

Article

Post-Harvest Chemical and Probiotic Interventions in Amazonian Fisheries: An Exploratory Evaluation of Trace Element Dynamics

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Abstract

Trace element contamination in Amazonian freshwater ecosystems threatens riverine food security. This study evaluated two post-harvest treatments—ethylenediaminetetraacetic acid (EDTA) and *Lactobacillus fermentum*—to assess cadmium (Cd) and lead (Pb) variations in edible muscle of four Amazonian fish species (*Cichla monoculus*, *Pimelodus jivaro*, *Platynemichthys notatus*, and *Serrasalmus rhombeus*). Concentrations were determined using ICP-OES. *Platynemichthys notatus* exhibited the highest baseline bioaccumulation, whereas *C. monoculus* showed the lowest. Although main treatment effects should be considered exploratory, distinct species-dependent trends emerged. EDTA achieved the highest Cd reduction in *P. notatus* (49.78%), while *L. fermentum* demonstrated notable Cd and Pb reductions in *C. monoculus*, *P. notatus*, and *S. rhombeus*. Conversely, relative concentration increases occurred in *P. jivaro* under both treatments, reflecting matrix-related effects. These findings suggest that post-harvest efficiency depends on fish species and metal type. Probiotic post-harvest processing represents a promising strategy for reducing dietary exposure to Cd and Pb in Amazonian fisheries, though optimizing operational parameters for community-level applications remains necessary.

Keywords: EDTA; *Lactobacillus fermentum*; subsistence fisheries

Key Contribution: This is the first study to explore the effectiveness of chemical (EDTA) and biological (*Lactobacillus fermentum*) treatments in reducing Cd and Pb concentrations in four economically and ecologically important Amazonian fish species. Our findings indicate a general trend where *Lactobacillus fermentum* may decrease both Cd and Pb levels, achieving apparent reductions of up to 36% for Pb and 34% for Cd in specific species.



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1. Introduction

Trace element contamination of aquatic ecosystems poses a major threat to both environmental integrity and human health, particularly in vulnerable regions such as the Amazon Basin. Toxic elements including cadmium (Cd) and lead (Pb), have been detected in rivers, lakes, and sediments affected by anthropogenic activities, especially agriculture, oil extraction, mining, industry, and domestic waste discharges [1]. In aquatic organisms, exposure to these heavy metals induces oxidative stress, tissue damage, metabolic disruptions, and impaired growth and reproduction, compromising overall ecosystem health. These contaminants accumulate in aquatic organisms and may biomagnify through food

webs, increasing human exposure in populations that depend on fish as their primary source of animal protein [2].

The accumulation of potentially toxic trace elements in fish is driven by bioaccumulation, a process influenced by physiological, ecological, dietary, and metabolic factors [2,3]. Species-specific differences in metal accumulation have been widely documented. Cd and Pb accumulation occurs mainly in inorganic ionic forms closely associated with environmental exposure, particularly through sediments, and their affinity for intracellular proteins [4,5]. Consequently, toxic element concentrations in freshwater fish may exceed international food safety standards. Reference standards, such as the Codex Alimentarius (CXS 193-1995) and European Union regulations (EU 2023/915), establish maximum permissible limits on a wet weight (w.w.) basis in fish muscle: 0.3 mg/kg w.w. for Pb, and 0.05 mg/kg w.w. for Cd [6]. Recent studies have reported elevated concentrations of these contaminants in fish from the Ecuadorian Amazon, largely associated with mining, oil extraction, agricultural activities, and other anthropogenic sources [7,8]. Specifically, trace element levels such as Pb in muscle tissue frequently exceed these international safety thresholds, reaching concentrations exceeding 1.0 mg/kg in high trophic level species from impacted Amazonian basins [7,8].

This contamination represents a serious food security concern for Indigenous and rural communities that rely on subsistence fishing. A recent assessment estimated that between 422,000 and 473,000 tons of fish are harvested annually across Brazil, Colombia, and Ecuador, with approximately 75% destined for household consumption rather than commercial markets [9]. Reducing dietary exposure to toxic metals is therefore essential, yet practical decontamination technologies suitable for remote Amazonian communities remain scarce. There is a clear need for affordable, effective, sustainable, and easily transferable approaches that can be implemented under local conditions.

Traditionally, heavy metal removal technologies have focused on environmental remediation or on farm aquaculture interventions. Although adsorption-based methods have demonstrated high removal efficiencies in water treatment, their application in rural settings is often constrained by infrastructure requirements, operational complexity, and maintenance costs [10]. Furthermore, *in vivo* dietary supplementation methods cannot be applied to wild caught fish obtained through subsistence fishing.

Consequently, post-harvest decontamination strategies directly applied *ex vivo* to edible fish muscle have emerged as an alternative approach with greater potential for community level implementation [11]. Despite its importance, scientific evidence regarding direct post-capture fish muscle decontamination remains extremely scarce, as most existing literature focuses on water remediation or *in vivo* interventions. Among these, chelating agents such as ethylenediaminetetraacetic acid (EDTA) have attracted attention because of their ability to bind metal ions and facilitate their removal from biological tissues [12]. Their application has been investigated through immersion treatments and dietary supplementation in aquaculture systems.

Biotechnological approaches have also gained increasing interest as environmentally friendly alternatives for reducing metal contamination. Certain lactic acid bacteria, particularly *Lactobacillus fermentum*, (renamed *Limosilactobacillus fermentum*, Bacilli, Lactobacillales, Lactobacillaceae, Gram-positive bacterium), are capable of binding heavy metals through biosorption mechanisms involving cell wall components and extracellular metabolites, thereby reducing metal concentrations [13]. In addition, microbial fermentation systems combining bacteria and yeasts have shown the potential to decrease Pb and Cd concentrations while simultaneously improving growth performance, organoleptic characteristics, and nutritional quality in cultured fish [14].

To address this knowledge gap regarding the direct decontamination of processed muscle tissue, the present study evaluated and compared two post-harvest decontamination strategies applied to fillets of four fish species from the Ecuadorian Amazon: a chemical treatment based on EDTA and a biotechnological treatment using *Lactobacillus fermentum*. We hypothesized that both treatments would reduce Cd and Pb concentrations in fish muscle *ex vivo*, although their effectiveness would vary among metals and fish species. By assessing accessible post-harvest interventions, this study seeks to contribute to the development of practical strategies to reduce dietary exposure to heavy metals and improve food security in Amazonian communities.

