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# Organic Micropollutants from an Agricultural Drainage Ditch Contaminate a Shrimp Farm in Sinaloa (Mexico)

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#### ABSTRACT

Among nutrients and pesticides, agricultural draining ditches also transport pollutants discharged with untreated wastewater from the municipalities adjoining the ditch. When the ditch water is used for irrigation and aquaculture, risks for the environment and food production are suggested. For the conducted field study, a shrimp farm in Sinaloa (Mexico) was used to trace organic pollutants (pesticides and pharmaceutical residues) on their way from an agricultural draining ditch to a shrimp farm fed partially by the drain water. The concentrations of pollutants in the drain water ranged from 10 ng  $L^{-1}$  to 453 ng  $L^{-1}$ . The pond water of the shrimp farm contained concentrations between  $\leq 10$  ng L<sup>-1</sup> and 177 ng L<sup>-1</sup>. The shrimps were contaminated by pollutants at concentrations between 40  $\mu$ g kg<sup>-1</sup> d.w. (dry weight) to 3.3 mg kg<sup>-1</sup> d.w. (fungicide Metalaxyl). Health risks for the cultivated shrimps cannot be excluded because some pesticides are known for their toxic effects to crustaceans. The concentrations of selected antibiotics in the shrimps were low and comparable with those found in the shrimps declared as seawater shrimps from a German supermarket. The incorporation of the antibiotics was probably caused by contact to the wastewater in the shrimp ponds and/or by contaminated shrimp feed. Additionally to the anthropogenic chemicals, coliforms were determined in the water (total coliforms: 30-50 CFU 100 mL<sup>-1</sup>; fecal coliforms: 0-20 CFU 100 mL<sup>-1</sup>). These values agree with the Mexican Norm NOM-242-SSA1-2009 representing a microbiological quality of water adequate for aquaculture. The number of coliforms measured in shrimp was higher than in pond water, suggesting bioaccumulation and a potential health risk for consumers.

Keywords: pesticides, pharmaceutical residues, antibiotics, coliforms, aquaculture, agricultural draining ditch.

### INTRODUCTION

Most of the shrimp farms in Mexico are located along the Pacific coast in Sonora, Sinaloa, and Nayarit States. This industry is growing rapidly since the 1980s, with some setbacks caused by disease outbreaks linked with high shrimp mortality (FAO, 2020). Shrimp farming in Sinaloa has been raised to about 70% of the total Mexican production of shrimps (DeWalt et al., 2012). Therefore, the semi-intensive shrimp production is an important economic factor in this region whereat Pacific white shrimps *Litopenaeus vannamei* are cultivated preferably. Shrimp farming has been documented to affect the coastal environment, for instance, by transferring coastal wetlands into flooded areas for aquaculture (Cortés et al., 2021) or by reducing mangrove zones that influences natural water flows and quality. Wildlife suffers from unsustainable farm practices causing eutrophication, silting, and algal blooms, because shrimp farms often discharge effluents highly loaded with nutrients and sludge (Hernández-Cornejo, 2000; Hansen, 2021). Furthermore, the fast-growing rural communities and the intensive agriculture in this region release a variety of bioactive chemicals, including pesticides, pharmaceutical residues, and diverse industrial chemicals that might interfere with marine environment. A view on the grade of chemical pollution in the Gulf of California considered the contamination of different environmental compartments and biota with focus on heavy metals and persistent organic pollutants (Páez-Osuna et al., 2017) but the data on the presence of human and veterinary drugs in the coastal area of Sinaloa are rare. In this region, a vast system of drain channels traverses the landscape to remove excess water from the fields and provide water for irrigation in dry seasons. These ditches also receive substantial amounts of untreated wastewater from adjoining municipalities (Moeder et al. 2017) and convey the water to coastal lagoons hosting aquaculture farms. Thus, diverse pollution sources may threaten the coastal ecosystems and may affect the cultivated animals (DeWalt et al., 2012). The presence of organic, wastewaterderived pollutants have been reported mainly for fish and related products, but the increasing importance of crustaceans for a healthy diet requires a more detailed consideration of the risks related to these production lines (NOAA, 2018). Some previous studies have investigated the occurrence of pesticides, metals, metalloids and antibiotics in shrimp tissue (He et al. 2012).

