

Dragonflies and Damselflies: A Comprehensive Review on their Role as Heavy Metal Bioindicators in Aquatic Ecosystems

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Abstract Aquatic ecosystems are seriously threatened by heavy metal contamination, which also endangers human health. The potential of damselflies and dragonflies (Odonata) as bioindicators of heavy metal pollution in freshwater habitats is examined in this review. They serve as excellent materials as the indicator of the wellbeing of the ecosystems they live in; because of their different life stages that have close ties to aquatic environments and their susceptibility to different metals. This comprehensive review aims to bridge gaps in the current understanding of the role of odonates, specifically dragonflies as bioindicators in the context of heavy metal contamination in aquatic ecosystems. Researchers analyse the presence and concentration of heavy metals in their tissues by various methods; this information can help understand their origin and dissemination and general health of the ecosystem. This bioindicator approach informs remediation tactics, provides early warning of emergent problems and ultimately directs efforts to protect freshwater resources. By consolidating diverse perspectives and synthesising the existing knowledge, this review aims to provide a valuable resource for researchers, environmentalists, and policymakers involved in safeguarding the delicate balance of aquatic environments.

Keywords Odonata, Dragonflies, Damselflies, Heavy

Metals, Bioindicators, Aquatic Ecosystems

1. Introduction

Heavy metals, elements with high density and atomic weight, are abundant in the environment due to their natural occurrence and widespread use in various industries. This widespread distribution raises concerns about their potential impact on both the environment and human health [1]. They include lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), zinc (Zn), arsenic (As), nickel (Ni), and manganese (Mn). Heavy metals can induce toxicity in various organs, including the kidneys, nervous system, liver, skin, and cardiovascular system. While some heavy metals play crucial roles in biological processes, others, like lead, thallium, cadmium and antimony, pose significant environmental and health risks. Some heavy metals, like lead and cadmium, are toxic and can have detrimental effects on various organs, even at low exposure levels. Antimony and chromium, in high exposure, are linked to increased cancer risk. Exposure to lead in children can lead to intellectual abnormalities. Mercury toxicity can cause Minamata disease, while

cadmium poisoning leads to itai-itai disease [1]. Figure 1 illustrates the various sources of these heavy metals in aquatic ecosystems [2]. Heavy metals enter aquatic environments, where they bind to particles, settle into sediments, and accumulate in the tissues of organisms [3].

Many insects, including dragonflies and damselflies (odonates), respond quickly and reliably to such accumulation, showing disruptions at the molecular and biochemical levels [4]. This sensitivity makes them ideal bioindicators for environmental pollution, alongside other living organisms like plants, plankton, and microbes. Bioindicators help us monitor the health of the environment, track changes, and assess the spread of contaminants in different areas [5]. They should be common, easy to sample and show clear, measurable responses to changing contaminant levels. However, their suitability varies across aquatic bodies due to differing sensitivities like the presence of other compounds in the environment, natural environmental stresses, and the length of exposure [6]. Selecting the right bioindicator depends also on the specific study goal; like focusing on habitat destruction, restoration or climate change [7]. The selection criteria for bioindicators are given in the Figure 2.

Larvae of odonate species are common in freshwater environments. They, in particular, dragonflies, play a crucial role in aquatic ecosystems as both top predators and indicators of environmental status. They are crucial to the operation of aquatic ecosystems. Being predators, they link terrestrial and aquatic ecosystems and have a significant top-down influence on food chains. Odonate nymphs can occasionally have extremely high densities, particularly during the monsoon season. In addition to their ability to effectively suppress mosquito populations, this group's economic significance is further highlighted by the fact that they form the food source for various vertebrates. Their vulnerability to various factors and close association with freshwater habitats make them valuable tools for assessing water quality [8].

This review aims to explore the sensitivity of odonates to heavy metals, envisaging their geographical and species-specific variations, which can be used to better understand and monitor environmental changes. By understanding how odonates respond to heavy metals, we can gain valuable insights into the health of our aquatic ecosystems and develop effective strategies for their conservation.

