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Determination of the Bioaccumulative Potential Risk of Emerging Contaminants in Fish Muscle as an Environmental Quality Indicator in Coastal Lagoons of the Central Mexican Pacific

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Abstract: This research proposes an environmental quality indicator to detect, predict and scientifically evaluate the environmental impact generated by chemical substances within the pollutant group of nonsteroidal anti-inflammatory drugs (NSAIDs) that are categorized as emerging contaminants (ECs) with endocrine disruptive action. The present study was carried out in two coastal lagoons affected by wastewater produced by urban and rural settlements in the states of Colima and Jalisco. Four pharmaceutical compounds were analyzed: diclofenac, ibuprofen, ketorolac and naproxen. The muscle tissues of 14 fish species were analyzed; all had measurable concentrations of the four contaminants. The presence of the ECs was confirmed in all the samples collected. The bioaccumulative potential risk (BPR) of the ECs is estimated by calculating the environmental risk factors and the potential risks to human health, evaluating the concentration and assessing the risk involved in the incorporation of the pollutants into the environment. The BPR indicates the potential impact of NSAIDs on the ecology of these coastal lagoons, and predicts whether a contaminant is likely to act and persist in the environment and bioaccumulate in organisms. Additionally, it estimates the possibility of contamination and risks to human health caused by residues of the chemical contaminants.

Keywords: ecotoxicology; NSAIDs; fish muscle tissue; bioaccumulation; coastal ecosystems

1. Introduction

As a consequence of the high levels of industrialization and urbanization along river basins, coastal ecosystems have been significantly impacted by a wide range of anthropogenic contaminants [1]. Chemical contaminants, particularly emerging organic pollutants, have been detected in coastal zones at trace concentration levels, which are still harmful to ecosystems [2]. Within this pollutant group are the nonsteroidal anti-inflammatory drugs (NSAIDs), which include the pharmaceuticals diclofenac, ibuprofen, ketorolac and naproxen. These products are biologically active and persistent substances [3]. Due to their volume of consumption and incomplete removal during the wastewater treatment

processes, NSAIDs are among the most frequently detected pharmaceuticals in treatment plants and surface waters worldwide [4]. This explains their occurrence in the environment, particularly in aquatic ecosystems, which they enter through municipal and hospital wastewater discharges [5].

The consumption of painkillers such as NSAIDs is predicted to increase [6] and their occurrence in the aquatic environment will increase accordingly [7]. The analgesic action of NSAIDs is mainly due to their ability to inhibit the enzymes that synthesize prostaglandins. These compounds have been designed to elicit a specific biological action in the body and often resist inactivation prior to inducement of their intended therapeutic effect, which causes toxicity and bioaccumulation in hydrobionts [8].

Although side effects have not yet been reported during clinical trials in fish, nephrotoxicity, hepatotoxicity and other toxic effects have been reported after chronic administration of diclofenac, ibuprofen, ketorolac and naproxen during ecotoxicological studies. Accordingly, they may accumulate in aquatic organisms and even bio-magnify through the food chain, thus threatening the aquatic ecosystem and potentially harming human health [9]. The toxicity of chemicals in aquatic organisms usually correlates with their uptake and accumulation in tissues [10]. Therefore, it is important to study the accumulation of chemicals in different biological tissues.

Bioconcentration is the accumulation of a substance (dissolved in water) within an aquatic organism. The bioconcentration factor (BCF) of a compound is defined as the ratio of the concentration of the chemical in the organism and in water at equilibrium [11]. BCF values have traditionally assumed that the concentration of a chemical in tissues of aquatic organisms is linearly and independently related to its concentration in water [12]. Bioaccumulation data can be used to support human health risk assessments and ecological risk assessments. Human health risk assessment is the method of estimating the probability of adverse health effects of toxic elements in humans; the human health risk factors of NSAIDs are usually estimated as oral ingestion (food and water), dermal contact (soil) and inhalation (dust), evaluating the ingestion rate, exposure frequency and exposure period. Because muscle tissue is the portion of the fish that humans typically consume, edible tissues (e.g., fish fillets) are recommended to measure chemical contamination [13].

