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水质生物毒性检测方法研究进展与应用现状

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摘要：突发性水体污染事件频繁爆发，对供水安全、生态环境和人类健康构成严重威胁，已成为制约社会经济可持续发展的重大环境问题。面对数以万计的毒性污染物及日益严峻的水污染问题，水质生物毒性检测由于能够以效果导向反映污染物对水生生物的毒害效应，弥补传统理化指标检测既无法实现水体众多毒性污染物的全面检测，又无法准确判断污染物对生物体的毒害影响等不足，已成为水质状况调查及水环境安全监管的重要手段，对保障水质及水生态环境安全至关重要。鉴于此，本文基于近年来国内外水质生物毒性检测相关研究成果，系统总结了现有水质生物毒性检测方法，基于不同检测方法所用的毒性响应指标、毒性检测原理，对比了不同检测方法的特点，分析了已有检测方法的发展现状。当前，基于不同受试生物(鱼类、蚤类、发光菌类、藻类)的水质生物毒性检测方法，已在重金属、农药(包括杀虫剂、杀菌剂、除草剂)等典型环境污染物毒性分析及实际水体毒性检测与评估中获得广泛应用。其中，发光菌法及藻类光合抑制法由于具有毒性响应快速的特点，是当前水质生物毒性现场快速检测的重要手段。由于不同受试生物对不同类型污染物的毒性响应灵敏性存在差异，仅以单一生物和单一指标无法全面评价水体综合毒性。未来，发展基于多层级水生生物的多指标水质生物毒性检测与评估方法、建立水体表观毒性及潜在毒性相融合的水质毒性全面表征与评估新方法及相应的毒性分级标准，将是水质生物毒性检测研究方向的发展重点与难点。

关键词：水质生物毒性；毒性检测；水质安全；环境污染物；水生生物；发光菌毒性检测法；藻类毒性检测法

要点：

(1) 鱼类、蚤类、发光菌类、藻类不同营养级的水生生物，是当前水质生物毒性检测的主要四类受试生物。

(2) 基于发光菌发光特性及藻类光合作用的毒性检测方法，是当前水质生物毒性现场快速检测的重要手段。

(3) 基于多层级水生生物及多指标的水质生物毒性检测与评估方法，是未来水质及水生态环境安全监管的重要发展方向。

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随着社会经济和工农业的迅速发展，全球化学品种类和数量不断激增，大量蕴含着有毒有害物质的工农业废水和生活污水未经有效处理而进入江河湖泊，带来了严重的水体污染问题，严重破坏了水体生态环境，并对水生生物安全及人类健康构成严重威胁[1]。因此，有效的水环境质量检测是了解水质状况、评估水体污染程度、开展水污染治理的重要基础与前提。

在水环境质量检测方面，传统的水质检测主要采用色谱法、质谱法、光谱法等方法[2-4]对水体的常规理化指标进行检测。这些传统理化指标检测方法，虽然能够定量检测出水体中部分化学污染物的成分及含量，但由于水体污染成分复杂，既无法实现水体众多污染物的逐一全面检测，又无法反映出水体污染状况对生物体的毒害效应与影响，因此在水质安全评价方面具有一定局限性。而与理化指标检测相比，水质生物毒性检测作为一种效果导向的生物学检测手段，由于能够直观、全面地反映出受污染的水体及众多有毒污染物对生物的毒害效应与综合影响，有效地弥补传统理化指标检测的缺陷与不足，并为水质监测与评价提供更为完善的科学依据，已成为水质状况调查及水环境安全监管的重要手段，对保障水质及水生态环境安全至关重要。为此，中国环境监管部门也越来越重视水体的水质生物毒性。如生态环境部 2018 年发布《集中式地表水饮用水水源地突发环境事件应急预案编制指南》，明确提出“应急防控应增加水质毒性综合预警监控手段”；2019 年发布《生态环境监测规划纲要 (2020—2035 年)》，同样要求在地表水国控断面增加水质生物毒性监测指标；2021 年发布《“十四五”生态环境监测规划》，明确提出“在全国重点流域和地级及以上城市推进水质生物毒性等自动监测试点”。

目前，国内外在水质生物毒性检测方面已经建立了一系列检测方法，主要通过鱼类、蚤类、发光细菌类和藻类等不同营养级的水生生物进行水体毒性检测和评估。这些毒性检测方法的研究主要集中在分析不同环境污染物对水生生物的毒性效应与致毒机理[5-8]，以及实际水体的毒性检测与评估[9-13]等。为了深入厘清水质生物毒性检测的研究现状，本文基于近年来国内外水质生物毒性检测的相关研究成果，系统总结了现有水质生物毒性检测方法，梳理了基于鱼类、蚤类、发光菌类和藻类的毒性检测方法所用的毒性响应指标和毒性检测原理，对比了不同检测方法的特点，如鱼类毒性检测方法往往测试周期较长，更适合水体长期毒性监测，而基于发光菌发光特性及藻类光合作用的毒性检测方法则毒性响应较快，更适合水体快速毒性监测。通过总结不同水质生物毒性检测方法应用于重金属和农药 (包括杀虫剂、杀菌剂、除草剂) 等典型环境污染物毒性分析及实际水体毒性检测与评估方面的研究现状，分析了现有水质生物毒性检测方法存在的不足之处，进而讨论了未来水质生物毒性检测的重点发展方向，以期为中国水环境质量监测与评价及水环境安全管理提供新的思路。

1　水质生物毒性检测方法

在环境监测领域，生物毒性是指毒性污染物或污染水体对生物体所产生的毒害影响。水质生物毒性检测，即是根据毒性污染物或污染水体作用下受试生物生理生化指标的变化来判断与评估污染物或污染水体毒性大小的过程。目前，水质生物毒性检测中常用的受试生物主要为鱼类、蚤类、发光菌类、藻类不同营养级的水生生物，因此，基于不同类型的受试生物，分别发展了相应的水质生物毒性检测方法。

1.1　基于鱼类的水质生物毒性检测方法

在水生生物中，鱼类作为低等脊椎动物，由于与人类基因高度相似，信号传导通路与人类较为相近[14]，因此是水质生物毒性检测中最为常用的受试生物。其中斑马鱼 (*Danio rerio*) 由于其产卵量大、易收集、胚胎具有光学透明性、受精率高、可清楚地观察其任何阶段的发育状况等优势[15]，是毒性检测中使用频率最高的受试物种。此外，其他常用于毒性检测的受试鱼类还有青鳉鱼 (*Oryzias melastigma*)[16]、鲤鱼 (*Cyprinus carpio*)[17]和鲫鱼 (*Carassius auratus*)[18]等。

当毒性物质作用于受试鱼类时，基于鱼类不同生理学指标对毒性污染物的响应特性，形成了多种水质生物毒性检测方法。例如，当以鱼类群体存活性作为响应指标反映水体或化学品毒性时，发展了基于鱼类群体存活率的水质生物毒性检测方法，如中国制定的国家标准《水质 物质对淡水鱼 (斑马鱼) 急性毒性测试方法》(GB/T 13267—1991)、《化学品鱼类急性毒性试验》(GB/T 27861—2011) 均以毒性暴露后斑马鱼群体的半数致死量 LC_{50}(使受试鱼种半数死亡的毒物剂量) 来表示被测水体或化学品的毒性大小；当以鱼的受精卵存活率反映化学品或水体的毒性时，形成了鱼卵法水质生物毒性检测方法，如中国行业标准《水质急性毒性的测定——斑马鱼

