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Bioaccumulation Study of Cadmium and Lead in *Cyprinus carpio* from the Colorado River, Using Automated Electrochemical Detection

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Abstract: The monitoring of heavy metals in aquatic ecosystems is of critical importance due to the toxic effects that these elements can have on wildlife and the potential risks that they pose to human health. Rivers situated in close proximity to agricultural regions are particularly susceptible to contamination from a combination of natural and anthropogenic sources. The study of bioaccumulation is of great importance for the early detection of environmental stressors. The combination of electrochemical techniques, such as square-wave anodic stripping voltammetry (SWASV), with automated flow-batch systems represents an efficient and cost-effective approach for the detection of trace metals in environmental samples. This study examines the bioaccumulation of cadmium and lead in *Cyprinus carpio*, a bioindicator of contamination in the Colorado River, Argentina. The fish were exposed to sublethal metal concentrations for 24, 48, and 96 h. Metal quantification was conducted using a novel automatic flow-batch system with SWASV and a bismuth film electrode. To the best of our knowledge, this constitutes the first application of this methodology on aquatic bioindicators for the assessment of metal accumulation in a natural environment. The technique demonstrated enhanced sensitivity and selectivity for the detection of trace metals. The bioaccumulation results demonstrated an increase in cadmium and lead concentrations in fish liver tissue after 96 h, reaching 10.5 μg g^{-1} and 11.9 μg g^{-1}, respectively. Validation with inductively coupled plasma–atomic emission spectrometry (ICP-AES) demonstrated a satisfactory correlation, confirming the reliability of the method. This novel electrochemical approach offers enhanced accuracy and efficiency, making it a promising tool for environmental monitoring. The results indicate that Colorado River water is within safe levels for aquatic life regarding these metals. However, continuous monitoring is recommended to detect changes in contamination levels and protect ecosystem health, especially during water crises and under climate change.

Keywords: environmental toxicology; heavy metal contamination; electrochemical analysis; aquatic bioindicators; aquatic pollution indicators; bismuth film electrode

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1. Introduction

Environmental pollution represents a substantial global challenge, mainly in the context of water contamination. This phenomenon has been identified as a crucial driver of deterioration in aquatic ecosystems, with adverse implications for water quality and biota [1–3]. Freshwaters are susceptible to contamination as a consequence of human activity. The introduction of waste and industrial waters, which are not always adequately subject to purification treatments, into discharge systems with a final destination of rivers and seas results in the deterioration of ecosystems [4]. The contamination of the environment can result in the poisoning, disease, and even death of fish, representing a significant environmental concern due to the prolonged presence of these pollutants and their high toxicity, even at low levels [5]. The presence of heavy metals in water can have severe and far-reaching consequences for the ecological balance of the environment, with the potential for biomagnification up the food chain. This phenomenon amplifies their impact, not only within the ecosystem and its wildlife but also on individuals who consume contaminated organisms. Consequently, this results in significant adverse effects on human health, whether through direct exposure or bioaccumulation [6–8].

The importance of bioindicators in biovigilance programs has grown significantly in recent years. They constitute a valuable, specific tool for the assessment of the physiological state of and stress in natural systems, thereby promoting an enhanced understanding of the mechanisms by which organisms respond to environmental changes [9–12]. Such methods provide information on the exposure and effects of substances, as well as on interactive processes occurring under natural conditions. As the apex of the food chain, fish are susceptible to direct and indirect pollution effects, rendering them a suitable bioindicator.

The common carp (*Cyprinus carpio*) is a well-documented fish species that has been observed in a variety of aquatic habitats over an extended period of time. Originally from Eurasia, the common carp was introduced to Argentina and was first recorded in Buenos Aires province, where it now coexists with local ichthyofauna in various aquatic settings, such as rivers and lakes [13]. Its adaptability has allowed it to establish itself in the Colorado River ecosystem, where its reproductive rate and feeding habits raise concerns regarding its status as an invasive species.

The Colorado River is a critical water resource for agriculture, livestock, and human consumption in the region. Its ecological importance lies in its role as a habitat for a diverse range of species and its contribution to the regional hydrological balance. However, the river faces increasing vulnerability to anthropogenic impacts, including pollution, land use changes, and water extraction for irrigation. In recent years, prolonged droughts and reduced flow rates have heightened the risk of salinization and the accumulation of pollutants, further endangering the ecosystem.

Due to its easy identification and capture, as well as the extensive knowledge of its life cycle, the carp serves as an ideal sentinel bioindicator in environmental studies. Consequently, it is of interest in studies on bioaccumulative metals of environmental and toxicological significance, such as cadmium (Cd) and lead (Pb) [14–17]. These metals are highly toxic heavy metals that accumulate in various tissues, including the liver, kidneys, and gills of fish [18]. Cadmium, recognized as a carcinogen, ranks among the most hazardous substances according to the United States Agency for Toxic Substances and Disease Registry (ATSDR), and it frequently contaminates aquatic environments. Studies have shown that Cd impairs essential functions in fish, such as hematological indices, iron metabolism, and antioxidant enzyme activity, while disrupting their reproductive capacities. Similarly, lead accumulates across multiple organs, where it impairs enzymatic activities, cellular structures, and physiological functions, adversely affecting growth, reproduction, and feeding efficiency. The 48 h median lethal concentration (LC_{50}/48 h) for

the combined effect of these metals is 50 μg mL^{-1} [19,20]. Given these harmful effects, the bioaccumulation study of Cd and Pb in *Cyprinus carpio* presents valuable insights into contamination dynamics and early warnings for ecosystem health, especially in the Colorado River watershed.

