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# **Emerging Contaminants (Acetaminophen, Oxytetracycline, Sulfamethoxazole and Trimethoprim) Detected in Aquaculture Sites in Java, Indonesia**

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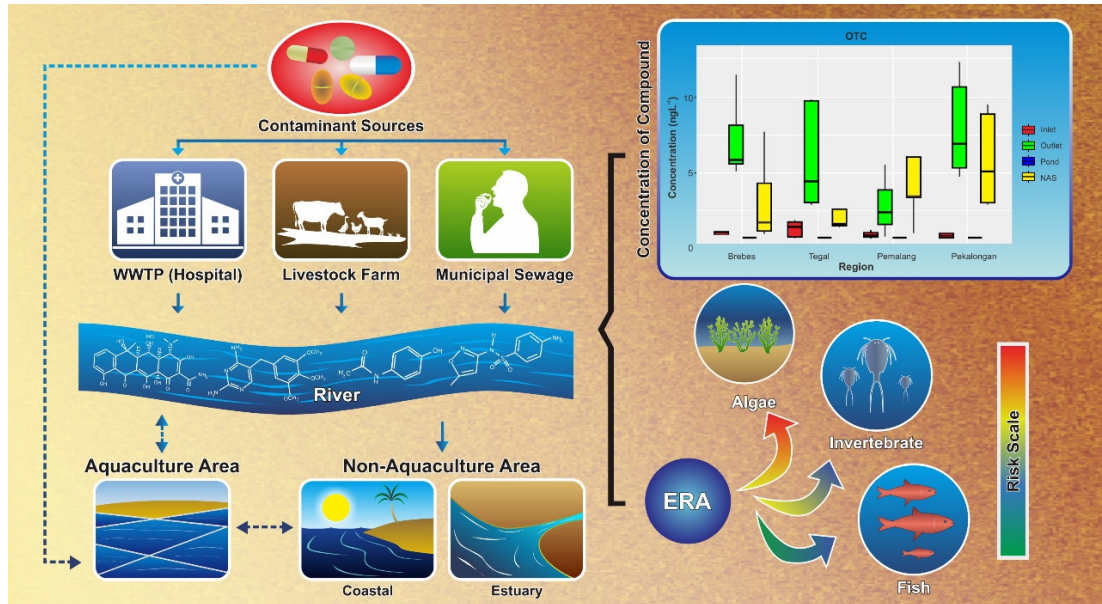
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# Graphical Abstract



## HIGHLIGHT

- High level and detection frequencies of oxytetracycline were found in outlet canal of aquaculture sites.
- Oxytetracycline posed a low risk for invertebrates and fish, and a high risk for algae.
- We found all targeted compounds in the nonaquaculture sites.
- Aquaculture wastewater, WWTP (hospital), municipal sewage (self-medication), and poultry and livestock farm were potential contaminants sources.

## Abstract

Pharmaceuticals of emerging concern (acetaminophen (ACM), trimethoprim (TMP), oxytetracycline (OTC), and sulfamethoxazole (SMX)) were detected in water samples from aquaculture environments and nonaquaculture sites in four regions located on the northern coast of Central Java. ACM was the most prevalent pharmaceutical, with a mean concentration ranging from not detected (n.d.) to  $5.5 \pm 1.9 \text{ ngL}^{-1}$  (Brebes). Among the target antibiotics (TMP, OTC, SMX), OTC was the most ubiquitous, with a mean concentration varying from n.d. to  $8.0 \pm 3.3 \text{ ngL}^{-1}$ . Correlation analysis demonstrated that there was a significant correlation between TMP and SMX concentrations. Based on ecological risk assessment evaluation, the use of OTC requires serious consideration, as it presented high health risks to algae, while ACM, TMP, and SMX posed an insignificant to moderate risk to algae, invertebrates, and fish. The findings obtained from this study highlight OTC as an emerging contaminant of prominent concern. More attention needs to be given to managing and planning for the sustainable management of shrimp farms, particularly in the northern part of Central Java.

**Keywords:** *emerging contaminants; Litopenaeus vannamei; ecological risk; antibiotics; matrix effect.*

## 1. INTRODUCTION

In the last few years, pharmaceuticals as emerging trace contaminants have become a matter of great concern due to their widespread distribution in the aquatic environment (Dhodapkar and Gandhi, 2019; Sangion and Gramatica, 2016), their pseudopersistence and high biological activity (Desbiolles et al., 2018; Fernández et al., 2014b; Verlicchi and Zambello, 2014), and their potential threat to aquatic life and human health (Chavoshani et al., 2020). These compounds enter the aquatic environment from multiple sources, such as municipal sewage, wastewater treatment plants (WWTPs) (Calisto and Esteves, 2009; Felis et al., 2020; Philip et al., 2018; Piram et al., 2008; Silva et al., 2011), effluents from hospitals and the pharmaceutical industry (Cardoso et al., 2014; Oliveira et al., 2015), livestock wastewater (Cycoń et al., 2019), and the utilization of animal manure as a fertilizer in the agriculture sector, animal waste storage, and disposal tanks (FAO & IWMI, 2018). (Felis et al., 2020; Scott et al., 2018) also reported that aquaculture activity is recognized as a main entry pathway for the release of pharmaceuticals into surface water.

Human and veterinary pharmaceuticals have been detected in the aquaculture environment (Burket et al., 2018; Kim et al., 2017; Xie et al., 2019). These pharmaceuticals have also been detected in groundwater (K'Oreje et al., 2016; Lapworth et al., 2012), river surface water (Philip et al., 2018; Rivera-Jaimes et al.,

2018; Silva et al., 2011; Wang et al., 2017), an estuary (Aminot et al., 2016), and coastal and sea waters (Ali et al., 2017; Brumovsky et al., 2017; Tahrani et al., 2015).

The presence of antibiotics in the aquatic environment represents a significant concern with respect to the development of antibiotic-resistant strains of bacteria (Cycoń et al., 2019; Manage and Liyanage, 2019). Furthermore, pharmaceuticals could pose a risk for nontarget aquatic organisms (Fent et al., 2006; Yan et al., 2014). Low- to high-risk pharmaceuticals are potentially adverse for aquatic organisms. Many acute and chronic effects due to the exposure of nontarget organisms to environmental concentrations of pharmaceutical residues have been observed (Guo et al., 2019; Oliveira et al., 2015). Thus, evaluating the potential environmental risk of pharmaceuticals needs to be performed.

From the aquaculture point of view, it is essential to evaluate antibiotic occurrence in the aquaculture environment, since aquaculture is one of the global prime industries and is becoming an essential sector for solving the challenge of global food security. The aquaculture industry is expanding rapidly, accounting for 46% of global fish and shrimp production in 2018. Of this production, 37.6% was mariculture and coastal aquaculture production, and Indonesia has become the second major shrimp producer in the world (FAO, 2020), with shrimp (*Litopenaeus vannamei*) becoming the main species of interest.

Several diseases have been reported in cultured *L. vannamei* in many countries, leading to a decrease in shrimp production and eventual global economic losses (FAO, 2013). As a consequence, antibiotics have been widely used to prevent and fight infectious diseases in aquatic organisms cultivated on a commercial scale (Chen et al., 2017; Nogueira-Lima et al., 2006; Vaughan et al., 2018). Although antibiotic application in aquaculture has been restricted and only a few antibiotics are licensed, it has been reported that some antibiotics are still detected in aquaculture farms in Vietnam (Braun et al., 2019; Thi Kim Chi et al., 2017), India (Saxena et al., 2018), China (Yuan et al., 2019), and Indonesia (Suprpto et al., 2015).

The presence of various pharmaceuticals in the aquatic environment has been reported worldwide; however, information and publications on pharmaceutical distribution in the aquatic environment in Indonesia are still lacking. To fill these gaps, four antibiotics (trimethoprim, TMP; oxytetracycline, OTC; sulfamethoxazole, SMX, and erythromycin, ERY) and an analgesic drug (acetaminophen, ACM) were chosen as our model target compounds. TMP, OTC, SMX, and ERY were selected for their

high utilization as both human and veterinary antibiotics (Alavi et al., 2015; Gu et al., 2019; Quaik et al., 2020; Wang et al., 2017), including in aquaculture activities (Choi et al., 2020; Holmstrom et al., 2003; Liu et al., 2017; Shimizu et al., 2013). Even though ACM is not used in aquaculture, we selected this drug as our target compound, as ACM is the most widely used over-the-counter pain reliever and is commonly used worldwide, including in Indonesia (Khetan and Collins, 2007; Kim et al., 2007; Perkins, 2006). The high potential ecological risks of ACM (Kim et al., 2007) and its toxicity to shrimp (Sung et al., 2014) also attracted our attention to its occurrence in aquaculture sites. Furthermore, it was also of interest to determine the presence of ACM in aquaculture sites since ACM can react with chlorine to form multiple products, two of which have been identified as the toxic compounds N-acetyl-p-benzoquinone imine and 1,4-benzoquinone. The latter is a toxicant related to lethality in ACM overdoses (Fernández et al., 2014a; Khetan and Collins, 2007). Chlorine is applied routinely to many shrimp ponds in Thailand (Husnah, 2001), and Indonesia (Mustafa et al., 2010; Putra and Manan, 2014; WWF-Indonesia, 2014) to disinfect water in storage ponds.

Therefore, this study aimed to determine the selected pharmaceuticals, namely, ACM, TMP, OTC, SMX, and ERY in water samples taken from aquaculture sites in Java based on an improved method. In this regard, we investigated and optimized the parameters influencing the matrix effect and extraction recovery of the solid-phase extraction procedure as well as optimizing LC-Q-TOF/MS analysis by modifying different ionic/acidic conditions in the mobile phase. Last, to the best of our knowledge, this study is the first work reporting the occurrence of these compounds in shrimp farming areas in Indonesia, as well as their potential ecological risk.

## **2. MATERIALS AND METHODS**

### **2.1. Chemicals and Reagents**

ACM, TMP, OTC, SMX, and ERY were purchased from Sigma-Aldrich (St. Louis, MO, USA), while the deuterated labeled compounds used as surrogate standards (acetaminophen-d4, sulfamethoxazole-d4, and trimethoprim-d3) and the internal standard (metoprolol-d7) were obtained from Dr. Ehrenstorfer GmbH, Germany. Acetonitrile (ACN) and water of Optima™ LC/MS Grade, formic acid (99.99%), acetic acid (100%) (ACS reagents) and ammonium acetate were obtained from Fisher Scientific (France), PTFE 0.22 µm syringe filters were from Thermo Fisher (USA), and disodium ethylenediamine tetraacetate (Na<sub>2</sub>EDTA) was from VWR (France). Glass fiber filters (GF/A, 1.6 µm and GF/F, 0.7 µm) were purchased from Whatman

(Maidstone, England). Oasis hydrophilic-lipophilic balanced (HLB) solid-phase extraction (SPE) cartridges (6 ml, 200 mg) were purchased from Waters Co. (Milford, MA, USA), while vacuum manifolds were obtained from Phenomenex (Torrance, CA, USA).

Artificial seawater was prepared by diluting artificial salt provided as Pure Ocean expert reef salt (Prodibio, France) in ultrapure water. Ultra-pure water with a resistivity of  $18.2 \text{ M}\Omega\text{cm}^{-1}$  was prepared by a Q5 Milli-Q water purification system, including an ultraviolet radiation source (Millipore, Massachusetts, USA). Each compound's individual stock standard solution was prepared in methanol at a concentration of  $1 \text{ gL}^{-1}$ , while the surrogate and internal standard solutions were prepared at  $100 \text{ mgL}^{-1}$  and stored at  $-20 \text{ }^\circ\text{C}$  in the dark. Working standard solutions were obtained by diluting each stock solution in ACN/Milli-Q water (5/95% v/v).

