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Biomonitoring using comet assay in fish: a scientometric approach

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Abstract

The goal of this paper is to analyze the knowledge development on biomonitoring using comet assay on fishes. Were selected 154 papers in the ISI Web of Science and PubMed databases. The comet assay was used by 24.7% of the papers with the purpose to evaluate genotoxicity of polluted water. Comet assay is many times associated with other techniques, offering good results to increase efficiency in environmental assessment or to evaluate genotoxicity of pollutants individually.

Keywords: Biomarkers, Environmental Pollution, Genotoxicity, Mutagenic, Single-Strand Breaks.

INTRODUCTION

In modern industrialized societies, a large number of newly generated chemicals have contributed to increase ecosystems' pollution (Eki, 2018). Rivers and lakes that are closer to more urbanized places are constantly affected by the discharge of industrial and domestic effluents and agricultural contamination (Bolognesi & Hayashi, 2011). Since 1988, with the launch of the comet assay, its use for assessing genetic toxicity increases and fish are clearly the most adopted group for ecotoxicological purposes (de Lapuente *et al.,* 2015).

The Comet Assay, or Single Cell Gel Electrophoresis (SCGE), is a standard and versatile method, adopted for ecotoxicological studies that can be applied to virtually any animal and plant tissue that can be disaggregated into single cells, measuring the breaks in the DNA chain caused by organic or inorganic pollutants (Al-Shami *et al.,* 2012; de Lapuente*, et al.,* 2015).

Considering the need of environmental biomonitoring and the wide use of the comet assay to evaluate DNA damage in organisms exposed to genotoxic agents, the purpose of this paper is to evaluate the development of knowledge about biomonitoring using the comet assay in fish aiming to provide information about which areas should be focused in subsequent studies.

MATERIALS AND METHODS

For the development of this study, papers indexed in the *ISI Web of Science* and *PubMed* databases were selected. The keywords *"Biomonitoring"* AND *"Fish"* AND "*Comet assay"* were used to select papers in which the two subjects were treated concomitantly. All the papers found until 2018 were considered. Publications that presented the following information were selected: (I) biomonitoring using comet assay in fish (II) year of publication; (III) country of affiliation; (IV) citation; (V) studied specie; (VI) techniques used in association to comet assay; (VII) evaluated pollutant. Through the gathering of this information a data collection was elaborated. From the data collected, the CiteSpace software was used to create maps with nodes that represent some chosen item (country, source, author, category, etc.), these nodes are linked to each other according to co-citations or co-occurrences (Xie, 2015).

RESULTS

The summary of the survey results is presented in Figure 1. Many articles found were deleted after reading the abstracts. Each study was analyzed to verify the data needed for integration in this study. The two main criteria for exclusion

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of articles at this stage were (1) studies that did not use fish in the comet assay (2) biomonitoring studies that did not use the comet assay. According to the criteria used, 154 papers were selected (Table S1 – Supplementary Material). The first was published in 1995 and accordingly to the technique's development the number of publications increased over the years (Figure 2). Most articles were published in 2014 and 2017, with 20 and 18 articles respectively. A total of 86 fish species were used in the papers. The articles selected were published by institutions from 41 countries, of which 20.7% were conducted by Brazilian institutions. Using CiteSpace it was possible to observe, by nodes size formed in the network of the co-authors' countries (Figure 3), that the top ranked item by citation counts is Brazil, with citation counts of 28, followed by India (14) and France (11). It means that Brazil is the country with the higher visibility in this field. In addition, the red center in Brazil indicates an explosion of citation that in this case reached a "Burst" of 5.45, demonstrating a very active country in this area. In spite of its good placement in the number of publications and in its activity, in the case of centrality (purple external rings), France and the United States of America are the only countries to contain it, with a value of 0.13 and 0.09 respectively, which shows a good influence of these in the area. 18.8% of the study was carried out in partnership between countries (Table S2; Table S3 – Supplementary Material). The article "*Alkaline single cell gel (comet) assay and genotoxicity monitoring using bullheads and carp*" was the most cited, 240 times. However, 33.8% of articles were cited less than 10 times (Table S4 – Supplementary Material).

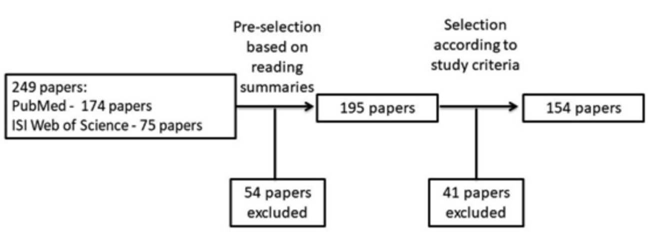

Figure 1. Results obtained from the search and papers used in this study.

