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Review

A Comprehensive Review of Characterization, Leaching, and Ecotoxicity of Coal Fly Ash to Aquatic Organisms

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Abstract

Upon entering aquatic ecosystems, coal fly ash (CFA) particles and their leachates distribute across all environmental compartments, from surface water to benthic sediments, rendering them accessible to aquatic organisms spanning multiple trophic levels. Although CFA is widely classified as non-hazardous waste, a growing body of toxicological evidence demonstrates its capacity to induce significant adverse biological effects. This discrepancy between laboratory-demonstrated toxicity and its current non-hazardous regulatory status highlights a critical knowledge gap in risk assessment frameworks and testing philosophies. Herein, we provide a comprehensive review of CFA characterization techniques, leaching methodologies and their environmental relevance, and the aquatic ecotoxicity of both particulates and leachates to primary, secondary, and tertiary consumers. Based on the findings from the review, we propose four paradigm shifts to more accurately characterize CFA ecotoxicological risks: from standardized leaching protocols to application-specific and maximum leachability tests, from single-species assays to multi-trophic-level bioassays, from single-metal analysis to comprehensive, whole-matrix testing that integrates particulate and dissolved fractions, and from standardizing the reporting of CFA provenance, coal types, and combustion conditions to meaningful meta-analyses and cross-study comparisons. It is believed that these suggestions will substantially advance the realistic ecotoxicological assessment of CFA and bridge the gap between laboratory toxicity data and regulatory classification.

Keywords: coal fly ash; aquatic ecotoxicity; leachability protocols; trophic transfer; particulate toxicity; hazardous trace elements

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1. Introduction

Over one third of global electricity generation continues to rely on coal, even as consumption declines in many regions in pursuit of carbon neutrality targets. In 2025, global coal production hit a record high of 9.1 billion tonnes, producing around 1 billion tonnes

of coal fly ash (CFA) [1,2]. According to the European Coal Combustion Products Association (ECOBA), CFA consists of fine particulates captured by electrostatic or mechanical precipitation from flue gas generated during the combustion of lignite or hard coal at 1100–1400 °C [3,4]. These predominantly spherical particles, typically 1–100 μm in diameter [5,6], are characterized by high specific surface areas and an aluminosilicate matrix that strongly sequesters hazardous trace elements (HTEs) such as Cr, Cu, Zn, As, Cd, and Pb during coal combustion [7].

Many countries currently classify coal fly ash as a non-hazardous solid residue, and about 70% of CFA is currently utilized [8]. Substantial country-level effort has been directed toward promoting sustainable CFA use, including applications in agricultural soil amendment, road base and pavement construction, and cement and concrete manufacturing. For example, India's Ash Utilization Notification (2021) mandates 100% utilization by coal-fired power plants [9], whereas China incentivizes CFA recycling through circular economy policies [10], the United States encourages safe reuse in construction [11], and the European Union promotes recycling under the Waste Framework Directive [12]. However, due to the large annual global production, a significant amount of unused CFA is still stockpiled in designated sites, such as ash ponds and surface impoundments [13].

Once placed in terrestrial disposal sites, CFA can enter aquatic ecosystems through multiple pathways, such as leaching from impoundments, accidental spills, atmospheric deposition, and runoff from reuse applications [14]. Ash ponds, in particular, allow the release of highly alkaline leachates enriched in soluble HTEs that can infiltrate into groundwater or enter adjacent surface waters [15]. Although infrequent, major coal ash spill incidents have resulted in severe environmental and economic damage, as exemplified by the Kingston Fossil Plant CFA slurry spill (1.1 billion gallons, USA, 2008) and Visakhapatnam CFA release into coastal waters (35,000 tons, India, 2020). Additionally, fine CFA particulates can be transported by wind and deposited on water surfaces, leading to widespread contamination [16]. Rainfall leaching of CFA-derived HTEs from embankments, roads, and other reuse structures constitutes another important pathway to streams and rivers [14]. Upon entering natural aquatic systems, CFA particles and HTEs become distributed across multiple environmental compartments, floating on the surface, dispersing through the water column, and accumulating in sediments, making them available to aquatic organisms occupying diverse habitats [17]. Moreover, CFA particulates resemble plankton in both size and appearance, which markedly increases the probability of dietary uptake and trophic transfer through aquatic food webs [18].

These exposure characteristics have raised growing concerns regarding the ecotoxicological risks posed by CFA to aquatic organisms. Toxicological studies have demonstrated that CFA elicits significant biological effects, such as chronic inflammation [19], lung injury, metabolic disruption [6], altered protein synthesis, and genotoxicity including DNA damage [20]. This disconnection between laboratory-demonstrated toxicity and the current non-hazardous regulatory designation highlights a critical knowledge gap in risk assessment frameworks and testing philosophies. To address this gap, the present study provides a comprehensive review of the characterization, leaching methodologies, and aquatic ecotoxicity of CFA. We critically synthesize recent advances in characterization techniques, compare standardized leaching protocols/guidelines and their environmental relevance, and evaluate the toxicity of CFA particulates and leachates based on recent experimental data. The overarching aim is to seek ways to refine existing assessment tools and make them sufficient to realistically capture the ecotoxicological risks of CFA.

2. Research Methodology

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [21]. In accordance with the established principles for literature evaluation, a structured approach was adopted to identify, select and synthesize relevant studies and associated data that satisfied the inclusion criteria. The comprehensive literature search was executed across Web of Science, ScienceDirect and Scopus from 1980 to 2026, spanning over 40 years to capture both the foundational studies on CFA leaching protocols and the more recent advances in nanotoxicology and ecotoxicological assessments. The search employed a combination of targeted keywords, including "coal fly ash", "coal ash", "leachability", "aquatic ecotoxicity", "hazardous trace elements," and "trophic transfer", linked by "AND" and "OR" to refine search results. To ensure comprehensive coverage, supplementary targeted searches were performed for specific topics including characterization techniques (e.g., "XRD", "SEM", "ICP-MS"), individual leaching protocols, and specific test organisms (e.g., "*Daphnia magna*", "*Vibrio fischeri*", "*Danio rerio*"). The reference lists of all retrieved review articles were manually examined to identify additional relevant studies that might have been missed by the database searches.

After the initial retrieval, all retrieved articles were downloaded, and duplicates were removed. The following criteria were further considered for exclusion: (1) conference abstracts, editorials, or non-peer-reviewed publications; (2) studies focusing exclusively on terrestrial organisms or human health effects without aquatic relevance; and (3) studies on other combustion residues, such as bottom ash, biomass ash, and municipal solid waste incineration (MSWI) fly ash, without explicit CFA data. Bottom ash refers to the coarser particles (246–604 μm) that accumulate at the bottom of the furnace and accounts for lower toxicity [22]. Biomass ash, the residual matter derived from the combustion of agricultural and forestry residues (e.g., wood, bamboo, and rice husks), typically contains high contents of alkali metals and chlorine [23]. It exhibits substantially lower concentrations of hazardous elements and thus lower overall heavy-metal toxicity than CFA [24]. MSWI fly ash, derived from the incineration of heterogeneous municipal solid waste, typically contains higher concentrations of heavy metals, soluble salts, and toxic organic compounds (e.g., dioxins) than CFA [25]. MSWI fly ash is therefore classified as hazardous solid waste [26]. The composition and morphology of the above combustion residues are significantly different from CFA, leading to different leaching behavior, bioavailability, and ecotoxicological effects [27,28]. The relevant articles were hence removed.