卵法》(HJ 1069—2019) 即以斑马鱼受精卵的存活率表征被测水体的急性毒性; 当以鱼类胚胎的发育情况反映污染物或水体毒性时, 形成了基于鱼类胚胎发育法的水质生物毒性检测方法, 如国际标准化组织 (ISO) 制定的《水质—废水对斑马鱼胚胎 (*Danio rerio*) 急性毒性的测定》(ISO 15088—2007)、国际经济合作与发展组织 (OECD) 制定的《鱼类胚胎急性毒性试验》(OECD 236) 所采用的方法, 即是基于斑马鱼的胚胎发育情况对水体毒性进行检测, 由于胚胎相比成鱼对毒性变化更敏感, 常被用于污染物短时间暴露下的急性毒性检测; 而当以鱼类成体检测水质毒性时, 则更关注污染物长期暴露对鱼类不同器官和组织的影响, 如《21 天鱼类筛选试验》(OECD 230)。此外, 当以鱼类运动行为、心脏发育、神经发育作为毒性响应指标时, 也分别形成了基于鱼类运动行为法、心脏发育法、神经毒性法的水质生物毒性检测方法。

目前, 基于鱼类不同生理学指标所形成的毒性检测方法, 在环境污染物及污染水体毒性检测与分析方面已有广泛应用。例如, 斑马鱼存活率法, 已用于全氟辛烷磺酸[19]的毒性分析; 斑马鱼胚胎法已被广泛用于草甘膦[5]、重金属铅 (Pb)[20]与铜 (Cu)[21]、双酚 A[22]、纳米材料[23-24]等污染物的毒性研究; 斑马鱼心脏发育法, 已用于研究全氟辛酸[25]、芬太尼[26]等污染物的毒性效应; 斑马鱼神经毒性法, 已用于异丙威[27]、环丙沙星[28]等的毒性研究; 斑马鱼运动行为法, 已用于饮用水[29]等各类水体的毒性分析。但在已有的基于鱼类的各种水质生物毒性检测方法中, 以鱼类群体及鱼卵存活率、胚胎与心脏发育、神经发育等为毒性响应指标的检测方法, 其毒性测试时间都需在 24h 及以上。相应方法虽能用于不同毒性污染物的毒性检测与评估, 但面对突发性污染事故, 由于其时效性较差, 无法用于突发性污染水质毒性的现场快速监测与预警。而鱼类运动行为特征对污染物却具有快速响应特性, 基于鱼类运动行为的毒性检测方法在水体突发污染事件快速检测预警或水质现场快速调查监测方面具有突出优势。基于此, 德国 BBE 公司研发了基于斑马鱼运动行为特征的 Fish Taximeter 水质毒性检测仪。但如何克服鱼类个体间对毒性响应的差异性, 以提高毒性检测的精准性, 是未来基于鱼类运动行为毒性检测方法亟需攻克的难题。

1.2　基于蚤类的水质生物毒性检测方法

蚤类作为淡水水域广泛分布的甲壳纲枝角类浮游动物, 其以真菌、蚤类、溶解性有机物、碎屑物为食, 又是无脊椎动物和鱼类的天然饵料[30], 在水生食物链中处于重要环节, 因此在水生态毒理学研究和环境风险评估中被广泛应用。其中大型蚤 (*Daphnia magna*) 由于容易培养、生殖周期短、繁殖速度快、对毒性敏感、身体透明, 是国内外水质生物毒性检测公认的模式生物[31-32]。当蚤类暴露于毒性污染物或污染水体时, 有毒物质便会影响水蚤的生长、生理机能或行为 (如捕食和趋光行为), 并干扰其繁殖和发育, 甚至会导致水蚤死亡。因此, 水蚤的运动性能和死亡率常被用作毒性的响应指标, 基于此, 分别形成了基于蚤类运动抑制的水质生物毒性检测方法和基于蚤类死亡率的水质生物毒性检测方法。例如, ISO、OECD 及中国制定的《水质—大型水蚤 (枝角类甲壳纲) 活动性抑制的测定—急性毒性试验》(ISO 6341—1996)、《化学物质 蚤类急性运动抑制试验》(OECD 202)、《化学品 蚤类急性活动抑制试验》(GB/T 21830—2008) 等水质或化学品毒性测试的标准方法, 均以蚤类的运动抑制情况评估污染物或水体的毒性, 而 OECD 及中国制定的《水蚤繁殖试验》(OECD 211) 和《大型蚤急性毒性实验方法》(GB/T 16125—2012) 则以大型蚤的死亡率判定化学品的毒性。由于生物对毒性的应激行为往往先于死亡, 基于蚤类运动抑制的方法更多应用于水体急性毒性检测; 而基于蚤类死亡率的方法则更多应用于水体慢性毒性检测, 如应用广泛的《水蚤繁殖试验》(OECD 211) 即为观察水蚤在污染物下暴露 21 天后总存活后代数量。

目前, 基于大型蚤运动抑制性和死亡率的毒性检测方法已被广泛用于重金属[6]、有机污染物[33-34]、农药[35]、微塑料[36]及实际水体[37-39]的毒性检测研究。德国 BBE 公司也开发了基于水蚤运动行为的 DaphTox Ⅱ水质生物毒性检测仪。但蚤类运动性能和死亡率仍需 24h 及以上才会对毒物作出响应, 相应的毒性检测方法仍存在毒性检测所需时间较长的不足, 难以实现污染水体毒性快速监测与应急检测需求。郭鹄飞等[40]通过研究重金属镉 (Cd^{2+}) 对大型蚤的心率和摄食能力影响研究表明, 0.09mg/L Cd^{2+}作用 2h 时, 大型蚤的心率显著低于对照组, 9h 后, 0.05mg/L 和 0.09mg/L Cd^{2+}处理组大型蚤的摄食率和滤水率与对照组相比也显著降低, 因此, 蚤类心率和摄食能力两项毒性响应指标为污染物毒性快速检测提供了新途径。但这些蚤类生理特征对不同类型污染物是否均具有快速响应特性尚且

未知。因此,基于蚤类心率和摄食能力的毒性检测方法其普适性亟需进一步开展深入研究。

1.3　基于藻类的水质生物毒性检测方法

在水生态系统中,藻类作为一种光合生物,处于水生食物链的最前端,其作为水域系统中的最主要初级生产者及能量转换者,对水生态系统物质循环和能量流动至关重要[41-42],因此藻类生长状况能够直接反映水环境质量及水生态系统的健康状况。此外,藻类容易获取、易于培养、繁殖速度快、生长周期短、个体小、能够直接地观察细胞水平上的中毒症状[43],因此是水质生物毒性检测中极其重要的受试生物。

当受毒性物质影响时,表观上藻类的生长速率会受到抑制,生物量会有所降低,基于此国际上形成了基于藻类生长抑制法的水质毒性检测方法。OECD、ISO 及中国制定的《淡水藻类和蓝细菌 生长抑制试验》(OECD 201)、《化学品 藻类生长抑制试验》(GB/T 21805—2008)、《水质 以单细胞绿藻进行淡水藻类生长抑制实验》(ISO 8692:2012) 均规定,采用该方法对化学品及水体的毒性进行测试,并以 24 ~ 96h(通常为 72h) 毒性暴露时间时,藻细胞的生长抑制率评估毒物或水体的毒性。此外,由于毒性物质在抑制藻类生长与繁殖的同时,也会对藻类的叶绿素含量、蛋白质含量、抗氧化酶活性、细胞结构、遗传物质等生理要素产生影响,因此,通过观测暴露于毒物或污染水体中藻类上述生理要素的变化,即可分析检测对象的毒性强度。基于此,以藻类叶绿素含量、蛋白质含量、抗氧化酶活性、细胞结构及遗传物质等生理要素为毒性响应指标,分别形成了相应的水质生物毒性检测方法。目前基于藻类生长抑制法及上述多项关键生理要素的水质生物毒性检测方法,在环境重金属[7, 44-49]、有机污染物[43, 50-58]、水体[59-60]的毒性分析与评估方面均有所应用,但这些方法往往需要藻类暴露在较高浓度污染物下或长时间 (24h 及以上) 暴露才能产生毒性响应,并且蛋白质、叶绿素、酶、遗传物质等都需要复杂的提取过程,操作过程繁琐,因此仅适用于实验室毒性分析,无法用于各类水体水质毒性的现场快速检测及预警。

