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Why is the Biotic ligand model so scarcely applied in Brazil? A review

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Why is the Biotic ligand model so scarcely applied in Brazil? A review

Abstract

Brazil boasts of large hydrographic basins, numerous lentic environments, and an extensive coastal region. These aquatic environments are susceptible to the presence of metals originated from both natural and anthropic activities, so methods to assess the ecological risk to these environments, such as the Biotic Ligand Model (BLM), are of immense value. This study comprises a systematic review of selected articles published from 2008 to 2020 to answer the following question: Why is BLM so scarcely applied in Brazil? Data was compiled to identify the origin, tests, methods, journal impact factor, and year of publication of all included papers retrieved from the Scopus database. The BLM was shown as efficient in predicting metal toxicity in both seawater and freshwater considering both organisms and environmental factors (speciation in water). Copper, cadmium, nickel, zinc, lead, and silver were the most reported throughout the years, with copper ranking first, reported in 133 publications. Other metals were also reported, but in a lower number of published papers. *Daphnia magna* was the most evaluated test organism. Several BLM papers were published in relatively high impact factor journals (4,93 on average), reinforcing the importance of the subject. Brazil ranked 7th in BLM publishing, participating with 4% of the published articles from the retrieved total, with most studies published by research groups in the South region. Some recommendations are raised in this review, such as the need for more interactions between research groups in Brazil, deeper connectivity between legislation and BLM studies and further BLM applications in the country, as each waterbody displays its own specific particularities.

Keywords: BLM, Copper, Metals. Water resources

1. INTRODUCTION

Pollutants of anthropic origin have become an integral part of global concerns with regard to aquatic environments. When assessing environmental health, the presence of these contaminants is so relevant to the point that metals and persistent organic pollutants have become potential geological Anthropocene marker candidates [1,2]

One of the main water contamination concerns worldwide comprises metal and metalloid contamination. Although many metals are essential to living organisms, they may become toxic above a certain threshold, while several toxic metals are also of concern, as they are dangerous to living organisms even at low concentrations [3]. Furthermore, metals and metalloids are highly persistent and cannot be metabolized, thus leading to bioaccumulation and, in some cases, biomagnification processes.

Predicting metal contamination risks in waterbodies is crucial for efficient risk assessments and aquatic biota protection. Mathematical modelling in this regard is important to connect all processes and biogeochemistry aspects involved in metal contamination scenarios, such as metal transport, binding, absorption, and biota effects [4].

Several mathematical modelling tools have been developed focusing on ecosystem protection in the ecotoxicology field [5, 6, 7]. One of these, in particular, has been widely investigated as a risk assessment predictor, termed the Biotic Ligand Model (BLM) [8, 9, 10, 11, 12, 13]. The BLM is a mathematical model developed to measure, assess, and understand how the chemical properties of a waterbody can affect metallic contaminant speciation, bioavailability, and consequent toxicity, comprising an important tool in understanding and predicting metal toxicity in different waterbodies [8, 10].

The BLM is based on three processes, as follows: (i) the interaction between water and dissolved chemical species, forming organic and inorganic compounds (2) competition between the bioavailable portion of dissolved chemical species with major cations and anions binding to gill surfaces (the main metal action site, commonly referred to as the biotic ligand), and (iii) metal uptake into the organism, resulting in mimetic processes, inhibiting enzymes responsible for sodium export, namely Na^+/K^+-ATPase and Ca^{+2}-ATPase, leading to toxic effects in case the exposed organism is not able to compensate for the ionic disturbance [10, 14].

In the presence of cations that naturally cross the gill membrane (Ca^{+2}, Mg^{+2}, Na^{+2}, H^{+}), metal cations (M^{z+}) will display a competitive behavior. Dissolved Organic Matter (DOM) complex with metals, forming compounds that do not cross the gill membrane. However, if metals cross this biotic ligand transport site, they may modify the concentrations of essential electrolytes, as they can mimic their functions, provoking biological disfunctions [15]. A BLM scheme overview is depicted in Figure 1.

