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Patterns of presence and concentration of pesticides in fish and waters of the Júcar River (Eastern Spain)

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HIGHLIGHTS

- Forty currently used pesticides were analyzed in fish by QuEChERS coupled with LC-MS/MS.
- Up to 23 pesticides were found along the Júcar River in fish and water samples.
- Eight pesticides, some of them forbidden in the EU, were in water.
- Most pesticides detected related to crop and livestock treatments in the floodplains.
- Different degrees of bio-concentration in fish, depending on the species and compound.

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ABSTRACT

The Júcar River, in a typical Mediterranean Basin, is expected to suffer a decline in water quality and quantity as a consequence of the climate change. This study is focused on the presence and distribution of pesticides in water and fish, using the first extensive optimization and application of the QuEChERS method to determine pesticides in freshwater fish. Majority pesticides in water – in terms of presence and concentration – were dichlofenthion, chlorfenvinphos, imazalil, pyriproxyfen and prochloraz (associated with a frequent use in farming activities), as well as buprofezin, chlorpyriphos and hexythiazox. In fish, the main compounds were azinphos-ethyl, chlorpyriphos, diazinon, dimethoate and ethion. The analysis of bio-concentration in fish indicated differences by species. The maximum average concentration was detected in European eel (a critically endangered fish species). The wide presence of pesticides in water and fish suggests potential severe effects on fish populations and other biota in future scenarios of climate change, in a river basin with several endemic and endangered fish species. The potential effects of pesticides in combination with multiple stressors require further research to prioritize the management of specific chemicals and suggest effective restoration actions at the basin scale.

1. Introduction

Rivers around the world are threatened by socioeconomic drivers that degrade environmental conditions by altering land use and climate, thereby affecting hydrology and water quality [1,2]. Climate change and human use both pose threats to the flow regime of water ecosystems, and altered flow regimes can have a high impact on the ecological and chemical status of waters [3]. In order to repair this situation, the European Parliament established the Water Framework Directive in 2000. Its ultimate objective is to achieve "good ecological and chemical status" for all Community waters by 2015. For this, priority substances (some of them pesticides) to be monitored and their limits have been established to control the pollution in surface waters [4].

However, the first round of the River Basin Management Plans in the EU show that more than half of Europe's surface water bodies are in less than good ecological status, and the reports about the Habitat Directive indicate that over two thirds of all river and lake habitats and inland water species are in unfavourable conservation status [3]. Furthermore, some regions of the EU are at risk of water scarcity, and the water ecosystems services upon which society depends may become more vulnerable to extreme events such as floods and droughts [5].

In the Júcar River basin (Spain), the last nationwide report on climate change estimated a 10–25% reduction of the mean annual flow [6], which indicates potential notable effects on water availability. Therefore, a reduction of water quality, which would produce severe risks for the ecosystem integrity, is probable [7]. Von der Ohe et al. [8] analyzed waters in four European rivers (including the

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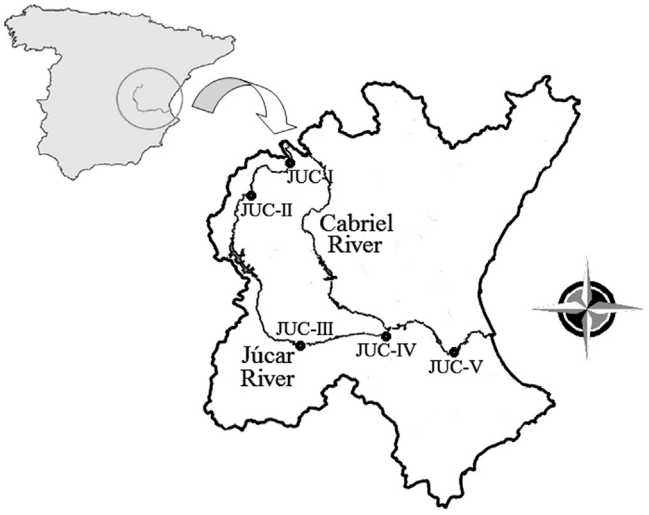

Fig. 1. Location of the Júcar River Basin (Eastern Spain) and the five sampling sites along the Júcar River.

Llobregat River in Spain), reporting that most of the high and very high risk substances detected were pesticides (74%). They reported that pollution with organic chemicals is a Europe-wide problem.

In a previous study on contaminants in Spain, different pesticides were detected, in the Duero, Ebro and Miño River basins (in decreasing order of quantity and concentrations) [9]. However, a review on the monitoring programs indicated that the analytical methods for most compounds were not sufficiently developed to consistently detect their often very low concentrations in the environment [10]. This lack of unified sample preparation and analytical methods in environmental matrices other than water and in particular in biota has been widely remarked in several reviews [11,12]. As a quick, easy, cheap, effective, rugged and safe sample preparation method, the QuEChERS method has attracted great attention for pesticide residue determination in fruit and vegetables. Recently, QuEChERS method was also applied on fish to detect pyrethrin and pyrethroid pesticides [13], as well as for the most commonly applied pesticides for cereals and oleaginous crops in France [14]. However, complementary research is needed to determine a wider range of pesticides in fish.