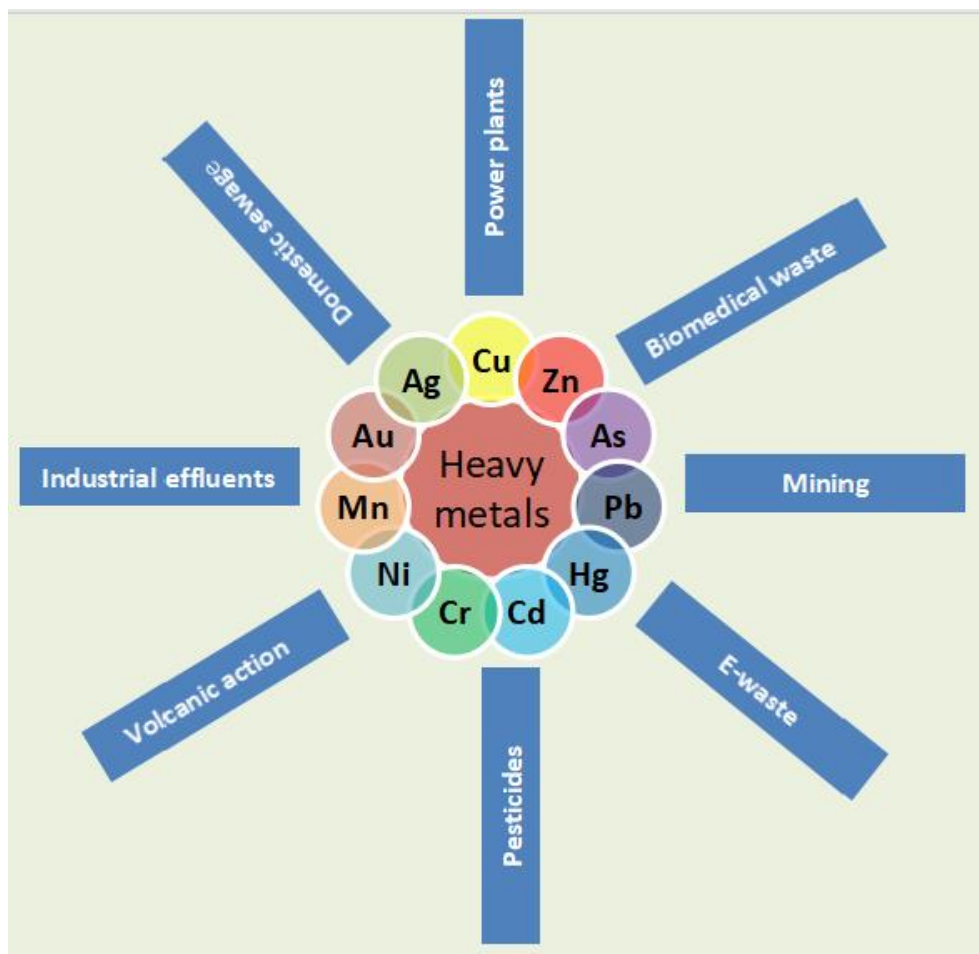


Figure 1. Various sources of major heavy metals in aquatic ecosystem

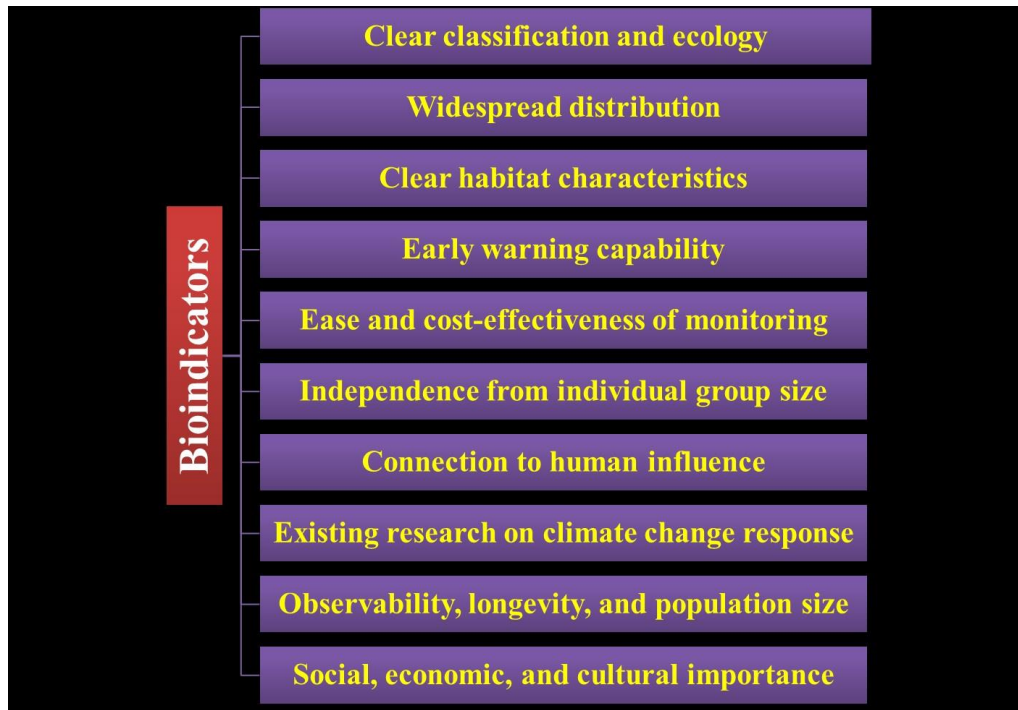


Figure 2. Selection criteria of bioindicators

2. Odonates and their Ecological Significance

The Odonata are an important group for environmental assessments because of their well-resolved taxonomy, noticeable diurnal adults, contact with both aquatic and terrestrial settings, and a wide range of environmental sensitivity across species. In aquatic ecotoxicological and bioaccumulation investigations, odonate nymphs are frequently used as test subjects. They are also frequently used in conjunction with other aquatic macroinvertebrate taxa in pollution-based biotic indices. The goal of some of the more recent research is to improve our understanding of the mechanisms underlying the impacts of contaminants and to clarify the evolutionary context of contamination risk. Typically, techniques like the Dragonfly Biotic Index, regional lotic quality indices, and coarse taxonomic metrics—which will be especially helpful in areas lacking descriptions and keys—are used to emphasise adults or exuviae in habitat quality assessments. Odonates, which are adapted to both aquatic and terrestrial environments, play a crucial role as ecological indicators in wetlands and rivers [9-11], and are distributed all over the world except in the polar region [12]. Their life cycle involves nymphal stages in water and adult stages on land, making them sensitive indicators of habitat quality, connectivity, and environmental changes in both aquatic and adjacent terrestrial habitats. Efficient sampling methods for Odonata should ideally encompass all three life stages: larvae, exuviae, and adults. Despite the predominance of methods focusing on the adult stage due to their cost-effectiveness and utility for rapid assessments [13],

