

Five decades of crocodilian ecotoxicology: a global scientometric analysis of environmental biomonitoring research

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Abstract

Crocodilians are valuable sentinel species for environmental biomonitoring because of their longevity, high trophic position, site fidelity, and capacity to bioaccumulate contaminants. This study presents a global scientometric assessment of crocodilian ecotoxicology by combining bibliometric analyses with a manually curated classification of ecotoxicological attributes. A PRISMA-based Scopus search retrieved 370 records, of which 187 publications from 1971 to May 2026 met the eligibility criteria. Bibliometrix, VOSviewer, and Python-based analyses were used to examine publication trends, conceptual structure, species, geographical distribution, contaminants, biomarkers, biological matrices, and study designs. Scientific production increased markedly after 2000. *Alligator mississippiensis* and *Caiman latirostris* were the predominant model species, while the United States, Argentina, Brazil, and South Africa were the principal research hubs. Species-level analyses associated *A. mississippiensis* mainly with heavy metals and endocrine disruption, *C. latirostris* with pesticide toxicology and multiple-biomarker approaches, and *Crocodylus moreletii* and *C. niloticus* with metals and persistent organic pollutants. The literature progressively shifted from contaminant quantification toward mechanistic and effect-based assessments. However, substantial taxonomic, geographical, and methodological gaps remain. These findings provide a global baseline for future biomonitoring, ecological risk assessment, and crocodilian conservation.

Introduction

Crocodilians are long-lived reptiles that occupy high trophic levels in aquatic and semi-aquatic ecosystems, where they play fundamental roles in ecosystem structure, nutrient cycling, and trophic regulation. Their ecological characteristics, including longevity, high site fidelity, delayed sexual maturity, and position as apex or mesopredators, make them particularly suitable as sentinel species for environmental monitoring (Campbell 2003; Gardner and Oberdörster 2005). Because they integrate contaminant exposure over long periods while occupying upper trophic levels, crocodilians provide valuable information on contaminant bioaccumulation, biomagnification, and ecosystem health.

Environmental contamination has intensified worldwide as a consequence of industrialization, agricultural expansion, mining activities, urbanization, and increasing chemical production. Aquatic ecosystems are now exposed to complex mixtures of contaminants, including heavy metals, persistent organic pollutants (POPs), pesticides, endocrine-disrupting chemicals (EDCs), pharmaceuticals, and other anthropogenic compounds capable of persisting in the environment and accumulating throughout food webs (van der Oost et al. 2003). These contaminants may induce chronic and sublethal effects that compromise wildlife health long before demographic consequences become apparent, highlighting the importance of biological monitoring programs based on sentinel organisms.

Over the last three decades, crocodilians have been increasingly incorporated into ecotoxicological investigations because of their ability to accumulate contaminants and exhibit measurable biological responses to chemical exposure (dos Santos et al. 2021). Studies have documented endocrine and

reproductive alterations, developmental abnormalities, immunotoxic responses, and genotoxic damage in several crocodilian species (Guillette et al. 1994; Crain et al. 1997; Crain et al. 1998; Gunderson et al. 2002; Siroski et al. 2016; Burella et al. 2017; López González et al. 2017; Marrugo-Negrete et al. 2019). Collectively, these findings have established crocodilians as valuable models for investigating the biological consequences of environmental contamination.

The growing application of biomarkers has substantially expanded the scope of crocodilian ecotoxicology. Contemporary studies increasingly combine chemical analyses with biomarkers that detect early physiological disturbances before population-level effects become evident. These biomarkers include biochemical responses, oxidative stress parameters, endocrine alterations, immune function, histopathological lesions, and genotoxic endpoints such as micronucleus frequency and DNA strand breaks (van der Oost et al. 2003; Galloway et al. 2004). Furthermore, contaminant exposure has been evaluated using diverse biological matrices, including blood, liver, muscle, adipose tissue, eggs, scutes, and claws, allowing the assessment of both recent exposure and long-term contaminant accumulation (Buenfil-Rojas et al. 2020; Schneider et al. 2015).

Despite the continuous growth of the literature, research on crocodilians as biomonitors of environmental contamination remains fragmented. Most studies have focused on individual contaminants, specific biomarkers, or single species under localized environmental conditions, limiting a broader understanding of how the field has evolved over time. Furthermore, research effort is geographically uneven, with a strong concentration of studies involving *Alligator mississippiensis* in North America, particularly those associated with historical contamination events in Florida (Guillette et al. 1994; Gunderson et al. 2004). Consequently, important taxonomic groups, contaminant classes, and biodiversity-rich regions remain comparatively underrepresented.

Scientometric approaches provide powerful quantitative tools for investigating the development, structure, and evolution of scientific research by integrating bibliometric indicators with network and thematic analyses (Aria and Cuccurullo 2017; Donthu et al. 2021). Beyond identifying publication trends, scientometric analyses allow the characterization of collaboration networks, conceptual structures, emerging themes, and knowledge gaps within scientific disciplines. When combined with domain-specific classifications, such as contaminant categories, biomarker types, biological matrices, and study designs, scientometric approaches can also reveal historical changes in research priorities and methodological development.

Although bibliometric studies have become increasingly common across environmental sciences, no global scientometric assessment has specifically investigated the evolution of crocodilian ecotoxicology. In particular, no previous study has integrated conventional bibliometric analyses with a detailed manual classification of ecotoxicological attributes, including contaminant categories, biomarkers, biological matrices, taxonomic models, geographic distribution, and experimental design. Such integration provides a more comprehensive understanding of how scientific knowledge has

developed within this field while simultaneously identifying research biases and opportunities for future investigation.

Therefore, this study provides a global scientometric analysis of research on crocodilians as biomonitors of environmental contamination. Specifically, we evaluated temporal trends in scientific production, identified the leading countries, journals, and taxonomic models, characterized the principal contaminant and biomarker categories investigated, and explored the conceptual structure and temporal evolution of the literature through bibliometric network and categorical analyses.

Material and Methods

Data collection and search strategy

The scientometric analysis was based on a systematic literature search conducted in the Scopus database (Elsevier) between January and May 2026. The final search update and bibliographic data export were performed in May 2026, and the dataset included all records indexed in Scopus up to that point. Scopus was selected because of its broad multidisciplinary coverage, high-quality bibliographic indexing, and extensive representation of peer-reviewed environmental science literature.

The search strategy was designed to retrieve studies investigating crocodilians in the context of environmental contamination, encompassing the principal contaminant classes and ecotoxicological biomarkers historically investigated in the literature. Crocodilian descriptors were searched in the title field, whereas contaminant and ecotoxicological descriptors were searched in titles, abstracts, and author keywords using the following query:

TITLE (crocodile OR alligator* OR caiman* OR "Crocodylia") AND TITLE-ABS-KEY ("heavy metal*" OR "trace element*" OR mercury OR lead OR cadmium OR arsenic OR pesticide* OR PCB* OR PAH* OR "persistent organic pollutant*" OR ecotoxicology OR "endocrine disrupt*" OR "endocrine disruption" OR genotoxic* OR micronucleus OR "comet assay")*

No publication-year restrictions were applied. Crocodilian descriptors were restricted to the title field to increase search specificity and reduce the retrieval of publications in which crocodilians were only incidentally mentioned. Contaminant and ecotoxicological descriptors were searched in titles, abstracts, and author keywords to retain studies addressing the principal chemical stressors and biological responses historically investigated in the field.

The search descriptors prioritized established contaminant groups and ecotoxicological endpoints to support historical consistency and comparability across five decades of scientific production. Emerging contaminants such as per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, and microplastics were not included as independent search descriptors. Nevertheless, studies addressing these contaminants could still be retrieved when they also included one or more of the ecotoxicological terms incorporated into the search strategy.

All retrieved records were exported in BibTeX and CSV formats, including authors, affiliations, publication year, source title, abstract, author keywords, cited references, and citation metrics. Study screening, data curation, manual ecotoxicological classification, and scientometric analyses were subsequently conducted through July 2026.

Eligibility criteria

Study selection followed a PRISMA-based workflow comprising identification, title and abstract screening, eligibility assessment, and final inclusion. All records were screened by a single reviewer (R.L.S.) using predefined eligibility criteria.

Original research articles, review articles, and scholarly book chapters investigating crocodilians in the context of environmental contamination were considered eligible. Records were included when they evaluated contaminant occurrence, bioaccumulation, environmental biomonitoring, biomarkers, or ecotoxicological effects associated with chemical exposure.