## **2.2. Study Area and Sampling**

A total of 42 sampling sites were selected from four shrimp producing areas located on the north coast of Central Java (Tegal, Pemalang, Brebes, Pekalongan). These regions were selected because they are the highest producers of shrimp in Central Java. Twenty-two sites were brackish shrimp farming areas, and 20 sites were nonfarming areas and referred to as nonaquaculture sites (NASs), as shown in **Fig. 1**.

Brebes Regency is a shrimp farming center in Central Java covering 14 ha of intensive shrimp farming and 403 ha of extensive shrimp farming. Similar to Brebes, Tegal is a shrimp farming center that has unfortunately shown declining shrimp production caused by the spread of “shrimp white feces” disease and environmental pollution. Pemalang has an aquaculture area of 1728 ha (including milkfish and shrimp farms), with the fisheries sector having the most important contribution to the gross regional domestic product in this region (Wahyuningrat et al., 2018). Pekalongan, with a coastline along the Java Sea of approximately 6 km from west to east, has a brackish farm area of 379 ha, including 752 aquaculture households (BPS, 2018).

## **2.3. Sample Collection and Analysis**

Surface water samples were collected in August 2017 during the dry season at three different sites in each pond, i.e., the inlet, pond, and outlet. The inlet samples were collected before water entered the pond; the pond samples were collected inside the pond, while the outlet samples were collected from a site after the discharge gates



(Hidayati et al., 2020). In the present study, we also collected water samples from 20 nonaquaculture sites. 800 ml of surface water (0.5 m below the surface) was collected at each site with precleaned amber glass bottles. Water samples from each site were prepared as composite samples by mixing water from three subsampling sites.

Water samples were then successively filtered through glass fiber filters (GF/A 1.6  $\mu\text{m}$ ) and GF/F 0.7  $\mu\text{m}$ ). All filtrates were stored in amber glass bottles that had previously been cleaned with methanol and distilled water. Then, the pH value of each sample was adjusted to 7 by using (1 M) HCl or NaOH solutions.  $\text{Na}_2\text{EDTA}$  200 mM solution (prepared in ultrapure water) was then added to the water samples to reach a final concentration of 10 mM (Capdeville, 2011). The samples were finally spiked with a standard surrogate solution before being transported to the laboratory in an insulated box with ice ( $<4$   $^{\circ}\text{C}$ ). The filtered water samples were then extracted using Oasis HLB SPE cartridges based on selected SPE procedures (Capdeville, 2011) with some modifications.

### **Optimization of the Solid-Phase Extraction (SPE) Procedure**

In many studies, SPE is the technique of choice for extracting pharmaceuticals from water samples (Mokh et al., 2017; Paíga et al., 2017; Pavlović et al., 2007); therefore, we employed and developed an SPE procedure for pharmaceutical analyses in water samples. In the present work, we investigated and optimized the parameters influencing the matrix effect and extraction recovery, i.e., (i) the final volume of extract for injection and (ii) the volume of washing solution used after sample loading on the SPE cartridge.

We employed three different final extract volumes, i.e., 200  $\mu\text{L}$ , 250  $\mu\text{L}$ , and 300  $\mu\text{L}$ , to determine a possible reduction in the matrix effect. The matrix effect was assessed according to the work of Paiga et al. (2015). Then, we studied three different volumes of cartridge washing solution, 10 ml, 20 ml, and 30 ml, to determine the possible reduction in the matrix effect as well as a reduction in the loss of the target compounds. The loss of target compounds was determined by comparing recovery rates. Recovery rates were calculated by comparing the peak areas from these samples to those from postextracted blank seawater samples fortified with the target compounds at the same concentration (Caban et al., 2012). Finally, a final sample injection volume of 300  $\mu\text{L}$  and a washing solution volume of 10 ml were selected as the optimal parameters.

### **SPE Procedure**

The filtered water samples were extracted using Oasis HLB SPE cartridges (200 mg, 6 mL) according to the selected procedure. Briefly, SPE cartridges were first successively conditioned with 5 ml of ACN, 5 mL ultrapure water, and 5 ml of 10 mM Na<sub>2</sub>EDTA at a 2 ml/min flow rate. After loading the sample at a 5–10 ml/min rate, two aliquots of 5 mL of ultrapure water were used to rinse the sample bottle twice and were then loaded onto the SPE cartridge. Then, the cartridges were dried for 1 h under vacuum to remove water. Vacuum manifolds (SUPELCO) were used for the SPE extraction. Five milliliters of ultrapure H<sub>2</sub>O/ACN 10/90 (% v/v) was used to elute the target compounds from the cartridges into a collection vial. The extract was evaporated under a gentle stream of nitrogen at 40 °C and redissolved in 0.3 ml of LC mobile phase A. Finally, the solution was filtered through a 0.45 µm filter for liquid chromatography quadrupole time-of-flight tandem mass spectrometry (LC-Q-TOF/MS) analysis.

#### **2.4. Optimization of LC-Q-TOF/MS Analysis**

To determine which mobile phase modifiers enabled the highest ESI-MS response for the analysis of the target pharmaceutical compounds, three different ionic (acidic) modifiers in the mobile phase, i.e., formic acid, acetic acid, and ammonium acetate, were tested. We also studied the analyte stability. The definition of stability was a measured change in the concentration of analytes by the given method (Lawson et al., 1982). In the present study, we examined the effect of the selected acidic modifier concentration in the mobile phase on the stability of target compounds. We employed multiple measurements over time, and the experiment was designed to repeatedly measure all analytes within one mixed standard at 9 different concentrations points.

#### **LC-Q-TOF/MS Analysis**

Chromatographic separation was performed by using an Agilent 1290 Infinity UHPLC system (Agilent, Palo Alto, USA) consisting of a vacuum degasser, an autosampler, a binary pump, and a thermostated column oven equipped with an Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 mm × 50 mm, 1.8 µm). Mass spectrometric detection was performed using an Agilent 6530 Accurate-Mass Q-TOF/MS (Palo Alto, CA, USA).

An Agilent Jet Stream Technology electrospray ionization source in positive mode (ESI+) was operated to analyze the target compounds with the following parameters: dry gas (N<sub>2</sub>) temperature, 325 °C; dry gas flow rate, 7 L min<sup>-1</sup>; nebulizer pressure,

45 psi; sheath gas temperature, 325 °C; sheath gas flow rate, 11 L min<sup>-1</sup>; capillary voltage, 3500 V; and nozzle voltage, 500 V. Data acquisition was executed in TOF MS full scan mode over a mass range of 50–1,200 m/z with an accuracy of <2 ppm. In addition to full scan spectra, product ion scan spectra of each target compound were acquired simultaneously using auto MS/MS mode. Internal mass correction was performed by continuous infusion of an Agilent reference mass calibration solution containing purine (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>), which provides m/z 121.0509, and hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazine, which provides m/z 922.0098, in positive ionization mode.

The mobile phase consisted of eluent A (0.5% acetic acid (AA) in 95/5% water/ACN, v/v) and eluent B (95/5% ACN/water, v/v, containing 0.5% A). The following gradient program was implemented: 0 min - 0% B; 10 min - 40% B; 10.10-12 min - 100% B; and 13.0-14 min - 0% B. The flow rate and injection volume were 0.4 mL min<sup>-1</sup> and 20 µL, respectively. After each analysis, a wash of the system was carried out for 10 minutes with ACN/water (80/20% v/v). The sample reservoir and column temperature were maintained at 4 °C and 45 °C, respectively.

To ensure the reliability of the data, procedural blanks were included in each batch of experiments. The instrument blank analysis was set up every nine samples to monitor the background signal of the instrument. Calibration standards were analyzed both at the beginning and the end of each sequence, and one calibration standard was analyzed repeatedly throughout the sequence to evaluate signal stability.

## 2.5. Method Validation

The method was validated according to the ICH guidelines of Q2 (ICH Guideline Q2(R1), 2005) in terms of linearity, instrument detection limits (IDLs) and instrument quantification limits (IQLs), selectivity, precision, accuracy, and extraction recovery. Calibration linearity was evaluated by analyzing standards in triplicate at eight concentrations in the range from 2 to 500 µg L<sup>-1</sup>, and satisfactory linearity was achieved with a correlation coefficient ( $R^2$ ) > 0.99. IDLs and IQLs were determined based on the standard deviation of the response and the slope. Taking into account a concentration factor of 2667 (whereby 800 mL of water sample was reconstituted to 0.3 mL for analysis) (Fang et al., 2019), the method of detection limits (MDLs) and the method of quantification limits (MQLs) of each target compound in water samples ranged from 0.29 to 0.96 ng L<sup>-1</sup> and 0.95 to 1.36 ng L<sup>-1</sup>, respectively (**Supplementary materials, SM, Table S1**). Compound selectivity was verified by the ratio of two

MS-MS transition ions per analyte and the accurate masses monitored for these ions. Agilent Mass Hunter Qualitative analysis software (version B.04.00) was used to acquire and process spectra for the accurate mass data of molecular ions. Positive detection of the analyte occurred at retention times within a range of 20% from the average value. **Table S2** shows key MS parameters for the detection of target compounds.

The precision and accuracy of the method were determined by repeated intraday and interday analyses at three different concentrations. The precision was described as the relative standard deviation (% RSD), while accuracy was evaluated by calculating the percent deviation of the nominal concentration and is reported as a relative error (RE). Intra- and interday precisions below 15% and an accuracy between 85 and 115% of the nominal concentrations indicated that target compounds could be determined with reasonable precision and accuracy. Extraction recovery of target compounds was assessed at three concentration levels, with three replicates for each level. The developed method showed the capability to simultaneously extract and enrich target compounds, resulting in excellent recovery rates of ACM, TMP, OTC, and SMX. The recoveries were between  $83.2 \pm 1.2$  and  $96.1 \pm 1.9\%$ , which met the acceptance criteria per method EPA-1694 for the determination of pharmaceuticals and personal care products (PPCPs) in environmental samples (EPA Method 1694, 2007) and (Association of Official Analytical Chemists (AOAC), 2016) for trace analysis.

## 2.6. Ecological Risk Assessment

The ecological risk of pharmaceutical compounds was estimated by applying the risk quotient (RQ) approach. Aquatic organisms from three different trophic levels of the ecosystem (algae/aquatic plants, crustaceans, and fishes) were the essential targets, as recommended by the European Commission (EUR 20418 EN/2) (European Chemicals Bureau-ECB, 2003). The risk quotient was evaluated based on the determination of the measured environmental concentrations (MECs) and the predicted no-effect concentrations (PNECs) and calculated according to the following equation (Biel-Maeso et al., 2018; Fang et al., 2019; Lu et al., 2019; Na et al., 2019):

$$RQ = \frac{MEC}{PNEC} \quad PNEC = \frac{EC_{50}}{AF}$$

where MEC is the measured concentration of pharmaceuticals in a water sample and EC50 is the concentration causing adverse effects on 50% of individuals exposed in a bioassay. PNEC was estimated using the reported lowest values of acute EC50 divided by a default assessment factor (AF). An AF value of 1000 was adopted following the “worst-case scenario” suggestion, following the Technical Guidance Document on Risk Assessment of the European Union (European Chemicals Bureau-ECB, 2003). In addition, the EC50 data from each of the three evaluated trophic levels were available in the Ecological Structure-Activity Relationship (ECOSAR) program (Biel-Maeso et al., 2018) (**Table S3**). The levels of ecological risks were classified into four categories according to the RQ values: insignificant risk ( $RQ < 0.01$ ), low risk ( $0.01 < RQ < 0.1$ ), moderate risk ( $0.1 < RQ < 1$ ), and high risk ( $RQ > 1$ ) (Biel-Maeso et al., 2018; European Commission, 2003).

