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Silkworms as bioindicators for assessing environmental toxicity and ecological risks

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Abstract

Environmental toxicity assessment is crucial for understanding the risks that pollutants pose to ecosystems. Traditional testing methods often rely on higher organisms like rodents and fish, which can be costly, time-consuming and raise ethical concerns. Silkworms (*Bombyx mori* L.) provide an effective, ethical and affordable alternative for environmental toxicity testing. With a short life cycle and well-understood biology, silkworms are highly sensitive to a range of pollutants, including heavy metals, pesticides and nanomaterials, making them valuable for Ecotoxicological studies. The advantages of using silkworms in toxicity testing, such as their rapid reproduction, ease of maintenance and ability to produce large datasets. Silkworms show clear biochemical and physiological responses to toxic substances, including changes in enzyme activity, oxidative stress markers and reproductive health. These responses offer important insights into the mechanisms of toxicity and help identify biomarkers for environmental monitoring. The use of silkworms in detecting pollutants in aquatic, terrestrial and atmospheric environments. Silkworms are particularly useful in high-throughput screening, bio monitoring and predictive modeling, which allow researchers to evaluate the long-term effects of contaminants. In conclusion, silkworms are an effective and ethical tool for ecological risk assessments, offering a scalable method for identifying environmental hazards and supporting more informed environmental management and policy decisions.

Keywords: Silkworms, toxicity testing, pollutants, biomarkers, ecotoxicology, bio monitoring, environment

Introduction

Environmental toxicity assessment plays an essential role in safeguarding ecosystems by understanding the harmful effects of pollutants and chemicals. These assessments are critical for maintaining the health of aquatic, terrestrial and atmospheric ecosystems, which are increasingly exposed to harmful substances due to human activities. Pollutants such as industrial waste, agricultural runoff and airborne toxins can severely impact living organisms, disrupting entire ecosystems (Singh *et al.*, 2021) [15]. This process involves evaluating the adverse effects of toxic substances on organisms at different levels of the food chain, starting from primary producers like plants to apex predators. Such comprehensive evaluations help identify risks, implement regulatory measures and develop strategies for environmental protection (Zhao *et al.*, 2021) [21].

Traditionally, environmental risk assessments have heavily relied on vertebrate models like rodents, fish and amphibians. These organisms provide valuable insights into toxicity levels and the potential ecological impacts of pollutants. However, the use of vertebrates is associated with several limitations. Firstly, these methods are resource-intensive, requiring significant financial investment and long testing durations. Secondly, there are ethical concerns

surrounding the use of higher animals for testing purposes, leading to increasing pressure from animal rights organizations and stricter regulatory frameworks (Zafar *et al.*, 2019; Makhija *et al.*, 2020) [19, 12]. Finally, reproducibility and scalability remain major challenges, particularly when dealing with a large number of pollutants or conducting high-throughput studies.

In recent years, researchers have sought alternatives to traditional models for environmental toxicity assessments. One such promising alternative is the use of *Bombyx mori*, commonly known as the silkworm (Benelli, 2018; Braeckman *et al.*, 1997) [2, 3]. Silkworms offer numerous advantages over vertebrate models. They are invertebrates, which eliminates ethical concerns associated with vertebrate testing. Additionally, silkworms have a well-documented biological and genetic framework, a short life cycle and are relatively easy and inexpensive to maintain (Benelli, 2018; Abdelli *et al.*, 2018) [2, 1]. These characteristics make silkworms an attractive option for researchers aiming to develop large-scale, cost-effective toxicity screening methods.