2. Materials and Methods

2.1. Sampling

The capture of fish and handling of samples were conducted under permits issued by the Ministry of Environment, Water, and Ecological Transition (MAATE), MAATE-ARSFC-2024.0411. Fish species were collected using gillnets and hooks within the northern Ecuadorian Amazon. The four Amazonian fish species were selected based on four standardized criteria: (1) high regional relevance for local indigenous communities as primary sources of dietary protein and subsistence fishing; (2) representation of contrasting trophic guilds (ranging from low trophic detritivores/herbivores to high-trophic predators) and habitat preferences; (3) variations in tissue physiology, particularly muscle lipid content, which govern differential trace element bioaccumulation; and (4) consistent availability in local Amazonian fish markets during the sampling framework [15].

In addition, the four Amazonian fish species were selected to represent distinct trophic guilds, dietary habits, and habitat preferences across the water column: three species, *Pimelodus jivaro* (a benthopelagic omnivore), *Platynemateichthys notatus* (a demersal piscivorous catfish), both belonging to the family Pimelodidae and the order Siluriformes, and the red-eye piranha *Serrasalmus rhombeus* (a predatory/scavenger, serrasalmidae, Characiformes), were collected from the Aguarico River, while the tucunare *Cichla monoculus* (a high-trophic pelagic predator, Cichlidae, Cichliformes) was collected from the Zancudococha Lagoon. Because *C. monoculus* was harvested from a distinct aquatic system, sampling location and species identity are naturally confounded for this group; thus, baseline metal comparisons for these species reflect the combined influence of habitat location and species-specific traits. The Aguarico River represents a major lotic system characterized by high hydrodynamic energy, continuous sediment dynamics, and surrounding land-use pressure, whereas the Zancudococha Lagoon is a low-energy, black-water lacustrine ecosystem situated within a protected conservation area, recognized for its high dissolved organic matter content and its critical role as a breeding and spawning habitat for regional fish species [15].

For each species, five individual fish ($n = 5$) were collected as independent biological replicates. Immediately after capture, three matched tissue subsamples of homogeneous size and weight were obtained from the dorsal muscle region of each specimen using a pre-sterilized ceramic knife, dorsal muscle samples were dissected, and the skin and scales were completely removed to isolate clean muscle tissue prior to experimental procedures. These three matched portions originating from the same fish were randomly assigned to the three treatment groups: (a) untreated control, (b) EDTA chemical treatment, and (c) *Lactobacillus fermentum* biotechnological treatment (as indicated by the triplet sample codes in Supplementary File S1). This matched block experimental design allowed for a direct evaluation of decontamination efficiency within the same biological individual, thereby minimizing inter-individual biological variability. Fish tissue samples were initially preserved in 97% ethanol for transport to the laboratory. Upon arrival, the samples were thoroughly rinsed with ultrapure water to remove residual alcohol to minimize potential

effects of ethanol preservation, transferred into new 15 mL Falcon tubes, and stored at -80°C until post-harvest treatment processing.

2.2. Experimental Design and Post-Capture Treatments

Two potentially toxic trace elements were analyzed in the laboratory: Cd and Pb. Initially, samples were washed with Type 1 water to remove surface residues. Fish muscle tissue samples weigh between 0.26 g and 0.87 g (dry weight), and 0.28 and 0.95 (wet weight) depending on species size and muscle lipid content. They were then divided into three treatment groups: control (no intervention), chemical treatment, and biotechnological treatment.

The chemical treatment consisted of applying a 4% EDTA solution with a pH of 7.5 at a temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 30 min under oscillating agitation at 250 rpm [16].

The biotechnological treatment was carried out by using a food-grade strain of *Lactobacillus fermentum*, which was isolated and maintained in-house within our laboratory collection. For this procedure, 500 mL of liquid MRS (Man, Rogosa, and Sharpe) culture medium was prepared, inoculated with the bacterial strain, and incubated at 37°C for 72 h in a 5% CO_2 incubator to achieve a standard cell density. Fish tissue samples were added to 15 mL Falcon tubes containing 10 mL of the bacterial suspension at 1×10^6 CFU total inoculum per tube; tissue mass to treatment volume ratio of approximately (0.18–0.8 g/10 mL), and incubated for 24 h at 37°C . Following the 24 h immersion, the treatment solution was discarded [4].

In both protocols, treated samples were transferred to a new, sterile 15 mL Falcon tube and subjected to three sequential 5-min washing cycles with 10 mL of Type 1 ultrapure water under gentle agitation at 150 rpm to eliminate residual treatment solutions.

2.3. Determination of Heavy Metal Concentrations

Following the treatments, the samples were ultra-frozen at -80°C and subjected to freeze-drying in a Büchi Lyovapor L-200 (BÜCHI Labortechnik AG, Flawil, Switzerland) for 2 to 4 days, depending on the moisture percentage of each tissue, at 0.2 mbar and -56°C to completely eliminate water content and obtain a homogeneous material suitable for acid digestion.

Sample digestion was performed by placing 0.3 to 0.5 g of the sample into high-temperature and high-pressure resistant TFM Teflon tubes compatible with a MARS 6 microwave-assisted digestion system CEM Corporation in Matthews, NC, USA. To each tube containing the sample, 7 mL of trace metal grade nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2), were added, following Mascia's protocol for animal tissues [17], using certified materials: ERM-BB422 Fish Muscle and ERM-CE464 Tuna Fish [18,19]. The total digestion process lasted approximately 40 min. Once completed, the digests were filtered and diluted to a final volume of 25 mL with Type 1 water.

Then, 10 mL of the digest was transferred to a polyethylene tube for metal quantification via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using an ICP 7400 ICP-OES Duo spectrophotometer (Thermo Fisher Scientific, Cambridge, UK), which allows precise detection of concentrations even at trace levels. The recovery range was 98.5–103.8%, and it corresponds to the yttrium (Y) internal standard, monitored throughout each analytical run to assess instrumental drift and matrix-induced signal effects.

