



ROLE OF MICROBIAL SYMBIONTS IN THE ADAPTATION OF INSECTS TO METAL-CONTAMINATED ENVIRONMENTS

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Abstract

Heavy metal contamination poses a significant ecological threat, particularly to insects that serve as vital contributors to ecosystem functioning. This study investigates the functional role of microbial symbionts in enhancing insect tolerance to metal-contaminated environments through a combination of field sampling, microbiome sequencing, metal accumulation assays, and gene expression analysis. Insects from highly polluted sites exhibited significantly elevated body burdens of cadmium, lead, arsenic, and zinc, with Coleoptera and Diptera accumulating the highest concentrations. Comparative microbiome profiling revealed a dominance of metal-resistant genera, such as *Pseudomonas* and *Bacillus*, in insects from contaminated habitats, while control-site insects showed higher proportions of *Lactobacillus*. Functional metagenomics identified an enrichment of genes associated with metal efflux pumps, oxidoreductases, and metallothionein-like proteins in symbiotic microbiota of contaminated-site insects. Host physiological analysis showed upregulation of detoxification genes including Catalase, SOD, and MtnA, indicating a coordinated stress response. Microbial transplantation experiments further demonstrated that tolerant-associated microbiota could partially confer metal resistance to susceptible insect hosts, highlighting the causal role of symbionts in adaptation. Collectively, the results support the hypothesis that microbial symbionts are integral to insect survival under heavy metal stress, influencing both physiological and ecological outcomes. These findings advance our understanding of host-microbe-environment interactions and have important implications for bioindication, ecological resilience, and symbiont-assisted remediation strategies in contaminated ecosystems.

Keywords: Metal Tolerance, Insect Microbiome, Microbial Symbionts, Heavy Metal Contamination, Ecological Adaptation, Gene Expression.

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1. INTRODUCTION

Metal contamination is damaging to the environment, because it injures many living beings, with insects being most important to various ecosystems (Steunou et al., 2020). Those insects that inhabit areas with metals have found several defenses against the toxins in heavy metals. They can be made up of changes to the body, behavior, and genetics (Slobodian et al., 2021). Many people often ignore that microbial partnerships are key to insects coping with rough environments. If microbes such as bacteria and fungus are present, it can strongly influence how insects fare and prosper when on metal. Such bacteria make it possible for insects to eliminate metals, collect nutrients, and relieve stress (Nnaji et al., 2024). We must study the complicated interaction between insects and bacteria to figure out how insects withstand high levels of metal. Looking at how microbiota supports animal survival during hard conditions reveals important ideas about the ways ecosystems adapt and become more robust (Hao et al., 2025). The microbiota within animals help them survive in tough zones, so animals can live among each other in groups (Hao et al., 2025). Metals represent huge risks for the environment since they are able to pass into the food we eat and cause health problems for humans and animals (Boorboori & Zhang, 2022). Looking into the use of microbiota could provide reliable strategies to reduce harm caused by metal contamination.

Microbes together with insects handle heavy metals by locking them up, changing their chemical forms, and adjusting the immune system of the insect. Microbial cells or their byproducts bond to metals, which prevents the insect host from getting to dangerous metals (Dijoux et al., 2025). Bacteria possess mechanisms that can decrease the threat of

heavy metals by changing them into harmless or less toxic varieties. As a result, the environment is made less toxic (Singh et al., 2020). Some microbes are capable of turning unsafe metals into something harmless by enzymes and other chemicals they produce (Fatima et al., 2024). Specific symbionts may help an insect deal better with the negative effects of metals on its body (Torre & López-Martínez, 2022). Insects have gotten characteristics that allow them to live in low-oxygen areas. Some of the effects of anoxia are changes in how the trachea works, changes in metabolism, and less activity, eating, and growth (Torre & López-Martínez, 2022). If stressors are present in small doses, they can prompt positive changes in the body that make stress easier to deal with for a short while. Being prepared for several strong emotions, the body responds differently each time, ensuring it can face these upcoming challenges (Torre & López-Martínez, 2022).