Silkworms are highly sensitive to a variety of environmental pollutants, including heavy metals, pesticides and nanomaterials. By studying their responses, researchers can

gain valuable insights into the effects of these substances on cellular, physiological and reproductive processes. For instance, exposure to heavy metals can lead to oxidative stress and cellular damage in silkworms, providing a model to study similar impacts on other organisms (Chen *et al.*, 2016) [4]. Similarly, the toxic effects of pesticides and nanomaterials on silkworm development and survival have been well-documented, showcasing their potential as bioindicators of environmental pollution (Du *et al.*, 2021) [6]. Silkworms offer several distinct advantages as a model for environmental toxicity assessment. One key benefit is their short life cycle, typically lasting 30 to 50 days, which enables researchers to study both acute and chronic toxic effects within a short timeframe (Benelli, 2018; Abdelli *et al.*, 2018; Gong *et al.*, 2016) [2, 1]. Their high reproductive rate further enhances their utility, as they produce large numbers of offspring, making high-throughput screening possible and allowing researchers to collect statistically significant data efficiently (Kaur *et al.*, 2020 [9]; Pan *et al.*, 2019). Moreover, their ability to reproduce quickly under laboratory conditions facilitates repeated testing across generations, providing insights into the potential long-term and generational effects of pollutants (Sun *et al.*, 2021).

Another major advantage is the silkworm's well-characterized genetics and physiological responses. With a fully sequenced genome, silkworms provide a robust platform for studying the molecular mechanisms underlying their responses to environmental pollutants (Yamamoto *et al.*, 2020; Xia *et al.*, 2014). Their physiological reactions to stressors such as changes in growth rates, feeding behavior, and metabolic function—make them an ideal model for understanding the cellular and biochemical impacts of toxic exposure (Benelli, 2018; Li *et al.*, 2018) [2]. For example, pollutants have been shown to significantly affect silkworm development and behavior, offering valuable insights into the broader ecological impacts of environmental stressors (Zafar *et al.*, 2019; Li *et al.*, 2018; Wang *et al.*, 2020) [19].

Silkworms are easy to maintain and ethically suitable. They thrive under simple laboratory conditions, without requiring specialized equipment or facilities, making them accessible to researchers worldwide (Kumar *et al.*, 2021) [15]. As invertebrates, silkworms bypass many ethical concerns associated with vertebrate testing, aligning with the growing scientific emphasis on humane research practices (Zafar *et al.*, 2019; Zhijian *et al.*, 2017; Zhao *et al.*, 2021) [21, 19, 22]. This ethical advantage, combined with their low maintenance requirements, underscores their suitability as an alternative to vertebrate models in toxicity assessments (Zhao *et al.*, 2021; Singh *et al.*, 2022) [15, 21].

The use of silkworms in environmental toxicity assessments. It delves into the mechanisms underlying their responses to various pollutants and discusses their broader applications in risk assessment. Additionally, the paper presents case studies where silkworms have been successfully employed to detect specific contaminants, such as heavy metals, pesticides and nanomaterials. These examples demonstrate the practical utility of silkworms in ecotoxicological research and underscore their potential to complement or replace traditional testing models. Silkworms represent a promising alternative for environmental toxicity studies, combining ethical compliance with cost-effectiveness and scientific relevance.

By leveraging their unique attributes, researchers can develop more efficient and sustainable approaches to assess the risks posed by environmental pollutants. This shift not only addresses the limitations of traditional methods but also opens new avenues for understanding and mitigating the impacts of pollution on global ecosystems.

Toxicity Assessment of Different Pollutants Using Silkworms: Heavy Metals: Heavy metals like cadmium, lead, and mercury are prominent environmental pollutants known for their toxicity to living organisms (Fan *et al.*, 2018) [7]. Silkworms are sensitive to these metals, which interfere with their growth, survival, and biochemical processes. Studies have shown that cadmium chloride exposure leads to oxidative stress in silkworms by altering enzyme activities, such as catalase and superoxide dismutase (Makhija *et al.*, 2020) [12]. Cadmium also disrupts silkworm growth and development, leading to delayed molting and smaller pupae, making silkworms valuable in assessing heavy metal toxicity and ecological risk (Tian *et al.*, 2021; Zhao *et al.*, 2021) [21]. Similarly, mercury and lead exposure in silkworms has been shown to reduce larval weight and lower reproductive success, further underscoring the adverse effects of heavy metals on population dynamics (Chen *et al.*, 2018; Wang *et al.*, 2020) [4].