Bioaccumulation refers to the continuous increase in the concentration of a chemical in an organism, compared to the chemical's concentration in the environment to which the organism is exposed, i.e., air, water, soil and food [14]. The bioaccumulative potential risk (BPR) is estimated by calculating the environmental risk factors and the potential risks to human health, evaluating the concentration and assessing the risk involved in the incorporation of the pollutants into the environment.

This research proposes an environmental quality indicator that evaluates the presence of emerging contaminants in fish muscle, their influence on the medium and the potential risks posed by the presence of these pollutants in the environment and possible effects to human health. Therefore, tools that contribute to the protection of water sources and the regulation of pollution in coastal zones can be provided.

2. Materials and Methods

2.1. Study Sites and Sample Collection

Four ECs of the NSAID group were analyzed: diclofenac, ibuprofen, ketorolac and naproxen. The samples were collected in two coastal lagoons located in the Central Mexican Pacific (Figure 1).

Sampling site "A" (Figure 1) includes the entire Barra de Navidad Lagoon located in the state of Jalisco, southeast of two settlements of 3200 and 4320 inhabitants [15]. Sampling site "B" (Figure 1) comprises the Cuyutlan Lagoon, located in the state of Colima, 8.8 km southeast of the commercial port of Manzanillo, which reports an approximate population of 184,541 inhabitants [15]. Both lagoons are designated Ramsar sites. They are estuarine environments that include mangroves, brackish/salty systems and are geologically classified as coastal lagoons. The altitude at the study sites varies from 0 to 10 m above sea level [16].

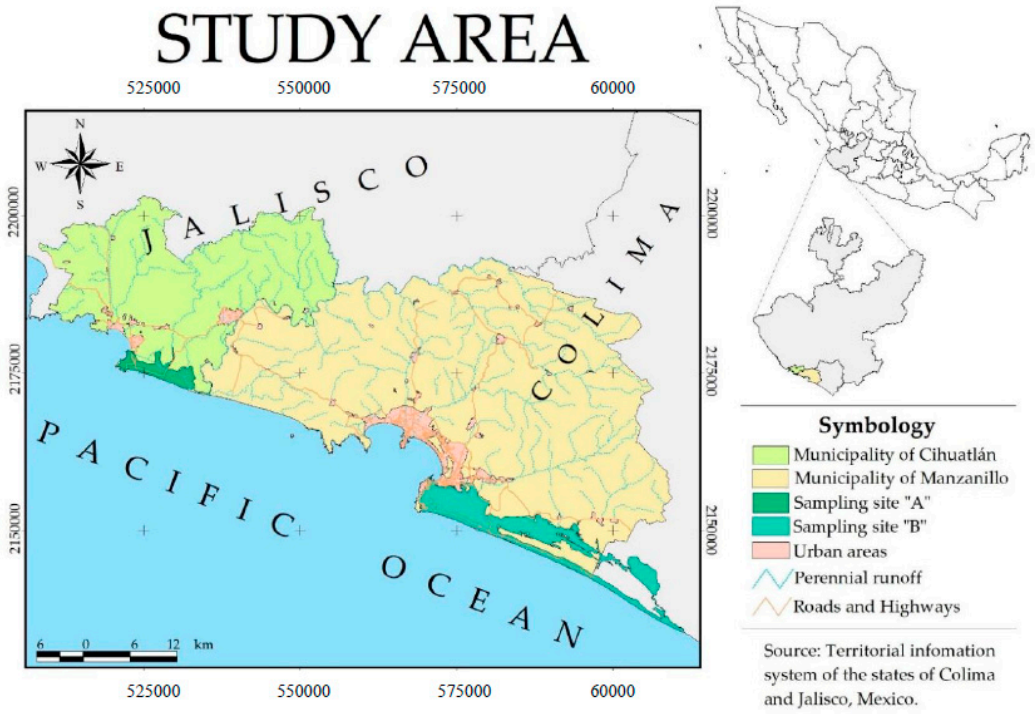

Figure 1. Location of the study area (states of Colima and Jalisco).

