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Effect of hexavalent chromium [Cr(VI)] on morphology and biochemical parameters of tasar silkworm (*Antheraea mylitta* D.)

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Abstract

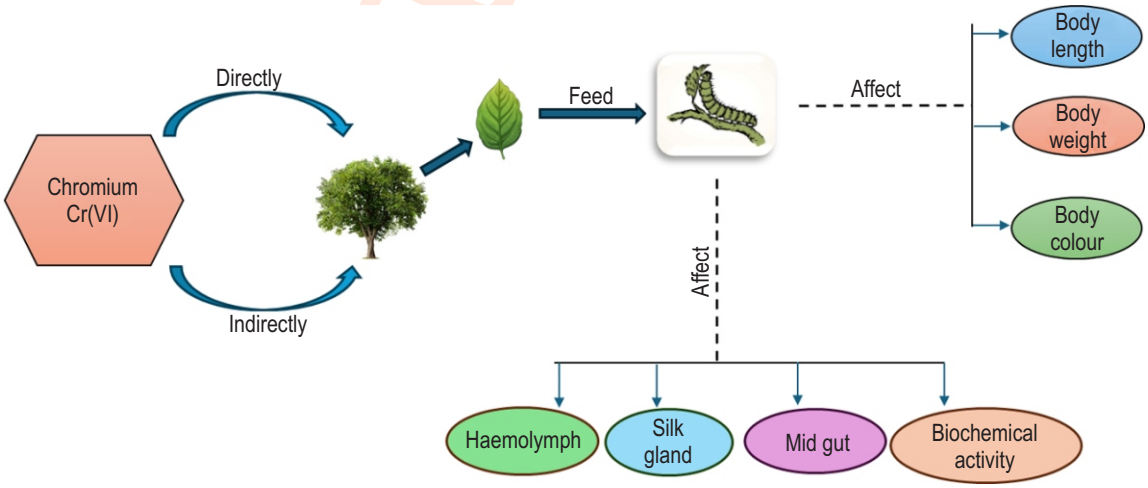
Aim: The present study focused on investigation the harmful effects of Cr (VI) on the 5th instar larvae of *Antheraea mylitta*.

Methodology: Silkworms were fed in an indoor setup with Arjuna (*Terminalia arjuna*) leaves treated with Cr (VI) at 10, 100 and 1000 ppm. The morphological study of larvae, along with biochemical assessments, was performed on larval and pupal haemolymph, as well as on silk gland and midgut tissues.

Results: The findings revealed a concentration-dependent reduction in larval body length and weight over 10 days and an alteration in larval coloration. The inhibitory effect of Cr(VI) was more pronounced in males than females, with treated males exhibiting a 28% reduction in length and a 20% reduction in weight. Biochemical assessments on larval and pupal haemolymph, silk gland, and midgut indicated significant physiological stress and metabolic disruption due to Cr (VI) exposure.

Interpretation: The findings suggest that chromium contamination affects the growth, development, and silk-producing potential of *A. mylitta,* with implications for the sustainability of the tasar silk industry.

Key words: *Antheraea mylitta,* Chromium (VI), Tasar silkworm



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Introduction

The tasar silkworm (*Antheraea mylitta* Drury), a widely found wild silkworm species, holds substantial economic and ecological importance in India, especially for tribal and forest-dependent communities. It thrives on host plants like *Shorea robusta, Terminalia arjuna* and *T. tomentosa* and contributes to the production of coarse but durable silk known for its natural gold sheen. Heavy metal contamination poses a pressing global environmental threat, largely attributed to the intensification of industrial activities such as mining operations, metal extraction and smelting, chemical manufacturing, and emissions from industrial facilities (Jaishankar *et al.,* 2014; Vareda *et al.,* 2019). These anthropogenic sources contribute significantly to the bioaccumulation of toxic metals in both aquatic and terrestrial ecosystems, leading to hazard to environment and human health. Chromium (Cr), a heavy metal of significant environmental concern, is known to exhibit phytotoxic effects on agricultural crops. Exposure to elevated levels of chromium has been reported to induce physiological stress symptoms such as pronounced wilting and chlorosis, particularly in the upper foliage of affected plants (Hossner *et al.,* 1998).

Chromium concentration in contaminated soils has been recorded at levels ranging from 50 to over 300 mg kg^{-1}, significantly above natural background level (Kabata-Pendias and Mukherjee, 2007). The presence of Cr(VI)- in soil poses a significant environmental risk due to its solubility and ability to be taken up by plants. Cr(VI) is among the most toxic forms of chromium, exhibiting significantly higher toxicity than trivalent chromium Cr (III) (Korshoj *et al.,* 2015). Studies have shown that exposure to Cr(VI) reduces mulberry leaf consumption in multiple races, with Qiufeng × *Baiyu* larvae exhibiting the maximum reduction in *B. mori* (Tucker *et al.,* 2003). Cr(VI) can enter the body through all major exposure routes, where it exerts lethal, carcinogenic, allergenic and irritation effects (Tan *et al.,* 2015). *T. arjuna,* commonly known as Arjun, is a medicinal tree, and food plant for *A. mylitta* native to the Indian subcontinent. Due to its widespread plantation in urban and peri-urban areas, it is frequently exposed to heavy metal contamination through soil and atmospheric deposition. Several studies have demonstrated that Arjun trees can absorb and accumulate chromium from contaminated soil. Chromium accumulation primarily occurs in roots and leaves, with leaves serving as an important bioindicator due to their direct exposure to airborne pollutants and systemic translocation from the root (Sundar and Chakravarty, 2010).

Elevated chromium levels in *T. arjuna* leaves have been reported in areas surrounding tannery and metal industries, with concentrations ranging from 5 to 30 mg kg^{-1} d.wt., depending on the exposure levels and soil pH (Shanker *et al.,* 2005). However, increasing industrialization and the emission of heavy metals into the environment, especially chromium from tanning and metallurgical industries, pose a growing threat to sericulture ecosystems. Chromium, especially in its hexavalent state Cr (VI), is a highly toxic heavy metal known to bioaccumulate in organisms and interferes with essential physiological processes. In sericulture, exposure to Cr (VI) through contaminated foliage can significantly impact the growth, developmental stages, survival rate, and cocoon quality of *A. mylitta,* thereby affecting silk yield and quality (Sinha *et al.,* 2006; Patra *et al.,* 2025).