Nanoparticles

Nanomaterials, especially nanoparticles, are increasingly used in various industries but raise concerns due to their persistence in the environment (Chen *et al.*, 2016) [4]. Silkworms serve as a sensitive model for assessing nanoparticle toxicity, as studies show that exposure to nanoparticles like graphene oxide and titanium dioxide results in oxidative stress and damage to cellular structures (Li *et al.*, 2018). These nanoparticles induce malformations, such as smaller body size and disrupted metamorphosis, indicating their potential harm to non-target organisms (Wu *et al.*, 2021). Further research shows that nanoparticles may accumulate in silkworm tissues, affecting key physiological functions and indicating the possible bioaccumulation risks of these materials in ecosystems (Zhang *et al.*, 2022; Shi *et al.*, 2019) [20].

Pesticides

Pesticides are widely used in agriculture, raising concerns over their effects on non-target species like silkworms (Du *et al.*, 2021) [6]. Silkworms exhibit high sensitivity to pesticides, making them a useful model for studying insecticide, herbicide and fungicide toxicity (Dawkar *et al.*, 2013) [5]. For instance, exposure to chlorpyrifos and cypermethrin has been linked to inhibited growth, lower reproductive success, and altered enzyme activities, including acetylcholinesterase inhibition, a marker of neurotoxicity (Zafar *et al.*, 2019) [19]. Long-term pesticide exposure has also been shown to decrease egg hatchability and larval survival in silkworms, indicating the severe ecological impacts of these chemicals (Kaur *et al.*, 2020; Tian *et al.*, 2021) [9]. Additionally, pesticides induce oxidative stress and damage cellular structures, with recent studies suggesting that pesticides may disrupt endocrine functions in silkworms, further emphasizing the need for safer pesticide alternatives (Zhang *et al.*, 2020; Lee *et al.*, 2019) [20].

Mechanism of Action of Toxicity Assessment in Silkworms: *B. mori*, exhibit a range of biochemical and physiological responses when exposed to environmental toxins. These responses can be used to identify the mechanisms of toxicity and assess the potential ecological risks posed by pollutants. Understanding the mechanisms of action through which toxicants affect silkworms helps

researchers identify specific biomarkers, predict environmental impacts and determine safe exposure levels. Below is an expanded section on the mechanisms of toxicity in silkworms, based on their responses to different environmental stressors such as chemicals, metals and nanoparticles.