## **2.7. Statistical Analysis**

Statistical analyses were performed using the R statistics package (v3.5.2, R Foundation for Statistical Computing, Vienna, Austria). Initially, the normality (Shapiro-Wilk normality test) and homogeneity (Bartlett homogeneity test) of the data were checked for parametric analysis, i.e., analysis of variance (ANOVA). A nonparametric analysis (Kruskal-Wallis test) was employed when the data did not meet the parametric test assumptions. Following a significant Kruskal-Wallis test, the Wilcoxon test was performed to explore the differences between the inlet, pond, outlet, and nonaquaculture site samples. The correlations among the four pharmaceuticals were analyzed by Spearman correlation analysis. The Spearman correlation coefficient of determination ( $R^2$ ) and its corresponding P-value were calculated. P values  $< 0.05$  were considered significantly different. Visualization of the antibiotic concentrations using box-and-whisker plots was also performed by using the R statistics package.

## **3. RESULTS AND DISCUSSION**

### **3.1. Optimization of LC-Q-TOF/MS Analysis**

Method development was conducted by employing various ionic modifiers in the mobile phase, i.e., formic acid, acetic acid, and ammonium acetate, at the same concentration, and chromatographic resolution along with ESI-MS response were

evaluated. **Fig. S4** shows the resulting chromatograms of the additive study. Acetic acid (0.5%) showed the greatest sensitivity and resolution for ACM and SMX, while ammonium acetate (0.5%) gave optimal sensitivity for the  $[M+H]^+$  ions of TMP and OTC.

In LC-MS, certain chemicals (organic acids) are often added to the mobile phase to influence analyte ionization for two fundamental reasons: (1) chromatographic separation is optimized in terms of retention and/or peak shape under acidic conditions because any silanol activity is suppressed, and (2) most mass spectrometric measurements are performed in positive ion mode, which is accomplished by the addition of a proton to form the molecular ion  $[M+H]^+$ . Certain organic acids, such as formic acid and acetic acid, have the necessary acidity and volatility to provide an excess of cations for this purpose. Acetic acid was finally selected as the best additive since it provided the best peak sensitivity and chromatographic resolution for ACM and SMX and could still produce good peak shape and sensitivity for TMP and OTC.

To conclude that analytes (pharmaceuticals) are stable under a particular condition, there should be no significant difference between the results for the sample at different time points and a corresponding fresh sample (initial time). To evaluate this, we analyzed samples at nine different time points. Mobile phases of 0, 0.1, 0.5, and 1% acetic acid were evaluated (**Fig. S5**).

The effect of the acetic acid percentage was statistically significant for ERY in the presence of acid, even at the lowest concentration (0.1%). Dehydro-erythromycin (ERY-H<sub>2</sub>O), a primary degradation product of ERY, might be formed during analysis, resulting in a rapid decrease in ERY concentration. As reported by Zhou et al. (2012), erythromycin is a weak base and unstable under acidic conditions, and ERY-H<sub>2</sub>O was obtained by adjusting the pH of an erythromycin solution to 3.0. A similar tendency was reported by Yang and Carlson (2004) and Terzic and Ahel (2011); that is, by using acidic eluents, approximately 10% of ERY was transformed into ERY-H<sub>2</sub>O. In contrast, OTC was stable at the highest percentage of acetic acid (1%). OTC is relatively stable under acidic conditions but not under basic conditions (Zhou et al., 2012). Finally, 0.5% ionic modifier was selected for subsequent analyses.

### **3.2. Optimization of SPE Procedure**

To select the most effective procedure (highest analyte recovery and lowest matrix effect), different volumes of washing solution during the preconcentration SPE step

and different volumes of extract were tested. Matrix effects (caused by the matrix coextracted with the analytes) are major concerns in quantitative LC-MS analysis since they detrimentally affect the accuracy, precision, and sensitivity of a method. The matrix complexity can cause signal suppression or enhancements during ESI, leading to false positive or negative results (Krakkó et al., 2019). Therefore, the presence of matrix effects needs to be evaluated during method development to achieve reliable analytical data. The practices commonly applied to overcome this problem are the use of matrix-matched calibration curves, which attempt to eliminate large matrix effects during the analysis and quantification process (Paíga et al., 2015; Paíga et al., 2017), the optimization of clean-up procedures by increasing the volume of washing solution (Wu et al., 2010) or the acidification of the washing solvent (Gonzalez-Gaya et al., 2018).

In this study, optimization of the clean-up procedure was our first choice and was implemented by testing three different final volumes of samples for injections (200-250-300  $\mu$ L). **Fig. S6** shows the matrix effect (%) as the effect of the final volume of extract. Ion signal enhancement for oxytetracycline and ion signal suppression for other compounds were found. The results showed that a final sample injection volume of 300  $\mu$ L was the optimal volume.

In the determination of organic compounds in brackish and seawater with LC-MS, the matrix effect resulting from salinity and the content of the suspended matter of these samples affects not only the sorption efficiency of (and analyte recovery from) SPE cartridges but also the ionization efficiency of ESI (Borecka et al., 2015). The authors found matrix effects of 54.23 for TMP and 56.49 for SMX from seawater samples collected from the Baltic Sea.

It must be kept in mind that after sample loading (during the preconcentration step), the SPE cartridge is washed with deionized water. In most studies, deionized water could altogether remove the impurities retained in the SPE cartridge (Kim et al., 2018). Thus, it is evident that the volume of deionized water as a washing solution played a crucial role in eliminating the matrix effect from seawater sample analysis when SPE combined with LC-Q-TOF/MS was employed. However, there is a possibility that target compounds will be lost; therefore, the relationships between the extraction recovery and washing solution volume must be investigated in relation to the matrix effect. Therefore, we tested three different volumes of washing solution, i.e., 10, 20, and 30 ml of ultrapure water. In this experiment, we investigated the elimination of the matrix effect, the effect of the sample solution volume and the possible loss of the

target compounds as the effect of the washing solution volume. The relationship between the extraction recovery, matrix effect, volume of extract and washing solution volume is displayed in **Fig. S7**.

A washing step is always recommended to flush interfering compounds from the sorbent while ensuring that the target analytes are immobilized until the elution step (Hernández et al., 2007). However, the increase in washing solution volume strongly influenced the extraction recovery of ACM, TMP, and OTC. There was concomitant loss of the target compounds indicated by the decrease in extraction recoveries. Compared to 10 ml of washing solution, a volume of 30 ml resulted in compound losses (%) of  $11.7 \pm 1.9$ ,  $7.3 \pm 1.1$ , and  $3.9 \pm 1.1$  for ACM, TMP, and OTC, respectively. A 10 ml washing solution volume was finally selected for subsequent analyses to maintain sufficient extraction recovery.

Based on the result of this optimization step, the matrix effect cannot be completely eliminated. Thus, we used an isotopically labeled standard to account for the matrix effect. As stated by the US EPA (2014) and FDA (2015), internal standards are used to account for matrix effects and/or variability in instrument response by normalizing the response of the target analytes and surrogates, thereby decreasing measurement bias. The isotopically labeled internal standard approach has also been recognized as the most effective approach to compensate for the matrix effect and avoid inaccurate quantification (Cortese et al., 2020; Gros et al., 2012; Paíga et al., 2015). Therefore, in this study, internal standard calibration using the isotopically labeled standard metoprolol-d7 was used to account for matrix effects.

### **3.3. The Occurrence of Pharmaceuticals Compound in Water**

**Fig. 2** presents the distribution of targeted pharmaceuticals in the farm (inlet-pond-outlet) and nonaquaculture sites along Central Java's northern coast. In general, the analgesic pharmaceutical ACM and the antibiotic OTC were detected most frequently (>80%) and in all regions.

The highest concentration of ACM was found in Brebes Regency, with values ranging from n.d. to  $11.3 \text{ ngL}^{-1}$  in the aquaculture area, while the ACM concentration in the nonaquaculture areas ranged between 2.1 and  $11.3 \text{ ngL}^{-1}$ . For Brebes, ACM was identified as the most ubiquitous pharmaceutical in Tegal (aquaculture area: n.d.- $9.2 \text{ ngL}^{-1}$ ; nonaquaculture areas: n.d.- $9.6 \text{ ngL}^{-1}$ ), Pemalang (aquaculture area: n.d.- $9.5 \text{ ngL}^{-1}$ ; nonaquaculture areas: n.d.- $9.8 \text{ ngL}^{-1}$ ), and Pekalongan (aquaculture area: n.d.- $9.5 \text{ ngL}^{-1}$ , nonaquaculture areas: n.d.- $10.9 \text{ ngL}^{-1}$ ). There was no significant



difference between ACM concentrations in aquaculture areas and nonaquaculture sites in all studied regions, suggesting widespread contamination of aquatic areas.

ACM, also named paracetamol, is used worldwide as an analgesic medicine (WHO, 2019) and is widely available in many prescription and nonprescription medications. FAO & IWMI (2018) reported that pharmaceuticals are released into the aquatic environment from a wide range of sources, such as wastewater treatment plants (WWTPs), agricultural fields, and the veterinary use of medicines in livestock production activity. Generally, conventional WWTPs are not designed to remove pharmaceuticals so that pharmaceuticals can enter water bodies directly or via surface runoff (OECD (2019). Furthermore, OECD (2019) reported that, globally, it is generally accepted that the main route for human pharmaceuticals to the aquatic environment is via the discharge of untreated or treated wastewater from households, particularly for painkiller and anti-inflammatory medicines (BIO Intelligence Service, 2013; Daughton and Ruhoy, 2009). These pharmaceuticals are excreted via urine and feces and then finally reach the water. In this study, the level of ACM was higher than those reported in the mariculture sites of the Pearl River Delta around the Pearl River estuary, Hong Kong ( $0.02 \text{ ngL}^{-1}$ ) (Xie et al., 2019), comparable to the levels detected in the aquaculture area in the western Portuguese Coast (Aveiro), Portugal ( $12 \text{ ngL}^{-1}$ ) (Gadelha et al., 2019) and cage culture areas in the Xiangjiang River Basin, China (n.d.- $16 \text{ ngL}^{-1}$ ) (Lin et al., 2018), but much lower than those in surface water in Mexico ( $354 - 4460 \text{ ngL}^{-1}$ ) reported by Rivera-Jaimes et al. (2018).

In the present study, among the three targeted antibiotics, OTC was the most abundant, irrespective of the studied regency. In Brebes, the concentration of OTC varied from n.d. to  $11.5 \text{ ngL}^{-1}$  in the farm area, while that in nonaquaculture sites ranged between n.d. and  $7.7 \text{ ngL}^{-1}$ . Similarly, OTC was the dominant antibiotic in Tegal (aquaculture area: n.d.- $9.7 \text{ ngL}^{-1}$ , nonaquaculture areas:  $1.4-5.8 \text{ ngL}^{-1}$ ), Pemalang (aquaculture area: n.d.- $5.5 \text{ ngL}^{-1}$ , nonaquaculture areas:  $1.0-12.9 \text{ ngL}^{-1}$ ), and Pekalongan (aquaculture area: n.d.- $10.6 \text{ ngL}^{-1}$ , nonaquaculture areas:  $2.9-9.5 \text{ ngL}^{-1}$ ) (**Table S8**).

The detection of OTC in shrimp farms suggests the application of this compound in aquaculture activities. Pond-based farms located in coastal areas have been identified as a source of antibiotics in the environment through the discharge of wastewater (Gaw et al., 2014; Le and Munekage, 2004). Antimicrobial use for disease prevention is a common practice in aquaculture systems, with OTC identified as one of the most frequently used antibiotics (Chiesa et al., 2018; Chuah et al., 2016; Kim et

al., 2018; Leal et al., 2019; Nogueira-Lima et al., 2006; Rigos et al., 2004; Tuševljak et al., 2013). In South Korea, OTC has a high consumption rate, and its use in domestic fisheries accounts for 80% of total antibiotic use (Kim et al., 2017). OTC, as the agent for the treatment and prevention of infectious diseases in fish and shrimp farms, was also found in Indonesia's aquaculture systems (A'yunin et al., 2019; Hatmanti, 2003). There was one reported incident where OTC residues were detected in a shipment of shrimp exported to Japan, which resulted in the shipment being rejected (FAO/NACA, 1995). OTC applications have also been reported in shrimp aquaculture regions in Thailand (Holmstrom et al., 2003), Vietnam (Shimizu et al., 2013), and China (Liu et al., 2017).