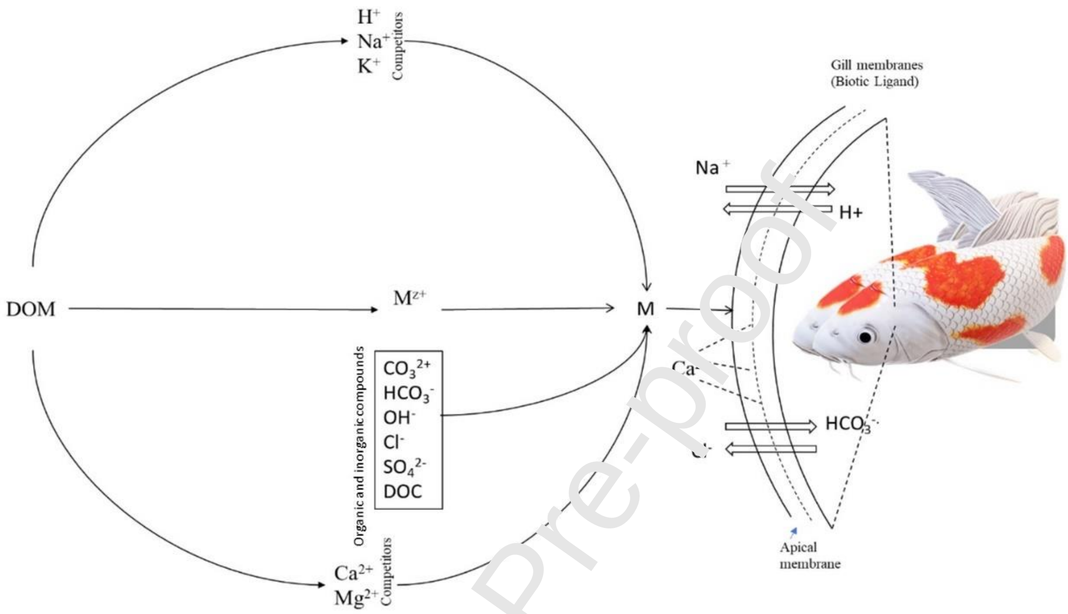

Fig. 1. BLM scheme overview: The biotic ligand (plasma membrane of gill cells); the ions that cross the biotic ligand barrier (Calcium - Ca^{2+}; sodium - Na^{+}; Chloride-Cl^{-}; Bicarbonate - HCO_3^{-}) and those that compete with metals (M^{2+}) for the biotic ligand (Hydrogen - H^{+}; Potassium - K^{+}; Carbonate - CO_3^{-2}; Hydroxyl - OH^{-}; Sulphate - SO_4^{-2}; Magnesium - Mg^{2+}), Dissolved Organic Carbon (DOC) and Dissolved Organic Matter (DOM) which also interfere with metal bioavailability (M^{2+}).

The BLM has long been used to predict the toxicity of several metals exclusively in freshwater and calibrated only for certain model-organisms. However, the most recent BLM WindWard Software versions can also carry out predictions in marine water. To run this software, specific water body parameters are required, such as temperature, to assess thermodynamic chemical equilibrium properties [17], pH, concerning the redox balance of several metals and DOM capacity for metal complexation [18, 12], DOM and humic acid concentrations, to investigate metal complexation processes, as the presence of these compounds commonly reduce metal bioavailability [18], the main cations present in water (calcium, magnesium, sodium, potassium) that compete with metals, reducing their bioavailability [19], and the main anions present in water (sulphate, chloride, sulphide, and carbonates), which directly influence charge equilibrium, ionic strength, and metals complexation [20].

The reliability and sturdiness of the BLM have led the US Environmental Protection Agency (USEPA) and the European Community (EU) to apply this model in establishing water quality criteria (WQC) for certain metals, such as Cu, in freshwater ecosystems [21]. In Brazil, several studies have employed the BLM to predict metal toxicity since 2002 [22, 23], although its application in the country is still scarce. To understand the reason for this limitation, a systematic review aiming at discussing the application of BLM in Brazil and other countries was conducted.

2. MATERIAL AND METHODS

In order to answer the main question of this review, "Why is BLM so scarcely applied in Brazil?" published papers on the subject available at the Scopus database (http://www.scopus.com.br) were identified considering only papers published from 2008 to 2020. A manual filtering was conducted and only papers on BLM were considered. Following study retrieval, a detailed database employing the Microsoft Excel 365 software was created according to Sampaio and Mancini [24] and Mengist *et al.* [25], highlighting the following relevant information: Authors, keywords, article, origin, publication year, organism, tested metal, type of test and Journal Impact Factor).

The systematic review was carried out following the guidelines and suggestions proposed by Sampaio and Mancini [24] and Mengist *et al.* [25]: a) Question refinement: The refined research questions were: (i) What is the impact factor of the published articles on BLM? (ii) Which metals are the most studied and (iii) Which ecotoxicological tests were conducted for inclusion in the BLM? The first question reveals the relevance of the topic, while the others deal with different BLM objectives. b) Database decision: ABS-key, screening: The ABS-keys applied to the search at the Scopus database were "blm" and "biotic AND ligand AND metal". A preliminary search indicated that some articles can be found exclusively by the BLM acronym, while others do not cite the acronym at all. c) Criteria definition: Inclusion criteria: 1- ABS-key present in the title, abstract or keywords; 2- Related papers published in any language; 3- Papers related to the Biotic Ligand Model; 4- Papers that indicate BLM use, validation and exemplification; Exclusion criteria: 1-Inaccessible papers; 2- Papers published before 2008 or after Jun/2020, d) Database following predefined strategies. e) Article selection based on predefined criteria. f) Conclusion presentation demonstrating the compiled evidence.

3. RESULTS AND DISCUSSION

3.1. Data search and retrieval

The first search for "BLM" in Scopus database retrieved 2.768 records. However, BLM is not only an acronym for Biotic Ligand Model, but also for other subjects. Thus, a manual screening was performed to assure all included articles were relevant. This step reduced the articles to a total of 120, discarding book chapters, presentations, and other publications that did not fit the applied inclusion criteria. The second search for "biotic AND ligand AND metal" followed the same pattern as the latter, with the word "metal" used for further refinement. Then, a second manual screening was carried out, also removing duplicates, resulting in a total of 124 articles. Both searches were then summed, totaling 244 articles.

3.2. Model organisms

Organisms may either resist or display sensitivity to environmental modifications. Thus, any water property alteration may result in deleterious effects in sensitive organisms [26]. Because of this, sensitive organisms should be employed as model organisms to predict metal effects. Model organisms are, in fact, extremely useful in ecotoxicology to understand toxicant effects on organism physiology [21] and, thus, provide efficient risk assessments. They are usually employed considering costs, transportability, response precision, necessary test volumes, manipulation difficulty and sensibility to toxic substances, among other factors [30]. Determined endpoints (LC_{50}, EC_{20}, among others) in these assays are then extrapolated to exposed populations and communities [27], the latter comprising a crucial regulatory purpose step. Furthermore, different toxicant concentration ranges are commonly tested to predict ecotoxicological consequences, thus evaluating if and how the results can be extrapolated to other organisms [29]. However, typical ecotoxicological assessments commonly depend on simplified metrics concerning species sensitivity, and do not consider several physiological or physical aspects, which may result in imprecisions during the extrapolation processes [28]. Figure 2 presents the five most employed model organisms in BLM assessments within the established publication range, where *Daphnia magna* ranked first.