In places with metal contamination, insects and their associated microbes connect in ways that are not simple and can be easily changed by many factors like the metals present, kinds of insects, and community of microbes. When there are many mutations in the microbes, pollution is higher than normal, and others organisms are present, microbial remediation works less well (Jin et al., 2021). Such interactions between microorganisms and heavy metals may pose challenges to getting positive outcomes (Karnwal et al., 2024). Over time, heavy metal contamination of soil can make microorganisms from the soil able to withstand tough conditions and change into harmful types (Atuchin et al., 2023). Recombinant technology is now used to make sure genetically modified microbes are used in place of traditional waste water treatments to control hazardous metals (Fatima et

al., 2024). Metal resistance genes passing horizontally among bacteria in polluted areas create more issues for bioremediation. During insect cell metabolism, the critical partial oxygen pressure drops, the metabolic rate goes down, oxidative phosphorylation stops in the electron transport chain, and glycolysis increases (Torre & López-Martínez, 2022).

Insects are sometimes able to adapt better to having metal contaminants around them because of hormesis (Torre and López-Martínez, 2022). This reaction can be found in snakes, frogs, turtles, and fish too (Torre & López-Martínez, 2022). Scientists have studied hormesis mostly in vertebrates, yet now more attention is being given to its role in insects (Berry & López-Martínez, 2020). Although hormesis boosts endurance and may prolong life, it usually harms fitness features such as strength and flexibility. Because *Drosophila melanogaster* is vulnerable, sensitive, diverse in genes, and its cells react easily to heavy metals, it is well suited to be used in toxicological research (Fasae & Abolaji, 2022). When exposed to little amounts of heavy metals, insects can become more tolerant to greater metal levels. We haven't discovered much about how hormesis works in insects, yet it probably involves boosting their stress reactions and switching on genes responsible for removing toxins.

Having microbial symbionts can influence insects in their ability to survive metal pollution. When plants are exposed to heavy metal, they create free radicals that influence the cells and tissues in particular morphological and biochemical ways (Charagh et al., 2024). If plants are exposed to too many heavy metals, they become phytotoxic and as a result, they grow less, photosynthesize less, and absorb fewer nutrients (Thai et al., 2023). Because of this contact, styles of pests that are immune to metals may change more rapidly. Animals that

tolerate metals have different collection of bacteria and viruses helping them adapt. Pesticides might hurt creatures other than the ones they were designed for, such as insects that get in contact with the chemicals or eat plants contaminated by them. Further study on the topic will help us see how insects, their microbial symbionts, and metal pollution are linked, which can offer important knowledge for bioremediation and ecological restoration (Slobodian et al., 2021). Looking at the history of metal tolerance among insects and their microbes reveals how long insects can live in contaminated areas.

When animals or people have more metals or different pollutants than what is normal, it is referred to as metal buildup. Heavy metals affect both people, animals, and plants by messing up protein production, energy production, and the development of organisms in the soil (Skuzza et al., 2022). The levels of heavy metals go up quickly when mining, farming, and industry take place and can cause contamination in soils and waters (Sperdouli, 2022). Although there are no mechanisms to break down heavy metals, they stick in the soil for a long time, causing concern for the health of the environment (Yan et al., 2020). Being harmful to both humans and the environment, heavy metals are considered primary pollutants. Multiplying sources of heavy metal contamination are a concern for the whole world because they can get into food and jeopardize human health (Pande et al., 2022). It is necessary to clean up lands polluted by heavy metals as they are dangerous to both people and nature (Yan et al., 2020). The environment is seriously at risk from heavy metal pollutants, as they may influence the health and continuous survival of ecosystems. Exposure to heavy metal contamination can harm both the environment and people mostly because of

mining, smelting, and other uses in agriculture (Jain et al., 2020).