Table 1: Mode of action of toxicity assessment of silkworm

Mechanism	Description	Examples/Case Studies
1. Biochemical Markers of Toxicity	Biochemical changes in silkworms reveal how toxicants interfere with cellular and metabolic functions.	Enzyme Activity Changes: Exposure to cadmium chloride disrupted catalase (CAT) and superoxide dismutase (SOD) activities, leading to oxidative stress in silkworms (Makhija <i>et al.</i> , 2020) ^[12a] .
		AChE Inhibition: Organophosphate pesticide chlorpyrifos caused significant inhibition of acetylcholinesterase activity, impairing neural function and development (Du <i>et al.</i> , 2021) ^[6] .
		Heavy Metals: Cadmium exposure led to altered enzyme activity, oxidative stress and delayed molting in silkworms, underscoring its ecological risks (Zhao <i>et al.</i> , 2021) ^[21] .
2. Oxidative Stress and Antioxidant Defense	Toxicants generate reactive oxygen species (ROS) leading to oxidative stress, prompting silkworms to activate antioxidant defenses.	Nanoparticle Toxicity: Graphene oxide nanoparticles increased ROS production, disrupting mitochondrial functions and elevating MDA levels, indicating oxidative damage in silkworm tissues (Wu <i>et al.</i> , 2021).
		Heavy Metals: Mercury exposure caused lipid peroxidation, protein oxidation, and DNA damage in silkworms, with increased malondialdehyde (MDA) levels serving as a biomarker for cellular damage (Wang <i>et al.</i> , 2020).
		Antioxidant Enzyme Activation: Silkworms exposed to lead showed upregulated SOD and CAT activity as a defense mechanism against ROS, though prolonged exposure reduced enzyme efficiency (Chen <i>et al.</i> , 2018) ^[4] .
		Pesticides: Cypermethrin induced oxidative stress in silkworms, significantly increasing MDA levels and disrupting mitochondrial functions (Lee <i>et al.</i> , 2019).
3. Development and Reproduction	Toxicants disrupt growth and reproductive functions, providing critical data on environmental impacts.	Developmental Delays: Cadmium exposure caused smaller pupae, delayed molting and abnormal cocoon formation, highlighting its developmental toxicity (Tian <i>et al.</i> , 2021).
		Reproductive Success: Mercury exposure reduced egg viability and larval survival rates, indicating potential impacts on silkworm population sustainability (Chen <i>et al.</i> , 2018) ^[4] .
		Nanoparticles: Titanium dioxide nanoparticles caused developmental malformations and reduced pupal weight, affecting silkworm metamorphosis and fitness (Shi <i>et al.</i> , 2019).
		Pesticides: Chlorpyrifos reduced fecundity and egg hatchability in silkworms, with decreased larval survival rates indicating long-term ecological impacts (Dawkar <i>et al.</i> , 2013; Kaur <i>et al.</i> , 2020) ^[9,5] .
4. Genetic and Epigenetic Responses	Toxicants induce changes in gene expression and epigenetic modifications, revealing molecular toxicity mechanisms.	Gene Expression Changes: Exposure to heavy metals like cadmium upregulated metallothionein genes, which are involved in metal detoxification and stress response (Li <i>et al.</i> , 2018).
		Epigenetic Changes: Pesticides like cypermethrin altered DNA methylation patterns in silkworms, potentially leading to heritable changes in gene expression (Johansson & Borg, 1988).
		Nanoparticles: Silkworms exposed to graphene oxide showed altered expression of stress-related genes and potential changes in histone modifications (Zhang <i>et al.</i> , 2022) ^[22] .
5. Cellular and Organismal Toxicity	Toxicants cause damage to organelles and disrupt cellular stability, leading to physiological impairments.	Lysosomal Integrity: Cadmium disrupted lysosomal membrane stability, leading to enzyme leakage and increased apoptosis in silkworm cells (Braeckman <i>et al.</i> , 1997) ^[3] .
		Mitochondrial Dysfunction: Nanoparticles like TiO ₂ and ZnO disrupted mitochondrial membrane potential, reducing ATP production and triggering oxidative stress in silkworm tissues (Chen <i>et al.</i> , 2016; Zhang <i>et al.</i> , 2022) ^[4,20] .
		Cell Membrane Damage: Arsenic exposure increased ion leakage from silkworm cells, impairing membrane function and overall cellular stability (Zhao <i>et al.</i> , 2021) ^[21] .

Applications of Silkworms in Eco-toxicological Risk Assessment: *B. mori* have emerged as valuable tools in ecotoxicological risk assessment due to their ability to respond to environmental pollutants in ways that are both quantifiable and reproducible. Their use has been integrated into environmental monitoring programs and toxicity

testing, aiding in the detection and evaluation of pollutants across various ecosystems. These organisms are particularly effective in studying pollutants such as heavy metals, pesticides, pharmaceuticals and nanomaterials, offering a wide range of applications that benefit both regulatory and ecological risk assessments.