Cadmium and lead determination in a variety of matrices is typically conducted using techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), or atomic fluorescence spectroscopy (AFS). These techniques necessitate the use of sophisticated instrumentation, entail the consumption of considerable quantities of reagents, and are typically conducted by an experienced analyst. In certain instances, the extraction procedures are lengthy and complex [21–24]. Conversely, electrochemical techniques, particularly those based on pulsed voltammetry and redissolution, are widely employed for the quantification of metals in samples of environmental interest. This is due to their high sensitivity, selectivity, rapidity of measurement and relatively inexpensive instrumentation. With regard to the indicator electrodes employed in these techniques, bismuth film electrodes (BiFEs) are widely utilized, given that they offer a number of advantages, including undistorted signals (well-defined peaks) and a highly reproducible, close peak resolution and a wide linear range [25,26]. Bismuth has the potential to form multicomponent alloys with the metals under investigation, which enables their accurate detection. Such alloys can be deposited on a variety of supports, including vitreous carbon electrodes, carbon paste, graphite, pencil leads, and screen-printed electrodes. The generation of the bismuth film on different supports can be achieved through the "in situ" modality, which allows for the simultaneous generation of the bismuth film and the presence of the metals under study [27,28]. Pierini et al. employed these systems for cadmium and lead determination in samples of bee products originating from the province of Buenos Aires, Argentina. The automation of electrochemical systems has the potential to enhance the efficiency and precision of analytical processes, facilitating a notable increase in the frequency, accuracy, and reliability of analyses. These systems permit the handling of unstable and toxic substances and facilitate continuous monitoring in any process, thereby reducing costs for large volumes of analysis and the control of equipment with minimal operator intervention. In this regard, Eggly et al. put forth a prototype comprising a potentiostat with an automated flow-batch system, which incorporates a voltammetric cell with a BiFE for the analysis of propolis samples [29]. The combination of flow-batch systems with electrochemical detection makes them an attractive option for routine environmental analysis laboratories.

In this work, the bioaccumulation of cadmium and lead in the livers of *Cyprinus carpio* specimens captured in the Colorado River (Argentina) was studied. These organisms are used as bioindicators of contamination. To assess the effectiveness of this approach, specimens of fish were subjected to controlled exposure to sublethal concentrations of the aforementioned metals for a period of 24, 48 and 96 h. The study was based on the hypothesis that this species exhibits a quantifiable and proportional accumulation of these metals in liver tissue, which could be effectively quantified by a novel electrochemical methodology. To verify this hypothesis, an automated flow-batch system was designed and developed using square-wave anodic stripping voltammetry with a bismuth film electrode as the analytical method. The results are intended to contribute to the development of sensitive tools for monitoring heavy metal contamination in freshwater ecosystems.

2. Materials and Methods

2.1. Chemicals and Reagents

All reagents were of analytical grade, and solutions were prepared employing ultrapure water (18 MΩ). The Bi (III) standard stock solution was prepared through the

dissolution of 0.0534 g of $Bi(NO_3)_3 \cdot 5\ H_2O$ (99.999%, Sigma-Aldrich, Buenos Aires, Argentina) in 5 mL of 20% ($v\ v^{-1}$) nitric acid, which was then brought up to 25.0 mL with water. Pb (II) and Cd (II) working solutions were obtained by diluting the corresponding standard solution (1000 mg L^{-1}, Merck, Buenos Aires, Argentina). The supporting electrolyte was 0.1 M acetate buffer (Cicarelli, Santa Fé, Argentina) with a pH of 4.50. Tris-HCl buffer with a concentration of 50 mM and a pH of 8.10 (Fluka, Buchs, Switzerland) was employed.

2.2. Study Area

The Colorado River basin is situated in the northern semi-arid region of Argentina's Patagonia. The river extends from its source in the Cordillera de los Andes to its point of discharge into the Atlantic Ocean, covering a total area of 47,459 km^2. The mean annual flow of the basin is 143.5 $m^3\ s^{-1}$, which is largely generated by snowmelt in the Cordillera de los Andes. This flows downstream through the basin's two major tributaries, the Rio Grande and the Barrancas. The Colorado River basin is characterized by the presence of three distinct climatic–terrestrial regions, which are distinguished by variations in climate, land cover, and land use. The aforementioned zones can be further subdivided as follows: upper, middle, and lower. The upper zone, situated to the northwest, encompasses a portion of the Cordillera de los Andes and plays a significant role in precipitation (mainly through snowfall) and runoff (mainly through snowmelt) within the basin. The middle region extends in a southeasterly direction to the Casa de Piedra Dam. The dam was constructed in 1990 with the objective of supplying water to local populations, generating hydroelectric energy, mitigating the impact of floods, and regulating the flow of water within the basin. It is the only basin to have a flow distribution agreement in place. The lower area, commonly designated the Valle Bonaerense del Río Colorado (VBRC), has been primarily developed as a significant irrigation zone for agricultural production. The irrigation water concession system is monitored and regulated by the Corporación de Fomento del Valle Bonaerense del Río Colorado (CORFO).