Finally, the selected articles underwent comprehensive full-text review, with relevant data extracted and entered into an Excel file. The extracted data contained information such as CFA source and characteristics, leaching protocol employed, test organism and exposure conditions, reported toxicity endpoints and principal conclusions. In addition to the peer-reviewed literature, policy documents and regulatory announcements were retrieved through directed manual searches on official agency websites, which were not subject to the PRISMA-based screening.

3. Characterization

Prior to sustainable utilization and toxicity studies of CFA, it is important to fully characterize the particulates, such as crystal structure, surface morphology, and chemical composition, to guarantee meaningful results [29]. This section summarizes the principal analytical techniques employed to probe these properties at both bulk and micro- or nanoscale (Figure 1).

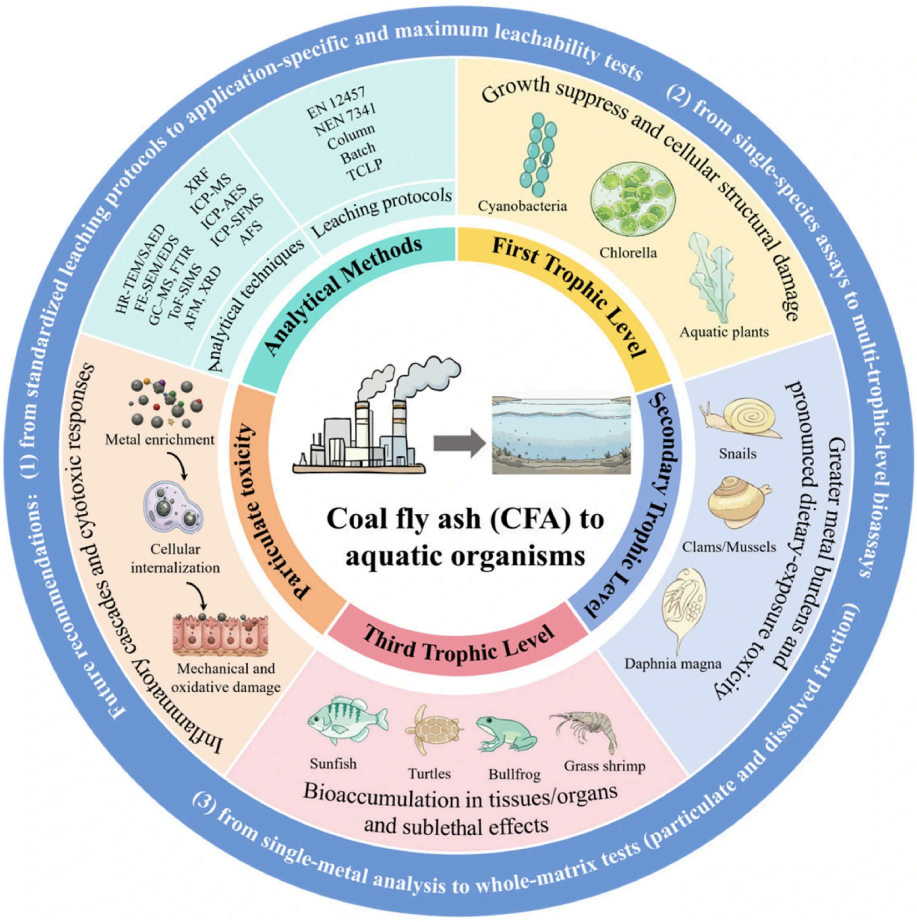


Figure 1. State-of-the-art understanding and future recommendations in the ecotoxicity of coal fly ash to aquatic organisms.

3.1. Crystal Structure

X-ray diffraction (XRD) is routinely used to identify the primary crystalline phases in CFA [30]. However, XRD has limited sensitivity for nano-minerals and ultrafine/nanoparticles enriched with HTEs. To resolve the crystallinity and symmetry of such phases, Silva et al. [31] employed microbeam diffraction (MBD), selected-area electron diffraction (SAED), and fast Fourier transform (FFT) analysis of the high-resolution transmission electron microscopy (HR-TEM) images. Crystalline phases can be identified by comparing measured d-spacings with the International Centre for Diffraction Data (ICDD) powder diffraction file (PDF) database (2026 version) [32].

3.2. Morphology

The surface morphology of CFA can be characterized by field emission scanning electron microscopy (FE-SEM), high-resolution transmission electron microscopy (HR-TEM), and atomic force microscopy (AFM) [29,30,33]. Fu et al. [34] classified CFA particles into several morphological types with SEM, including monolithic irregular particles with spheroidal or drossy surface precipitates, fused amorphous aggregates, irregular skeleton or spongy particles, and unburnt carbon. Various SEM images of CFA particles are shown in Figure 2 [35–38]. Dwivedi et al. [30] used AFM to characterize CFA nanoparticles and observed quartz crystallites embedded within an amorphous matrix, reporting an average particle size of 21 nm and a surface roughness of 24 nm.

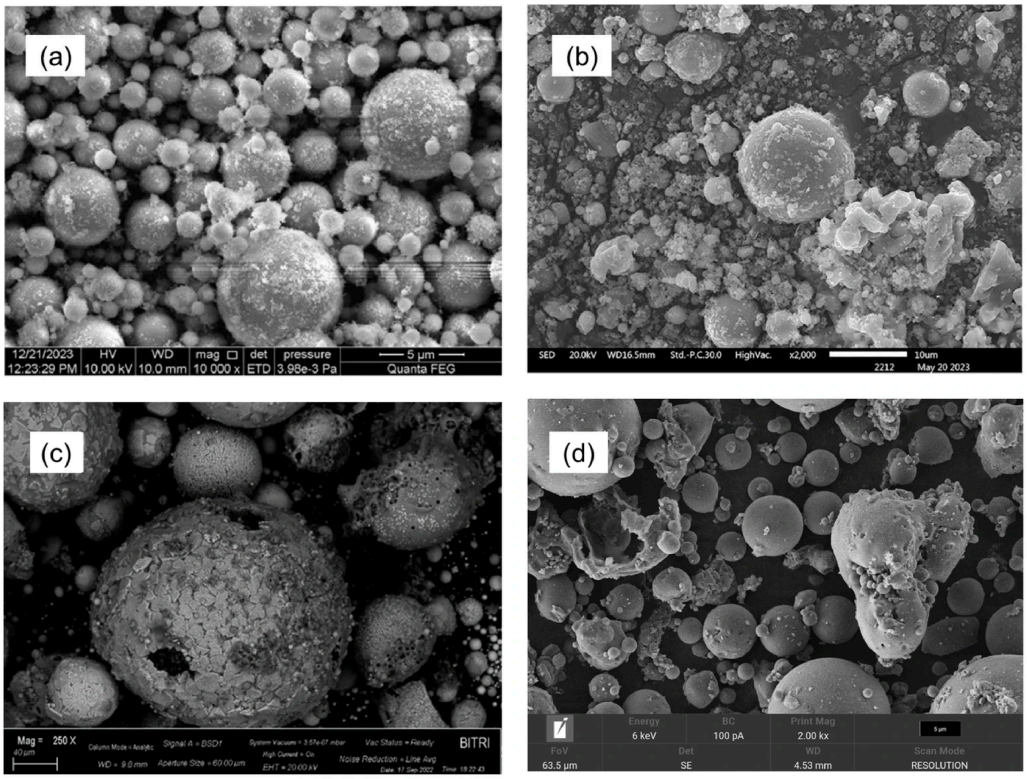


Figure 2. SEM images showing various particle morphologies of CFA from (**a**) Huaneng Beijing Thermal Power Co., Ltd. [35], (**b**) Inner Mongolia YiYai Group, Ltd., Ordos city, Inner Mongolia province, China [36], (**c**) collected from coal-fired power [37], and (**d**) coal-fired power plants in Guizhou Province [38]. Panels (**a**–**d**) were adapted from refs. [35–38], respectively, under the Creative Commons Attribution 4.0 International License. (**a**) Microstructural morphology of raw fly ash sourced from Huaneng Beijing Thermal Power Co., Ltd., adapted from Xu et al. [35], (**b**) SEM photographs of CFA was collected from Inner Mongolia YiYai Group, Ltd., Ordos city, Inner Mongolia province, China. Adapted from Luo et al. [36] (**c**) smooth, glassy surface of individual cenospheres, adapted from Manyepedza et al. [37] (**d**) SEM image of fly ash samples collected from three coal-fired power plants in Guizhou Province, adapted from Deng et al. [38].