In this context, the aims of this study were: (i) to test the effectiveness of the QuEChERS method for determining the presence and concentration of pesticides in freshwater fish; (ii) to establish general patterns of presence and concentration of pesticides in water and fish along the Júcar River; and (iii) to assess the potential risk for the health of freshwater fish species, based on bio-concentration and fish condition. This is to our knowledge the first study that simultaneously monitors a large number of pesticides in both water and fish.

2. Materials and methods

2.1. Study area and sampling

The Júcar River is 497.5 km long and its mean annual flow is 10 m^3/s; it flows through three provinces (Teruel, Cuenca and Valencia) in Eastern Spain, under a typical Mediterranean climate. Sampling was performed at five sites distributed along the main stream of the Júcar River (Fig. 1) in October 2010. The site (JUC-I) is located at the basin headwaters, showing the natural flow regime. In the other sites, a great percentage of flat lands are dedicated to agriculture and the river flow is regulated by small and large dams.

The sampling was carried out, as much as possible, following the Environmental Quality Standards Directive 2008/105/EC (EQSD) [15]. October was the month selected for several reasons, (i) it coincides with the end of the growing season period, which is the appropriate for monitoring of fish, and (ii) there are not very recent applications of pesticides, which allow to establish what pesticides are constantly present in the environment because its capacity of accumulation and/or its persistence.

Physical and chemical characteristics of water (temperature, pH, total soluble salts, dissolved O_2 and redox potential) were recorded at the sampling sites using a Multiparameter Eutech Instrument CyberScan PCD 650 (Thermo Fisher Scientific, Basel, Switzerland). Water samples were collected in glass bottles (2.5 L) and transferred immediately to the laboratory for analysis. The samples were stored at 4 °C for no more than 10 days before analysis. Five hundred millilitres of water samples were filtered to remove any floating or insoluble materials.

Fish were sampled using electrofishing for approximately 1 h at each site, with standard equipment, following the recommendations of the Norm UNE-EN 14011:2003 regarding sampling of fish with electricity. This norm states that in general the sampling should take place at the end of the growth period, when the juveniles are large enough to be captured by electrofishing. In this river, the best time approximately corresponds to October, although water temperature differs from the upper to lower study sites. Accordingly, a sampling campaign was carried out by the Water Authority of the Jucar River Basin in October 2010, in order to monitor pesticides concentration in fish; such data allowed the comparison of results. The sampling in water was performed in the same month to show potential relations between concentrations in fish and water.

According to the aforementioned European norm, the weight (g) and fork length (mm) of each fish were measured in the field. In total, 172 individuals belonging to nine fish species were collected. The different fish species were distributed as follows. In JUC-I: Iberian gudgeon (n = 8) and brown trout (n = 9); in JUC-II, Iberian gudgeon (n = 24), brown trout (n = 2) and Iberian nase (n = 6); in JUC-III, Iberian gudgeon (n = 28) and largemouth bass (n = 6); in JUC-IV, European eel (n = 3), bleak (n = 4), pumpkinseed (n = 1), Iberian gudgeon (n = 14), Eastern Iberian barbel (n = 1) and largemouth bass (n = 5); in JUC-V, Iberian gudgeon (n = 7), pumpkinseed (n = 1), bleak (n = 27), northern pike (n = 2), largemouth bass (n = 2), European eel (n = 13) and Eastern Iberian barbel (n = 6).

The collected fish samples were transported to the laboratory in a cool-box and classified depending on the site and species. Then, the entire fishes were grinded using a Oster BPST02-B00 (London, United Kingdom). The wet weights were recorded and fish samples then stored in aluminium wrappers, freeze-dried at −80 °C and lyophilized.

2.2. Extraction procedures

The full list of chemicals and reagents used, as well as the pesticides selected as target compounds are provided in the Supplementary Material (Table S1). Very briefly, water samples were extracted by solid-phase extraction (SPE) with Oasis HLB cartridge using a previously published procedure [16]. The limits of detection (LODs) and quantification (LOQ) ranged from 0.1 to 2 ng/L and from 0.3 to 6 ng/L, respectively, depending on the pesticides. Calibrations curves were linear in the concentration range of 10 ng/L to 10 μg/L and the matrix effect was always ≤20%. Recoveries varied from 48.50% to 70% and precision was below 20% for all pesticide.

The fish samples were prepared with the modified QuEChERS method. Two grams of lyophilized fish were placed in a 50 ml Falcon tube and added with 8 ml of H_2O MiliQ and 15 ml of acetonitrile and shaken vigorously for 30 s. Six grams of magnesium sulphate ($MgSO_4$) and 1.5 g of sodium chloride (NaCl) were then added and the tube was shaken again for 1 min. The tube was