Since August 2015, a collaboration and assistance agreement has been in place between four institutions (Universidad Nacional del Sur (UNS), Instituto Nacional de Tecnología Agropecuaria (INTA Hilario Ascasubi), Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC), and CORFO) with the objective of promoting the development of research and carrying out studies for the preservation of the VBRC's water resources. The aforementioned agreement designated the Paso Alsina station (39°22′2.60″ S 63°14′16.26″ W) as a sampling station (Figure 1).

2.3. Water Samples

Water samples were collected on a monthly basis over the course of the period between August 2015 and February 2021. The procedure was conducted in accordance with the established guidelines, employing the standard methods for the examination of water and wastewater [30]. The water samples were obtained directly from the Paso Alsina station and stored in high-density polyethylene bottles with a nominal volume of 500 milliliters. In the laboratory, all samples were stored at a temperature of 4 °C until analysis, which was conducted within a seven-day period. The following parameters were analyzed: total dissolved solids (TDS), pH, electrical conductivity (EC), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), carbonates (CO_3^{2-}), bicarbonates (HCO_3^-), chlorides (Cl^-), sulfates (SO_4^{2-}), cadmium (Cd), and lead (Pb). The pH, temperature, EC, and TDS were determined in situ.

Figure 1. Study area, with the Paso Alsina station as the monitoring site.

2.4. Fish Samples and Pre-Treatment

Cyprinus carpio experiments were carried out in accordance with the international laws (EU Directive 2010/63/EU for animal experiments) and institutional guidelines for the protection and welfare of research animals. Following these criteria, the volume of water, the size of the container, the temperature, and the lighting of the fish were appropriate for the species [31].

The freshwater *Cyprinus carpio* were caught exclusively at the Paso Alsina station using beam trawls. This site was selected as it represents a typical habitat for local population of *Cyprinus carpio* and is frequently used for fishing activities. Local fishermen were consulted to identify the most suitable area for capture within this station, thus ensuring controlled conditions with minimal environmental variability. *Cyprinus carpio* specimens were identified using standard ichthyological keys and morphological characteristics, such as body shape, fin structure, the presence of two pairs of barbels, and large, thick scales, as outlined in Kottelat and Freyhof's *Handbook of European Freshwater Fishes* [32]. Additionally, local fishermen with extensive experience in catching *Cyprinus carpio* in the region contributed to confirming the species identification, ensuring accuracy through their practical knowledge. The fish were subsequently transported in ice-cold water to the laboratory, where they were acclimated for a period of seven days in a 500 L aquarium [33]. A total of 36 specimens were employed in the course of the experiments. The fish were provided with standard powdered food and were fasted for a period of 24 h prior to the commencement of experimentation. A 12 h photoperiod was maintained throughout the course of the experimental work. A continuous airflow was maintained, and the fish were provided with artificial dry food on a daily basis. During the course of the experiment, the pH of the water was maintained at 8.00 ± 0.30, with a temperature of 20 ± 1 °C. It is noteworthy that the water used for this experiment was sourced directly from the Paso Alsina station in the Colorado River.

The fish were separated into four distinct groups: the first served as the control group, while the other three were designated as experimental groups. The fish were randomly

exposed to a solution of cadmium and lead, with a combined sublethal concentration of 5.0 µg mL^{-1} (1/10th of LC_{50}/48 h) [19]. The exposure period was 24, 48, and 96 h, respectively, in experimental tanks designated as T1, T2, and T3 (Table 1) [19]. Each experimental group consisted of nine fish, with three fish assigned per exposure period, ensuring three replicates for each condition. The water was maintained at a consistent level of aeration and refreshed at 24 h intervals. Prior to anesthesia and dissection of the organs, the weights and lengths of the fish were determined (Table 1).

Table 1. Experimental data for bioaccumulation of cadmium and lead.

	Experimental Tank 0 (Control)	Experimental Tank 1 (T1)	Experimental Tank 2 (T2)	Experimental Tank 3 (T3)
Average length	38.7 ± 1.8 cm			
Average weight	810 ± 35 g			
Cd	-	2.5 mg L^{-1}	1.0 mg L^{-1}	4.0 mg L^{-1}
Pb	-	2.5 mg L^{-1}	4.0 mg L^{-1}	1.0 mg L^{-1}
Total	-	5.0 mg L^{-1}	5.0 mg L^{-1}	5.0 mg L^{-1}

Subsequently, their livers were separated and weighed for analysis. An appropriate quantity of tissue was homogenized using ultrasound in a 50 mM Tris-HCl solution (pH 8.10), employing a piezoelectric ultrasonic device for 4 min, followed by a 10 min centrifugation at 2500 rpm. The resulting supernatant was heated to 95 °C for a period of 5 min on a hot plate and then filtered. The resulting homogenate was subjected to analysis using the voltammetric technique, as previously described by Fan et al. [34]. Figure 2 shows a flow chart of liver sample collection.