3.3. Elemental Composition

Bulk elemental concentrations of CFA are typically determined by X-ray fluorescence spectroscopy (XRF), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), sector field ICP-MS (ICP-SFMS), and atomic fluorescence spectroscopy (AFS) [34,39–41]. Deonarine et al. [42] used micro XRF mapping and reported that As(V) was only weakly adsorbed onto iron mineral surfaces in the ash, which is characteristic of outer-sphere complexes and explains their rapid desorption immediately upon contact between fly ash and the buffer solution. For leachate analysis, ICP-AES has been used to quantify As, Ba, Cd, Cr, Cu, Mo, Ni, Pb, Sb, Se, and Zn [43]. Nevertheless, these bulk techniques often lack the spatial resolution required for certain HTEs. Energy-dispersive X-ray spectroscopy (EDS) coupled with FE-SEM or HR-TEM overcomes this limitation by providing spatially resolved elemental information. Using this approach, Ribeiro et al. [44] demonstrated that CFA consists of: (i) large (~100 μm) thick- and thin-walled cenospheres and dense spheroidal particles, frequently with embedded quartz, (ii) irregular agglomerates of small glassy spheres, quartz, and iron-rich phases, (iii) ultrafine (~100 nm) or dense thick-walled glassy spheres, and (iv) carbonaceous nanospheres (~50 nm) that host HTEs such as As, Hg, Pb, and Se, often as multi-nanomineral assemblages.

3.4. Surface Chemistry and Molecular Structure

While the foregoing techniques yield morphological and average compositional data, they do not reveal the chemical state or molecular architecture at the outermost surface where critical environmental interactions occur. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) addresses this gap by mapping elemental and molecular species at the surface [44]. Nagao et al. [45] applied ToF-SIMS to image the surface distribution of boron and fluorine on CFA particles, observing a fine, heterogeneous distribution of boron and a more homogeneous distribution of fluorine across larger grains, albeit with localized concentration gradients. Wang et al. [46] employed ToF-SIMS to probe the surface distribution of Ga and Ge on CFA particles. The spatial coincidence of Ga/Ge-rich areas with Ca/Mg-rich domains implies a close association, which further suggests that Ga and Ge may share similar leaching behaviors with Ca and Mg when CFA is exposed to aqueous environments. Surface functional groups and organic pollutants are typically characterized by Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) [34,47].

4. Leaching Guidelines

In most laboratory-scale ecotoxicological studies, test organisms are exposed to leachates obtained by leaching CFA with either a single static batch or periodically renewed volumes of leachate, using either column/flow-through or bulk/flow-around systems. The resulting leachate is subsequently filtered (e.g., 0.45 μm or 0.22 μm) and prepared for toxicity testing, with or without pH adjustment. Several standardized protocols for laboratory-scale leaching experiments are summarized below.

4.1. Dynamic/Column Leaching

Dynamic column leaching tests are widely employed in ecotoxicological studies. In these procedures, the leachant is continuously passed through a packed column of the test material and is renewed periodically or continuously [48–51]. This approach simulates the leaching behavior of waste materials under conditions that approximate real environmental scenarios across short, medium, and long-time scales, yielding information on contaminant release at predetermined times under acid–base conditions, flow patterns (e.g., continuous or interrupted), and flow rates [33,52]. A commonly adopted dynamic column procedure for waste materials is the NEN 7343 column test (The Netherlands) [53], which models the release of contaminants as acidified water (pH 4) percolates through a porous layer of CFA [52].

4.2. Batch Extraction

Batch extraction tests involve mixing the test material with a fixed amount of leachant without renewal [54,55]. These tests can predict the concentrations of selected constituents when anticipated field conditions (e.g., pH, liquid-to-solid ratio) match those of the laboratory test.

The Toxicity Characteristic Leaching Procedure (TCLP), a standard toxicity measure specifically designed by the U.S. Environmental Protection Agency (EPA Method 1311) for leaching hazardous materials, determines the mobility of both organic and inorganic analytes present in wastes [54,56]. Prior to the leaching experiment, the solid waste is mixed with DI water, and its initial pH is measured. Extraction fluid #1 (0.1 N sodium acetate buffer, pH 4.93 ± 0.05) is used when the initial pH is <5.0. If the initial pH > 5.0, 3.5 mL of 1 M hydrochloric acid is added, and the mixture is heated to 50 °C and held at this temperature for 10 min. After cooling to room temperature, the pH is re-measured. Extraction fluid #2 (glacial acetic acid diluted with water to pH 2.88 ± 0.05) is used if the measured pH remains above 5.0, whereas extraction fluid #1 is selected otherwise [54,57].

The Serial Batch Leaching Procedure (SBLP), developed at the National Energy Technology Laboratory (NETL), is designed to provide information on the leachable fraction within 2–3 days. The SBLP encompasses two approaches: a serial batch procedure conducted at four different pH values to estimate cumulative elemental availability, and a serial batch procedure using increasing liquid-to-solid ratios to estimate long-term elemental leaching [54].

The EN 12457 series of leaching methods targets inorganic constituents over 24 h, without accounting for the specific behavior of non-polar organic compounds or microbially mediated transformations in biodegradable organic wastes [43,58]. Tsiridis et al. [43] compared the single-stage test EN 12457-2 (L/S = 10 L kg^{-1}, 24 h) [59] with the cumulative-stages test EN 12457-3 and found that the single-stage protocol may underestimate the leaching potential of certain constituents. In contrast, the cumulative procedure removes a portion of sulfates during the first stage (L/S = 2 L kg^{-1}, 6 h) [60], thereby promoting further release of other constituents in the second stage (L/S = 10 L kg^{-1}, 18 h) [43].

According to the availability leaching test NEN 7341 [61], the maximum leaching capacity and diffusive release of constituents from particles into the bulk solution are determined under static conditions at two fixed pH levels (7 and 4), with pH constantly maintained by addition of HNO_3 buffer. The resulting leachate fractions are then combined to derive the total constituent concentration. NEN 7341 is generally regarded as representing the worst-case scenario, as it is designed to reveal maximum leachability without requiring pH manipulation of the test suspension. This absence of pH adjustment is confirmed by Tsiridis et al. [43], who applied the method to fly ash samples from six different sources and observed that the resulting leachates consistently exhibited a pH of approximately 6, a value within the acceptable range for ecotoxicity testing, eliminating the need for further pH adjustment. However, the conclusion of maximum leachability has been challenged by Darakas et al. [62], who concluded that the NEN 7341 test underestimated the release of the oxyanion Cr(VI). This underestimation likely arises because the resulting leachate pH was 5.5, whereas the maximal Cr(VI) release occurs between pH 7 and 8. Consequently, caution is required when applying NEN 7341 to elements whose pH for maximum leachability falls outside the 4–7 range. A summary of the leaching methods discussed in this section, along with other relevant protocols, is provided in Table 1 and Figure 3.