To express trace element concentrations on a tissue-mass basis, digestion-solution concentrations were converted to tissue concentrations using the following equation, where

Vdigestion is the fixed digestion volume (25 mL) and msample is the mass of freeze-dried tissue digested for each individual sample:

$$C_{\text{tissue}} (\text{mg/kg, dry weight}) = [CICP (\text{mg/L}) \times V_{\text{digestion}} (\text{L})] / m_{\text{sample}} (\text{kg})$$

Limits of detection (LOD) and quantification (LOQ) were calculated on a solution basis (mg/L) from $n = 10$ full-procedure blanks (deionized water carried through the complete acid-digestion protocol described above), following $LOD = \bar{x}_{\text{blank}} + 3 \times SD_{\text{blank}}$ and $LOQ = \bar{x}_{\text{blank}} + 10 \times SD_{\text{blank}}$. These ten blanks constituted the formal quality-control batch for this analytical run; additional digestion blanks were processed separately for instrument line-conditioning and carry-over control and were not included in the LOD/LOQ calculation.

2.4. Statistical Analysis

First, we make the data conversion from dry weight to wet weight was performed using the following formula:

$$\% \text{moisture} = ((\text{wet weight} - \text{dry weight}) / (\text{wet weight})) \times 100$$

The obtained moisture percentage values were multiplied by the concentration of each trace element. To determine the wet weight concentration value, the following formula was applied:

$$\text{Wet weight concentration} = \text{dry weight concentration} \times (1 - ((\% \text{ moisture}) / 100))$$

To calculate the percentage of reduction in each metal the following formulas were applied:

$$\text{Differences in concentrations} = (\text{Control concentration} - \text{Treatment concentration})$$

$$\% \text{ reduction} = (\text{Differences in concentrations} / \text{Control concentration}) \times 100$$

To analyze the effects of the treatments on the samples, statistical analyses were performed using generalized linear and mixed-effects models to accommodate the specific distributions of the trace element concentrations, which were expressed as bounded continuous proportions strictly within the (0, 1) interval. Thus, beta regression models were implemented. Specifically, mixed-effects beta regressions with a logit link function were fitted using the glmmTMB package [20,21], incorporating the sampled individual as a random intercept, and species and treatment as fixed factors. Statistical tests were executed using RStudio software version 4.4.3.

Even though 80% of Cd and 66.7% of Pb concentrations were above the LOD but below the LOQ (File S1), these were retained because they represent detectable analytical signals and contain information about the presence and relative magnitude of the analyte, albeit with greater quantitative uncertainty.

3. Results

3.1. Concentrations of Trace Elements Across Species and Treatments

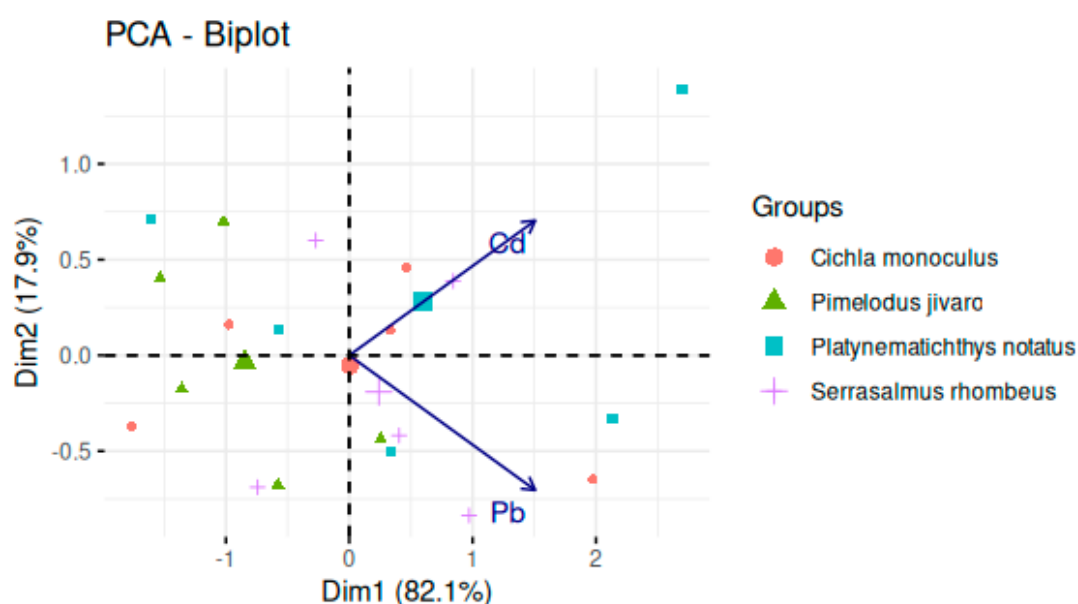
Mean control concentrations of Cd were highest in *Platynemachthys notatus* (0.078 mg/kg), whereas the highest control concentrations of Pb were observed in *Serrasalmus rhombeus* (1.228 mg/kg) (Table 1). Individual concentration values for all analyzed samples are included in File S1, together with LOD and LOQ.

Table 1. Concentration (mg/kg) means and standard deviations (SD) of four trace elements for control, EDTA and *Lactobacillus fermentum* treatments.

Species	Treatment	Cd		Pb	
		Mean	SD	Mean	SD
<i>Cichla monoculus</i>	Control	0.061	0.027	1.087	0.603
	EDTA	0.059	0.011	1.029	0.352
	<i>L. fermentum</i>	0.054	0.027	0.693	0.535
<i>Pimelodus jivaro</i>	Control	0.045	0.011	0.757	0.437
	EDTA	0.066	0.034	1.562	1.004
	<i>L. fermentum</i>	0.127	0.719	1.761	1.060
<i>Platynemathichthys notatus</i>	Control	0.078	0.039	1.185	0.705
	EDTA	0.039	0.030	1.407	1.499
	<i>L. fermentum</i>	0.052	0.0040	0.757	0.701
<i>Serrasalmus rhombeus</i>	Control	0.062	0.018	1.228	0.378
	EDTA	0.056	0.018	0.658	0.442
	<i>L. fermentum</i>	0.043	0.016	0.799	0.792

The PCA allowed for the identification of bioaccumulation patterns of the trace elements based on species prior to treatment application (Figure 1). Data clustered into two principal components, which explained 100% of the total data variability (Dim1: 82.1%; Dim2: 17.9%).