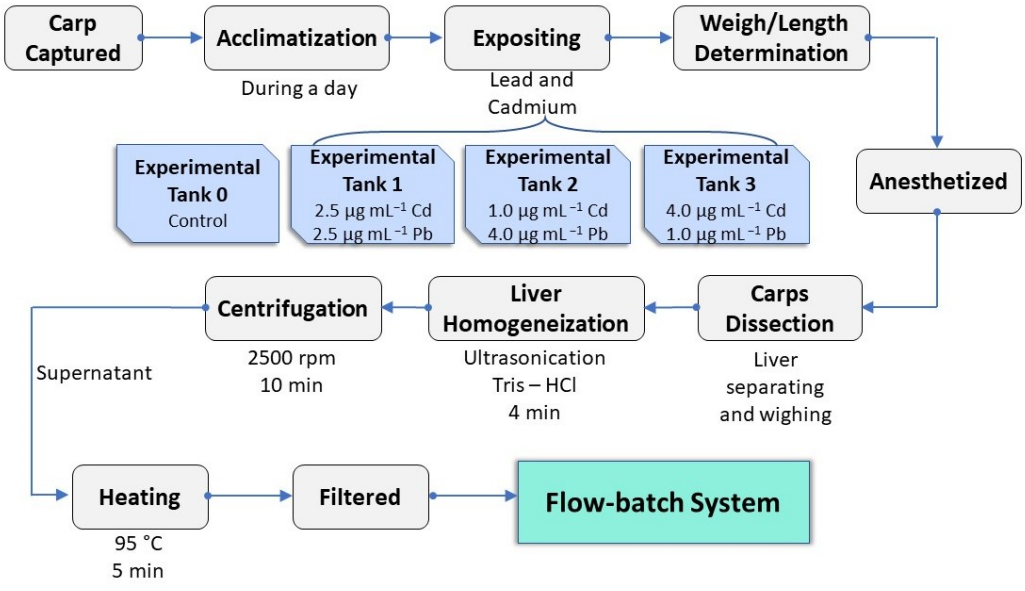

Figure 2. Flow diagram of the experimental procedure: fish acclimatization, metal exposure, liver collection, and sample preparation for analysis.

2.5. Flow-Batch System with Voltammetric Detection

The SWASV was performed using a lab-made prototype (Figure 3) consisting of a lab-made embedded flow-batch system with a potentiostat for electrochemical detection [29]. This device employs a low-cost microcontroller-based platform on an open-hardware Arduino kit. The electrochemical reaction takes place in a 25.0 mL electrochemical cell

(EC), which contains a PTFE cap with three holes for the attachment of a three-electrode configuration. This configuration includes a bismuth film prepared in situ on a glassy carbon as the working electrode, an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode.

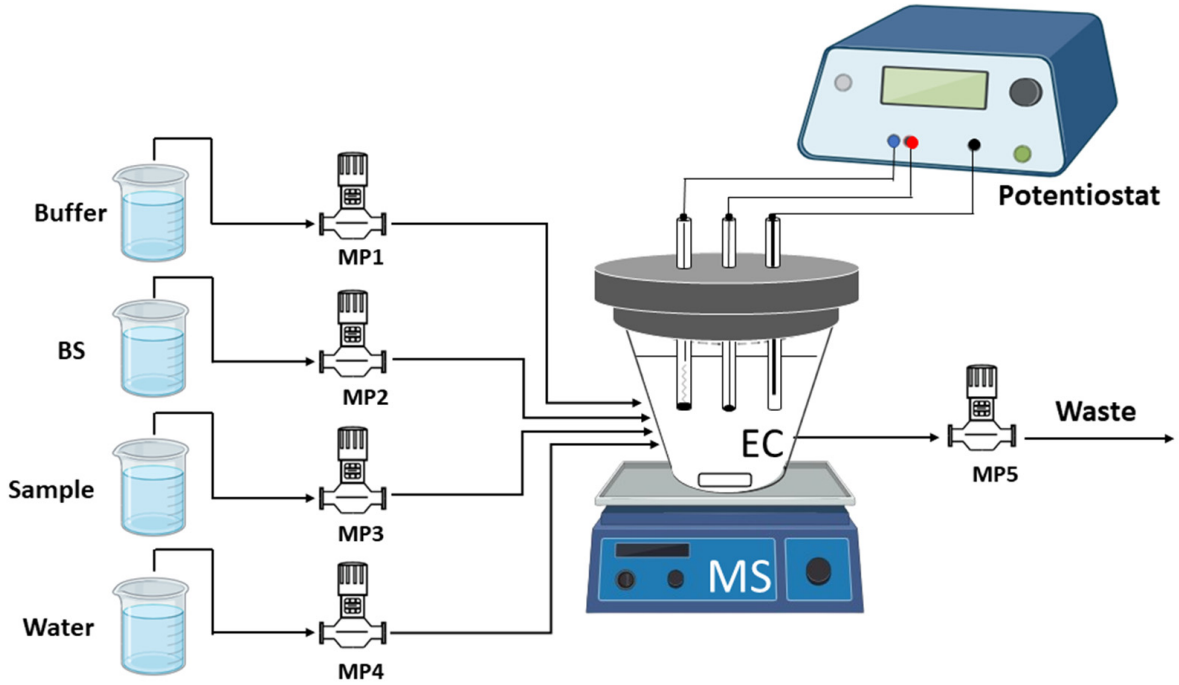

Figure 3. Embedded flow-batch system with electrochemical detection. MP: metering pump; EC: electrochemical cell; MS: magnetic stirrer; Buffer: buffer solution of 0.1 M of sodium acetate; BS: bismuth solution acidified with 5 mL of nitric acid; Sample: cadmium and lead standard solution or sample; Water: ultrapure water (18 MΩ).