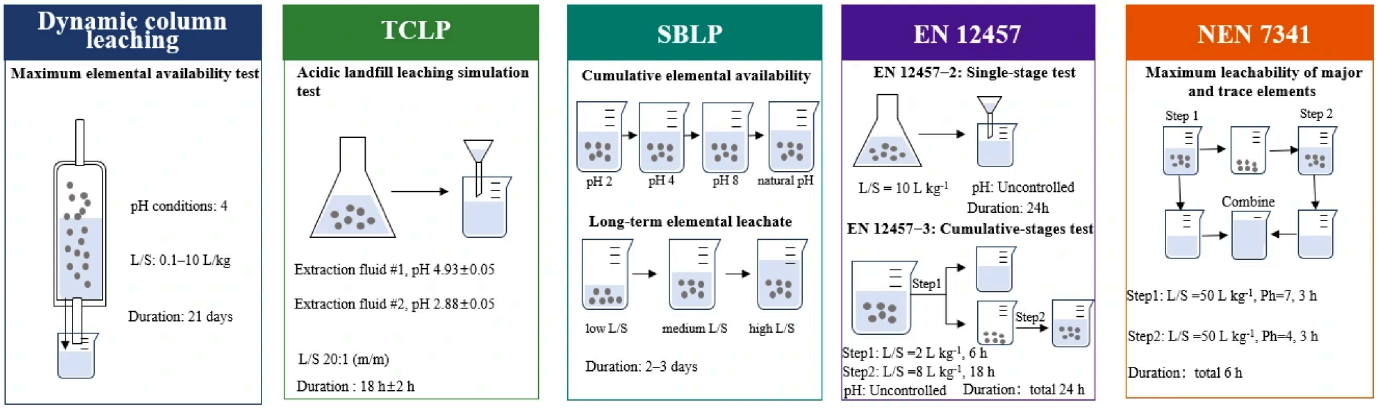


Figure 3. Conceptual diagram illustrating typical leaching protocols, including dynamic column leaching, TCLP, SBLP, EN 12457 series and NEN 7341 tests.

Table 1. Summary of key experimental conditions for internationally standardized coal fly ash leaching methods. * means that the pH of the leachant was used as is, without any further adjustment.

Type of Test	Test Procedure	Country	Leaching Target	Leachant	pH	L/S	Duration	Merits	References
Column	NEN 7343	NL	Inorganic components	Demineralized acidified water	4	0.1 to 10 L kg^{-1} (v/m)	21 d	The determination of inorganic components from acidic, flow-through environments	[33]
Batch	TCLP	US	Both organic and inorganic analytes	Extraction fluid #1 (0.1 N sodium acetate buffer, pH 4.93 ± 0.05); Extraction fluid #2 (glacial acetic acid diluted with water to pH 2.88 ± 0.05)	2.88 or 4.93	20:1 (m/m)	18 h	The determination of heavy metals and organics in wastes under acidic conditions	[7,57,63–65]
Batch	SBLP	US	Non-volatile organics and inorganics	deionized water	2, 4, 8, and natural pH	A series of ratios	2–3 d in total	Intended to give information on cumulative elemental availability during long-term elemental extraction	[54]
Batch	EN 12457-2	EU	Inorganic constituents	Distilled water, demineralized water, DI water or water of equivalent purity (5 < pH < 7.5) with conductivity < 0.5 mS m^{-1} (Grade 3 as specified in EN ISO 3696 [66])	Uncontrolled *	10 L kg^{-1} (v/m)	24 h	Balances ease of operation with data reliability	[7,43,58,67]
Batch	EN 12457-3					Step 1, 2 L kg^{-1} (v/m), Step 2, 8 L kg^{-1} (v/m) (cumulative)	Step 1: 6 h Step 2: 18 h	More closely resembles actual landfill multi-stage leachate scenarios	[43]
Batch	Availability test NEN 7341	NL	Inorganic components	Demineralized water, HNO_3	4, 7	50:1 (v/m)	Step 1, 3 h Step 2, 3 h	Maximizes constituent aqueous solubility and particulate-to-bulk diffusion at two defined pH values.	[62]

4.3. Leaching Method Selection

The choice of leaching method critically influences subsequent ecotoxicological evaluations. Different protocols generate leachates with varying pH and HTE concentrations, which in turn induce different redox transformations, precipitation/dissolution, and adsorption/desorption reactions, ultimately leading to different ecotoxicological responses [68].

Beyond reaction time, liquid-to-solid ratio, and particle size, the type and pH of the leachant can significantly affect leachate composition and behavior. Karuppiah and Gupta [63] demonstrated that the type of leachant governed the HTEs' leaching capacity. At equivalent pH (4, 5, 6 or 7), HCl leached higher concentrations of all tested metals (Cd, Cu, Fe, Ni, and Pb) than CH_3COOH, because HCl provides a higher concentration of hydrogen ions available for dissolving metal oxides. For example, at pH 4, HCl leached approximately 10-fold more Pb (30.0 vs. 3.0 μg L^{-1}), 6.3-fold more Fe (518.7 vs. 82.3 μg L^{-1}), and 3.7-fold more Cu (277.3 vs. 74.3 μg L^{-1}) than CH_3COOH. Additionally, leached metal concentrations were strongly correlated with toxicity (r = 0.98–0.79 for HCl, r = 0.97–0.59 for CH_3COOH).

The pH of the leachant can also influence ecotoxicological outcomes by affecting HTEs' leaching capacity and the final pH of the leachate. First, pH strongly affects the speciation and availability of HTEs in the solid matrix [64]. For instance, Cd is leached only under strongly acidic conditions (pH 1.2–2.9, < 0.2 mg kg^{-1}) [69]. The leaching of Cr(VI) is markedly pH-dependent. It increases with pH under acidic conditions, reaches a maximum range between pH 7 and 8, and then declines under more alkaline conditions [62]. Between pH 4 and 8, the leaching profile of Cr(III) is V-shaped, exhibiting minimal leachability near neutral pH (0.02 mg kg^{-1}) and reaching a plateau (~5 mg kg^{-1}) between pH 8 and 12 [70]. As an oxyanionic species, As is mobile over a wide pH range, and its leaching profile displays an N-shape, peaking at 1 mg kg^{-1} over the pH range of 7–11 and decreasing under more acidic or more alkaline conditions [71]. Ca exhibits consistently high release regardless of leachant pH. The release of several metals (Al, Be, Co, Cu, Fe, Mn, Ni, Pb, Zn) is suppressed until the leachant is neutralized and the leachate subsequently becomes acidic [43,72].

Second, the pH of the leachant directly influences the final leachate pH. For example, when deionized water is used as the leachant, Tsiridis et al. [44] reported that the leachate pH ranged from 9.3 to 12.6, while when an acidic leachant is employed, the leachate pH can drop below 5 [52]. Conducting toxicity tests directly on these leachates may confound the attribution of toxic effects solely to heavy metals, since the elevated pH of the leachate itself imposes an additional physiological stress on test organisms. In most studies, the leachate pH is adjusted to 7 before toxicity testing. Such pH adjustment, however, may alter heavy metal and nutrient speciation, thereby affecting their bioavailability and introducing potential bias into toxicity outcomes [65,73]. Neither of the above two methods can accurately reflect the actual toxicological impact of CFA in natural environments. It is therefore recommended that future ecotoxicological studies should report toxicity endpoints for both pH-adjusted and unadjusted leachates, or document metal speciation changes induced by pH adjustment.