dragonflies remain the most reliable ecological indicators in riparian and aquatic environments [14]. The significance of odonates is highlighted in Figure 3, emphasising their role as indicators of heavy metal and waste bioaccumulation in aquatic ecosystems [15,16]. Odonate nymphs, serving as aquatic predators feeding on various small aquatic organisms, such as mosquito larvae and other insect larvae, contribute significantly to the regulation of insect populations in aquatic ecosystems, thereby maintaining ecological balance [17]. Odonata are particularly valuable for long-term monitoring, efficiency control in planning and constructing water bodies, describing succession processes and assessing the ecological capability of wetlands [9-12,18]. Their counts can be effectively used for studying heavy metal bioaccumulation [19], with the presence or absence of dragonflies being closely tied to the chemical properties of the water in sewage lagoons [20]. Dragonfly richness is higher in ponds with a natural bottom compared to those with an artificial bottom (PEHD membrane) [21]. Locations lacking mine tailings exhibited greater abundance, species richness, and endemism compared to those with mine tailings suggesting that the presence of mine tailings has a detrimental impact on the species diversity of Odonata [22]. Exuviae, or shed exoskeletons of dragonflies, can serve as indicators for the restoration of degraded areas [23]. The fact that they accumulate various substances, including microplastics, lead, arsenic, and cadmium from their diets and habitats; further emphasizes their importance in ecological monitoring [24]. Odonata emerge as a valuable tool for bioassessment and biomonitoring; encompassing biodiversity measurement;

appraisal of health of water bodies and the detection of impacts of global warming. Benthic diatom abundance and nutritional quality drive the nutritional quality of larval dragonfly predators, with sewage pollution having the strongest negative impact [25]. Odonata play a significant role in providing aquatic resources, both quantitatively and qualitatively, to ecosystems [26]. For an integrated environmental assessment, dragonfly nymphs can be effectively used in genotoxicity assays, and detection of potential water quality degradation in high-anthropogenic areas, emphasising the absence of sensitive insect groups as valuable bioindicators [27,28].