The issue of this study was to trace the way of organic pollutants from a draining ditch into the ponds of a selected shrimp farm and into the shrimps cultivated. The organic target pollutants selected for this study represent typical markers for the input of domestic wastewater and field runoff into the water of a selected draining ditch. For the field study in the northwest of Mexico, the shrimp farm called "Bataoto" was studied, which received water from the "Bataoto" drain crossing a region with intensive agriculture and many little municipalities discharging their untreated wastewater into the ditch.

Among the uptake of anthropogenic chemicals, shrimps and consumers can suffer from pathogens entering the foodweb. Hence, the determination of coliforms in water and shrimps was performed to characterize the sanitation status of the pond water and to identify possible risks for consumers.

## MATERIALS AND METHODS

### Sampling sites

The coastal region of Sinaloa in Altata-Ensenada del Pabellón (Mexico) is used intensively for agri- and aquaculture. The objective of the conducted investigation was a semi-intensively operating shrimp farm called "Bataoto" that received water from a draining ditch that crossed an agricultural area with small municipalities and manufacturing companies. The farm ponds were fed additionally by coastal intertidal water coming from the Altata Ensenda del Pabellón area. Thus, the water in the aquaculture hatcheries was composed of brackish water delivered from the "Bataoto" drain and from the coastal lagoon. The ponds monitored were about 45 cm deep with a culture density of about 4 shrimps per square meter. The cultivation period ranged between 120 and 150 days. The shrimps bought from a German supermarket were declared as wild sea shrimp of Southeast Asia provenience.

### **Target substances**

The target pollutants were partially related from a previous study that monitored the pollution in a comparable agricultural ditch in Sinaloa (Mexico) during 2013 (Moeder et al., 2017). The pesticides, food additives, pharmaceutical residues including some antibiotics were selected to characterize the input of field runoff as well as that of untreated municipal wastewater. All target substances were available as reference compounds at 98-99% purity delivered by Sigma-Aldrich, Merck (both Darmstadt, Germany) and HPC Standards GmbH (Cunnersdorf, Germany).

#### Sampling, sample preparation and analysis

All samples were taken on May 5<sup>th</sup> in 2013. Seven grab samples were taken from the ditch water where it entered the shrimp farm and from water of 3 ponds. These composite samples were analyzed for organic pollutants and coliforms. At the sampling time, the water has not been refreshed by sea water. For sample preparation, 250 mL of water samples were extracted after filtration. The subsequent solid-phase extraction used an "Oasis HLB" sorbent (200 mg , 5 mL cartridge, Waters Corp., Eschborn, Germany). About 500 g of shrimps were collected from each pond ( $\cong$  50 individual shrimps). Shrimp muscle was frozen for transport, later lyophilized, and milled. Then, 50 pooled shrimps were prepared for analysis. The target analytes in the shrimp extracts were quantified by standard addition procedure. The analysis of the target pollutants was carried out by High Pressure Liquid Chromatography (HPLC) coupled to tandem mass spectrometry (MS-MS) in positive and negative electrospray ionization mode and multiple reaction monitoring (MRM) for mass analysis. Stock solutions of the reference substances were prepared in acetonitrile (at 100  $\mu$ g mL<sup>-1</sup>) and diluted with the initial eluent mixture used for HPLC. Calibration curves used for quantification were based on 7 concentration points measured in duplicates. The full method protocol has been described previously by Moeder et al. (2017). Counting of coliforms was carried out as reported by Ahumada-Santos et al. (2014).