The diversity of leaching methods adopted across the European Union, the United States, Canada, and Asian countries hinders the harmonization of risk assessments and cross-study comparisons for CFA. Consequently, the development of unified leaching protocols has been repeatedly advocated to facilitate ecotoxicological comparisons [74]. However, each trace element possesses unique solubility characteristics and affinity for release and transport during leaching, leading to element-specific leaching behaviors. A unified standard leaching test may not be equally effective for all elements [75]. While standardized protocols are valuable for general comparisons, they may not adequately capture the specific geochemical conditions encountered in a given disposal or reuse

scenario for site-specific ecological risk assessments. Leachability testing under application-relevant conditions is therefore recommended when evaluating the actual ecological risk of CFA, as it provides a more realistic assessment and enables a more accurate prediction of environmental impact. Moreover, to evaluate the maximum possible environmental burden posed by CFA, optimized leaching conditions and the determination of the saturation leachability of each HTE are urgently needed. It is recommended that ecotoxicological studies incorporate both maximum leachability tests and application-specific leachability tests, using leachates with and without pH adjustment. This dual approach will provide a comprehensive understanding of the environmental impact of CFA, encompassing both the maximum release potential and the behavior under realistic field conditions.

5. Aquatic Toxicity

5.1. First Trophic Level

5.1.1. Accumulation of Heavy Metals

In aquatic ecosystems, the first trophic level is primarily represented by photosynthetic organisms, including cyanobacteria, algae, and other phytoplanktonic taxa, which are directly exposed to CFA-derived metals through both waterborne and sediment-associated pathways. Metals released from CFA leachates can be taken up and accumulated in their tissues. In addition, heavy metals are capable of trophic transfer. Figure 4 shows the relationships and discrepancies in CFA-related exposure pathways across different trophic levels. In a study involving nine aquatic plants and two algal species of primary producers, a significant positive correlation ($p < 0.001$) was observed between the bioaccumulation of CFA-derived metals and the metal availability in CFA leachates [76]. Among different metal species, the cyanobacterium *Anabaena variabilis* exhibited the highest accumulation of Cr and Zn under high CFA exposure levels due to its specific uptake selectivity (10% and 20%) [77]. Such accumulation may contribute to subsequent toxic responses, including photosynthesis inhibition, oxidative stress, membrane damage, and metabolic disruption, when internal metal burdens exceed organismal tolerance thresholds, thereby resulting in growth suppression and cellular structural damage of the organisms, as shown in Figure 1.

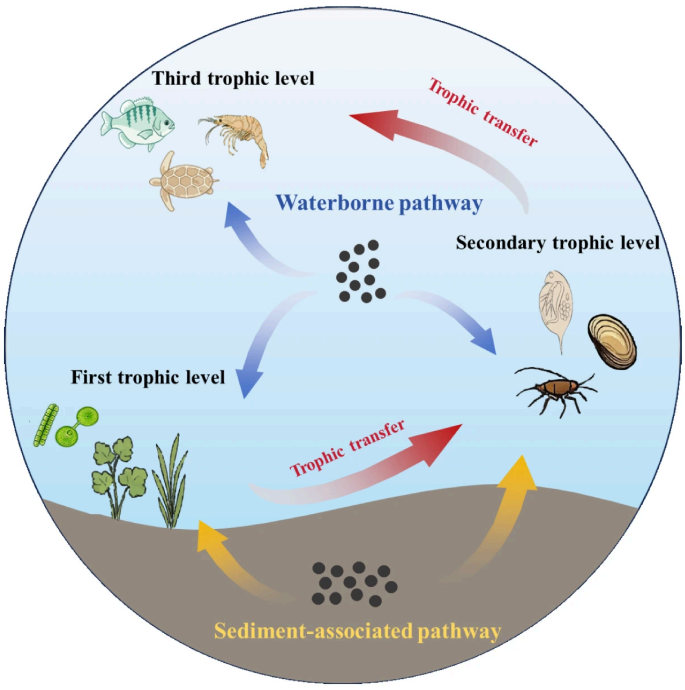


Figure 4. Schematic illustration of contaminant exposure pathways and trophic transfer across different trophic levels in aquatic ecosystems.

5.1.2. Photosynthesis Inhibition

Photosynthetic impairment induced by CFA is mechanistically associated with photosystem II (PSII) dysfunction, chlorophyll degradation, chloroplast membrane damage, enzyme activity alteration, and consequent suppression of biomass accumulation. Specifically, CFA-derived metals (e.g., Cd and Cu) may inhibit PSII activity in *Chlorella*, as evidenced by a significant reduction in the maximum quantum efficiency of PSII photochemistry (F_v/F_m) and other chlorophyll fluorescence parameters [78,79]. This inhibition follows distinct dose- and time-dependent characteristics, with pronounced aggravation observed at elevated metal concentrations. In a concentration range of 0.1–1000 ppm, total chlorophyll content of the alga decreased continuously with increasing exposure duration, reaching zero on the 8th day post-inoculation at 1000 ppm and on the 11th day at 500 ppm [80]. Similar inhibitory responses have been observed across cyanobacteria, green algae, and diatoms when exposed to leached Cd at concentrations $\geq$ 5 mg L^{-1}. Under such conditions, these organisms exhibited severe growth retardation, concomitant with compromised PSII functionality, altered activities of antioxidant enzymes, and disrupted cell wall structural integrity [81]. As summarized in Table 2, the reported growth inhibition rates were 10–35% for *Scenedesmus quadricauda*, 51.5–93% for *Pseudokirchneriella subcapitata*, and below 63.6% for *Vibrio fischeri* [43,82].

Table 2. Comprehensive summary of ecotoxicological responses of coal fly ash (CFA) exposure on aquatic organisms across multiple trophic levels: inclusive of various standard test organisms and different extraction methods.

Trophic Level	Test Organism	Source	Extraction Method	Toxicity Effects	Reference
First trophic level	*Scenedesmus quadricauda*	From thermal power plants in Russia	Contacted with aerated tap water (1–3 days)	Growth inhibition rate: 10% to 35%	[82]
	Pseudokirchneriella subcapitata	From Swedish industry	ER-H method, L/S = 10 L kg^{-1}	EC_{50}: 11% leachate	[40]
		From 6 power plants in Europe	EN 12457-3, L/S = 0.1 L kg^{-1}	Growth inhibition rate: 51.5–93%	[43]
	Vibrio fischeri	From Swedish industry	ER-H method, L/S = 10 L kg^{-1}	EC_{50}: >90% leachate	[40]
		From Agios Dimitrios thermal power plant, Northern Greece.	NEN 7341 L/S = 10, 20, 30, 40, 50, 100 and 200 L kg^{-1}	Inhibition rate: < 63.6%	[62]
		From coal power plants in Europe	TCLP eluates (pH adjusted to 7) L/S = 20 L kg^{-1}	Prior to pH adjustment, higher pH (4 vs. 7) reduced mortality EC_{50}: (0.32% to over 100% leachate) After pH adjustment, no toxic effect on the eluate	[65]
		From 6 power plants in Europe	EN 12457-2, L/S = 0.1 L kg^{-1} EN 12457-3, L/S = 0.1 L kg^{-1}	Inhibition rate: 18.3% to 40%	[43]
Second Trophic Level	*Brachionus calyciflorus*	From coal power plants in Europe	TCLP eluates (pH adjusted to 7) L/S = 20 L kg^{-1}	EC_{50}:45% to 71% leachate	[65]
	Daphnia magna	From coal power plants in Europe	TCLP eluates (pH adjusted to 7) L/S = 20 L kg^{-1}	*Daphnia magna* is the most sensitive species EC_{50}:34% to 67% leachate	[65]
		Anhui Suzhou Huiyuan Power Generation Co., Ltd, China	Horizontal Oscillation Method (HJ 557–2010) L/S = 300 L kg^{-1}	Mortality: 42.5%; the mortality was linearly positively correlated to leached Cr and Ni	[83]

