Environmental Pollution 208 (2016) 830–839

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Pollutant exposure at wastewater treatment works affects the detoxification organs of an urban adapter, the Banana Bat

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ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form
18 September 2015
Accepted 21 September 2015
Available online 18 November 2015

Keywords:
Neoromicia nana
Wastewater pollutants
Metallothionein
Hepatosomatic/renalsomatic index
Histopathology

ABSTRACT

The Banana Bat, *Neoromicia nana*, exploits pollution-tolerant chironomids at wastewater treatment works (WWTWs). We investigated how pollutant exposure impacts the detoxification organs, namely the liver and kidney of *N. nana*. (i) We performed SEM-EDS to quantify metal content and mineral nutrients, and found significant differences in essential metal (Fe and Zn) content in the liver, and significant differences in Cu and one mineral nutrient (K) in the kidneys. (ii) We performed histological analysis and found more histopathological lesions in detoxification organs of WWTW bats. (iii) We calculated hepatosomatic/renalsomatic indices (HSI/RSI) to investigate whole organ effects, and found significant increases in organ size at WWTWs. (iv) We quantified metallothionein 1E (MT1E), using Western Blot immunodetection. Contrary to predictions, we found no significant upregulation of MT1E in bats at WWTWs. Ultimately, *N. nana* exploiting WWTWs may suffer chronic health problems from sublethal damage to organs responsible for detoxifying pollutants.

1. Introduction

The global human population has been growing at an unprecedented rate and is projected to reach over 9 billion by the year 2050 (United Nations Population Fund, State of World Population (2014)). To accommodate the growing numbers, natural land is rapidly becoming urbanized. In fact, in India, China and Africa, urban land expansion rates have now exceeded or are equal to urban population growth rates (Seto et al., 2011). The typical infrastructure of cities is designed to cater for the needs of the urban population, with the most basic requirement being sanitation. Thus, a common physical feature of the urban landscape is wastewater treatment works (WWTWs). WWTWs receive industrial and household waste which is treated in large, open-top tanks (Gagnon and Saulnier, 2003). Wastewater treatment tanks usually contain particularly high levels of metals (Karvelas et al., 2003). In the UK, an estimated 12,508 tonnes of the toxic elements Cd, Cu, Cr, Ni, Pb and Zn is received at a typical urban WWTW per year (Crane et al., 2010). Despite advances in metal treatment techniques, the removal of certain metals such as copper and zinc at WWTWs, have shown little improvement in the last three decades (Crane et al., 2010). A prominent biotic characteristic of WWTWs is the proliferation of pollution-tolerant chironomid midge swarms (Boonstra et al., 2009). Chironomid midges often contain high concentrations of metal pollutants from WWTWs without showing adverse effects on survival (Krantzberg and Stokes, 1990). Predators that feed on these midges may however, accumulate metals in their tissues with acute or chronic effects on their health (Hare, 1992).

The Banana Bat, *Neoromicia nana*, is an urban adapter (Jung and Kalko, 2011; Monadjem et al., 2010) that exploits the swarms of pollution-tolerant chironomid midges that occur at WWTWs (Naidoo et al., 2013). At wastewater-polluted sites, we previously found that chironomid midges were the most abundant prey type in the diet of resident bats (Naidoo et al., 2013), compared to diverse insect diets at unpolluted sites (Naidoo et al., 2013; Schoeman and Jacobs, 2011). Significant correlations between insects captured and diet composition at the wastewater-polluted sites suggested that *N. nana* fed opportunistically on the abundant chironomid prey (Naidoo et al., 2013). There is a considerable body of literature showing the transfer of metals and other pollutants from chironomid prey to predators, including bats (Goodyear and McNeill, 1999; Lescord et al., 2015; Park et al., 2009; Reinhold et al., 1999; Timmermans et al., 1992). In addition, metal concentrations in the water at the WWTW sites were significantly correlated with the metal concentrations in the kidney tissue of the bats (Naidoo et al., 2013). We have previously shown that pollutant

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http://dx.doi.org/10.1016/j.envpol.2015.09.056
0269-7491/

exposure from this abundant food resource carries physiological costs for *N. nana*, specifically sub-lethal haematological and genotoxic responses (Naidoo et al., 2015). *N. nana* at WWTWs had significantly lower antioxidant capacity and significantly higher levels of DNA damage and haematocrits than bats from unpolluted sites (Naidoo et al., 2015). An accumulation of DNA damage, especially from double-stranded breaks as observed in *N. nana* at WWTWs, ultimately leads to tissue aberrations and disease (Jackson and Bartek, 2009). These longer-term effects may occur in various tissue types and organs in the body, however the organs where sub-lethal effects of chronic pollutant exposure would be most evident are the liver and kidneys (Clark and Shore, 2001).

The liver and kidneys are the main organs responsible for detoxification in the body. The liver has a wide range of functions including detoxification of the blood by excretion in bile, phagocytosis and chemical transformation of toxic molecules (Fox, 1991). The kidneys regulate the extracellular fluid environment in the body and the concentrations of waste products that are filtered from the blood and returned into circulation (Fox, 1991). Thus, when bats ingest pollutants including the metals found at WWTWs, they are either metabolized, excreted, accumulated or stored in a less toxic form (Baker et al., 2003). An accumulation of metals in the organs may, however, cause various types of tissue damage including inflammation, necrosis, hyperplasia or hypertrophy. These lesions in the tissue may further lead to altered organ size and impaired organ function (Ma, 1989).