## **RESULTS AND DISCUSSION**

# Occurrence of organic pollution in an agricultural ditch

Particularly in the studied region of Sinaloa, shrimp farms receive drain water from intensive agriculture and untreated wastewater discharged permanently from adjoining municipalities and industries situated alongside the draining ditches. In a previous study, the input and transport of pollutants, such as pesticides, pharmaceutical residues and coliforms in a vegetated agricultural ditch in Sinaloa was evaluated (Moeder et al., 2017). For studying the shrimp farm, a similar set of pollutants was chosen that allowed a comparison to previous investigations.

The analysis of the water of the "Bataoto"drain that feed the shrimp farm indicated comparable concentrations of pollutants as found in the drain "Michoacana" (median values of a one-year monitoring). As shown by Moeder et al. (2017), the concentrations of pollutants can vary strongly due to season-dependent pesticide input at multiple crop rotations per year. Thus, short pulses of rather high concentrations reach the draining ditch and even acute exposure of organisms increases the probability for bioaccumulation and respective toxic effects. Particularly, persistent and polar pollutants (-1.33 < logP < 4.7) can be transported over a long distance, for instance the metalaxyl fungicide or the bentazone herbicide. These pesticides are very stable in water/sediment systems with dissipation half times ( $DT_{50}$ ) of 80-716 days (PPDB, 2018) and may reach aquafarms fed by the ditch water.

Semipolar compounds like chlorpyrifos (water solubility of 1.5 mg L<sup>-1</sup>) are transported mainly via particles which precipitate and can become available for benthonic aquatic organisms, such as shrimp.

Considering the acute toxicity of some pesticides to crustaceans, adverse effects to shrimps cannot be ruled out as, for instance, for chlorpyrifos or thiacloprid (Sucahyo et al., 2008).

While pesticides cause an often timely limited threat, many wastewater derived substances like pharmaceutical residues and food related compounds enter the ditch water continuously in changing amounts due to consumers' behavior. In the "Bataoto" drain, the concentrations of pharmaceutical residues and food ingredients ranged from <LOQ to 0.453 µg L<sup>-1</sup> (acesulfame) with elevated concentrations in the hot season due to water evaporation and variation in consumption (Moeder et al., 2017). In comparison, surface water in Brazil contained about 0.25-7.3 µg L<sup>-1</sup> acesulfame and 20-90 µg L-1 in influents of wastewater treatment plants whereat the number of contributing inhabitants was much higher than in the municipalities along the draining ditch in Sinaloa (da Costa et al., 2021). While some studies suggest biological effects of pharmaceutical residues particularly for drug combinations (Wojcieszyńska et al., 2020, Mathias et al., 2018), little is known about their actions to shrimp (Garcia et al., 2020) and other aquatic organisms. When the contaminated water is used to feed a shrimp farm, the animals will be exposed to a mixture of pollutants of varying concentrations. Health risks for the cultivated shrimps and for wild marine organism cannot be excluded when the draining ditches ends up in the sea. While the analyzed pollutants represent only a small cut of the cocktail of chemicals in the water, their mixture toxicity is hardly to predict due to the very complex and mostly unknown interactions (Fritts, 2016).

# Organic pollutants in the pond water of the shrimp farm

The ponds with shrimp cultivation received water from the "Bataoto"- drain and at tidal rhythm sea water (about 10% of the pond water is exchanges). Table 1 shows the concentrations