To obtain muscle samples, eleven species of fish were selected at site "A" and seven species of fish were selected at site "B". The fish were collected in the lagoons through traditional fishing arts according the "Fish sampling protocol in continental waters" established in the Mexican Official Standard, NOM-159-SCFI-2012. Four specimens per species were collected; the specimen processing was conducted in accordance with the American Fisheries Society's Guidelines for The Use of Fishes in Research [17]. The complete fish of each species were transported to the laboratory in frozen conditions (near −10 °C) where biometric data (weight and height) were reported. Each fish was partially defrosted (until muscle felt soft) and then muscle tissue samples (all pieces approximately 5.0 × 3.0 × 0.5 cm with skin and scale) were extracted from the ventral muscle of the fish [18]. Samples were individually stored at −16 °C to −18 °C in Nalgene airtight containers until use.

The fish collected include both resident and transient species in the studied lagoons. Species were selected according to their frequency of occurrence, type of feeding and those that represent commercial interest in the study area.

2.2. Sample Processing

The tissue samples were thawed inside Nalgene airtight containers and then minced with an industrial immersion blender (KEF Industrial® BL-40C, İstanbul, Turkey) and finally homogenized as a slurry with a disperser and homogenizer (IKA® UltraTurrax-3593001, Staufen, Germany). All processes were carried out at temperatures below 4 °C. Approximately 25 to 30 g of slurry from each fish specimen were mixed with 200 mL of dicholoromethane-methanol solvent mixture (40:60), with Supelco HPLC gradient [19].

Each fish slurry sample was extracted in a 500 mL Pyrex borosilicate reflux system for 4 h and then ultrasonic (US) extractions were performed with an ultrasonic probe device (UP400S, Hielscher, Teltow®, Teltow, Germany) with a glass reactor double chamber (with water cooling system) for ultrasonic flow. The US extractions were performed under controlled temperature conditions (20 ± 1.5 °C) and a fixed 20 kHz, with a maximum power of 400 W and a surface emitter of 3.8 cm^2. The ultrasonic probe was immersed in the slurry and the container was kept in the dark. US operations ran for 1 min followed by 2 min pauses, for half an hour. Weighed extracted samples

were transferred into 50 mL polytetrafluoroethylene centrifuge tubes and centrifuged at 4000 rpm for 10 min and the supernatant was decanted into a 50 mL test tube. The remaining sample precipitate was extracted and centrifuged again. Two extraction solvents were filtered through 0.45 and 0.20 µm filters (Sartorius AG®, Gottingen, Germany) on ice, combined and then evaporated with a rotary evaporator at high vacuum pressure to almost dryness and re-dissolved in 10 mL of dichloromethane-methanol (40:60). The 10 mL samples were kept on ice until use.

The samples were then concentrated within C18SPE columns in fractions of 5 mL. The C18 SPE column was equilibrated with type 1 (ultra pure) water from a Milli-Q water purification apparatus and methanol using the vacuum pump at minimum pressure. After passing the entire sample through the column it was eluted with 20 mL of a methanol-water (80:20, *v/v*) solvent mixture and then with a 30 mL dichloromethane-methanol (40:60) solvent mixture. Both eluates were collected separately and dried with a rotary evaporator at high vacuum pressure, then re-dissolved to 1 mL of methanol for analysis by the ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) [20].

2.3. Analysis of ECs

The ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS) equipment and chromatographic conditions were used to characterize the ECs as analytes [21]. Eluted analytes were monitored by MS/MS using a Varian model 1200 L triple-quadrupole mass analyzer equipped with an electrospray interface (ESI). Additional instrumental parameters held constant for all analytes were as follows: nebulizing gas, N_2 at 60 psi; drying gas, N_2 at 19 psi; temperature, 300 °C; needle voltage, 5000 V ESI+, 4500 V ESI−; declustering potential, 40 V; collision gas, argon at 2.0 mTorr. The Ionization method selected for all the analytes was the ESI negative MS/MS analysis.