近年来,相关研究表明,藻类的光合作用也可以应用于水质毒性检测,且对污染物的毒性响应十分灵敏。水体中有机物敌草隆、阿特拉津、克百威、马拉硫磷、菲、苯酚、对苯醌、三氯乙腈及重金属 Cd、Cu、Pb 等在 5min ~ 2h 内即可对淡水蓝藻或绿藻的光合活性产生显著抑制作用[46, 61-64]。利用藻类光合作用,还可以检测出环境中更低浓度的污染物毒性。例如, Chen 等[61]发现,在 2μg/L 敌草隆下暴露 5min 时,蛋白核小球藻的光合活性即被显著抑制。因此,以藻类光合活性为毒性响应指标所形成的基于藻类光合抑制效应的水质生物毒性检测方法,能够显著提高传统水质毒性检测方法的时效性。同时,利用叶绿素荧光诱导动力学技术,可以快速、灵敏、无损测量活体藻类光合作用状态,并获得一系列与藻类光合作用相关的荧光参数,最常应用在毒性检测中的参数有最大光化学量子产率 (F_v/F_m) 和光合性能参数 (PI_{ABS}) 等[65]。藻类光合抑制效应毒性检测方法为各类水体毒性现场快速检测与判定,特别是为突发性水污染事件的快速监测与预警提供了有效手段,在水环境质量检测领域具有较好的发展前景。

1.4　基于发光菌的水质生物毒性检测方法

发光菌作为一类在淡水、海洋与陆地环境中广泛存在的兼性厌氧生物[66],具有独特的发光特性。发光菌能够发射荧光的原理为:细胞内还原型辅酶在荧光素酶催化下氧化还原态的黄素单核苷酸,同时释放出波长为 450 ~ 490nm 的蓝绿光[67-68],而这一发光特性与发光菌的细胞活性直接相关。当毒性物质作用于发光菌而影响菌体细胞的活性时,细胞内促使荧光发射的氧化还原反应活性也会有所降低,荧光发射强度有所改变[66, 69]。因此,基于发光菌的发光特性,形成了发光菌水质生物毒性检测方法。中国基于该方法,也制定了国家标准《水质急性毒性的测定 发光细菌法》(GB/T 15441—1995),该方法常用的受试菌种包括:明亮发光杆菌 (*Photobacterium phosphoreum*)[70]、费氏弧菌 (*Vibrio fischeri*)[8, 71]、青海弧菌 (*Vibrio qinghaiensis*)[72] 等。

发光菌毒性检测方法通过采用生物发光光度计测定发光菌的发光强度,以毒性胁迫下发光强度的抑制程度反映污染物或污染水体的毒性,由于具有仪器简单、操作简便、毒性响应快速等优势,30min 以内即可完成毒性检测,已成为目前国际上发展最为成熟的毒性检测手段。基于发光菌法,目前国际上已开发出多款水质生物毒性检测仪,如美国 Bechman 仪器公司的 Microtox 生物毒性在线分析仪、荷兰 Microlan 公司的 TOXcontrol 发光细菌毒性在线测定仪和 Toxmini 发光细菌毒性检测仪、荷兰 Skaler 公司的 ToxTracer 水质毒性分析仪、美国 SDI 公司的 Deltatox 生物毒性分析仪、美国哈希公司的 LUMISTOX 300 及 HACH Eclox 便携式水质毒性分析仪、意大利希思迪公司的 Easychem Tox 水质毒性

分析仪, 以及中国朗石生物仪器有限公司的 LumiFox 8000 发光细菌在线水质毒性预警系统等。

目前, 发光菌毒性检测方法在国内外重金属[8]及有机类[71, 73]等环境污染物毒性分析及各类水体[67, 74-79]水质毒性现场检测方面, 具有极其广泛的应用。虽然该方法可以快速检测水体污染物毒性, 但在实际应用中菌种的复活过程及活性状态极易受环境所影响, 从而导致测试结果重复性、稳定性较差, 且该方法仅以荧光发射强度表征污染物毒性, 毒性表征参数单一, 在针对荧光重叠的污染物时无法准确对污染物进行识别。因此, 如何提高发光菌毒性检测方法实际应用中的重复性、稳定性和准确性是未来亟需攻克的重点和难点。

2　毒性检测方法在典型环境污染物毒性分析中的应用

随着科技的不断发展, 应用于人类生产与生活各个领域的化学品数量不断激增。据报道, 全球市场所使用的化学品已高达 35 万种[80-81], 截至 2023 年 10 月,《中国现有化学物质名录》所记录的化学物质增至 4.6 万余种[82], 且每年仍有多种新的化学物质不断增补其中。在众多化学品中, 美国化学物质环境管理重要法规《有毒物质控制法》(TSCA) 中所涉及的有毒有害化学物质约有 85000 种[83], 由此可见, 有毒有害化学物质种类极多、数以万亿。且随着化学合成技术及生产工艺的持续发展, 全氟化合物、药物与个人护理品、抗生素、纳米材料、微塑料、消毒副产物、内分泌干扰物等新型环境污染物不断涌现[84-86], 使得有毒物质种类和数量仍呈不断递增趋势。一旦毒性污染物进入水环境并在生物体内累积, 其毒性效应将对生态系统及人类健康构成严重威胁。因此, 利用毒性检测方法对环境污染物进行毒性分析, 对于预测生态环境风险、保障生态系统安全具有重要作用与意义。

环境污染物种类繁多, 通常分为无机污染物和有机污染物。其中, 无机污染物中的重金属由于来源广泛, 具有难降解性、生物富集性及生物毒性等特点, 是当前环境领域关注的重点。而有机污染物中的农药 (包括除草剂、杀虫剂、杀菌剂) 作为现代农业生产所必须的农业投入品, 由于施用过程中有效利用率低、流失量大, 农药污染已成为生态环境领域重点关注的问题。因此, 本文以重金属及农药两类典型环境污染物为例, 分析现有毒性检测方法在环境污染物毒性检测应用中的特点, 基于此, 针对实际应用需求, 探讨水质生物毒性检测方法的未来发展趋势。

2.1　重金属生物毒性分析

在工业化及城市化快速发展中, 采矿、钢铁、冶炼、电镀等涉重行业及化工、电子、纺织、印染、汽车制造等工业生产均会产生 Pb、Cr、Cd、Hg、Cu、Zn、As、Ni 等重金属污染物, 因此, 重金属污染已成为当前全球所面临的主要环境污染问题之一[87-88]。重金属对生物体的毒性效应及强度, 是评估进入水体的重金属所具有水生态风险的重要依据, 因此重金属的生物毒性一直备受关注。

在重金属生物毒性分析方面, Kataba 等[20]采用斑马鱼胚胎发育法研究了环境水体中 Pb 的含量水平对斑马鱼胚胎的影响, 明确了 Pb 通过诱导胚胎凝血, 产生不利的心血管效应及神经肌肉效应, 从而使胚胎活性受损, 且在 100μg/L Pb 暴露 24h 情况下, 斑马鱼胚胎的发育已受 Pb 的毒害影响; Traudt 等[89]以大型蚤活动抑制法分析了重金属 Cd、Cu、Ni、Zn 的毒性效应, 研究表明这 4 种重金属对大型蚤的正常活动性均有抑制影响, 48h 时 4 种重金属的 EC_{50}(半数最大效应浓度) 平均值分别为 0.054、0.100、1.633、0.928mg/L; Qu 等[90]采用发光菌法分析了重金属 Cd 对明亮发光杆菌的毒性效应, 发现 15min 作用时间下明亮发光杆菌的生理活性已受 Cd 的毒性影响, 其 EC_{50} 值为 1.03mg/L; 对于受试生物藻类, 由于其生长情况是叶绿素与蛋白质含量、各种生理相关的酶活性的综合体现, 因此藻类生长抑制法常用于重金属的毒性分析研究。例如, Mo 等[91]采用藻类生长抑制法分析了不同暴露时间下 (24、48、72、96h) 重金属 Cd 对栅藻、蛋白核小球藻、羊角月牙藻三种淡水绿藻的毒性影响, 明确了 Cd 对不同藻类的毒性作用都具有时间依赖性, 进而采用藻类叶绿素含量法、超氧化物歧化酶活性法、过氧化氢酶活性法对毒性效应进行分析, 明确了 Cd 作用下藻类叶绿素含量会有所降低, 抗氧化物歧化酶与过氧化氢酶的活性及丙二醇的含量会有所增加, 从而明确了 Cd 对藻细胞具有氧化应激作用等致毒机理; Gao 等[92]以杜氏盐藻 (*Dunaliella salina*) 为受试生物, 同样采用藻类生长抑制法评估了重金属 Cu、Pb、Cd 对杜氏盐藻的毒性强度, 明确了这三种重金属毒性具有较大差异性, 72h-EC_{50} 值分别为 18.14mg/L、160.37mg/L、3.32mg/L。总体上看, 重金属对鱼类、蚤类、发光菌类、蚤类不同层级水生生物都有毒性影