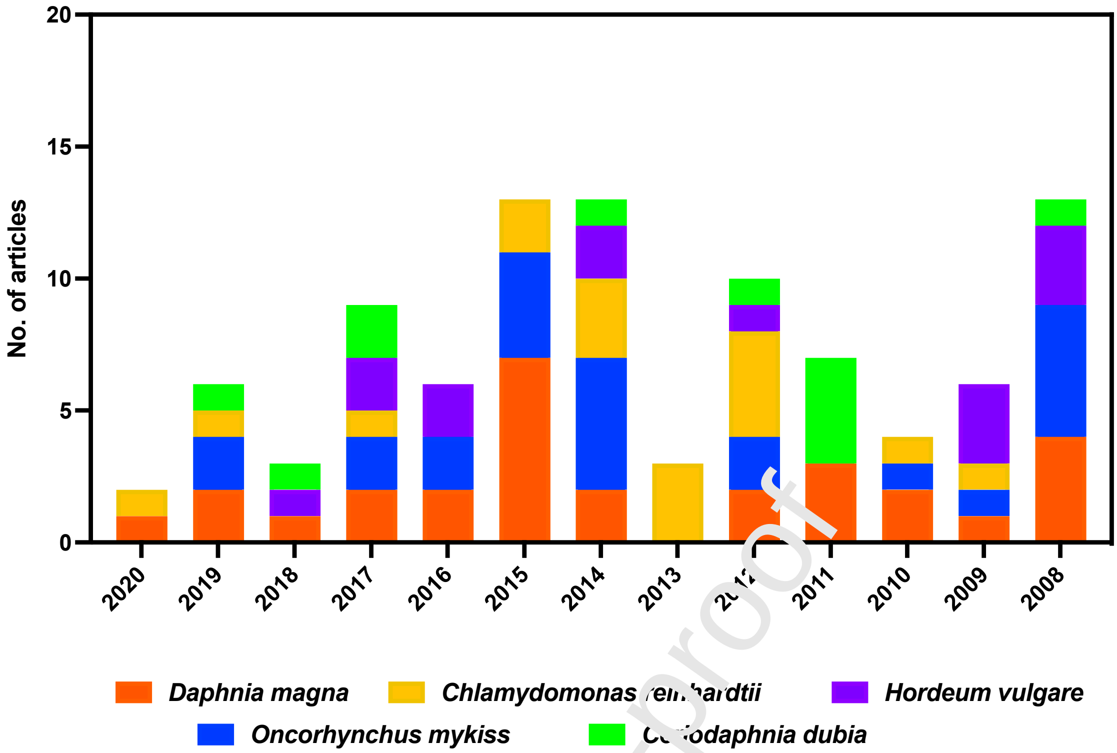

Fig. 2. The five most employed model organisms in BLM assessments and their frequencies of publication from 2008 to 2020 available at the Scopus database.

Daphnia magna (waterflea), is a freshwater crustacean comprising an important food source for fishes and other aquatic organisms. It is an excellent biological indicator, easy to find, maintain, and manipulate [31] and commonly employed worldwide in ecotoxicological EC_{50} 24 h assessments [32]. *Oncorhynchus mykiss* (trout), is a cold-water fish, commonly found in temperate climate regions [33]. *Chlamydomonas reinhardtii* is a unicelular green algae, also commonly used as a model organism in aquatic toxicity assessments [34], due to specific photosynthetic responses and a short lifecycle [35]. Interestingly, *H. vulgare*, barley, a member of the grass family and major cereal grain grown globally, ranked fourth, revealing BLM applications concerning land resource protection, in contrast to its original water application. In this regard, several authors have investigated metal toxicity effects in barley roots, reporting speciation and bioaccumulation and evaluating whether BLM should be used for land contamination predictions [11, 36, 37]. *Ceriodaphnia dubia* (another waterflea), is a freshwater microcrustacean very sensitive to environmental changes, also easy to find, maintain, and manipulate [38].

It is important to note that many organisms used in BLM assessments have not yet been validated. Figure 3 depicts only papers published employing validated organisms according to the BLM Windward Software 2.1.

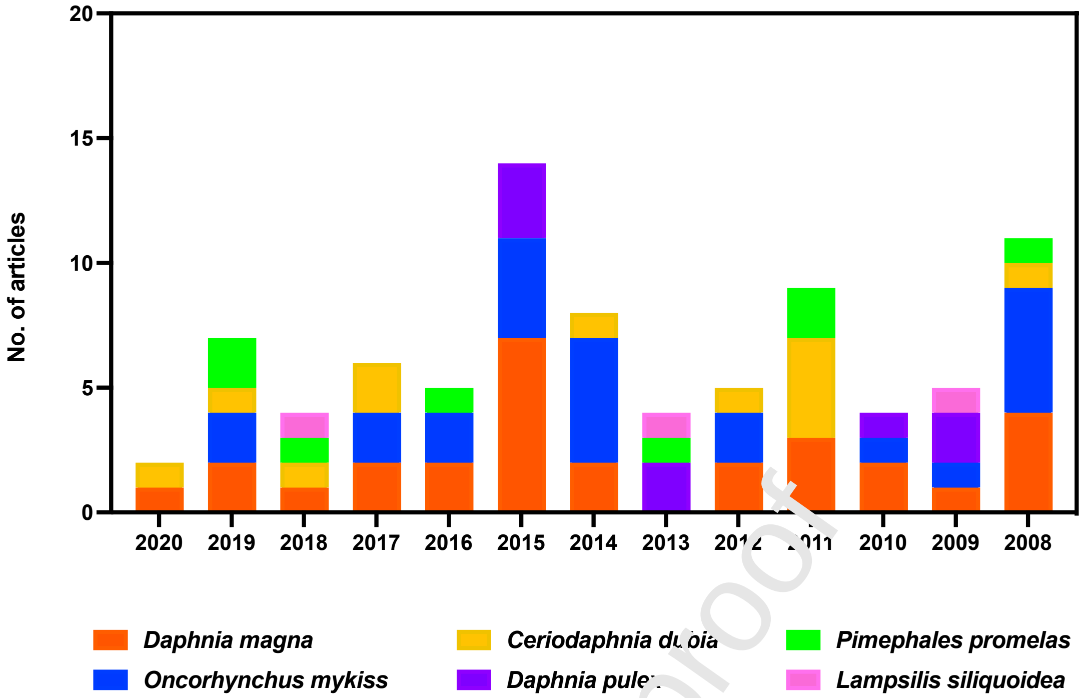

Fig. 3. Papers published employing validated organisms according to the BLM Windward Software 2.1 and their frequencies of publication from 2008 to 2020 available at the Scopus database.