The identification and the quantification of these molecules was carried out using calibration curves and mass spectrum comparison with MERCK analytical standards: diclofenac sodium salt (329770242), ibuprofen sodium salt (329815360), ketorolac tris salt (24278503) and naproxen sodium salt (57654352). Standard stock solution of 2 mg/mL for each analyte was prepared by dissolving 200 mg in 100 mL of methanol. In addition, intermediate standard solutions of 400 µg/mL, 50 µg/mL and 2 µg/mL were prepared by diluting with methanol from the solution and stored at 4 °C. Internal standards and surrogates for recovery rates were isotopically labeled ibuprofen-d_3 solution 100 µg/mL (Merck I-032-1 mL), (±)-naproxen-(*methoxy*-d_3) (Merck 32104-10MG), diclofenac-d_4 (Biosynth Carbosynth) FD165151-1MG and ketorolac tromethamine certified reference material cerilliant corp (Merck PHR1140-500MG). The latter was also used as an internal standard. Internal standards were used for each one of the analytes with 10 ng/mL for the determinations of %Rec and -%ME. Analytes specific limits of detection (LODs), limits of quantitation (LOQs) and method detection limits (MDLs) are defined and reported in Table 1. LOD was calculated as three times the standard deviation in the background signal observed for replicate analysis of a tissue blank. LOQ was calculated as ten times the standard deviation in the background signal observed for replicate analysis of a tissue blank. MDL was determined by multiplying the one-sided Student's *t*-statistic at the 99% confidence limit times the standard deviation observed for eight replicate analyses of a matrix spike (spiking level 10 × MDL).

Table 1. Linear range, limits of detection (LOD), limits of quantitation (LOQ) and method detection limit (MDL) for target analytes in fish tissue samples.

Analyte	Linear Range (ng/g)	LOD (ng/g)	LOQ (ng/g)	MDL (ng/g)
Diclofenac	38.5–3000	1.15	3.83	33.65
Ibuprofen	25.1–2700	3.21	10.66	45.92
Ketorolac	14.0–2500	1.32	4.44	40.88
Naproxen	45.8–3200	0.97	3.25	27.93

Mean limit of quantification for the analytical method: 0.5 ng/g. Mean equipment detection limit: 0.02 ng/g. Recovery rate for the four analytes (upper and lower limit values): (92%, 95%) ± 1.5%. Matrix effect for the four analytes (upper and lower limit values): (−9%, −2%) ± 0.5% and correlation of determination R^2 for the four analytes (upper and lower limit values): (0.992, 0.998).

3. Calculation

To quantify the bioaccumulative potential risk (BPR) of the studied pollutants in the environment, the human and ecological risk factors were calculated using Equations (1)–(5) [9,22,23].

3.1. Ecological Risk Factors

$$\text{BCF} = \frac{\text{Ct}}{\text{Ce}} \times 1000 \tag{1}$$

where BCF is the bioconcentration factor. Ct is the EC concentration in tissue; Ce is the EC concentration in the effluent.

$$C_f = \frac{C_m}{C_{ref}} \tag{2}$$

where C_f is the contamination factor. C_m is the mean EC concentration in the medium; C_{ref} is the EC_{50} for each pharmaceutical.

$$E_p = BCF \times C_f \tag{3}$$

where E_p is the ecological potential risk for individual NSAIDs.

3.2. Human Health Risk Factor

$$HRF: \ I_r \times T_e \times T_p \tag{4}$$

where *HRF* is the human health risk factor for each contaminant; I_r is the ingestion rate, T_e is the exposure frequency, T_p is the exposure period.

3.3. Bioaccumulative Potential Risk

$$BPR = \log_{10}\left(\sum_{i=1}^{m} E_p \times HRF\right) \tag{5}$$

where *BPR* is the bioaccumulative potential risk; *HRF* is the average human health risk factor for the NSAIDs detected.

For the calculation of the bioaccumulative potential risk, the pharmacokinetic properties of nonsteroidal anti-inflammatory drugs were considered (Table 2).

Table 2. Pharmacokinetic characteristics of contaminants.

