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Metals and metalloids in blood and feathers of common moorhens (*Gallinula chloropus*) from wetlands that receive treated wastewater☆

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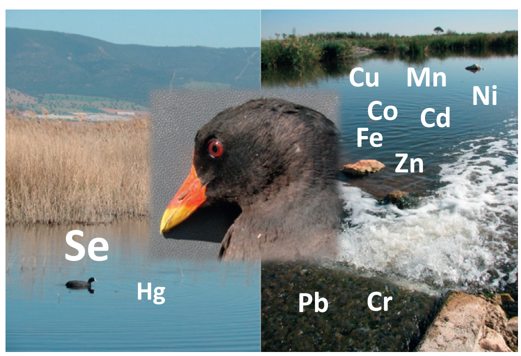
HIGHLIGHTS

- Higher sediment metal levels were found in Navaseca that receives sewage effluent.
- Birds from Navaseca did not show higher levels of metals in blood and feathers.
- Sediment of Tablas de Daimiel showed some points with elevated Se levels.
- 24% of moorhens from Tablas exhibited Se levels associated with toxicity in birds.

GRAPHICAL ABSTRACT



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ABSTRACT

We addressed the hypothesis that birds in eutrophic wetlands receiving wastewater treatment plant (WWTP) effluents are exposed to high levels of metals and metalloids and this may drive an ecological trap in some species attracted to these highly productive ecosystems. Levels of metals and metalloids were determined in sediment and in blood and feathers of common moorhens (*Gallinula chloropus*) from two wetlands in Central Spain: Navaseca Pond, which receives directly the effluent of a WWTP; and Tablas de Daimiel National Park, which is a floodplain less affected by urban discharges. Sediment concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb were higher in Navaseca Pond than in Tablas de Daimiel; only Se was higher in Tablas de Daimiel than in Navaseca. Blood levels of Hg and Se were higher in moorhens from Tablas de Daimiel than those from Navaseca. In the case of Hg these levels were below the threshold of adverse effect, but Se levels in 24% of moorhens from Tablas de Daimiel were above the threshold value associated with Se toxicity in birds (1000 ng/mL). In feathers, Hg, Se, Mn, Cu and As levels were higher in Tablas de Daimiel than in Navaseca. Body condition of moorhens was negatively associated with blood Se levels in the moorhens from Tablas de Daimiel. We can reject the hypothesis of a higher accumulation of metals and metalloids in birds associated with the WWTP effluent, but Se levels may need further research considering the nature of the floodplain of Tablas de Daimiel National Park.

1. Introduction

Wetlands are ecosystems with an important value because they provide benefits and services for the maintenance and conservation of the biodiversity and have an important role as carbon traps (Turner et al.,

☆ Capture and sample collection of wild birds were authorized by the Regional Government of Castilla-La Mancha (Ref JCCM 49/13 and 46/14).

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2000). Natural wetlands occupy between 700 and 1024 million hectares worldwide (Mitsch and Gossilink, 2000), which represents only 4.6% of the global land mass (Jiménez-Ballesta et al., 2016). Several human actions like the increased demand for land and water for agriculture or livestock production and the creation of infrastructures are threatening the conservation of wetlands worldwide (Cazcarro et al., 2016). The conservation of wetlands in Spain is particularly difficult in arid and semi-arid zones of the Center and South of the country because of conflicts for water resources demanded for crop production (Casado et al., 1992). The excessive use of groundwater for agriculture together with the pollution caused by poorly treated sewage and the run-off of fertilizers seriously threaten Doñana National Park and Tablas de Daimiel National Park (TDNP) (Green et al., 2017).

An important source of contamination in wetlands is the inflow of poorly treated sewage water of urban and industrial origin. This is especially relevant in water systems with reduced capacity to dilute contamination, as is the case for many Mediterranean rivers. Moreover, the use of wastewater treatment plant (WWTP) effluents for the maintenance of wetlands is a common practice in flat semiarid regions in Spain (Navarro et al., 2011). In fact, these natural wetlands are used as a tertiary treatment of WWTP effluents and even become the final receivers of the treated water in semiarid flat areas with no rivers around. The wetlands that receive the effluents of WWTP normally tend to eutrophication and, at some stages, to a high productivity that makes these sites very attractive for waterbirds that feed on aquatic invertebrates (Okafor, 2011). This is the case of Navaseca Pond, a seminatural wetland located close to TDNP, which is permanently flooded with the WWTP effluent of the town of Daimiel (Anza et al., 2014). However, these wetlands also tend to concentrate a wide range of urban and industrial contaminants (Bueno et al., 2012; Rivetti et al., 2017), so birds attracted to these wetlands could fall into an ecological trap driven by contaminants, a concept barely explored yet in the field of wildlife toxicology (Huang et al., 2018), but commonly used in the study of other anthropogenic impacts on wildlife (Robertson et al., 2013).