Daphnia pulex, yet another waterflea, similar to *D. magna,* is widely distributed in a variety of habitats, with a short lifecycle and easy to manipulate and store in lab conditions [32]. *Pimephales promelas*, the fathead minnow, is commonly used in ecotoxicological assays as it easy to reproduce in lab conditions and very sensitive to stressors [39]. *Lampsilis siliquoidea*, is the most sensitive bivalve among those available for use in BLM [40] for many metals such as copper, nickel and zinc [41]. *Chironomus tetans, Daphnia pulicaria, Lampsilis fasciola, Lepomis macrochirus, Oncorhynchus tshawytscha* and *Utterbackia imbecillis* are also validated for use in BLM software, although no publications related to their use for BLM within the research period criteria were obtained.

3.3. Metals associated to BLM publications

Some publications report on more than one metal, considered herein as individual occurrences. Within the 244 included articles, 333 occurrences for metal toxicity predictions employing the BLM were noted for individual metals. The 10 main metals employed in BLM assessments in the included articles are displayed in Figure 4.

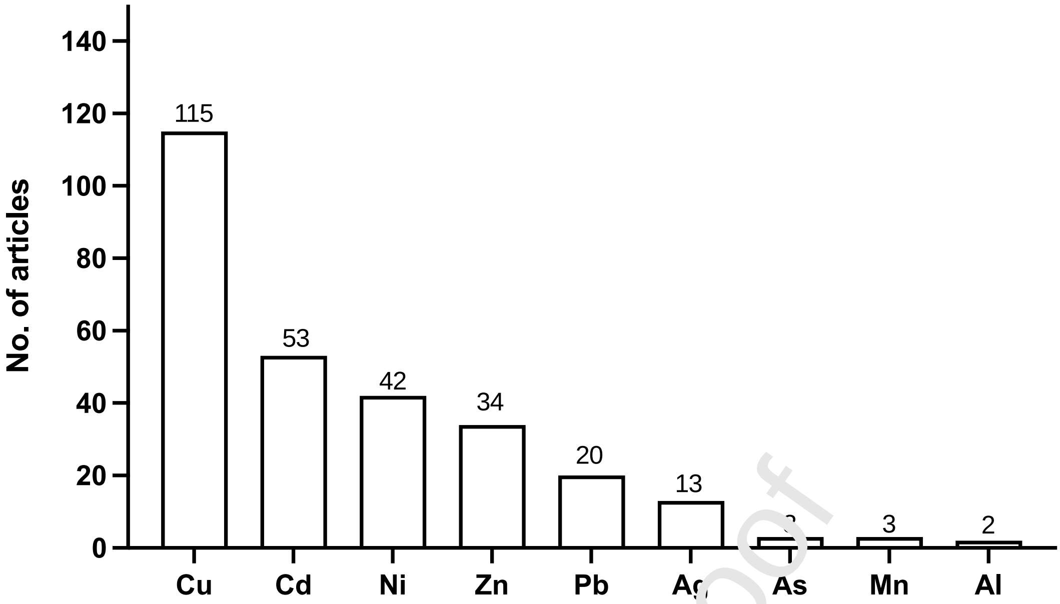

Fig. 4. The 10 main metals employed in BLM assessments in the included articles retrieved in this systematic review published from 2008 to 2020.

Only three essential metals were among the 10 main metals employed in BLM assessments, namely Cu, comprising the most assessed, followed by Zn and Mn. The other seven metals are all toxic elements.

Concerning validation, several metals reported in BLM articles are not validated for BLM usage and, thus, not found in BLM software databases. The only validated metals in this regard are Cu, Cd, Zn, Pb and Ag, depicted in Figure 5 according to their reports in the published BLM papers.

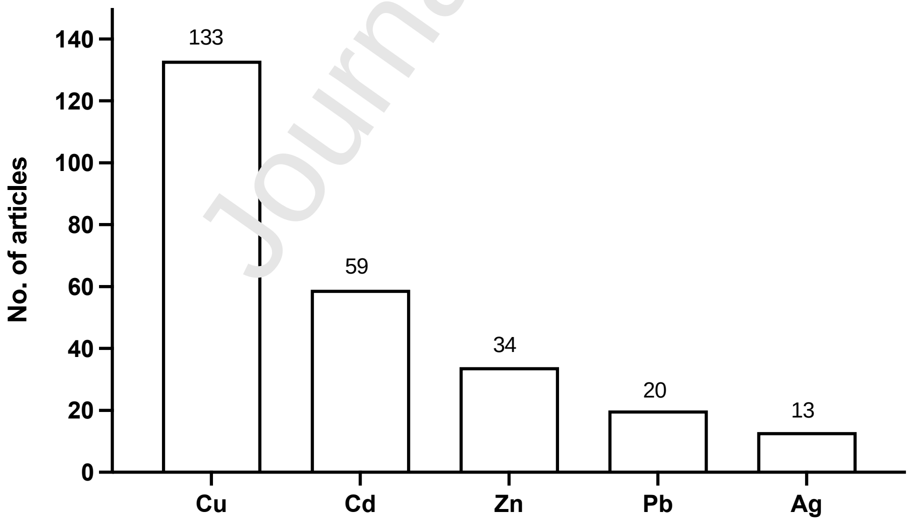

Fig. 5. Validated metals employed in BLM assessments in the included articles retrieved in this systematic review published from 2008 to 2020.