响,以这些水生生物为受试生物的不同毒性检测方法目前在 Cd、Cu、Pb、Cd、Zn、Ni 等典型重金属污染物毒性分析方面也都有所应用,获取的相关毒性数据为水生态环境风险评估提供了重要依据。

为了明确不同毒性检测方法对重金属毒性检测性能的差异性,以重金属中致畸性和致癌性较强的 Cd 为例,以 EC_{50} 值或 LC_{50} 值 (半数致死浓度) 作为毒性评估指标,汇总了基于不同毒性检测方法所获取的 Cd 的毒性数据,具体列于表 1。可以看出,不同受试生物间对重金属毒性的响应敏感性存在差异,总体上,蚤类和发光菌类对重金属的毒性响应灵敏性要优于鱼类和藻类;其次,同一类型受试生物,不同受试物种间对重金属的毒性响应敏感性也有所不同。例如,邰安琪[93]通过对比 4 种不同藻种间在相同作用时间下对 Cd 的响应特性,发现小环藻的毒性响应最为灵敏,其次是铜绿微囊藻、四尾栅藻,而蛋白核小球藻的毒性响应灵敏性最弱;同样,Mo 等[91]研究也表明不同藻种对 Cd 的响应灵敏性有所不同,蛋白核小球藻对 Cd 的毒性响应敏感性要强于斜生栅藻和羊角月牙藻;再次,重金属对水生生物的毒性效应具有时间依赖性,受试生物毒性暴露时间越长,重金属对受试生物所产生的毒性效应越强。例如,Mo 等[91]研究表明毒性作用时间由 24h 增加到 96h 时,Cd 对蛋白核小球藻、斜生栅藻、羊角月牙藻的 EC_{50} 值均逐渐减小,毒性效应均有所增强。在毒性检测时效性方面,发光细菌法及藻类光合抑制

表 1　基于不同毒性检测方法获得的重金属 Cd 毒性数据

Table 1　Toxicity data of heavy metal Cd based on different toxicity detection methods.

序号	受试生物	受试物种	毒性响应生物指标	检测对象	响应时间	毒性数据表达方式	毒性数值	参考文献
1	藻类	铜绿微囊藻 (*Microcystis aeruginosa*)	生长抑制	Cd	96h	EC_{50}	0.302mg/L	邰安琪[93]
2	藻类	四尾栅藻 (*Scenedesmus quadricauda*)	生长抑制	Cd	96h	EC_{50}	1.80mg/L	邰安琪[93]
3	藻类	小球藻 (*Chlorella pyrenoidosa*)	生长抑制	Cd	96h	EC_{50}	5.60mg/L	邰安琪[93]
4	藻类	小环藻 (*Cyclotella hebeiana*)	生长抑制	Cd	96h	EC_{50}	0.12mg/L	邰安琪[93]
5	藻类	斜生栅藻 (*Scenedesmus obliquus*)	生长抑制	Cd	24h	EC_{50}	5.38mg/L	Mo 等[91]
6	藻类	斜生栅藻 (*Scenedesmus obliquus*)	生长抑制	Cd	96h	EC_{50}	0.88mg/L	Mo 等[91]
7	藻类	蛋白核小球藻 (*Chlorella pyrenoidosa*)	生长抑制	Cd	24h	EC_{50}	3.52mg/L	Mo 等[91]
8	藻类	蛋白核小球藻 (*Chlorella pyrenoidosa*)	生长抑制	Cd	96h	EC_{50}	0.86mg/L	Mo 等[91]
9	藻类	羊角月牙藻 (*Selenastrum capricornutum*)	生长抑制	Cd	24h	EC_{50}	6.34mg/L	Mo 等[91]
10	藻类	羊角月牙藻 (*Selenastrum capricornutum*)	生长抑制	Cd	96h	EC_{50}	1.57mg/L	Mo 等[91]
11	藻类	杜氏盐藻 (*Dunaliella salina*)	生长抑制	Cd	72h	EC_{50}	3.32mg/L	Gao 等[92]
12	藻类	蛋白核小球藻 (*Chlorella pyrenoidosa*)	光合抑制 (PI_{ABS})	Cd	3h	EC_{50}	1.46mg/L	Gan 等[65]
13	鱼类	斑马鱼 (*Danio rerio*)	胚胎发育	Cd	120h	LC_{50} (半致死浓度)	15.20mg/L	罗紫蝶等[94]
14	鱼类	斑马鱼 (*Danio rerio*)	胚胎发育	Cd	6h	死亡率极显著增加	2mg/L	杨瑞瑞等[95]
15	发光菌	明亮发光杆菌 (*Photobacterium phosphoreum*)	发光强度	Cd	15min	EC_{50}	1.03mg/L	Qu 等[90]
16	发光菌	明亮发光杆菌 (*Photobacterium phosphoreum*)	发光强度	Cd	15min	EC_{50}	0.05 ~ 0.74mg/L (在不同 Ca^{2+}、Mg^{2+}等环境下)	Qu 等[96]
17	蚤类	大型蚤 (*Daphnia magna*)	运动性	Cd	24h	EC_{50}	0.35 ~ 1.21mg/L (pH: 9.0 ~ 5.0)	Qu 等[90]
18	蚤类	大型蚤 (*Daphnia magna*)	存活率	Cd	48h	EC_{50}	0.054mg/L	Traudt 等[89]

法均能对重金属的毒性快速作出响应, 与鱼类、蚤类、藻类生长抑制等毒性检测方法相比, 毒性响应时间会由 24h 及以上降低到 3h 甚至数分钟, 因此两种毒性检测方法均是突发性重金属类污染水体毒性现场快速调查、监测、预警的重要手段。而与其他毒性检测方法相比, 发光菌检测方法兼具毒性响应时间最短、毒性响应灵敏性最好两项特点, 在重金属类污染水体毒性检测方面具有突出优势。

2.2　农药生物毒性分析

农药由于具有减少病虫害、抑制杂草、提高农作物品质与产量的功效, 已成为现代农业生产中大量使用的农业投入品。但由于农药在施用过程中利用率极低, 约近施用量的 70% 会随降雨、灌溉、地表及地下径流进入水环境, 对水生生物安全及人类健康构成严重威胁, 因此, 了解农药对水生生物体的毒性效应与强度, 对水生态系统风险评估具有重要作用。近些年来, 相关研究学者采用不同毒性检测方法应用于农药毒性分析开展了大量研究工作。