In the present study, the OTC levels in the aquaculture areas were significantly different among inlet, pond, and outlet samples. The highest level of OTC was found in the outlet canals, with mean concentrations of  $7.2 \pm 2.7 \text{ ngL}^{-1}$ ,  $5.9 \pm 3.6 \text{ ngL}^{-1}$ ,  $2.8 \pm 1.0 \text{ ngL}^{-1}$ , and  $8.0 \pm 2.0 \text{ ngL}^{-1}$  in Brebes, Tegal, Pemalang, and Pekalongan, respectively (**Table S9**). The detected concentration of OTC in this study (outlet canals) was nevertheless lower than that in shrimp aquaculture wastewater in Vietnam ( $18 \text{ ngL}^{-1}$ ) (Shimizu et al., 2013).

Regarding antibiotics, the same trends observed in this study were reported by previous works (Choi et al., 2020). These authors also found that among the investigated antibiotics, OTC was the most often detected (93%). Furthermore, the OTC concentration in the effluents (outlet) was higher than that in aquaculture influents. The accumulation of this compound probably caused a higher concentration of OTC in the outlet canal than in the influent due to the water discharge of multiple shrimp ponds. In the study area, most aquaculture farms were designed and constructed with one common discharge canal system receiving water from many ponds. Furthermore, these systems had no wastewater treatment system for the effluent, so the effluents of the aquaculture facilities were likely discharged with little or no treatment into the canal system (**Fig. S10**).

By comparing aquaculture areas to nonaquaculture areas, statistical analysis revealed that generally, there was no significant difference in pharmaceutical concentration between aquaculture areas and nonaquaculture sites in the studied region (except for OTC in Pemalang). It is worth noting that in Pemalang, the locations of the five nonaquaculture sites consisted of two estuaries (Comal estuary and Kaliprau estuary), two rivers (Comal River upstream and Comal River middle stream), and one beach site (Joko Tingkir beach). In addition, flowing through seven districts

within the regency, the Comal River is the largest river in Pemalang and empties into the Java Sea. Several authors have reported that estuarine environments have been dramatically affected by increasing anthropogenic activities because of the large number of contaminants released into estuaries (Guo et al., 2019; Zhao et al., 2015), with antibiotics becoming a great research concern. Although tetracyclines are not persistent organic pollutants (POPs), they are widely used, and consistent inputs arise from various sources, e.g., domestic sewage, livestock areas, and the aquaculture industry, resulting in their occurrence in rivers and estuaries (Chen et al., 2017; Cheng et al., 2013; Gao et al., 2012; Guo et al., 2019; Liu et al., 2016; Luo et al., 2010; Zhou et al., 2011).

The same level of OTC detected in aquaculture sites and nonaquaculture sites indicated that the source of those compounds in the aquaculture environment may derive from varied sources. FAO & IWMI (2018) reported that pharmaceuticals are released into the aquatic environment from a wide range of sources, such as WWTPs (municipal, hospital, industry), the veterinary use of medicines in livestock production activity, and agricultural fields.

It is well documented that WWTPs have been recognized as a primary source of multiple pharmaceuticals entering aquatic environments (Michael et al., 2013; Petrovic et al., 2006; Piram et al., 2008; USGS, 2020). Sources of antibiotics in WWTPs include human use in the community (self-medication) and discharges from hospitals. In the Southeast Asian region, self-medication with antibiotics is broadly practiced, as antibiotics can be purchased and used without a physician's prescription. Antibiotic usage in self-medication in Indonesia is relatively high (Fatmah et al., 2019), and it is a common practice among residents in Pekalongan (Restiyono, 2016). After ingestion, these compounds are excreted by the human body and enter sewage treatment plants or are discharged directly into the aquatic system. Tetracyclines, sulfonamides, and trimethoprim were abundantly detected in the effluent of residential areas (Li, 2014). These authors also detected antibiotics in the effluent of hospitals and a municipal WWTP system. In traditional WWTPs, SMX is only partially remediated, and since it is persistent in the environment, SMX is regularly detected in river surface waters (Peller et al., 2014). Petrovic et al. (2006) also claimed that ACM and TMP were the most frequently detected compounds in WWTPs. This result is also in line with the work of Borecka et al. (2015), who pointed out that SMX and TMP were the most frequently detected compounds in the semienclosed bodies of water close to

the output of WWTPs. Thus, rivers play a crucial role as pharmaceutical residue transport media and eventually reach coastal regions.

Antibiotics are also widely used for disease prevention in global livestock systems and enter the aquatic environment through direct application in aquaculture and manure application (Wang et al., 2017). Antibiotics are primarily added to animal feeds for disease prevention (subtherapeutic levels), treatment (therapeutic levels), and growth promotion (Haller et al., 2002; Van Epps and Blaney, 2016). Farm profitability, disease prevention, and mortality rate reduction were identified as drivers of antibiotic use in livestock systems in Indonesia, Vietnam, and Thailand (Coyne et al., 2019). In 25 European countries, antibiotics are also used in food-producing animals, as reported by Grave et al. (2014) and EMA (2016), with tetracyclines, penicillin, and sulfonamides as the top three most consumed antibiotics. Similarly, Shimazu et al. (2017) found that SMX was the predominant antibiotic in livestock wastewater in Vietnam. Tetracyclines are also presented as the most frequently used in poultry farms in Iran (Alavi et al., 2015), Austria, Egypt (Van Epps and Blaney, 2016), the USA (Gonzalez Ronquillo and Angeles Hernandez, 2017), Albania, Kosovo (Topi and Spahiu, 2020) and China (Yang et al., 2020; Zheng et al., 2020).

In the present study, the two highest detection frequencies of OTC were found in Brebes (40%) and Pekalongan (45%) regencies. Similarly, we found that the highest TMP detection frequency of 40% was in Brebes. It is well known that Brebes is the region with the highest duck population compared to other regions in Central Java (Sumekar et al., 2013). Ducks are a type of waterfowl that are commonly farmed with the primary purpose of producing eggs and meat and grow from rural to suburban areas in Indonesia (Iriyanti et al., 2018). Duck farming areas Brebes are mostly located in the lands along the riverbank and surrounding the aquaculture area, directly connected to the Java Sea (**Fig. S11**). Ducks are also commonly farmed in Tegal Regency, the region with the highest detection frequency of SMX (25%). The occurrence of various diseases frequently reduces duck health and productivity, and antibiotics have long been used to overcome this problem (Iriyanti et al., 2018; Tugiyanti et al., 2017). Hidayah et al. (2018) investigated the use of antibiotics in duck farming in East Java and found that among the types of veterinary medicines, vaccines, and chemicals that are often used in duck farming, antibiotics contributed 18% of the application. Ten types of antibiotics were used, including SMX and TMP. In light of the above, we assumed that poultry farms, particularly duck farming in the Brebes area, were one of the antibiotic sources entering the aquaculture system. As a partial conclusion, duck farming needs to be monitored, and regulation needs strengthening.

Another source of antibiotics in the present study is livestock and poultry waste applications in agricultural practice. Since antibiotics as medicated feed are not completely metabolized, a significant amount of antibiotics are excreted in feces and urine and eventually end up in manure (Gu et al., 2019; Quaik et al., 2020; Wang et al., 2017). The most commonly reported antibiotics in animal manure are fluoroquinolones, sulfonamides, and tetracyclines (Van Epps and Blaney, 2016). Alavi et al. (2015) detected OTC in chicken manure at a concentration up to 13.77 mg kg<sup>-1</sup>, while Topi and Spahiu (2020) found sulfonamides with the highest concentration of 10.1 mg kg<sup>-1</sup> in poultry manure. In Brebes, chicken manure is traditionally widely applied to land as fertilizer in onion farming (Firmansyah et al. (2015), as manure is a nutrient-rich fertilizer and essential for onion production (Sitompul et al., 2017). The correlations among the concentrations of detected pharmaceuticals in four regions were analyzed, and significant positive correlations ( $p < 0.05$ ) were observed between SMX and TMP in Tegal, Brebes, and Pekalongan, suggesting that these compounds shared similar contamination sources (**Fig. S12**).

Haller et al. (2002) reported that TMP is an antibiotic commonly used together with sulfonamide (including SMX) to give a more significant synergistic effect than either of these components individually in treating bacterial infections. Hidayah et al. (2018) also reported the administration of SMX in combination with TMP to prevent and/or treat bacterial disease in poultry.

### **3.4. Ecological Risk Assessment**

In the present study, the obtained results provide information on the potential ecological effects of the target compounds. The potential adverse effects of the detected pharmaceuticals were estimated based on the risk quotients (RQs) and are displayed in **Fig. 3**. According to the calculated RQs, generally, ACM, TMP, and SMX posed a low risk for algae, invertebrates, and fish. In comparison, OTC posed a low risk for invertebrates and fish and a high risk for algae. Philip et al. (2018) also reported that algae were more sensitive than invertebrates and fish to antibiotics. Whereas, algae plays an important role in *L. vannamei* culture as it can improve the immune response of *L. vannamei* against *Vibrio parahaemolyticus* and white spot virus (WSSV) (Anaya-Rosas et al., 2019).

## **4. Conclusion**

The key environmental interactions of coastal shrimp aquaculture are related particularly to the habitat and aquatic resources in shrimp aquaculture areas. Impacts of the surrounding environment on aquaculture, aquaculture on the environment, and aquaculture on aquaculture itself may increase the overall environmental impacts.

The present study demonstrates the widespread occurrence of ACM, OTC, TMP, and SMX in aquaculture surface water and the surrounding area, with ACM and OTC as the most prominent compounds. The relatively high level of OTC detected in the effluent indicated OTC application in aquaculture activities, which have become the source of antibiotics in the aquatic system. However, since we also found all target compounds at the nonaquaculture sites, we concluded that the presence of pharmaceuticals is also derived from many other sources. WWTPs, municipal sewage, poultry farms, and livestock systems can be seen as potential sources of pharmaceutical contamination in water bodies. In this context, aquaculture areas have become impacted by the surrounding environment.

Although the concentrations of the target compounds in the aquaculture surface water were not high, the risk assessment results based on the calculated risk quotients showed that SMX posed a moderate risk for algae and OTC posed a high risk. The findings obtained from this study highlight OTC and SMX as alarming emerging contaminants.

In conclusion, regarding codes of conduct and best management practices in the aquaculture sector, more attention needs to be given to managing and planning strategies for the development of sustainable shrimp aquaculture, particularly in the northern part of Central Java.

