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Dr. Shashi Parmar

Associate Professor, Department
of Zoology, Shree Bhogilal
Pandya Government College,
Dungarpur, Rajasthan, India

Dr. Rekha Salvi

Assistant Professor, Department
of Zoology Seth Mathuradas
Binani Government PG College,
Nathdwara Rajasthan, India

Vikram Singh

Assistant Professor, Department
of Botany, Shree Bhogilal
Pandya Government College,
Dungarpur, Rajasthan, India

Corresponding Author:

Dr. Shashi Parmar

Associate Professor, Department
of Zoology, Shree Bhogilal
Pandya Government College,
Dungarpur, Rajasthan, India

Assessing the fate of antibiotics and heavy metals in biofloc systems: Removal efficiency and bioaccumulation risk

Shashi Parmar, Rekha Salvi and Vikram Singh

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Abstract

The intensification of aquaculture has led to concerns over the use of antibiotics and the accumulation of heavy metals in aquaculture systems, posing risks to environmental security and food safety ^[1]. Biofloc Technology (BFT), a sustainable aquaculture approach, is renowned for its ability to maintain water quality through microbial conversion of nitrogenous wastes². However, its role in mitigating chemical contaminants like antibiotics and heavy metals remains complex and inadequately synthesised. This comprehensive review assesses the dual role of BFT systems in influencing the fate of these pollutants. We examine the mechanisms of removal, primarily through biosorption, bioaccumulation, and biodegradation facilitated by the rich microbial community and extracellular polymeric substances (EPS) of bioflocs. Evidence indicates that BFT can significantly reduce antibiotic concentrations and sequester heavy metals from the water column, enhancing overall system water quality. Conversely, this very efficiency presents a significant risk: the bioaccumulation of these contaminants within the biofloc biomass and their subsequent transfer into the tissues of cultured species, potentially amplifying exposure. This review synthesises current literature on removal efficiencies for various antibiotics and metals, analyses the factors affecting their fate (e.g., C/N ratio, floc maturity, pH), and evaluates the associated bioaccumulation risks. We conclude that while BFT shows promise as a bioremediation tool, its implementation necessitates careful management to prevent it from becoming a reservoir and vector for contaminants. Future research directions should focus on understanding the microbial degradation pathways, the long-term stability of sequestered metals, and the development of safety standards for the reuse of biofloc biomass.

Keywords: Biosorption, Biofloc technology, antibiotics, heavy metals, biodegradation, bioaccumulation, aquaculture safety, circular bio economy

1. Introduction

Global aquaculture production must expand to meet the protein demands of a growing population, but its sustainability is challenged by environmental impacts, including chemical pollution ^[1]. The prophylactic and therapeutic use of antibiotics, along with the presence of heavy metals in feed and water sources, are significant concerns. Antibiotic residues can promote antimicrobial resistance (AMR) ^[2], while heavy metals like copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) are toxic to aquatic life and can pose human health risks through consumption ^[2].

Biofloc Technology (BFT) has emerged as a revolutionary, environmentally sustainable aquaculture system³. By maintaining a high carbon-to-nitrogen (C/N) ratio, BFT promotes the growth of a heterogeneous microbial community (bacteria, micro-algae, protozoa) that forms aggregates, or "bioflocs." These flocs assimilate ammonia from fish waste, improving water quality and providing a supplemental feed source ^[3]. The core principles of BFT microbial activity and biosorption logically extend to the management of other pollutants.

The fate of antibiotics and heavy metals in BFT is a double-edged sword. On one hand, the system's components can act as a biofilter, removing contaminants from the water. On the other hand, the accumulated pollutants may persist within the system, leading to bioaccumulation in aquaculture species and creating a potential "sink" for hazardous substances. Therefore, a critical assessment is needed to evaluate the efficacy of BFT as a bioremediation tool while quantifying the associated risks. This review paper aims to:

- Synthesise the mechanisms by which BFT systems remove antibiotics and heavy metals.
- Summarise reported removal efficiencies for different classes of contaminants.
- Evaluate the evidence for bioaccumulation in biofloc biomass and cultured species.
- Identify key knowledge gaps and propose future research directions for the safe application of BFT.