Exposure to heavy metals like arsenic, lead, cadmium, and chromium significantly alters the morphological characteristics of silkworm (*Bombyx mori*). These metals disrupt normal growth and development by interfering with physiological processes at cellular level. Morphological deformities observed include reduced larval body size, shriveled or malformed cocoon structures, irregular spinning behavior, and delayed pupation. Histological examinations have revealed degeneration of silk glands and epidermal tissues in silkworms exposed to heavy metals, indicating a detrimental effect on silk production and quality (Sinha *et al.,* 2006).

Furthermore, bioaccumulation of these toxic elements may trigger oxidative stress, resulting in membrane damage and impaired metabolic function, which collectively compromise the structural integrity and overall morphology of the silkworm (Krishnaraj *et al.,* 2012). Preliminary studies suggest that ingestion of chromium-contaminated foliage may result in reduced larval weight, altered cocoon morphology, decreased silk yield, and increased mortality in silkworms. However, these observations are predominantly inferred from studies on *B. mori* and other Lepidopterans, with minimal direct investigation on tropical Tasar silkworm. The absence of comprehensive toxicological assessments on Tasar silkworm under chromium stress presents a significant research gap. The current study focuses on evaluating the toxic effects of hexavalent chromium exposure on *Antheraea mylitta* to safeguard Tasar sericulture, promote sustainable silk production, and protect biodiversity in forest ecosystems.

Materials and Methods

Collection of *A. mylitta*: The fifth-instar larvae of the Daba bivoltine ecorace of *A. mylitta* were selected for the experiments, as this stage is critical for silk protein synthesis. The larvae of this ecorace were collected from the Department of Sericulture, Baripada, Odisha, India and raised in the Zoology laboratory of KKS Women's College, Balasore. Rearing cages, nylon nets, wooden rods, scale, electronic weighing balance, and chromium (VI) ($K_2Cr_2O_7$) were used for conducting the experiment. The chemical used in this study was procured from Merck, New Delhi.

Experimental design for toxicity test on *A. mylitta*: Fifth-instar larvae of equal size were selected and divided into four groups of 10 larvae each. The control and experimental groups were exposed to Cr (VI) concentrations for 120 hr. Group-I control larvae (no chemical exposure), Group-II, Group-III, Group-IV larvae were exposed to lethal hexavalent chromium concentrations of 10, 100 and 1000 ppm, respectively. Fresh Arjun leaves were separately soaked for 15 min in different

concentrations of Cr (VI) ($K_2Cr_2O_7$) and air dried for 10 min. The treated Arjun leaves were fed to fifth-instar *A. mylitta* larvae. Larvae were monitored at 24, 48, 72, 96, and 120 hrs to determine the Lethal Concentration (LC_{50}) at 325 ppm. Death rates were recorded, and a sublethal dose of 162.5 ppm was used for morphological and biochemical studies.

Rearing of wild silkworm *A. mylitta*: The larvae were divided into four experimental groups along with the control, each group comprising 25 male and 25 female larvae. Fresh Arjun leaves (*T. arjuna*) were separately treated in different Cr (VI) concentrations. The fifth-instar *A. mylitta* larvae were exposed to treated Arjun leaves, and reared upto cocoon formation.

Sample preparation: After 10 days of completing the 5th instar stage, the larvae were collected and dissected. The procedures for sample preparation were carried out following the methodologies described by Nesa *et al.* (2022). Haemolymph was collected from the abdominal legs by puncturing and transferred into a pre-cooled sterile Eppendorf tube with 1 ml phenylthiourea. For pupae, haemolymph was obtained by puncturing the ventral intersegmental membrane with a sterile needle and stored on ice until analysis. Following the collection of haemolymph, the samples were centrifuged at 1000 rpm for 5 min at 4°C. Thereafter, the collected supernatant was preserved at -20°C. Tissue samples from silk gland and midgut were homogenized in ice-cold 50 mM phosphate buffer (pH 7.4). The homogenate was then centrifuged at 10,000 rpm for 10 min at 4°C, and the resulting supernatant were utilized for the subsequent biochemical analysis.

Morphometric assessment of *A. mylitta* larvae: Ten days after feeding, the weight, length, and coloration of 5th instar *A. mylitta* larvae were evaluated. Ten larvae per group were randomly selected for weight measurement using a digital monopan balance (Sartorius). Larval length was measured with a standard ruler and thread method.

Biochemical assessment of haemolymph, mid gut and silkgland: Biochemical indices, including proteins, were determined according to the procedure of Lowry *et al.* (1951), and carbohydrate levels were assessed by the anthrone reagent method (Seifter *et al.,* 1950). The concentrations of total lipids were evaluated by Vanillin-phosphoric acid reagent method (Knight *et al.,* 1972).These assessments were conducted in the haemolymph and different functional tissues, such as the midgut and silk gland, of the 5th instar larva and pupae of both male and female of the tasar silkworm (*A. mylitta*) for Cr(VI) treatments.

Statistical analysis: All the data were taken in four replicates. The arithmetic mean, standard deviation and standard error of means were determined through MS-Excel program. The difference between two sets of mean was compared with Student's t-test. The data were analyzed by One-way ANOVA and comparison of means of control and treated group was done by Duncans Multiple Range Test.

Results and Discussion

Exposure of *A. mylitta* larvae to Cr (VI) showed a clear dose and time-dependent rise in mortality. No deaths occurred in the control group. The highest concentration (1000 ppm) caused 20% mortality at 24 h, reaching 70% by 96 h. LC_{50} was 325 ppm, and the sublethal concentration was 162.5 ppm. Such mortality trends indicate that Cr (VI) exerts acute toxicity in tasar silkworms, possibly through oxidative stress, midgut epithelial damage, and interference with essential metabolic pathways (Rong *et al.,* 2023). Similar dose-dependent patterns have been reported in *B. mori* and other lepidopterans exposed to Cd, Pb, and Hg (Zhang *et al.,* 2021; Liu *et al.,* 2021; Wen *et al.,* 2024). According to Ali *et al.* (2021) higher the Cr (VI) amount in mulberry leaves, causes more toxicity to the *B. mori* population. At 300 mg l^{-1} concentration of Cr (VI) in *B. mori* resulted in poor digestive rate, reduced weight gain and significant larval mortality within a few days of exposure, indicating acute toxicity. Shoukat *et al.* (2014) also reported that Cr (VI) affects on *B. mori* life cycle.