Copper is a is found naturally as a free ion metal in its ionic form or associated to other elements. This is an essential metal to many taxa, comprising a vital component of many proteins and enzymes, and, specifically in crustaceans, is also a component of hemocyanin oxygen-carrying proteins [34]. As a free metal it usually poses no risk to aquatic biota, but speciation processes may result in toxic effects [42, 43]. In this regard, Cu speciation is mainly controlled by DOM and HA water contents, as these compound bind to free Cu and decrease the amount of bioavailable Cu ions in aquatic environments [47, 48]. If Cu is not successful in competing with other cations (Ca^{2+}, Mg^{2+} Na^{+} and K^{+}), it cannot reach the gill membrane and performs its essential roles in living organisms [49, 10]. On the other hand, if reaching the gills in excess or in toxic speciated form, toxicity may take place, typically in the form of inhibited cellular Na^{+2} and Ca^{+2}/K^{+}-ATPase and carbonic anhydrase [16, 46]. Toxic Cu effects include orientation system alterations, intestinal issues, and reduced growth, among others [44]. Furthermore, ionic and osmoregulation disfunctions in crustaceans, have also been reported, due to ammonia influx alterations [45].

Cadmium, the second most frequent element employed in BLM studies is highly toxic, although most assessments are carried out in freshwater fishes, with less studies on estuarine and saltwater species [50]. This element is found in coal and phosphate fertilizers [34], leading to high runoff into waters surrounding agricultural areas [51]. High Cd concentrations (>2 mg kg^{-1}, in some cases) have been reported as associated to hypo and hyperpigmentation, ocular hypoplasia, retinal ganglionic and optic neuronal projection reductions in *Danio rerio* embryos, while low concentrations (ranging from 1 to 2000 ug kg^{-1} may lead to neurological disfunctions [52].

Zinc is commonly present in protective coatings and used in galvanization to prevent corrosion [21] and is reported in BLM papers as highly associated to industrial effluents [53, 54]. Biologically, it is an essential metal that plays an important role in the activation of several enzymes [34], becoming toxic at higher concentrations (*i.e.,* 1000 $\mu g.L^{-1}$), for example interfering with Ca^{+2} flow in certain fish species *Galaxias maculatus* [55]. As zinc and calcium compete for the same binding site on the membrane, increased concentrations in water can cause hypocalcemia in aquatic organisms. In addition to impairing acid-base regulation in sublethal concentrations by inhibiting carbonic anhydrase and in osmoregulation by affecting sodium and chloride flow through the membrane [53].

Lead is toxic to all living organisms [56], highly associated to industrial effluents [34]. Its effects depend on exposure time, exposed organism, tolerance, concentrations, and water properties (for example, hardness and pH) [57]. It has been reported as causing neurodegenerative diseases in zebrafish, even at low concentrations (<100 ug kg^{-1}) and as affecting genes associated to nervous system development [52]. Lead uptake and pH have been reported as inversely proportional [57].

Silver is a very toxic metal [58, 59]. widely used in home appliances and daily life products and in electrical, and medical equipment [60], resulting in bioaccumulation and severe liver damages in several aquatic organisms [60]. However, Ag contamination reports have has reduced significantly over the years, mainly due to the reduction in the use of traditional photographs that used silver salts in their preparation, which are now replaced by digital photographs [34].

3.4. Types of assay

The distinction between acute and chronic toxicity is required in BLM assessments, as this depends on exposed species sensitivity, water properties and exposure intervals [21].

It is interesting to note that most of the evaluated BLM studies apply acute toxicity assays (58 %), followed by chronic assays (19 %), while simultaneous acute and chronic assays were reported in 23% of the studies. Simultaneous acute and chronic toxicity tests are often reported when evaluating and comparing BLM predictions for one specific xenobiotic, possibly due to the fact that lower concentrations of a specific toxicant may be insufficient for acute toxicity results, while exposures at the same concentration may indicate interesting and understudied sublethal effects.

The preference for acute tests is justified by their quick response time, up to the endpoint (in general lethality), and by some less progressive environmental legislation that disregards the importance of chronic effects. In this case, because they anachronistically consider that the role of dilution that the environment can play at low concentrations of pollutants would prevent further damage. For example, by Brazilian ecotoxicology groups was noted from a brief search at the Scopus database for any ecotoxicological test conducted in the country involving toxicant, which retrieved 46 acute toxicity publications and only 25 chronic assays from 2008 to 2020 (unpublished data).

3.5. Journal impact factor

Although the use of bibliometric indicators does not represent an adequate way to measure scientific merit [61], the Thomson Reuters Journal Citation Report Impact Factors to detail scientific impact of the included studies were considered herein. The 244 included BLM studies were published in 54 scientific journals, with only four published in non-indexed journals (Figure 6). The impact factor averaged 4.93 and 50% (122 articles) of the studies were published in journals with IF above this mean [62].