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## References

- A'yunin Q., Kartikaningsih H., Andayani S., Arifin N.B. (2019) Efikasi Oxytetracycline Terhadap Kesehatan Ikan Lele yang Diinfeksi Bakteri Edwardsiella tarda. *JFMR - Jurnal Riset Perikanan dan Kelautan* 2.
- Alavi N., Babaei A.A., Shirmardi M., Naimabadi A., Goudarzi G. (2015) Assessment of oxytetracycline and tetracycline antibiotics in manure samples in different cities of Khuzestan Province, Iran. *Environmental Science and Pollution Research* 22:17948-17954. DOI: 10.1007/s11356-015-5002-9.
- Ali A.M., Rønning H.T., Alarif W., Kallenborn R., Al-Lihaibi S.S. (2017) Occurrence of pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea. *Chemosphere* 175:505-513. DOI: <https://doi.org/10.1016/j.chemosphere.2017.02.095>.
- Aminot Y., Le Menach K., Pardon P., Etcheber H., Budzinski H. (2016) Inputs and seasonal removal of pharmaceuticals in the estuarine Garonne River. *Marine Chemistry* 185:3-11. DOI: <https://doi.org/10.1016/j.marchem.2016.05.010>.
- Anaya-Rosas R.E., Rivas-Vega M.E., Miranda-Baeza A., Piña-Valdez P., Nieves-Soto M. (2019) Effects of a co-culture of marine algae and shrimp (*Litopenaeus vannamei*) on the growth, survival and immune response of shrimp infected with *Vibrio parahaemolyticus* and white spot virus (WSSV). *Fish & Shellfish Immunology* 87:136-143. DOI: <https://doi.org/10.1016/j.fsi.2018.12.071>.
- Association of Official Analytical Chemists (AOAC). (2016) Official methods of analysis of AOAC international ((20th ed.), Association of Official Analytical Chemists Inc, Arlington.
- Biel-Maeso M., Baena-Nogueras R.M., Corada-Fernandez C., Lara-Martin P.A. (2018) Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). *Sci Total Environ* 612:649-659. DOI: 10.1016/j.scitotenv.2017.08.279.
- BIO Intelligence Service. (2013) Study on the environmental risks of medicinal products, Final Report prepared for Executive Agency for Health and Consumers.
- Borecka M., Siedlewicz G., Halinski L.P., Sikora K., Pazdro K., Stepnowski P., Bialk-Bielinska A. (2015) Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: Method development and field studies. *Mar Pollut Bull* 94:62-71. DOI: 10.1016/j.marpolbul.2015.03.008.
- BPS. (2018) Pekalongan municipality in figures 2018, BPS-Statistics of Pekalongan Municipality, Pekalongan.
- Braun G., Braun M., Kruse J., Amelung W., Renaud F.G., Khoi C.M., Duong M.V., Sebesvari Z. (2019) Pesticides and antibiotics in permanent rice, alternating rice-shrimp and permanent shrimp systems of the coastal Mekong Delta, Vietnam. *Environment International* 127:442-451. DOI: <https://doi.org/10.1016/j.envint.2019.03.038>.
- Brumovsky M., Becanova J., Kohoutek J., Borghini M., Nizzetto L. (2017) Contaminants of emerging concern in the open sea waters of the Western Mediterranean. *Environ Pollut* 229:976-983. DOI: 10.1016/j.envpol.2017.07.082.
- Burket S.R., Sapozhnikova Y., Zheng J.S., Chung S.S., Brooks B.W. (2018) At the Intersection of Urbanization, Water, and Food Security: Determination of Select Contaminants of Emerging Concern in Mussels and Oysters from Hong Kong. *Journal of Agricultural and Food Chemistry* 66:5009-5017. DOI: 10.1021/acs.jafc.7b05730.
- Caban M., Migowska N., Stepnowski P., Kwiatkowski M., Kumirska J. (2012) Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of beta-blockers and beta-agonists in environmental samples. *J Chromatogr A* 1258:117-27. DOI: 10.1016/j.chroma.2012.08.029.



- Calisto V., Esteves V.I. (2009) Psychiatric pharmaceuticals in the environment. *Chemosphere* 77:1257-74. DOI: 10.1016/j.chemosphere.2009.09.021.
- Capdeville M.-J. (2011) Études Des Cycles Biogéochimiques Des Contaminant Organiques Dits « ÉMERGENTS » dans Les Systemes Aquatiques, ÉCOLE DOCTORALE DES SCIENCES CHIMIQUES, L'UNIVERSITÉ BORDEAUX 1, Bordeaux. pp. 474.
- Cardoso O., Porcher J.-M., Sanchez W. (2014) Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: Review of evidence and need for knowledge. *Chemosphere* 115:20-30. DOI: <https://doi.org/10.1016/j.chemosphere.2014.02.004>.
- Chavoshani A., Hashemi M., Mehdi Amin M., Ameta S.C. (2020) Chapter 2 - Pharmaceuticals as emerging micropollutants in aquatic environments, in: A. Chavoshani, et al. (Eds.), *Micropollutants and Challenges*, Elsevier. pp. 35-90.
- Chen C.Q., Zheng L., Zhou J.L., Zhao H. (2017) Persistence and risk of antibiotic residues and antibiotic resistance genes in major mariculture sites in Southeast China. *Sci Total Environ* 580:1175-1184. DOI: 10.1016/j.scitotenv.2016.12.075.
- Cheng W., Chen H., Su C., Yan S. (2013) Abundance and persistence of antibiotic resistance genes in livestock farms: A comprehensive investigation in eastern China. *Environment International* 61:1-7. DOI: <https://doi.org/10.1016/j.envint.2013.08.023>.
- Chiesa L.M., Nobile M., Malandra R., Panseri S., Arioli F. (2018) Occurrence of antibiotics in mussels and clams from various FAO areas. *Food Chemistry* 240:16-23. DOI: 10.1016/j.foodchem.2017.07.072.
- Choi S., Sim W., Jang D., Yoon Y., Ryu J., Oh J., Woo J.-S., Kim Y.M., Lee Y. (2020) Antibiotics in coastal aquaculture waters: Occurrence and elimination efficiency in oxidative water treatment processes. *Journal of Hazardous Materials* 396:122585. DOI: <https://doi.org/10.1016/j.jhazmat.2020.122585>.
- Chuah L.-O., Effarizah M.E., Goni A.M., Rusul G. (2016) Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture. *Current Environmental Health Reports* 3:118-127. DOI: 10.1007/s40572-016-0091-2.
- Cortese M., Gigliobianco M.R., Magnoni F., Censi R., Di Martino P.D. (2020) Compensate for or Minimize Matrix Effects? Strategies for Overcoming Matrix Effects in Liquid Chromatography-Mass Spectrometry Technique: A Tutorial Review. *Molecules* 25. DOI: 10.3390/molecules25133047.
- Coyne L., Arief R., Benigno C., Giang V.N., Huong L.Q., Jiamsripong S., Kalpravidh W., McGrane J., Padungtod P., Patrick I., Schoonman L., Setyawan E., Harja Sukarno A., Srisamran J., Ngoc P.T., Rushton J. (2019) Characterizing Antimicrobial Use in the Livestock Sector in Three South East Asian Countries (Indonesia, Thailand, and Vietnam). *Antibiotics (Basel)* 8. DOI: 10.3390/antibiotics8010033.
- Cycoń M., Mroziak A., Piotrowska-Seget Z. (2019) Antibiotics in the Soil Environment—Degradation and Their Impact on Microbial Activity and Diversity. *Frontiers in Microbiology* 10. DOI: 10.3389/fmicb.2019.00338.
- Daughton C.G., Ruhoy I.S. (2009) Environmental footprint of pharmaceuticals: The significance of factors beyond direct excretion to sewers. *Environmental Toxicology and Chemistry* 28:2495-2521. DOI: 10.1897/08-382.1.
- Desbiolles F., Malleret L., Tiliacos C., Wong-Wah-Chung P., Laffont-Schwob I. (2018) Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment? *Science of The Total Environment* 639:1334-1348. DOI: <https://doi.org/10.1016/j.scitotenv.2018.04.351>.
- Dhodapkar R.S., Gandhi K.N. (2019) 3 - Pharmaceuticals and personal care products in aquatic environment: chemicals of emerging concern?, in: M. N. V. Prasad, et al.

- (Eds.), *Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology*, Butterworth-Heinemann. pp. 63-85.
- EMA. (2016) Sales of veterinary antimicrobial agents in 29 European countries in 2014. Trends from 2011 to 2014. Sixth ESVAC Report, European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption.
- EPA Method 1694. (2007) Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS.
- European Chemicals Bureau-ECB. (2003) Technical guidance document on risk assessment, Part II, EUR 20418 EN/2, in: J. r. c. European Commission (Ed.), *Office for Official Publications of the European Communities*, Luxembourg.
- European Commission. (2003) European Commission Technical Guidance Document in Support of Commission Directive 93//67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substance, Part II, Italy.
- Fang T.H., Lin C.W., Kao C.H. (2019) Occurrence and distribution of pharmaceutical compounds in the Danshuei River Estuary and the Northern Taiwan Strait. *Mar Pollut Bull* 146:509-520. DOI: [10.1016/j.marpolbul.2019.06.069](https://doi.org/10.1016/j.marpolbul.2019.06.069).
- FAO. (2013) Report of the FAO/MARD Technical Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of Cultured Shrimp (under TCP/VIE/3304), FAO Fisheries and Aquaculture Report No. 1053, Food and Agriculture Organization of the United Nation (FAO), Rome. pp. 54 pp.
- FAO. (2020) *The State of World Fisheries and Aquaculture 2020. Sustainability in action*, Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.
- FAO & IWMI. (2018) *More people, more food, worse water? A global review of water pollution from agriculture* Food and Agriculture Organization of the United Nations and The International Water Management Institute on behalf of the Water Land and Ecosystems research program of the CGIAR, Rome.
- FAO/NACA. (1995) Report on a Regional Study and Workshop on the Environmental Assessment and Management of Aquaculture Development (TCP/RAS/2253), NACA Environment and Aquaculture Development Series, Bangkok, Thailand
- Fatmah S., Aini S.R., Pratama I.S. (2019) Tingkat Pengetahuan Mahasiswa Tahun Pertama Bersama (TPB) tentang Penggunaan Antibiotik dalam Swamedikasi *Jurnal Sains Farmasi & Klinis* 6:200-205. DOI: <http://dx.doi.org/10.25077/jsfk.6.3.200-205.2019>.
- FDA. (2015) *Guidelines for the Validation of Chemical Methods for the FDA FVM Program (Second Edition)* Food and Drug Administration (FDA) - Foods and Veterinary Medicine (FVM)
- Felis E., Kalka J., Sochacki A., Kowalska K., Bajkacz S., Harnisz M., Korzeniewska E. (2020) Antimicrobial pharmaceuticals in the aquatic environment - occurrence and environmental implications. *Eur J Pharmacol* 866:172813. DOI: [10.1016/j.ejphar.2019.172813](https://doi.org/10.1016/j.ejphar.2019.172813).
- Fent K., Weston A.A., Caminada D. (2006) Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* 76:122-159. DOI: <https://doi.org/10.1016/j.aquatox.2005.09.009>.
- Fernández C., Beltrán E.M., Tarazona J.V. (2014a) Pharmaceuticals Effects in the Environment, in: P. Wexler (Ed.), *Encyclopedia of Toxicology (Third Edition)*, Academic Press, Oxford. pp. 844-848.
- Fernández M., Fernández M., Laca A., Laca A., Díaz M. (2014b) Seasonal occurrence and removal of pharmaceutical products in municipal wastewaters. *Journal of Environmental Chemical Engineering* 2:495-502. DOI: <https://doi.org/10.1016/j.jece.2014.01.023>.
- Firmansyah I., Lukman L., Khaririyatun N., Yufdy M.P. (2015) The Growth and Yield of Shallots with Organic Fertilizers and Biofertilizers Application in Alluvial Soil (Pertumbuhan