Metallothionein 1 E (MT1E) is a protein produced primarily in the liver and kidney that protects against metal damage by binding to and detoxifying metal ions (Sakulsak, 2012). Metallothionein has a high affinity for non-essential metals such as Cd and Hg and some essential metals such as Zn and Cu, with its metal binding affinity in the order: Cd > Pb > Cu > Hg > Zn > Ag > Ni > Co (Waalkes et al., 1984). When the metal ions exceed metallothionein binding capacity, they may cause physical damage such as histopathological alterations (Goyer et al., 1989) to tissue as observed in both the liver and kidney (Sánchez-Chardi et al., 2009) of shrews (*Crocidura russula*) inhabiting an abandoned pyrite mining site. Thus, metallothionein is generally upregulated in animals exposed to excess metal levels (Dai et al., 2013; Sakulsak, 2012).

Metallothionein protein expression is however, highly species-specific (Henry et al., 1994). For instance, humans have metallothionein concentrations per gram of liver up to 100 times the levels of that found in rat and mouse (Henry et al., 1994). To date, of the limited number of reports of metallothionein levels in bats, Pikula et al. (2010) found that species and foraging habitat influences metallothionein content. Bats foraging in aquatic habitats had higher levels of metallothionein than bats foraging in terrestrial or terrestrial/aquatic-habitats (Pikula et al., 2010). Habitat quality and diet play a significant role in eliciting physiological coping responses. Thus, given that WWTWs form an integral part of the urban landscape and an important prey base for urban adapters, it is important to understand the potential sub-lethal effects in organs of *N. nana* foraging at these sites. Furthermore, the effect of exposure to the cocktail of pollutants at WWTWs has not been elucidated in wild bats or laboratory based studies.

The aim of our study was to therefore investigate how pollutant exposure impacts the detoxification organs, namely the liver and kidney of *N. nana* foraging at WWTWs: (i) We performed SEM-EDS metal imaging to quantify the content of metals and mineral nutrients in liver and kidney tissue; (ii) We performed histological analysis to investigate the extent of tissue damage in the detoxification organs; (iii) We calculated hepatosomatic/renalsomatic indices (HSI/RSI) to investigate whole organ effects and (iv) We quantified metallothionein 1E (MT1E) in the liver and kidney, using Western Blot immunodetection. We predicted that, compared to *N. nana* foraging at unpolluted sites, *N. nana* foraging at WWTWs should have (i) higher levels of toxic non-essential metals, (ii) a greater extent of histopathological lesions in the liver and kidney tissue, (iii) higher hepatosomatic/renalsomatic indices (characteristic of organ swelling due to metal damage (Ma, 1989)), and (iv) upregulated metallothionein protein content in the liver and kidney.

2. Methods

2.1. Sample collection

We captured *N. nana* bats at sludge tanks in three WWTWs within Durban, South Africa (S29°58′; E30°57′): Umbilo Wastewater Works (S29°50.44′; E30°53.31′), the Verulam Wastewater Works (S29°38.38′; E31°03.49′), and the Kingsburgh Wastewater Works (S30°04.29′; E30°51.26′) (Fig. 1). These WWTWs use open-top sludge tank systems that contain high levels of wastewater–associated metals (lead, cadmium, chromium, nickel, copper, zinc and iron; Naidoo et al., 2013). We captured *N. nana* bats at two unpolluted reference sites in the forest of Umdoni Park (S30°41.15′; E30°23.35′), Pennington about 80 km south of Durban (Fig. 1). Umdoni Park covers an area of 210 ha comprising mainly dense coastal forest representative of the Indian Ocean Coastal Belt biome (Mucina et al., 2006). There are no WWTWs located in the immediate vicinity of the park, with the closest WWTWs situated >8 km away. We sampled two sites within the forest: Unpolluted site 1 (S30°40.36′; E30°23.31′), located close to the border of the park, and unpolluted site 2 (S30°41.15′; E30°23.35′) located further inside the park. Because *N. nana* has a relatively small home range – 300 m from the roost (LaVal and LaVal, 1977) – individual turnover between unpolluted sites and contamination from the nearest WWTWs was unlikely.

We used mist nets and harp traps to capture bats at the sites during the summer (January–March 2013). We recorded their sex,

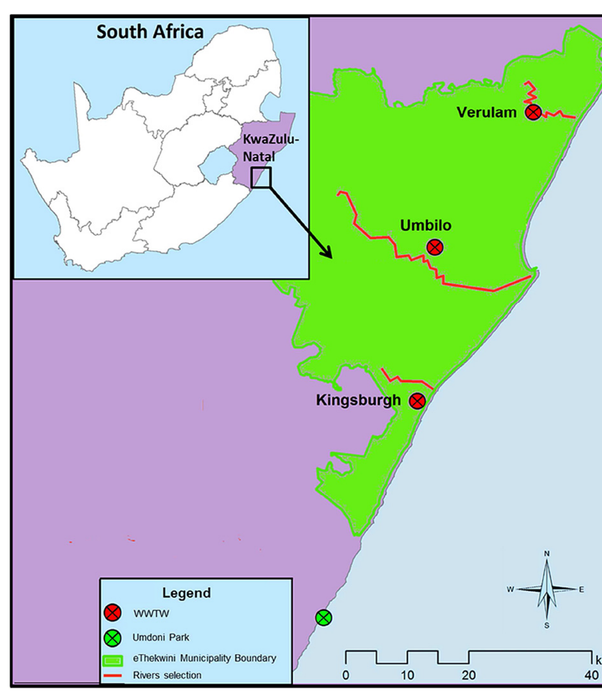

Fig. 1. Map of the study area in Durban, South Africa, showing the location of the Verulam, Kingsburgh and Umbilo Wastewater Works and Umdoni Park (Unpolluted sites 1 and 2).