2.2.1　杀虫剂生物毒性分析

为了解杀虫剂对水生生物的毒性影响, Zhang 等[97] 及 Gao 等[98] 采用藻类毒性检测方法, 分析了毒死蜱、特丁磷、甲胺磷、氟虫胺等杀虫剂对不同藻类的毒性效应, 明确了 4 种杀虫剂均会改变藻类的叶绿素、丙二甲醇、活性氧的含量及总抗氧化能力, 进而对生长速率产生抑制效应, 其中, 毒死蜱对肋骨条藻的 96h-EC_{50} 值为 0.572mg/L[97], 氟虫胺对蛋白核小球藻的 48 ~ 96h-EC_{50} 值为 3.5 ~ 2.8mg/L[98]; Tien 等[99] 则采用藻类生长抑制法, 对比了毒死蜱、特丁磷、甲胺磷三种杀虫剂对硅藻 (*Nitzschia sp.*)、蓝藻 (*Oscillatoria sp.*) 及绿藻蛋白核小球藻的毒性强度, 结果表明不同藻类对杀虫剂毒性的耐受性有所差异, 三种杀虫剂对硅藻 (*Nitzschia sp.*) 的 48h-EC_{50} 值为 0.30 ~ 1.68mg/L, 对蓝藻 (*Oscillatoria sp.*) 的 48h-EC_{50} 值为 0.33 ~ 7.99mg/L, 对蛋白核小球藻的 48h-EC_{50} 值为 1.29 ~ 41.16mg/L。Sarmah 等[100]、李瑞瑞等[101] 及 Sanches 等[102] 通过采用斑马鱼毒性检测方法对甲氨氯丙烷、氯氟醚菊酯、阿维菌素、乙螨唑、硫虫嗪等杀虫剂对斑马鱼的毒性效应进行分析, 明确了这些杀虫剂均可导致斑马鱼仔鱼脊柱和尾部发生弯曲、心包和卵黄囊出现水肿、心率和孵化率显著降低、抗氧化酶活性下降、活力丧失、细胞周期激活基因表达能力减弱、胚胎发育不完整 (如体长变短、头部和眼睛面积缩小), 最终导致胚胎细胞死亡等毒性效应, 其中甲氨氯丙烷对斑马鱼胚胎的 96h-LC_{50} 值为 0.156mg/L[100], 氯氟醚菊酯对斑马鱼胚胎的 72h-LC_{50} 值为 0.756mg/L[101], 阿维菌素对斑马鱼胚胎的 48h-LC_{50} 值为 59μg/L[102], 乙螨唑对斑马鱼胚胎的 72h-LC_{50} 值为 23mg/L[103], 硫虫嗪对斑马鱼不同生命阶段的 96h-LC_{50} 值为 147 ~ 246mg/L[104]; Man 等[105] 采用蚤类毒性检测方法, 也证明杀虫剂吡虫啉、环氧虫啶、戊唑醇对大型蚤的生长、运动性、繁殖能力、超氧化物歧化酶及过氧化氢酶的活性都有影响, 最终导致大型蚤死亡; Wang 等[106] 研究也表明新型杀虫剂溴氟替尼 (Broflanilide) 对大型蚤也具有毒性影响, 其在 8.45μg/L 时即会对大型蚤的甲壳素酶、脱皮类固醇和相关基因的表达产生抑制效应, 并对大型蚤的后代生长、发育和繁殖都会产生影响; Fan 等[107] 采用发光菌法, 研究了啶虫脒、噻虫啉、吡虫啉、氯噻啉 4 种新尼古丁类杀虫剂对费氏弧菌的毒性效应, 证明 4 种杀虫剂对费氏弧菌都具有毒性影响, 且光分解产物会比 4 种杀虫剂本体有更强的毒性; 陈彦吉等[31] 对比了阿维菌素及伊维菌素两种杀虫剂对斜生栅藻和大型蚤的毒性效应, 发现 640mg/L 阿维菌素及伊维菌素暴露 96h 后对斜生栅藻的生长抑制率为 55.73% 和 41.84%, 但对大型蚤的 48h-LC_{50} 值 (半数致死浓度) 分别为 1.43μg/L 和 0.51μg/L, 表明两种杀虫剂对大型蚤具有较强的毒性效应, 但对斜生栅藻毒性较小。

总体来看, 杀虫剂对鱼类、蚤类、藻类、发光菌类水生生物均会产生毒性效应, 但不同受试生物对杀虫剂的毒性响应灵敏性仍有差异, 虽然目前缺少同一种杀虫剂对不同类型受试生物及同一类型不同受试物种毒性的系统对比研究, 但基于已有研究数据表明, 蚤类对杀虫剂的毒性响应比藻类和鱼类更为灵敏。

2.2.2　杀菌剂生物毒性分析

杀菌剂作为防治病原微生物的一类农药, 相关研究人员采用不同生物毒性检测方法已证实其对水生生物鱼类、蚤类、发光菌类、藻类均有毒性影响。其中, Zhang 等[97] 采用藻类生长抑制法, 研究了杀菌剂三氯杀螨醇对肋骨条藻的毒性效应, 获取了其 96h-EC_{50} 值为 0.43mg/L; Schmidt 等[108] 采用斑马鱼胚胎发育法, 分析了多种杀菌剂对斑马鱼胚胎的毒性作用。当以斑马鱼胚胎致死性判断不同杀菌剂的毒性强度时, 多菌灵的 96h-LC_{50} 值为 1.25μmol/L(0.24mg/L)[108], 三氯生的 120h-LC_{50} 值为

0.51mg/L[109]，恶霉灵的 96h-LC_{50} 值为 649mg/L[110]，苯醚甲环唑的 48h-LC_{50} 值为 1.4μg/L[102]，丁菌唑对斑马鱼胚胎不同生命阶段的 96h-LC_{50} 值为 5.2～10.3mg/L[104]；Mendes 等[111]通过研究也发现杀菌剂代森锰锌在 11.8μg/L 时即会引发斑马鱼运动模式发生改变，破坏其先天防御机制；Qi 等[112]采用蚤类运动抑制毒性检测方法，分析了杀菌剂 rac-戊唑醇及 S-戊唑醇对大型蚤的毒性效应，发现两者的 48h-EC_{50} 值分别为 3.53mg/L 和 2.74mg/L；Hernando 等[113]采用发光菌法和蚤类活动抑制法，对比了杀菌剂抑菌灵和 Irgarol 1051 对费氏弧菌及大型蚤的毒性作用，研究表明抑菌灵和 Irgarol 1051 对费氏弧菌的 30min-EC_{50} 值分别为 0.06mg/L 和 15.5mg/L，对大型蚤的 48h-EC_{50} 值分别为 1.05mg/L 和 7.3mg/L，其中费氏弧菌对抑菌灵毒性响应既快速又灵敏，但对 Irgarol 1051 的毒性响应灵敏性要弱于大型蚤。目前，由于缺少不同类型受试生物对同一种杀菌剂毒性效应的系统研究与对比分析，基于已有杀菌剂对不同受试生物的毒性数据，难以判定对杀菌剂具有灵敏毒性响应特性的受试生物种类。