The screening process involved the sequential assessment of titles, abstracts, and full texts when necessary. Records for which eligibility could not be confidently determined from the title and abstract were retained for full-text assessment. Publications primarily focused on taxonomy, ecology, geographic distribution, captive management, or veterinary medicine without a direct environmental contamination context were excluded. Studies addressing analytical method development without direct ecotoxicological application were also excluded, as were conference abstracts, dissertations, theses, editorials, and other documents lacking sufficient information for classification.

Of the 370 records screened, 183 were excluded after title and abstract assessment because they did not meet the predefined eligibility criteria. The remaining 187 reports were retrieved and assessed for eligibility, and all were included in the final curated dataset. No reports were unavailable for retrieval, and no reports were excluded after full-text assessment.

Data processing and standardization

Bibliographic records were imported into the Bibliometrix package (Aria and Cuccurullo 2017) through its Biblioshiny graphical interface within the R environment for bibliometric processing and descriptive analyses. Subsequently, all records underwent manual verification and computational standardization to ensure consistency across the ecotoxicological dataset. Taxonomic nomenclature, contaminant terminology, author keywords, and biological matrices were standardized to minimize redundancy and harmonize synonymous descriptors.

Because individual studies frequently investigated multiple species, contaminant classes, biomarkers, or biological matrices, a dedicated data-processing workflow was implemented in Python (version 3.10). Multi-valued fields were expanded using an "explode" procedure implemented in the pandas library,

allowing each ecological attribute to be analyzed independently while preserving the integrity of the original bibliographic records and preventing inflation of publication counts.

Scientometric analysis

Scientometric analyses were performed using the Bibliometrix package (Aria and Cuccurullo 2017) through the Biblioshiny interface and complemented by VOSviewer version 1.6.20 (van Eck and Waltman 2010). Descriptive bibliometric indicators included annual scientific production, source productivity, and journal impact based on the local h-index. The conceptual structure of the field was explored through author-keyword co-occurrence analysis. Network visualization was generated using the association strength normalization algorithm implemented in VOSviewer.

Keyword network analysis

Keyword co-occurrence networks were constructed using author keywords extracted from the selected publications. Analyses employed the Full Counting method with a minimum occurrence threshold of three keywords. Prior to network construction, keywords were manually refined to remove taxonomic redundancies, geographic descriptors, spelling variations, and non-informative terms. The final network retained the thirty most representative keywords describing contaminants, biomarkers, ecotoxicological responses, and crocodilian species. Network and overlay visualizations were generated using the association strength normalization algorithm implemented in VOSviewer.

Manual ecotoxicological classification

To complement the bibliometric analyses, each of the 187 selected studies was manually classified according to ecotoxicological attributes extracted directly from the original publications. The classification included: crocodilian species; country of study; study design; contaminant category; specific contaminants; biological matrices; biomarker categories; biological endpoints.

This manually curated dataset enabled ecological analyses that are not directly available through conventional bibliometric software, providing a more detailed characterization of research specialization and methodological evolution within crocodilian ecotoxicology.

Categorical data analysis

The manually classified dataset was analyzed using Python (version 3.10) through the pandas and matplotlib libraries. Descriptive categorical analyses included absolute frequencies, contingency tables, and cross-tabulations examining country × contaminant category, species × contaminant category, species × biomarker category, and biomarker category × publication period.

Species–contaminant associations were evaluated using a contingency matrix derived from the standardized multi-valued records generated during data processing. Each unique publication was counted only once within each species–contaminant pair, thereby preventing duplicate counts when the same association appeared more than once within a record. Publications investigating more than one species or contaminant category contributed to each corresponding association. Records classified as “Multiple crocodilian species” were retained in the overall descriptive analyses but excluded from the species-level matrix because they could not be assigned to a single taxon. The resulting frequencies, expressed as the number of publications reporting each species–contaminant association, were visualized using a heatmap.

Species–biomarker associations were evaluated using a second contingency matrix following the same counting procedure. Each unique publication was counted only once within each species–biomarker category pair. Publications investigating more than one species or biomarker category contributed to each corresponding association, whereas records classified generically as “Multiple crocodilian species” were excluded from the species-level matrix. Frequencies were expressed as the number of publications reporting each association and visualized using a heatmap.

Temporal analyses grouped publications into four chronological intervals: 1971–2000, 2001–2010, 2011–2020, and 2021–2026. These analyses were descriptive and were used to identify historical changes in contaminant priorities, biomarker application, and research specialization.

PRISMA flow and reproducibility

The study-selection process followed the PRISMA 2020 framework (Page et al. 2021). The Scopus search identified 370 records. No duplicate records, automation-based exclusions, or other pre-screening removals were recorded. All 370 records were screened by title and abstract, resulting in the exclusion of 183 records. The remaining 187 reports were sought for retrieval, successfully retrieved, assessed for eligibility, and included in the final curated dataset. The complete selection process is summarized in the PRISMA flow diagram (Fig. 1).

To support methodological transparency, the complete search strategy, eligibility criteria, data-processing procedures, and analytical methods are described in the manuscript. Bibliographic metadata, the manually curated ecotoxicological dataset, contingency matrices, and analytical code were retained in structured formats and are available from the corresponding author upon reasonable request.

Results

Database retrieval and study selection

The Scopus search identified 370 records. No records were removed before screening. Following title and abstract assessment, 183 records were excluded because they did not meet the predefined eligibility

criteria. The remaining 187 reports were retrieved and assessed for eligibility, and all were included in the final curated dataset. No reports were excluded after full-text assessment.

[Insert Fig. 1 here]

Figure 1 PRISMA 2020 flow diagram illustrating the study-selection process. The Scopus search identified 370 records, of which 183 were excluded after title and abstract screening. The remaining 187 reports were retrieved, assessed for eligibility, and included in the final curated dataset.

Temporal evolution of scientific production

Scientific production on crocodilians as biomonitors of environmental contamination covered the period from 1971 to May 2026 and exhibited a marked increase over time (Fig. 2). Between 1971 and 2000, publication output remained consistently low, generally comprising no more than one or two studies per year. A noticeable increase began after 2000, followed by continuous growth throughout the following two decades. The highest publication rates were observed during the 2011–2020 period, with publication activity remaining elevated through 2021–2026. Overall, the temporal distribution demonstrates a sustained increase in scientific interest in crocodilian ecotoxicology during the last two decades.

Source impact and journal distribution

The selected studies were published across a broad range of environmental science journals. However, publication output was concentrated in a relatively small group of specialized journals. According to the local h-index calculated from the dataset, *Environmental Pollution*, *Chemosphere*, and *Ecotoxicology and Environmental Safety* were among the principal publication sources represented in the dataset. The remaining publications were distributed across a broad range of environmental toxicology, ecology, physiology, and wildlife journals.

Taxonomic and geographical distribution

Marked taxonomic asymmetry was observed among crocodilian species included in the literature. *Alligator mississippiensis* was the most frequently investigated species, appearing in 84 studies (44.9%), followed by *Caiman latirostris* (39 studies; 20.9%), *Crocodylus moreletii* (17 studies; 9.1%) and *Crocodylus niloticus* (15 studies; 8.0%). The geographical distribution of scientific production also showed clear regional specialization (Fig. 3). Regional differences in contaminant research priorities are presented in Online Resource 1.

The United States accounted for the largest number of publications (86 studies) and investigated the widest diversity of contaminant categories, particularly heavy metals and endocrine-disrupting compounds. Argentina represented the second principal research center, with pesticide-related studies predominating, especially those involving glyphosate- and atrazine-based formulations. Brazilian

publications were primarily associated with heavy-metal contamination, whereas studies conducted in South Africa focused predominantly on heavy-metal exposure in *Crocodylus niloticus* populations.

Species-specific contaminant profiles

Cross-tabulation of crocodilian species and contaminant categories revealed marked species-specific research profiles (Fig. 4a). *Alligator mississippiensis* exhibited the broadest contaminant coverage, with studies focusing primarily on heavy metals (n = 26) and endocrine-disrupting compounds (n = 22), followed by persistent organic pollutants (POPs; n = 11). In contrast, research involving *Caiman latirostris* was strongly concentrated on pesticides (n = 23), which substantially exceeded the number of studies addressing endocrine disruptors (n = 6), heavy metals (n = 3), or POPs (n = 2).

