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Case Report

First report of a fish kill episode caused by pyrethroids in Italian freshwater

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ABSTRACT

Introduction: Fish kills are events of strong emotional impact on the population because of the frequent suspicion that they can be the result of serious pollution accidents. As a matter of fact, they are often due to natural occurrences, such as low levels of dissolved oxygen in the water, but in many cases the causes remain unknown. Fish are particularly sensitive to pesticides and pyrethroids are reported to be the most ecotoxicologically active in the aquatic environment. Nevertheless, the reported cases of massive wild fish mortalities due to these toxicants are very few. This paper describes a fish kill episode occurred in the Padua Province (Veneto Region — North Eastern Italy) which involved several fish species and for which it was possible to identify the cause in the presence of pyrethroids in the water.
Case presentation: When a whitish liquid coming from the rainwater drain of an industrial area was seen to be spilling into a drainage channel, a fish massive mortality was noticed and investigated. The collected water samples showed the presence of relevant concentrations of cypermethrin, permethrin, deltamethrin and tetramethrin. Analyses on the fish tissues revealed the presence of cypermethrin and permethrin at a concentration range of 476–2834 μg/kg and 346–2826 μg/kg on a lipid basis, respectively.
Discussion: According to the results of the performed analyses, we can reasonably state that the described episode had been caused by the exposure of biota to high concentrations of pyrethroids. The present case report significantly contributes to the limited literature available on pesticides-related fish kills. Moreover, it highlights the importance of sharing protocols for fish kill management at a national level, as this would help to better define the roles of the different institutions involved and to improve the investigation and the reporting of these events.

1. Introduction

A fish kill is defined as the sudden and unusual death of non-mammalian wild aquatic animals in terms of either number or type of subjects involved [1].

These events may cause a significant economic loss, both for the depletion of wild fish stocks important for commercial and recreational fishery activities and for the carcasses disposal costs; in addition, they can represent a sanitary threat for human health and cause environmental degradation [2].

Fish kills, both in fresh and salted waters, are events of strong emotional impact as they are believed to be the result of serious pollution accidents [3]. Instead, these events are mostly due to natural biological occurrences such as low levels of dissolved oxygen in the water [2,3]. This often happens during sustained periods of hot weather, coupled with low-flow conditions, when the total demand for oxygen by biological and chemical processes in the water medium exceeds the oxygen input by aeration and photosynthesis [4]. Other frequent causes of fish mortality are the proliferation of toxic algal blooms [5–8] and fish diseases as well [9–13]. Fish are particularly sensitive to chemical pollutants and many fish kill events could be caused by toxic substances. As a matter of fact, there are different kinds of insecticides that, if accidentally or maliciously introduced into aquatic habitats, may harmfully interact with biochemical and physiological processes of

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the biota leading to massive fish deaths [14–17]. Among the many existing pesticides, pyrethroids are reported to be the most ecotoxicologically active in the aquatic environment [18]. They have been shown to be, at comparable levels, up to 1000 times more toxic to fish than to mammals and birds [19,20] and they result to be lethal even at minimum concentrations [17,21]. Pyrethroids toxicity is mainly caused by their interaction with the sodium channels of cells, but chloride and calcium channels are also affected. The extension of their opening and closing time results in a sustained excited state of the cell membrane and of the nervous system. Moreover, some pyrethroids have an effect on the gamma-aminobutyrric acid (GABA) receptors in the nervous filaments [20,22,23].

At the heart of the matter is the importance of identifying what causes fish kills, which would allow to better manage fish populations [2] and to protect the environment. However, investigating the causes of fish kill incidents can often prove to be a complicated matter. When these events occur, different agencies might get involved in the investigation process, even within individual jurisdictions [1], with a consequent overlapping of skills and roles. Furthermore, the frequent lack of prompt intervention, the collection of samples in poor conditions or incomplete data collection, frequently lead to a failure in the identification of the triggering cause. The lack of diagnostic findings and the effect on the public opinion contribute to demotivate all the actors involved in the management of these events. In Italy this happens quite frequently, as there is only one Region, namely Tuscany, which has implemented and officially adopted proper procedures for managing fish kills (Decreto Regionale no. 6481, 15/12/2009).

This paper describes a case of massive fish mortality in the Padua Province (Veneto Region — North Eastern Italy), which involved several fish species and for which the possible cause was identified as being the presence of pyrethroids in the water.

2. Analytical methods for pyrethroids determination

2.1. Water samples

2.1.1. Samples preparation

Water samples (1 mL), previously filtered by means of 0.22 μm nylon disposable filter, were added with 10 μL of Triphenylphosphate 50 μg/L ACN-formic acid 9:1 ISTD solution, and then analysed by UPLC/MSMS system. Two spiked (0.05 $\mu g\,L^{-1}$) blank samples and one blank sample were included in each analytical batch.