| Specification | Concentration in water  |                            |  |                            |   |                            |                              |                |  |  |
|---------------|---|----------------------------|--|----------------------------|---|----------------------------|------------------------------|----------------|--|--|
|               | inlet from "Bataoto"-drain  |                            | pond 1   |                            | pond 2  |                            | pond 3                       |                |  |  |
|               | μg L-1  | ± SD<br>µg L <sup>.1</sup> | µg L⁻¹   | ± SD<br>μg L <sup>-1</sup> | µg L⁻¹  | ± SD<br>μg L <sup>-1</sup> | µg L⁻¹                       | ± SD<br>µg L-1 |  |  |
| Ketoprofen    | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Naproxen      | 0.040   | 0.003                      | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Propranolol   | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Caffeine      | 0.029   | 0.0002                     | 0.034  | 0.001                      | 0.024   | 0.001                      | 0.018                        | 0.0005         |  |  |
| Thiacloprid   | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Carbamazepine | 0.016   | 0.002                      | 0.006  | 0.001                      | 0.005   | 0.001                      | 0.006                        | 0.001          |  |  |
| Atrazine      | 0.019   | 0.0001                     | 0.01   | 0.0001                     | 0.025   | 0.0001                     | 0.036                        | 0.0002         |  |  |
| Metolachlor   | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Chlorpyrifos  | 0.007   | 0.001                      | 0.002  | 0.0001                     | 0.004   | 0.0003                     | 0.003                        | 0.0002         |  |  |
| Carbofuran    | 0.001   | 0.0001                     | <loq< td=""><td></td><td><loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<></td></loq<> |                            | <loq< td=""><td></td><td><loq< td=""><td></td></loq<></td></loq<> |                            | <loq< td=""><td></td></loq<> |                |  |  |
| Metalaxyl     | 0.038   | 0.002                      | 0.064  | 0.004                      | 0.090   | 0.006                      | 0.087                        | 0.006          |  |  |
| Dimethoate    | 0.034   | 0.001                      | 0.004  | 0.0001                     | 0.003   | 0.0001                     | 0.003                        | 0.0001         |  |  |
| Acesulfame    | 0.453   | 0.048                      | 0.172  | 0.016                      | 0.176   | 0.019                      | 0.177                        | 0.018          |  |  |
| Ibuprofen     | 0.081   | 0.004                      | 0.005  | 0.0002                     | <loq< td=""><td></td><td>0.004</td><td>0.0002</td></loq<>         |                            | 0.004                        | 0.0002         |  |  |
| Diclofenac    | 0.012   | 0.001                      | <loq< td=""><td></td><td>0.004</td><td>0.0002</td><td><loq< td=""><td></td></loq<></td></loq<>         |                            | 0.004   | 0.0002                     | <loq< td=""><td></td></loq<> |                |  |  |
| Bentazone     | 0.036   | 0.009                      | 0.030  | 0.001                      | 0,024   | 0.001                      | 0.026                        | 0.001          |  |  |

 Table 1. Concentrations of the pollutants in the "Bataoto" drain and in ponds 1-3 with shrimp cultivation. Standard deviation was related from the analytical method

detected in the drain water and in water of the pond 1-3. The "Bataoto"- drain water contained comparable pollutants as e.g. diclofenac in the range between 0.1-2 µg L<sup>-1</sup> as in effluents and surface water reported from France and Germany (Ferrari et al., 2004). Carbamazepine was higher concentrated in the European water samples (1-6) $\mu$ g L<sup>-1</sup>) whereat the number of population equivalents contributing to the wastewater is clearly higher in both European countries than in the little communities in Sinaloa. The homogeneity of the water conditions in the ponds was measured by standard water parameters. The pH of the pond water ranged from 8.7 to 8.9 ( $\pm 0.04\%$ , n = 16). The redox potentials varied slightly within the ponds but were negative in all cases as in the feeding "Bataoto"-drain, too. This corresponded with low content of dissolved oxygen  $(0.2 \text{ mg } \text{L}^{-1} < \text{DO} < 1.39 \text{ mg } \text{L}^{-1}, \pm 0.12\%, \text{ n} = 16)$ and in sum, with less optimum conditions for shrimps' health. Furthermore, these anoxic conditions cause inefficient biodegradation of many pollutants, thus a longer exposure of the shrimps' results, and spreading of the pollutants into environment is possible. The antibiotic chloramphenicol is an exception, because it is preferably degraded under anaerobic conditions (half-life of 0.4 days, Gräslund & Bengtsson, 2001).