Researchers adopt various methods to assess sensitivity of organisms to anthropogenic disturbances. The Kruskal-Wallis test can be used for calculating species richness differences across disturbance regimes. *Pantala flavescens* indicates high disturbance; *Orthetrum abboti* and *Africallagma vaginale* indicate moderate disturbance; *Gynacantha bullata*, *Tramea limbata*, *Olpogastra lugubris*, *Neodythemis klingi*, *Brachythemis leucosticta*, *Chlorocypha curta*, *Chlorocypha luminosa*, *Chlorocypha radix*, and *Agriocnemis* sp. indicate low disturbance [28]. Odonate assemblage conservation priority can be analysed by measuring the correlation between Dragonfly Biotic

Index (DBI) and the magnitude of climate change and human perturbation [29].

Odonate-based biomonitoring should focus on species composition, taxonomic diversity, and taxonomic distinctness for optimal assessment of habitat health [30,31]. Different odonate suborders have varying habitat preferences and tolerances; the species richness of damselflies is a good indicator of urbanisation levels, and the specific odonate species can be used as "flags" for healthy or degraded environments [32]. Different Odonata species indicate different water quality levels. Genera like *Megalestes*, *Copera*, and *Ischnura* are associated with specific water quality grades. Odonate diversity and richness decrease with declining water quality. Dissolved oxygen positively influences Odonata diversity, while total phosphorus has a negative effect [33]. Some, like *Ischnura ramburii*, exhibit high sensitivity to specific metals like chromium, copper, manganese, and zinc [34]. This makes them suitable bioindicators for monitoring environmental contamination levels. By investigating the benthos assemblage (bottom-dwelling organisms), researchers discovered 37 species of Odonata, offering valuable insights into the health of the river [35].