Larval length and weight was recorded on day 1 and day 10 in both control and hexavalent chromium [Cr (VI)] exposed groups. In the control group, female larvae exhibited a significant ($P \leq 0.0001$) increase in length from 8.12±0.095 cm to 9.97±0.198 cm and weight 18.05±0.185 g to 22.56±0.175 g. In contrast, Cr (VI)-treated females showed insignificant growth (Fig.1b,2b). Similarly, Cr(VI)-exposed male larvae showed minimal growth in comparison to control, which significantly ($P \leq 0.0001$) grew from 7.64±0.211 cm to 9.86±0.132 cm in length and 17.27±0.107g to 20.92±0.379 g weight (Fig.1a, 2a). These findings indicate that exposure to Cr(VI) impairs normal larval growth in both sexes, with a more pronounced effect was observed in males. Al-Misned (2001) reported that, at a cadmium concentration of 400 µg g^{-1}, the males of *Chrysomyaal biceps* exhibited shorter larval and overall developmental periods compared to females. Similar observations were made by Moe *et al.* (2001), who found that male blowfly *Lucilia sericata* also had reduced larval development times relative to females under similar heavy metal exposure (Raise and Gemmellaro, 2024). Female silkworms exhibited greater sensitivity to heavy metals such as graphene oxide compared to males. At low concentrations, graphene oxide exposure led to a reduction in whole cocoon and shell weights in females, whereas higher concentrations primarily decreased shell weights in males (Xin *et al*., 2024). Chromium, especially in its hexavalent form, is known to produce reactive oxygen species, leading to cell damage, impaired nutrient assimilation, and growth retardation in aquatic organisms (Fergusson, 1990; Wong, 2012). Previous studies have shown that Cr(VI) can disrupt endocrine function and interfere with enzymatic pathways critical for growth and development in fish besides invertebrates (Wu *et al.,* 2016).

Treated larvae showed slower growth and blackening of abdominal prolegs by day 10, a sign of Cr(VI) toxicity not seen in control (Fig. 3). This discoloration may indicate tissue necrosis or stress responses like melanization, often elevated by heavy metal stress, including chromium toxicity (Suwalsky *et al.,* 1998).

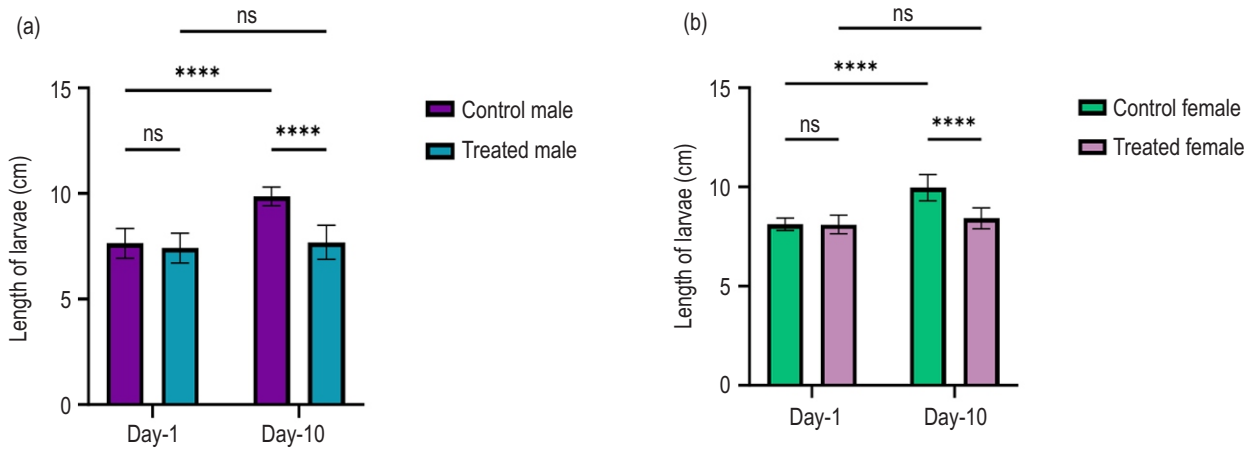

Fig. 1: Effect of Cr(VI) on the length of (a) male and (b) female *A. mylitta* larvae on day 1 and day 10. **** (P≤0.0001); ns (non significant).

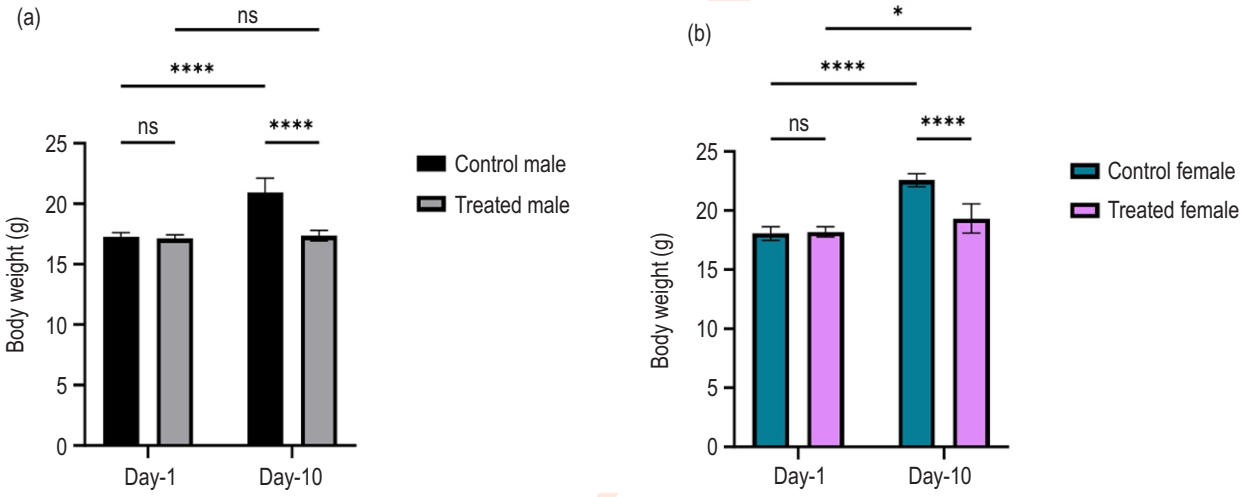

Fig. 2: Effect of Cr(VI) on weight (g) of (a) male and (b) female *A. mylitta* larvae on day 1 and 10. **** (P≤0.0001); ns (non significant).