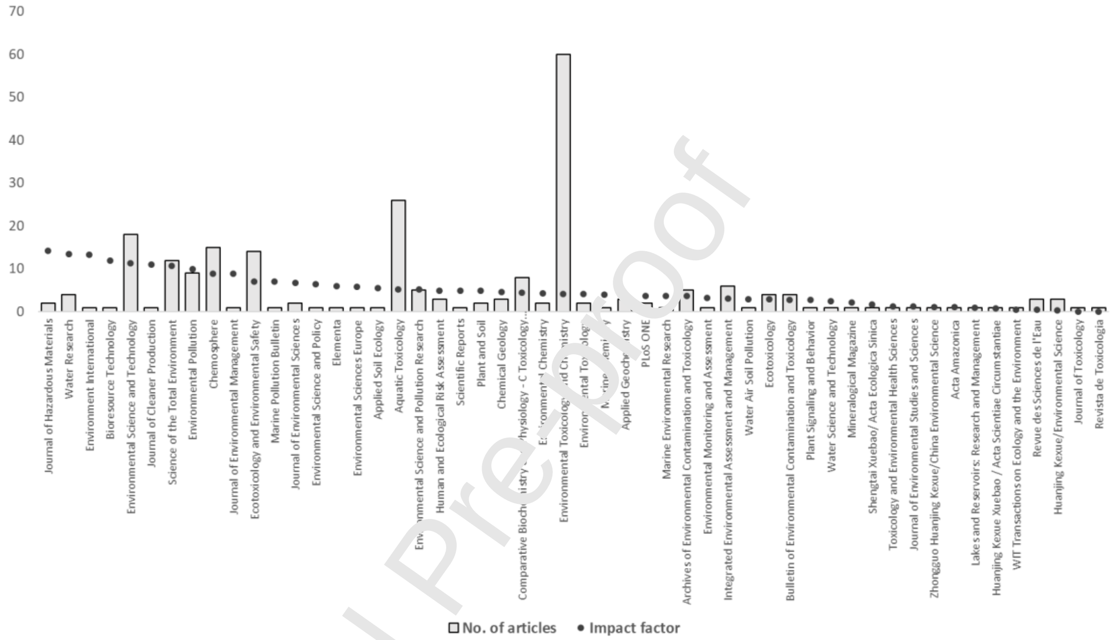

Fig. 6. Journals where the included articles ron BLM etrieved in this systematic review were published from 2020. Impact Factor JCR of 2022 [62].

3.6. BLM publications worldwide

The included BLM studies were published in 26 countries, with 16 presenting over two published articles and only seven had over 10 articles published on the subject (Figure 4).

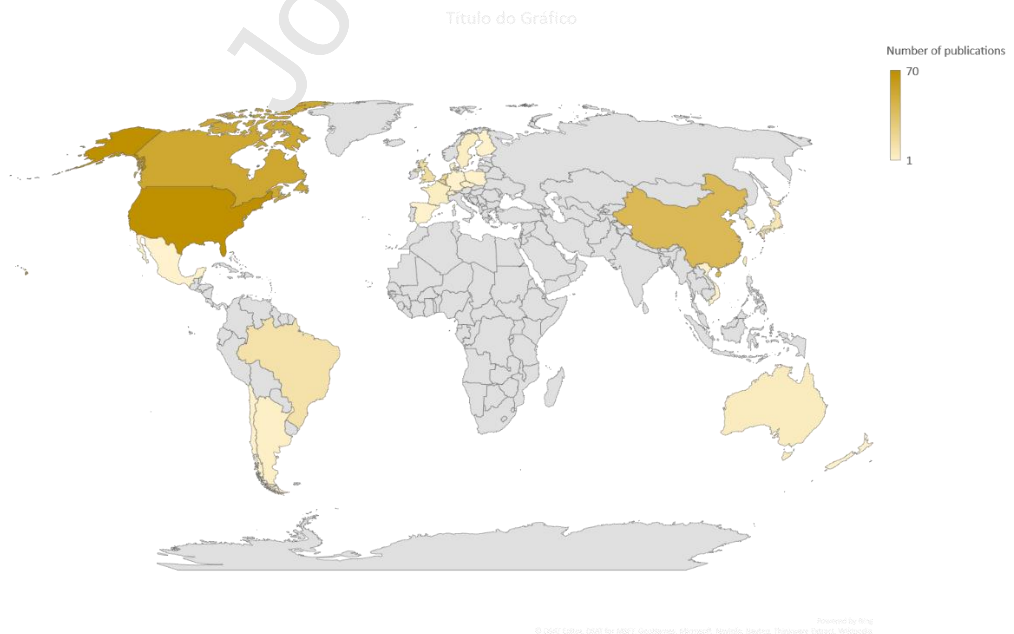

Fig. 4. Worldwide distribution and frequency of BLM publications included in this systematic review published from 2008 to 2020.

The top 10 countries with the most BLM publications among the included articles in this systematic review published from 2008 to 2020 were the USA (25%), followed by Canada (19%), China (14%), then the Netherlands and Belgium (7% each), the UK (5%) and, finally Brazil (4 %) The USA and Canada, participated with 70 and 53 publications in this topic, with North America contributing with a total of 43% of published articles from 2008 to 2020. The high number of publications from these specific countries may be due to the fact that the USEPA has established a requirement for BLM since 2007 to evaluate freshwater WQC and Cu toxicity to specific organisms [63] Brazil was responsible for only 4% of the total number of publications, ranking 7th, a rather inexpressive position, in face of the country's high hydric availability and the historical relationship of Brazilian environmental policies with sustainability.

3.6.1 BLM publications in Brazil

Despite the growing number of ecotoxicological studies in Brazil, only ten papers related to BLM published between 2008 and 2020 were from Brazil (Table 1), mostly concerning crustaceans (60 %), followed by bivalve molluscs (20 %), fishes (10 %) and amphibians (10 %). Furthermore, most studies were carried out to investigate Cu toxicity and were conducted in acute conditions (80 % of the publications), which may be due to the international regulation trend mostly based on acute toxicity assays. Surprisingly, most studies were conducted under marine and estuarine environments conditions, even though the freshwater BLM (the only version available at the time the papers were published) was used. This is probably due to the fact that most aquatic ecotoxicology research groups in Brazil are located in coastal regions. Studies employing the BLM concept in aquatic environments in Brazil published from 2008 to 2020 are listed in Table 1.

Table 1 –Studies employing the BLM concept in aquatic environments in Brazil in the publications included in this systematic review published from 2008 to 2020.