2. Mechanisms of Removal in Biofloc Systems

The removal of antibiotics and heavy metals in BFT is facilitated by the complex structure of the biofloc, which includes live microorganisms, dead organic matter, and a matrix of extracellular polymeric substances (EPS).

2.1 Removal of Antibiotics

The primary mechanisms for antibiotic removal are biodegradation and biosorption.

- **Biodegradation:** The diverse microbial consortium in bioflocs harbours bacteria capable of utilising antibiotics as carbon, nitrogen, or energy sources. Enzymatic breakdown is a key pathway for compounds like sulfamethoxazole and tetracyclines ^[4]. The high microbial density and activity in mature flocs enhance the probability of degradation.
- **Biosorption:** Antibiotics can be adsorbed onto the surface of bioflocs, particularly to the EPS. EPS, composed of polysaccharides, proteins, and humic substances, contains functional groups (e.g., carboxyl, hydroxyl, amine) that can bind antibiotic molecules through ionic, hydrophobic, and hydrogen bonding interactions ^[5]. This is often the initial rapid phase of removal.

2.2 Removal of Heavy Metals

Heavy metals are primarily removed through biosorption and bioaccumulation, as they are not biodegradable.

- **Biosorption:** This passive, metabolism-independent process is highly efficient in BFT. Metal ions in the water (e.g., Cu²⁺, Zn²⁺, Pb²⁺) bind to negatively charged sites on the EPS and microbial cell walls ^[6]. The large surface area of the flocs provides ample binding sites, effectively concentrating metals from the water column ^[6].
- **Bioaccumulation:** Some microorganisms within the floc can actively transport metals into their cells, a metabolism-dependent process. This can lead to intracellular sequestration.

3. Removal Efficiency: Evidence from Literature

- **Antibiotics:** The efficacy of BFT in removing antibiotics is highly variable and depends on the antibiotic's molecular structure, hydrophobicity, and the specific microbial community present in the biofloc. Studies demonstrate varying removal efficiencies based on the antibiotic class and system conditions. For instance:
- **Tetracyclines:** This class of antibiotics has a strong affinity for binding to calcium and magnesium ions and organic matter. Indian research on *Penaeus vannamei* culture has confirmed the rapid adsorption of oxytetracycline (OTC) onto bioflocs. A study by Kumar *et al.* ^[7] reported that in a biofloc system, over 70% of OTC was removed from the water column within the first 24 hours, primarily through adsorption to the floc

particles. However, the study also cautioned that this rapid removal from the water does not equate to degradation; the OTC remains bioactive within the floc matrix, posing a risk for the development of antibiotic-resistant genes (ARGs). Further work by Rathore *et al.* ^[8] compared OTC removal in biofloc and clear-water systems, finding that the presence of bioflocs accelerated the initial removal phase but noted that the overall mineralization rate was slow, leading to persistent residues.

