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Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: Compound prioritization, mixture characterization and relationships with biological descriptors

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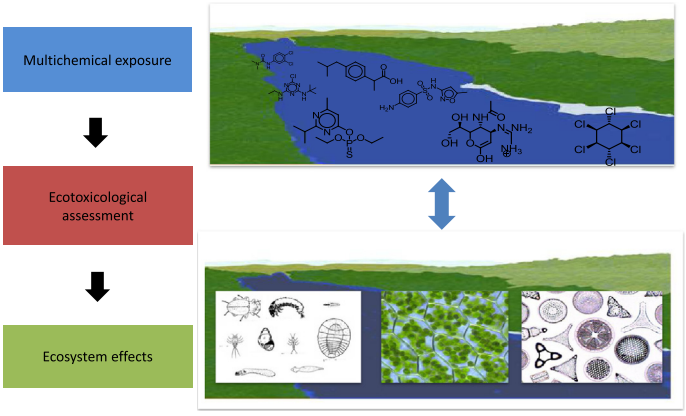
HIGHLIGHTS

- Ecosystem biological variables are correlated with toxic units' related parameters.
- Relative contribution of pharmaceuticals and pesticides to total ecotoxic pressure is site dependent.
- Environmental pollutant mixtures are characterized according to the lognormal distribution of the constituents' toxic units.
- Occurring pollutants are prioritized using toxic units as ecotoxicological criteria.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemical pollution is typically characterized by exposure to multiple rather than to single or a limited number of compounds. Parent compounds, transformation products and other non-targeted compounds yield mixtures whose composition can only be partially identified by monitoring, while a substantial proportion remains unknown. In this context, risk assessment based on the application of additive ecotoxicity models, such as concentration addition (CA), is rendered somewhat misleading. Here, we show that ecotoxicity risk information can be better understood upon consideration of the probabilistic distribution of risk among the different compounds. Toxic units of the compounds identified in a sample fit a lognormal probability distribution. The parameters characterizing this distribution (mean and standard deviation) provide information which can be tentatively interpreted as a measure of the toxic load and its apportionment among the constituents in the mixture (here interpreted as mixture complexity). Furthermore, they provide information for compound prioritization tailored to each site and enable prediction of some of the functional and structural biological variables associated with the receiving ecosystem. The proposed approach was tested in the Llobregat River basin (NE Spain) using exposure and toxicity data (algae and *Daphnia*) corresponding to 29 pharmaceuticals and 22

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pesticides, and 5 structural and functional biological descriptors related to benthic macroinvertebrates (diversity, biomass) and biofilm metrics (diatom quality, chlorophyll-a content and photosynthetic capacity). Aggregated toxic units based on *Daphnia* and algae bioassays provided a good indication of the pollution pattern of the Llobregat River basin. Relative contribution of pesticides and pharmaceuticals to total toxic load was variable and highly site dependent, the latter group tending to increase its contribution in urban areas. Contaminated sites' toxic load was typically dominated by fewer compounds as compared to cleaner sites where more compounds contribute.

1. Introduction

Pollution in surface waters is considered one of the main causes of impairment of aquatic ecosystems and biodiversity loss (Vorosmarty et al., 2010). Advances in environmental chemistry (Barceló and Petrovic, 2007) have shown that typical environmental scenarios are characterized by continuous exposure to many contaminants at low doses, except in specific situations (i.e., accidental spills). This is due not only to the high number of chemicals used that can potentially be released into the environment (Muir and Howard, 2006), but also to the biotic and abiotic transformations that they may undergo once there. Therefore, parent compounds, transformation products and other non-targeted compounds yield complex mixtures whose composition can only be partially identified by monitoring, while a substantial proportion essentially remains unknown (Daughton, 2004; Hendricks, 2013). Of special concern is the fact that a non-negligible number of pollutants is constituted by bio-active compounds (i.e., pesticides, pharmaceuticals, illicit drugs, endocrine disruptors, etc.) which are continuously released into the environment and whose long-term effects on the receiving ecosystems are relatively unknown. In this scenario, mutual interactions (synergistic/antagonistic) among the co-occurring compounds can also take place, further complicating environmental assessment.