Among the chemical contaminants entering wetlands, metals and metalloids are especially relevant because of their persistence and capacity to enter the food chain by diverse routes. Anthropogenic sources of pollution can increase the natural concentrations of metals and metalloids present in the sediment of wetlands originating in the geologic sources located within the same hydrological basin (Casas et al., 2003). Jiménez-Ballesta et al. (2016) found elevated concentrations of Cu, Se, Sn, Sb, Pb in TDNP soils compared with background levels of the region of Castilla-La Mancha (Jiménez-Ballesta et al., 2010). Jiménez-Ballesta et al. (2016) attributed the high content of some of these elements to the use of fertilizers, the municipal waste disposal and urban WWTP effluent discharged into TDNP. Sewage discharges into the Azuer, Cigüela and Guadiana rivers from villages located upstream TDNP have had consequences in the quality of the water of this floodplain (Cirujano et al., 1996; Berzas et al., 2000; Sanchez-Ramos et al., 2016), and we may expect a larger impact on wetlands flooded with WWTP effluents. In fact, Rivetti et al. (2017) detected much higher levels of microcontaminants (i.e. triazoles, polycyclic aromatic hydrocarbons, organophosphorus flame retardants, alkylphenols and parabens) in water of Navaseca Pond than in TDNP.

However, there is not yet an ecotoxicological assessment of the effects on birds of the contaminants accumulated in semiarid wetlands that are permanently supplied with WWTP effluents like Navaseca Pond. In this study, we determined the levels of metals and metalloids in sediments and in blood and feather samples of common moorhens (*Gallinula chloropus*) from two wetlands that receive the effluents from WWTPs at different intensities. Our hypothesis is that birds from a wetland receiving directly the WWTP effluent accumulate higher concentrations of metals and metalloids than those from TDNP and this could drive a mechanism of an ecological trap for some species because of their attraction to eutrophic wetlands in the case that these contaminants produce adverse effects on birds.

2. Materials and methods

2.1. Study area

Samples were taken in two wetlands in Castilla-La Mancha (C Spain; Fig. 1). One of these sites is the floodplain of TDNP, located at the junction of Cigüela and Guadiana rivers, with a maximum flooded surface of 2000 ha. TDNP is declared a Biosphere Reserve (MAB Programme, UNESCO), it is listed as a Wetland of International Importance by the Ramsar Convention and it was recognised as a Special Protection Area for Birds in 1988 (Directive 79/409/CEE). TDNP occasionally receives the input of poorly treated wastewater from towns located upstream, but the levels of organic contaminants in water are low (Rivetti et al., 2017). The other study site is Navaseca Pond (24.3 ha), which is located at 6.5 km from TDNP and only overflows into Guadiana river during exceptional heavy rains. This wetland was a seasonal pond in the past, but now it is permanently flooded and highly eutrophic because it receives the effluents of the WWTP of Daimiel town (Anza et al., 2014).

2.2. Sediment sampling

Sediments from TDNP and Navaseca Pond were collected in flooded areas 1 m from the shoreline. Sediment samples were collected with a core sampler (Ø = 33.5 mm) and the upper 5 cm were stored in polypropylene flasks at −4 °C before treatment. Sediment samples were air-dried at ambient temperature, stones and plant debris were removed by sieving with a 2 mm steel mesh, and then mixed thoroughly to obtain a representative sample. Samples were manually ground with a Teflon hammer in a mortar and then finally sieved through a 0.5 mm stainless steel mesh.

2.3. Bird sampling

The studied species was the common moorhen, which is a medium-sized member of the family Rallidae with worldwide distribution (i.e. North and South America, Africa, Europe and Asia) in aquatic environments with its relative and conspecific until recently *G. galeata*. Moorhens feed on plants (sedges, reeds, cattails, submerged plants, various sprouts, fruit and cereals) and animals (predominantly aquatic invertebrates, but also small vertebrates and even carrion) (Taylor et al., 2018). The widespread distribution of common moorhens, its sedentary habits and its omnivorous diet make this species appropriate as a bioindicator of pollution in wetlands. Bird trapping was done between October 2013 and March 2015 by using funnel traps on the shore of the lake where groups of moorhens usually feed. In total, 137 moorhens were captured and marked with a metal ring, weighted, and their tarsus and wing length measured. Age (juvenile or adult) was determined using plumage criteria (Baker, 1993) and the sex was determined in whole blood with a conventional PCR (Phusion Blood Direct PCR Kit from Thermo Scientific, see details in supplementary material). Blood from jugular vein (1 mL in heparin) and secondary wing feathers were taken for the analysis of contaminants. Blood was stored at −80 °C and feathers were stored in plastic bags at −20 °C. The body condition of moorhens was calculated according to the Scaled Mass Index (SMI) proposed by Peig and Green (2009) with wing length as a measure of body size. Capture and sample collection of wild birds were authorized by the Regional Government of Castilla-La Mancha (Ref JCCM 49/13 and 46/14).

2.4. Analysis of metals and metalloids

Sediment samples were subjected to acid digestion using the methodology described in Rodríguez-Estival et al. (2012). Each sediment sample (0.2 g) was digested with 2 mL of nitric acid (68% Normapur, Panreac) in a glass tube (Pyrex) heated in a thermoblock (Micro, Selecta) with an electronic temperature controller (RAT-s, Selecta). The tubes were heated at 70 °C for 5 h, then 105 °C for an additional