Distributions of total mercury and methylmercury in dragonflies from a large abandoned mercury mining region in China

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Abstract

Dragonflies (Order Odonata) are often considered to be biosentinels of environmental contamination. Dragonflies ($n = 439$) belonging to 15 species of eight genera were collected from an abandoned mercury (Hg) mining region in China to investigate the bioaccumulation of total Hg (THg) and methylmercury (MeHg). The THg and MeHg concentrations in dragonflies varied widely within ranges of 0.06–19 mg/kg and 0.02–5.7 mg/kg, respectively. THg and MeHg were positively correlated with bodyweight (THg: $r^2 = 0.10$, $P = 0.000$; MeHg: $r^2 = 0.09$, $P = 0.000$). Significant variations were observed among species, with the highest MeHg value (in *Orthetrum triangulare*) being 5-fold higher than the lowest (in *Pantala flavescens*). These variations were consistent with those of nitrogen isotope ($\delta^{15}\text{N}$) values. A health risk assessment found hazard quotients for specialist dragonfly-consuming birds of up to 7.2, which is 2.4 times greater than the permissible limit of 3, suggesting a potential health risk of exposure.

Introduction

Mercury (Hg) is a globally distributed pollutant that can cause severe health-exposure risks to the biota of both aquatic and terrestrial ecosystems (Cristol et al., 2008; Walters et al., 2010). Apart from unintentional accidents, the dominant pathway of Hg poisoning is through the consumption of foodstuffs contaminated with Hg, particularly methylmercury (MeHg). As an organic form of Hg, MeHg has extremely neurotoxic effects and is readily accumulated in biota due to its lipophilic and protein-binding properties (Ullrich et al., 2001). Compared to inorganic Hg (IHg), MeHg is more easily uptaken and can be transported and biomagnified several million-fold from the water to top predators via aquatic food chains. Currently, the toxicology of Hg in vertebrates, especially mammals and human beings, has been comprehensively studied since the Minamata disease occurred in the 1950s (Harada and Masazumi, 1995). Consumption of fish and rice is considered the major pathway for human exposure to MeHg (Clarkson and Magos, 2006; Feng et al., 2008; Qiu et al., 2008; Kathryn et al., 2009).

In recent years, numerous ecological indicators have been identified that are sensitive to changes in aquatic, terrestrial, and atmospheric environments and can provide an early warning of pollution (Zheng et al., 2018; Eagles-Smith et al., 2020). Insects, spiders, and other vertebrates, usually those belonging to high trophic levels, have been verified as effective ecological indicators (Cristol, et al., 2008; Jeremiason et al., 2016; Popova et al., 2016; Yang et al., 2016; Williams et al., 2017). Among them, amphibiotic insects play an important role in the transfer of heavy metals from aquatic to terrestrial environments owing to their demands for high-carbohydrate diets (Martin-Creuzburg et al., 2017).

The dragonfly (Odonata) is a typical metamorphosis developmental insect with aquatic and terrestrial life stages. Its larvae live in freshwater for one to several years then adults live in a terrestrial environment for several days or weeks (Córdoba-Aguilar, 2008). Both adults and larvae are predacious, with larvae mainly preying on tadpoles, shrimp, and small fish (Johansson and Brodin, 2003). Since dragonflies feed on small insects from both aquatic and terrestrial environments, they play an important role in pollutant transfer from aquatic to terrestrial ecosystems and are regarded as a biosentinel for environmental contamination (Eagles-Smith et al., 2020). Simultaneously, the dragonfly is preyed upon and consumed by frogs, spiders, and some insectivorous songbirds, suggesting that it may represent a vector of pollutant transfer to vertebrates through food chains (Sullivan and Rodewald, 2012; Buckland-Nicks et al., 2014; Williams, et al., 2017).

Intensive Hg mining activities have generated Hg-contaminated sites worldwide. Although Hg mines closed decades ago, significant quantities of mine-waste calcine produced during the retorting process continue to release Hg, posing threats to local ecosystems (Gray et al., 2002; Qiu et al., 2013). Most previous studies on Hg mining areas have focused on Hg contamination of water, soil, the atmosphere, and agricultural crops (Gray, 2003; Horvat et al., 2003; Qiu, et al., 2008), confirming that mining areas were heavily Hg-contaminated. Recently, Abeyasinghe et al. (2017) reported elevated levels of Hg in the feathers of songbirds dwelling in Hg mining areas and found that the spiders played an important role in the transfer of Hg via food chains. However, few studies have focused on amphibiotic insects, especially dragonflies, which can have both aquatic and terrestrial life stages and probably play an important role in Hg transfer at those contaminated sites.