Table 2. *Cont.*

Trophic Level	Test Organism	Source	Extraction Method	Toxicity Effects	Reference
Second Trophic Level	*Nitocra spinipes*	From Agios Dimitrios thermal power plant, Northern Greece.	NEN 7341 L/S = 10, 20, 30, 40, 50, 100 and 200 L kg^{-1}	TU: 1.47–28.6 The *Daphnia magna* was more sensitive than *Vibrio fischeri*. The toxicity of the leachates was related to Cr(VI)	[62]
		From 6 power plants in Europe	EN 12457-3: L/S = 0.1 L kg^{-1}	Immobilization rate: 85–100%	[43]
		From thermal power plants in Russia	Contacted with aerated tap water (1–3 days)	Mortality rate: 50–80%	[82]
		From Swedish industry	ER-H method, L/S = 10 L kg^{-1}	Acute LC_{50}: 16% leachate NOECs: 0.5% leachate	[40]
	Benthic macroinvertebrate	Savannah River Project	An eight-year field monitoring investigation	96 h-LC_{50} = 0.27	[84]
Third Trophic Level	*Danio rerio*	From Swedish industry	ER-H method, L/S = 10 L kg^{-1}	NOECs: > 10% leachate	[40]
	Juvenile *Largemouth Bass*	Permitted and unpermitted leaks from coal fly ash pond	Site investigation of Herrington Lake by Downstream Strategies in 2016	12.2% spinal and/or craniofacial malformations	[85]
	Largemouth Bass	Permitted and unpermitted leaks from coal fly ash pond	Site investigation of Herrington Lake by Downstream Strategies in 2016	Selenium (10 μg L^{-1}) induced >25% mortality every year and 75% abnormal survivors	[85]
	Fish communities	CFA spill to Emory River of Watts Bar Reservoir in 2008	Site investigation of Watts Bar Reservoir in 2010	Ash and ash-related constituents pose negligible risks to the fish communities.	[86]
	Pimephales promelas	CFA spill to Emory River of Watts Bar Reservoir in 2008	Dredging elutriate test: elutriates were prepared by bubbling collected fly ash with air for > 2 days, 2009	No toxicity was observed in larvae and juveniles	[87]
	Gambusia holbrooki	CFA at the D-Area coal-fired power plant, South Carolina, USA	Site investigation of Savannah River Site Aiken	No toxicity was observed in *Gambusia holbrooki*.	[88]

CFA-derived metals may also inhibit photosynthesis through the reduction in photosynthetic pigment content and disruption of chloroplast membrane integrity. For instance, in the submerged macrophyte *Ceratophyllum demersum*, exposure to Cu at concentrations of 2 μM and 4 μM resulted in a 16.4% and 23.1% decrease in total chlorophyll content after 24 h, respectively. This may be attributed to Cu-mediated peroxidation of chloroplast membranes, as Cu^{2+} is known to stimulate lipid peroxidation and enhance membrane permeability, ultimately disrupting thylakoid integrity [89,90]. Similar effects on chlorophyll content and chloroplast ultrastructure have been documented in other plant species following CFA exposure, wherein the deposition of heavy metals suppresses chlorophyll-family activities and leads to chloroplast expansion, grana layer-stack disorder, and even chlorophyll membrane damage [91,92].

In addition to photosynthetic impairment, CFA exposure may also disturb cellular metabolism. For example, exposure to the CFA sample from the first row of the electrostatic precipitator in Republika Thermal Power Plant (5.0 g L^{-1}) resulted in a reduction in bioluminescence intensity to approximately 20% within 1 min. Given that bacterial luminescence depends on $FMNH_2$, long-chain aldehydes, and O_2, this rapid inhibition may suggest impaired metabolite synthesis, altered enzymatic reactions, or disruption of the cellular electron transport chain [93].

5.1.3. Oxidative Stress and Membrane Damage

Oxidative stress constitutes a key mechanism by which CFA compromises plant physiological performance, photosynthetic efficiency, gas exchange, and chloroplast structure through the generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), and superoxide anions ($O_2^{-}\cdot$) [94]. Excessive ROS may disrupt antioxidant defense and promote the formation of lipid peroxidation products (e.g., malondialdehyde, MDA), ultimately leading to membrane damage [95]. For example, treatment at 25% CFA increased MDA by 94.6% and electrolyte leakage by 16.8% in mint leaves, indicating membrane disruption. Similarly, in *Ceratophyllum demersum*, MDA increased with Pb concentration up to 10 μM, but declined markedly above 20 μM after 14–21 days of exposure, suggesting the exceedance of the tolerance threshold. Comparable patterns of MDA elevation and concomitant oxidative damage have also been reported in ferns and other plants exposed to CFA-amended soils [95,96]. However, when metal toxicity surpasses detoxification capacity, antioxidant systems collapse, and the subsequent decline in MDA may indicate severe cellular injury beyond the plant's survival threshold.

5.2. Second Trophic Level

Bioassays employing the primary trophic level (e.g., *Vibrio fischeri*) tend to underestimate the ecological toxicity of CFA because of their simple metabolism, lower sensitivity, and limited structural complexity [65]. For instance, the EC_{50} of *Vibrio fischeri* was observed when the CFA leachate concentration exceeded 90% (Table 2) [40], indicating that substantial leachate fractions are required to elicit a measurable response in this test organism. Such test systems are incapable of capturing the process of pollutant bioaccumulation or the trophic transfer effects. In contrast, secondary consumers exhibit higher metal burdens and more pronounced dietary-exposure toxicity [97], owing to their exposure pathways via both direct CFA particle ingestion and trophic transfer from contaminated first-trophic-level organisms [98]. Consequently, an increasing number of studies have adopted second-trophic-level organisms to evaluate the environmental risk of CFA. A synthesis of studies indicates that CFA exposure exerts multiple adverse effects on second-trophic-level organisms, including alterations of water chemistry, bioaccumulation of major and trace elements, induction of oxidative stress, and disruption of osmotic homeostasis.