2.6. Procedure

The experiments were conducted in the EC. The glassy carbon electrode (GCE) was polished with 0.05 µm alumina and subsequently rinsed with deionized water. In order to stabilize the surface of the working electrode, cyclic voltammograms were performed between −1.4 and −0.2 V using the supporting electrolyte solution (buffer acetic acid–sodium acetate, pH 4.50), at a scan rate of 100 mV s^{-1}, with the objective of stabilizing the surface of the working electrode. SWASV measurements were conducted by immersing the three electrodes in the EC, which contained the supporting electrolyte, a solution of 1000 µg L^{-1} of Bi^{3+}, and the sample. This process allows the bismuth film to be generated in situ on the surface of the GCE. The deposition potential was set at −1.4 V for a period of 120 s, with a rotation rate of 6000 rpm for the working electrode. Subsequently, the stirring was concluded, and following a 10 s interval, the voltammogram was recorded in square-wave voltammetry mode between −1.4 and 0.2 V. The SWASV parameters were as follows: step potential (ΔEs) 2 mV, amplitude of the square wave (ΔESW) 40 mV, and frequency (f) 25 Hz [27]. These parameters allowed for the effective resolution of the lead and cadmium peaks. Subsequently, in order to clean the surface of the working electrode, a potential of 0.2 V was applied for 30 s under stirring conditions of 6000 rpm. All experiments were conducted at room temperature and without the removal of oxygen.

Table 2 illustrates the flow-batch parameters employed for the cadmium and lead determination in the samples. Prior to the initiation of the analytical process, the metering pumps (MP1, MP2, MP3, MP4) were activated to facilitate the filling of the channels with the requisite solutions. Subsequently, the solution within the EC was discarded to the waste

by activating the MP5. Afterward, MP1 was activated for 75 s to introduce the buffer, to stabilize the pH; MP2 for 25 s to add the bismuth solution, which serves as a film-forming agent on the electrode surface; and MP3 for 5 s to incorporate the sample into the EC. These steps were conducted with the continuous agitation of a magnetic stirrer.

Table 2. Flow-batch procedure steps for sample preparation and metal detection using SWASV.

Steps	Events	Time (s)	Pump Setup (µL) @ 1 Hz	Volume (mL)
1	Buffer (V1)	75	200	15.0
2	Bismuth (V2)	25	200	5.0
3	Sample (V3)	5	20	0.1
4	Deposition	120	-	-
5	Quiet time	10	-	-
6	Detection (SWASV)	32	-	-
7	Cleaning (V4)	480	200	20.0

Once all the reagents were introduced into the EC, the deposition step was performed at −1.4 V for 120 s, allowing cadmium and lead to accumulate on the bismuth film electrode. A resting time of 10 s followed to stabilize the system. Finally, the detection step was carried out for 32 s, recording the voltammograms between −1.4 and 0.2 V. After completing this stage, the EC underwent a cleaning process. First, MP5 was activated to remove waste solutions, followed by MP4 to introduce washing water, and MP5 again to eliminate the water. The total cleaning time was 480 s, preparing the EC for subsequent analyses.

3. Results and Discussions

3.1. Water Quality

Table 3 presents the results of the hydrosaline analysis of the Colorado River water, evaluated at the Paso Alsina station. The data set comprises the concentrations of the ions sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), carbonate (CO_3^{2-} + HCO_3^-), and sulfate (SO_4^{2-}), the total hardness (TH), electrical conductivity (EC), pH, and total dissolved solids (TDS). The common carp were collected in July 2021, a period with particular implications in terms of water quality. This was due to the high levels of dissolved salts and metals resulting from drought and the flow regulation by the Casa de Piedra Dam [35,36]. The effects of these extreme conditions on the health of aquatic species, particularly carp, are of particular interest. Additionally, water samples were collected at the fishing site the same day and analyzed using ICP-AES. Metal concentrations (Cd and Pb) in the water were below the detection limit.

With regard to heavy metals, the Pb levels observed during the study period exhibited a range between 0.6 and 28.5 µg L^{-1}, while the Cd levels ranged between 0.22 and 1.3 µg L^{-1}. In some cases, these concentrations exceeded the guideline values for the protection of aquatic life established by the Canadian Environmental Quality Guidelines, which specify 7 µg L^{-1} for lead and 0.37 µg L^{-1} for cadmium under conditions of total water hardness above 180 mg L^{-1} and 280 mg L^{-1}, respectively [37]. From a biological perspective, increased salinity levels necessitate greater energy expenditure by fish to maintain osmotic balance, which subsequently reduces their capacity to effectively process toxic metals [38]. This additional effort may result in saline stress, which in turn affects the bioaccumulation of metals such as Cd and Pb, primarily affecting organs such as the liver, where the majority of accumulation occurs [39].

Table 3. Physicochemical characteristics of Colorado River basin in Paso Alsina station.