Contaminant	Molecular Formula	pKa	Solubility in Water (mg/L)	EC_{50} *Daphnia Magna* 48 h (mg/L)
Diclofenac	$C_{14}H_{11}NCl_2O_2$	4.15	19.4	68.30 [24]
Ibuprofen	$C_{13}H_{18}O_2$	5.2	21	101.2 [25]
Ketorolac	$C_{15}H_{13}NO_3$	3.84	15	109.2 [24]
Naproxen	$C_{14}H_{14}O_3$	4.15	15.9	166.3 [25]

The index proposed by Hakanson (Table 3) was used to establish the level of bioaccumulative potential risk of the contaminants.

Table 3. Hakanson classification.

BPR	Risk Classification
<1	Low
1–10	Moderate
11–100	Considerable
>100	Very High

4. Results and Discussion

As suspected, it was possible to identify the presence of the four targeted NSAIDs within all samples. The average concentrations found within the tissues of the fish species at both sites are presented in the following graphs (Figures 2 and 3). The standard error of the mean (SEM) was calculated using N = 16, which represents the number of analytical samples per specimen.

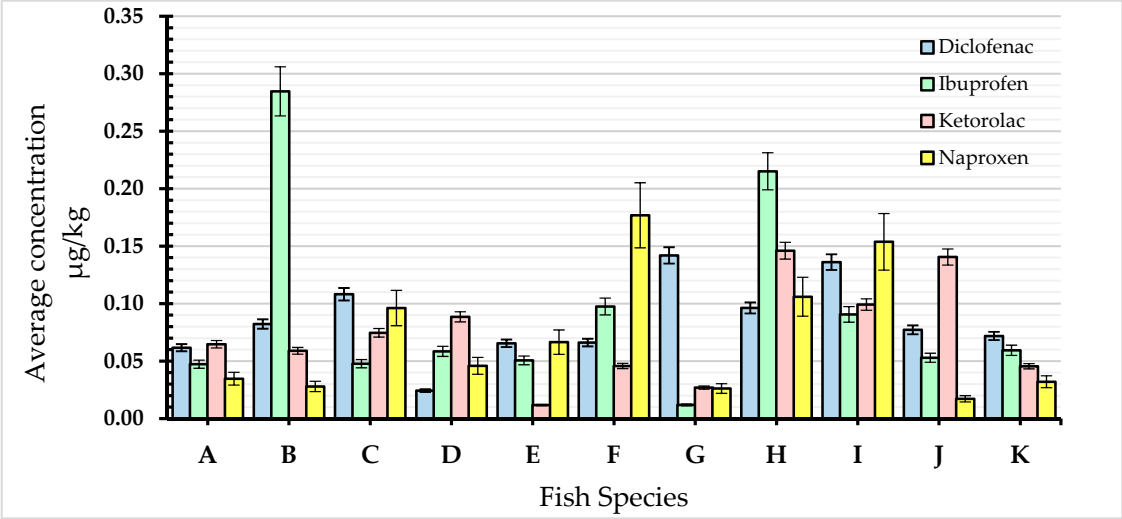

Figure 2. Concentration of nonsteroidal anti-inflammatory drugs (NSAIDs) at site "A". Fish species: A: *Acanthurus xanthopterus*, B: *Ariopsis felis*, C: *Caranx caninus*, D: *Chanos chanos*, E: *Gerres cinereus*, F: *Haemulopsis elongatus*, G: *Lutjanus peru*, H: *Mugil curema*, I: *Paralichthys californicus*, J: Pseudupeneus *grandisquamis*, K: *Selene peruviana*.