The first component (Dim1) was positively correlated with Cd and Pb concentrations and was associated with *Platynemathichthys notatus*, indicating a higher concentration of these metals in this species. On the other hand, *Pimelodus jivaro* was positioned in the second quadrant (negative Dim1 values), indicating lower metal concentrations, while *Cichla monoculus* was located near the origin (central zone), representing intermediate or lower trace element bioaccumulation. *Serrasalmus rhombeus* exhibited a broad dispersion along Dim2, with its centroid positioned near the origin. This suggests moderate overall bioaccumulation of Cd and Pb with high intraspecific variability among individuals compared to the other species studied.

**Figure 1.** PCA Biplot showing data variability of control samples.

3.2. Treatments' Effects

Figure 2 illustrates the distinct response patterns of trace element concentrations Cd and Pb in the four evaluated fish species under Control, EDTA, and *Lactobacillus fermentum* treatments.

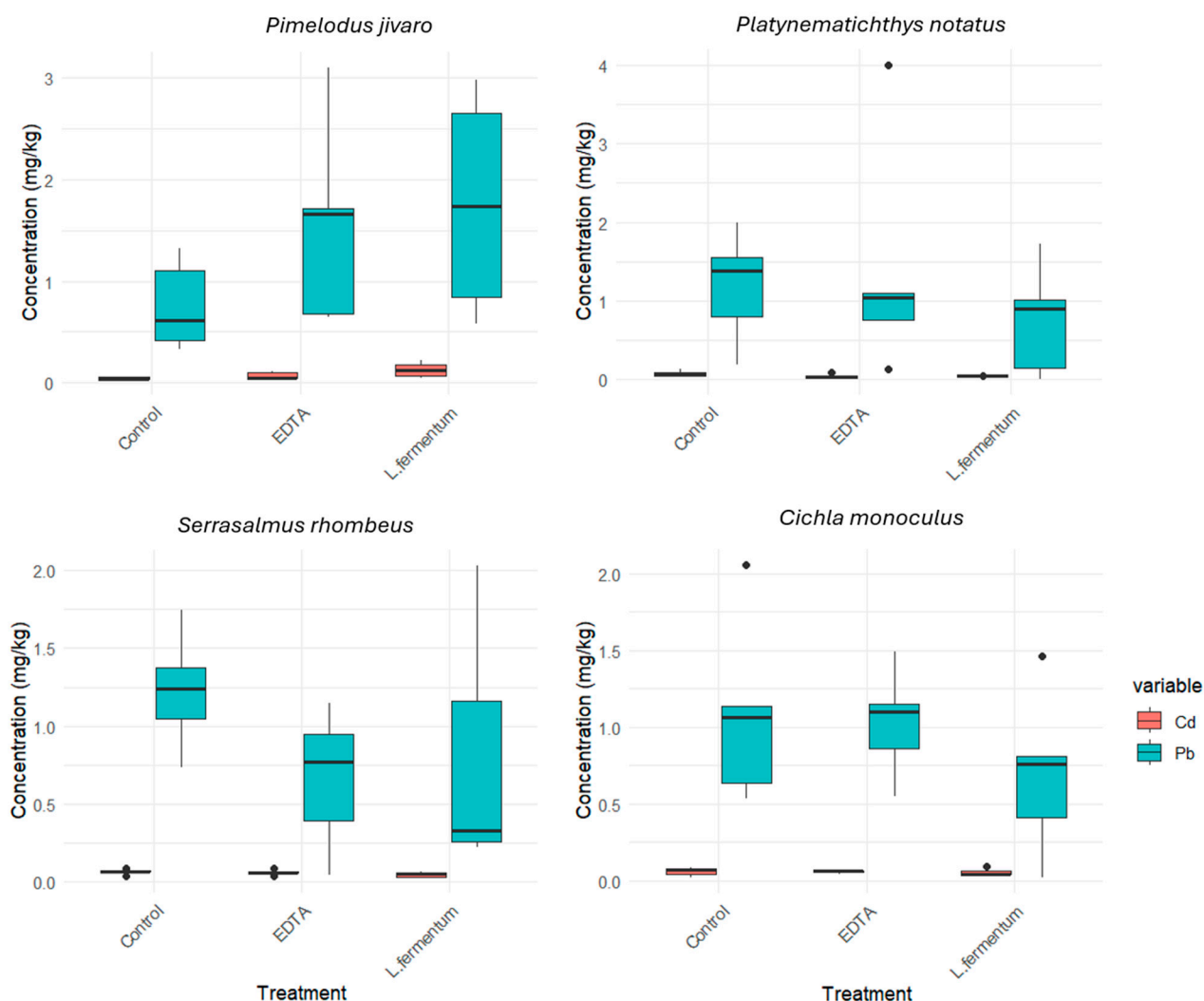


Figure 2. Boxplot chart shows metal variation based on species and treatments.

For *Pimelodus jivaro*, Cd concentrations remained consistently very low across all groups, whereas Pb concentrations showed a marked increase in median levels in both the EDTA and *L. fermentum* treatment groups compared to the Control. In *Cichla monoculus*, Cd levels remained low and stable across all treatments; Pb concentrations were highest in the Control and EDTA groups, while *L. fermentum* yielded the lowest median Pb concentration.

For *Serrasalmus rhombeus*, Cd concentrations were consistently low, whereas Pb levels were highest in the Control group and markedly decreased with both EDTA and *L. fermentum* treatments, although *L. fermentum* exhibited higher overall variability. Finally, for *Platynemachthys notatus*, Cd concentrations remained low and stable, whereas Pb levels were highest in the Control group and were lower in both the EDTA and *L. fermentum* treatment groups, with *L. fermentum* showing a wider range of variation.