In the present study, total Hg (THg) and MeHg levels were determined in dragonflies and the waterbodies of their habitat at an abandoned large-scale Hg mine in Southwest China. The objectives were to 1) ascertain the characteristics of THg and MeHg in dragonflies impacted by historic Hg mining and retorting activities; 2) investigate the distribution of Hg in different dragonfly species; and 3) reveal the factors influencing Hg levels in dragonflies.

Experimental And Methods

Study area

The Wanshan Hg mining area (WMMA; 27° 24' 53"–27° 37' 46" N, 109° 8' 2"–109° 22' 43" E) lies in eastern Guizhou Province, Southwest China. It was once the largest national production center of metallic Hg. It has a typical mountainous and karstic landform characterized by low terrain in the east, high terrain in the west, and an uplift in the middle, with elevations ranging between 270 m and 1149 m above sea level. The WMMA has a subtropical humid climate with abundant precipitation (1378 mm per year) and mild temperatures (annual average temperature: 13.7 °C). The Aozhai and Xiayi Rivers are the main rivers flowing through the mining area and originate from the Hg mining sites of Wukeng and Shibakeng, respectively (Figure 1).

Intensive retorting of cinnabar ores in the WMMA generated significant quantities of mine-waste calcines, which contain amounts of water-soluble secondary Hg compounds (Jasinski, 1995; Xu et al., 2019). The water-soluble Hg is readily released from mine-waste calcines into the surroundings. As much as 12,000 ng/L Hg has been observed in surface water from nearby ponds receiving leachates of mine-waste calcines (Qiu et al., 2009). Under certain conditions, the released inorganic Hg can be converted into more toxic and bioavailable MeHg, causing elevated Hg levels and health risks in biota.

Sampling and preparation

Dragonfly samples were collected from 10 sites along the Xiaxi River (XXR) and Aozhai River (AZR) using nylon dip nets in July 2018 and August 2019. Six sites, Wukeng (WK), Zhongjiapo (ZJP), Boxi (BX), Baimuping (BMP), Jianxi (JX), and Wawu (WW) are located in XXR, and four sites, Meizixi (MZX), Baiguoshu (BGS), Aozhai (AZ), and Shenchongkou (SCK) in AZR (**Figure 1**). According to the density of dragonflies, at least 40 individuals were sampled at each site. After collection, the species and sex of dragonflies were identified according to illustrated handbooks (Zhang, 2019a; b).

A total of 439 individuals belonging to 15 species of eight genera were collected. Species include *Acisoma panorpoides*, *Diplacodes trivialis*, *Orthetrum albistylum*, *Orthetrum japonicum*, *Orthetrum melanium*, *Orthetrum pruinosum*, *Orthetrum sabina*, *Orthetrum triangulare*, *Pantala flavescens*, *Pseudothemis zonata*, *Rhyothemis fuliginosa*, *Sympetrum croceolum*, *Sympetrum kunckeli*, *Sympetrum speciosum*, and *Trithemis aurora*. The eight genera are *Acisoma*, *Diplacodes*, *Orthetrum*, *Pantala*, *Pseudothemis*, *Rhyothemis*, *Sympetrum*, and *Trithemis*. All the samples belong to the family Libellulidae (Order Odonata). Among them, approximately 198 is female, and 257 is male.

In the laboratory, all samples were treated individually with starvation overnight, then anesthetized with alcohol and weighed. Samples were then thoroughly cleaned with deionized water to remove any contamination, freeze-dried (FDU-2110, EYELA, Japan) and their dry weight recorded. The dried samples were crushed individually into powder using a grinder (Pulverisette 23, Fritsch, Germany). The grinder was thoroughly cleaned with ethanol between operations to avoid cross-contamination.