- **Fluoroquinolones:** Antibiotics like enrofloxacin are widely used in shrimp farming due to their efficacy against bacterial pathogens. Their strong tendency to adsorb to solids makes them a significant concern in BFT. Research conducted by Verma *et al.* ^[9] demonstrated that while biofloc systems could remove over 85% of enrofloxacin from the aqueous phase, a substantial portion was recovered intact from the floc biomass. The study highlighted that the bioavailability of the antibiotic to the shrimp decreased in the BFT system, but the risk of selective pressure for enrofloxacin-resistant bacteria within the floc community was high.
- **Sulfonamides:** Being more hydrophilic, sulfonamides like sulfamethazine show lower adsorption but are susceptible to biodegradation. A lab-scale study by Sharma and Datta ^[10] investigated the role of a mature, diverse biofloc microbiome in breaking down sulfamethazine. They identified specific bacterial genera, including *Bacillus* and *Pseudomonas*, enriched in the BFT system that were capable of utilising the antibiotic as a nutrient source, achieving a 65% degradation rate over a 10-day period, compared to less than 20% in the control system.
- **Heavy Metals:** The biofloc system acts as an efficient bio-sorbent for heavy metals, a property of immense importance in regions like India where industrial effluent can contaminate water sources. The extracellular polymeric substance (EPS) matrix is the primary site for this sequestration.
- **Copper (Cu) and Zinc (Zn):** These metals are common in aquaculture, originating from feed supplements and algacides. A seminal study by Gauns *et al.* ^[11] systematically evaluated the biosorption capacity of bioflocs for Cu and Zn. They reported removal efficiencies of 85-95% from synthetic aquaculture wastewater, with the process following a Langmuir adsorption isotherm, indicating monolayer adsorption onto a surface with a finite number of identical sites. Their research pinpointed the protein and polysaccharide components of the EPS as the key binding sites through ion exchange and complexation. Similarly, Patel *et al.* ^[12] working with *Macrobrachium rosenbergii* (giant freshwater prawn), found that biofloc systems effectively mitigated the toxic effects of sub-lethal Cu exposure by rapidly sequestering the metal, resulting in significantly improved survival and growth rates compared to prawns in clear water with the same Cu concentration.
- **Arsenic (As) and Lead (Pb):** Research has extended to more toxic, non-essential metals. Mahananda *et al.* ^[13], in a carp polyculture model, demonstrated that bioflocs could accumulate significant amounts of arsenic and lead. Their study found that the concentration of these metals in the floc biomass was 200-300 times higher than in the

water column. This high bioaccumulation factor underscores the effectiveness of BFT as a polishing treatment for water but also flags the floc biomass as a hazardous material if the system is sourced from contaminated water.

- **Chromium (Cr):** Investigating the fate of chromium (Cr VI), Reddy and Shaikh ^[14] demonstrated that bioflocs not only adsorbed the metal but also facilitated its bioreduction to the less toxic Cr (III). Their research attributed this to the activity of specific sulfate-reducing bacteria and other anaerobic microbes present within the anoxic microsites of the larger floc particles. This highlights an advanced remediation function of BFT beyond simple biosorption.

4. The Bioaccumulation Risk

The remarkable efficiency of Biofloc Technology (BFT) in stripping antibiotics and heavy metals from the water column creates a critical paradox: the process of remediation concentrates these contaminants within the system's living biomass, creating a potent vector for exposure and raising significant food safety concerns. Indian research has been instrumental in quantifying this risk across various species and contaminants.

- **Accumulation in Floc Biomass:** The Primary Sink The biofloc aggregate is the first and most significant repository for contaminants. Indian studies have consistently demonstrated high Bioconcentration Factors (BCFs) in floc biomass.
- **Heavy Metals:** The work of Mahananda *et al.* ^[13] in carp polyculture provided stark evidence, showing that the concentration of metals like lead (Pb) and chromium (Cr) in the biofloc solids was 200-300 times higher than in the ambient water. They described the biofloc system as a "living filter" that effectively scrubs metals from the water but warned that the resulting metal-laden floc becomes a hazardous material. Similarly, a study by Das *et al.* ^[15] focusing on shrimp (*Penaeus vannamei*) in BFT systems exposed to ambient levels of zinc (Zn) and copper (Cu) found that the floc biomass accumulated these metals to levels that exceeded safe limits for direct use as a feed ingredient for other animals, complicating the circular economy potential of spent biofloc.
- **Antibiotics:** Research led by Kumar *et al.* ^[7] provided crucial pharmacokinetic data for oxytetracycline (OTC). They found that while OTC concentration in water decreased rapidly, the floc biomass contained high and persistent levels of the antibiotic. The study calculated a high adsorption coefficient (Kd) for OTC on bioflocs, confirming that the flocs act as a long-term reservoir, slowly desorbing the antibiotic back into the water or making it directly available for ingestion.
- **Trophic Transfer and Tissue Residues:** The Food Safety Threat The core principle of BFT that flocs are consumed by the cultured species becomes the primary pathway for contaminant transfer. Indian research has directly measured this transfer into edible tissues.
- **Antibiotic Residues in Shrimp:** The study by Kumar *et al.* ^[7] went beyond floc accumulation to track OTC in shrimp tissues. They reported detectable residues in the muscle (the edible portion) of *Penaeus vannamei* that exceeded the Maximum Residue Limit (MRL) for several days post-exposure. This was a critical finding,

demonstrating that even with rapid water clearance, the consumption of contaminated flocs leads to violative tissue residues. Furthermore, Verma *et al.* ^[9] found enrofloxacin and its metabolite ciprofloxacin in the hepatopancreas and muscle of shrimp reared in a biofloc system. Their research highlighted that the bioavailability of the antibiotic via floc consumption was significant enough to pose a selection pressure for antimicrobial resistance (AMR) in gut bacteria, even at sub-therapeutic concentration.

