F).

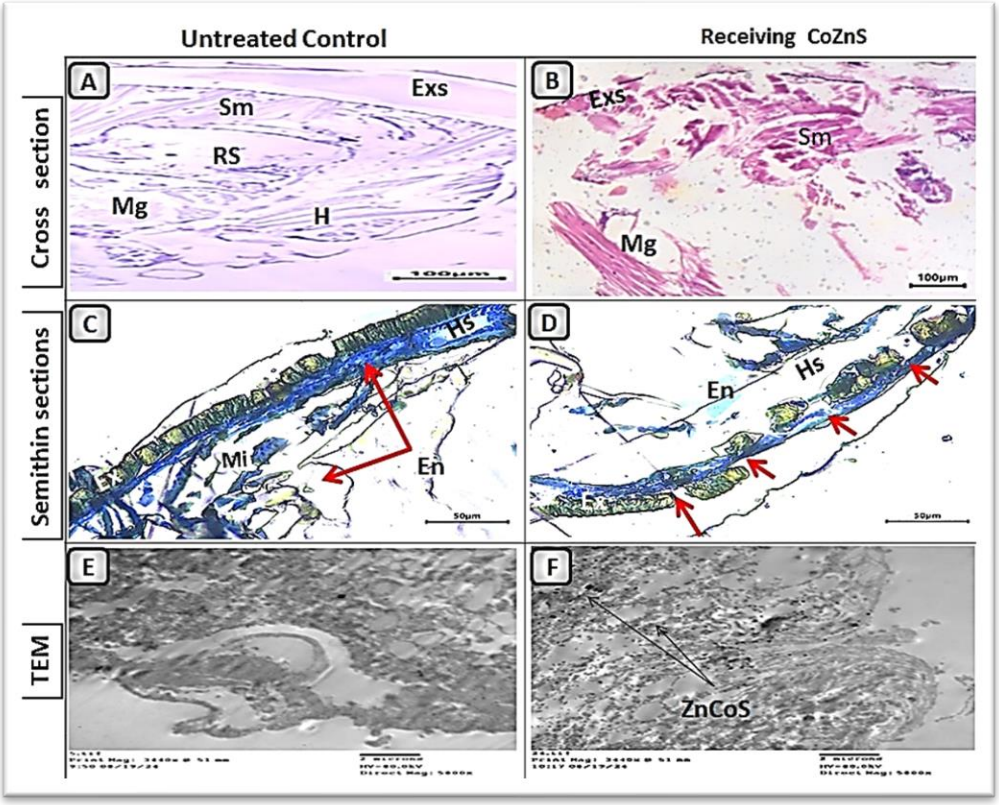

Fig. 7: Transverse sections of the different groups of beetle (*Dermestids maculatus*),(A) normal group,(B) treated group. Photomicrographs of semithin sections of the different groups of beetle (*Dermestids maculatus*) elytron, (C) normal group, (D) treated group. Transmission electron microscopy micrographs of the different groups of beetle (*Dermestids maculatus*) showing dryness, damage and erosions (arrows),(E) normal group,(F) treated group.

Exs: Exoskeleton, **Ex**: Exocuticle, **En:** endocuticle, **H:** Hemocoel , **Hs**: haemolymph space, **Mi**:Microtrichia, **Mg:** Midgut, **Sm**: Skeletal muscle, **RS:** Rectal sac.

DISCUSSION

Forensic investigators employ the study of insect populations to analyze the cause of death, such as poisoning (Joseph et al., 2011). Therefore, determining the cause of death is a crucial aspect of criminal inquiry, especially when dealing with a severely decomposed body (Waghmare *et al*., 2015). Arthropods are useful in ecotoxicology as they can serve as indicators of the toxins effects on terrestrial environments. Therefore, we chose isopods to assess the effect of hydrogen cyanamide toxicity on arthropods, particularly hide beetle (*Dermestids maculatus*), visiting uncovered decomposing rat carrions using several analyses.

This study identified five stages of decomposition in rat carcasses, with variations based on the type of carcass and the duration of each stage (Moretti *et al*., 2008). In contrast, Farag and coauthors (Farag *et al*., 2021) documented four decomposition stages in rats during the dry (remains) phase. In our investigation, we recorded a higher number of arthropod taxa compared to most previous studies, identifying 14 distinct taxa. These included one Calliphoridae, one Apidae, one Muscidae, three Sarcophagidae, two Dermestidae, one Histeridae, one Pteromalidae, one Lycosidae, one Pyroglyphidae, one Cimicidae, and one Porcellionidae, which is consistent with the findings of Horenstein and his colleagues (Horenstein *et al*., 2012).

Estimating the PMI using entomological techniques involves estimating the minimal PMI based on the age of the oldest fly larvae found on decomposing remains (Zou *et al.,* 2013). However, intoxicated carcasses possess a unique circumstance when it comes to determining the PMI. Verma and Paul (Verma & Paul, 2013) highlighted the importance of taking into account the potential impact of pharmaceuticals found in carrion on the growth rates of insects at various stages.