To evaluate the variation patterns of trace element concentrations across treatments, a generalized linear mixed-effects model (GLMM) and log-link function was applied. Individual fish identity (ID) was included as a random intercept to account for repeated

measurements with a variance of 6.528×10^{-10} , a SD of 2.55×10^{-5} , and a dispersion parameter of 0.189 (Table 2).

Table 2. Results from the linear mixed-effects model for Cd Conditional model:

Estimate	Estimate	Std. Error	z Value	Pr(> z)	
(Intercept)	−2.7955	0.19444	−14.377	$<2 \times 10^{-16}$	***
Species <i>Pimelodus jivaro</i>	−0.3008	0.27497	−1.094	0.27399	
Species <i>Platynemichthys notatus</i>	0.25338	0.27497	0.921	0.35680	
Species <i>Serrasalmus rhombeus</i>	0.02973	0.27497	0.108	0.91391	
Treatment EDTA	−0.01869	0.27497	−0.068	0.94581	
Treatment <i>L. fermentum</i>	−0.1172	0.27497	−0.426	0.66995	
Species <i>Pimelodus jivaro</i> : Treatment EDTA	0.40454	0.38887	1.040	0.29820	
Species <i>Platynemichthys notatus</i> : Treatment EDTA	−0.67022	0.38887	−1.724	0.08480	
Species <i>Serrasalmus rhombeus</i> : Treatment EDTA	−0.10174	0.38887	−0.262	0.79361	
Species <i>Pimelodus jivaro</i> : Treatment <i>L. fermentum</i>	1.15175	0.38887	2.962	0.00306	**
Species <i>Platynemichthys notatus</i> : Treatment <i>L. fermentum</i>	−0.29792	0.38887	−0.766	0.44362	
Species <i>Serrasalmus rhombeus</i> : Treatment <i>L. fermentum</i>	−0.25295	0.38887	−0.650	0.51539	

** and *** indicate highly significant and very highly significant results respectively.

For Pb, the model scored a variance of 2.711×10^{-10} , a SD of 1.647×10^{-5} , and a dispersion parameter of 0.61 (Table 3).

Table 3. Results from the linear mixed-effects model for Pb.

Conditional Model:				
Estimate	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.08343	0.34934	0.239	0.8112
Species <i>Pimelodus jivaro</i>	−0.36086	0.49404	−0.730	0.4651
Species <i>Platynemichthys notatus</i>	0.08660	0.49404	0.175	0.8609
Species <i>Serrasalmus rhombeus</i>	0.12201	0.49404	0.247	0.8049
Treatment EDTA	−0.05446	0.49404	−0.110	0.9122
Treatment <i>L. fermentum</i>	−0.45009	0.49404	−0.911	0.3623
Species <i>Pimelodus jivaro</i> : Treatment EDTA	0.77796	0.69868	1.113	0.2655
Species <i>Platynemichthys notatus</i> : Treatment EDTA	0.22586	0.69868	0.323	0.7465
Species <i>Serrasalmus rhombeus</i> : Treatment EDTA	−0.56936	0.69868	−0.815	0.4151
Species <i>Pimelodus jivaro</i> : Treatment <i>L. fermentum</i>	129.362	0.69868	1.851	0.0641
Species <i>Platynemichthys notatus</i> : Treatment <i>L. fermentum</i>	0.00165	0.69868	0.002	0.9981
Species <i>Serrasalmus rhombeus</i> : Treatment <i>L. fermentum</i>	0.01991	0.69868	0.028	0.9773

Variations in trace element concentrations of Cd were dependent on specific species and treatment combinations. According to the generalized linear mixed-effects model (GLMM), neither main species effects nor main treatment effects differed significantly from

the baseline reference (*Cichla monoculus* under control conditions; $p > 0.27$). However, a statistically significant interaction was detected between *Pimelodus jivaro* and *Lactobacillus fermentum* ($p = 0.0031$), suggesting a distinct species-specific response to the probiotic treatment. Additionally, a marginally significant interaction trend was observed for *Platynemachthys notatus* exposed to EDTA ($p = 0.0848$). Nonetheless, these results should be considered exploratory and not conclusive. In contrast, interaction effects for *Serrasalmus rhombeus* across both treatments remained non-significant ($p > 0.51$), indicating high response stability or intra-treatment variability. Our Cd model showed potential specific species–treatment interactions rather than uniform main effects. In the case of Pb, our exploratory models showed a marginally significant interaction between *Pimelodus jivaro* and *Lactobacillus fermentum* ($p = 0.0641$). In contrast, interaction effects for *Platynemachthys notatus* and *Serrasalmus rhombeus* across both treatments remained non-significant ($p > 0.41$), suggesting high response stability or intra-treatment variability.

EDTA and *L. fermentum* treatments resulted in measured concentration increases for Cd in *Pimelodus jivaro*, as indicated by negative reduction percentages (Table 4). For this trace element, EDTA and *L. fermentum* treatments resulted in measured concentration increases in *Pimelodus jivaro* and *Platynemachthys notatus*, as indicated by negative reduction percentages. Nevertheless, the highest percentage of trace Cd reduction for EDTA was observed in *Platynemachthys notatus* (49.78%), followed by *Serrasalmus rhombeus* (11.34%). With the treatment with *Lactobacillus fermentum* the highest trace of Cd reduction was also in *Platynemachthys notatus* (33.97%), followed by *Serrasalmus rhombeus* (30.93%). In the case of Pb, the highest reductions were observed in *Cichla monoculus* (36.25%) and *Platynemachthys notatus* (36.13%) for the treatment with *Lactobacillus fermentum*. In contrast, for the EDTA treatment, the highest Pb reduction was observed in *Cichla monoculus* and *Platynemachthys notatus* (36%) both (Table 4).

Table 4. Percentages of trace element reduction by treatment for the analyzed species.

Trace Element	Treatment	<i>Cichla monoculus</i>	<i>Pimelodus jivaro</i>	<i>Platynemachthys notatus</i>	<i>Serrasalmus rhombeus</i>
Cd	EDTA	1.85	−47.08	49.78	11.34
	<i>L. fermentum</i>	11.06	−181.38	33.97	30.93
Pb	EDTA	5.30	−106.16	−18.69	46.41
	<i>L. fermentum</i>	36.25	−132.45	36.13	34.96

In general, *Lactobacillus fermentum* displayed lower percentage variations for Cd and Pb compared to EDTA, with the widest negative variations observed under the bacterial treatment, extending below −150% for Cd (Figure 3). Conversely, EDTA exhibited positive reduction variations for Cd reaching up to 46.41% while displaying a wider range of negative values for Pb.