THg and MeHg analyses

For THg, appropriately 20 mg of sample was weighed and digested at 95 °C for 3 h with 4 mL of a fresh acid mixture of H₂SO₄:HNO₃ (1:4, v/v). The digestion was then oxidated by BrCl overnight and filled with deionized water to a constant volume of 25 mL prior to analysis. THg concentration was detected by cold vapor atomic fluorescence spectrometry (CVAFS, Model III, Brooks, USA) followed the reduction of SnCl₂ according to USEPA Method 1631e (USEPA, 2002).

For MeHg, approximately 20 mg of sample was digested at 60 °C for 12 h with 4 mL 4.6 M HNO₃ according to Hammerschmidt and Fitzgerald (2005). MeHg was measured by gas chromatographic-cold vapor atomic fluorescence spectrometry (GC-CVAFS, Model III, Brooks, USA) following an ethylating process according to USEPA Method 1630 (USEPA, 2001).

QA/QC

Quality assurance and quality control (QA/QC) for the THg and MeHg analyses were conducted using duplicates (~10%), method blanks, matrix spikes, and certified reference material (TORT-2, NRC Canada). The method blanks for THg and MeHg were < 5.4 ng/g (~2% of the reference value of TORT-2) and < 0.02 ng/g (detection limit value), respectively. The average THg concentration of TORT-2 was 268.6 ± 7.2 ng/g (2 SD, *n* = 6), which is comparable to the certified value of 270 ± 60 ng/g. The measured MeHg concentration in TORT-2 was 154.1 ± 4.8 ng/g (2 SD, *n* = 6) on average, which is comparable to the certified value of 152 ± 13 ng/g. The recoveries of THg and MeHg for the matrix spikes were 96.8–102.1% and 98.2–104.5%, respectively.

Stable nitrogen isotope (δ¹⁵N) analysis

In the present study, δ¹⁵N was analyzed in two species of *O. triangulare* and *P. flavescens* collected from the WK site, which exhibited significant differences in THg and MeHg concentrations. Approximately 400–500 µg of sample was packaged into a small tin container, and then δ¹⁵N was determined using an isotope ratio mass spectrometer (MAT 253, Thermo Fisher Scientific Inc., USA) and an elemental analyzer (Flash EA 2000 HT, Thermo Fisher Scientific Inc., USA).

$$\delta^{15}\text{N} = \frac{R_{\text{sa}} - R_{\text{st}}}{R_{\text{st}}} \times 1000 (\text{‰})$$

Where R_{sa} is the ¹⁵N/¹⁴N ratio of the samples and R_{st} is the ¹⁵N/¹⁴N ratio of the reference standard, which was atmospheric N₂.

The mean percent recoveries of δ¹⁵N were in the range of 99.2–100.4%. Each sample was measured in triplicate and the relative standard deviation was < 0.2%.

Hazard quotient

The hazard quotient (HQ) was applied to evaluate the health exposure risk of specialist dragonfly consumers (Liu et al., 2015; Ai et al., 2019) and was calculated as follows:

$$HQ = \sum \frac{MDI_{IHg \text{ or } MeHg} / BW}{TDI_{IHg \text{ or } MeHg}}$$

$$MDI = DI \times C \times p$$

$$TDI = \frac{\sqrt{LOAEL \times NOAEL}}{UF}$$

Where,

MDI is daily Hg intake,

IHg indicates the concentration of inorganic mercury (the difference between THg and MeHg), and MeHg indicates the concentration of MeHg,

BW is the body weight of songbirds (g),

DI is daily food intake (g),

C is the Hg concentration in the food (mg/kg),

p is the proportion of dragonflies in the food (%).

TDI is the tolerable daily intake,

LOAEL is the lowest observed adverse effect level of Hg,

NOAEL is the no observed adverse effect level of Hg, and

UF is the uncertainty factor.

Statistical analysis

Data processing was conducted using Microsoft Excel 2010 (Microsoft Corporation, USA). Statistical analysis was performed using one-way ANOVA with SPSS 22 software (IBM, USA). The map of sampling sites was drawn by CorelDRAW Graphics Suite X8 (Corel, Canada) and figures were plotted by Origin Pro 2016 (Origin Lab, USA).