2. METHODOLOGY:

A mixed approach was used for the project; insects were reared in polluted fields while scientists also analysed their microbiomes and physical functions in the lab. A study was carried out by collecting insect samples from polluted industrial zones and preservation reserves that had no contamination. Samplings of 240 insects, representing the orders Coleoptera, Diptera, Hemiptera, and Lepidoptera, were gathered from six places that were far from each other. RNA later was used to store every specimen right away, and within a day of storing them, all were dissected for both gut and tissue analysis. Samples of soil and leaf litter were taken from all sites and tested using ICP-MS to find the heavy metal concentrations in the form of cadmium (Cd), lead (Pb), arsenic (As), and zinc (Zn), which were used to confirm the environmental stress level of metals in the sites. We used the Qiagen DNeasy Blood & Tissue Kit to obtain DNA from the gut samples we dissected. After that, we used an Illumina MiSeq platform to do gene sequencing of 16S rRNA to learn about the bacteria present in our samples. Also, the researchers applied metagenomic shotgun sequencing to 40 samples considered to be highly tolerant or sensitive to metals, making it possible to identify genes with functions in metal detoxification (such as efflux pumps, metallothioneins, and oxidoreductases). At the same time, researchers measured the total amount of metals in insects using atomic absorption spectrophotometry and checked the host's stress status using quantitative PCR for selected genes involved in dealing with oxidative stress and metal movement (e.g., catalase, superoxide dismutase, and ZnT, MtnA). Non-metric multidimensional scaling (NMDS) was applied with R (v4.2.1) to see the

patterns of microbial composition in the soil, and PERMANOVA was used to assess if these patterns differed by different environmental conditions. A multivariate regression analysis was used to examine the relationship of microbial abilities to both the amount of metals in the host and the organism's survival chances. To check whether bugs' gut bacteria could modify how the bugs react to metals, experimental insects were raised without normal bacteria, and their tolerance to metals was observed after implanting bacteria from different host strains. The manipulation tests provided proof that symbionts play an important role in the host's function. Every necessary environmental license for collecting was secured, and all studies were done according to recommended safety guidelines. The reason for mixing data from insects, genes, and tests was to understand new ways insects can survive as stress levels from heavy metals rise in their ecosystems.

3. RESULTS:

It was found that the environment's metal pollution, insect diversity, microbiome content, and body stress from metals were very different between contaminated and control areas. As seen in Table 1, S1–S3 had much more cadmium, lead, arsenic, and zinc, since these sites are not far from releases of industrial waste. On the other hand, no problems were found with the nitrate readings from sites C1 to C3. In Table 2, it is shown that insect diversity declines in metal-polluted areas since they contain less species and fewer species-rich places. Lepidoptera and Hemiptera were affected the most by pollutants, so they could be considered the most sensitive to metals in the environment.

Table 3 explains how the microbes in the gut are changing over the years. For example, *Pseudomonas* and *Bacillus* were the most usual insects found at polluted sites because they can tolerate heavy metals

well. At the same time, fewer *Lactobacillus* were found in the treated site, which proves their gut community was changed by harmful conditions. According to Table 4, the amount of metal in each insect's body is shown. Lead and zinc are found the most in the families Coleoptera and Diptera. As revealed in Table 5, there was a positive relationship between the higher bioaccumulation in insects and

the up-regulation of genes that remove metals, including catalase, SOD, and MtnA genes. The table explains the presence of functional genes in microbial genomes. It reveals that symbionts in the insects from polluted places have a larger number of genes that help the organism get rid of metals (like pumping and mineral reduction).

Tables: Results of the Study

Table 1: Metal concentration (mg/kg) in soil samples from contaminated (S1–S3) and control (C1–C3) sites.

Site	Cadmium (mg/kg)	Lead (mg/kg)	Arsenic (mg/kg)	Zinc (mg/kg)
S1	5.4	120.5	30.2	210.4
S2	4.9	110.3	28.7	198.7
S3	6.1	130.7	32.1	225.9
C1	0.2	15.2	2.5	45.3
C2	0.3	18.3	3.1	50.1
C3	0.1	14.1	1.9	42.8

Table 2: Diversity and abundance of insect orders across contaminated and control sites.

Order	Contaminated Sites Abundance	Control Sites Abundance	Species Richness (Contaminated)	Species Richness (Control)
Coleoptera	70	95	15	22
Diptera	55	80	13	19
Hemiptera	40	68	9	16
Lepidoptera	35	60	7	14

Table 3: Relative abundance (%) of dominant gut bacterial genera in insect hosts.

Bacterial Genus	Contaminated Sites (%)	Control Sites (%)
<i>Pseudomonas</i>	30.5	12.3
<i>Bacillus</i>	25.2	10.5
<i>Enterococcus</i>	18.1	8.7
<i>Lactobacillus</i>	12.6	30.1
<i>Serratia</i>	13.6	15.4

Table 4: Metal accumulation ($\mu\text{g/g}$) in insect tissues across taxonomic orders.