Studies involving *Crocodylus moreletii* focused predominantly on heavy metals (n = 9) and POPs (n = 8), whereas *Crocodylus niloticus* was investigated mainly in relation to heavy metals (n = 8) and, to a lesser extent, POPs (n = 4). Heavy metals also dominated the literature involving *Caiman crocodilus* (n = 8) and nearly all less frequently studied crocodilian species. Overall, the matrix demonstrates that the taxonomic distribution of crocodilian ecotoxicology is accompanied by pronounced specialization in the contaminant categories investigated for each principal model species.

Species-specific biomarker profiles

The distribution of biomarker categories also revealed marked methodological specialization among crocodilian species (Fig. 4b). *Alligator mississippiensis* exhibited the broadest biomarker profile, with bioaccumulation (n = 30) and endocrine disruption (n = 23) representing the most frequent categories, followed by reproductive effects (n = 11) and multiple-biomarker approaches (n = 10).

In contrast, studies involving *Caiman latirostris* were characterized by greater emphasis on integrated and effect-based assessments. Multiple-biomarker approaches represented the most frequent category (n = 11), followed by genotoxicity (n = 8), endocrine disruption (n = 7), and reproductive effects (n = 5). Bioaccumulation alone was reported in only one publication involving this species.

Research involving *Crocodylus moreletii* and *Crocodylus niloticus* was dominated by bioaccumulation studies (n = 12 for each species), with comparatively limited application of effect-based biomarkers. A similar predominance of bioaccumulation was observed among most less frequently investigated species. Overall, the matrix demonstrates that methodological diversification has been concentrated in a small number of established model species, while much of the remaining taxonomic diversity has been assessed primarily through contaminant accumulation measurements.

Keyword co-occurrence network

The keyword co-occurrence analysis identified four major thematic clusters within the literature. The largest cluster was organized around mercury, selenium, reproduction, and maternal transfer. A second cluster grouped terms associated with bioaccumulation, heavy metals, biomonitoring, and organochlorine pesticides. A third cluster included endocrine disruption, biomarkers, and pesticides,

whereas the fourth cluster was primarily composed of genotoxicity, micronucleus test, comet assay, glyphosate, and *Caiman latirostris*. Together, these clusters describe the principal thematic organization of crocodilian ecotoxicology and illustrate the relationships among contaminants, biomarkers, and biological responses.

Temporal evolution of contaminants and biomarkers

The chronological distribution of biomarker categories revealed important changes in research focus over time. Bioaccumulation remained the most frequently investigated endpoint throughout the study period, comprising 80 studies (42.8%). In contrast, studies adopting multiple-biomarker approaches increased substantially after 2000, reaching 13 publications during 2011–2020 and remaining at the same level during 2021–2026. Genotoxic biomarkers were absent from publications prior to 2000 but became progressively incorporated into the literature during the following decades. Overall, the temporal distribution demonstrates increasing diversification in the biological endpoints investigated in crocodilian ecotoxicology (Fig. 5).

Discussion

The emergence and consolidation of crocodilian ecotoxicology

The temporal evolution identified in the present scientometric analysis demonstrates the progressive consolidation of crocodilian ecotoxicology as a distinct research field within environmental sciences. Although studies investigating contaminant exposure in crocodilians have been published since the early 1970s, scientific production remained limited for nearly three decades, with a marked increase only after the beginning of the twenty-first century. This publication trajectory closely follows the historical development of ecotoxicology itself, which evolved from descriptive contaminant monitoring toward increasingly interdisciplinary investigations integrating environmental chemistry, toxicology, physiology, molecular biology, and ecological risk assessment (van der Oost et al. 2003; Walker et al. 2012).

The acceleration in scientific production after 2000 likely reflects the convergence of scientific, technological, and regulatory advances. International environmental agreements, particularly the Stockholm Convention on Persistent Organic Pollutants, stimulated long-term environmental monitoring and encouraged the development of standardized approaches for contaminant assessment in wildlife. Simultaneously, improvements in analytical instrumentation, including inductively coupled plasma mass spectrometry (ICP-MS), gas chromatography coupled to mass spectrometry (GC-MS), and liquid chromatography tandem mass spectrometry (LC-MS/MS), substantially increased analytical sensitivity, allowing contaminants to be quantified at environmentally relevant concentrations. These methodological advances expanded the scope of wildlife ecotoxicology by enabling researchers to investigate not only contaminant occurrence but also the physiological mechanisms underlying toxic effects.

The temporal pattern observed in the present dataset indicates that the expansion of crocodilian ecotoxicology occurred within a broader period of methodological and conceptual transformation in environmental sciences. Growing concern regarding chemical pollution, biodiversity loss, ecosystem degradation, and anthropogenic pressures on freshwater ecosystems increased the demand for approaches capable of integrating contaminant occurrence with biological effects. Therefore, the growth observed in crocodilian ecotoxicology should be interpreted as part of a broader scientific movement toward interdisciplinary environmental assessment rather than as an isolated increase in research involving reptiles.

The evolution of research objectives throughout the study period further illustrates the maturation of the discipline. Early investigations frequently emphasized contaminant occurrence and bioaccumulation. Rainwater et al. (2007), for example, quantified metals and organochlorine pesticides in caudal scutes of crocodiles from Belize and Costa Rica, illustrating the use of keratinized tissues for contaminant monitoring. As ecotoxicological concepts evolved, studies increasingly incorporated biomarkers capable of detecting biological responses before population-level effects became apparent. Consequently, biochemical, endocrine, immunological, histopathological, oxidative-stress, and genotoxic biomarkers became progressively integrated into contaminant assessment, reflecting the transition from exposure-based monitoring toward effect-based ecotoxicology. Similar shifts have been described for aquatic vertebrates, in which biomarker-based approaches link contaminant exposure to alterations in biological function (van der Oost et al. 2003; Galloway et al. 2004).

The increasing use of crocodilians as sentinel organisms represents another important milestone in the historical development of the field. Their exceptional longevity, high trophic position, delayed sexual maturity, relatively restricted home ranges, and capacity to bioaccumulate contaminants enable individuals to integrate environmental exposure over extended periods while reflecting contaminant transfer through aquatic food webs (Campbell 2003; Gardner and Oberdörster 2005). Unlike short-lived vertebrates that primarily reflect recent exposure, crocodilians provide long-term ecological records of contaminant dynamics, making them particularly valuable for evaluating chronic pollution in freshwater and estuarine ecosystems.

Our findings also corroborate previous reviews highlighting the increasing importance of reptiles in environmental biomonitoring. For example, dos Santos et al. (2021) reported a continuous expansion of studies using crocodilians and freshwater turtles to investigate inorganic contaminants, particularly mercury, cadmium, and lead. By adopting a broader search strategy encompassing multiple contaminant classes and biological responses, the present study extends those observations and demonstrates that the growth of crocodilian ecotoxicology has been accompanied by substantial diversification in contaminants, biomarkers, and methodological approaches. This diversification reflects the progressive integration of wildlife toxicology with molecular biology, environmental chemistry, and conservation science.

Despite the sustained increase in scientific production, important limitations remain evident. Ecotoxicological research continues to be concentrated in a relatively small number of crocodilian species and geographical regions, indicating that the expansion of the discipline has resulted primarily from the intensification of research on well-established model species rather than from a proportional increase in taxonomic coverage. Consequently, many crocodilian species inhabiting tropical ecosystems remain poorly represented in the scientific literature despite facing increasing pressures associated with mining, agricultural intensification, urbanization, and climate change. Expanding ecotoxicological investigations to these underrepresented taxa and regions will therefore be essential for improving the global representativeness of future biomonitoring efforts.

Overall, the temporal evolution documented in this study demonstrates that crocodilian ecotoxicology has progressed from a niche research topic into a mature and internationally recognized discipline. The convergence of advances in analytical chemistry, environmental legislation, molecular toxicology, and ecological risk assessment has transformed crocodilians into strategic sentinel organisms capable of supporting long-term environmental monitoring. Future progress will likely depend on integrating conventional contaminant analyses with emerging molecular tools, standardized biomarker frameworks, and interdisciplinary approaches capable of evaluating contaminant effects across multiple levels of biological organization.