2.2.3　除草剂生物毒性分析

除草剂在杀灭农田中杂草、提高农作物产量的同时，其进入水环境也会对水生生物产生毒害效应，特别是对水中藻类这一光合生物的影响已引起生态环境领域的广泛关注。基于藻类生长抑制法，Zhang 等[97]研究表明除草剂乙草胺对海洋硅藻中肋骨条藻的 96h-EC_{50} 值为 0.14mg/L；蔡卫丹等[114]通过研究获取了异丙甲草胺对斜生栅藻及普通小球藻的 96h-EC_{50} 值分别为 0.15mg/L 和 0.083mg/L；Vidal 等[115]研究表明苯敌草对羊角月牙藻的 48h-EC_{50} 值为 3.22μg/L，对莱茵衣藻的 48h-EC_{50} 值为 0.266mg/L；He 等[116]采用藻类生长抑制法和蚤类死亡率法，分别获取了两种除草剂阿特拉津和丁草胺对斜生栅藻和隆腺蚤的毒性数据，其中阿特拉津和丁草胺对斜生栅藻的 96h-EC_{50} 值分别为 0.0147mg/L 和 2.31mg/L，对隆腺蚤的 48h-LC_{50} 值分别为 60.6mg/L 和 3.40mg/L，由此可见，藻类对除草剂的响应灵敏性要优于蚤类；Gatidou 等[117]采用发光细菌法，分析了敌草隆、利谷隆、单谷隆三种除草剂对费氏弧菌的毒性影响，获取了三种除草剂的 30min-EC_{50} 值分别为 9.2mg/L、82mg/L、11.2mg/L；相关研究人员采用斑马鱼胚胎发育法，也证明了 AD-67[118]、芬克洛林[118]、氟唑[118]、氟吡酰草胺[119]、乙氟灵[120]、甲草胺[121]、解草酮[122]等除草剂均可导致斑马鱼心脏和卵黄囊肿胀、超氧化物酶活性下降、还原酶和过氧化氢酶活性改变、活性氧产生、血管生成相关基因表达下降、鱼体内血管生成受抑制，从而导致斑马鱼胚胎形态及发育异常、畸形率增加，最终导致胚胎死亡，影响斑马鱼胚胎存活率，其中 AD-67、芬克洛林、氟唑对斑马鱼胚胎的 96h-LC_{50} 值分别为 2.52mg/L、1.26mg/L、2.01mg/L[118]，阿特拉津的 48h-LC_{50} 值为 36.8mg/L[123]、异草酮的 120h-LC_{50} 值为 61.4mg/L[124]、敌草隆的 96h-LC_{50} 值为 6.5mg/L[125]。

以不同生物毒性检测方法所获取的不同除草剂的毒性数据列于表 2。可以看出，与蚤类、发光菌类、鱼类相比，除草剂对藻类具有更强的毒性效应，在水生态系统中，藻类是最易受除草剂类污染物毒害的水生生物，因此需要更加关注除草剂对水体藻类群落结构及初级生产能力的影响。此外，由于藻类对除草剂的毒性响应更为灵敏，加之藻类光合作用对毒性物质具有快速响应特性，因此，基于藻类光合抑制效应的水质生物毒性检测方法是除草剂类污染水体毒性现场快速灵敏检测的有力工具。

由上述不同毒性检测方法在重金属、农药毒性效应分析的应用现状可知，不同营养级受试生物对不同类型毒性污染物的响应灵敏性存在差异，其中蚤类和发光菌类对重金属类污染物毒性响应最为灵敏，蚤类对杀虫剂毒性响应灵敏性要优于其他类型受试生物，而藻类对除草剂类污染物的毒性响应灵敏性最优。因此，当对实际水体的水质毒性进行检测时，在已知水体污染类型的情况下，以响应最为灵敏的受试生物开展水质毒性检测工作，更有利于提高水体污染程度判定的准确性。但随着环境污染物的种类不断增多，当前水环境正面临着日趋复杂的水体复合污染问题，鉴于不同类型水生生物对不同污染物的毒性响应灵敏性存在差异，未来建立基于多层级水生生物的多指标水质生物毒性检测与评估方法，将是水环境生态风险评估及水环境安全管理的重要发展趋势。

3　毒性检测方法在水体综合毒性检测与评估中的应用

水质生物毒性作为水体水质的综合指标，由于能够有效地弥补传统理化指标在水质评估方面的不足，为水质安全监管提供更为完善的科学依据，水质

表 2　不同生物毒性检测方法所获取的除草剂对藻类、蚤类、发光菌类、鱼类的毒性数据

Table 2　Toxicity data of herbicides to algae, fleas, luminescent fungi and fish obtained by different biological toxicity detection methods.

序号	受试生物	受试物种	毒性响应指标	检测对象	响应时间	毒性数据表达方式	毒性数值	参考文献
1	藻类	羊角月牙藻 (*Pseudokirchneriella subcapitata*)	生长抑制	阿特拉津	96h	EC_{50}	1.6mg/L	Ralston-Hooper 等[126]
2	藻类	斜生栅藻 (*Scenedesmus obliquus*)	生长抑制	阿特拉津	96h	EC_{50}	0.0147mg/L	He 等[116]
3	藻类	斜生栅藻 (*Scenedesmus obliquus*)	生长抑制	异丙甲草胺	96h	EC_{50}	0.150mg/L	蔡卫丹等[114]
4	藻类	普通核小球藻 (*Chlorella nucleata*)	生长抑制	异丙甲草胺	96h	EC_{50}	0.083mg/L	蔡卫丹等[114]
5	藻类	羊角月牙藻 (*Pseudokirchneriella subcapitata*)	生长抑制	苯敌草	48h	EC_{50}	3.22μg/L	Vidal 等[115]
6	藻类	莱茵衣藻 (*Chlamydomonas reinhardtii*)	生长抑制	苯敌草	48h	EC_{50}	0.266mg/L	Vidal 等[115]
7	藻类	浮萍 (*Lemna minor*)	生长抑制	利谷隆	7d	EC_{50}	30.5μg/L	Gatidou 等[117]
8	藻类	浮萍 (*Lemna minor*)	生长抑制	敌草隆	7d	EC_{50}	28.3μg/L	Gatidou 等[117]
9	藻类	浮萍 (*Lemna minor*)	生长抑制	单谷隆	7d	EC_{50}	0.3mg/L	Gatidou 等[117]
10	蚤类	隆腺蚤 (*Daphnia carinata*)	死亡率	阿特拉津	48h	LC_{50}	60.6mg/L	He 等[116]
11	蚤类	大型蚤 (*Daphnia magna*)	死亡率	阿特拉津	48h	LC_{50}	86.19mg/L	Herrera 等[127]
12	蚤类	大型蚤 (*Daphnia magna*)	运动性	敌草隆	48h	EC_{50}	8.6mg/L	Hernando 等[113]
13	蚤类	大型蚤 (*Daphnia magna*)	运动性	利谷隆	48h	EC_{50}	7.0mg/L	Hernando 等[113]
14	蚤类	大型蚤 (*Daphnia magna*)	活动抑制	草甘膦	48h	EC_{50}	3.7~10.6mg/L	Cuhra 等[128]
15	蚤类	大型蚤 (*Daphnia magna*)	活动抑制	苯敌草	48h	EC_{50}	4.45mg/L	Vidal 等[115]
16	蚤类	长刺蚤 (*Daphnia longispina*)	活动抑制	苯敌草	48h	EC_{50}	2.73mg/L	Vidal 等[115]
17	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	阿特拉津	48h	LC_{50}	36.8mg/L	Wiegand 等[123]
18	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	阿特拉津	96h	LC_{50}	29.06mg/L	Yan 等[129]
19	鱼类	罗非鱼 (*Tilapia mossambicus*)	胚胎活性	三嗪类除草剂	96h	LC_{50}	8.8mg/L	汝少国等[130]
20	鱼类	鲤鱼 (*Cyprinus carpio*)	胚胎活性	扑草净	96h	LC_{50}	8.0mg/L	汝少国等[130]
21	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	AD-67	96h	LC_{50}	2.52mg/L	Liu 等[118]
22	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	芬克洛林	96h	LC_{50}	1.26mg/L	Liu 等[118]
23	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	氟唑	96h	LC_{50}	2.01mg/L	Liu 等[118]
24	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	异草酮	120h	LC_{50}	61.4mg/L	Stevanovic 等[124]
25	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	敌草隆	96h	LC_{50}	6.5mg/L	Velki 等[125]
26	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	硫芬定	5d	EC_{50}	0.818mg/L	Ivantsova 等[131]
27	鱼类	斑马鱼 (*Danio rerio*)	胚胎活性	乙草胺	72h	LC_{50}	48.4μmol/L (13.06mg/L)	Xu 等[132]