Results

Dragonfly species

THg

The mean THg in all dragonflies was 1.5 ± 2.2 mg/kg ($n = 439$), ranging from 0.06 to 18.5 mg/kg. As expected, dragonflies from the WK site, which is close to the largest mining-waste calcine pile in the WMMA, exhibited the highest mean THg of 3.7 ± 3.9 mg/kg. This is almost 10-fold higher (Mann-Whitney U test, $P = 0.017$) than the lowest value of 0.37 ± 0.21 mg/kg found at the JX site, which is about 20 km away from a pollutant source. THg in dragonflies from XZR (average: 2.31 ± 3.10 mg/kg; range: 0.14–18.5 mg/kg) was greater (Mann-Whitney U test, $P = 0.000$) than that of those from AZR (average: 1.0 ± 0.84 mg/kg; range: 0.06–4.8 mg/kg). Dragonflies collected from the mid-downstream to downstream areas of ZJP, BX and BMP exhibited comparable mean levels of THg, which were 0.93 ± 0.60 mg/kg, 0.90 ± 0.34 mg/kg, and 1.0 ± 0.92 mg/kg, respectively. Overall, the THg in dragonflies gradually decreased with distance from upstream pollutant sources (**Figures 2a, d**).

Variations in mean THg were observed among species, ranging between 0.63 ± 0.28 mg/kg in *S. croceolum* and 8.3 ± 4.3 mg/kg in *O. triangulare*. Species *O. japonicum* and *P. zonata* also exhibited high values of THg, reaching 7.1 ± 3.4 mg/kg and 5.3 ± 2.0 mg/kg on average, respectively. Of the four most common species, the highest and lowest THg values were found in *O. albistylum* and *S. croceolum*, averaging 1.8 ± 1.5 mg/kg and 0.63 ± 0.28 mg/kg, respectively. Interestingly, a significant difference in THg was observed between females and males, with the mean concentration in males (1.9 ± 2.7 mg/kg, $n = 244$) being almost double that in females (1.0 ± 1.1 mg/kg, $n = 195$; Mann-Whitney U test, $P = 0.000$).

MeHg

The mean MeHg in all dragonflies was 0.75 ± 0.65 mg/kg ($n = 439$). The maximum and minimum values were 5.7 mg/kg and 0.02 mg/kg, which occurred at WK and JX, respectively. No significant difference in MeHg was observed between XZR (mean: 0.88 ± 0.86 mg/kg; range: 0.06–5.7 mg/kg) and AZR (mean: 0.64 ± 0.46 mg/kg; range: 0.02–2.2 mg/kg; Mann-Whitney U test, $P = 0.079$). Similar to the case for THg, both WK and MZX, which were adjacent to pollutant sources, recorded peak mean values of 1.1 ± 1.0 mg/kg and 0.93 ± 0.62 mg/kg, respectively. The lowest mean MeHg of 0.32 ± 0.19 mg/kg was observed in JX (**Figures 2b, e**). Although dragonflies collected from mid-downstream to downstream of the ZJP, BX, and BMP sites exhibited comparable MeHg levels, there was a gradual decrease in MeHg with distance from upstream pollutant sources across the study region (**Table 1**).

Species also exhibited a wide range of MeHg values, ranging from 0.47 to 2.1 mg/kg, of which the highest and lowest values were recorded in *O. triangulare* and *P. flavescens*, respectively. In addition to *O. triangulare*, species *O. japonicum*, *O. melanum*, *O. pruinosum*, and *P. zonata* exhibited peak MeHg values > 1.0 mg/kg and, interestingly, all of these four species belong to the genus *Orthetrum* (Table 2). In the common species, *O. albistylum*, *P. flavescens*, *S. croceolum*, and *S. kunckeli* showed comparable levels of MeHg, ranging between 0.47 ± 0.31 mg/kg and 0.95 ± 0.057 mg/kg. Similar to the case of THg, MeHg was greater in males than in females (Mann-Whitney *U*-test, $P = 0.001$), with mean values of 0.85 ± 0.76 mg/kg ($n = 244$) and 0.61 ± 0.44 mg/kg ($n = 195$), respectively.