- dan Hasil Bawang Merah dengan Aplikasi Pupuk Organik dan Pupuk Hayati pada Tanah Alluvial). *Jurnal Hortikultura* 25:133-141. DOI: <http://dx.doi.org/10.21082/jhort.v25n2.2015.p133-141>.
- Gadelha J.R., Rocha A.C., Camacho C., Eljarrat E., Peris A., Aminot Y., Readman J.W., Boti V., Nannou C., Kapsi M., Albanis T., Rocha F., Machado A., Bordalo A., Valente L.M.P., Nunes M.L., Marques A., Almeida C.M.R. (2019) Persistent and emerging pollutants assessment on aquaculture oysters (*Crassostrea gigas*) from NW Portuguese coast (Ria De Aveiro). *Science of The Total Environment* 666:731-742. DOI: <https://doi.org/10.1016/j.scitotenv.2019.02.280>.
- Gao P., Mao D., Luo Y., Wang L., Xu B., Xu L. (2012) Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Research* 46:2355-2364. DOI: <https://doi.org/10.1016/j.watres.2012.02.004>.
- Gaw S., Thomas K.V., Hutchinson T.H. (2014) Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. *Philos Trans R Soc Lond B Biol Sci* 369. DOI: 10.1098/rstb.2013.0572.
- Gonzalez-Gaya B., Cherta L., Nozal L., Rico A. (2018) An optimized sample treatment method for the determination of antibiotics in seawater, marine sediments and biological samples using LC-TOF/MS. *Sci Total Environ* 643:994-1004. DOI: 10.1016/j.scitotenv.2018.06.079.
- Gonzalez Ronquillo M., Angeles Hernandez J.C. (2017) Antibiotic and synthetic growth promoters in animal diets: Review of impact and analytical methods. *Food Control* 72:255-267. DOI: <https://doi.org/10.1016/j.foodcont.2016.03.001>.
- Grave K., Torren-Edo J., Muller A., Greko C., Moulin G., Mackay D., Group o.b.o.t.E. (2014) Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. *Journal of Antimicrobial Chemotherapy* 69:2284-2291. DOI: 10.1093/jac/dku106.
- Gros M., Rodríguez-Mozaz S., Barceló D. (2012) Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal of Chromatography A* 1248:104-121. DOI: <https://doi.org/10.1016/j.chroma.2012.05.084>.
- Gu D., Feng Q., Guo C., Hou S., Lv J., Zhang Y., Yuan S., Zhao X. (2019) Occurrence and Risk Assessment of Antibiotics in Manure, Soil, Wastewater, Groundwater from Livestock and Poultry Farms in Xuzhou, China. *Bulletin of Environmental Contamination and Toxicology* 103:590-596. DOI: 10.1007/s00128-019-02692-0.
- Guo X., Feng C., Gu E., Tian C., Shen Z. (2019) Spatial distribution, source apportionment and risk assessment of antibiotics in the surface water and sediments of the Yangtze Estuary. *Sci Total Environ* 671:548-557. DOI: 10.1016/j.scitotenv.2019.03.393.
- Haller M.Y., Müller S.R., McArdell C.S., Alder A.C., Suter M.J.F. (2002) Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography–mass spectrometry. *Journal of Chromatography A* 952:111-120. DOI: [https://doi.org/10.1016/S0021-9673\(02\)00083-3](https://doi.org/10.1016/S0021-9673(02)00083-3).
- Hatmanti A. (2003) Bacterial Pathogen in Crustacean Culture and the Handling (Bakterial Pada Budidaya Krustasea Serta Cara Penanganannya). *Oseana XXVIII*:1-10.
- Hernández F., Sancho J.V., Ibáñez M., Guerrero C. (2007) Antibiotic residue determination in environmental waters by LC-MS. *TrAC Trends in Analytical Chemistry* 26:466-485. DOI: 10.1016/j.trac.2007.01.012.
- Hidayah N., Tristiono H., Surjaningdyah T., Hardjito A., Setyawan E., Kompudu A., Sukarno A.H., Nugroho E. (2018) The use of Antibiotics in Small-Scale Duck Farm at Mojokerto District, East Java. *Hemera Zoa*.

- Hidayati N.V., Prudent P., Asia L., Vassalo L., Torre F., Widowati I., Sabdono A., Syakti A.D., Doumenq P. (2020) Assessment of the ecological and human health risks from metals in shrimp aquaculture environments in Central Java, Indonesia. *Environmental Science and Pollution Research*. DOI: 10.1007/s11356-020-09967-8.
- Holmstrom K., Graslund S., Wahlstrom A., Pongshompoo S., Bengtsson B.-E., Kautsky N. (2003) Antibiotic use in shrimp farming and implications for environmental impacts and human health. *International Journal of Food Science and Technology* 38:255-266. DOI: 10.1046/j.1365-2621.2003.00671.x.
- Husnah H. (2001) Chlorine demand and bacterial abundance of shrimp pond water under different suspended solid concentrations. *Indonesian Fisheries Research Journal* 7:16-24.
- ICH Guideline Q2(R1). (2005) Validation of analytical procedures: text and methodology.
- Iriyanti N., Hartoyo B., Suhermiyati S. (2018) Performance and Intestinal Profiles of Tegal Duck Fed Ration Supplemented with Prebiotics. *Tropical Animal Science Journal* 41:15-21. DOI: 10.5398/tasj.2018.41.1.15.
- K'Oreje K.O., Vergeynst L., Ombaka D., De Wispelaere P., Okoth M., Van Langenhove H., Demeestere K. (2016) Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere* 149:238-244. DOI: <https://doi.org/10.1016/j.chemosphere.2016.01.095>.
- Khetan S.K., Collins T.J. (2007) Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry. *Chemical Reviews* 107:2319-2364. DOI: 10.1021/cr020441w.
- Kim C., Ryu H.D., Chung E.G., Kim Y., Lee J.K. (2018) A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass spectrometry. *J Environ Manage* 217:629-645. DOI: 10.1016/j.jenvman.2018.04.006.
- Kim H.-Y., Lee I.-S., Oh J.-E. (2017) Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. *Science of The Total Environment* 579:940-949. DOI: <https://doi.org/10.1016/j.scitotenv.2016.10.039>.
- Kim Y., Choi K., Jung J., Park S., Kim P.-G., Park J. (2007) Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. *Environment International* 33:370-375. DOI: <https://doi.org/10.1016/j.envint.2006.11.017>.
- Krakkó D., Licul-Kucera V., Záray G., Mihucz V.G. (2019) Single-run ultra-high performance liquid chromatography for quantitative determination of ultra-traces of ten popular active pharmaceutical ingredients by quadrupole time-of-flight mass spectrometry after offline preconcentration by solid phase extraction from drinking and river waters as well as treated wastewater. *Microchemical Journal* 148:108-119. DOI: 10.1016/j.microc.2019.04.047.
- Lapworth D.J., Baran N., Stuart M.E., Ward R.S. (2012) Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environmental Pollution* 163:287-303. DOI: <https://doi.org/10.1016/j.envpol.2011.12.034>.
- Lawson N.S., Haven G.T., Williams G.W. (1982) Analyte Stability in Clinical Chemistry Quality Control Materials. *CRC Critical Reviews in Clinical Laboratory Sciences* 17:1-50. DOI: 10.3109/10408368209107031.
- Le T.X., Munekage Y. (2004) Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Viet Nam. *Marine Pollution Bulletin* 49:922-929. DOI: <https://doi.org/10.1016/j.marpolbul.2004.06.016>.
- Leal J.F., Santos E.B.H., Esteves V.I. (2019) Oxytetracycline in intensive aquaculture: water quality during and after its administration, environmental fate, toxicity and bacterial resistance. *Reviews in Aquaculture* 11:1176-1194. DOI: 10.1111/raq.12286.

- Li W.C. (2014) Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environ Pollut* 187:193-201. DOI: 10.1016/j.envpol.2014.01.015.
- Lin H., Chen L., Li H., Luo Z., Lu J., Yang Z. (2018) Pharmaceutically active compounds in the Xiangjiang River, China: Distribution pattern, source apportionment, and risk assessment. *Science of The Total Environment* 636:975-984. DOI: <https://doi.org/10.1016/j.scitotenv.2018.04.267>.
- Liu S., Zhao H., Lehmler H.J., Cai X., Chen J. (2017) Antibiotic Pollution in Marine Food Webs in Laizhou Bay, North China: Trophodynamics and Human Exposure Implication. *Environ Sci Technol* 51:2392-2400. DOI: 10.1021/acs.est.6b04556.
- Liu W.-X., Wang Y., He W., Qin N., Kong X.-Z., He Q.-S., Yang B., Yang C., Jiang Y.-J., Jorgensen S.E., Xu F.-L. (2016) Aquatic biota as potential biological indicators of the contamination, bioaccumulation and health risks caused by organochlorine pesticides in a large, shallow Chinese lake (Lake Chaohu). *Ecological Indicators* 60:335-345. DOI: <https://doi.org/10.1016/j.ecolind.2015.06.026>.
- Lu J., Zhang Y., Wu J., Wang J., Zhang C., Lin Y. (2019) Occurrence and spatial distribution of antibiotic resistance genes in the Bohai Sea and Yellow Sea areas, China. *Environ Pollut* 252:450-460. DOI: 10.1016/j.envpol.2019.05.143.
- Luo Y., Mao D., Rysz M., Zhou Q., Zhang H., Xu L., P J.J.A. (2010) Trends in antibiotic resistance genes, occurrence in the Haihe River, China. *Environ Sci Technol* 44:7220-5. DOI: 10.1021/es100233w.
- Manage P.M., Liyanage G.Y. (2019) 18 - Antibiotics induced antibacterial resistance, in: M. N. V. Prasad, et al. (Eds.), *Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology*, Butterworth-Heinemann. pp. 429-448.
- Michael I., Rizzo L., McArdell C.S., Manaia C.M., Merlin C., Schwartz T., Dagot C., Fatta-Kassinos D. (2013) Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research* 47:957-995. DOI: <https://doi.org/10.1016/j.watres.2012.11.027>.
- Mokh S., El Khatib M., Koubar M., Daher Z., Al Iskandarani M. (2017) Innovative SPE-LC-MS/MS technique for the assessment of 63 pharmaceuticals and the detection of antibiotic-resistant-bacteria: A case study natural water sources in Lebanon. *Science of The Total Environment* 609:830-841. DOI: <https://doi.org/10.1016/j.scitotenv.2017.07.230>.
- Mustafa A., Sapo I., Paena M. (2010) Study on the use of chemical and biological products in whiteleg shrimp (*Litopenaeus vannamei*) culture in brackishwater ponds of Pesawaran Regency Lampung Province. *J. Ris. Akuakultur* 5:115-133.
- Na T.W., Kang T.W., Lee K.H., Hwang S.H., Jung H.J., Kim K. (2019) Distribution and ecological risk of pharmaceuticals in surface water of the Yeongsan river, Republic of Korea. *Ecotoxicol Environ Saf* 181:180-186. DOI: 10.1016/j.ecoenv.2019.06.004.
- Nogueira-Lima A.C., Gesteira T.C.V., Mafezoli J. (2006) Oxytetracycline residues in cultivated marine shrimp (*Litopenaeus vannamei* Boone, 1931) (Crustacea, Decapoda) submitted to antibiotic treatment. *Aquaculture* 254:748-757. DOI: <https://doi.org/10.1016/j.aquaculture.2005.11.021>.
- OECD. (2019) *Pharmaceutical Residues in Freshwater*.
- Oliveira T.S., Murphy M., Mendola N., Wong V., Carlson D., Waring L. (2015) Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. *Science of The Total Environment* 518-519:459-478. DOI: <https://doi.org/10.1016/j.scitotenv.2015.02.104>.
- Paíga P., Lolić A., Hellebuyck F., Santos L.H.M.L.M., Correia M., Delerue-Matos C. (2015) Development of a SPE-UHPLC-MS/MS methodology for the determination of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawater. *Journal*