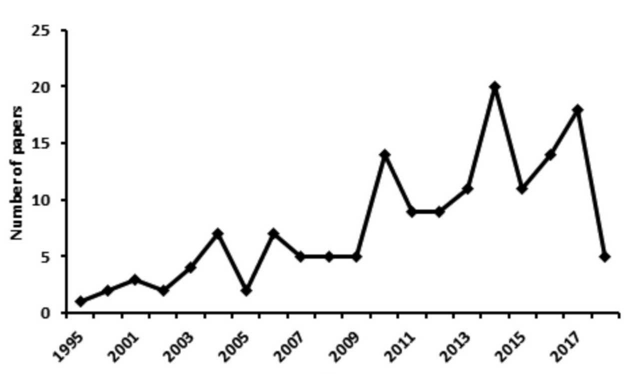

Figure 2. Number of papers by year about biomonitoring using comet assay in fish.

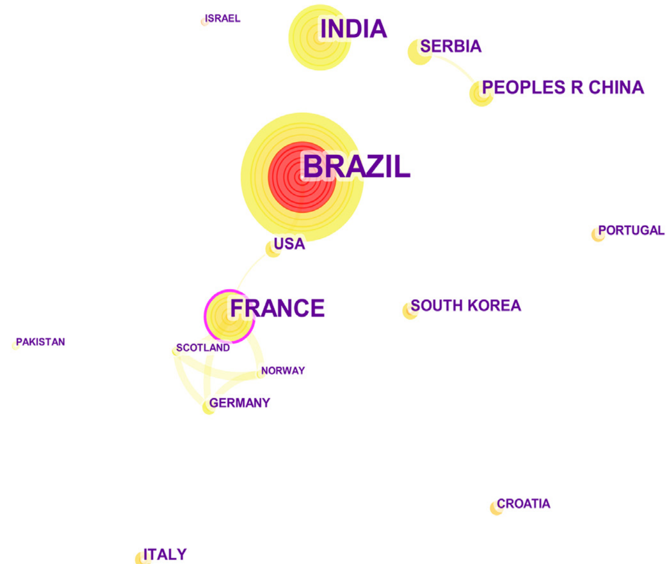

Figure 3. Visualization of frequency, centrality and burst of citations according to publications by countries obtained by CiteSpace.

As for the categories of research (Figure 4), "Environmental Sciences" and "Environmental Sciences & Ecology" have the highest frequency, with 109 publications in the area, followed by "Toxicology" (85), "Genetics & Heredity" Marine &

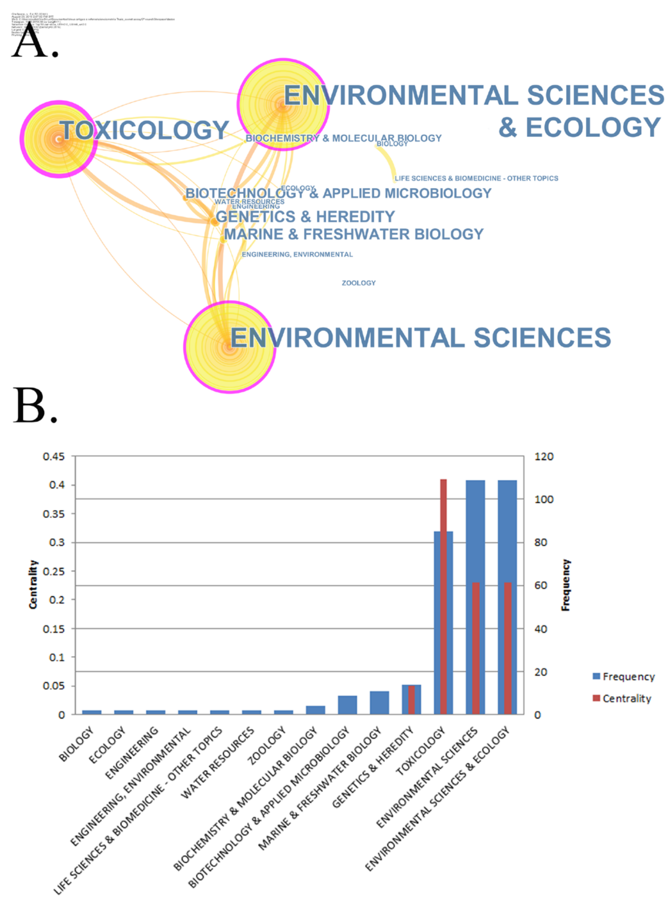

Figure 4. A. Category display by frequency (node size), centrality (light gray), and citation burst (dark grey) obtained by CiteSpace. B. Frequency and centrality of categories with comet assay publications.

Freshwater Biology "(11). The "Toxicology" category has the greatest influence in the area with a centrality of 0.41, followed by "Environmental Sciences" and "Environmental Sciences & Ecology" with a centrality of 0.23. However, the most active areas with burst of 6.27 and 4.88 are, respectively, "Genetics & Heredity" and "Biotechnology & Applied Microbiology".