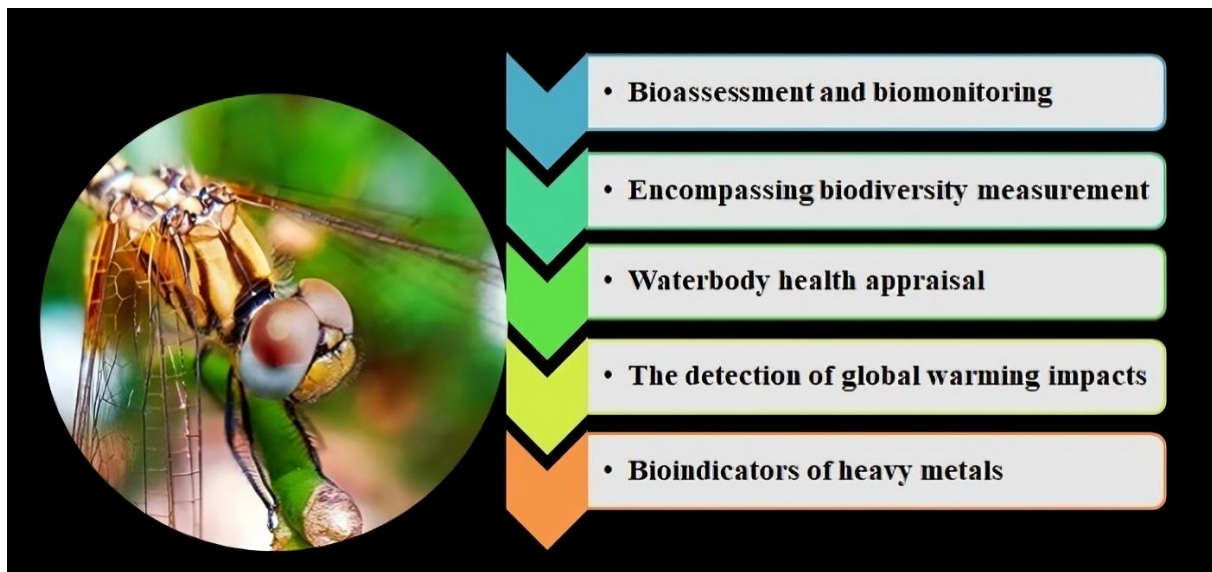


Figure 3. Ecological significances of odonates

3. Odonates Shine a Light on Heavy Metals in Aquatic Ecosystems

Dragonflies and damselflies, along with other macroinvertebrates, serve as widely recognised indicators of heavy metals in streams and wetlands. They play a significant role in the bioaccumulation of methylmercury (MeHg) in aquatic environments [9,11,36,37]. Dragonfly larvae, particularly those of the Aeshnidae family, emerge as valuable sentinels of methylmercury (MeHg) contamination in aquatic systems, including those marred by mining activities [38]. Investigation of heavy metal exposure in mosquito larvae and subsequent feeding to dragonfly nymphs (*Tramea cophysa*) revealed that mercury caused the highest mortality, indicating significant bioaccumulation and biotransfer within the food chain [39]. Their remarkable ability to bioaccumulate mercury intensifies with the size of the lakes they inhabit, hinting at a link between lake volume and biomagnification within these organisms [40]. This bioaccumulation extends beyond MeHg, encompassing total mercury as well, and varies across species, body size, and even the food web's potential for magnification. This cocktail of factors culminates in a potential toxicological risk not only for the dragonflies themselves but also for their predators, such as birds, raising an alarm about the far-reaching consequences of mercury contamination in aquatic ecosystems [41].

4. Odonates Are Bioindicators of Various Heavy Metals

Odonata tend to accumulate iron, manganese, copper, and zinc, with lower amounts of cadmium and cobalt, and the accumulation levels are related to the nutritive base [42]. *Libellula luctosa* accumulated higher cadmium and lead levels [43]. The study on damselfly nymphs, *Enallagma boreale*, [44] indicated that damselfly nymphs accumulate Mn, Pb, and As. A significant correlation between iron, lead, zinc, manganese, and nickel levels in Azure damselflies and the environment was reported, suggesting Azure damselflies as biomarkers [45]. In another investigation [46], the bioaccumulation patterns of trace elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were studied in three dragonfly larvae species and river sediments using scanning electron microscopy (SEM) and a desorption test; it was found that dragonfly larvae readily take up various metals and trace elements (including Cu, Cd, Zn, As, Cr, Fe, Mn, and Ni) from their aquatic environment. Different dragonfly species exhibit varying bioaccumulation tendencies, with *Ischnura ramburii* showing highest sensitivity to Cr, Cu, Mn, and Zn. Inner organs accumulate primarily Cu, Cd, and Zn. Outer body (exoskeleton) accumulated As, Cr, Fe, and Mn due to oxyhydroxide formation [34,46]. Gomphid dragonfly larvae can bioaccumulate elements like Mn, Zn, Cu, and

As, promising bioindicators for river sediment contamination [47]. *Pantala* dragonfly nymphs, rich in protein, amino acids, fatty acids, and minerals need investigation regarding the presence of potentially harmful heavy metals like arsenic, cadmium, and lead because they are widely consumed by humans [24]. Damselfly larvae (*Pseudagrion microcephalum*, *Pruinosum fraseri*, and *Copera marginipes*) are effective bioaccumulators of heavy metals (Mn, Zn, Cd, and Cu) [48]. Odonates effectively bioaccumulate Zn, Cu, Pb, and Mn from the agricultural areas [49]; metals like Cd, Pb, Cu, and Zn primarily accumulate in arthropods that dwell in agroecosystems and undergo minimal bioaccumulation and transfer to higher trophic levels, except for lead [50]. *Gomphus flavipes* larvae accumulated distinct levels of heavy metals depending on the river they inhabited, reflecting the pollution levels in each river [51]. Accurate estimation of metal concentrations in both larvae and adults is a non-invasive method for monitoring ecosystem health [23]. Studies have shown that specific metals accumulate in different regions of the larvae. For example, copper, cadmium, and zinc tend to be localized in the inner organs while; arsenic, chromium, iron, and manganese are often found deposited on the exoskeleton [46]. This compartmentalization helps minimize the harmful effects of metal exposure.