5.2.1. Water Chemistry Alteration and Bioaccumulation

Regarding water chemistry alteration and bioaccumulation, both an eight-year field investigation [84] and a laboratory-based study [65] have demonstrated that acute acidification events (pH < 5.5) cause significant mortality among aquatic fauna, particularly small-bodied invertebrates. Pouil et al. [18] found a strong positive correlation between CFA-derived elemental concentrations in the Emory and Clinch Rivers (e.g., metallic elements Ca, Mg, Zn, Cd, Cr, and nonmetallic elements As, Se) and bioaccumulation, although the magnitude of uptake varied among the examined species. For example, As accumulation in *Pleurocera canaliculatum* and *Hexagenia bilineata* tissues was 16.4 mg kg^{-1}, but dropped rapidly to 3.7 mg kg^{-1} (BAF < 1) following depuration. Cr mainly exists as Cr(III) in CFA and can be leached in substantial quantities under acidic conditions [99,100]. In laboratory exposure with *Daphnia magna*, Cr accumulation reached 150 μg kg^{-1} (BAF > 1) at an exposure concentration of 72.04 μg L^{-1} [83], exceeding the 16 μg L^{-1} criterion (U.S. EPA, 2025). In contrast, Cd burdens in *Stenonema pudicum* tissues (0.11–0.14 mg kg^{-1}) remained sublethal [84], whereas Se levels > 5 mg kg^{-1} (BAF > 1) in aquatic invertebrates were linked to selenosis [85].

5.2.2. Oxidative Stress and Osmotic Disruption

The leached elements mediate toxic effects primarily through oxidative stress and the disruption of osmotic pressure. Metallic constituents can catalyze one-electron reductions in O_2 to generate reactive oxygen species [101,102], while associated HTEs may amplify free-radical formation near DNA, inducing oxidative damage and inflammatory responses [30]. In organisms exposed to leachate-derived metals and metalloids, significant upregulation of key biomarkers was observed, including increased superoxide dismutase (SOD) and catalase (CAT) activities, elevated lipid peroxidation (LPO) levels, confirming pathological manifestations including lipid peroxidation, DNA damage, and mitochondrial dysfunction [83].

The extensive accumulation of major elements (e.g., >2000 mg kg^{-1} Ca, K) poses latent risks to *Nitocra spinipes* through osmotic stress [40,103]. The initial hyper-saline conditions force organisms to expend substantial energy maintaining osmotic balance, which is a physiological demand that chronically disrupts cellular regulation [104]. This sustained energetic burden compromises osmoregulatory capacity, ultimately triggering either cellular swelling and cytolysis or dehydration-induced plasmolysis, culminating in organismal mortality.

5.3. Third Trophic Level

Compared with organisms at the first and second trophic levels that are directly exposed to CFA, those at the third trophic level are indirectly affected through dietary uptake via the food web. Otter et al. [105] observed As and Se accumulation in largemouth bass, white crappie, bluegill, and redear sunfish, with the highest Se concentrations (24.83 mg kg^{-1} in liver, 10.40 mg kg^{-1} in ovaries, dry weight) detected in redear sunfish. This is likely because redear sunfish primarily feed on bivalves, which exhibit approximately 90% lower Se depuration capacity than other invertebrates, leading to high Se accumulation in bivalves and redear sunfish [106]. This trophic transfer reinforces the concern that elevated Se in lower trophic organisms can translate into toxic burdens in higher vertebrates.

Additionally, metal accumulation in organisms varies across tissue types [107,108]. Robin et al. [109] reported respective Se concentrations of 17.3, 32.5, and 37.1 μg g^{-1} (dry weight) in whole body, ovary, and testes tissues of bluegill, all exceeding the proposed toxicity thresholds for fish whole body (7.9 μg g^{-1}) and ovary (17 μg g^{-1}) by approximately 2–3 times [109]. Furthermore, comparable Se accumulation patterns across tissues have been observed in turtles, with blood and claw tissues showing differential increases in metal concentration upon exposure [110,111]. This differential response is due to the distinct turnover period of these tissues. For instance, the turnover period of trace elements in claws is approximately 12 months [112], allowing them to reflect long-term exposure, while blood metal concentrations serve as indicators of recent exposure [111]. These results from tissues with varying turnover periods enable a more comprehensive reconstruction of exposure history.

Despite the accumulation of trace elements from CFA in the third-trophic-level organisms, acute toxicity was not observed [13,113,114], possibly due to their more developed detoxification and excretory systems. Furthermore, the relative contributions of direct waterborne exposure, dietary transfer, and heritable tolerance cannot be readily distinguished. To our knowledge, exposure manifests primarily as sublethal effects, including oxidative stress, hepatotoxicity, immunotoxicity, reproductive and developmental toxicity, and genotoxicity.

5.3.1. Oxidative Stress

Following 24 h of exposure to CFA leachate (1 mL L^{-1} of leachate representing 100 mg mL^{-1} of CFA in original slurry), lipid peroxidation was induced in all examined fish organs, with the gills showing the most pronounced response [115]. This susceptibility is

likely attributed to the weak antioxidant capacity of gill tissue against xenobiotics [116,117]. In detail, the uptake of As in CFA leachate via gills disrupts the antioxidant system by elevating glutamate cysteine ligase activity and glutathione levels [118]. The induction of enzymes and glutathione exhibited the highest activity levels in the liver, activating antioxidant defense to counteract ROS during the initial exposure period [117,119].

5.3.2. Hepatotoxicity

Since the liver is the primary organ for trace element metabolism and detoxification, hepatic physiological and histopathological changes provide sensitive and integrative indicators of organismal health under pollutant stress [120]. Lohner et al. [121–123] reported significantly elevated Se, Cu, and As concentrations in sunfish from CFA-polluted areas relative to unexposed groups, accompanied by hematological and metabolic disturbances, indicative of nutritional stress and impaired hepatocyte function. These findings are corroborated by cellular-level mechanistic studies. Trace elements in CFA can induce hepatomegaly through glycogen deposition and intracellular vacuolation [16,115,123], impair hepatocytes' proliferation, and promote apoptosis [124]. Upon CFA exposure, *Channa punctata* hepatocytes generated ROS, resulting in cell membrane damage and cytochrome c release, subsequent apoptosome formation, and ultimately caspase activation and cleavage of essential cellular proteins [124].

5.3.3. Immunotoxicity

Immunotoxicity has emerged as a critical endpoint in assessing the ecological risks of CFA contamination, due to the increased susceptibility of organisms to diseases upon immune impairment [125]. Trace elements from CFA, such as Mn, V, As, and Cu, have been shown to inhibit lymphocyte division, thereby compromising innate immune capacity [126]. However, some studies have reported no significant immunological impairment in organisms exposed to CFA contamination [127]. This discrepancy may be attributed to the compositional complexity of CFA, wherein certain elevated elements (e.g., Se, Cu) can enhance bacterial killing capacity and upregulate immune-related gene expression [110].

5.3.4. Reproductive and Developmental Toxicity

CFA-induced reproductive and developmental toxicity has profound consequences for the third-trophic-level organisms [128]. Bullfrog larvae from CFA-contaminated sites exhibited a significantly higher incidence of lateral spinal curvature than those from uncontaminated areas [129]. Similarly, juvenile largemouth bass inhabiting CFA-polluted sites displayed a 12.2% incidence of spinal and/or craniofacial malformations (Table 2) [85]. Although malformed individuals can survive, their compromised swimming performance and increased predation susceptibility often preclude them from reaching reproductive maturity, ultimately leading to population reproductive failure [130,131]. CFA contamination can also impair reproductive success via maternal transfer. Metts et al. [132] reported that female southern toads from CFA-contaminated sites transferred elevated Cu, Pb, Se, and Sr to their eggs, resulting in a 27% reduction in overall reproductive success. A similar mechanism has been documented in fish, indicating that Se translocation to oocytes drives teratogenic effects and population-level failure in CFA-contaminated waters [133,134].