The blackening of larval tissues may serve as an early sign of oxidative damage and cellular stress from Cr (VI) accumulation, which disrupts enzymatic and metabolic functions (Bagchi *et al.,* 2002). These changes, linked to growth retardation, highlight chromium's severe developmental impact on insect physiology. Chromium, a pervasive environmental contaminant, poses significant risks to insect physiology, notably affecting protein metabolism in economically vital species like silkworm, *B. mori*. It was found that the protein level in both larval and pupal haemolymph of *A. mylitta* declined significantly following exposure to Cr (VI), with differences observed between control and treated groups as well as between sexes. In female larvae, the protein content decreased from 19.604±0.272 mg ml^{-1} in control to 15.376±0.063 mg ml^{-1} following chromium exposures. Similarly, in pupae, levels significantly (P≤ 0.05) declined from 21.00±0.06 to 15.54±0.092 mg ml^{-1} in treated individuals (Table 1). In male larvae, the control group showed a protein content of 13.404±0.580 mg ml^{-1}, which decreased sharply to 9.404±0.232 mg ml^{-1} under Cr(VI) exposure. In pupae, the protein content dropped from 19.02±0.23 mg ml^{-1} in control to 11.733±0.14 mg m l^{-1} in treated samples. Statistical analysis indicated significant differences (P ≤ 0.05) in protein level among control and treated groups in both developmental stages. Similar observations have been reported in other invertebrate models, where heavy metal exposure led to altered haemolymph biochemistry (Patra *et al.,* 2011; Impellitteri *et al.*, 2022).

The decline could be attributed to protein denaturation, increased proteolysis, or impaired protein synthesis due to toxic effects of chromium ions (Shanker *et al.,* 2005). Chromium is

Fig. 3: Female larval morphology of *A. mylitta* compared with control (a) and Cr(VI) treatment (b), (c); control male larval morphology compared with Cr (VI) treatment (d), (e); control larval abdominal leg compared with Cr (VI) treatment (f) larval abdominal leg.

known to induce oxidative stress by generating reactive oxygen species (ROS), which can damage cellular proteins, lipids, and nucleic acids (Bagchi *et al.,* 2002). In silkworm, the fat body, a key organ for protein synthesis and storage, may be directly affected, leading to reduced export of proteins into the haemolymph. Interestingly, a few previous studies have also reported compensatory mechanisms where moderate metal stress can initially elevate certain stress-related proteins such as heat shock proteins or metallothioneins (Bian *et al.,* 2025). However, prolonged or high-concentration exposure typically overwhelms these protective responses, resulting in a net protein decrease, corroborates with the findings of this study. Su *et al.* (2024) reported at cadmium exposure levels of 3.2 and 51.2 mg kg^{-1}, the fecundity in the female-only stressed group was significantly lower than in the male-only stressed group. These findings highlight that Cr (VI) stress causes substantial depletion of protein reserves across developmental stages, with pupae showing more severe reductions than larvae, especially in males. The protein level in the silk gland and midgut tissues of *A. mylitta* larvae varied notable between control and Cr(VI) treated groups, with distinct differences observed between sexes and tissue types (Fig. 4a,4b). In control female larvae, the silk gland showed the highest protein content (44.459 ± 0.093 mg g^{-1}), while the midgut had 37.84 ± 0.752 mg g^{-1}. Under Cr (VI) exposure, these dropped to 27.382 ± 0.31 mg g^{-1} and 28.703 ± 0.052 mg g^{-1}, respectively. Male larvae showed a similar but lower trend.

The results showed significant differences ($P \leq 0.05$) between control and treated groups in both tissues and sexes. In silkworm midgut epithelium, essential for digestion, oxidative stress disrupts enzymes and proteins, impairing nutrient absorption. Metal-induced stress can also denature proteins and inhibit key metabolic enzymes (Wen *et al.*, 2024). In *B. mori*, oxidative stress disrupts the production of digestive enzymes and structural proteins required for larval growth. It degrades existing proteins and impairs protein synthesis machinery, including ribosomes, in midgut epithelial cells (Shi *et al.*, 2024). Chromium disrupts amino acid metabolism, limiting protein synthesis precursors (Mala and Vijila, 2017). The midgut of *B. mori,* essential for digestion and nutrient absorption, depends on balanced proteins (Shinbo *et al*., 1996). Heavy metals like chromium can also cause midgut cell apoptosis and necrosis,

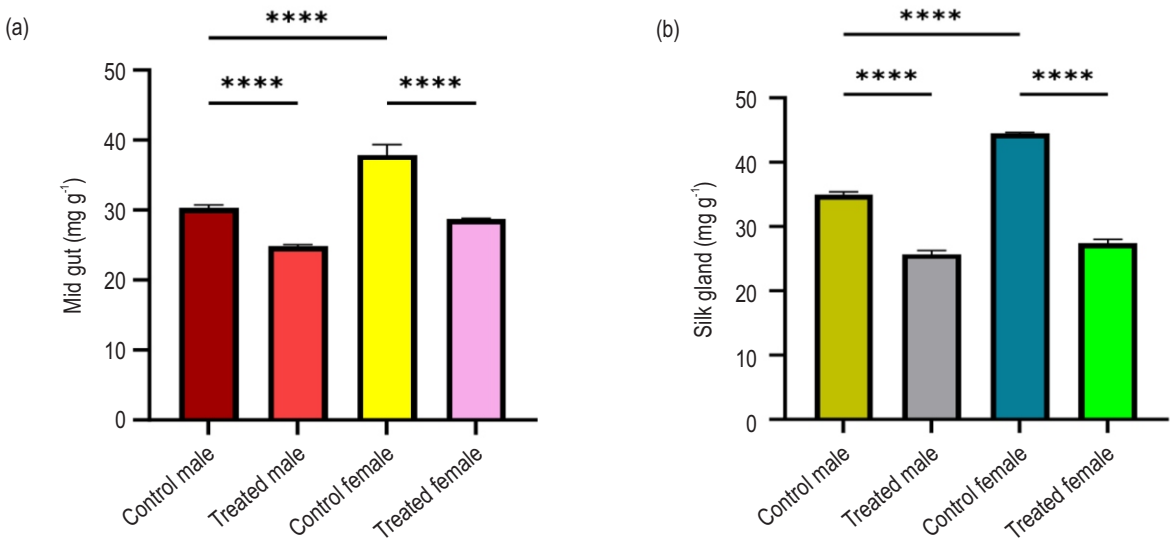

Fig. 4: Effect of Cr(VI) on total protein content in the (a) midgut tissues of *A. mylitta* and (b) silkgland tissues of *A. mylitta* .