Apart from ketoprofen, propranolol and thiacloprid, all selected substances were detected in the water of the "Bataoto"-drain above their LOQ (limit of quantification, Moeder et al. 2017. Most of the concentrations did not exceed 0.1  $\mu$ g L<sup>-1</sup> except for the artificial sweetener acesulfame (0.453  $\mu$ g L<sup>-1</sup>) but it is low compared to concentration between 10  $\mu$ g L<sup>-1</sup> and 100  $\mu$ g L<sup>-1</sup> detected in wastewater influents and river water (Castronovo, 2017; Kahl, 2018). Its long lasting presence in aqueous environment is caused by an inefficient biodegradation that needs special degrader and conditions (Kahl et al., 2018; Belton et al., 2019), but toxicological effects are unknown for crustacean. As shown in Table 1, the concentrations of

As shown in Table 1, the concentrations of most target pollutants decreased slightly from inlet to the ponds 1-3, whereat dilution by sea water and degradation seem to be the main reasons. Comparable concentrations of acesulfame and bentazone in all three ponds suggested their persistence under the prevailing anoxic conditions.

#### Selected substances in shrimps

While the shrimps in pond 1 contained the highest concentrations of all the target substances, those of ponds 2 and 3 showed smaller concentrations, and not all of the selected compounds were detected above LOD (Fig. 1).

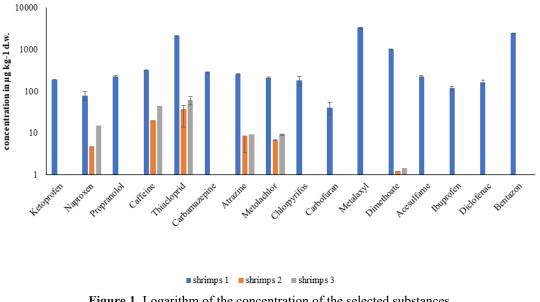


Figure 1. Logarithm of the concentration of the selected substances in shrimps of the different ponds in  $\mu g kg^{-1} d.w.$ 

Thus, the primary source of the pollutants seems to be the inlet of the "Bataoto"- drain. Ketoprofen, propranolol, and thiacloprid were detected neither in the ditch influent nor in the pond's water, but they were found in the shrimps. The analgesic ketoprofen can be bioaccumulated by shrimp but may disappear rapidly in pond water due to its low persistence (98% disappearance within 5 days in river water, Camacho-Muñoz et al., 2019). A long exposure towards low concentrated persistent compounds such as the anti-inflammatory drug naproxen, the insecticide thiacloprid, and the herbicide metolachlor with dissipation half times of 99 d, 1000 d, and 90 d can result in noticeable uptake by shrimp (PPBD; Näslund et al., 2017; Xie et al., 2015).

For pond 1 with the highest concentration of pollutants, a direct relation between water concentration and concentration in the shrimps became obvious (Table 1). It is known that pollutants with neutral properties are more efficiently absorbed by organisms than ionic compounds like diclofenac, naproxen and ibuprofen. Thus, the environmental conditions such as pH predestine the uptake behavior of ionic pollutants as in the case of acesulfame. At pH 8.9 in the pond water, acesulfame exists in about 39% as anion and 61% as neutral molecule (estimated by ACD "Perseptive" program) and was taken up by the shrimp despite its highly polar and anionic nature (Trapp, 2004). The shrimps in ponds 2 and 3 contained only 6 of the 16 target pollutants although some of them were detected in the pond water at low concentrations. Thus, the