- **Heavy Metal Accumulation in Fish and Prawns:** The risk is not limited to crustaceans. Sharma and Krishnan ^[16] investigated the bioaccumulation of mercury (Hg) in Nile tilapia (*Oreochromis niloticus*) reared in a biofloc system. They found that tilapia in the BFT system had significantly higher mercury concentrations in their liver and muscle tissues compared to fish in a clear-water system, despite identical waterborne mercury levels. This was directly linked to their continuous grazing on the contaminated flocs. In a study on the giant freshwater prawn, Patel *et al.* ^[12] confirmed that while BFT mitigated the acute toxicity of copper (Cu), it led to a 3-4 fold increase in Cu accumulation in the hepatopancreas of *Macrobrachium rosenbergii* compared to the control group. The hepatopancreas, a detoxification organ, became a major storage site, raising concerns about the long-term health of the prawns and the safety of consuming whole prawns, where this organ is often ingested.
- **The Risk of Antimicrobial Resistance (AMR):** Gene Proliferation Beyond direct toxicity and tissue residues, Indian researchers are beginning to document a more insidious risk: the role of BFT as a hotspot for AMR. The work of Verma *et al.* ^[9] demonstrated that the enrofloxacin-enriched biofloc environment led to a significant shift in the bacterial community, favouring populations with known resistance traits. They observed a marked increase in the abundance of plasmid-mediated quinolone resistance (PMQR) genes like qnrS and aac(6)-Ib-cr within the floc microbiome. This creates a scenario where the biofloc system, while treating the water, inadvertently becomes a bioreactor for the amplification and horizontal gene transfer of resistance genes, which can then be transferred to pathogens affecting the shrimp or the surrounding environment when water is discharged.

5. Factors Influencing Fate and Risk

The ultimate fate of antibiotics and heavy metals whether they are successfully remediated or become a bioaccumulation hazard is not predetermined. It is heavily influenced by key operational parameters of the BFT system. Among these, the Carbon-to-Nitrogen (C/N) ratio is arguably the most masterful control lever, directly steering the microbial community responsible for the system's function.

The Central Role of the Carbon-to-Nitrogen (C/N) Ratio

The C/N ratio dictates the dominant metabolic pathway in the aquaculture system. A low C/N ratio promotes the autotrophic nitrification process (converting ammonia to nitrate). In contrast, the core principle of BFT is to maintain a high C/N ratio (typically 10:1 to 20:1) to stimulate heterotrophic bacterial growth³. These bacteria assimilate ammonia nitrogen

directly into their cellular biomass, requiring carbon for energy. This fundamental control has profound implications for contaminant management.

A) Influence on Antibiotic Fate

A high C/N ratio promotes a dense, fast-growing heterotrophic bacterial community, which can enhance contaminant removal through two primary mechanisms:

- **Enhanced Biodegradation:** A diverse and metabolically active heterotrophic community has a higher probability of hosting species capable of utilizing antibiotic molecules as a carbon, nitrogen, or energy source. Indian research supports this; Sharma and Datta ^[10] observed that maintaining a C/N ratio of 15:1 was optimal for enriching *Bacillus* and *Pseudomonas* populations, which were directly correlated with the increased biodegradation of sulfamethazine. At lower C/N ratios (e.g., 6:1), the microbial community was less dense and less effective at breaking down the antibiotic.
- **Dilution via Biomass Production:** The high C/N ratio encourages the rapid production of massive amounts of bacterial biomass (the bioflocs). Even antibiotics that are not fully degraded can be effectively "diluted" by being adsorbed onto and distributed throughout this large volume of floc. Kumar *et al.* ^[7] noted that in systems with a stable high C/N ratio, the initial rapid adsorption of oxytetracycline onto the prolific floc biomass was a key removal mechanism from the water, reducing the immediate bioavailability to shrimp.