At the species level, the most prominent negative percentage variations for Cd and Pb were recorded in *Pimelodus jivaro* under *Lactobacillus fermentum* (Figure 3). In contrast, *Cichla monoculus*, *Platynemachthys notatus*, and *Serrasalmus rhombeus* consistently exhibited positive reduction percentage variations under *Lactobacillus fermentum*. Lastly, *Platynemachthys notatus* displayed positive reduction values under *L. fermentum*, but exhibited a wider range of variation extending into negative values when exposed to EDTA.

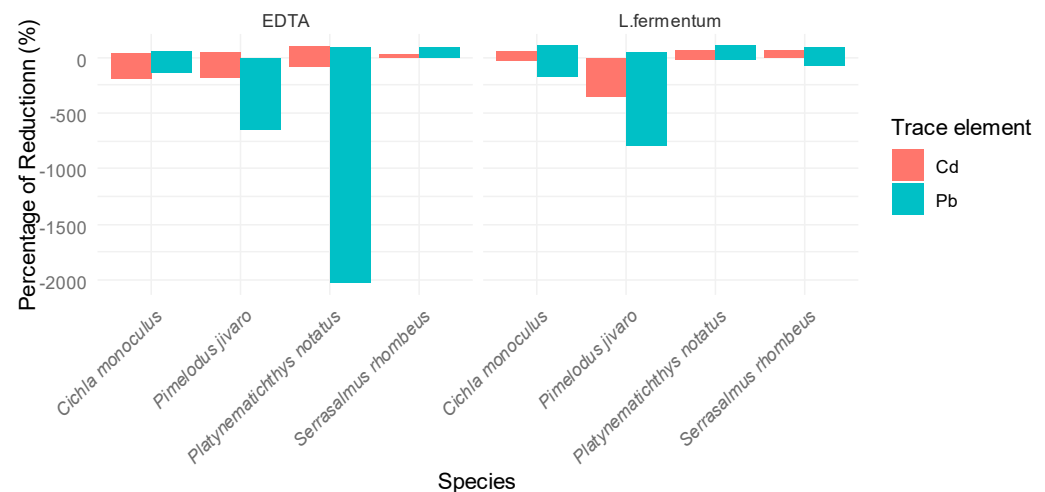


Figure 3. Barplots depicting the reduction percentages of trace element concentrations by species treatment and trace element.

4. Discussion

4.1. Bioaccumulation Patterns

Differences in potentially toxic trace element concentrations among the four fish species suggest a potential influence of ecological and physiological traits on metal bioaccumulation. The ICP-OES analyses confirmed the presence of Cd and Pb in the muscle tissue of all species, although their concentrations varied considerably. Such variability is commonly attributed to multiple interacting factors, including metabolic activity, feeding behavior, habitat use, and exposure history. For instance, fish with higher metabolic rates or greater feeding frequency may be exposed to larger quantities of contaminants over time, theoretically increasing metal accumulation likelihood [22].

Species-specific ecological characteristics offer plausible working hypotheses for the observed baseline patterns. Benthic species such as *Pimelodus jivaro* maintain close contact with contaminated sediments, a primary reservoir for Cd and Pb [23]. Prolonged sediment contact and physiological differences in metal uptake may contribute to higher accumulation in benthic groups [24]. Although biological parameters such as fish length, weight, sex, and maturity stage were recorded and are provided in the Supplementary Material File S1, specific biochemical metal-binding targets (sulfhydryl groups or metallothionein-like proteins) and muscle proximate composition were not evaluated. Thus, while individual variations are documented, the specific biochemical mechanisms underlying metal retention require future validation.

In our exploratory models, distinct species-dependent response trends were observed. In *Cichla monoculus*, *Platynemachthys notatus*, and *Serrasalmus rhombeus*, *Lactobacillus fermentum* consistently achieved positive percentage reductions for both Cd and Pb concentrations, with *Platynemachthys notatus* reaching up to 33.97% for Cd and 36.13% for Pb. Under EDTA treatment, the highest positive Cd reduction was recorded in *Platynemachthys notatus* (49.78%), whereas the highest positive Pb reduction occurred in *Serrasalmus rhombeus* (46.41%). Conversely, *Pimelodus jivaro* displayed negative percentage variations for both metals under both treatments, reflecting higher measured concentrations in the remaining tissue matrix alongside high intra-specific variability. These results suggest that treatment efficiency may depend not only on metal physicochemical properties but also on species-specific tissue characteristics that influence metal binding, retention, and release [12,13,23], a hypothesis that warrants dedicated tissue-level investigations.

Of the four species, *Cichla monoculus* consistently exhibited the lowest baseline metal concentrations. Unlike the other sampling sites, this species was captured in Zancudococha,

an area located further away from main mining and oil extraction activities. Although water quality analyses were conducted at the sampling sites, environmental parameters were not integrated into the present study, as the primary objective was to evaluate post-capture metal reduction in fish muscle tissue. Nevertheless, these observations suggest that local contamination levels might contribute to the lower baseline metal burden observed in this species. However, because sampling site and species identity are confounded by *Cichla monoculus*, it remains uncertain whether these lower metal concentrations are driven primarily by species-specific biological factors or by lower environmental contamination in the lagoon. Overall, these findings suggest that post-capture treatment efficiency may be influenced by species-specific biological characteristics as well as environmental factors associated with fish origin.

4.2. Effectiveness of the Treatments

The contrasting responses observed between EDTA and *Lactobacillus fermentum* indicate that the effectiveness of post-harvest decontamination may depend on both the physicochemical properties of individual metals and the biological characteristics of the treated fish. Although neither treatment produced significant reductions across all species and metals, consistent trends suggest that each approach operates through different mechanisms, resulting in distinct removal efficiencies.