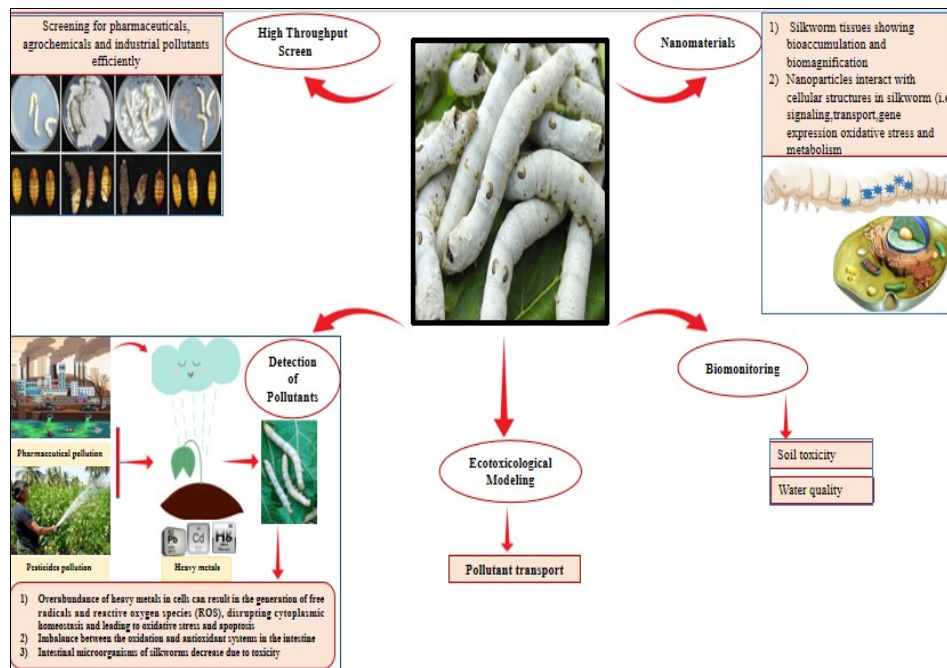


Fig 1: Visual representation of applications of silkworms in ecotoxicological risk assessment

Detection of Pollutants in Aquatic and Terrestrial Environments: Silkworms are sensitive to a variety of environmental contaminants, making them ideal candidates for use in pollution detection across both aquatic and terrestrial ecosystems. Given their biological relevance, silkworms can help assess the bioavailability and toxicity of pollutants in different ecological compartments, such as soil, water, and air.

Silkworms as Indicators of Environmental Pollution Ramasamy *et al.* (2020) ^[14] conducted a comprehensive review on the role of *Bombyx mori* in evaluating environmental pollution. The study consolidated evidence of silkworms’ ability to bioaccumulate pollutants and exhibit measurable physiological and molecular changes in response to contamination. It underscored their use in assessing a wide range of environmental pollutants, including heavy metals, pesticides, and organic chemicals. The review advocated for integrating silkworm-based models in ecological risk assessments due to their environmental relevance, ease of maintenance and high sensitivity to contaminants, which can provide actionable insights for pollution management.

Heavy Metals: Heavy Metal Toxicity and Biochemical Responses in Silkworms: Varela *et al.* (2020) ^[17] investigated the biochemical responses of silkworms to heavy metal contamination. Their findings demonstrated that exposure to heavy metals, including cadmium and lead, caused significant oxidative stress and disruptions in metabolic processes within silkworms. The study highlighted the potential of silkworms as bioindicators for heavy metal pollution due to their pronounced biochemical changes and measurable toxicological responses. These findings contribute to the growing body of evidence supporting the use of silkworms in ecological and environmental safety assessments. Silkworms have been used to detect heavy metal

contamination, such as lead (Pb), cadmium (Cd) and mercury (Hg), in water and soil. Research has demonstrated their sensitivity to metal exposure, as indicated by changes in growth, development, enzyme activity and reproduction (Fan *et al.*, 2018) ^[7]. By assessing the response of silkworms to these metals, researchers can establish the environmental threshold levels of contamination and predict the impact on non-target organisms in the ecosystem.

Pesticides and Herbicides: Silkworms have shown to be particularly sensitive to pesticides and herbicides used in agriculture. Bioassays involving silkworms have been used to evaluate the toxicological effects of common pesticides like chlorpyrifos, imidacloprid and glyphosate. These chemicals often have sub-lethal effects, such as impaired growth or altered reproduction, which can affect the long-term viability of populations (Zafar *et al.*, 2019; Dawkar *et al.*, 2013) ^[19, 5]. Silkworms can help assess the impact of pesticide runoff in agricultural landscapes and its potential spread into non-target organisms within adjacent ecosystems.