B) Influence on Heavy Metal Fate: The C/N ratio's effect on heavy metals is primarily mediated through the production of Extracellular Polymeric Substances (EPS).

- **Stimulation of EPS Production:** Heterotrophic bacteria under high C/N conditions produce copious amounts of EPS to form the floc matrix. This EPS is rich in functional groups (e.g., carboxyl, hydroxyl, sulfate, amine) that are primary binding sites for metal cations. Research by Gauns *et al.* ^[11] explicitly linked a higher C/N ratio (15:1) to greater EPS production and a correspondingly higher biosorption capacity for copper (Cu) and zinc (Zn) in lab-scale BFT systems. They found that systems with a C/N of 10:1 had approximately 30% lower metal removal efficiency compared to those at 15:1, directly linking floc quality to remediation performance.
- **Floc Structure and Binding Capacity:** A mature, stable floc cultivated at an optimal C/N ratio has a more robust structure with a greater surface area for metal binding. Patel *et al.* ^[12] observed that in their prawn culture studies, systems where the C/N ratio was allowed to fluctuate exhibited poorer floc formation and higher free Cu²⁺ ion concentrations in the water, leading to increased stress in the animals. Maintaining a consistently high C/N ratio was key to creating a resilient floc that could sequester the metal effectively.

C) The Double-Edged Sword and Management Imperative: It is crucial to understand that while a high C/N ratio enhances removal, it simultaneously amplifies the bioaccumulation risk within the floc. The very process that makes BFT effective at water purification concentrating contaminants into the biomass is intensified. Therefore, the

C/N ratio must be managed not just for water quality, but for contaminant load. A study by Reddy and Shaikh¹⁴ on chromium remediation highlighted this balance. They found that a C/N ratio of 15:1 was optimal for both Cr(VI) reduction and biosorption. However, they cautioned that in systems with high initial contaminant loads, the resulting floc biomass became so heavily contaminated that its safe disposal became a major challenge. This underscores that the C/N ratio is not a "set-and-forget" parameter; it is a dynamic tool that must be calibrated based on the known or suspected presence of antibiotics and heavy metals.

In summary, the C/N ratio is the cornerstone of managing contaminant fate in BFT. It directly governs the engine of the system the heterotrophic microbial community thereby controlling the rates of biosorption, biodegradation, and ultimately, the concentration of hazards in the biofloc biomass. Optimal C/N ratio management is essential for harnessing the bioremediation potential of BFT while developing strategies to mitigate the inherent bioaccumulation risk.

6. Conclusions and Future Perspectives

Biofloc Technology presents a paradoxical scenario for managing antibiotics and heavy metals in aquaculture. It is highly effective at stripping these contaminants from the water, thereby improving the immediate rearing environment. However, this comes at the cost of concentrating pollutants within the system's biomass, creating a significant risk of bioaccumulation in the final product and turning the biofloc into a hazardous waste if not managed properly.

To harness the benefits of BFT while mitigating risks, future research must focus on:

- **Microbial Pathways:** Identifying and enriching specific microbial strains or consortia capable of complete mineralization of antibiotics.
- **Safe Disposal/Valorisation:** Developing safe protocols for the treatment or disposal of contaminated biofloc sludge. Research is needed on methods to detoxify the sludge before its use as a fertiliser or feed ingredient.
- **Real-time Monitoring:** Establishing guidelines for monitoring contaminant levels in both water and floc biomass during production cycles.
- **Species-Specific Risk Assessment:** Conducting detailed risk assessments for different commercially important species reared in BFT under realistic exposure scenarios.

In conclusion, while BFT holds promise as a bioremediation tool, a precautionary approach is essential. Its application must be coupled with rigorous management practices and a clear understanding of contaminant fate to ensure the production of safe seafood and truly sustainable aquaculture.

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