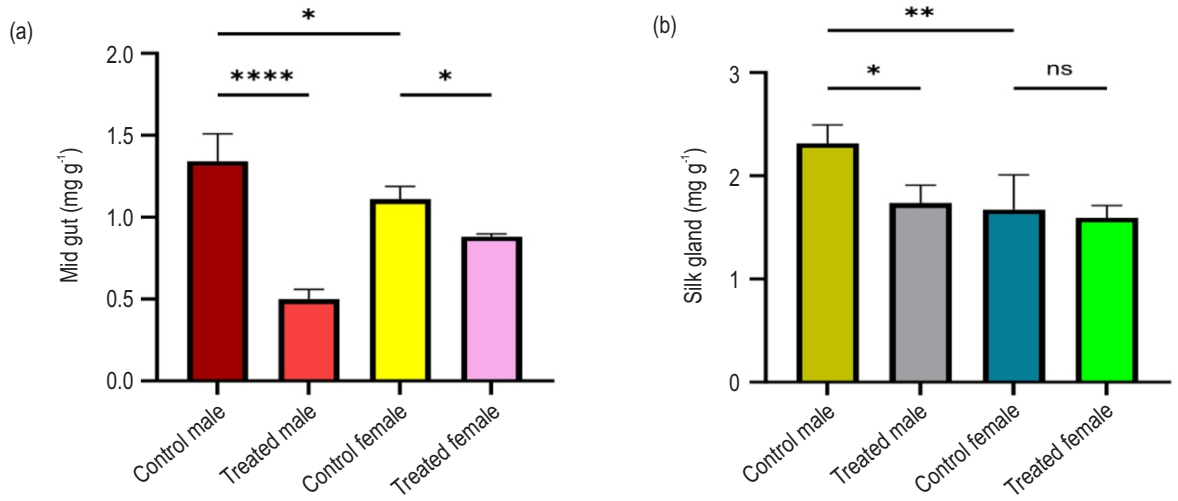

Fig. 5: Effect of Cr (VI) on carbohydrate content in the (a) midgut tissues of *A. mylitta* and (b) silk gland tissues of *A. mylitta*.

reducing tissue protein content (Rong *et al*., 2023; Yu *et al*., 2021). Midgut tissues show vacuolization, epithelial disruption, and nuclear pyknosis under heavy metal exposure (Fang *et al*., 2025). The midgut acts as a detox site, with metallothioneins and heat shock proteins up regulated to counter metal toxicity (Zhao *et al.,* 2022).

Two novel stress-responsive genes were identified, offering insights into silkworm digestion and stress adaptation to chromium (Rong *et al.,* 2023). However, chronic or high-dose exposure to chromium may counter the protective mechanisms, leading to cellular damage and reduced protein synthesis capacity. In the present study, chromium-treated groups exhibited a marked decline in haemolymph carbohydrates level compared to the control group. In female larvae, insignificant different level of carbohydrate content was noticed in control and Cr (VI) treated group (Table 1). Whereas a significant reduction ($P \leq 0.05$) was observed in the treated pupae group (0.514 ± 0.003 mg ml^{-1}) compared to the control group (0.67 ± 0.002 mg ml^{-1}) (Table 1). Among male larvae, the Cr (VI) treatment led a significant ($P \leq 0.05$) reduction to 0.498 ± 0.001 mg ml^{-1}. In male pupa, the

Table 1: Effect of of Cr(VI) on total protein, carbohydrate and lipid content of haemolymph of silkworm larvae and pupae of male and female *A. mylitta*

Haemolymph	Silkworm larvae		
	Protein (mg ml^{-1})	Carbohydrate (mg ml^{-1})	Lipid (mg ml^{-1})
Control Female	19.604±0.272****	0.672±0.000	1.835±0.019****
Treated Female	15.376±0.063	0.660±0.005	0.967±0.003
Control Male	13.404±0.580****	0.665±0.004****	0.875±0.004****
Treated Male	9.404±0.232	0.498±0.001	0.388±0.022
	Silkworm Pupae		
Control Female	21.00±0.23****	0.670±0.01****	2.258±0.141**
Treated Female	15.542±0.10	0.514±0.01	1.853±0.027
Control Male	19.02±0.23****	0.610±0.005****	1.506±0.003*
Treated Male	11.733±0.14	0.396±0.004	1.201±0.011

Each value represents the measurement from one larva, with four replicates, expressed as mean ± S.E. of the mean. Significance levels ****P<0.0001, **P<0.05, and *P<0.05

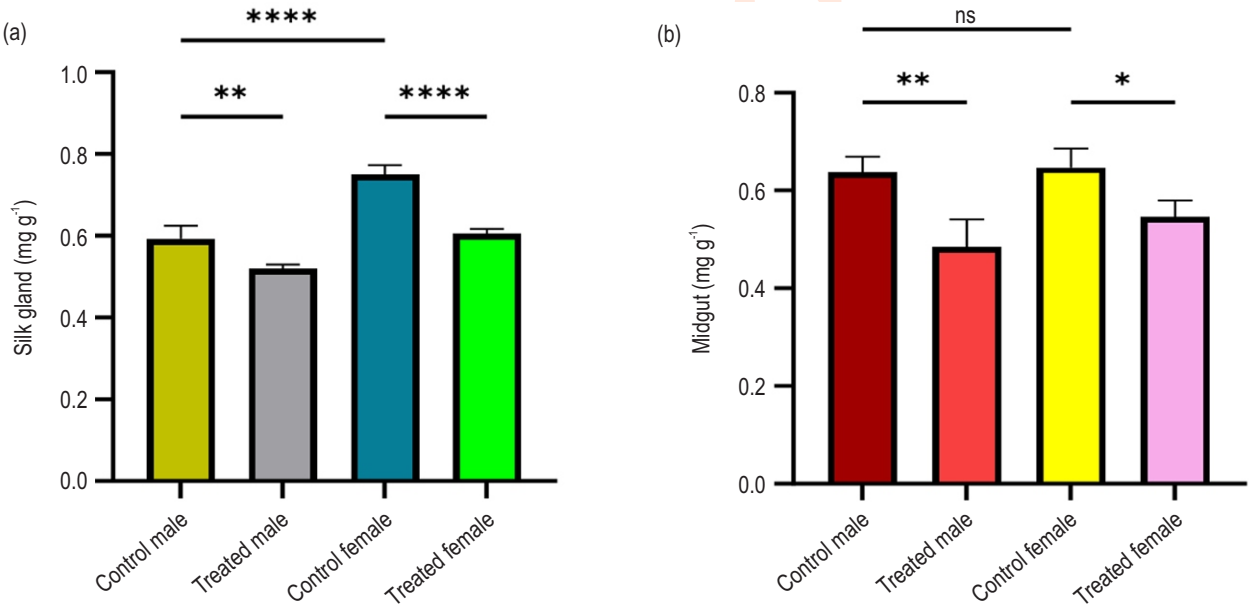

Fig. 6: Effect of Cr(VI) on total lipid content in (a) silk gland tissues of *A. mylitta* and (b) midgut tissues *A. mylitta*.