Drugs can exert diverse impacts on the developmental rates of flies, with certain substances accelerating specific life stages, while others result in delayed development (Bhardwaj *et al.,* 2020). Therefore, the existence of a toxin serves as a potential factor that can introduce inaccuracies in determining the PMI, necessitating careful consideration. Malathion significantly postponed the arrival of different arthropod species and consequently delayed their egg-laying process for several days (Gunatilake & Lee Goff, 1989). In addition, the inclusion of ethanol in pig carcasses caused a postponement in the growth phases of Diptera (Tabor *et al*., 2005). Moreover, methamphetamine and cocaine accelerate the growth of developing insects (Verma & Paul, 2013), as opposed to other findings. The impact of medications on these flies is influenced by the concentration of the drugs in some cases, but in many cases, it is solely determined by the presence of the drugs. Furthermore, a crucial aspect to consider when estimating PMI is the disparity in temperature between covered/uncovered carrions. Monthei attributed the discrepancy between his findings and previous investigations to variations in the temperature and the duration of exposure to cyclic light in the case of exposed carrion (Monthei, 2009). In addition to medicines and temperature, several other parameters must be considered when estimating PMI, including particular species characteristics, meteorological conditions, maggot mass, food type, and geographic region (Byrd & Tomberlin, 2019).

Here, we established a significant reduction in invading blowflies, houseflies, and flesh flies between Groups II and III ~~from~~ versus Group I. This can be attributed to the similar impact of malathion on the developing insect due to the poisonous properties of ZnCoS NPs. Nevertheless, this significant disparity was not detected between Groups II and III. This demonstrates the influence of covering in safeguarding the carcass from flies. Air serves as a medium by which flies are transported to corpses. Therefore, covering had a substantial protective effect in reducing the number of flies in Groups II and III. This finding is aligned with El-Aziz, *et al.*, (2022 c) and Abd El-Aziz, (2024) who observed a decrease in flies infiltrating scorpion venom and digoxin on rat carrions. Additionally, they documented the

total absence of certain fly families.

In this investigation, various arthropods were identified in the rat cadavers, representing 14 different species, which have drawn the attention of forensic researchers (Abd El-Aziz *et al.*, 2022; a,b). These arthropod species are essential for determining the time, manner, and causes of death.

Temperature is regarded as the second most critical factor influencing the decomposition of carrion, following the arrival of arthropods at the remains. In the present study, the effects of ZnCoS NPs were examined, revealing a reduction in the duration of the five stages of decomposition: fresh, bloated, active decay, advanced decay, and dry. Additionally, observations using photographs, SEM, semithin, and TEM techniques on beetles (*Dermestid maculatus*) showed visible damage in the chitin wings of the group exposed to ZnCoS NPs, as evident in TEM micrographs. These findings align with our recent studies, which confirm that ZnCoS NPs induce significant cell death *in vitro*, with apoptosis being the predominant death mechanism triggered. These results are consistent with the previous work (Abd El-Aziz *et al*., 2022 a,b,c).

The histological analysis of the hide beetle (*Dermestes maculatus*) revealed distinct anatomical features. In the control group, the stomach was clearly divided into dorsal, lateral, and ventral chambers, with a well-defined filter region. The typhlosole in the hindgut showed the gut tube was extensive and filled with diverse food particles. The pairs of laterally aligned midgut gland diverticula showed the anterior dorsal caecum and four endodermal hepatopancreatic tubules. Histopathological changes in beetle (*Dermestids maculatus*) of treated groups showed a breakdown of typhlosole and necrotic epidermis, circular muscle, and longitudinal muscle, destroyed epidermis and circular muscle, with only the longitudinal muscle is present. Moreover, the results showed severe damage in the stomach and endodermal hepatopancreatic.

Furthermore, the head was absent, and the epidermis thickened. Specifically, we employed SEM to identify the effects of ZnCoS NPs toxicity on the decomposition of rat carcasses in the context of arthropod succession. These findings align with the data of Mustafa, who verified the very toxic and harmful impacts of ZnCoS NPs on beetle (*Dermestids maculatus*) using histological and SEM examinations (Mustafa, 2012).

The current study highlights the necessity of conducting fundamental research in taxonomy, ecology, succession process, and other relevant domains utilized in forensic investigations. The presence of specific animal species and their state can provide valuable information in determining the circumstances surrounding a deceased individual. The postmortem evidence varies depending on different factors such as environment, location, season, drug, and toxin.

CONCLUSION

The findings of this study revealed that ZnCoS NPs are highly toxic, leading to significant damage and mortality in both adult and larval *Dermestes maculatus* found on decomposing rat carcasses. The relationship between the insects' presence and the stages of decomposition was critical, particularly in terms of forensic relevance. The application of SEM and TEM provided valuable insights into nanoparticle-induced toxic effects. Furthermore, ZnCoS NPs delayed the development of certain fly species by 22 to 33 hours, an important factor (PMI) estimation. Given the potential environmental and health hazards posed by these nanoparticles, more extensive studies are essential to fully understand their long-term effects and potential ecological disruptions.

Declarations:

Ethics Approval and Consent to Participate: The faculty of Veterinary medicine, Assiut University Ethical Committee, approved this study (06/ 2024/0268). All animal procedures followed ARRIVE guidelines and the relevant guidelines and regulations.

Competing interests: The authors have declared that no competing interests exist.

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