Understanding these interactions and their effects on the biota requires addressing the toxicity of pollutant mixtures. There are currently two accepted models for ecotoxicity prediction, respectively termed *concentration addition* (CA) (Loewe and Muischnek, 1926) and *independent action* (IA) (Bliss, 1939). These are considered to account for the most general relationships between the effects of single substances and those of their corresponding mixtures. Use of these models allows calculation of the toxicity of a mixture on the basis of known toxicities of the mixture's individual components (Barata et al., 2006). The CA model is founded on the assumption that the mixture components possess a similar toxicological mode of action which is thus applicable to toxic substances that have the same molecular target site, whereas the IA model assumes that the compounds present in the mixture have dissimilar mechanisms of action. Although both of them have been proven acceptable if the corresponding mechanistic assumptions are fulfilled, the exact modes of action are often unknown for the majority of compounds, and both CA and IA must be regarded as two special extreme cases (Backhaus et al., 2003; Vighi et al., 2003; Backhaus and Faust, 2012) useful to set up a "prediction window", as stated by Altenburger et al. (2004). In practice, they have both been applied. The results obtained with the two models are not very different, CA tending to overestimate toxicity in controlled experiments as compared to IA (Junghans et al., 2006; Altenburger et al., 2004). Furthermore, Backhaus and Faust (2012) quantified the maximum possible ratio between the mixture EC50s predicted by CA and IA models (see Section 2.2.). Because of this, and its simplicity as regards calculation, CA is often preferred and constitutes a recommended first step on a tiered process (Backhaus and Faust, 2012). De Zwart and Posthuma (2005) have extended the foregoing concepts by using the so called multi-substance Potentially Affected Fraction (msPAF), which is defined as the fraction of taxa in a community that would potentially suffer from exposure to a local mixture of toxicants. It is a statistical approach based on the Species Sensitivity Distribution (SSD) method (Posthuma et al., 2002), where the msPAFs express the acute toxic pressure of whole mixtures of contaminants and estimate the fractions of taxa that would potentially be affected by local mixtures.

However, our ignorance concerning the exact composition of environmental mixtures poses a severe limitation on the application of CA to real cases. Our approach is based on the assumption that monitoring provides, at most, a statistical representation of the sample. Rather than aiming to predict the exact ecotoxicity value of the mixture (where a major part is unknown) (Hendricks, 2013), we can only describe how a compound's ecotoxicity is statistically distributed. This seems a reasonable assumption at least for transformation products (i.e., unknown products related to the parent compounds monitored). In the present study, we sought to extend the scope of the CA model by exploring this direction.

Here, we define a procedure whereby the compounds identified in a sample are ranked in descending order according to their toxic load expressed in terms of toxic units (Sprague, 1970), and their distribution is characterized. The corresponding parameters obtained after fitting the appropriate probability distributions provide valuable information concerning how a compound's toxic load is distributed in the mixture in an easily understandable and succinct form, and can be interpreted as a quantitative measure of its complexity (see Sections 3.2.). The use of ranked lists will also enable identification of the most relevant compounds associated with each site in terms of their relative toxicity contribution to that of the whole mixture. A preliminary outline of such approach is described in Ginebreda et al. (2012), and the present method may be seen as an added value in the optimisation of monitoring efforts.

While it might be accepted that both the CA and IA models provide reasonably good estimates for mixture ecotoxicity, extrapolating their results to biological environmental variables and finally to entire ecosystems is not straightforward. Bridging the gap between multichemical ecotoxicity and ecosystem impairment remains a challenge, and is a topic of active research (Schäfer et al., 2007; Clements and Rohr, 2009). Although the information derived from the application of mixture ecotoxicity models (total ecotoxicity) has been related to some biological variables (Liess and Von der Ohe, 2005; Posthuma and De Zwart, 2006), the role of mixture complexity by itself has not yet been explored.

The present study aimed to: (a) extend the application domain of the CA ecotoxicity model beyond the measure of the whole compound's total toxic load, taking into consideration distribution patterns as an alternative quantitative characterisation of the mixture load and complexity; (b) identify relevant compounds at each sampling site, in order to provide some orientation for monitoring; and (c) examine the predictive relationship between the ecotoxicological risk of chemical compounds (in terms of total ecotoxic load and distribution) with functional or structural biological variables characteristic of the receiving ecosystem. The above approach was tested in a highly polluted system (Llobregat River, NE Spain) (Sabater et al., 2012) for which both chemical and biological data were available (Muñoz et al., 2009; Ricart et al., 2010; Ginebreda et al., 2010). In this particular case study, the predictions obtained using the new proposed approach were compared against invertebrate and biofilm descriptors.