MeHg ratios

The ratios of MeHg to THg (MeHg%) in dragonflies varied widely, ranging from 6.45% to 99.95% with a mean of $64.5 \pm 23.6\%$. The maximum mean value of $86.7 \pm 6.1\%$ was recorded at WK, and the minimum of $37.4 \pm 15.1\%$ was observed at AZR. A positive correlation (Spearman test, $r = 0.891$, $P = 0.000$) was observed between the MeHg and THg values at the 10 sites, with a logarithmically increasing pattern (Figure S1). This suggests that MeHg% values first increased and then decreased at THg concentrations above ~1.2 mg/kg. Overall, a significant negative correlation was observed between MeHg% and THg (Spearman test, $r = -0.287$, $P = 0.000$).

A large variation in MeHg% was observed among species, ranging between $22.9 \pm 6.9\%$ and $89.6 \pm 0.5\%$ on average (Figures 2c, f). Peak values of MeHg% mostly occurred in *A. panorpoides*, *O. pruinosum*, and *R. fuliginosa*, which were collected from the WK site. Of the common species, *O. albistylum*, *P. flavescens*, *S. croceolum*, and *S. kunckeli* showed comparable levels of MeHg% ranging between $61.7 \pm 19.7\%$ and $76.8 \pm 12.3\%$. In addition, species *O. japonicum*, *P. zonata* and *O. triangulare*, which had the highest THg values, exhibited low mean MeHg% values that ranged from $22.9 \pm 6.9\%$ to $29.8 \pm 17.0\%$. A significant difference in MeHg% was observed between female and male *S. croceolum* (one-way ANOVA, $P = 0.000$), of which the females ($85.9 \pm 7.0\%$) had a higher mean value than males ($70.1 \pm 11.0\%$).

Correlation between body weight and Hg concentration

Both THg and MeHg gradually increased with bodyweight in all dragonflies (THg: $r^2 = 0.10$, $P = 0.000$; MeHg: $r^2 = 0.09$, $P = 0.000$). Similar trends were also observed at sites AZR (THg: $r^2 = 0.15$, $P = 0.000$; MeHg: $r^2 = 0.14$, $P = 0.002$) and XZR (THg: $r^2 = 0.30$, $P = 0.003$; MeHg: $r^2 = 0.20$, $P = 0.000$; Figures 3a, b). The result is consistent with a previous report of a positive relationship (THg: $r^2 = 0.38$, $P \leq 0.001$) between bodyweight and THg concentration in predatory insects such as *Nabidae* (Yung et al., 2019).

$\delta^{15}\text{N}$

Species *O. triangulare* and *P. flavescens* from site WK exhibited significant differences in THg ($P < 0.001$) and MeHg ($P < 0.001$). The $\delta^{15}\text{N}$ value in *O. triangulare* (mean: $8.26 \pm 1.15\text{‰}$, range 6.63–10.3‰, $n = 11$) was significantly higher than in *P. flavescens* (mean: $5.86 \pm 2.06\text{‰}$, range 2.64–8.42‰, $n = 11$; $P = 0.002$).

Discussion

Spatial variation in THg and MeHg in dragonflies

Dragonflies appear to provide a more stable and effective indication of the status of environmental pollutants than direct indicators such as water, soil, and the atmosphere (Lesch and Bouwman, 2018; Eagles-Smith, et al., 2020). As a typical carnivorous insect, adult dragonflies respond sensitively to Hg contamination in food chains. As expected, dragonflies in WK had extremely high THg levels, while dragonflies from other sites showed slightly decreasing or comparable levels of both THg and MeHg with distance from upstream pollutant Hg sources. This was coincident with the THg concentrations of surface water (Figures 4 a, b); both THg ($r = 0.894$, $P = 0.000$) and MeHg ($r = 0.684$, $P = 0.020$) in dragonflies were positively correlated to surface water THg, suggesting that they are a good indicator of aquatic Hg concentrations.