- of Pharmaceutical and Biomedical Analysis 106:61-70. DOI: <https://doi.org/10.1016/j.jpba.2014.06.017>.
- Paíga P., Santos L.H.M.L.M., Delerue-Matos C. (2017) Development of a multi-residue method for the determination of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* 135:75-86. DOI: <https://doi.org/10.1016/j.jpba.2016.12.013>.
- Pavlović D.M., Babić S., Horvat A.J.M., Kaštelan-Macan M. (2007) Sample preparation in analysis of pharmaceuticals. *TrAC Trends in Analytical Chemistry* 26:1062-1075. DOI: 10.1016/j.trac.2007.09.010.
- Peller J.R., Mezyk S.P., McKay G., Watson E. (2014) 11 - Hydroxyl Radical Probes for the Comparison of Secondary Treated Wastewaters, in: S. Ahuja (Ed.), *Water Reclamation and Sustainability*, Elsevier, Boston. pp. 247-263.
- Perkins J.D. (2006) Acetaminophen sets records in the United States: Number 1 analgesic and number 1 cause of acute liver failure. *Liver Transplantation* 12:682-686. DOI: <https://doi.org/10.1002/lt.20735>.
- Petrovic M., Gros M., Barcelo D. (2006) Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry. *Journal of Chromatography A* 1124:68-81. DOI: <https://doi.org/10.1016/j.chroma.2006.05.024>.
- Philip J.M., Aravind U.K., Aravindakumar C.T. (2018) Emerging contaminants in Indian environmental matrices – A review. *Chemosphere* 190:307-326. DOI: <https://doi.org/10.1016/j.chemosphere.2017.09.120>.
- Piram A., Salvador A., Gauvrit J.-Y., Lanteri P., Faure R. (2008) Development and optimisation of a single extraction procedure for the LC/MS/MS analysis of two pharmaceutical classes residues in sewage treatment plant. *Talanta* 74:1463-1475. DOI: <https://doi.org/10.1016/j.talanta.2007.09.038>.
- Putra F.R., Manan A. (2014) Monitoring of water quality on rearing ponds of vannamei shrimp (*Litopenaeus vannamei*) in Situbondo, Jawa Timur. *Jurnal Ilmiah Perikanan dan Kelautan* 6.
- Quaik S., Embrandiri A., Ravindran B., Hossain K., Al-Dhabi N.A., Arasu M.V., Ignacimuthu S., Ismail N. (2020) Veterinary antibiotics in animal manure and manure laden soil: Scenario and challenges in Asian countries. *Journal of King Saud University - Science* 32:1300-1305. DOI: <https://doi.org/10.1016/j.jksus.2019.11.015>.
- Restiyono A. (2016) Analisis Faktor yang Berpengaruh dalam Swamedikasi Antibiotik pada Ibu Rumah Tangga di Kelurahan Kajen Kabupaten Pekalongan (Analysis of Influential Factors in Antibiotic Medicine for Housewives in Kajen Village, Pekalongan Regency). *Jurnal Promosi Kesehatan Indonesia* 11:14-26.
- Rigos G., Nengas I., Alexis M., Troisi G.M. (2004) Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. *Aquatic Toxicology* 69:281-288. DOI: <https://doi.org/10.1016/j.aquatox.2004.05.009>.
- Rivera-Jaimes J.A., Postigo C., Melgoza-Aleman R.M., Acena J., Barcelo D., Lopez de Alda M. (2018) Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: Occurrence and environmental risk assessment. *Sci Total Environ* 613-614:1263-1274. DOI: 10.1016/j.scitotenv.2017.09.134.
- Sangion A., Gramatica P. (2016) Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity. *Environment International* 95:131-143. DOI: <https://doi.org/10.1016/j.envint.2016.08.008>.
- Saxena S.K., Rangasamy R., Krishnan A.A., Singh D.P., Uke S.P., Malekadi P.K., Sengar A.S., Mohamed D.P., Gupta A. (2018) Simultaneous determination of multi-residue and

- multi-class antibiotics in aquaculture shrimps by UPLC-MS/MS. *Food Chemistry* 260:336-343. DOI: <https://doi.org/10.1016/j.foodchem.2018.04.018>.
- Scott T.M., Phillips P.J., Kolpin D.W., Colella K.M., Furlong E.T., Foreman W.T., Gray J.L. (2018) Pharmaceutical manufacturing facility discharges can substantially increase the pharmaceutical load to U.S. wastewaters. *Sci Total Environ* 636:69-79. DOI: 10.1016/j.scitotenv.2018.04.160.
- Shimizu A., Takada H., Koike T., Takeshita A., Saha M., Rinawati, Nakada N., Murata A., Suzuki T., Suzuki S., Chiem N.H., Tuyen B.C., Viet P.H., Siringan M.A., Kwan C., Zakaria M.P., Reungsang A. (2013) Ubiquitous occurrence of sulfonamides in tropical Asian waters. *The Science of the total environment* 452-453:108-115. DOI: 10.1016/j.scitotenv.2013.02.027.
- Silva B.F.d., Jelic A., López-Serna R., Mozeto A.A., Petrovic M., Barceló D. (2011) Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. *Chemosphere* 85:1331-1339. DOI: <https://doi.org/10.1016/j.chemosphere.2011.07.051>.
- Sitompul G.S.S., Yetti H., Murniati M., . (2017) The Effect of Manure and KCl Fertilizer on Growth and Productivity of Shallot ( *Allium ascalonicum* L.) (Pengaruh Pemberian Pupuk Kandang dan KCl Terhadap Pertumbuhan dan Produksi Tanaman Bawang Merah (*Allium ascalonicum* L.)). *JOM FAPERTA* 4.
- Sumekar W., Isbandi I., Atmomarsono U., Susilowati I. (2013) BUSINESS PERFORMANCE OF DUCK FARMERS IN BREBES REGENCYCENTRAL JAVA. *Journal of the Indonesian Tropical Animal Agriculture*:171-175.
- Sung H.-H., Chiu Y.-W., Wang S.-Y., Chen C.-M., Huang D.-J. (2014) Acute toxicity of mixture of acetaminophen and ibuprofen to Green Neon Shrimp, *Neocaridina denticulate*. *Environmental Toxicology and Pharmacology* 38:8-13. DOI: <https://doi.org/10.1016/j.etap.2014.04.014>.
- Suprpto H., Sulmartiwi L., Sudarno S. (2015) Detection of Antibiotic Residues from Shrimp Pond and Their Environment in East Java Province Indonesia. *Aquacultura Indonesiana*:29-32.
- Tahrani L., Van Loco J., Ben Mansour H., Reyns T. (2015) Occurrence of antibiotics in pharmaceutical industrial wastewater, wastewater treatment plant and sea waters in Tunisia. *Journal of Water and Health* 14:208-213. DOI: 10.2166/wh.2015.224.
- Terzic S., Ahel M. (2011) Nontarget analysis of polar contaminants in freshwater sediments influenced by pharmaceutical industry using ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry. *Environmental Pollution* 159:557-566. DOI: <https://doi.org/10.1016/j.envpol.2010.10.009>.
- Thi Kim Chi T., Clausen J.H., Van P.T., Tersbøl B., Dalsgaard A. (2017) Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. *Aquaculture Reports* 7:40-47. DOI: <https://doi.org/10.1016/j.aqrep.2017.05.003>.
- Topi D., Spahiu J. (2020) Presence of veterinary antibiotics in livestock manure in two Southeastern Europe countries, Albania and Kosovo. *Environmental Science and Pollution Research*. DOI: 10.1007/s11356-020-10341-x.
- Tugiyanti E., Mawarti N., Rosidi R., Harisulistyan I. (2017) The Effect of Soursop (*Announa Muricata* L.) Leaves Powder on Diameter of Muscle Fiber, Lipid Cell, Body Weight Gain and Carcass Percentage of Tegal Duck. *ANIMAL PRODUCTION*:47-54.
- Tuševljak N., Dutil L., Rajić A., Uhländ F.C., McClure C., St-Hilaire S., Reid-Smith R.J., McEwen S.A. (2013) Antimicrobial Use and Resistance in Aquaculture: Findings of a Globally

- Administered Survey of Aquaculture-Allied Professionals. *Zoonoses and Public Health* 60:426-436. DOI: 10.1111/zph.12017.
- US EPA. (2014) SW-846 Compendium: Project Quality Assurance and Quality Control (Update V, Rev. 2).
- USGS. (2020) Pharmaceuticals in Water, U.S. Geological Survey (USGS)
- Van Epps A., Blaney L. (2016) Antibiotic Residues in Animal Waste: Occurrence and Degradation in Conventional Agricultural Waste Management Practices. *Current Pollution Reports* 2:135-155. DOI: 10.1007/s40726-016-0037-1.
- Vaughan D.B., Grutter A.S., Hutson K.S. (2018) Cleaner shrimp are a sustainable option to treat parasitic disease in farmed fish. *Scientific Reports* 8:13959. DOI: 10.1038/s41598-018-32293-6.
- Verlicchi P., Zambello E. (2014) How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of The Total Environment* 470-471:1281-1306. DOI: <https://doi.org/10.1016/j.scitotenv.2013.10.085>.
- Wahyuningrat, Haryanto T., Rosyadi S. (2018) Practices of Illegal Fishing in Pemalang Region: A Policy Analysis. *E3S Web Conf.* 47:06010.
- Wang W., Wang H., Zhang W., Liang H., Gao D. (2017) Occurrence, distribution, and risk assessment of antibiotics in the Songhua River in China. *Environmental Science and Pollution Research* 24:19282-19292. DOI: 10.1007/s11356-017-9471-x.
- WHO. (2019) World Health Organization Model List of Essential Medicines, 21st List, Geneva: World Health Organization.
- Wu J., Qian X., Yang Z., Zhang L. (2010) Study on the matrix effect in the determination of selected pharmaceutical residues in seawater by solid-phase extraction and ultra-high-performance liquid chromatography–electrospray ionization low-energy collision-induced dissociation tandem mass spectrometry. *Journal of Chromatography A* 1217:1471-1475. DOI: <https://doi.org/10.1016/j.chroma.2009.12.074>.
- WWF-Indonesia. (2014) Better Management Practices (BMP) Budidaya Udang Vannamei (*Litopenaus vannamei*), Tambak Semi Intensif dengan Instalasi Pengolahan Air Limbah (Better Management Practices (BMP) in Vannamei Shrimp Cultivation (*Litopenaus vannamei*), Semi-Intensive Pond with Installation Wastewater Treatment) WWF-Indonesia, Jakarta.
- Xie H., Hao H., Xu N., Liang X., Gao D., Xu Y., Gao Y., Tao H., Wong M. (2019) Pharmaceuticals and personal care products in water, sediments, aquatic organisms, and fish feeds in the Pearl River Delta: Occurrence, distribution, potential sources, and health risk assessment. *Science of The Total Environment* 659:230-239. DOI: <https://doi.org/10.1016/j.scitotenv.2018.12.222>.
- Yan Z., Yang X., Lu G., Liu J., Xie Z., Wu D. (2014) Potential environmental implications of emerging organic contaminants in Taihu Lake, China: Comparison of two ecotoxicological assessment approaches. *Science of The Total Environment* 470-471:171-179. DOI: <https://doi.org/10.1016/j.scitotenv.2013.09.092>.
- Yang S., Carlson K.H. (2004) Solid-phase extraction–high-performance liquid chromatography–ion trap mass spectrometry for analysis of trace concentrations of macrolide antibiotics in natural and waste water matrices. *Journal of Chromatography A* 1038:141-155. DOI: <https://doi.org/10.1016/j.chroma.2004.02.084>.
- Yang Y., Qiu W., Li Y., Liu L. (2020) Antibiotic residues in poultry food in Fujian Province of China. *Food Additives & Contaminants: Part B* 13:177-184. DOI: 10.1080/19393210.2020.1751309.