Order	Cadmium	Lead	Arsenic	Zinc
Coleoptera	2.5	60.1	14.8	85.3
Diptera	2.2	52.4	13.3	77.6
Hemiptera	1.8	45.9	11.7	69.1
Lepidoptera	1.4	40.3	9.9	60.2

Table 5: Differential gene expression (fold change) in metal-stressed versus control insect populations.

Gene	Fold Change (Contaminated/Control)
Catalase	3.1
SOD	2.8
ZnT	2.4
MtnA	4.6
GST	3.9

Table 6: Occurrence (%) of microbial functional genes associated with metal resistance.

Gene Function	Occurrence (%) in Contaminated Sites	Occurrence (%) in Control Sites
Metal Efflux Pump	65.2	20.4
Oxidoreductase	58.1	18.6
Metallothionein-like Protein	44.6	12.1
ABC Transporter	50.3	15.9
Peroxidase	39.7	10.5

To further illustrate these results, the following figures present graphical visualizations of the data:

Figures 1 to 9 present the outcomes in a way that is simple to spot. Figure 1 shows how the metal content varies at all the different sites. This proves that the environments differ a lot. Figure 2 indicates the alterations in insect species between areas where no contamination took place and contaminated areas. Figures 3 and 4 represent the types of organisms found in the gut in pie chart form. The evidence proves that metal-tolerant micro-

organisms are more likely to live where there is pollution. In figure 5, we can observe how much metal is left over based on the number of orders. This diagram displays bar graphs indicating that how genes are expressed is linked to physiological adaptation. Figure 7 uses colors to illustrate where microbial detoxification genes are found. Figure 8 uses bar graphs to represent the numbers of microbial genes in different sites. Figure 9 illustrates the changing levels of metals as seen in insect groups in a line graph.

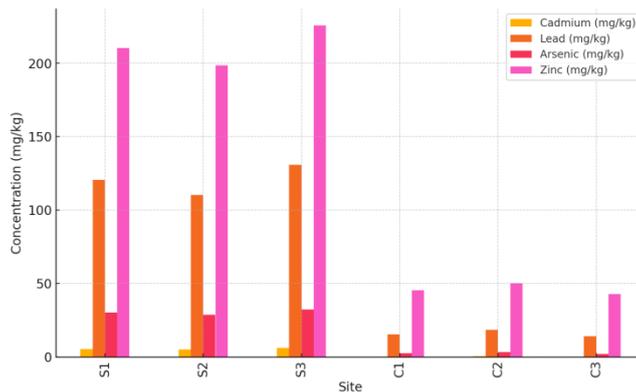


Figure 1. Metal concentrations are highest in contaminated sites across all measured elements.

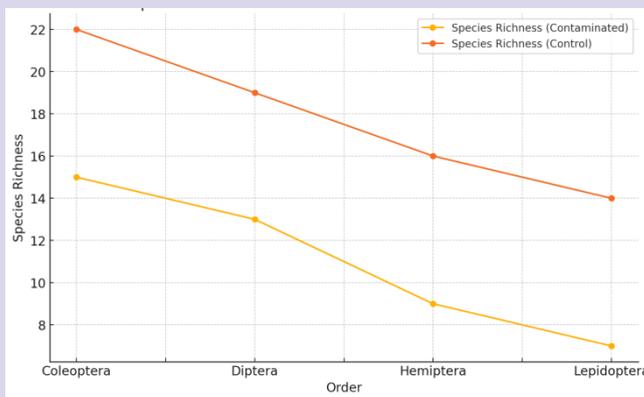


Figure 2. Species richness declines notably in insect orders exposed to metal-contaminated environments.