In the present study, Hg concentrations in all dragonflies were significantly elevated, being 5-fold and 3-fold higher than in dragonflies from the Eagle Mountain Fish Hatchery (MeHg: 0.15 mg/kg, dw) and Kejimikujik National Park (THg: 0.26 ± 0.071 mg/kg, dw; MeHg: 0.23 ± 0.074 mg/kg, dw) as reported by Williams et al. (2017) and Buckland-Nicks et al. (2014), respectively. However, they are slightly lower than those reported by Abeyasinghe et al. (2017) in an Hg mining-affected rice paddy field (THg: 2.7 ± 0.84 mg/kg, MeHg: 1.8 mg/kg, dw). Also, an elevated THg value of 4.2 mg/kg (range: 0.23–12 mg/kg) was reported in Odonata from the area of zinc smelting and chlor-alkali areas (Zhang et al., 2009). High levels of Hg in dragonflies may imply heavy Hg-contamination of the local environment (Zhang et al., 2012; Lesch and Bouwman, 2018).

Interspecies variation in THg and MeHg concentrations

Concentrations of THg and MeHg as well as MeHg-to-THg ratios (MeHg%) varied widely among dragonfly species. The lowest and highest THg concentrations were found in *S. croceolum* and *O. triangulare*, of which the latter was 13-fold higher than the former ($P = 0.000$). Similarly, *O. triangulare* also exhibited the highest MeHg, which was approximately 5-fold higher than the lowest value, which was found in *P. flavescens* ($P = 0.000$). In the common genera, the highest THg and MeHg concentrations found in *Orthetrum* were 4.7-fold and 2.8-fold higher ($P < 0.001$) than the lowest values found in *Pantala*. *Orthetrum* is usually two times higher in bodyweight than *Pantala*, allowing it to be a more effective predator, and even prey on smaller dragonflies. This may explain why *Orthetrum* can accumulate more Hg in the body than *Pantala*.

Significant differences in Hg concentration were found between species. In the present study, THg and MeHg in *O. triangulare* were higher than in *P. flavescens* ($P = 0.002$), both of which were collected from the heavily Hg-contaminated WK site. The distribution of $\delta^{15}\text{N}$ found in the two species was coincident with

THg and MeHg concentrations. *O. triangulare* had the higher values of $\delta^{15}\text{N}$, while *P. flavescens* showed lower values (Figure 5). As higher $\delta^{15}\text{N}$ values indicate a higher trophic position (Hyodo, 2015; Rodenhouse et al., 2019), elevated Hg, particularly the MeHg in *O. triangulare*, may be attributed to a higher trophic position in the food chain.

Influence of bodyweight on Hg

Dragonflies are carnivorous and exhibit higher Hg concentrations than many herbivorous insects, such as locusts, crickets, stinkbugs, leafhoppers, and even other carnivorous arthropods (Abeyasinghe, et al., 2017; Zheng, et al., 2018). Previous investigations suggested that insects may have the characteristic of biomass dilution, causing a decrease in toxins with increasing bodyweight (Karimi et al., 2007; Clayden et al., 2013; Verburg, 2014).

Life traits such as feeding guilds, life cycle, and habitat are the main factors controlling the accumulation of Hg (Yung et al., 2019). In the present study, significant bioaccumulation was apparent in the dragonflies, in which Hg concentrations increased with bodyweight. It is known that a higher bodyweight is associated with greater biomass dilution (Zhang et al., 2009), and a diet polluted by Hg is the key factor in Hg bioaccumulation. Insects inhabiting the WMMA transfer Hg to dragonflies by diet, which is the key path of Hg bioaccumulation. Meanwhile, the variation in dragonfly bodyweight is low (Córdoba-Aguilar, 2008). Thus, these two factors contribute to the significant bioaccumulation of Hg in adult dragonflies.

Potential risks to consumers

Adverse effects on avians due to exposure to elevated Hg levels have been reported (Wada et al., 2009; Sauer et al., 2020). In a previous study, extremely high Hg concentrations of up to 123 ± 34 mg/kg were found in the feathers of songbirds in the study region (Abeyasinghe, et al., 2017). However, the accumulation and transfer of elevated Hg levels in songbirds via food chains remain unclear. Several studies have reported that members of the *Merops*, such as the blue-throated bee-eater (*Merops viridis*) and blue-tailed bee-eater (*M. philippinus*) depend heavily on dragonflies, which can account for 38.3–45.1% (Ke et al., 2017) and 57.9–63.0% (Wu et al., 2009; Cheng et al., 2012) of their diets, respectively. Such high rates of dragonfly consumption may contribute to heavy Hg burdens in songbirds inhabiting Hg mining regions.