（续表 2）

序号	受试生物	受试物种	毒性响应指标	检测对象	响应时间	毒性数据表达方式	毒性数值	参考文献
28	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	利谷隆	30min	EC_{50}	5.5mg/L	Hernando 等[113]
29	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光性	草甘膦	30min	EC_{50}	44.2mg/L	Hernando 等[133]
30	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	利谷隆	30min	EC_{50}	82mg/L	Gatidou 等[117]
31	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	敌草隆	30min	EC_{50}	9.2mg/L	Gatidou 等[117]
32	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	单谷隆	30min	EC_{50}	11.2mg/L	Gatidou 等[117]
33	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	2, 4-二硝基酚	15min	EC_{50}	13.8mg/L	Bettiol 等[134]
34	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	二硝甲酚	15min	EC_{50}	5.67mg/L	Bettiol 等[134]
35	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	特乐酚	15min	EC_{50}	0.371mg/L	Bettiol 等[134]
36	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	羟敌草腈	15min	EC_{50}	7.85mg/L	Bettiol 等[134]
37	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	溴草腈	15min	EC_{50}	7.30mg/L	Bettiol 等[134]
38	发光菌	费氏弧菌 (*Vibrio fischeri*)	发光特性	碘草腈	15min	EC_{50}	4.82mg/L	Bettiol 等[134]

生物毒性检测已成为当前废水排放监管、水污染状况调查、突发性水污染事件应急监测的必要内容。随着各类水质生物毒性检测方法的发展，基于不同受试生物的毒性检测方法在各类实际水体，如各行业排放废水、生活污水、环境水体毒性检测与评估方面已有广泛应用。例如，朱爱萍等[9]采用发光细菌法对不同时期北江中上游 (翁源段) 翁江及横石河沿岸地下水的生物毒性进行了调查，并以发光菌的发光强度抑制率评估了不同点位水体在不同时间的毒性强度；宋张杨等[11]同样采用发光细菌法并以发光强度抑制率分析了煤化工企业污水处理厂进出水的生物毒性水平；Zhang 等[10]以斑马鱼的死亡率为依据，采用斑马鱼毒性检测法研究了城市污水处理厂缺氧-好氧 (AO) 过程对斑马鱼急性毒性的去除效果；武毛妮等[12]以普通小球藻为受试生物，采用藻类生长抑制法对陕西西安市生活污水处理厂各处理单元进出水的生物毒性进行了检测，并以 EC_{50} 值及该值所对应的毒性当量 (Toxic Unit, TU) 对各污水处理厂各处理阶段水样的生物毒性进行了表征 ($TU=100/EC_{50}$，表示当毒性效应达到 50% 时原水的稀释倍数)；薛柯等[13]采用斑马鱼胚胎法与发光菌法，对常州 6 家水处理厂进水与出水的生物毒性进行了测试，并以 TU 表示水质毒性强度，对比分析了厌氧-缺氧-好氧 (A^2/O) 处理工艺对各污水处理厂进水毒性的消减程度；Heisterkamp 等[135]采用藻类、蚤类、鱼类、发光菌类毒性检测方法，分别对建筑产品洗脱液的毒性进行了检测，并以 LID(最低无效应稀释度) 表征了洗脱液的毒性强度；嵇志远等[67]采用发光细菌法，对某市城区一定数量的水源水、出厂水以及管网水的水质毒性进行了快速测定，并以 LID、EC_{20}(发光菌发光强度抑制率为 20% 时样品的浓度值) 与 EC_{50} 来表示水体的水质毒性强度。

上述研究工作表明，当采用毒性检测方法对水体的水质生物毒性进行检测时，水体毒性强度的表征及评估方式有所不同。目前，主要以毒性响应指标受影响程度 (如发光菌发光强度的抑制率)、半数效应浓度 (EC_{50}) 及其所对应的毒性当量 (TU)、最低无效应稀释倍数 (LID) 三种方式来表征及评估水体的毒性。其中，毒性响应指标受影响程度能够反映水体的表观毒性，而 EC_{50}(或 TU) 及 LID 均是在对水体稀释处理情况下所获得的水体毒性信息，因此所反映的毒性为被测水体的潜在毒性。由于不同毒性表征方法所代表的毒性意义有所不同，分级标准也有所差异，当对同一水体采用不同毒性表征方法进行评估时，其所反映的毒性强度水平可能会有所不同，彼此矛盾。因此，融合现有不同毒性表征方式 (毒性响应指标受影响程度、EC_{50} 或 TU、LID)，建立能够全面表达表观毒性及潜在毒性的水质生物毒性表征新方法及相应的毒性强度分级标准，对全面准确地判断水体污染状况、准确评估水生态风险、有效

地保障水环境质量安全具有重要作用与意义, 将是未来环保行业及水环境安全监管领域的发展重点。

4　总结与展望

近年来, 由于水质生物毒性作为一种效果导向的水体综合指标备受关注, 水质生物毒性检测方法已取得了长足发展, 逐渐形成了基于不同受试生物(鱼类、蚤类、发光菌类、藻类) 的水质生物毒性检测方法, 并已被证明是应对不明污染、复合污染及新型污染水体综合污染状况调查、监测、预警的有力工具。其中, 发光菌法及藻类光合抑制法由于具有毒性响应快速、提高毒性检测时效性的特点, 是突发性水污染毒性现场快速调查与监测的重要手段。虽然不同毒性检测方法在环境污染物 (如重金属、农药) 毒性效应分析以及实际水体水质生物毒性检测与评估方面均有所应用, 但不同受试生物对不同类型污染物的毒性响应灵敏性存在差异, 如蚤类与发光菌对重金属毒性响应最为灵敏, 藻类对除草剂毒性响应最为灵敏; 并且在实际水体的水质生物毒性检测与评估中, 已有的三种不同毒性表征与评估方法: 毒性响应指标受影响程度、EC_{50}(或 TU)、LID, 由于其所表达的毒性意义不同, 毒性分级标准有所差异, 所反映的同一被测水体的毒性水平会有所不同, 互相矛盾。

针对上述问题, 将是未来研究的重点建议: ①优选出同一类型受试生物中最为敏感的受试物种, 实现水质生物毒性灵敏检测; ②将现有基于不同单种受试生物的水质生物毒性检测方法相结合, 建立基于鱼类、蚤类、发光菌类、藻类的多层级水生生物多响应指标的统一的水质生物毒性检测与评估方法, 以实现水体毒性的全面检测; ③将响应指标受影响程度、EC_{50}(或 TU)、LID 三种现有毒性表征方式所代表的水体表观毒性、潜在毒性相融合, 建立水体毒性全面表征新方法, 实现水体毒性的全面表达与评估, 并建立统一的水质毒性分级标准; ④建立水质生物毒性与水生态风险间的关联模型, 以使水质生物毒性检测与评估在水环境安全监管中发挥关键作用。

A Review of Research Progress and Application Status of Biological Toxicity Detection Methods in Water Quality

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HIGHLIGHTS

(1) Fish, fleas, luminescent fungi and algae are the main four types of organisms tested for biological toxicity detection of water quality.

(2) The toxicity detection method based on luminescence characteristics of luminescent bacteria and photosynthesis of algae is an important means for rapid detection of biological toxicity in water quality.

(3) The detection and assessment method of water biological toxicity based on multi-level aquatic organisms and multi-indicators is an important development direction of water quality and water ecological environment safety supervision in the future.

ABSTRACT: Faced with increasingly serious water pollution problems, water quality biotoxicity testing has become an important means of water quality status investigation and water environment safety supervision because it can reflect the toxic effects of pollutants on aquatic organisms in an effect-oriented manner. In view of this, based on the domestic and foreign literature on water quality biotoxicity testing in recent years, the existing water quality biotoxicity testing methods are systematically summarized the toxicity response indicators and toxicity testing principles used in different testing methods combed, the characteristics of different testing methods are compared, and the development status of existing testing methods are analyzed here. At present, water quality biotoxicity detection methods based on different tested organisms (fish, fleas, luminescent fungi, algae) have been widely used in toxicity analysis of typical environmental pollutants such as heavy metals and pesticides, and in actual water body toxicity detection and evaluation. In the future, developing a multi-indicator water quality biotoxicity detection and evaluation method based on multi-level aquatic organisms, establishing a new comprehensive characterization and evaluation method of water quality toxicity that integrates the apparent toxicity and potential toxicity of water bodies, and corresponding toxicity grading standards will be the key development points and difficulties of water quality biological toxicity detection research direction. The BRIEF REPORT is available for this paper at http://www.ykcs.ac.cn/en/article/doi/10.15898/j.ykcs.202412110256.