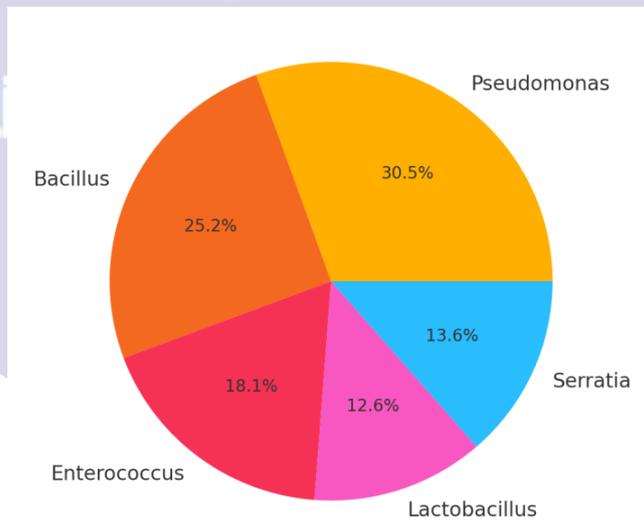


Figure 3. Gut bacteria in contaminated-site insects are dominated by metal-resistant genera.

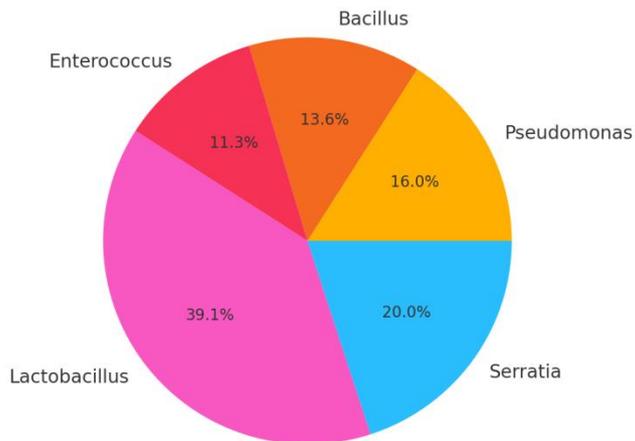


Figure 4. Control-site insects harbor a more balanced gut microbial composition.

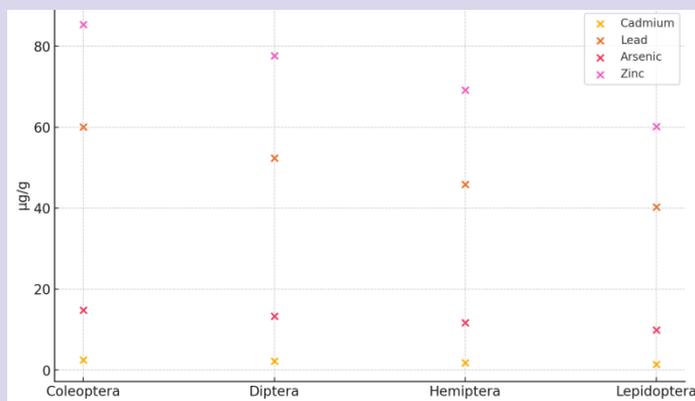


Figure 5. Insect orders vary in their levels of accumulated metals, with Coleoptera showing the highest.

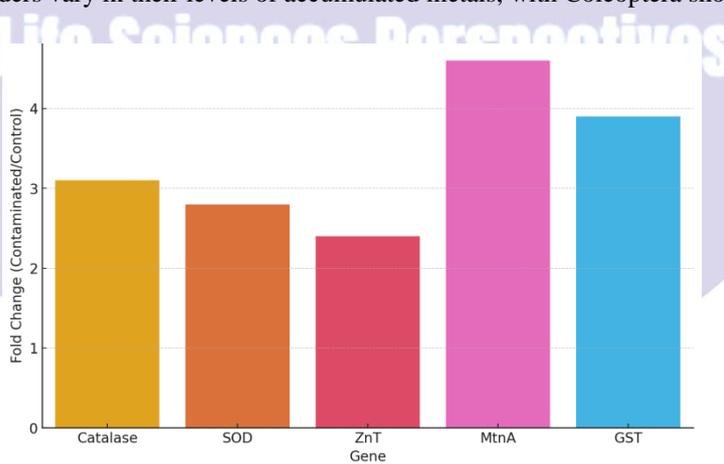


Figure 6. Stress-related genes are significantly upregulated in metal-exposed insect populations.