Parameters	Units	Min	Max	Median	Mean	Std	Method [30]	Instruments and Equipment
Na^+	mg L^{-1}	119	227	158	164	28	3500-Na^+ B	Photometer Metrolab 315. Manufacturer: Metrolab. Buenos Aires, Argentina
K^+	mg L^{-1}	3	6	4	4	0.6	3500-K^+ B	Photometer Metrolab 315. Manufacturer: Metrolab. Buenos Aires, Argentina
Ca^{2+}	mg L^{-1}	73	174	134	135	19	3500-Ca^{2+} B	Sartorius Model Biotrate 50 mL. Manufacturer: Sartorius. Göttingen, Germany
Mg^{2+}	mg L^{-1}	3	42	19	20	7.5	3500-Mg^{2+} B	Sartorius Model Biotrate 50 mL. Manufacturer: Sartorius. Göttingen, Germany
Cl^-	mg L^{-1}	171	400	238	248	48	4500-Cl^- B	Sartorius Model Biotrate 50 mL. Manufacturer: Sartorius. Göttingen, Germany
CO_3^{2-} + HCO_3^-	mg L^{-1}	91	161	121	121	15	2320-B	Sartorius Model Biotrate 50 mL. Manufacturer: Sartorius. Göttingen, Germany
SO_4^{2-}	mg L^{-1}	83	455	312	293	83	4500-SO_4^{2-} E	Spectrophotometer Lambda 35 UV–Vis Perkin Elmer. Manufacturer: Perkin Elmer. Waltham, MA, USA
TH	mg L^{-1}	330	517	418	419	46		
EC	µs cm^{-1}	870	1940	1400	1454	240	2520 B	Hanna 991301, Manufacturer: Hanna Instruments. Woonsocket, RI, USA,
pH	-	7.7	8.6	8.2	8.2	0.2	4500-H^+ B	Hanna 991301. Manufacturer: Hanna Instruments. Woonsocket, RI, USA,
Temperature	°C	16	26	21	20	3	2550 A	Hanna 991301. Manufacturer: Hanna Instruments. Woonsocket, RI, USA,
TDS	mg L^{-1}	600	1300	1000	983	166	2540 B	Gravimetrics 321 LX 220A. Manufacturer: Precisa. Buenos Aires, Argentina

Furthermore, the joint impact of salinity and heavy metals can result in synergistic interactions, whereby the toxicity of both stressors is amplified [40,41]. It is possible that the toxic effects of heavy metals on aquatic organisms may be intensified by drought stress and water regulation. Consequently, this period represents a significant context for the evaluation of bioaccumulation risks under conditions of environmental adversity, which could intensify metal accumulation and impact the health of fauna.

3.2. Analytical Parameters

Experimental calibration curves were constructed for the cadmium and lead quantification in the range between 1.00 and 30.00 µg L^{-1}. The resulting calibration curves were as follows: Ipn = (0.244 ± 0.009) C* Cd + (0.192 ± 0.072) (R^2 = 0.996) and Ipn = (0.211 ± 0.012 C* Pb) + (0.151 ± 0.065) (R^2 = 0.998), where Ipn represents the maximum current peak and C* the concentration (µg L^{-1}). The estimated detection limits,

calculated as three times the standard deviation of the signal divided by the slope, were 0.92 μg L^{-1} and 0.88 μg L^{-1} for cadmium and lead, respectively. The reproducibility was evaluated as the percentage of variation relative to standard deviation of six independent measurements of 15.00 μg L^{-1} of cadmium and lead solutions, respectively. The resulting values were 6.2% for cadmium and 5.3% for lead.

3.3. Bioaccumulation Study

Cadmium and lead concentrations in liver samples of *Cyprinus carpio* were quantified using SWASV with a BiFE, a method known for its ability to detect trace concentrations of metals. In contrast to traditional techniques, SWASV offers high sensitivity and is particularly effective for distinguishing between metals in complex biological samples, making it a promising alternative to conventional methods like atomic absorption spectrometry or ICP-AES. This method is especially useful in bioaccumulation studies of metals in fish, where cadmium and lead levels can be low but still pose significant toxicological risks.

The initial analysis of the control group demonstrated the absence of quantifiable metal concentrations, as illustrated in Figure 4A (the signal at −0.11 V corresponds to Bi^{3+} ions), thereby confirming the initial conditions of the experiment. The subsequent analysis of samples exposed to metals at varying time points revealed a notable increase in lead and cadmium levels, with discernible signals for both elements in samples collected after 48 h (Pb at −0.52 V and Cd at −0.74 V). This is illustrated in Figure 4B. These findings demonstrate a clear accumulation of the metals in the liver tissues following exposure.