Specific insect species are associated with certain metals. Difference can also be observed in different body parts of a species [52,53]. Dragonfly nymphs, especially *Libellula* and *Pachydiplax*, emerged as valuable bioindicators for widespread metal exposure in wetlands [52].

Anthropogenic activities impacting a river are indicated by elevated heavy metal levels and changes in benthic invertebrate community composition. Monitoring of heavy metal levels and benthic invertebrate communities could be helpful to track changes and implement mitigation measures [54]. Odonate groups were identified as potential bioindicators of metal contamination streams near sugarcane cultivation [55]. *Platycnemis pennipes* and *Aeschna juncea*, when monitored in the two-way indicator species analysis [56]; showed high concentrations of cadmium, lead, copper, and boron. There exists a correlation between the concentration of heavy metals in invertebrate and vertebrate populations in the contaminated freshwaters [57]. Cadmium accumulation was documented highest in the Libellulid, *Crocothemis servilia* [58]. Tropical dragonfly nymphs (*Tramea cophysa*) were found to be less sensitive to metal toxicity compared to ostracod species and metal-specific variations in sensitivity were observed in them [59]. A comparison of heavy metal bioaccumulation in aquatic insects across trophic groups in streams highlighted the potential of aquatic insects as bioindicators for detecting metal pollution [60]. *Orthetrum pruinosum neglectum*, *O. anceps*, and *O. chrysostigma luzonicum* accumulate heavy metals; abundance patterns of these species are influenced by

environmental factors and the accumulation of metals exhibits species-specific variations [61]. Odonate larvae assemblages, abundance, richness, and diversity show a positive relationship with sediment metal content [62]. When heavy metal distribution (Cd, Cu, Pb, Mn, and Zn) in *Austroaeschna inermis* dragonflies and sediments was assessed, a widespread heavy metal presence, particularly elevated Mn and Zn in sediments, was observed. Oxidative stress biomarkers in *A. inermis* exhibited a positive correlation with Pb concentrations [63]. The study emphasises the significance of *A. inermis* as a pollution indicator. There is an urgent need for research in this direction to fill existing knowledge gaps, address potential environmental threats, and foster sustainable practices for the well-being of ecosystems and their inhabitants [64].

5. Morphological Changes and Decline in River Water Quality

High fluctuating asymmetry (FA) levels in the selected traits - first and second antennal segments of *Onychothemis* sp. and last tarsal segment of hind legs of *Pseudagrion* sp. were found associated with deterioration in the water quality (WQI) of the river [65]. The study suggests that certain traits in these odonate species could serve as effective bioindicators. The previously assumed teratogenic (malformation-causing) thickenings on the anal pyramid of dragonfly exuviae in the species *Libellula quadrimaculata* are actually associated with heavy metal contamination in the aquatic environment [66].

6. Physiological Markers of Water Quality

Dragonfly larvae are resilient against lead and cadmium; their varying metal tolerance makes them good tools for toxicity studies [10]. Enzyme biomarkers AChE (acetylcholine esterase) and GST (glutathione S-transferase) help monitor seasonal variations in water pollution through metal bioaccumulation [67]. GST activity influences the detoxification response in the damselfly larva of *Mnesarete aenea*, a promising sentinel species for biomonitoring environmental pollution [68]. Textile dyeing effluents and heavy metals disrupt sugar metabolism in *Bradinopyga geminata* and *Lymantria dispar*, indicating interference with sugar metabolism pathways by heavy metals [69]. Elevated sediment pollution levels, particularly polycyclic aromatic hydrocarbons (PAHs) and zinc, lead to significantly higher DNA damage in dragon fly nymphs in highway sedimentation ponds [70]. Northern damselflies, *Coenagrion hastulatum*, undergo distinct metabolic shifts during their life cycle, responding to environmental stress like wastewater exposure [71].