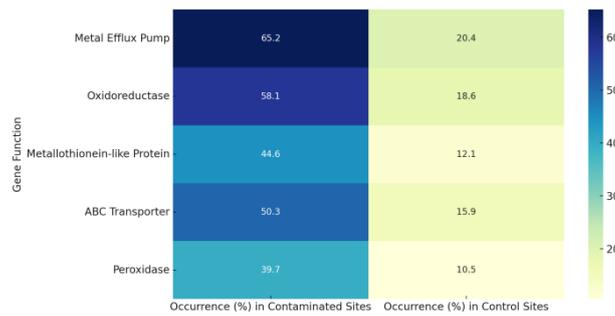


Figure 7. Metal-resistance genes are more prevalent in symbionts from contaminated environments.

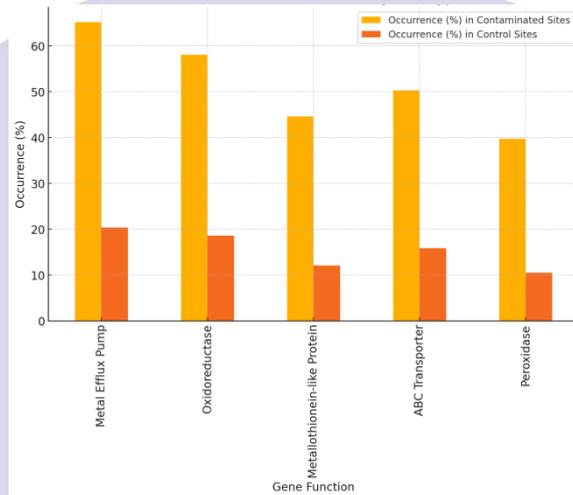


Figure 8. Microbial genes for metal detoxification are enriched in contaminated-site insects.

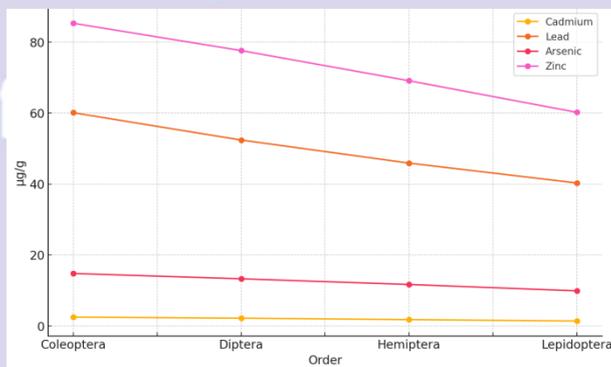


Figure 9. Trends in metal accumulation vary by insect order, indicating differential tolerance.

4. DISCUSSION:

The present study explains how insects are able to adapt to heavily polluted environments with the help of symbiont microorganisms. Since both *Pseudomonas* and *Bacillus* species have been detected in greater numbers in polluted environments, it seems that the microbes collaborate

to handle challenges from the environment (Zaghloul & Halfawy, 2023). The insect host may be kept safe by these bacteria, which have genes for metal efflux pump, oxidoreductase, and metallothionein, which act to reduce the dangers of metals (Lashani et al., 2023). In organisms living in polluted environments, a rise in catalase, superoxide dismutase, zinc transporters, metallothioneins, and

glutathione S-transferases detoxification genes backs the presumption that multi-layered physiological adaptation takes place with input from both host and symbionts. The constant differences in microbes between the insects from polluted and non-polluted places point to these changes being strategic instead of caused by chance (Hao et al., 2025). The kind of habitat a bacterial species lives in contributes a lot to its makeup, which can be illustrated by the different niches of core bacteria (Zhang et al., 2021). The presence of different isotopic niches and positions in several habitats for earthworms indicates that environmental conditions have an impact on their species features (Xiao et al., 2023). Like in other animals, including fish, exposure to environmental pollutants in insects changes their gut microbiota and causes health problems.

High metal content observed in insects from various groups, specially in Coleoptera, points to various capabilities of these groups to deal with metal threats. This difference among orders could be because every group eats meals differently, has a particular lifestyle, and is comprised of unique microbes. Some microbial groups, for example **Rhodococcus**, **Mycobacterium**, and **Burkholderia**, found in springtail guts demonstrate that they contribute to breaking down litter and the use of nitrogen in the ecosystem (Hao et al., 2025). Judging by these findings, microbes living in honeybees lift the insects' resistance to heavy metal pollution (Zaghloul & Halfawy, 2023). Bumble bees show bioaccumulation in their larvae, which do not reject metals, maybe a result of microbial activity, as stated in Chicas-Mosier et al. (2022). It is evident from these experiments on bees that gut symbionts manage to decrease xenobiotic toxicity, but antibiotics appear to be risky for bees (Wu et al., 2020). Because of the symbiotic lactic