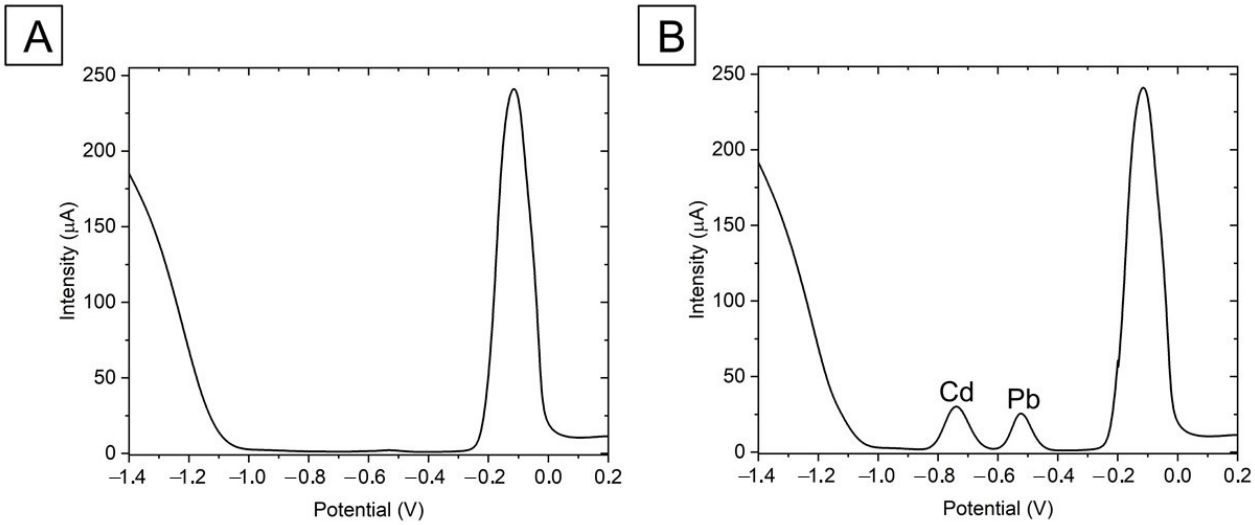

Figure 4. (**A**) Voltagram for a resulting homogenate of *Cyprinus carpio* taken from the Colorado River belonging to the control group. (**B**) Voltagram for a resulting homogenate of *Cyprinus carpio* after 48 h of exposure to metals.

The experimental tanks were subjected to different treatments, as was detailed in Table 1:

- T1: Equal concentrations of Cd and Pb at sublethal levels (1/10th of LC50/48 h for both metals) for exposure periods of 24, 48, and 96 h;
- T2: A higher concentration of Pb four times greater than Cd, with the same sublethal exposure times of 24, 48, and 96 h;
- T3: A higher concentration of Cd four times greater than Pb, with the same sublethal exposure times of 24, 48, and 96 h.

The results of the bioaccumulation study of these analytes are presented in Table 4. With regard to the temporal progression of bioaccumulation, a considerable increase in the

concentration of metals in the liver was observed during the initial 24 h period, followed by a period of stabilization in the subsequent periods. This pattern indicates an initial phase of rapid uptake and accumulation in tissues, which is likely associated with the high permeability of liver membranes to metal ions under conditions of stress. Prior research has substantiated that the bioaccumulation of metals in fish exhibits considerable variability contingent on the specific metal in question. Additionally, factors such as the age, size, and physiological state of the fish also exert a significant influence [42,43].

Table 4. Bioaccumulation of metals in each experimental tank for the different times measured (n = 3).

	Experimental Tank 0 (Control)		**Experimental Tank 1 (T1)**		**Experimental Tank 2 (T2)**		**Experimental Tank 3 (T3)**	
	Cd	**Pb**	**Cd**	**Pb**	**Cd**	**Pb**	**Cd**	**Pb**
				μg g^{-1} Dry wt.				
24 h	<LD	<LD	8.5 ± 0.3	9.9 ± 0.1	2.0 ± 0.3	14.0 ± 0.6	12.9 ± 0.3	2.5 ± 0.2
48 h	<LD	<LD	10.4 ± 0.8	11.6 ± 0.6	2.0 ± 0.1	14.7 ± 0.5	13.2 ± 0.1	2.5 ± 0.1
96 h	<LD	<LD	10.5 ± 0.6	11.9 ± 0.7	2.4 ± 0.2	15.1 ± 0.7	13.4 ± 0.2	2.6 ± 0.1

The results of the study demonstrated that the concentration of lead was higher than that of cadmium when both metals were present in equal concentrations (T1). This finding is consistent with the results of previous studies that have suggested a greater affinity of liver tissue for lead (Table 5). These studies also highlight the variability in metal accumulation depending on environmental conditions, analytical methods, and sampling sites.

Table 5. Accumulation of Cd and Pb in liver tissue of *Cyprinus carpio* reported in different studies.

Metal	**Tissue**	**Liver Concentration μg g^{-1} Dry wt.**	**Analytical Method**	**Source**	**Reference**
Cd, Pb, Cu, Cr, Ni	Liver, gills, and muscle	Cd: 1.51 Pb: 9.45	Atomic absorption spectrophotometry	Seyhan River, Turkey	[14]
Cd, Pb, Cr, Ni	Liver, gills, kidney, and flesh	Cd: 1.69 Pb: 2.00	Atomic absorption spectrophotometry	Ponds of Tamilnadu, India	[19]
Cd, Pb, Zn, Cr, Ni, Cu	Liver, muscle, intestine, and gills	Cd: 58.0 Pb: 261	Atomic absorption spectrophotometry	Kabul River, Afghanistan	[44]
Cd, Pb, Cu, Cr, Zn	Liver, muscle, intestine, and gills	Cd: 39.8 Pb: 307	Atomic absorption spectrophotometry	Kabul River, Afghanistan	[45]
Cd, Pb, Fe, Cu, Zn, Hg	Liver, stomach, intestine, gills, skin, and muscle	Cd: 0.010 Pb: 0.024	ICP-MS	Kızılırmak River, Turkey	[46]
Cd, Pb	Liver	Cd: 10.0 Pb: 11.0	Proposed method	Colorado River, Argentina	This study

The concentrations of Cd and Pb reported in different studies show considerable variability, ranging from the lowest values observed in the Kızılırmak River, Turkey (Cd: 0.01 μg g^{-1} dry wt.; Pb: 0.024 μg g^{-1} dry wt., [46]), to the highest concentrations recorded in the Kabul River, Afghanistan (Cd: 39.8–58.0 μg g^{-1}; Pb: 261–307 μg g^{-1}, [44,45]). This wide range may be attributed to differences in environmental conditions, contamination levels, or even genetic and ecological variations among *Cyprinus carpio* populations. Factors such as water salinity, pH, and hardness are known to play a critical role in metal bioavailability and bioaccumulation.