Fungicide Toxicity in Silkworms: Jiang *et al.* (2020) evaluated the toxicological effects of fungicides on silkworm growth and reproduction. Their research identified significant disruptions in developmental processes and reproductive success due to fungicide exposure. The study revealed that silkworms exhibited alterations in enzyme activities and oxidative stress levels, making them a valuable model for assessing the ecological risks of fungicides. The findings provided a foundation for further research into the mechanisms of fungicide toxicity and the development of safer pest control methods.

Use of Silkworms for Assessing Organic Pollutant Toxicity: The potential of silkworms (*Bombyx mori*) as a model organism for evaluating the toxicity of organic pollutants. Their study highlighted the sensitivity of

silkworms to various chemical contaminants, emphasizing their suitability as an alternative to traditional mammalian models in toxicity testing. By assessing physiological and biochemical responses, the study provided a comprehensive understanding of how organic pollutants affect silkworms, thus offering a cost-effective and ethical approach for environmental monitoring. The findings also underscored the applicability of silkworm-based bioassays for assessing risks associated with exposure to hazardous organic compounds (Park *et al.* 2019).

Pharmaceuticals and Personal Care Products (PPCPs): The rising concern about the environmental impact of pharmaceuticals and personal care products (PPCPs) has led to investigations into how silkworms can be used to detect their presence in the environment. Silkworm bioassays have shown promise in detecting contaminants such as antibiotics, anti-inflammatory drugs and other chemicals commonly found in water sources. By observing changes in behavior, growth, and reproduction, silkworms can offer insights into the long-term ecological impacts of PPCPs on wildlife and humans (Braeckman *et al.*, 1997) ^[3].

Understanding the Impact of Nanomaterials on Ecosystems: Nanomaterials, due to their increasing use in industry, consumer products, and agriculture, present a growing challenge for environmental risk assessment. Their unique properties, such as small size and high reactivity, allow them to interact with biological systems in ways that are not fully understood, posing potential risks to both human health and the environment (Chen *et al.*, 2016) ^[4]. Silkworms have become invaluable in studying the ecological impacts of nanomaterials, particularly in relation to toxicity.

Nanoparticles in Pollution: The toxicity of nanoparticles, such as titanium dioxide (TiO₂), carbon-based nanoparticles and silver nanoparticles, has been assessed using silkworms. These nanoparticles can cause oxidative stress, DNA damage and disruption of development in silkworms. Studies have shown that exposure to nanoparticles leads to a reduction in growth, increased mortality and reproductive toxicity, making silkworms a critical tool in understanding how these materials interact with the environment (Li *et al.*, 2018). Silkworms can be used to evaluate the potential risks of nanoparticles when released into the environment through industrial discharge or agricultural runoff.

Bioaccumulation and Biomagnification: Silkworms are also used to study bioaccumulation and biomagnification processes. Due to their ability to ingest contaminated particles, silkworms serve as indicators of how nanomaterials may accumulate in organisms at different trophic levels. Understanding how nanoparticles accumulate in silkworm tissues can offer valuable insights into the transfer of contaminants through food webs and their potential to reach higher organisms, including humans.

Biomonitoring of Pollutants in Ecological Risk Assessment: Silkworms are increasingly being incorporated into biomonitoring programs to assess the health of ecosystems in regions exposed to environmental pollutants.

Biomonitoring involves the continuous or periodic collection of data from biological indicators, such as silkworms, to track the presence and impact of pollutants.

Silkworm-Based Bioassays for Water Pollutants: Lin *et al.* (2021) examined the potential of silkworm-based bioassays for the risk assessment of water pollutants. The study evaluated how waterborne contaminants impacted silkworms at various developmental stages, revealing their significant sensitivity to pollutants. The authors discussed how specific biochemical markers in silkworms, such as oxidative stress and enzyme activity, could serve as reliable indicators of water quality. This research emphasized the applicability of silkworm bioassays in real-world scenarios, particularly in monitoring and mitigating the effects of water pollution on ecosystems.