Title	Author/Date	Organism	Chemical Species	Environment	Test Type	Journal	JCR
Acute copper toxicity in the euryhaline copepod *Acartia tonsa*: Implications for the development of an estuarine and marine biotic ligand model	PINHO; BIANCHINI, 2010	*Acartia tonsa*	Copper	Estuarine	Acute/48 h	Environmental Toxicology and Chemistry	4.218
Acute toxicity, accumulation and tissue distribution of copper in the blue crab *Callinectes sapidus* acclimated to different salinities: *In vivo* and *in*	MARTINS, et. al., 2011	*Callinectes sapidus*	Copper	Marine	Acute/96 h	Aquatic Toxicology	5.202

vitro studies							
Does sulfide or water hardness protect against chronic silver toxicity in *Daphnia magna*? A critical assessment of the acute-to-chronic toxicity ratio for silver	BIACHINI; WOOD, 2008	*Daphnia magna*	Silver	Freshwater	Acute/48 hr and Chronic/21 d	Ecotoxicology and Environmental Safety	7.129
Effect of copper on ion content in isolated mantle cells of the marine clam *Mesodesma mactroides*	LOPES, et. al., 2011	*Mesodesma mactroides*	Copper	Marine	Acute/1 h and 3 h	Environmental Toxicology and Chemistry	4.218
Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper	JORGE, et al., 2013	*Lampsilis siliquoidea*	Copper	Freshwater	Chronic/28 d	Aquatic Toxicology	5.202
Potential of the Biotic Ligand Model (BLM) to Predict Copper Toxicity in the White-Water of the Solimões -Amazon River	PONT et al., 2017	*Otocinclus vittatus*	Copper	Freshwater	Acute/96 h	Bulletin Environmental Contamination Toxicology	2,807
Prediction of toxicity of zinc and nickel mixtures to Artemia sp. at various salinities: From additivity to antagonism	DAMASCENO, et al., 2017	Artemia sp	Zinc and Nickel	Marine	Acute/24 h	Ecotoxicology and Environmental Safety	7.129

Sediment quality in a metal-contaminated tropical bay assessed with a multiple lines of evidence approach	RODRIGUES, et. al., 2017	Tiburonella viscana; Tisbe biminiensis	Cadmium, Lead and Zinc	Estuarine	Acute/10 d and Chronic 7 d	Environmental Pollution	9,988
Toxicidade do cobre em scinax ruber e *Rhinella granulosa* (Amphibia: Anura): Potencial do modelo do ligante biótico para predizer a toxicidade em igarapés urbanos	FRANCO-DE-SÁ, et. al., 2014	*Scinax ruber* and *Rhinella granulosa*	Copper	Estuarine	Acute/96 h	Acta Amazonica	1,09
Copper accumulation and toxicity in isolated cells from gills and hepatopancreas of the blue crab (*Callinectes sapidus*)	PAGANINI; BIANCHINI, 2009	*Callinectes sapidus*	Copper	Marine	Gill cells culture/1, 3 and 6 h	Environmental Toxicology and Chemistry	4.218

The Federal University of Rio Grande (FURG) research group, located in the estuarine city of Rio Grande, was responsible for 60 % of all BLM publications in the studied period [64, 65, 66, 67, 68, 69]. These studies exposed several crustaceans (*Callinectes sapidus, Acartia tonsa* and *Daphnia magna*) and molluscs (*Lampsilis siliquoidea* and *Mesodesma mactroides*) to Cy for short periods of type comprising acute toxicity assays, and evaluated the influence of salinity [66, 67], sodium, potassium, calcium, and chloride ions [65], sulphide and hardness [69] on Cu and Ag toxicity upon the biotic ligand, comprising gills or similar structures. Two studies used *in vitro* assays to assess isolated gill cells and hepatopancreas cultures from *Callinectes sapidus* and the mantle cells of *Mesodesma mactroides*, supporting an interesting assessment strategy with the use of animal cultures and not as many exposed organisms. The remaining papers were published by different groups studying different metals (Cd, Pb and Zn) in different organisms [70], namely from the Federal University of Rio de Janeiro (IFRJ) and the Federal Fluminense Universoty (UFF), as well as assessments on the additive and antagonistic effects Zn and Ni of mixtures in *Artemia sp* [71] by a group from the Federal University of Ceará, and two studies in the Amazon region applying BLM to assess Cu toxicity towards native fish (*Otocinclus vittatus*) and anurans (*Scinax ruber*, *Rhinella granulosa*) [72,73].

3.6.2 Metal parameter regulations in Brazil

Toxicity test method standardization dates to the 1970s [75] and opened space for the development of aquatic toxicity test and prediction models [76]. The BLM application was first introduced as a regulation by the USEPA in the document entitled “EPA’s 2007 aquatic life and freshwater quality criteria for copper” [63]. Although vast evidence on BLM usefulness and validation is available, few regulations recommending the use of BLM worldwide are noted. A massive number of papers refers to the USEPA [63] and European Union Commission in the form of its Water Framework Directive 441/2016 [77]. Guidelines have also established in

Australia [78] and New Zealand [78], and Canada [79].

In Brazil, the Brazilian Association for Technical Normalization (ABNT) is responsible for normalizing guidelines for different uses, including ecotoxicity tests. However, to date, the current guidelines available in the country do not mention the BLM. Furthermore, the Brazilian National Council of Environment (CONAMA), responsible for setting environmental regulations has established the CONAMA guideline no. 357/05 [80], which presents the limits for 86 chemical substances in different water classes, and many articles in this guideline set the requirement for ecotoxicological tests, regardless of physico-chemical water analyses. However, they too do not mention the BLM or any other risk assessment model. Both Brazilian and International accredited institutions may set the parameters to be analyzed for toxicants not listed in CONAMA guidelines, as far as its toxicity is proven by accredited. However, we note the Brazilian National Institute of Science and Technology in Aquatic Toxicity (INCT-TA) has been increasingly disclosing model applications for Brazilian environments [74].

Some metals established in the CONAMA guideline 357/05 are validated for BLM assessments, listed in Table 2.

Table 2. Metals validated for BLM assessments in different aquatic environments in accordance with CONAMA 357/2015 [80] for special class waters alongside their maximum permitted concentrations.