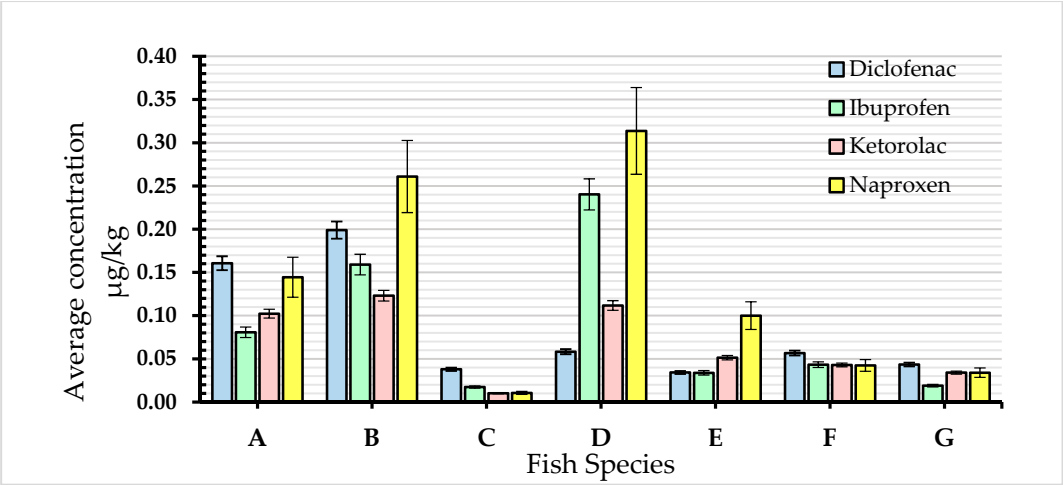

Figure 3. Concentration of NSAIDs at site "B". Fish species A: *Ariopsis felis*, B: *Caranx caninus*, C: *Centropomus robalito*, D: *Synodus lacertinus*, E: *Lutjanus peru*, F: *Mugil curema*, G: *Sarda orientalis*.

The results of the analysis performed on the muscle tissue of the fishes demonstrated the presence of the pharmaceuticals diclofenac, ibuprofen, ketorolac and naproxen in all the species analyzed at both study sites. As shown in Figures 2 and 3, the pollutants found in higher concentrations were ibuprofen for the "A" study site (0.01–0.31 µg/kg) and naproxen for the "B" study site (0.01–0.28 µg/kg), which suggests that these are the most persistent compounds in the studied group, or they are the most consumed drugs at the study sites.

Adverse effects can be inferred in fish exposed to environmental concentrations of NSAIDs at the study sites, like mild to moderate alterations in fish liver and gills [26]. These compounds also have the potential to affect biomarkers associated with sexual differentiation and gametogenesis of fish, acting as estrogenic endocrine disruptors (EDC) [27]. Other studies show that ibuprofen modulates the production of hormones and the transcription of related genes of the hypothalamic–pituitary–gonadal axis in a sex-dependent manner, which could cause adverse effects on reproduction and offspring development in the fish species analyzed [28]. In blood, ketorolac acts as a genotoxic, increasing the frequency of micronuclei for some fish species and inducing oxidative damage (increased lipid peroxidation, hydroperoxide content and carbonyl protein content) [8].

Tables 4 and 5 present the average values of the bioconcetration (BCF) and bioaccumulative potential risk (BPR) for the NSAIDs, calculated using Equations (1)–(5) and classified using the Hakanson index. Values were calculated using the tissue sample analysis results, with the average values of NSAID concentrations in effluents reported in 2019 in [21] and the parameters in Table 2.

Table 4. Average values of bioconcentration (BCF) and bioaccumulative potential risk (BPR) at site "A".

Site "A"			
Fish Species	**BCF**	**BPR**	**Classification**
Acanthurus xanthopterus	8.4	26.7	Considerable
Ariopsis felis	19.3	0.0	Nd
Caranx caninus	9.3	0.0	Nd
Chanos chanos	11.1	7.1	Moderate
Gerres cinereus	3.6	0.0	Nd
Haemulopsis elongatus	9.0	0.0	Nd
Lutjanus peru	3.1	1.9	Moderate
Mugil curema	24.1	0.0	Nd
Paralichthys californicus	13.7	0.0	Nd
Pseudupeneus grandisquamis	15.7	0.5	Low
Selene peruviana	7.1	0.0	Nd

Nd: Not able to determine.

Table 5. Average values of BCF and BPR at site "B".

Site "B"			
Fish Species	**BCF**	**BPR**	**Classification**
Ariopsis felis	13.5	26.7	Considerable
Caranx caninus	19.3	0.0	Nd
Centropomus robalito	1.8	0.0	Nd
Synodus lacertinus	22.1	7.1	Moderate
Lutjanus peru	6.5	0.0	Nd
Mugil curema	6.1	0.0	Nd
Sarda orientalis	4.1	1.9	Moderate

Nd: Not able to determine.