Water Quality Assessment: Silkworms are commonly used to monitor water quality, especially for the detection of pollutants in freshwater systems. For instance, bioassays using silkworm larvae have been employed to assess the toxicity of industrial effluents and wastewater from mining activities. By measuring the survival rates, growth and developmental stages of silkworms exposed to these water samples, researchers can identify potentially hazardous substances and gauge the overall health of aquatic ecosystems (Song *et al.*, 2021) ^[16].

Soil and Sediment Toxicity: Silkworms can be used to assess soil and sediment quality by conducting bioassays in which they are exposed to contaminated substrates. Studies have shown that exposure to polluted soils can lead to changes in silkworm behavior and health, including altered feeding patterns, developmental delays and reproductive impairment. These assays are particularly valuable in evaluating the long-term effects of persistent pollutants, such as heavy metals and pesticides, in the soil environment (Zhao *et al.*, 2021) ^[21].

High-Throughput Screening for Toxicity Assessment
One of the greatest advantages of using silkworms in ecotoxicological risk assessment is their suitability for high-throughput screening. Due to their rapid life cycle, large reproductive output and ease of maintenance, silkworms are ideal for testing large numbers of compounds in a relatively short amount of time. This high-throughput capability makes them particularly useful for large-scale environmental monitoring programs that need to assess the impact of various pollutants on ecosystems quickly and cost-effectively.

Pharmaceuticals, Agrochemicals and Industrial Chemicals: High-throughput silkworm assays have been employed to screen various industrial chemicals, pharmaceuticals and agrochemicals for their toxic effects. By exposing silkworm larvae to different concentrations of these chemicals and observing changes in survival, growth and behavior, researchers can identify the most hazardous substances and evaluate their potential environmental risks. This approach has been successfully used to screen pesticide formulations and herbicides that may be used in large quantities, offering valuable insights for regulatory agencies and policymakers.

Ecotoxicological Databases: High-throughput silkworm bioassays can also contribute to the creation of ecotoxicological databases that provide critical information for risk assessments. These databases can help regulatory bodies set safe exposure limits for chemicals and pollutants, protecting environmental health and biodiversity. Furthermore, these databases can be used to identify emerging contaminants that may pose a risk to ecosystems but have not yet been widely studied (Makhija *et al.*, 2020) ^[12].

Silkworms in Ecotoxicological Modeling: In addition to their direct applications in toxicity testing, silkworms can also be used to develop and validate ecotoxicological models. These models can predict the behavior and fate of pollutants in ecosystems, helping to assess the potential long-term impacts of contaminants.

Modeling Pollutant Transport

Silkworms can be integrated into models that simulate the transport and fate of pollutants in ecosystems. These models can predict how pollutants move through food webs, how they accumulate in organisms and how they affect biodiversity. By incorporating silkworm toxicity data into such models, researchers can develop more accurate risk assessments for complex environmental scenarios (Zhao *et al.*, 2021) ^[21].

Predictive Toxicity Modeling

Silkworm-based assays have been used to build predictive models for toxicity based on various environmental factors, including exposure duration, pollutant concentration and the age and developmental stage of the silkworm. These models can be applied to predict the risk of environmental contamination in new regions and forecast the potential ecological damage of pollution events before they occur.

Table 2: Significant Studies Related to Pollution Detection and Risk Assessment Using Silkworms