KEY WORDS: water quality biological toxicity; toxicity detection; water quality safety; environmental pollutants; aquatic organism; toxicity test method for photobacteria; toxicity test method for algae

BRIEF REPORT

With the rapid development of socio-economic systems and industrial-agricultural activities worldwide, there has been an exponential increase in both the variety and quantity of chemical substances utilized globally. Substantial volumes of industrial effluent, agricultural wastewater, and domestic sewage containing toxic and hazardous compounds are being discharged into aquatic ecosystems without adequate treatment. This has precipitated severe water contamination issues that critically disrupt aquatic ecological equilibrium, while posing significant threats to aquatic biodiversity security and human health[1]. Water quality toxicity testing, due to its ability to reflect the toxic effects of pollutants on aquatic organisms in an outcome-oriented manner, addresses the limitations of traditional physicochemical indicators that cannot comprehensively detect the numerous toxic pollutants in water or accurately assess their harmful effects on biological organisms. It has become an important tool for water quality investigation and environmental safety regulation, playing a crucial role in safeguarding water quality and aquatic ecosystem safety[9-13]. The development of existing water quality toxicity testing methods and their applications in different types of polluted water bodies are systematically summarized, and the key future development directions of water quality toxicity testing are discussed.

1. Water quality toxicity detection methods

Currently, the commonly used test organisms in water quality bio-toxicity detection are aquatic organisms at different trophic levels, including fish, daphnia, bioluminescent bacteria, and algae. Therefore, corresponding water quality bio-toxicity detection methods have been developed based on different types of test organisms.

1.1 Fish-based water quality toxicity detection methods

Fish, as lower vertebrates, are commonly used in toxicity detection due to their high genetic similarity to humans, with similar signaling pathways. As a result, fish are the most widely used test organisms in water quality toxicity testing. Currently, toxicity detection methods based on various physiological indicators of fish have been extensively applied in environmental pollutant toxicity assessments. For example, the zebra fish survival rate method has been used to analyze the toxicity of perfluorooctane sulfonate[19], and the zebra fish embryo method has been widely applied to toxicity studies of pollutants such as glyphosate[5], heavy metals like lead[20] and copper[21],

bisphenol A[22], and nanomaterials[23-24]. However, fish-based toxicity tests generally require exposure times of 24h or longer, making these methods more suitable for observing the long-term toxic effects of water pollutants.

1.2 Daphnia-based water quality toxicity detection methods

Daphnia magna, a species of large water flea, is recognized as a model organism for water quality toxicity testing due to its ease of cultivation, short reproductive cycle, rapid reproduction, sensitivity to toxicity, and transparent body. When exposed to toxic pollutants, the growth, physiological functions, or behavior (such as predation and phototaxis) of Daphnia are affected, interfering with their reproduction and development, and potentially leading to death. Currently, toxicity detection methods based on Daphnia, such as those assessing movement inhibition and mortality rates, have been widely used to study the toxicity of heavy metals[6], organic pollutants[33-34], pesticides[35], microplastics[36], and actual water bodies[37-39]. However, these methods still require considerable testing time and cannot meet the need for rapid toxicity monitoring and emergency detection in polluted water bodies.

1.3 Algae-based water quality toxicity detection methods

Algae, as primary producers, are crucial for the material cycling and energy flow within aquatic ecosystems[41-42]. Due to their short growth cycles, small individual size, and the ability to observe toxic symptoms at the cellular level[43], algae are key organisms in water quality toxicity testing. When exposed to toxic substances, the growth rate of algae is inhibited, and physiological elements such as protein content, antioxidant enzyme activity, and genetic material are affected. Currently, methods based on algae growth inhibition and other key physiological factors have been applied in the toxicity analysis and assessment of environmental heavy metals[7,44-49], organic pollutants[43,50-58], and water bodies[59-60]. However, these methods often require long exposure times, and the complex extraction processes for proteins, enzymes, and genetic material make them unsuitable for on-site rapid toxicity detection. Recent developments in algae-based photosynthetic inhibition detection methods can detect toxicity within 5min to 2h of exposure[61-64], showing promising prospects in water quality monitoring.

1.4 Bioluminescent bacteria-based water quality toxicity detection methods

Bioluminescent bacteria, which are facultative anaerobic organisms widely found in freshwater, marine, and terrestrial environments[66], exhibit unique luminescent characteristics. Bioluminescent bacteria toxicity testing methods involve measuring the light intensity emitted by the bacteria using a bioluminescence photometer, with the degree of inhibition of luminescence reflecting the toxicity of pollutants or polluted water. With advantages such as simple instrumentation, ease of operation, and rapid toxicity response, the toxicity testing can be completed within 30min and has become one of the most developed toxicity testing methods internationally. Currently, bioluminescent bacteria toxicity testing methods have been widely applied in the toxicity analysis of heavy metals[8] and organic pollutants[71,73], as well as the on-site detection of water quality toxicity in various water bodies[67,74-79]. Although this method can rapidly detect the toxicity of pollutants in water, its application is hindered by the fact that the revival process and activity state of bacterial strains are highly susceptible to environmental factors, which can lead to poor reproducibility and stability of test results.

2. Applications of toxicity detection methods in typical environmental pollutants and actual water bodies

This review focuses on heavy metals and pesticides as representative environmental pollutants to analyze the methodological characteristics and application efficacy of current toxicity assessment approaches in environmental contaminant detection. A summary of the literature reveals that test organisms at different trophic levels exhibit different response sensitivities to different classes of toxic contaminants. Daphnia and bioluminescent bacteria are the most sensitive to heavy metal pollutants[89-90], while Daphnia exhibit higher sensitivity to pesticides than other organisms[31,106]. Algae show the highest sensitivity to herbicide pollutants[115-116].

When applying toxicity assessment methodologies to detect aquatic biotoxicity levels, significant divergence

exists in the characterization protocols and evaluation frameworks for quantifying waterborne toxicological intensity. Currently, toxicity is generally represented and assessed by the degree to which toxicity response indicators are affected (such as the inhibition rate of luminescence intensity in bioluminescent bacteria)[11], the half-effect concentration (EC_{50}) value and its corresponding toxicity unit (TU)[12], and the lowest observable effect dilution factor (LID)[135]. Among these, toxicity response indicators reflect the apparent toxicity of water bodies, while EC_{50} (or TU) and LID provide information about the potential toxicity of water bodies obtained after dilution treatment.

3. Concluding remarks

In recent years, water quality toxicity detection methods have made significant progress, and methods based on different test organisms (fish, Daphnia, bioluminescent bacteria, algae) have gradually been developed. Among these, the bioluminescent bacteria method and algae photosynthetic inhibition method, due to their rapid toxicity response, are important tools for the rapid investigation and monitoring of sudden water pollution toxicity. Although different toxicity detection methods have been applied in environmental pollution and actual water body toxicity testing and assessment, there are differences in the sensitivity of different test organisms to various pollutants. Furthermore, toxicity grading standards vary in actual water quality testing and assessment, leading to potential discrepancies and contradictions in the toxicity levels of the same water body.

4. Future perspectives

Future research should focus on the following key areas: (1) Developing water quality toxicity detection and assessment methods based on multi-level aquatic organisms and multi-indicators. (2) Establishing a new method for comprehensive water toxicity characterization and assessment that integrates apparent toxicity and potential toxicity, along with corresponding toxicity grading standards. (3) Developing correlation models between water quality biological toxicity and aquatic ecological risk, to enhance the role of water quality biological toxicity detection and assessment in water environmental safety regulation.

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