Metal	**Maximum permissible concentrations in special class waters (mg L^{-1})**		
	Estuarine	**Freshwater**	**Marine**
Cu	0.005	0.009	0.005
Cd	0.005	0.001	0.005
Pb	0.01	0.01	0.01
Ag	0.005	0.01	0.005
Zn	0.09	0.18	0.09

In this regard, Lima *et al*. [81] assessed metal concentration in several fishes sampled from the Amazonas-Cassiporé river and identified limiting concentrations for Cd (0.000164±0.00004 mg.L^{-1}), Cu (0.00[illegible]69±0.00216 mg.L^{-1}) and Pb (0.00118±0.00077 mg. $^{-1}$) employing the BLM higher than the regulated by the CONAMA agency, except for Zn (0.000134 ± 0.00007 mg.L^{-1}), whose average did not exceed legal parameters. On the other hand, Gurgel et al. [82], identified employed *Mysidopsis juniae* and *Pomacea lineata* to identify the bioavailability of certain metals at the Jundiaí river, State of Sao Paulo, Brazil, employing BLM, reporting the following concentrations for Pb (0.050 mg L^{-1}), Cd (0.002 mg L^{-1}), Cu (0.044 mg L^{-1}), Ag (0.002 mg L^{-1}) and Zn (0.139 mg.L^{-1}) evidencing contamination by these metals in the assessed environment, and higher levels than established by legal regulations for all except for Zn.

In Brazil, states and municipalities have the power to create more restrictive laws than Federal ones to ensure environmental protection due to specific local industrial and/or agricultural economies, which increase water contamination risk. An example of this comprises the Rio Grande municipality, in the state of Rio Grande do Sul, through its Environmental Defense City Council (COMDEMA), which decided that the BLM should be used as a complementary tool for the investigation of metal toxicity in surface water and effluents, providing rules for specific use and guidelines, through Resolution 002/2014 [83]. This is the first and an important step ahead for Brazil to disseminate the regulated use of BLM. Furthermore, this resolution supports the use of native Brazilian species for regulatory interests based on biological pollutant effects.

It is important to note that, as indicated previously, most toxicity tests in the included articles were carried out following international standards, possibly due to the low number of standard procedures for Brazilian native species. This may lead to a WQC which may not be so efficient for the protection of native Brazilian biodiversity and the great variety of ecosystems present throughout the country. Thus, the use of native species in BLM are likely to create more realistic simulations and more precise predictions [75].

4. BLM limitation and future perspectives

The BLM is a reliable predictive tool routinely applied in several contamination scenarios. However, some limitations are still observed, such as the fact that this model is commonly associated to toxicants absorbed by the gills, not considering other uptake means, such as the dietary route. Age and sex of the employed species are also usually not reported, which may lead to imprecise LC_{50} evaluations, as these parameters are known to significantly affect toxicant uptake and effects in some species. Different salinities are also important, as the BLM still does not consider this parameter, even though the complex effects of salinity are well-known [84, 85].

Chemical speciation, both in freshwater and marine water, is also significant in the prediction of xenobiotic contamination effects. Real-life situations, however, comprise the effects and dynamics between several contaminants at the same time, so understanding the influence one contaminant has on another, *i.e.,* synergic or antagonic effects, is extremely useful for more precise predictions [86]. Some recommendations for next generations of this model following updates include the validation of more metals, a better understanding of the employed model organisms, Brazilian legal guideline updates, the identification, quantification and evaluation of other important means of xenobiotic uptake, the validation of Brazilian native species in BLM assessments, the inclusion of salinity as a model parameter and a better understanding on and further assessments employing mixed metals effects. These will, in turn, reduce the number of test organisms required for ecotoxicological assays, due to organism and contamination validation.

5. CONCLUSION

The BLM is a fast, reliable, and low-cost tool employed to assess and monitor water quality criteria, although some caveats are noted, which can be easily and rapidly solved if more involvement in this subject is achieved. These limitations, however, do not reduce the protection effectiveness and the quality that BLM is able to provide for Brazilian aquatic environment, either as a complementary or a decision-making tool. BLM publications are of interest in the field, as most available studies have been published in high impact-factor journals.

However, the reality of different waterbody features in Brazil makes specific tools for monitoring and assessing risks of aquatic contamination necessary. In this regard, more groups from other regions should participate in BLM assessment and use. Brazilian (native) organisms should be validated, and more metals of interest should be calibrated. In addition, the legislation should recommend BLM use at the Federal level, not only for higher tool effectiveness, but also to motivate studies to increasingly adapt this tool to specific Brazilian needs.

6. AUTHOR CONTRIBUTIONS

Nikolas Gomes Silveira de Souza, Manildo Marcião de Oliveira- Conceptualization, Literature review; **Laura Isabel Weber da Conceição, Victor Barbosa Saraiva, Maria Inês Paes Ferreira, Vicente de Paulo Santos de Oliveira-** Formal analysis; **Nikolas Gomes Silveira de Souza** Investigation; Methodology; **Jader Lugon Júnior, Laura Isabel Weber da Conceição**-Project administration; **Nikolas Gomes Silveira de Souza, Samantha Eslava Martins** - Resources; **Nikolas Gomes Silveira de Souza, Samantha Eslava Martins-** Software; **Manildo Marcião de Oliveira** - Supervision; **Rachel Ann Hauser-Davis, Renato Matos Lopes** - Visualization, **Nikolas Gomes Silveira de Souza, Victor Barbosa Saraiva, Vicente de Paulo Santos de Oliveira** Writing - original draft; **Manildo Marcião de Oliveira**, **Rachel Ann Hauser-Davis, Renato Matos Lopes** - Writing - review editing.

7. CONSENT FOR PUBLICATION

All authors have read and agreed to the published version of the manuscript.

8. DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

HIGHLIGHTS

- Brazil has several aquatic environments that are impacted by metals;
- Research with Biotic Ligand Model (BLM) is compatible with the Brazilian environments;
- Copper is the most studied metal and *Daphnia magna* the most used organism in papers;
- Brazil is only the 7th in BLM paper production;
- BLM implementation is recommended in Brazilian environmental legislation.