S. No.	Application	Pollutants/Focus	Key Findings	References
1.	Detection of Pollutants in Aquatic and Terrestrial Environments	Heavy Metals	Silkworms exposed to lead (Pb), cadmium (Cd) and mercury (Hg) show growth inhibition, altered enzyme activities (CAT, SOD) and reproductive toxicity, providing thresholds for environmental contamination in water and soil.	Fan <i>et al.</i> , 2018; Zhao <i>et al.</i> , 2021 ^[21, 7]
		Pesticides and Herbicides	Chlorpyrifos and imidacloprid exposure impaired silkworm growth, reproduction, and enzyme activity (e.g., acetylcholinesterase), highlighting their sensitivity to agricultural runoff.	Dawkar <i>et al.</i> , 2013; Zafar <i>et al.</i> , 2019 ^[19, 5]
		Pharmaceuticals and Personal Care Products	Silkworms detect PPCPs such as antibiotics and anti-inflammatory drugs in water by exhibiting behavioral and developmental changes, providing insights into their long-term ecological effects.	Braeckman <i>et al.</i> , 1997 ^[3]
2.	Understanding the Impact of Nanomaterials on Ecosystems	Nanoparticles	Titanium dioxide (TiO ₂) and graphene oxide nanoparticles cause oxidative stress, DNA damage, and developmental malformations in silkworms, with effects such as reduced body size and mortality, indicating nanoparticle toxicity and bioaccumulation risks.	Chen <i>et al.</i> , 2016 ^[4] ; Li <i>et al.</i> , 2018
		Bioaccumulation	Silkworms accumulate nanoparticles, providing insights into trophic transfer and biomagnification processes in ecosystems, helping to predict risks for higher organisms.	Shi <i>et al.</i> , 2019; Zhang <i>et al.</i> , 2022 ^[20]
3.	Biomonitoring of Pollutants in Ecological Risk Assessment	Water Quality	Silkworm bioassays detect industrial effluents and mining pollutants in water by measuring survival, growth, and developmental metrics, assisting in freshwater ecosystem health assessments.	Song <i>et al.</i> , 2021 ^[16]
		Soil and Sediment Quality	Exposure to polluted soil leads to developmental delays and altered feeding behaviors in silkworms, enabling long-term assessments of persistent pollutants like heavy metals and pesticides in terrestrial environments.	Zhao <i>et al.</i> , 2021 ^[21]
		Pharmaceuticals and Agrochemicals	High-throughput silkworm assays identify toxicity thresholds for various pesticides, herbicides, and industrial chemicals, providing rapid data for environmental regulatory frameworks.	Makhija <i>et al.</i> , 2020 ^[12]
		Ecotoxicological Databases	Data from silkworm bioassays contribute to databases for setting safe exposure limits and identifying emerging contaminants, enabling policy makers to mitigate risks to biodiversity.	Dawkar <i>et al.</i> , 2013; Lee <i>et al.</i> , 2019 ^[5]
5.	Silkworms in Ecotoxicological Modeling	Pollutant Transport	Silkworms provide critical data for models simulating pollutant transport through ecosystems, aiding in predicting contaminant behavior and long-term ecological impacts.	Zhao <i>et al.</i> , 2021 ^[21]
		Predictive Toxicity	Predictive models built from silkworm assays assess toxicity under varied conditions (e.g., pollutant concentrations, exposure durations), offering tools to forecast pollution impacts in new regions.	Tian <i>et al.</i> , 2021

Conclusion

Silkworms (*Bombyx mori*) have proven to be a groundbreaking model organism in the field of ecotoxicology, offering a fresh perspective on assessing environmental pollutants. Their unparalleled sensitivity to toxins, such as heavy metals, pesticides and nanoparticles, enables scientists to detect even subtle ecological threats. What sets silkworms apart is their well-mapped genetic and physiological systems, which open the door to understanding toxicity at cellular, biochemical and molecular levels with remarkable accuracy. Beyond their scientific utility, silkworms are practical, they are easy to rear, require minimal resources and provide results quickly, making them ideal for high-throughput studies. In a world increasingly focused on sustainability and ethical research, silkworms embody a forward-thinking approach to environmental science. Their use circumvents the ethical dilemmas tied to vertebrate models, offering a humane and innovative solution to toxicity testing. Moreover, their adaptability to various types of pollutants ensures they remain relevant as new environmental challenges arise. Silkworm-based studies not only deepen our understanding of ecological impacts but also support the development of policies and technologies aimed at creating a cleaner, safer planet. By integrating silkworms into ecotoxicological research, we move closer to a future where environmental protection is informed by science that is both precise and compassionate.

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