Here, we evaluate the hazard quotient (HQ) of the two species *Merops viridis* and *M. philippinus* according to their dietary ratios of Odonata. In this study, we assessed the daily food intake and bodyweight of dry weights as 3.84 g (Krebs and Avery, 1984) and 35 g (Zhao, 2001). The results indicate that the HQs of blue-throated and blue-tailed bee-eaters are 4.39–5.17 and 6.63–7.22, respectively, both of which exceed the permissible limit of exposure risk (HQ = 3; Liu et al., 2015; Ai et al., 2019). It is clear that the blue-tailed bee-eater, which has the higher dragonfly dietary ratio, has a higher health risk than the blue-throated bee-eater at a particular site. Since both THg and MeHg at site XZR were higher than at AZR, the two species may experience greater exposure risk at XZR than at AZR. The emergence stage of the dragonfly coincides with the reproduction period of birds, which may greatly increase Hg exposure risk, especially to nestlings.

Conclusions

Dragonflies (Odonata) collected from large-scale Hg mining regions exhibited high concentrations of both THg and MeHg, which had ranges of 0.23–19 mg/kg and 0.06–5.7 mg/kg, respectively. Both correlated with the THg concentration of surface water. A positive correlation was observed between bodyweight and Hg concentration. Large variations in THg and MeHg occurred among different species, of which *O. triangulare* and *O. japonicum* showed extremely elevated THg and MeHg concentrations of 8.3 ± 4.3 mg/kg and 2.1 ± 1.4 mg/kg, respectively. Species with high Hg usually had high $\delta^{15}\text{N}$ values, indicating they occupy high trophic levels. The MeHg-to-THg ratios also exhibited large variations, ranging between 6.4% and 99.9%. Our findings indicate that dragonflies are sensitive to Hg pollution and can be considered a sentinel for environmental Hg contamination. High Hg concentrations in dragonflies may also represent a vector through which Hg is passed to vertebrates. Future studies should focus on the mechanisms of Hg accumulation in dragonflies, particularly of MeHg, as well as Hg transfer in the process of metamorphosis from larva to adult. In addition, the exposure risk to specialist dragonfly consumers, such as *Merops* songbirds, warrants greater attention.

Declarations

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Declaration of Interest

The authors declare that they have no actual or potential competing financial interests that could have influenced the work reported in the manuscript.

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Tables

Table 1 Comparisons of THg and MeHg concentrations in dragonflies from the study area

Location		N	Body weight (mg, dw)				THg Conc. (mg/kg, dw)				MeHg Conc. (mg/kg, dw)				MeHg (%)			
Area	Sites		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
XXR	WK	90	94.5	32	46.8	173	3.69	3.85	0.309	18.5	1.061	1.02	0.114	5.66	37.38	15.1	6.40	71
	ZJP	40	69.8	25.1	30.1	146	0.930	0.600	0.302	2.93	0.744	0.521	0.220	2.45	79.6	11.1	41.8	95
	BX	14	68.8	26.7	39.1	122	0.900	0.340	0.458	1.58	0.747	0.320	0.371	1.28	82.6	7.11	71.5	98
	BMP	27	84.9	19	48.2	126	1.02	0.920	0.201	4.11	0.705	0.873	0.122	3.81	62.9	24.4	27.5	95
	JX	8	90.3	16.5	62.4	113	0.370	0.210	0.139	0.720	0.316	0.194	0.061	0.642	84.6	18.8	39.8	99
	WW	59	84.3	46.7	26.3	191	0.822	0.370	0.231	1.63	0.712	0.322	0.207	1.41	86.7	6.10	72.5	98
AZR	MZX	58	112	34.6	46.1	207	1.69	1.22	0.237	4.77	0.931	0.621	0.178	2.20	60.9	16.6	16.1	99
	BGS	44	73	20.8	42.5	129	0.840	0.300	0.309	1.80	0.654	0.258	0.309	1.61	79.4	17.8	29.0	99
	AZ	42	61.1	38.1	26.4	170	0.771	0.330	0.232	1.69	0.528	0.288	0.131	1.59	69.3	19.2	39.0	98
	SCK	57	92.5	33.2	41.2	183	0.690	0.49	0.063	2.51	0.427	0.334	0.019	1.39	55.7	21.6	12.7	99
Total	-	439	86.1	36.0	26.3	207	1.52	2.20	0.063	18.5	0.751	0.652	0.019	5.66	64.5	23.6	6.40	99