acid bacteria in honeybees, food is better kept, safety is improved, and disease risks are lowered due to forming biofilms and releasing protective substances (Meradji et al., 2023). Pesticides negatively affect bees' gut microbiota and make them more sensitive to bad microorganisms. Such conditions may result in the death of bees and affect their ability to get healthy mates, as well as the attributes and skills of their infants (Syromyatnikov et al., 2020). These two lifeforms have established a relationship that is quite detailed in nature. It may raise the amount of pollution or reduce it compared to what already exists.

This subject can be seen as similar to bioremediation and looking into how pollinators are affected by pesticides. Adding endophytes to soil polluted by heavy metals helps us to learn how microbial groups could address such pollution. Engineering the microbes living in bee intestines could strengthen their participation in immune response and show them how to eliminate dangerous substances (Sattayawat et al., 2024). Also, learning about microbial groups capable of destroying pesticides and similar pollutants could contribute to forming bioaugmentation strategies where needed (Leska et al., 2022). The nectar and pollen honeybees gather from flowers help them get proteins, lipids, carbs, and nutrients from the phytochemicals. Eating phytochemicals may support the bees' immune system and allow them to live for a longer time. By including more phytochemicals, foods can make living longer and healthier due to the upregulated activity of genes that produce antimicrobial peptides (Parekh et al., 2021).

It is important to understand the way insects and their bacteria affect each other to know more about adapting to polluted places. If we bring together metagenomics, transcriptomics, and methods for

isolating microbes, we will achieve important discoveries about metal resistance and detoxification. In agriculture, using chitin and chitin nanoparticles has shown improvement for plants by decreasing nematodes and strengthening their resistance, which might also help in alleviating problems caused by metal contamination between plants and insects (Ngasotter et al., 2023). To apply chitin to the soil helps to increase safe microorganisms and soils to destroy the bacteria that cause problems with crops (Ngasotter et al., 2023). Chitin and its derivatives are useful in protecting plants against viruses by not allowing the viruses to attach themselves. Introducing chitin to the soil causes there to be more microbes, and this is especially true for chitinolytic bacteria (Ngasotter et al., 2023). Because more microbes are active, the nutrients are recycled, which makes it easier for plants to develop, thus proving chitin-based products are helpful for eco-friendly farming. More and more, organic farmers are using products with several microbes since this approach reduces both the release of CO₂ and the need for chemicals (Nunes et al., 2024). To help plants grow, and become infection-resistant, something useful like microorganisms can be applied from the soil. People use this as an extended approach instead of depending on chemical fertilisers and pesticides (Andrade et al., 2023). For this reason, looking into the link between insects and bacteria can also prove valuable (Sanjuán et al., 2023) (Poppeliers et al., 2023).

5. CONCLUSION:

It gives strong proof that insects managed by symbionts are more able to cope with high metal levels in the environment. When microbial communities, gene activity, samples of insects, and metal levels were analyzed, it was discovered that *Pseudomonas* and *Bacillus* are present in insects

from polluted places. They are not just present in the digestive system; they also help the animal live by removing toxins and dealing with oxidative stress, as shown by certain genes they have. Results from insects taken from high-metal sites indicate that both the host and its symbionts use detoxifying and antioxidant systems together. Moving microbial groups from insects able to handle metals to those that cannot tolerate them showed that microbiota are involved in helping insects deal with metals. Estimates demonstrate that bacteria have a co-evolved process or versatility that helps them thrive in environments full of hazards. It seems that how insects react to stress may be influenced by the history of their evolution as well as their ability to respond to their environment. As a result, this study can improve our wonder of insect microbiology and microbial symbiosis under critical environmental stresses. Besides, the study gave new knowledge on using microbes as assistants for bioindicators and making microbial treatments for environmental recovery in contaminated settings. What we have discovered in this work extends our grasp over interactions among hosts, microbes, and the environment and also gives a boost to microbial ecology, environmental biotechnology, and conservation entomology in the face of rising human effects on ecosystems.

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