Scientific specialization and geographical asymmetries

The publication landscape identified in this scientometric analysis reveals that crocodilian ecotoxicology has developed around a relatively small but highly specialized scientific community. Rather than being broadly distributed across journals dedicated to herpetology or zoology, the literature is concentrated in internationally recognized environmental science journals, demonstrating that research involving crocodilians has progressively become integrated into the broader fields of ecotoxicology, environmental chemistry, and ecological risk assessment. This publication profile reflects the interdisciplinary nature of contemporary environmental sciences, in which analytical chemistry, wildlife toxicology, ecology, and conservation biology increasingly converge to investigate the ecological consequences of chemical contamination (Walker et al. 2012; Newman 2023).

The predominance of *Environmental Pollution*, *Chemosphere*, and *Ecotoxicology and Environmental Safety* further illustrates the scientific maturity of the discipline. These journals have historically served as major publication platforms for studies integrating contaminant quantification with biological responses, particularly those involving wildlife biomonitoring and environmental risk assessment. Similar publication patterns have been reported in recent bibliometric studies of aquatic ecotoxicology, in which these journals consistently rank among the principal outlets for research on contaminant exposure and biomarker responses in aquatic vertebrates (Moral-Muñoz et al. 2020). Their central position in the present dataset therefore indicates that crocodilian ecotoxicology has become firmly embedded within mainstream environmental science rather than remaining a niche area of reptile biology.

The geographical distribution of scientific production demonstrates equally pronounced asymmetries. The United States accounted for nearly half of all publications included in the dataset and investigated the greatest diversity of contaminant classes. This leadership largely reflects the long-term use of *Alligator mississippiensis* as a sentinel species in freshwater ecosystems, supported by continuous monitoring programs conducted in regions such as Florida and the southeastern United States. Historical contamination events, particularly those associated with Lake Apopka, stimulated decades of interdisciplinary research investigating endocrine disruption, reproductive abnormalities, contaminant bioaccumulation, and physiological responses, establishing one of the most comprehensive wildlife ecotoxicology datasets currently available for reptiles (Guillette et al. 1994; Guillette and Gunderson 2001; Milnes et al. 2005).

Regional environmental pressures strongly influenced the thematic specialization observed in other countries. Argentina has become a major research center for pesticide ecotoxicology involving crocodilians, particularly through the use of *Caiman latirostris* as an experimental model. Studies conducted by Argentine research groups have investigated genotoxic and immunological responses following embryonic or post-hatching pesticide exposure under controlled conditions (Poletta et al. 2009; Latorre et al. 2016; Burella et al. 2017). These investigations have contributed substantially to the mechanistic understanding of pesticide toxicity in reptiles and have established *C. latirostris* as an important experimental model in wildlife ecotoxicology.

Brazil exhibited a distinct research profile in the curated dataset, characterized by a predominance of studies involving trace elements, particularly mercury. Brazilian investigations were mainly associated with contaminant occurrence, bioaccumulation, and biomonitoring in tropical freshwater ecosystems. This pattern reflects the regional importance of metal contamination in aquatic food webs and highlights the contribution of Brazilian research to the use of crocodilians as biomonitoring models in biodiversity-rich environments.

South African research demonstrated another regional specialization centered on *Crocodylus niloticus*. Studies have documented population decline and pollution-related concerns in the Loskop Dam system (Botha et al. 2011), as well as high lead exposure and clinical signs of toxicosis in wild Nile crocodiles from the Lake St Lucia estuarine system (Humphries et al. 2022). Although the number of studies remains smaller than that observed in North America, these investigations have expanded the taxonomic and geographical scope of crocodilian ecotoxicology beyond the traditionally studied American species.

The marked taxonomic concentration identified in the present study reflects the unequal distribution of scientific effort across the order Crocodylia. Nearly two-thirds of the published literature focuses on only two species, *Alligator mississippiensis* and *Caiman latirostris*, whereas many extant crocodilian species remain virtually absent from ecotoxicological investigations. This pattern is not necessarily associated with ecological relevance but rather with the existence of established research infrastructure, long-term monitoring programs, and accessibility of study populations. Similar taxonomic biases have been

reported across wildlife ecotoxicology, where a limited number of model species often dominate scientific production despite the considerably broader biological diversity available (Newman 2023).

The species–contaminant matrix further demonstrates that the taxonomic imbalance in crocodilian ecotoxicology is accompanied by pronounced thematic specialization. *Alligator mississippiensis* presented the broadest contaminant profile, reflecting its long-term use in monitoring programs investigating metals, persistent organic pollutants, endocrine-disrupting compounds, and more recently, emerging contaminants. In contrast, *Caiman latirostris* was strongly associated with pesticide toxicology, particularly experimental investigations of glyphosate- and atrazine-based formulations. Research involving *Crocodylus moreletii* and *Crocodylus niloticus* was concentrated mainly on heavy metals and POPs, consistent with the principal contamination pressures investigated in Mexico and southern Africa, respectively (Poletta et al. 2009; Botha et al. 2011; Burella et al. 2017; Humphries et al. 2022).

These profiles should not be interpreted as evidence of intrinsic species-specific susceptibility to particular contaminant classes. Rather, they reflect the geographical distribution of the species, regional pollution sources, accessibility of study populations, established research infrastructure, and the historical specialization of individual research groups. Nevertheless, this thematic compartmentalization limits direct comparisons among species and restricts the extrapolation of ecotoxicological responses across Crocodylia. Comparative studies applying standardized contaminant and biomarker frameworks to multiple species would therefore be particularly valuable for distinguishing biological variation from differences generated by research design and regional scientific priorities.

The geographical and taxonomic asymmetries identified here have important implications for environmental monitoring and conservation. Large regions of Southeast Asia, Central Africa, and Oceania support highly diverse crocodilian assemblages while simultaneously experiencing rapid agricultural expansion, mining, dam construction, and urbanization. Nevertheless, ecotoxicological information from these biodiversity hotspots remains scarce, limiting our understanding of contaminant exposure under different ecological and climatic conditions. Expanding international collaborations and strengthening research capacity in these underrepresented regions should therefore constitute a major priority for future crocodilian ecotoxicology.

Collectively, these findings demonstrate that the global development of crocodilian ecotoxicology has been driven by geographically distinct research programs responding to regional environmental challenges. Rather than representing a homogeneous scientific field, the literature reflects multiple complementary research traditions, each contributing unique perspectives on contaminant exposure, biomarker development, and ecological risk assessment. Recognizing these regional research signatures is essential for identifying knowledge gaps and promoting a more balanced global understanding of contaminant impacts on crocodilian populations.

Evolution of contaminants and biological responses

The thematic structure and temporal evolution identified in the present scientometric analysis demonstrate that crocodilian ecotoxicology has undergone a profound conceptual transformation over the past five decades. Rather than representing isolated changes in research priorities, the shifts observed in keyword networks and biomarker categories reflect the broader evolution of environmental toxicology from descriptive contaminant monitoring toward mechanistic investigations capable of linking chemical exposure to physiological dysfunction and ecological risk. This trajectory parallels the development of ecotoxicology as a scientific discipline, in which advances in analytical chemistry, molecular biology, and environmental risk assessment have progressively expanded the ability to evaluate both contaminant occurrence and their biological consequences (van der Oost et al. 2003; Walker et al. 2012; Newman 2023).

The co-occurrence network revealed that heavy metals remain the conceptual backbone of crocodilian ecotoxicology. Mercury, selenium, cadmium, and lead have historically dominated the literature because of their persistence, bioaccumulative potential, and capacity to biomagnify through aquatic food webs. As long-lived apex predators, crocodilians integrate contaminant exposure over decades, making them particularly suitable for investigating trophic transfer and long-term environmental contamination. Consequently, early studies focused primarily on quantifying trace elements in blood, liver, muscle, adipose tissue, eggs, and keratinized tissues, generating the baseline datasets that still underpin contemporary ecotoxicological investigations (Rainwater et al. 2007; Schneider et al. 2013; Buenfil-Rojas et al. 2020).

Although contaminant quantification continues to represent an essential component of environmental biomonitoring, the temporal analysis clearly demonstrates that research priorities have progressively shifted toward biological responses. The remarkable increase in studies employing multiple biomarkers after 2010 indicates that contaminant concentrations are no longer interpreted as isolated indicators of environmental quality. Instead, recent investigations increasingly combine chemical analyses with physiological, biochemical, endocrine, histopathological, immunological, and genetic endpoints to obtain a more comprehensive understanding of contaminant toxicity. This transition reflects one of the principal advances in modern ecotoxicology, where biological responses are recognized as more ecologically informative than contaminant concentrations alone because they provide direct evidence of adverse effects at different levels of biological organization (van der Oost et al. 2003; Galloway et al. 2004).