uptake of the different pollutants by shrimps depends on the pollutant concentration in water, and the physico and microbial degradation conditions in water. The physico-chemical properties of the pollutants influence their bioavailability; and the health and developmental status of the shrimps influence their uptake efficiency. The shrimp bought from the supermarket contained some target pollutants, such as carbofuran (111.27 ng kg<sup>-1</sup> d.w.), ketoprofen (198.62 ng kg-1 d.w.), and caffeine (1390.75 ng kg<sup>-1</sup> d.w.) at higher concentrations than those of the "Bataoto" shrimp farm. Therefore, it was assumed that the shrimps of the supermarket declared as seawater shrimp had contact with domestic wastewater and agricultural runoff. Particularly complex mixtures of organic pollutants incorporated in aquatic animals can cause developmental and health problems (Stancova et al., 2014; Yokota et al., 2018), and secondary infections due to an impaired immune system (Näslund et al., 2017). At least, the acute toxic properties of selected substances e.g. chlorpyrifos and thiacloprid can trigger health risks for the cultivars and the natural ecosystems in the adjacent coastal areas (Sucahyo et al., 2008).

# Coliforms in the ditch, pond water, and shrimp samples

Among the seasonality of the occurrence of coliforms in the ditch water depending on temperature and dissolved oxygen, bacteria and viruses continuously enter the ditch via untreated domestic

| Water sample      | Total coliforms | Fecal coliforms       | Shrimp in pond | Total coliforms                       | Fecal coliforms |  |
|-------------------|-----------------|-----------------------|----------------|---------------------------------------|-----------------|--|
| water sample      | (CFU 10         | 00 mL <sup>-1</sup> ) |                | (number of colonies g <sup>-1</sup> ) |                 |  |
| Pond 1            | 30              | 10                    | 1              | 4800                                  | 640             |  |
| Pond 2            | 50              | 20                    | 2              | 50                                    | 20              |  |
| Pond 3            | 30              | 0                     | 3              | 1850                                  | 465             |  |
| "Bataoto" – ditch | 1700            | 600                   | —              | —                                     | -               |  |

 Table 2. Results of the microbiological analyses of the pond water, the "Bataoto"- ditch water and the shrimp in the different ponds

wastewater. Thus, they serve as marker for the input of untreated wastewater. As reported by Ahumada-Santos et al. (2014), vegetated drainage ditches are able to mitigate pathogens to a certain extend. As mean of a one-year observation over a distance of 3.6 km total (TC), fecal coliforms (FC) were reduced by 86% but coliform bacteria were still present in the ditch water at TCs  $770 - 9.4 \times 10^4$ CFU (colony forming units) 100 mL<sup>-1</sup> and FC at 100 to 2.7×104 CFU 100 mL<sup>-1</sup> and moved downstream. Thus, the load of coliforms and pathogens may affect the health of cultivars in aquafarms receiving this ditch water. The number of coliforms in the "Bataoto"-drain, which fed the shrimps ponds of the studied farm, was TC at 1.7×10<sup>3</sup> CFU 100 mL<sup>-1</sup> and FC at 600 CFU 100 mL<sup>-1</sup>, proving that this ditch received untreated wastewater (Table 2).

The number of coliforms in the different shrimp ponds agreed with the Mexican Norm NOM-242-SSA1-2009 representing a microbiological quality of water that is adequate for aquaculture. The rather low content of dissolved oxygen (0.31-1.39 mg L<sup>-1</sup> in the ponds could be beneficial for reducing coliform bacteria. Despite the homogeneity of the pond water, the number of coliforms in shrimps varied clearly (Table 2) but coliforms are normal intestinal inhabitants, and the whole shrimp was used for the coliform analysis, but bioaccumulation of coliforms has been described in crustacean recently (Farrapeira et al. 2010). Only the shrimp of pond 2 matched with the Mexican Norm NOM-242-SSA1-2009 (maximum limit 443 CFU/g) and could be considered microbiologically safe. A high load of coliforms in shrimp may affect their health, and the owners of the shrimp farm observed a higher shrimp lethality in the studied season.