A systematic literature review of 80 peer-reviewed papers was done, the vast majority of which related to studies of changing patterns of biodiversity in urban odonate communities. Odonates such as *Aeshna juncea* and *Platycnemis pennipes* may be candidate indicators for particular contaminants [72]. Multi-element trace element analysis in dragonfly nymphs showed greater variations in accumulation patterns between genera than within genera; suggesting that the genus is an appropriate unit of comparison in dragonfly nymphs [73]. Dragonflies accumulate metallic elements effectively, with concentrations associated with specific pollution sources [74]. When the impact of copper, cadmium, mercury, and manganese on the survival of tropical invertebrates, including the odonate, *Tamea cophysa*, was assessed [59], manganese exhibited the least toxicity, and the selected species were found suitable for toxicity evaluations. When the impact of heavy metals and boron on aquatic insect communities (polluted with organic and industrial waste) was assessed using multivariate analysis (canonical correspondence analysis), elevated levels of cadmium, lead, copper, and boron were found [56].

Dragonflies, particularly *Ischnura elegans*, can tolerate a wide range of urban stressors, including the urban heat island effect [75]. Odonata larvae are suitable bioindicators for water pollution, and the measured biomarkers (GSH, TBARS, Na⁺, K⁺-ATPase) provide valuable insights into the oxidative stress response of organisms to pollution [76].

7. Metallothioneins in Dragonfly Larvae: Guardians of the Aquatic Kingdom

To combat the challenge of heavy metals, dragonfly larvae possess a powerful weapon – metallothioneins (MTs). They are small, cysteine-rich, multifunctional proteins with a remarkable affinity for binding with metal ions. Dallinger [77] highlighted the preferential binding of Cu by metallothioneins in dragonfly larvae and influence bioaccumulation dynamics. However, the larvae were found to be less tolerant to copper, indicating a nuanced interplay between metallothioneins and specific metals. They act as cellular detoxifiers, sequestering harmful metals like cadmium, zinc, copper, and mercury, preventing them from causing damage to vital organs and biochemical processes. This has made MTs of great interest in environmental monitoring and ecotoxicology [34,46,77,78,79]. Different dragonfly species vary in their MTs expression and metal binding capacities. MTs synthesis in dragonfly larvae can be significantly up regulated in response to metal exposure. This inducible response allows larvae to adapt to fluctuating metal concentrations in their environment; further enhancing their resilience. Recent research suggests that MTs in

dragonfly larvae might play additional roles beyond metal sequestration. MTs may exhibit antioxidant properties, scavenging free radicals generated by environmental pollutants and cellular metabolism. MTs could participate in the immune response against pathogens and parasites by regulating metal availability for microbial growth. MTs might be involved in regulating metal homeostasis during the complex molting and metamorphosis stages of dragonfly development [77-79].

8. Heat Shock Proteins (HSPs) - A Vital Defence Mechanism of Odonates against Heavy Metals

Dragonfly larvae possess a vital defence mechanism in the form of heat shock proteins (HSPs) also. These molecular chaperones act as a cellular shield, protecting proteins from the damaging effects of various stressors, including high temperatures, heavy metals, and oxidative stress. In the context of heavy metal exposure, HSPs play a crucial role in protein stabilization, metal binding and chaperone-mediated folding. HSPs and metallothioneins (MTs) may work synergistically. HSPs could facilitate the transport of metals to MTs for binding and sequestration. Studies have shown that dragonfly larvae exposed to heavy metals exhibit increased expression of specific HSPs, indicating their role in the stress response and tolerance of the organism to heavy metals [77-80]. This suggests potential for using HSPs responses as bioindicators for environmental contamination. Dragonfly larvae of the species *Orthetrum albistylum* effectively bioaccumulate heavy metals from their aquatic environment, their HSPs gene expression and antioxidant enzyme activity serve as sensitive biomarkers for water quality and environmental stress [80]. By combining their potent detoxifying arsenal of HSPs and MTs, dragonfly larvae demonstrate remarkable resilience against heavy metal stressors. Further research into these mechanisms can unlock valuable insights for environmental monitoring, ecotoxicological studies, and even biomedical applications.

9. Conclusions

Dragonflies and damselflies (Odonata) emerge as critical bioindicators for heavy metal pollution in freshwater systems. Their life stages intimately linked to aquatic habitats, analyzing their tissues reveals metal presence, origins, and ecosystem health. This review bridges understanding gaps, emphasizing Odonata's role in biomonitoring. Synthesizing knowledge informs targeted remediation and serves as an early warning system for environmental threats, ultimately aiming to safeguard precious freshwater resources.

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