The species–biomarker matrix demonstrates that this methodological transition has not occurred uniformly across Crocodylia. *Alligator mississippiensis* supported the broadest range of biomarker applications, combining contaminant bioaccumulation with endocrine, reproductive, biochemical, immunological, and genotoxic responses. This diversity reflects its long-standing use in both environmental monitoring and mechanistic ecotoxicology, particularly in North American research programs. *Caiman latirostris* exhibited a distinct methodological profile, characterized by the predominance of multiple-biomarker approaches and genotoxic endpoints rather than isolated contaminant quantification. This pattern is consistent with its extensive use in controlled pesticide-

exposure studies, in which biochemical, immunological, endocrine, reproductive, and genetic responses are frequently assessed simultaneously. In contrast, research involving *Crocodylus moreletii*, *Crocodylus niloticus*, and most less frequently studied species remained strongly centred on bioaccumulation.

These differences should be interpreted primarily as consequences of regional research traditions, study designs, laboratory capacity, and the historical objectives associated with each model species, rather than as evidence of intrinsic differences in biomarker responsiveness. Nevertheless, the concentration of effect-based and multi-biomarker approaches in only a few species limits comparative interpretation across Crocodylia. Applying standardized biomarker frameworks to a broader range of taxa would help distinguish genuine interspecific variation from methodological and geographical research biases.

One of the clearest examples of this conceptual transition is the growing prominence of endocrine disruption within the keyword network. Beginning in the 1990s, the pioneering investigations conducted in *Alligator mississippiensis* inhabiting Lake Apopka fundamentally altered the direction of wildlife ecotoxicology by demonstrating that chronic exposure to organochlorine pesticides and other endocrine-disrupting compounds could profoundly affect reproductive development, steroid hormone regulation, gonadal differentiation, and reproductive success without necessarily causing immediate mortality (Guillette et al. 1994; Crain et al. 1997; Guillette and Gunderson 2001). These studies established endocrine biomarkers as essential tools for evaluating sublethal contaminant effects and significantly expanded the ecological interpretation of environmental biomonitoring.

The increasing prominence of pesticides, particularly glyphosate- and atrazine-based formulations, represents another important thematic shift identified in this study. Unlike the earlier predominance of heavy metals and legacy persistent organic pollutants, pesticide research has expanded considerably during the last two decades, especially in South America. This pattern closely reflects agricultural intensification throughout the Neotropics and the growing concern regarding chronic exposure of non-target wildlife to herbicides used in large-scale crop production. Experimental studies using *Caiman latirostris* have demonstrated genotoxic effects following embryonic exposure to glyphosate-based formulations (Poletta et al. 2009; Burella et al. 2017; López González et al. 2017). Other pesticide formulations have also been associated with immunological alterations in this species (Latorre et al. 2016). Consequently, *C. latirostris* has emerged as one of the principal reptilian models for experimental ecotoxicology involving contemporary agricultural contaminants.

The thematic evolution also highlights the rapid incorporation of genotoxic biomarkers into crocodilian ecotoxicology. The appearance of micronucleus assays and comet assays as central keywords reflects increasing recognition that chromosomal damage and DNA strand breaks represent sensitive early-warning indicators of contaminant exposure. Unlike contaminant quantification alone, these biomarkers directly evaluate biological damage at the cellular level, frequently preceding detectable alterations in physiology, reproduction, or survival. Their growing application therefore represents an important methodological advance, allowing ecotoxicologists to identify sublethal effects before population-level consequences become evident. The strong association among genotoxicity, glyphosate, and *Caiman*

latirostris observed in the keyword network reflects the contribution of South American studies applying micronucleus and comet assays to evaluate embryonic pesticide exposure (Poletta et al. 2009; Burella et al. 2017; López González et al. 2017).

An equally important pattern emerging from the temporal analysis is the progressive decline in studies focused exclusively on persistent organochlorine pollutants. During the 1980s and early 2000s, compounds such as DDT, DDE, PCBs, and related persistent organic pollutants represented a major research priority because of their persistence, biomagnification, and endocrine-disrupting properties. Following regulatory restrictions established under international agreements, particularly the Stockholm Convention, research attention gradually shifted toward currently used pesticides and other contaminants of emerging concern. This transition is consistent with broader changes in chemical pollution assessment and regulatory priorities described by Brack et al. (2017) and Bernhardt et al. (2017).

Despite this diversification, the retrieved literature indicates that studies addressing contaminants of emerging concern (including pharmaceuticals, personal care products, per- and polyfluoroalkyl substances (PFAS), nanomaterials, and microplastics) remain comparatively scarce within crocodilian ecotoxicology. Because these contaminant classes were not explicitly incorporated as independent search descriptors, this finding should be interpreted as a trend observed within the retrieved dataset rather than as an exhaustive estimate of global research activity. Nevertheless, the increasing environmental relevance of these contaminants highlights important opportunities for future investigations. The keyword network additionally demonstrates that contemporary crocodilian ecotoxicology has become increasingly interdisciplinary. Rather than replacing previous research themes, new methodological approaches have progressively incorporated traditional contaminant monitoring, generating a highly interconnected conceptual structure in which environmental chemistry, physiology, molecular biology, toxicology, and conservation biology converge. This cumulative pattern suggests that future advances are more likely to emerge through methodological integration and the incorporation of new analytical approaches than through the complete replacement of established research themes.

Overall, the thematic evolution documented in the present study demonstrates that crocodilian ecotoxicology has evolved from a discipline centered on contaminant detection into an increasingly mechanistic science focused on understanding how environmental contaminants affect biological systems. The progressive incorporation of multiple biomarkers, coupled with advances in analytical chemistry and molecular toxicology, has considerably strengthened the ecological relevance of crocodilians as sentinel organisms. As environmental contamination becomes increasingly complex, integrating contaminant chemistry with multi-biomarker and multi-omics approaches will be essential for improving ecological risk assessment and supporting evidence-based conservation of freshwater ecosystems.

Research gaps and future perspectives

Despite the substantial expansion of crocodilian ecotoxicology over the last five decades, the scientometric patterns identified in this study reveal persistent knowledge gaps that continue to limit the global representativeness and predictive capacity of the field. Although publication output has increased steadily, scientific production remains highly concentrated in a limited number of countries, research groups, and model species. Consequently, current knowledge largely reflects regional environmental realities rather than providing a comprehensive understanding of contaminant exposure across the ecological diversity of the order Crocodylia.

One of the most evident limitations concerns the marked taxonomic bias observed throughout the literature. Nearly two-thirds of the studies included in this analysis focused on *Alligator mississippiensis* and *Caiman latirostris*, while many extant crocodilian species remain virtually unrepresented. Species occurring in Southeast Asia, Central Africa, Oceania, and parts of Central America are particularly under-investigated despite inhabiting regions undergoing rapid agricultural expansion, mining activities, hydrological alteration, and urban development. Because contaminant exposure is strongly influenced by local environmental conditions, trophic interactions, and habitat characteristics, this taxonomic imbalance restricts our capacity to extrapolate ecotoxicological responses across the diversity of crocodilian species.

The geographical distribution of research exhibits a similar asymmetry. North and South America dominate the scientific literature, largely reflecting the existence of long-term monitoring programs, well-established analytical laboratories, and sustained research funding. In contrast, biodiversity-rich tropical regions remain poorly represented despite supporting several threatened crocodilian species and experiencing some of the fastest rates of environmental change worldwide. Expanding international collaboration and strengthening scientific capacity in these regions will therefore be essential for producing a more geographically balanced understanding of contaminant dynamics and wildlife health.

The present scientometric analysis also demonstrates that the field remains centered on a relatively restricted group of contaminant classes. Heavy metals, organochlorine pesticides, and endocrine-disrupting compounds collectively accounted for a substantial proportion of the investigations included in the curated dataset. In contrast, pharmaceuticals, personal care products, PFAS, flame retardants, nanomaterials, and microplastics were rarely represented among the retrieved publications. Because these compounds were not included as independent search descriptors, their apparent scarcity should be interpreted cautiously. Nevertheless, expanding the assessment of contaminants of emerging concern represents an important direction for future crocodilian ecotoxicology.