carbohydrate content dropped from 0.61± 0.005 mg ml^{-1} in control to 0.396± 0.004 mg ml^{-1} in treated individuals. Cr (VI) exposure reduced carbohydrate metabolism in *A. mylitta*, with the maximum decline in male pupae, indicating higher metabolic sensitivity during metamorphosis. Heavy metal stress decreases trehalose levels, aligning with reduced carbohydrate content in haemolymph (Bischof, 1995). Chromium toxicity may disrupt carbohydrate balance by hindering insulin-like peptide (ILP) activity, leading to altered glucose and trehalose levels, and affecting growth and development (Shi *et al*., 2021; Suljević *et al*., 2024).

Chromium (VI) exposure resulted in a noticeable reduction in carbohydrate content in the silk gland and midgut tissues of *A. mylitta* tissue with sex specific patterns. In control female larvae, the carbohydrate content was 1.673±0.167 mg g^{-1} in the silk gland and 1.11± 0.039 mg g^{-1} in the midgut. Following Cr (VI) treatment, these values significantly (P≤ 0.05) declined to 1.594±0.059 and 0.882±0.008 mg g^{-1} respectively. Carbohydrate levels in male larvae significantly (P≤0.05) decreased after Cr(VI) exposure (silk gland: 2.310± 0.091 to 1.734± 0.087 mg g^{-1}; mid gut: 1.315±0.194 to 0.503±0.073 mg g^{-1}) (Fig 5a, b). The midgut of the silkworm plays a crucial role in nutrient absorption and metabolism; thus, it is highly susceptible to toxic insults. Previous studies have documented similar effects of heavy metals on carbohydrate metabolism in insects and other invertebrates. Vijayavel (2007) reported a significant depletion of glycogen and soluble sugars in the tissues of *Perna viridis* exposed to chromium. In silkworms, heavy metals like cadmium and lead

have also been shown to disrupt carbohydrate homeostasis, leading to metabolic imbalance (Bian *et al.,* 2025; Jiang *et al.*, 2020). The present results are consistent with the previous studies indicating that sub-lethal doses of heavy metals can significantly alter intermediary metabolism in insects, potentially affecting growth, development, and silk production in *B. mori* (Rao and Smitha, 2011). The result indicates that Cr (VI) exposure significantly impairs carbohydrate metabolism, with a more pronounced effect observed in midgut tissues, particularly in male larvae. This suggests a higher metabolic vulnerability of male midgut tissue Cr (VI) toxicity.

In *A. mylitta*, the haemolymph lipid content of larvae and pupae exhibited marked reductions following Cr(VI) exposure, with distinct differences observed between control and treated groups, as well as between sexes. Lipids are fundamental to insect metabolic processes, including energy storage, membrane structure, and the synthesis of hormones essential for growth and development (Arrese and Soulages, 2010). In control female larvae, lipid the content was 1.835±0.019 mg ml^{-1} and 2.258±0.141 mg ml^{-1} in pupae. Whereas Cr(VI) exposure significantly decreased ($P \leq 0.05$) the lipid levels to 0.967±0.003 mg ml^{-1} in larvae and 1.853±0.027 mg ml^{-1} in pupae, respectively (Table 1). Similarly lipid content in male larvae and pupae significantly ($P \leq 0.05$) decreased from 0.875±0.004 to 0.388±0.022 mg ml^{-1} and 1.506±0.003 to 1.201±0.011 mg m l^{-1}, respectively, after Cr(VI) exposure (Table 1). The effect was more pronounced in male larvae, indicating a possible sex specific vulnerability to Cr (VI) induced metabolic stress. The comparatively smaller reduction on pupal lipid content suggests some level of metabolic adjustment or storage compensation occurring during metamorphosis. Previous studies corroborate these findings. Bian *et al.* (2025); Rong *et al*. (2023) reported that exposure to heavy metals like cadmium and lead in *B. mori* led to significant metabolic disturbances, including alterations in lipid profiles. Similarly, chromium exposure has been shown to affect lipid metabolism in other invertebrates, such as *Drosophila melanogaster* and aquatic insects, indicating a conserved toxicological impact across species (Lee *et al.*, 2018).

In female controls, the lipid concentrations were highest in the silk gland (0.749±0.012 mg g^{-1}) and moderately lower in the midgut (0.647±0.019 mg g^{-1}). Exposure to Cr(VI) elicited a significant reduction, with lipid levels decreasing significantly ($P \leq 0.05$) to 0.606±0.006 and 0.546±0.017 mg g^{-1} in the silk gland and midgut, respectively. In control male larvae, lipid levels were slightly higher in the midgut (0.637±0.016 mg g^{-1}) than in the silk gland (0.593±0.016 mg g^{-1}) (Fig. 6b). Cr(VI) exposure resulted in significant ($P \leq 0.0$) reductions, with levels decreasing to 0.519±0.005 mg g^{-1} in the silk gland and 0.485±0.028 mg g^{-1} in the midgut (Fig 6a). The observed reduction in lipid levels suggests a potential disruption of metabolic activity and energy storage mechanism in Cr (VI) exposed larvae.

The findings of this study demonstrates that sub-lethal Cr(VI) exposure under controlled laboratory conditions can be effectively correlated with contamination levels found in chromite mining regions. Previous reports by Das *et al.* (2013) reported chromium concentrations as high as 1715.85 mg kg^{-1} in Sukinda chromite mine soils, exceeding ecotoxicological threshold established by both the European Community Commission (Mushtaq and Khan, 2010) and Indian Standards (Awashthi, 2000). The observed effects in *A. mylitta* highlight the risk to non-target invertebrates in chromium-contaminated areas, with potential ecological and economic impacts. The sub-lethal doses used reflect real environmental exposure, offering insights into field-level effects.