EDTA acts as a chelating agent, forming stable, water-soluble complexes with divalent and trivalent metal cations through its carboxyl and amine functional groups. This mechanism facilitates the mobilization of metals from biological tissues into the surrounding solution and has been widely exploited in soil and environmental remediation [23]. In the present study, EDTA was more effective at reducing Pb concentrations in certain species, supporting its capacity for metal removal. However, EDTA binding efficacy in biological tissues is strongly governed by conditional stability constants, local pH, competing endogenous cations (Ca^{2+} , Mg^{2+}), metal oxidation state, and the strength of metal-protein matrix binding [25].

Furthermore, increases in Cd concentrations were observed in certain treated samples. Rather than indicating metal concentrations increased, which was not directly assessed in this study, this pattern represents a higher measured concentration in the remaining tissue matrix. Potential explanations include tissue matrix redistribution, relative dry matter loss during processing, or incomplete removal of metal EDTA complexes. However, because metal levels were evaluated exclusively in the fish tissue, a complete mass-balance analysis (accounting for metal contents in untreated tissue, treated tissue, exposure solutions, and post-treatment rinsing fractions) was not performed. Consequently, the precise fate of metals and the exact mechanisms underlying these concentration increases remain speculative and warrant future mass-balance investigations. Similar limitations have been described in previous studies, where EDTA efficacy depended heavily on treatment conditions, including pH, exposure time, temperature, and subsequent washing or cooking procedures [12,16]. Thus, while EDTA represents a promising decontamination strategy, its application requires careful optimization to avoid unintentional increases in measured metal levels within fish tissues [26].

Conversely, *L. fermentum* generally reduced targeted trace element concentrations across most species, although this trend reflects an estimate since concentrations fell between the LOD and LOQ; additionally, localized increases were observed, particularly in *Pimelodus jivaro*. Species belonging to the genus *Lactobacillus* are known to remove heavy metals through biosorption processes, in which metal ions bind to negatively charged functional groups on the bacterial cell wall, including carboxyl, phosphate, hydroxyl, and sulfhydryl groups [4]. Live bacterial cells may also contribute to metal immobilization

through active metabolic processes, facilitating metal sequestration [27]. While these mechanisms provide plausible explanations for the reductions observed following probiotic treatment, the experimental design of the present study did not differentiate among passive cell-wall biosorption, active intracellular uptake, extracellular precipitation, acid-mediated tissue leaching, or binding to metabolites present in the MRS medium. Because metal levels were evaluated exclusively in the fish tissue without analyzing the treatment supernatants or bacterial pellets, and without non-inoculated, pH-adjusted MRS controls the precise relative contribution of bacterial sequestration versus non-specific tissue extraction remains to be determined in future mechanistic studies.

The ability of different *Lactobacillus* species, including *L. fermentum*, *L. plantarum*, *L. rhamnosus*, and *L. casei*, to bind Cd, Pb, Zn, and Cu has been widely demonstrated under experimental conditions [28]. Heavy metal biosorption by bacterial cell walls relies primarily on interactions with carboxyl and phosphate groups present in cell-wall proteins, peptidoglycan, and extracellular polymeric substances (EPS), which act as key binding sites for metal ions under standardized treatment conditions [29].

In addition to their metal binding capacity, probiotic bacteria have been reported to improve intestinal barrier function, modulate oxidative stress, and enhance host health in fish species such as *Oncorhynchus mykiss* and *Oreochromis niloticus* [11,12]. Although these physiological benefits were not assessed in the present study, they highlight the broader potential of probiotic-based approaches as multifunctional tools for reducing contaminant exposure while supporting fish health. The relatively consistent metal reductions observed with *Lactobacillus fermentum*, particularly in *Platynemichthys notatus* and *Pimelodus jivaro*, suggest that probiotic treatments merit further investigation. Nevertheless, because specific metal-binding kinetics and biomass retention were not directly quantified, proposed removal pathways remain hypothetical and warrant specific mechanistic validation.

4.3. Implications for Food Security

The practical application of post-harvest decontamination strategies should be evaluated not only in terms of their ability to alter metal concentrations but also considering their feasibility, safety, and potential implementation in rural communities. Although EDTA showed higher positive reductions for specific metals in certain species (such as Cd in *Platynemichthys notatus*), its performance was inconsistent across species and, in some cases, was associated with higher measured concentrations after treatment [23]. Similarly, while *Lactobacillus fermentum* achieved positive reductions in species such as *Cichla monoculus*, *Platynemichthys notatus*, and *Serrasalmus rhombeus*, it also exhibited substantial negative variations in *Pimelodus jivaro*, highlighting that species-specific tissue characteristics require careful consideration for both treatments [14].

The four fish species analyzed are commonly consumed by Indigenous and rural communities in the Ecuadorian Amazon, where fishing remains an essential source of dietary protein and food security [9,30]. In these contexts, post-harvest interventions must be not only effective, but also economically affordable, technically simple, and compatible with local food preparation practices. From this perspective, probiotic-based treatments represent an attractive approach, as they require relatively simple processing conditions and could be integrated into fish handling practices at the community scale.

Despite the metal variations observed after treatment, the implications for food safety varied depending on the element and its initial contamination levels in the tissue. Cd concentrations in untreated fish were close to or slightly above international regulatory thresholds established for edible fish muscle (0.05 mg/kg wet weight, Codex Alimentarius/EU Regulation 2023/915). Following treatment, positive Cd reductions reached up to 33.97% with *Lactobacillus fermentum* and 49.78% with EDTA, whereas *Pimelodus jivaro* displayed

fluctuating efficacy and unexpected concentration increases under both treatments [31]. Regarding Pb, baseline concentrations in several analyzed species exceeded the maximum permissible limit for fresh fish muscle (0.3 mg/kg wet weight, Codex/EU), representing a potential dietary hazard for local consumers [6]. Both treatments achieved positive Pb reductions in *Cichla monoculus*, *Platynemichthys notatus*, and *Serrasalmus rhombeus*, reaching up to 46.41% with EDTA. However, these results should be interpreted with caution, considering the limitations with the quantification of the trace elements in our study. These species-specific differences suggest that tissue structure, membrane permeability, and baseline metal distribution strongly influence binding capacity and release kinetics during washing protocols. Overall, these findings demonstrate that post-harvest biotechnological interventions can contribute to mitigating dietary exposure to heavy metals such as Cd and Pb.