Another significant gap concerns methodological integration. Although the temporal evolution identified in this study demonstrates a clear increase in the use of biomarkers, relatively few investigations combine contaminant quantification with multiple complementary biological endpoints. Most studies continue to evaluate isolated biomarkers, limiting the interpretation of contaminant effects across different levels of biological organization. Future research would benefit from integrative frameworks combining bioaccumulation data with biochemical, endocrine, immunological, histopathological,

reproductive, and genotoxic biomarkers within the same experimental or field-based investigations. Such multi-endpoint approaches provide a more realistic assessment of contaminant toxicity and improve the ecological relevance of biomonitoring programs (van der Oost et al. 2003; Galloway et al. 2004).

The incorporation of molecular ecotoxicology also remains incipient within crocodilian research. High-throughput approaches, including transcriptomics, proteomics, metabolomics, epigenomics, and environmental DNA (eDNA), have become increasingly common in studies involving fishes, amphibians, and marine vertebrates but remain seldom applied to crocodilians (Walker et al. 2012; Newman 2023). These techniques offer unprecedented opportunities to identify molecular pathways affected by contaminant exposure, discover novel biomarkers, and establish mechanistic links between chemical stressors and adverse biological outcomes. Their integration with conventional ecotoxicological methods has the potential to considerably enhance the sensitivity and predictive capacity of crocodilian biomonitoring.

The scientometric results further highlight the need for greater methodological standardization across studies. Considerable variation persists regarding sampling matrices, analytical techniques, contaminant classification, biomarker selection, and reporting practices, making direct comparisons among studies challenging. Harmonized protocols for contaminant quantification and biomarker assessment would facilitate reproducibility, improve data comparability, and strengthen future systematic reviews and meta-analyses. The development of standardized reporting guidelines would also contribute to establishing global reference values for different crocodilian species and environmental contexts.

Finally, the conceptual evolution observed throughout the present analysis suggests that crocodilian ecotoxicology is progressively converging with broader environmental assessment frameworks such as One Health, EcoHealth, and Planetary Health. Because crocodilians are long-lived apex predators capable of integrating contaminant exposure across aquatic food webs, they represent valuable indicators of ecosystem integrity and environmental change. Future research should therefore move beyond single-contaminant approaches toward integrated assessments that simultaneously consider chemical pollution, habitat degradation, climate change, pathogen emergence, and biodiversity loss. Such interdisciplinary perspectives will strengthen the contribution of crocodilians to environmental monitoring while supporting more effective conservation strategies under increasingly complex anthropogenic scenarios.

Taken together, the gaps identified in this scientometric analysis indicate that the next stage of crocodilian ecotoxicology will depend less on expanding the number of contaminant surveys than on increasing taxonomic coverage, geographical representativeness, methodological standardization, and mechanistic integration. Addressing these challenges will substantially improve the use of crocodilians as globally relevant sentinel organisms and reinforce their role in evidence-based environmental management and biodiversity conservation.

Limitations

The present study has several limitations that should be considered when interpreting the results. First, crocodilian descriptors were restricted to the title field to increase search specificity. Consequently, potentially relevant publications in which crocodilians were mentioned only in the abstract or author keywords may not have been retrieved. Studies whose titles contained only species-level scientific names not represented by the adopted descriptors may also have been omitted. Therefore, the curated dataset should be interpreted as a high-specificity representation of the Scopus-indexed literature rather than as an exhaustive census of all publications related to crocodilian ecotoxicology.

Second, the search strategy prioritized historically established contaminant groups and ecotoxicological endpoints to ensure comparability across five decades of scientific production. Some studies addressing contaminants of emerging concern may consequently be underrepresented because pharmaceuticals, personal care products, PFAS, nanomaterials, and microplastics were not included as independent search descriptors. Findings concerning these contaminant classes should therefore be interpreted within the scope of the adopted search strategy rather than as a complete representation of global research activity.

Third, the literature search relied exclusively on the Scopus database. Publications indexed only in other bibliographic databases, non-indexed regional journals, or grey literature may not have been retrieved. The geographical and linguistic distribution of the dataset may therefore partly reflect the coverage characteristics of the selected database.

Fourth, study screening and manual ecotoxicological classification were conducted by a single reviewer. Consequently, reviewer-related selection and coding bias cannot be completely excluded. To minimize this limitation, predefined eligibility criteria, standardized terminology, and consistent classification rules were applied throughout the screening and data-curation processes. Records for which eligibility could not be confidently determined from the title and abstract were retained for full-text assessment before the final inclusion decision.

Fifth, although classification criteria were standardized, the manual categorization of contaminant groups, biomarkers, biological matrices, study designs, and biological endpoints necessarily involved interpretive decisions. Studies investigating multiple species, contaminants, matrices, or biomarkers were assigned to all applicable categories. Therefore, totals across categories represent frequencies of associations and should not be interpreted as mutually exclusive publication counts.

Finally, the literature search was updated through May 2026. Consequently, publication counts for 2026 represent only a partial year and should not be directly compared with complete annual publication totals. Temporal patterns involving the final study period should therefore be interpreted cautiously.

Conclusion

This study provides a global scientometric assessment of crocodilian ecotoxicology across more than five decades of scientific production. By integrating conventional bibliometric indicators with a manually

curated classification of species, contaminant categories, biomarkers, biological matrices, study designs, and geographic distribution, the analysis offers a standardized synthesis of how crocodilians have been used in environmental contamination research. The results demonstrate that the field has evolved from an initial emphasis on contaminant quantification toward a more interdisciplinary framework incorporating biomarker-based ecotoxicology, molecular responses, physiological effects, and ecological risk assessment.

Scientific production increased markedly after 2000 and was accompanied by substantial thematic and methodological diversification. Heavy metals remained the most frequently investigated contaminant group, whereas endocrine disruption, genotoxicity, reproductive effects, and multiple-biomarker approaches became increasingly prominent in recent decades. Species-level analyses further revealed that the field is structured around distinct research profiles, with *Alligator mississippiensis* spanning multiple contaminant categories, *Caiman latirostris* strongly associated with pesticide toxicology, and other principal models investigated predominantly in relation to metals and persistent organic pollutants. Biomarker applications were similarly uneven, with integrated and effect-based assessments concentrated mainly in *A. mississippiensis* and *C. latirostris*, while most other species were investigated predominantly through bioaccumulation measurements.

Despite this expansion, the literature remains strongly concentrated in a limited number of countries, research groups, journals, and model species. These geographical, taxonomic, and methodological asymmetries restrict the global representativeness of current knowledge and limit direct comparisons across Crocodylia. Future research should therefore expand investigations to underrepresented species and biodiversity-rich tropical regions, incorporate contaminants of emerging concern, and apply standardized multi-biomarker frameworks capable of linking contaminant exposure to biological effects across different levels of organization.

Overall, this study establishes an updated global baseline for crocodilian ecotoxicology and reinforces the value of these long-lived reptiles as sentinel organisms for environmental biomonitoring. Greater taxonomic coverage, methodological standardization, broader geographical representation, and integration of emerging molecular approaches will be essential for strengthening their application in ecological risk assessment, environmental management, and evidence-based conservation.

Declarations

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Authors' Contributions

Rayssa Lima dos Santos was responsible for conceptualization, methodology, literature search and screening, data curation, formal analysis, writing - original draft, and writing - review and editing.

Ethical Approval

This is not applicable. This study analyzed previously published scientific literature and did not involve human participants or live animals.

Consent to Participate

This is not applicable.

Consent to Publish

This is not applicable.

Competing Interests

The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The manually curated ecotoxicological dataset and the contingency matrices generated during this study are available from the corresponding author upon reasonable request. Bibliographic metadata were retrieved from Scopus under its terms of access.

Code Availability

The analytical code used for data processing and categorical analyses is available from the corresponding author upon reasonable request.

Declaration of Generative AI Use

During the preparation of this manuscript, the author used ChatGPT (OpenAI) to improve the readability and language of the text. The author reviewed and edited all AI-assisted content and takes full responsibility for the content of the publication.