The estimated bioaccumulation factors indicate that the species classified as carnivores (*Caranx caninus*, *Synodus lacertinus*) and detritivores (*Ariopsis felis*, *Mugil curema*) accumulated the greatest quantity of NSAIDs (Tables 4 and 5), indicating the presence of these compounds in lagoon sediments, as well as the accumulation of these drugs along the trophic network of these aquatic ecosystems. The results shown in Tables 4 and 5 suggest that the species of commercial interest, *Lutjanus peru* and *Chanos chanos* [29] at site A, and *Synodus lacertinus* and *Sarda orientlais* [30] at site B, pose a moderate BPR.

To estimate the risk to human health, the consumption standards developed by the EPA were used. These guidelines assume a person to be 70 kg with a 70-year lifespan, consuming an average of 17.5 and 142.4 g of fish per day for recreational and subsistence fishers, respectively [12]. The calculated average values for the human health risk factor (HRF) were 0.1 (±0.03) for recreational fishers and 0.2 ± (0.02) for subsistence fishers in all species studied.

The obtained HRF values indicate no direct risk to human health, as these values represent consumption averages over a 70-year lifespan. However, the constant ingestion of food contaminated with NSAIDs, in this case the fish muscle, can gradually decrease the effect of the drugs [31] as well as increase resistance and/or tolerance to the same compounds [32]. This could result in increased consumption of these compounds, causing higher concentrations of these pollutants in wastewater discharges and subsequently in natural water bodies.

5. Conclusions and Comments

The BPR, estimated by calculating the environmental risk factor and the potential risk to human health, is used as an environmental indicator to evaluate the toxicity of four NSAIDs (diclofenac, ibuprofen, ketorolac and naproxen) in wastewater discharged into estuarine coastal ecosystems in the

central Mexican Pacific. The muscle tissues of 14 species of fish were analyzed, all of which presented the four contaminants at different concentrations (0.1–0.31 μg/kg).

The calculation of the BPR revealed the potential impact of the NSAIDs on the ecology of these coastal lagoons, as well as the possibility of contamination and risks to human health caused by residues of the same chemical contaminants in food.

This study emphasizes the importance of carrying out additional research on the toxic effects of these types of pollutants in higher organisms that inhabit estuarine and coastal ecosystems. Future research should focus on the analysis of other contaminants considered as emergent (hormones, analgesics, psychotropic drugs and antibiotics), especially those that are released into the environment in large quantities and that are expected to have environmental effects, as well as focus on the study of origins, means of propagation and physicochemical characteristics.

To corroborate and identify potential effects produced by NSAIDs in the biota of these ecosystems, the unknown effects and potential risks for other organisms (invertebrates, fish, birds that feed on fish) must be assessed. We propose the development of bioassays that evaluate environmental concentrations in species of interest. Another line of research should focus on the effects of these contaminants for a better understanding of the species that are at risk.

In order to evaluate the contamination and exposure of the ECs in surface water bodies in the near future, research should be carried out to define the sampling period and duration, the type of species (and trophic levels) to be monitored and the tissues/organs that should be collected for a better understanding of the pharmaceutical's destination. Although many organizations have established toxicological and environmental health standards, there are no environmental toxicity indicators of this type in Mexico. This study provides valuable information to establish new environmental quality criteria, as well as to propose preventive and corrective measures in order to restrict and reduce the amounts of these emerging pollutants released into the environment.

Author Contributions: M.Á.A.-P.: writing—original draft preparation, methodology and investigation. E.R.-A.: methodology and investigation. J.A.M.-P.: conceptualization, resources and supervision. M.M.M.-M.: formal analysis and resources. M.V.-G.: formal analysis and resources. C.L.-C.: formal analysis and resources. E.G.-D.: visualization and supervision F.d.A.S.-B.: investigation and validation. A.T.-G.: conceptualization, supervision and validation. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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