First detected in China in 2009, farmed juvenile shrimps suffered from a disease with a high mortality (Putth and Polchana, 2016). The Early Mortality Syndrome (EMS) or acute hepatopancreatic necrosis spread over Vietnam, Malaysia, Thailand, also to Mexico. *Vibrio parahaemolyticus* was finally identified as the trigger of EMS and regulations for better farming practices were introduced. EMS was mainly observed in ponds with pH range between 8.5 and 8.8 which corresponds with the measured pH values in the shrimp ponds in the conducted study. Thus, a good water quality and a high sanitation status in the ponds are essential to reduce the risk for an outbreak and spread of EMS and other diseases (Morales-Covarrubias, 2009, FAO, 2020).

The bacteria strain Vibrio parahaemolyticus causing the EMS disease was not identified in the conducted study, but other bacteria, such as Enterobacter agglomerans, Hafnia alvei, Klebsiella ozonae, Serratia liquefaciens, and Escherichia coli in pond 1; Hafnia alvei, Enterobacter agglomerans, Providencia stuartti, and Ewingella americana in pond 2; and in pond 3: Escherichia coli, Providencia stuarti, and Yersinia enterocolitica. Salmonella spp. was not detected in the "Bataoto" shrimp, although often reported as inherent part of the microflora of brackish cultured shrimp and linked with major concern for consumers, (Okocha et al. 2018). Most bacteria identified previously in the "Michoacana" drain (Ahumada-Santos et al., 2014) were also found in the shrimp of the "Bataoto" farm. Providencia stuartti, Ewingella americana, and Yersinia enterocolitica have been already isolated from marine organisms (Ababouch et al. 1991; Farrapeira et al. 2010; Ripabelli et al. 2004) which can reach the shrimp ponds during the tidal exchange with seawater.

In case of disease outbreaks, antibiotics are the preferred and increasingly used tools to treat the cultivars (Schar et al. 2020). Some antimicrobial reagents and drugs, such as oxytetracyline are still approved for use in aquaculture by e.g. the U.S. Food and Drug Administration (FDA); others, like chloramphenicol, nitrofuran and fluoroquinolones have been prohibited but their application is sometimes tolerated due to missing alternatives to prevent or treat diseases and to protect wild populations (Bôto et al., 2016). As consequence of less controlled use of antibiotics, the spread of antimicrobial resistant bacteria increases rapidly and risks for human health become of global concern (Okocha et al., 2018; Thornber et al., 2020).

#### Selected antibiotics in shrimps

As shown in Figure 2, the concentrations of the selected antibiotics in the shrimps were found much lower than those of the micro pollutants in ponds 1-3 and all were detected also in the shrimps of the supermarket.

Amoxicillin one of the most used antibiotics worldwide was detected slightly higher in the "Bataoto"- shrimps (Bottoni et al., 2005). However, this antibiotic is quite instable in water ( $DT_{50}$  0.27–0.57 days, Cycoń et al., 2019). The prohibited antibiotics flumequine and ciprofloxacine were detected in the shrimps with concentrations between 1.4 to 2.5 µg kg<sup>-1</sup> d.w. whereat ciprofloxacine was found significantly higher in the shrimps of the "Bataoto"-farm. Flumequine and chloramphenicol were detected in both types of shrimps at comparable concentrations of 1.4 µg kg<sup>-1</sup>and 1.3 µg kg<sup>-1</sup> d.w.

Muñoz et al. (2010) reported for a Spanish marine fish farm e.g. for flumequine  $3.5 \times 10^{-5} \ \mu g \ kg^{-1}$  in fish at a water concentration of 0.13 ng L<sup>-1</sup>, and atrazine was found in the fish at  $1 \times 10^{-3} \ \mu g \ kg^{-1}$  at 0.2–1.5 ng L<sup>-1</sup>. Both examples exhibited lower concentrations in the cultivated fish than were found in the shrimps.