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Figures

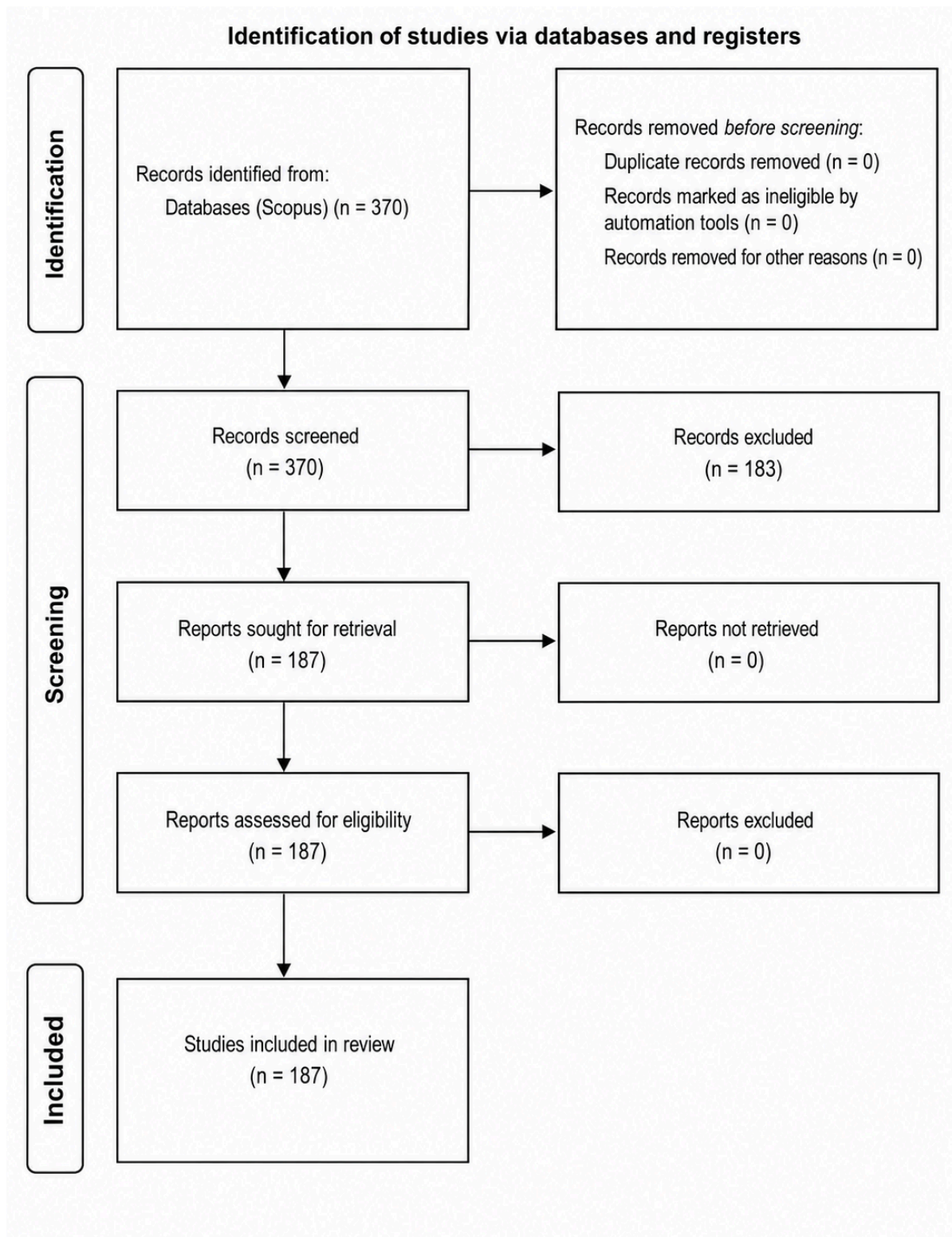


Figure 1

PRISMA 2020 flow diagram illustrating the study-selection process. The Scopus search identified 370 records, of which 183 were excluded after title and abstract screening. The remaining 187 reports were retrieved, assessed for eligibility, and included in the final curated dataset.

Annual Scientific Production

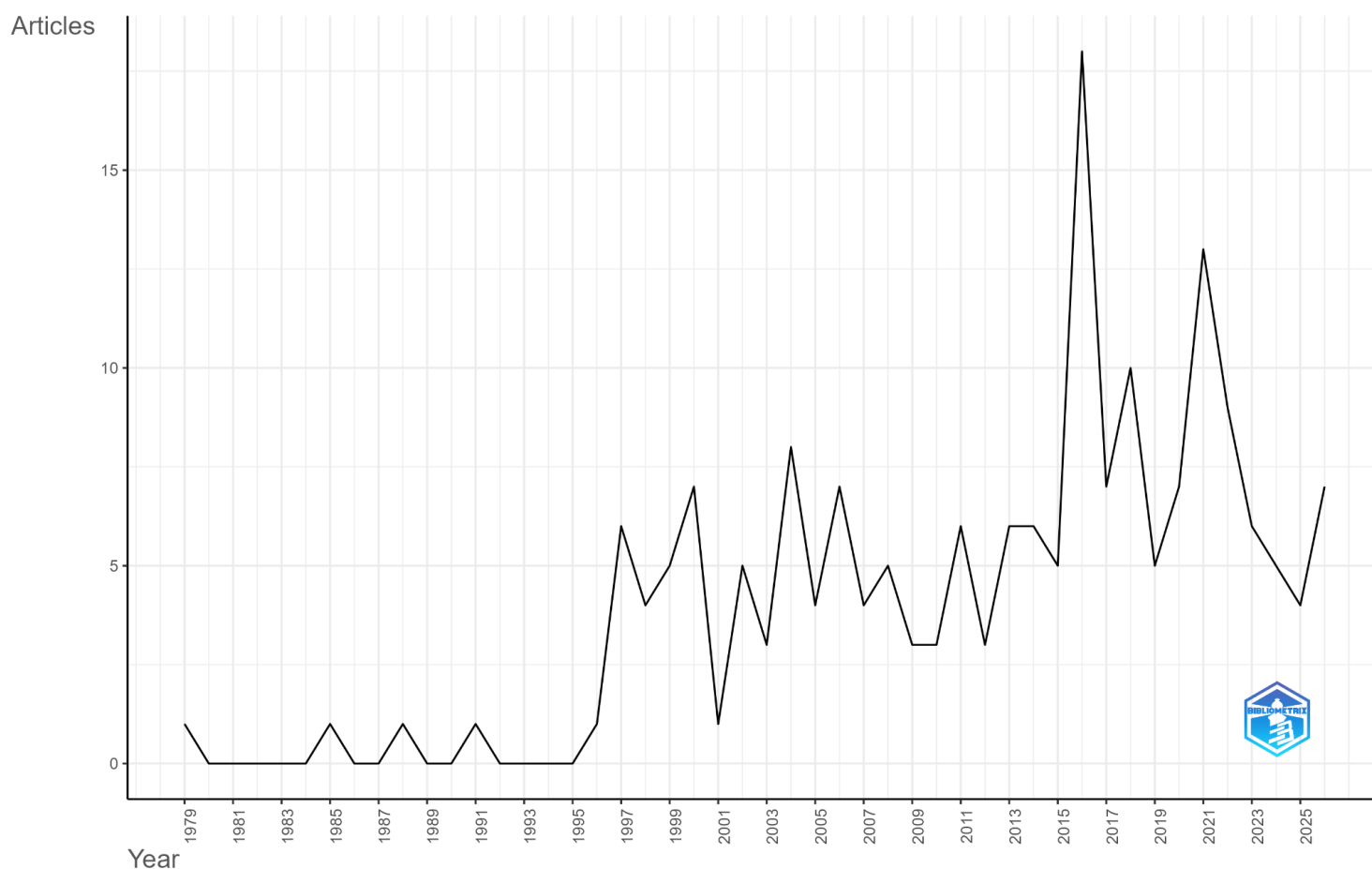


Figure 2

Annual scientific production on crocodilian environmental biomonitoring from 1971 to May 2026, highlighting the progressive growth post-2000.