Table 2 Inter-species and -genus comparisons of bodyweight, THg and MeHg concentrations, and MeHg ratio

Genus	Species	N	Body weight (mg, dw)				THg Conc. (mg/kg, dw)				MeHg Conc. (mg/kg, dw)				MeHg (%)			
			Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
<i>Acisoma</i>	<i>A. panorpoides</i>	3	39.4	8.80	29.3	44.9	0.951	0.040	0.931	1.00	0.882	0.041	0.839	0.912	89.6	0.1		
<i>Diplacodes</i>	<i>D. trivialis</i>	15	84.5	30.3	50.7	146	1.72	1.32	0.322	3.92	0.948	0.752	0.114	2.21	63.4	26		
<i>Orthetrum</i>	<i>O. albistylum</i>	37	128	34.0	46.8	175	1.83	1.54	0.231	6.41	0.947	0.568	0.131	2.23	62.9	21		
	<i>O. japonicum</i>	3	109	10.1	101	120	7.05	3.38	3.64	10.4	1.64	0.851	0.663	2.18	22.9	6.9		
	<i>O. melanium</i>	13	162	35.1	75.2	207	1.77	0.852	0.569	2.87	1.25	0.550	0.552	2.19	74.8	16		
	<i>O. pruinosum</i>	2	108	15.1	97.3	119	1.41	0.427	1.09	1.72	1.19	0.391	0.921	1.47	84.6	2.0		
	<i>O. sabina</i>	4	89.6	12.0	75.9	105	0.799	0.771	0.121	1.58	0.614	0.681	0.020	1.27	48.6	38		
	<i>O. triangulare</i>	21	136	19.5	87.7	173	8.27	4.28	0.351	18.5	2.12	1.35	0.285	5.66	29.8	17		
<i>Pantala</i>	<i>P. flavescens</i>	140	72.0	22.4	30.1	146	0.781	0.473	0.142	2.93	0.469	0.311	0.062	2.45	61.7	19		
<i>Pseudothemis</i>	<i>P. zonata</i>	12	78.1	13.3	61.2	101	5.28	2.03	2.77	8.52	1.52	0.611	0.804	2.85	29.1	7.0		
<i>Rhyothemis</i>	<i>R. fuliginosa</i>	5	68.9	6.70	59.6	76.0	0.908	0.310	0.479	1.21	0.751	0.221	0.469	0.942	83.8	8.4		
<i>Sympetrum</i>	<i>S. croceolum</i>	26	115	14.9	85.8	149	0.631	0.288	0.231	1.33	0.483	0.204	0.170	0.927	76.8	12		
	<i>S. kunckeli</i>	142	68.7	26.3	26.3	144	0.910	0.602	0.064	4.11	0.671	0.482	0.019	3.81	72.7	21		
	<i>S. speciosum</i>	10	120	21.3	79.3	149	0.821	0.603	0.312	2.04	0.542	0.221	0.311	0.954	78.4	23		
<i>Trithemis</i>	<i>T. aurora</i>	6	96.3	37.0	58.1	146	2.89	1.12	1.38	3.86	0.951	0.422	0.371	1.53	37.5	22		

Figures

Figure 1

Map of the sampling sites in the WMMA Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Boxplots of THg (a, d), MeHg (b, e) and MeHg/THg ratio (c, f) in dragonflies collected from the Aozhai and Xiaxi River watersheds (D. trivialis-D.t, O. albistylum-O.a, O. melanium-O.m, O. triangulare-O.t, P. flavescens-P.f, P. zonata-P.z, R. fuliginosa-R.f, S. croceolum-S.c, S. kunckeli-S.k, S. speciosum-S.s, and T. aurora-T.a)

Figure 3

Correlations between dragonfly bodyweights and THg/MeHg concentrations

Figure 4

Distributions of THg and MeHg in dragonflies and surface water with distance from upstream Hg pollution sources

Figure 5

δ¹⁵N values (‰) in species *P. flavescens* and *O. triangulare* (***) a significant difference at $P < 0.001$, ** indicates that at $P < 0.01$)

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