Most articles used only one species in the analysis and only 14.28% of the articles used more than one species (Table S5 -Supplementary Material). *Danio rerio* was the most studied species, evaluated in 14 studies. *Oreochromis niloticus* and *Cryprinus carpio* were studied in 13 and 12 articles, respectively. All species analyzed are listed in table S6 (Supplementary Material).

The comet assay was used by 24.7% of the studies to evaluate the genotoxicity of polluted water. The genotoxic effects of Polycyclic aromatic hydrocarbons (PAHs) were evaluated in 14 studies. In the other papers, many genotoxic compounds were evaluated (Table S7 - Supplementary Material).

Some biomonitoring programs used multiple biomarkers to evaluate toxicity. Considering the selected papers 31.8% used exclusively the comet assay in the analysis, 37.7% of papers associated comet assay and piscine micronucleus test (MN) (Figure 5).

DISCUSSION

The problem related to pollution is growing over the years (Lu *et al.,* 2015), and this can be a justification for the two years with the largest publications (2014 and 2017) to be so recent. The intensive use of fertilizers and pesticides, pressured by the need to produce more and more food, generates contamination by toxic residues to living organisms (Carvalho, 2017), which may also be one of the reasons for increased biomonitoring to assess the health of ecosystems.

The significant number of Brazilian publications in this study can be justified because the South America has the most diverse fish fauna on Earth, with approximately 9,100 species of fish (Reis *et al.,* 2016). Brazil also participates actively in world agricultural production, which may be related to the use of agrochemicals.

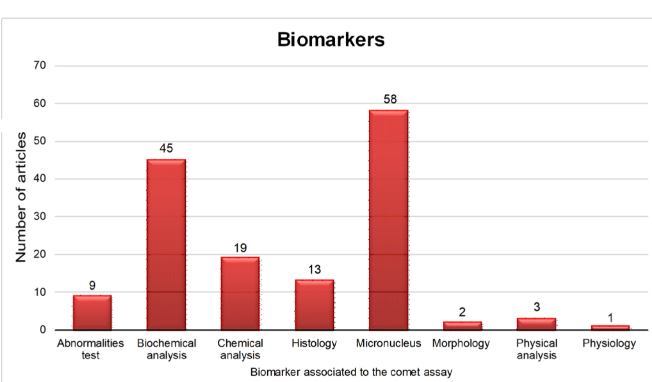

Figure 5. Types of biomarkers associated to comet assay in papers about biomonitoring using fish.

Knowing that the location, preservation state of riparian forest, as well as the economic activities developed in each river basin are decisive for surface water pollution control. The monitoring of metals and pesticides in rivers surrounded by intensive industrial and agricultural activities is very important (Machado *et al*., 2016). Still, it is expressive the concern of Brazilian research with tannery (Rocha & Oliveira, 2017), copper (Simonato *et al*., 2016), heavy metals (Paula Filho *et al.,* 2015) and microplastics (Santana *et al.,* 2016).

Most of the articles were few cited, while few papers were cited, and the most cited articles are the oldest. The H index was 37, according to the Web of Science. It means that in our dataset there were 37 papers with at least 37 citations each.

The use of more than one species is interesting, considering the different behaviors between species. Gonzalez-Mille *et al.,* (2010) used five fish species of different trophic levels to determine the level of persistent organic pollutants. The omnivore species were the ones that presented the greater damages probably because these organisms can incorporate available pollutants by many sources. Second Gajski *et al.* (2019), when it comes to using the comet assay for environmental risk assessments of water pollutants in vertebrates, fish are among the most studied organisms and until now more than 300 papers describing DNA damage in fish cells with the comet assay have been published. It makes fish by far the most adopted animal group in the framework of environment health assessments, and the method was adopted for more than 90 different species (De Lapuente *et al* 2015).

Most of the studies evaluated the environmental pollution nonetheless many compounds can be evaluated by using comet assay and the list of these compounds can help to guide and give support to new researches. Comet assay is many times associated with other techniques, especially MN test (37.7%), offering good results to increase efficiency in analyzes (Garcia-Nieto *et al.,* 2014).

To corroborate our data, the working group in the 6th International Workshop on Genotoxicity Testing (IWGT) conclude that the standard comet assay in combination with the MN assay is useful as part of a test battery to detect DNA damaging agents including crosslinking agents. In this sense, it is important to discuss the importance of the Comet assay in biomonitoring programs. Second Speit *et al.* (2015), in specific cases, the *in vivo* comet assay had higher sensitivity for the detection of carcinogens better than *in vitro* comet assay. Second the same authors the standard protocol for the alkaline version detects a broad spectrum but not all types of DNA damage. With this, it is possible to detect induced DNA strand breaks, alkali-labile sites and strand breaks associated with incomplete excision repair sites. But, for example, to detect DNA crosslinking it is necessary to perform modifications in Comet assay. Bulky adducts are another example of a lesion class which might be missed. While DNA crosslink can decrease the migration, the bulky adducts increase the DNA migration. Therefore, we can conclude that in biomonitoring studies comet assay technique is truly efficient and must continue to be used.

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