The extreme concentrations reported for the Kabul River highlight the severity of environmental pollution in that region. As noted in previous studies, industrial and urban runoff significantly impact the Kabul River, leading to sublethal effects on *Cyprinus carpio* populations and potentially reducing their development. This underscores the influence of anthropogenic activities on metal accumulation in aquatic ecosystems.

Notably, our study is the first to employ an automated electrochemical system with a bismuth film electrode for the detection of cadmium and lead in aquatic bioindicators. The system's automation enables precise analyses with minimal operator intervention, offering a more accessible, cost-effective alternative to traditional techniques. This innovation eliminates the need for expensive instrumentation, while maintaining high sensitivity for trace metal detection, and it allows for continuous monitoring, making it a promising tool for routine environmental monitoring and toxic substance management.

In experimental T2, where the concentration of lead was four times greater, this trend of prioritizing lead accumulation over cadmium was maintained. These results further confirm the suitability of *Cyprinus carpio* as a bioindicator, particularly in ecosystems where species may be exposed to heavy metals and fluctuations in water conditions can favor toxin accumulation in critical tissues.

In contrast, in experimental T3, where the concentration of cadmium was four times greater than that of lead, the cadmium accumulation exceeded sublethal levels, demonstrating an accumulation proportional to the concentration present in the medium. This shift reinforces the capacity of *Cyprinus carpio* to respond predictably to variations in environmental metal concentrations, highlighting its utility in monitoring aquatic ecosystems [47].

Furthermore, while previous studies predominantly employed atomic absorption spectrophotometry (AAS) for metal analysis, our study utilized an automated flow-batch system with SWASV. This novel approach represents a significant advancement in analytical methodology, offering a comparable sensitivity while reducing operator intervention. Its automation and ease of use make it a promising tool for routine environmental monitoring.

3.4. Validation with Reference Method

The cadmium and lead content in the samples with 48 h of bioaccumulation in the three experimental tanks were validated by the inductively coupled atomic emission spectrometry technique (ICP-AES), and the results obtained were deemed satisfactory (Table 6). The results derived from the proposed method were compared with the ICP-AES values for both analytes. With regard to the element of cadmium, the identity line demonstrates a linear correlation, with an intercept of 0.376 μg L^{-1}, a slope of 0.979, and a correlation coefficient of r = 0.993. In the case of lead, the identity line yielded a linear correlation with an intercept of 0.023 μg L^{-1}, a slope of 0.994, and a correlation coefficient r = 0.988. These results confirm that no notable discrepancy exist between the proposed method and the reference technique, further validating the robustness of the automated system.

Table 6. Comparison of the proposed method with the ICP-AES reference method.

	Experimental Tank 1		**Experimental Tank 2**		**Experimental Tank 3**	
	Cd	**Pb**	**Cd**	**Pb**	**Cd**	**Pb**
	μg g^{-1} Dry wt.					
Proposed method	10.4 ± 0.8	11.6 ± 0.6	2.0 ± 0.1	14.7 ± 0.5	13.2 ± 0.1	2.5 ± 0.1
ICP-AES	11.1 ± 0.5	10.8 ± 0.3	2.2 ± 0.4	15.2 ± 0.5	12.9 ± 0.2	2.7 ± 0.1
RSD %	6.3	7.4	9.1	3.3	2.3	7.4

4. Conclusions

This study demonstrates the bioaccumulation of cadmium and lead in *Cyprinus carpio*, thereby substantiating its value as a sensitive bioindicator for environmental contamination. The utilization of sophisticated electrochemical techniques, such as square-wave anodic stripping voltammetry (SWASV) with a bismuth film electrode integrated into an automated flow-batch system, offers a cost-effective and reliable methodology for the detection of trace metal concentrations. The results were validated against those obtained using traditional techniques, such as inductively coupled plasma–atomic emission spectrometry (ICP-AES), confirming the accuracy and potential of this innovative approach for monitoring heavy metals in aquatic ecosystems.

The findings revealed a significant degree of bioaccumulation of lead and cadmium in the liver of *Cyprinus carpio*, with lead demonstrating a stronger affinity and higher accumulation levels. These findings are particularly pertinent given the environmental stressors present during the study, including high salinity and altered flow conditions resulting from drought and the Casa de Piedra Dam. It is probable that elevated salinity exacerbated the toxic effects of metal exposure, thereby increasing bioaccumulation and the potential risks to aquatic organisms. These findings highlight the necessity for continuous monitoring of the Colorado River in order to mitigate the impacts of water crises and climate change on vulnerable ecosystems and to ensure the sustainable management of water resources.

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