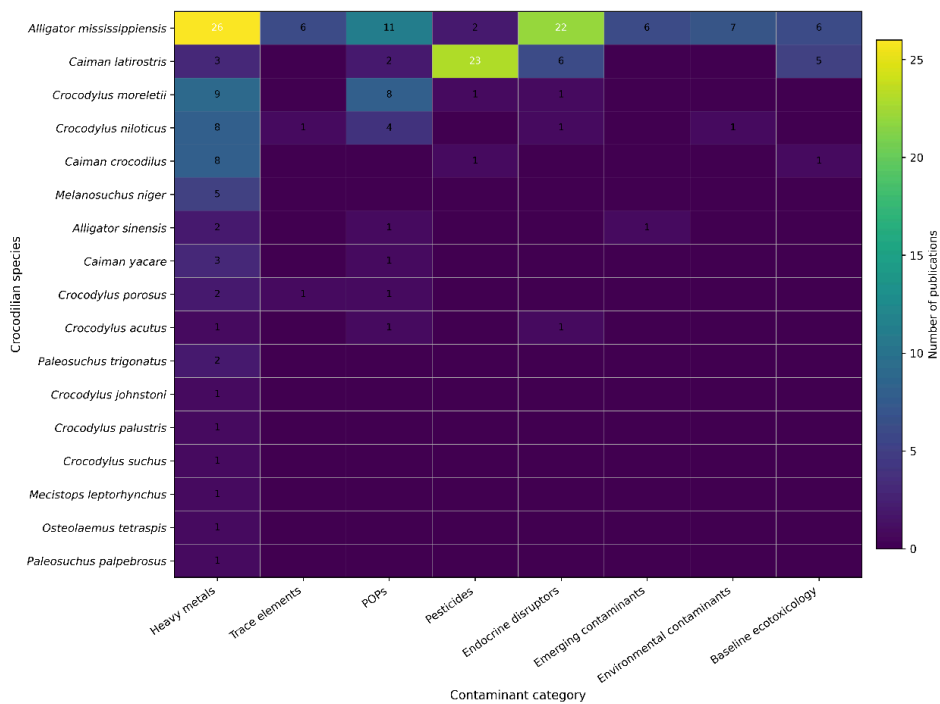
Global Distribution of Ecotoxicological Studies with Crocodilians



Figure 3

Global distribution of ecotoxicological studies involving crocodilian species. The map illustrates the number of studies identified in the curated Scopus dataset according to the country where the research was conducted. Warmer colors, ranging from yellow to red, indicate greater research activity, whereas countries shown in light blue had no studies identified in the curated dataset. Research efforts were concentrated in the Americas, southern Africa, and Australia, while large portions of Asia, Europe, and northern regions were not represented.

A. Contaminant categories



B. Biomarker categories

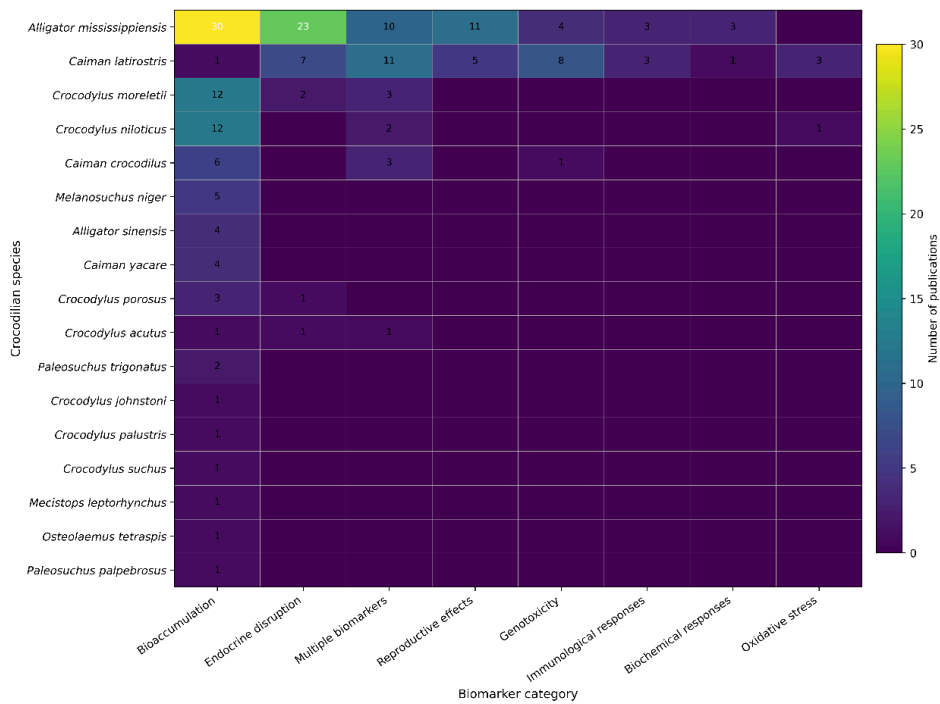


Figure 4

Species-specific ecotoxicological profiles across crocodilian taxa. **a** Distribution of environmental contaminant categories across crocodilian species included in the curated dataset. **b** Distribution of biomarker categories across crocodilian species included in the curated dataset. Cell values indicate the number of unique publications reporting each species–category pair. Multi-valued records were disaggregated, and each publication was counted only once within each corresponding pair. Records

classified generically as “Multiple crocodilian species” were excluded from the species-level matrices. Together, the two heatmaps illustrate species-specific research specialization in both contaminant focus and biomarker application within crocodilian ecotoxicology.

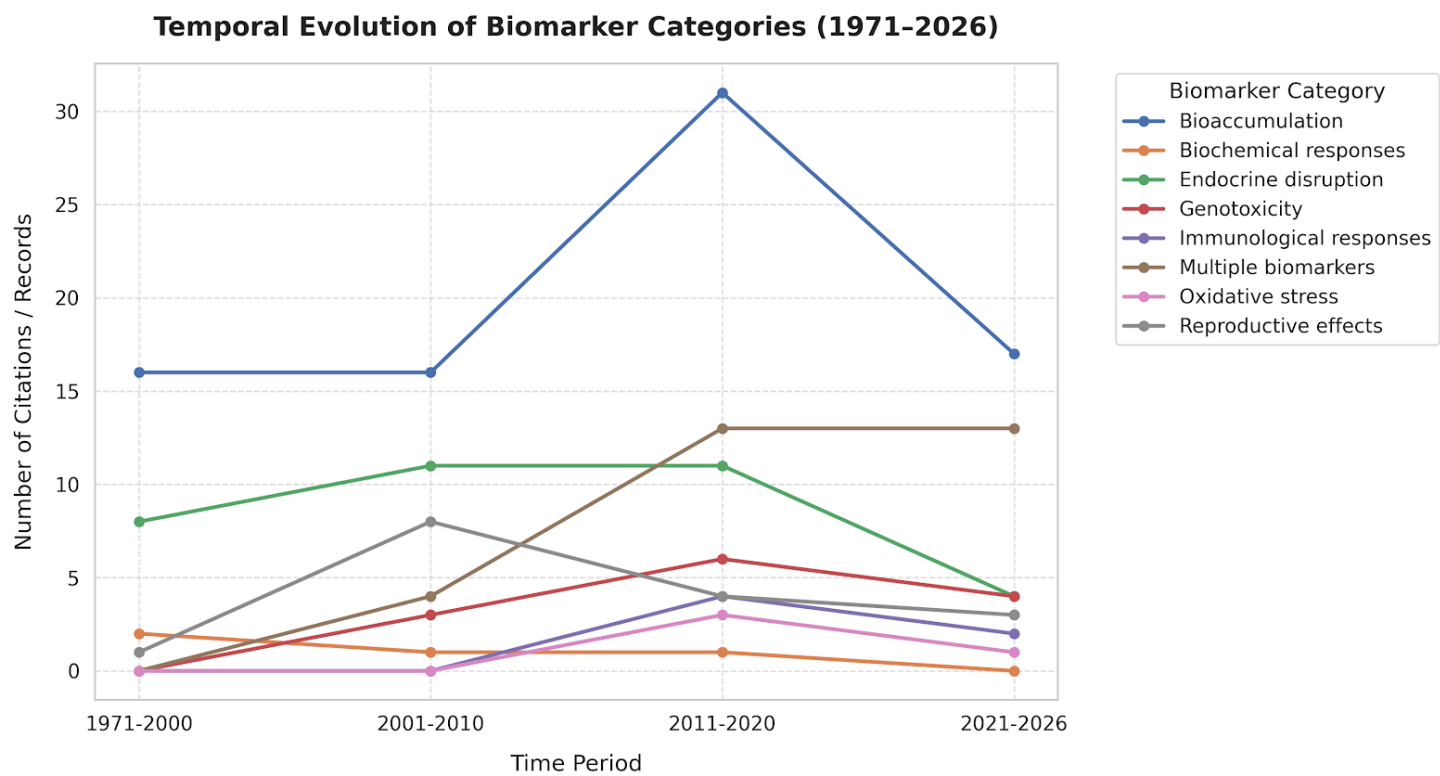


Figure 5

Temporal evolution of biomarker categories from 1971 to May 2026, illustrating the historical shift from basic bioaccumulation assessments toward integrated multi-biomarker approaches.

Supplementary Files

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