The current study provides compelling evidence that Cr(VI) exposure induces significant alterations in both biochemical and morphological characteristics of *A. mylitta.* These changes adversely affect key developmental parameters and lead to the production of low-quality tasar silk. Consequently, the cultivation of tasar silk in industrial areas may be adversely affected, leading to the production of substandard tasar silk products. Further studies are essential to elucidate the chronic and transgenerational effects of Cr(VI) on *A. mylitta,* including physiological, biochemical, and reproductive endpoints. Such investigations would enhance the overall understanding of heavy metal ecotoxicology in Lepidopteran species and aid in formulating mitigation strategies for sericulture practices in mining-affected regions.

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References

Ali, M., M. Alamgeer, M.A. Qayyum, K. Zia, M. Ashfaq and M.A. Saleem: Transportation of Chromium (VI) to *Bombyx mori* L. from mulberry plant (*Morus alba* L.) grown at soil irrigated with Chromium (VI) containing effluents. *J. Sci. Agric.,* **5**, 36-43 (2021).

Al-Misned, F. A.: Biological effects of cadmium on life cycle parameters of *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae), Kuwait. *J. Sci. Eng.,* **28**, 179-188 (2001).

Arrese, E.L. and J.L. Soulages: Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.,* **55**, 207–225 (2010).

Awashthi, S.K.: Prevention of Food Adulteration Act no 37 of 1954. Central and State Rules as Amended for 1999, Ashoka Law House, New Delhi, 367 pages (2000).

Bagchi, D., S.J. Stohs, B.W. Downs, M. Bagchi and H.G. Preuss: Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology*, **180**, 5-22 (2002).

Bian, D. D., Y. X. Shi, K. W. Shi, H. C. Du, B. P. Tang and Q. N. Liu: Insights into lead toxicity and detoxification mechanisms in the silkworm, *Bombyx mori. Insects*, **16**, 699 (2025).

Bischof, C.: Effects of heavy metal stress on carbohydrate and lipid concentrations in the haemolymph and total body tissue of parasitized *Lymantria dispar* L. larvae (Lepidoptera). *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.,* **112**, 87-92 (1995).

Das, S., S.S. Ram, H.K. Sahu, D.S. Rao, A. Chakraborty, M. Sudarshan and H.N. Thatoi: A study on soil physico-chemical, microbial and metal content in Sukinda chromite mine of Odisha, India. *Environ. Earth Sci.,* **69**, 2487–2497 (2013).

Fang, Y., X. Li, F. Zhang, Y. Liu, L. Ma, X. Zeng and Q. Xie: Oxidative stress-mediated midgut damage induced by antimony tin oxide inhibits growth and development in *Bombyx mori., SSRN*, **27**, 5531657 (2025).

Fergusson, J.E.: The Heavy Elements: Chemistry, Environmental Impact and Health Effects. *Pergamon Press: Oxford, UK,* pp. 85–547 (1990).

Hossner, L.R., R.H. Loeppert, R.J. Newton and P.J. Szaniszlo: Literature review: phytoaccumulation of chromium, uranium, and plutonium in plant systems. Office of Scientific and Technical Information. GOV (U.S.) Page No. 20, 21 (1998).

Impellitteri, F., A. S. Curpăn, G. Plăvan, A. Ciobica and C. Faggio: Hemocytes: a useful tool for assessing the toxicity of microplastics, heavy metals, and pesticides on aquatic invertebrates. *Int. J. Environ. Res. Pub. Hlth.*, **19**, 16830 (2022).

Jaishankar, M., B.B. Mathew, M.S. Shah and K.R.S. Gowda: Biosorption of few heavy metal ions using agricultural wastes. *J. Environ. Poll. Human Hlth.,* **2**, 1-6 (2014).

Jiang, Y., S. Jiang, X. Yan, Z. Qin, C. Jia, Z. Li, J. Zhang and R. Huang: The mobility of cadmium and lead in the soil-mulberry-silkworm system. *Chemosphere*, **242**, 125179 (2020).

Kabata-Pendias, A. and A.B. Mukherjee: Trace elements of group 12 (Previously group IIb). *Trace Elements Soil Human,* pp. 283-319 (2007).

Knight, J.A., S. Anderson and J.M. Rawle: Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clin. Chem.,* **18**, 199-202 (1972).

Korshoj, L.E., A.J. Zaitouna and R.Y. Lai: Methylene blue-mediated electrocatalytic detection of hexavalent chromium. *Anal. Chem.,* **87**, 2560-2564 (2015).

Krishnaraj, C., E.G. Jagan, R. Ramachandran, S.M. Abirami, N. Mohan and P.T. Kalaichelvan: Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. *Process Biochem.,* **47**, 651-658 (2012).

Lee, M. C., J. C. Park and J. S. Lee: Effects of environmental stressors on lipid metabolism in aquatic invertebrates. *Aquat. Toxicol*., **200**, 83-92 (2018).

Liu, Y., C. Yang, L. Sun, A. Wang, X. Lan, W. Xu, Y. Liang, S. Ma and Q. Xia: In-depth transcriptome unveils the cadmium toxicology and a novel metallothionein in silkworm. *Chemosphere,* **273**, 128522 (2021).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Mala, N. and K. Vijila: Changes in the activity of digestive enzymes produced from the gut microflora of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) in response to fortification of mulberry leaves. *Int. J. Curr. Microbiol. App. Sci.*, **6**, 225-236 (2017).

Moe, S.J., N.C. Stenseth and R.H. Smith: Effects of a toxicant on population growth rates: Sublethal and delayed responses in blowfly populations. *Funct. Ecol.,* **15**, 712-721 (2001).

Mushtaq, N. and K.S. Khan: Heavy metals contamination of soils in response to waste water irrigation in Rawalpindi region. *Pak J Agr. Sci.,* **47**, 215–224 (2010).

Nesa, J., S.K. Jana, A. Sadat, K. Biswas, A. Kati, O. Kaya, R. Mondal, P. Dam, M. Thakur, A. Kumar and M. Hossain: Antimicrobial potential of a ponericin-like peptide isolated from *Bombyx mori* L. hemolymph in response to *Pseudomonas aeruginosa* infection. *Sci. Rep.*, **12**, 15493 (2022).

Patra, B., B. Baisakhi and J. Pradhan: Heavy metals and sericulture: Implications for industry sustainability. *Int. J. Ecol. Environ. Sci.,* **51**, 287-295 (2025).

Patra, R.C., A.K. Rautray and D. Swarup: Oxidative stress in lead and cadmium toxicity and its amelioration. *Vet. Med. Int.,* **2011**, 457327 (2011).