However, such treatments should be integrated into broader, multi-barrier risk-mitigation frameworks rather than relied upon as stand-alone solutions for contaminated fisheries. Ultimately, safeguarding public health and preserving aquatic resources will necessitate comprehensive management strategies aimed at directly controlling point and non-point pollution sources across Amazonian watersheds.

4.4. Limitations of the Study and Recommendations

The present study has several limitations that should be considered when interpreting the results. First, although the experimental design allowed comparison of two post-harvest decontamination strategies, the sample size and number of biological replicates ($n = 60$) may have limited the statistical power to detect subtle treatment effects, particularly given the natural variability in metal accumulation among fish species. In addition, only four species commonly consumed in the Ecuadorian Amazon were evaluated. Because fish differ markedly in trophic position, feeding ecology, habitat use, and tissue composition, the effectiveness of both treatments may not be directly transferable to other species exploited by Amazonian communities [7,9].

Relative standard deviations (RSDs) for several metal–species–treatment combinations exceeded 50%, and in some cases 100%. This is consistent with the expected analytical behavior of trace-element quantification near the LOQ, where %RSD increases as concentration decreases [32,33]. Residual variability not attributable to proximity to the LOQ may additionally reflect genuine inter-individual physiological variability (e.g., lipid content, body size) among wild-caught specimens, which the matched-block design used here minimizes but cannot eliminate. On the other hand, fish tissue samples were initially preserved in 97% ethanol prior to laboratory processing. No parallel fresh-tissue comparison was performed in this study, and we identify this as a limitation and priority for future validation work.

Another limitation is that only a single treatment protocol was evaluated for each decontamination strategy. Treatment performance is likely to depend on variables such as EDTA concentration, exposure time, pH, temperature, bacterial cell density, and fermentation conditions. Further differences in treatment performance could arise from the specific specimen location. For instance, *Cichla monoculus* was collected in the Zancudococha Lagoon, far from the potential sources of pollution.

Likewise, the study quantified total metal concentrations Cd and Pb but did not assess specific chemical speciation or binding states in tissue. Furthermore, metal content associated with the bacterial biomass and treatment supernatants was not directly measured, preventing a definitive resolution of the precise mechanism (surface biosorption via cell-wall functional groups vs. extracellular chelating mechanisms) underlying the ob-

served reductions in Cd and Pb [34]. These factors should be considered when interpreting treatment efficiency and assessing potential reductions in human health risk.

Future studies should therefore expand the number of fish species and sampling locations while leveraging biometric parameters (length, body weight, sex, and maturity stage), which were recorded in this study and are available in Supplementary Material File S1, alongside additional ecological variables such as trophic position, habitat, diet, and age to better explain interspecific differences in bioaccumulation. Evaluating metal distribution among different organs would also improve our understanding of species-specific retention patterns and the mechanisms underlying treatment responses. In addition, comparative studies involving different *Lactobacillus* strains, as well as optimization of EDTA concentration, exposure time, and treatment conditions, would help identify protocols with greater decontamination efficiency and reproducibility.

While environmental water parameters were analyzed but not included in this study, future research should integrate fish-based studies with comprehensive environmental monitoring of water and sediments to establish clearer links between contamination sources and trace element accumulation in aquatic food webs. From an applied perspective, the feasibility of implementing these post-harvest treatments in Amazonian communities should be assessed. Because *Lactobacillus fermentum* is a food-grade microorganism, probiotic-based decontamination offers a promising, low-cost strategy that could be adapted into simple, household- or artisanal-scale immersion procedures prior to fish preparation. However, community-level translation requires optimizing operational parameters (such as soaking duration and microbial biomass availability) and establishing participatory training programs to ensure safe handling, proper hygiene, and correct application.

Additionally, safe management and disposal of treatment residues must be developed to prevent secondary environmental contamination. Because these interventions are ultimately intended to improve food safety, future investigations should evaluate their effects on sensory quality, nutritional composition, and consumer acceptance, thereby providing a comprehensive framework for practical community adoption [8,11].

Finally, the relatively high proportion of measurements below the LOQ, particularly for Cd and Pb, represents an important analytical limitation. Concentrations in the LOD–LOQ range should therefore be interpreted as estimated rather than fully quantitative values, and the associated high variability should be considered when interpreting treatment effects. However, even though interval-censored results represent detectable analyte concentrations whose numerical precision is insufficient for full quantification, excluding these observations would discard information on the lower end of the concentration distribution and may bias analyses toward higher concentrations [35].

5. Conclusions

This study suggests that the effectiveness of post-harvest decontamination strategies for managing toxic metal levels in Amazonian fish might strongly depend on both fish species and metal type. Although statistically significant differences were not detected across all main treatments, relevant reductions in Cd and Pb were observed in specific species–treatment combinations; however, as measurements frequently fell between the LOD and LOQ, these patterns should be considered exploratory and not interpreted as conclusive, despite highlighting distinct species-dependent responses. Among the evaluated approaches, *Lactobacillus fermentum* achieved notable reduction variations across *Cichla monoculus*, *Platynemichthys notatus*, and *Serrasalmus rhombeus*, while EDTA displayed the highest single Cd reduction in *P. notatus*. These findings highlight the potential of biotechnological and chelating interventions to help mitigate dietary exposure to heavy metals in fish consumed by Amazonian communities. Further research is needed to

optimize treatment conditions, clarify matrix-binding mechanisms, evaluate practical applicability in community settings, and integrate these post-harvest interventions within broader efforts to reduce environmental contamination at its source.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11090528/s1>, File S1. Concentrations of Cd and Pb in 60 samples for control, EDTA and *Lactobacillus fermentum* treatments of four species of the subsistence fisheries of the Ecuadorian Amazon, LODs, LOQs and biological data of the analyzed specimens.

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Abbreviations

The following abbreviations are used in this manuscript:

EDTA	Ethylenediaminetetraacetic acid
HNO ₃	Nitric acid
H ₂ O ₂	Hydrogen peroxide
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
LD	Detection limit
LD/2	Half detection limit
PCA	Principal Component Analysis
w. w	Wet weight

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