Pharmacokinetic investigations of ciprofloxacine in shrimp *Litopenaeus vannamei* (muscle tissue) reported a clearance time of about 4 days (Flores-Miranda et al., 2012). When 200 mg kg<sup>-1</sup> of enrofloxacine was applied via shrimp feed, 30 to 360 µg kg<sup>-1</sup> ciprofloxacine were found in muscle and hepatopacreas tissue. The concentration of ciprofloxacine determined in the "Bataoto"-shrimp was much lower by factor 10 to 100. Thus, a treatment with enro- or ciprofloxacin in the "Bataoto"farm was not assumed or was carried out weeks ago. More probable was the exposure to the antibiotics contained in the drain water feeding the shrimp ponds. As Ahumada-Santos et al. (2022) reported, antibiotic-resistant bacteria (E. coli) occur in draining ditch water in Sinaloa. It was found that 47.5% of the bacterial strains were resistant and 5.9% even multidrug resistant, particularly to  $\beta$ -lactam (39.6%) and quinolone (9.9%) such as ciprofloxacin.

Antibiotics find their way into environment via municipal wastewater e.g. ciprofloxacine 3-5 µg L<sup>-1</sup> (Ellingwood and Sanchez, 2010), with common feed for shrimp farms (Chen et al. 2015), or the prophylactic use of antibiotics during the shrimp breeding process (Islam et al., 2014). Compared to the concentrations found in refused entry lines of shrimps (e.g. 114 µg kg<sup>-1</sup> ciprofloxacine, FDA, 2021), the antibiotics determined in the "Bataoto" shrimps seem to be more associated with the exposure to contaminated pond water. In both, the "Bataoto"- shrimps and the shrimps from the supermarket, chloramphenicol was determined at concentrations of 0.65  $\mu$ g kg<sup>-1</sup> and 0.46  $\mu$ g kg<sup>-1</sup>, respectively. Even low concentrations are of concern and associated with risks for human health and the spread of antibiotic resistance currently considered as an important threat to global health (Hatosy and Matiny, 2015; Hanekamp 2020).

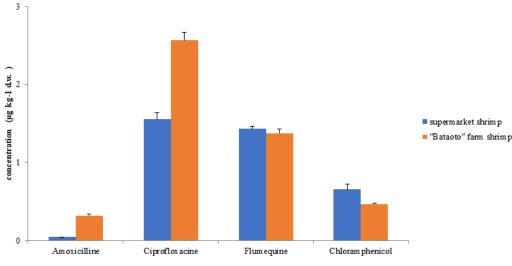


Figure 2. Selected antibiotics detected in shrimps of the "Bataoto"-farm shrimp of pond 3 and of shrimps bought from a German supermarket

# CONCLUSIONS

The cultivation of shrimp as fast growing industry generates problems in land use, water management and impacts natural habitats. The shrimp farm studied in Sinaloa received water from an agricultural draining ditch that contained untreated municipal wastewater from adjoining municipalities among pesticides released from the fields. Thus, wastewater related pollutants such as pharmaceutical residues, food ingredients and coliforms were introduced into the drain water that feeds a shrimp farm, and the cultivated shrimps were exposed to a great variety of biological active pollutants. The shrimp investigated in the conducted study contained traces of the selected pollutants and even antibiotics but the low concentrations suggested their exposure to contaminated drain water and less to an intentional application of antibiotics in the farm.

When in 2022, at least in the European Community, new regulations coming into force that prohibit all routine and prophylactic applications of antibiotics in (aqua)farming (Regulation (EU) 2019/6 on Veterinary Medicines, Regulation (EU) 2019/4 on Medicated Feed), other strategies than antibiotic use have to be established as for instance an improved quality and sanitation status of the water in the farm ponds. An efficient wastewater treatment in rural areas and in the shrimp farms would help to reduce the spread of anthropogenic pollutants and pathogens, contribute to more healthy cultivars, less demand to apply antibiotics, reduce risks for consumers, and the formation of superbugs.

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