Raise, G. and M.D. Gemmellaro: A review on the effects of heavy metals on the development of blow flies (Diptera: Calliphoridae). *Wiley Interdisciplinary Reviews: Foren. Sci.,* **6**, e1503 (2024).

Rao, A. V. B. and S. Smitha: Alterations in the nutritional parameters of silkworm *Bombyx mori* L. on exposure to selenium. *Nat. Environ. Pollut. Technol.*, **10**, 559-562 (2011).

Rong, W., Y. Chen, J. Lu, S. Huang, L. Xin, D. Guan and X. Li: Effects of chromium exposure on the gene expression of the midgut in silkworms, *Bombyx mori. Genes,* **14**, 1616 (2023).

Seifter, S., S. Dayton, B. Novic and E. Muntwyler: The estimation of glycogen with the anthrone reagent. *J. Arch. Biochem.,* **25**, 191-200 (1950).

Shanker, A.K., C. Cervantes, H. Loza-Tavera and S. Avudainayagam: Chromium toxicity in plants. *Environ. Int.,* **31**, 739-753 (2005).

Shi, B., X. Tao, M. B. Betancor, J. Lu, D. R. Tocher, F. Meng, C. Figueiredo-Silva, Q. Zhou, L. Jiao and M. Jin: Dietary chromium modulates glucose homeostasis and induces oxidative stress in Pacific white shrimp (*Litopenaeus vannamei*). *Aquat. Toxicol.*, **240**, 105967 (2021).

Shi, Y.X., D. D. Bian, F. X. Chen, Y. Jin, Q. Jiang, Q. N. Liu, B. P. Tang and L. S. Dai: Effect of lead exposure on silkworm midgut: insights into oxidative gene expression, digestive function, and microbial community structure. *Process Saf. Environ. Prot.*, **190**, 481- 494 (2024).

Shinbo, H., K. Konno, C. Hirayama and K. Watanabe: Digestive sites of dietary proteins and absorptive sites of amino acids along the midgut of the silkworm, *Bombyx mori*. *J. Insec. Physiol*., **42**, 1129-1138 (1996).

Shoukat, M.A., S. Ashraf, N. Sial, M. Ali, Z. Iqbal, M.I. Shahzad and H.R. Chaudary: The effect of Cr (VI) on silk worm (*Bombyx mori*) fed on in vitro accumulated mulberry leaves. *Asian J. Agric. Biol.,* **2,** 119-128 (2014).

Sinha, S., A.K. Gupta, K. Bhatt, K. Pandey, U.N. Rai and K.P. Singh: Distribution of metals in the edible plants grown at Jajmau, Kanpur (India) receiving treated tannery wastewater: relation with physico-chemical properties of the soil. *Environ. Monit. Assess.,* **115**, 1-22 (2006).

Su, H., M. Wu, Y. Yang, Y. Deng, Y. Yang and Q. Sun: Tissue distribution of cadmium and its effect on reproduction in *Spodoptera exigua*. *J. Integr. Agric.,* **23**, 195-204 (2024).

Suljević, D., M. Fočak and A. Alijagic: Assessing chromium toxicity across aquatic and terrestrial environments: a cross-species review. *Drug Chem. Toxicol.*, **47**, 1312-1324 (2024).

Sundar, S. and J. Chakravarty: Antimony toxicity. *Int. J. Environ. Res. Pub. Hlth.,* **7**, 4267–4277(2010).

Suwalsky, M., B. Ungerer, L. Quevedo, F. Aguilar and C.P. Sotomayor: Cu^{2+} ions interact with cell membranes. *J. Inorg. Biochem.,* **70**, 233-238 (1998).

Tan, C., Z. Zeyu, X. Sai, W. Hongtao and L. Wenjing: Adsorption behavior comparison of trivalent and hexavalent chromium on biochar derived from municipal sludge. *Bioresour. Technol.,* **190**, 388- 394 (2015).

Tucker, F.B., K.X. Wang, S.L. Lu and L.J. Xu: Influence of form and quantity of chromium on the development and survival of two silkworm (*Bombyx mori* L.) races. *J. Environ. Sci. (China),* **15**, 744-748 (2003).

Vareda, J.P., A.J. Valente and L. Durães: Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review. *J. Environ. Manag.,* **246**, 101-118 (2019).

Vijayavel, K., S. Gopalakrishnan, A. Chezhian and M. P. Balasubramanian: Biochemical constituents and bioaccumulation as biomarkers in the green mussel *Perna viridis* with reference to silver and chromium toxicity. *Toxicol. Environ. Chem*., **89**, 353-361 (2007).

Wen, H., Y. Wang, Y. Ji, J. Chen, Y. Xiao, Q. Lu, C. Jiang, Q. Sheng, Z. Nie and Z. You: Effect of acute exposure of Hg on physiological parameters and transcriptome expression in silkworms (*Bombyx mori*). *Front. Vet. Sci.,* **11**, 1405541(2024).

Wong, M.H.: Environmental Contamination: Health Risks and Ecological Restoration. CRC Press: Boca Raton, FL, USA, pp. 193-208 (2012).

Wu, M., Y. Xu, W. Ding, Y. Li and H. Xu: Mycoremediation of manganese and phenanthrene by *Pleurotus eryngii* mycelium enhanced by Tween 80 and saponin. *Appl. Microbiol. Biotechnol.,* **100**, 7249–7261 (2016).

Xin, Y., J. Liang, C. Ren, W. Song, B. Huang, Y. Liu and S. Zhang: Physiological and transcriptomic responses of silkworms to graphene oxide exposure. *Ecotoxicol. Environ. Saf.,* **278**, 116434 (2024).

Yu, Z., S. F. Xu, J. L. Zhao, L. Zhao, A. Z. Zhang and M. Y. Li: Toxic effects of hexavalent chromium (Cr^{6+}) on bioaccumulation, apoptosis, oxidative damage and inflammatory response in *Channa asiatica*. *Environ. Toxicol. Pharmacol*, **87**, 103725 (2021).

Zhang, C.F., Y.X. Liu, L.F. He, F.J. Shi, W.T. Yao and X.G. Luo: Effects of uranium on the antioxidant responses of Chinese oak silkworm, *Antheraea pernyi*. *Pakistan J. Zool.,* **54**, 339-346 (2021).

Zhao, L., W. Zhang, X. Xue and H. Wang: Molecular responses of insects to heavy metals: Insights into detoxification and tolerance mechanisms. *J. Hazard. Mater.,* **424**, 127370 (2022).