5.3.5. Genotoxicity

Genotoxicity represents a critical mode of toxicity induced by CFA. In a study conducted at the U.S. Department of Energy's Savannah River Site, grass shrimp exposed over a multi-year period exhibited significant increases in tail DNA content, tail torque, and tail length, which were strongly correlated with elevated concentrations of Se (9.44 mg L^{-1}) and Cd (2.15 mg L^{-1}) [135]. These observations are indicative of DNA single-strand breaks,

likely resulting from base damage caused by chronic exposure to these metal (loid)s. Nevertheless, it remains unclear whether such genetic damage arises from direct metal–DNA interactions or from indirect oxidative stress mediated by other cellular pathways [136].

5.4. Particulate Toxicity

As far as we know, most studies on aquatic ecotoxicity of CFA focused on the CFA leachant, while the intrinsic particle toxicity of nano- or micro-scale CFA particles has been largely overlooked. Similar to the studies on PM10 [137] and PM2.5 [138], fine CFA fractions warrant special attention since they can penetrate the alveolar epithelial lining and enter the pulmonary interstitium more efficiently. They are also more capable of crossing the pulmonary and skin barriers, entering the circulatory and lymphatic systems, and even accumulating in organelles such as mitochondria [139,140].

In addition, fine CFAs have a large specific surface area, concentrating trace metals more effectively than coarser particles [141]. Lanzerstorfer et al. [141] showed that most heavy metals and trace constituents (As, Br, Cu, Ga, Ge, Hg, P, Pb, Sn, Sr, Tl, V, Zn) are enriched in fine CFA fractions but depleted in coarse ones. Sherrard et al. [142] agreed that peak As leaching from fine fractions of CFA (>40% fly ash) drove the observed toxicity in *Chironomus dilutes*. Ball et al. [143] further concluded that CFA particle size is the governing factor, generating substantially divergent risk profiles for each size class.

Furthermore, CFA particles (<40 μm) fall within the optimal filter-feeding by zooplankton such as *Daphnia magna*, facilitating enhanced ingestion, gastrointestinal retention, and consequent mechanical and oxidative damage to gut epithelia (Figure 1) [13,30]. Even in the absence of dietary exposure, CFA particles can directly invade cells upon contact with external biological surfaces by hijacking clathrin- and caveolin-mediated endocytic pathways, triggering biofilm disruption, protein oxidation, genotoxicity, impaired energy transduction, and oxidative stress [13,30]. These cellular-level mechanisms are consistent with observations from mammalian cell studies, in which internalized ultrafine particles initiate inflammatory cascades and cytotoxic responses.

In the context of climate change, elevated temperature and acidified surface waters may influence the aggregation, dissolution, and ultimately the biological uptake of nano/micro CFA particles, thereby either mitigating or enhancing their toxicity [144]. On the mitigating side, elevated temperature promotes the aggregation of CFA nanoparticles in aqueous solutions, which reduces their cellular uptake and consequently lowers their bioavailability and toxicity to organisms [145]. Conversely, elevated temperature accelerates HTEs dissolution and diffusion (e.g., Cu, Co, Ni) [145,146], and acidification further promotes metal release [147,148], jointly enhancing toxicity. Moreover, the formation of large agglomerates at higher temperatures may induce shading effects that suppress photosynthesis in algae, representing an additional stress.

No matter what, a holistic evaluation of CFA particle-size effects on aquatic organisms remains lacking. Future studies should systematically investigate the interplay between particle size, metal enrichment, surface reactivity, and biological uptake pathways. Moreover, because organisms at higher trophic levels may be exposed to CFA particles through dietary transfer, research should extend beyond direct aqueous exposure to include particle trophic transfer and its contribution to overall toxicity.

6. Future Recommendations and Perspectives

Future ecotoxicological assessments of CFA should move beyond single-species and single-metal paradigms. Studies summarized in Table 2 demonstrated that the ecotoxicological impacts of CFA leachates are trophic level-dependent. Ribé et al. [41] showed that the first-trophic-level *Vibrio fischeri* is insufficient for evaluating the toxicity of CFA

leachates, exhibiting only marginal inhibition. The second-trophic-level crustacean *Daphnia magna* consistently displays higher sensitivity [7,62], likely due to the physical overlap between CFA particle size and its optimal filter-feeding range (<45 μm) [41,67]. The third-trophic-level *Brachionus calyciflorus*, however, shows blunted toxic responses and lower susceptibility than *Daphnia magna.* This is possibly due to its more developed detoxification and excretory systems. These findings underscore the necessity of employing a battery of organisms representing different trophic levels for robust CFA risk evaluation. To overcome this limitation, future research must extend beyond individual-level, single-generation tests. Multi-generational exposure experiments, together with assessments at population, community, and ecosystem levels, are urgently needed to distinguish between innate species tolerance and local adaptive evolution, and to elucidate the ecological consequences of CFA leachates in real-world aquatic environments.

Furthermore, the interactive mechanisms governing the co-released metals remain poorly understood. Combined low levels of Cr, Ni, and As have been reported to elicit additive or synergistic toxic effects [62,83,142]. Conversely, certain metals may exceed regulatory thresholds without eliciting proportional toxicity [7,149], a phenomenon that may be attributable to competitive ionic uptake suppressing the bioavailability of more toxic species [150]. This observation is likely linked to the leaching protocols (Table 1) employed prior to aquatic bioassays. Mild procedures, such as TCLP or EN 12457, tend to underestimate metal mobilization under realistic environmental stressors. Moreover, the intrinsic toxicity of nano- or micro-scale CFA particles themselves has been largely neglected in current hazard assessments.

Last but not least, the reporting of CFA properties should be standardized to allow for cross-study comparisons. CFA exhibits considerable heterogeneity arising from variations in coal rank, combustion conditions, flue gas cleaning system, and aging [151]. Coal rank profoundly influences elemental composition. For instance, subbituminous coal-derived fly ash captures more Hg than bituminous-derived ash [152], while low-sulfur coals yield lower concentrations of As, Cd, Hg, Mo, Ni, and Pb [153]. Combustion technology also plays a key role. Conventional pulverized coal combustion predominantly generates spherical glassy particles, whereas fluidized-bed combustion produces ashes with distinct mineral phases due to lower operating temperatures [154]. The efficiency of flue gas cleaning systems further modifies CFA composition by controlling volatile element retention. For example, Hg is more enriched in baghouse-filter ash than in electrostatic precipitator ash [153]. Aging also introduces additional complexity. In alkaline CFA, CO_2 dissolution, Ca^{2+} carbonation, and hydration progressively lower pH and suppress amphoteric metal (e.g., Pb, Zn) release [155], while secondary Ca-bearing minerals (e.g., calcite, ettringite) form surface barriers that retard B and As mobilization from the glassy phase [156]. For CFA with low pH (e.g., pH ≈ 4), inhibited Ca mineral precipitation weakens immobilization and may increase leaching [157]. Thus, aging effects must be assessed on a case-by-case basis according to the initial physicochemical properties.

Therefore, we propose four paradigm shifts to accurately characterize the ecotoxicological risks associated with CFA: (1) moving from standardized leaching protocols to application-specific and maximum leachability tests, covering both pH-adjusted and unadjusted leachates. (2) transitioning from single-species assays to multi-trophic-level bioassays, (3) shifting from single-metal analysis to comprehensive, whole-matrix testing that integrates both particulate and dissolved fractions, and (4) standardizing the reporting of CFA provenance, coal types, and combustion conditions to facilitate meaningful meta-analyses and cross-study comparisons. Such a refined framework will substantially advance the realistic ecotoxicological assessment of CFA and provide a robust foundation for risk management in aquatic environments.

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