2.1.2. Apparatus

UPLC separation was carried out by means of a Shimadzu UFLC XR-20AC UPLC System equipped with a binary solvent manager and a sample manager. The column was a Supelco Ascentis Express RP-Amide 2.6 μm, 150 mm × 2.1 mm i.d. The flow rate was 0.45 $mL\,min^{-1}$ with an injection volume of 100 μL. The mobile phase was composed as follow: phase A — aqueous ammonium formate (315 mg ammonium formate + 500 μl formic acid made up to 1000 mL with deionised water); phase B — HPLC grade methanol. Gradient conditions are reported in Supporting information 1.

Mass spectrometric analysis was performed on a TQD tandem mass spectrometer Applied Biosystem API 4000 operating in ESI positive ion mode (desolvation temperature 350 °C, capillary voltage 55 KV). MS/MS optimized conditions for each analyte are listed in Supporting information 2.

The quantification of analytes was carried out using a matrix-matched calibration curve in the concentration range of 0.05 to 1.00 μg/L. The estimated limit of quantification (LOQ) was found to be 0.05 μg/L for each analyte.

2.2. Fish samples

2.2.1. Sample preparation

Target tissues (muscle, liver, gonads), were homogenised and weighed for analysis. 10 g of homogenised sample was added of 40 mL of cyclohexane-dichloromethane 50:50 *v/v* organic mixture and then extracted by means of automatic stirring; this procedure was repeated three times. The assembled organic phase was filtered through sodium sulphate, after which the solvent had evaporated. The lipid content was determined by weighing.

2.2.2. GPC clean up

1 mL of fat solution (1 g of fat dissolved in 5 mL of cyclohexane-dichloromethane 50:50 *v/v* organic mixture) was injected into a Gel Permeation Chromatograph (Dedicated Sample Cleanup System GPC12S LabService Analytica), equipped with glass column 50 × 1ϕ cm packed with 15 g Bio Beads SX3 200–400 mesh (BioRad Laboratories); mobile phase: cyclohexane-dichloromethane 50:50 *v/v;* flow rate: 1 mL/min; dumping cycle: 17 min; collecting cycle: 18 min; washing cycle: 5 min.

After evaporation of eluate under a gentle stream of nitrogen, the residue was finally re-dissolved in 200 μl of *n*-hexane.

2.2.3. GC–μECD analysis

An Agilent 6890 Plus gaschromatograph equipped with double μECD ^{63}Ni detectors, split/splitless injection port and an 7683B autosampler injector was used. Capillary column apparatus: DB5 cross-linked 5% phenyl methyl polysiloxane (30 m × 0.25 mm i.d. × 0.25 μm f.t.) and DB1701 cross-linked 14% cyanopropylphenyl methyl polysiloxane (30 mt × 0.25 mm i.d. × 0.25 μm f.t.). Both columns were connected to a fused deactivated silica "Y" union connector splitter; the splitter to injector connection was made with a Retention Gap 5 m × 0.25 mm i.d. uncoated guard column.

GC system was operated under the following conditions: 2 μl injected splitless, splitless time 1 min. Injector held at 240 °C. Temperature programme: 60 °C for 2 min, 80 °C/min to 230 °C, 7 °C/min to 280 °C, 3 °C/min to 300 °C held for 15 min. Total run time 32.93 min. Detectors temperature 310 °C. Helium served as carrier gas at 3.5 mL/min. Quantification of all target analytes according to their peak area was performed using a three point calibration curve (25–100–200 ng/ml). Each sample batch was analysed concurrently with control samples (spiked blank samples). Recoveries of the considered compound were found to be between 70 and 120%; limit of quantification (LOQ) was 25 ng/g for each analyte.

3. Case presentation

The episode hereinafter described occurred in October 2014, over a stretch of about 6 km along the Sorgaglia drainage Channel, which flows in the south of Padua Province (Veneto Region, north eastern Italy — Fig. 1), in an area traditionally devoted to intensive agriculture activities and where nowadays many industrial facilities are located. Therefore, this channel receives not only the water coming from many other farmland drainages, but also the stormwater runoff from different squares and parking of the nearby industrial areas and the wastewaters of a water treatment plant. There are no specific studies or information concerning the fish species living in the Sorgaglia Channel, but they are likely to be the same of other rivers nearby, such as: Common Bleak (*Alburnus alburnus*), Common Rudd (*Scardinius eritrophtalmus*), Crucian carp (*Carassius carassius*), Common carp (*Cyprinus carpio*), Stone moroko (*Pseudorasbora parva*), European bitterling (*Rhodeus amarus*), Sheatfish (*Silurus glanis*), Black Bullhead Catfish (*Ameiurus melas*), Pumpkinseed (*Lepomis gibbosus*), Tench (*Tinca tinca*) and Eastern mosquitofish (*Gambusia holbrooki*).