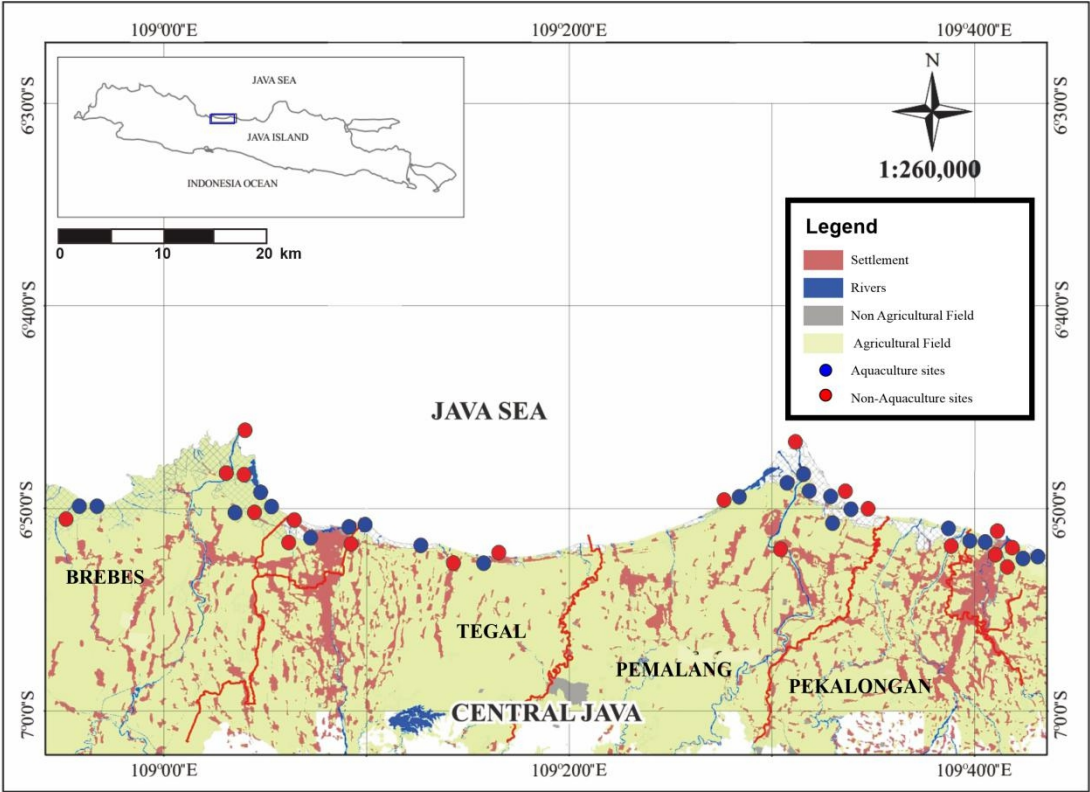
- Yuan J., Ni M., Liu M., Zheng Y., Gu Z. (2019) Occurrence of antibiotics and antibiotic resistance genes in a typical estuary aquaculture region of Hangzhou Bay, China. *Mar Pollut Bull* 138:376-384. DOI: 10.1016/j.marpolbul.2018.11.037.
- Zhao H., Zhou J.L., Zhang J. (2015) Tidal impact on the dynamic behavior of dissolved pharmaceuticals in the Yangtze Estuary, China. *Science of The Total Environment* 536:946-954. DOI: <https://doi.org/10.1016/j.scitotenv.2015.06.055>.
- Zheng J., Zhang J., Gao L., Kong F., Shen G., Wang R., Gao J., Zhang J. (2020) The Effects of Tetracycline Residues on the Microbial Community Structure of Tobacco Soil in Pot Experiment. *Scientific Reports* 10:8804. DOI: 10.1038/s41598-020-65203-w.
- Zhou L.-J., Ying G.-G., Liu S., Zhao J.-L., Chen F., Zhang R.-Q., Peng F.-Q., Zhang Q.-Q. (2012) Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry. *Journal of Chromatography A* 1244:123-138. DOI: <https://doi.org/10.1016/j.chroma.2012.04.076>.
- Zhou L.-J., Ying G.-G., Zhao J.-L., Yang J.-F., Wang L., Yang B., Liu S. (2011) Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. *Environmental Pollution* 159:1877-1885. DOI: <https://doi.org/10.1016/j.envpol.2011.03.034>.

## **Emerging Contaminants (Acetaminophen, Oxytetracycline, Sulfamethoxazole and Trimethoprim) Detected in Aquaculture Sites in Java, Indonesia**

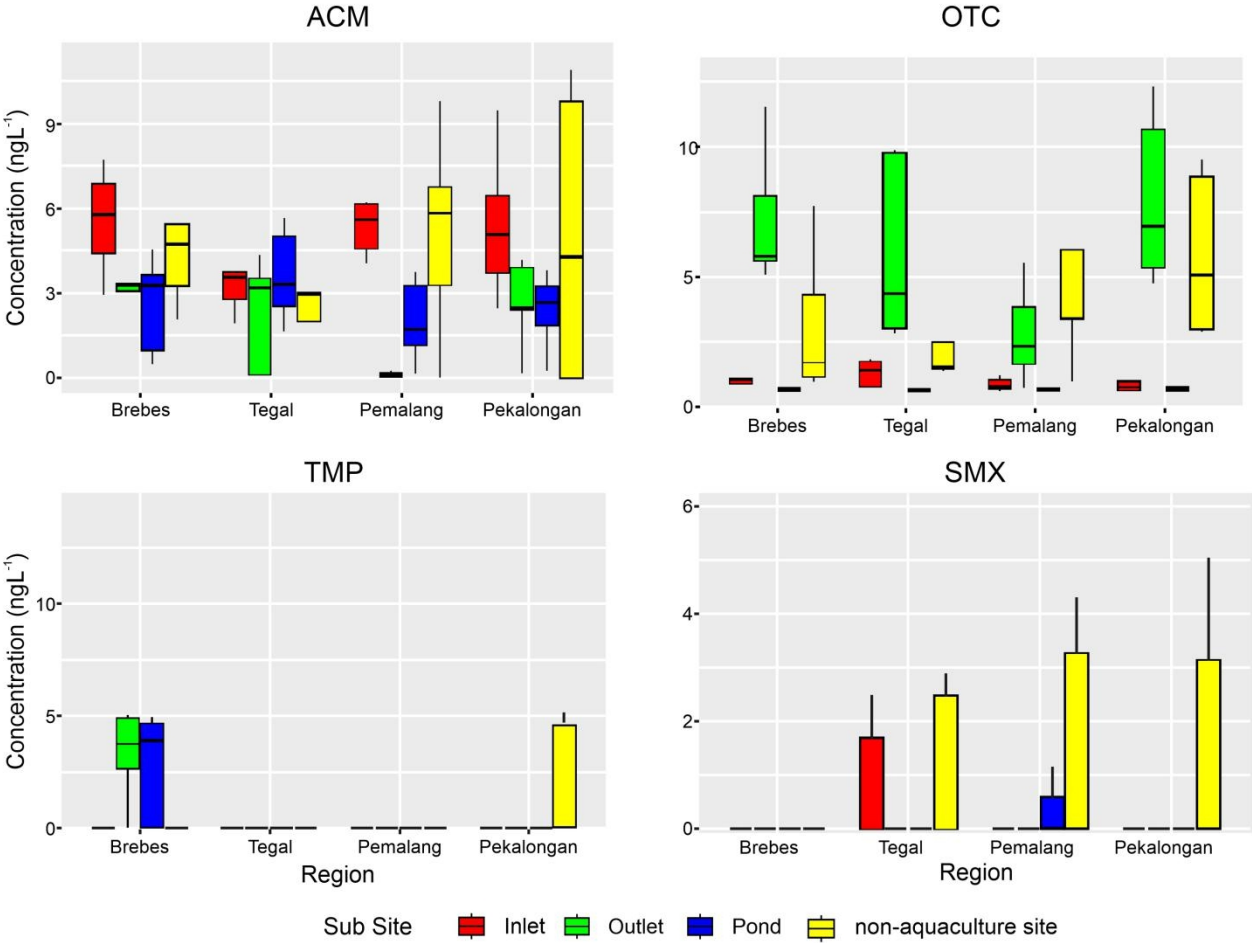
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- Fig. 2.** The concentration range ( $\text{ngL}^{-1}$ ) of selected pharmaceuticals in the inlet, outlet, pond of shrimp farm area and nonaquaculture area
- Fig. 3.** Heat map of the ecological risks of antibiotics for aquatic organisms in water samples (green = insignificant risk; blue = low risk; yellow = moderate risk; red = high risk; NAS = nonaquaculture sites)

**Fig. 1.** Map of the Northern Coast of Central Java showing sampling stations, indicated with blue (aquaculture sites) and red circles (nonaquaculture sites) and major types of land cover (source: <http://portal.ina-sdi.or.id/>)



**Fig. 2.** The concentration range (ngL<sup>-1</sup>) of selected pharmaceuticals in the inlet, outlet, pond of shrimp farm area and nonaquaculture area



**Fig. 3.** Heat map of the ecological risks of antibiotics for aquatic organisms in water samples (green = insignificant risk; blue = low risk; yellow = moderate risk; red = high risk; NAS = nonaquaculture sites)

Organism	Algae				Invertebrate				Fish			
	ACM	TMP	OTC	SMX	ACM	TMP	OTC	SMX	ACM	TMP	OTC	SMX
<b>Brebes</b>												
inlet	blue	green	red	green	blue	green	blue	green	blue	green	green	green
pond	blue	blue	red	yellow	blue	blue	blue	green	blue	blue	green	green
outlet	blue	blue	red	yellow	blue	blue	blue	green	blue	blue	green	green
NAS	blue	blue	red	yellow	yellow	green	yellow	green	blue	green	green	green
<b>Tegal</b>												
inlet	blue	green	red	yellow	yellow	green	blue	green	blue	green	green	green
pond	blue	green	red	yellow	yellow	green	blue	green	blue	green	green	green
outlet	blue	green	red	yellow	blue	green	yellow	green	green	green	green	green
NAS	blue	green	red	yellow	yellow	green	yellow	green	blue	green	green	green
<b>Pemalang</b>												
inlet	blue	green	red	green	yellow	green	blue	green	blue	green	green	green
pond	blue	green	red	yellow	blue	green	blue	green	blue	green	green	green
outlet	green	green	red	yellow	green	green	yellow	green	green	green	green	green
NAS	blue	green	red	yellow	yellow	green	yellow	green	blue	green	green	green
<b>Pekalongan</b>												
inlet	blue	green	red	green	yellow	green	blue	green	blue	green	green	green
pond	blue	green	red	yellow	blue	green	blue	green	green	green	green	green
outlet	blue	green	red	yellow	blue	green	yellow	green	blue	green	green	green
NAS	blue	blue	red	yellow	yellow	blue	yellow	green	blue	blue	green	green

## Supplementary Material

### Emerging Contaminants (Acetaminophen, Oxytetracycline, Sulfamethoxazole and Trimethoprim) Detected in Aquaculture Sites in Java, Indonesia

#### LIST OF SUPPLEMENTARY MATERIALS:

- Table S1.** Instrument detection limit (IDL), Instrument quantification limit (IQL), method detection limit (MDL), and method quantification limit (MQL) of the pharmaceuticals from water samples
- Table S2.** The acquisition parameters and the key data used for identification and quantification the target compounds
- Table S3.** Half maximum effective concentrations ( $EC_{50}$ ) of selected pharmaceuticals ( $\mu\text{gL}^{-1}$ ) for algae, invertebrate and fish for each detected compound.
- Fig. S4.** Chromatographic resolution and peak sensitivity of target compounds with various ionic mobile phase additives (*green: formic acid; blue: acetic acid; purple: ammonium acetate*)
- Fig. S5.** Stability of pharmaceuticals in various acetic acid percentage in mobile phase
- Fig. S6.** Matrix effect (%) as the effect of final volume of extract.
- Fig. S7.** Extraction recovery, matrix effect, volume of extract and washing solution volume
- Table S8.** Concentration of targeted compounds in studied area ( $\text{ngL}^{-1}$ )
- Fig. S9.** The concentration of OTC ( $\text{ngL}^{-1}$ ) detected in farm area (inlet, pond, outlet)
- Fig. S10.** Design of shrimp farm areas in the studied area (Tegal)
- Fig. S11.** Duck farming area in Brebes Regency located in the surroundings of the aquaculture area
- Fig. S12.** Spearman correlation coefficients based on antibiotic concentrations in water.

Total number of Pages: 11

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**Table S1.** Instrument detection limit (IDL), Instrument quantification limit (IQL), method detection limit (MDL), and method quantification limit (MQL) of the pharmaceuticals from water samples

Compounds	Water			
	IDL ( $\mu\text{g L}^{-1}$ )	IQL ( $\mu\text{g L}^{-1}$ )	MDL ( $\text{ng L}^{-1}$ )	MQL ( $\text{ng L}^{-1}$ )
Acetaminophen	1.51	4.57	0.65	1.97
Trimethoprim	0.73	2.20	0.29	0.88
Oxytetracycline	2.19	6.63	0.96	2.90
Sulfamethoxazole	1.57	4.76	0.61	1.86

**Table S2.** The acquisition parameters and the key data used for identification and quantification the target compounds

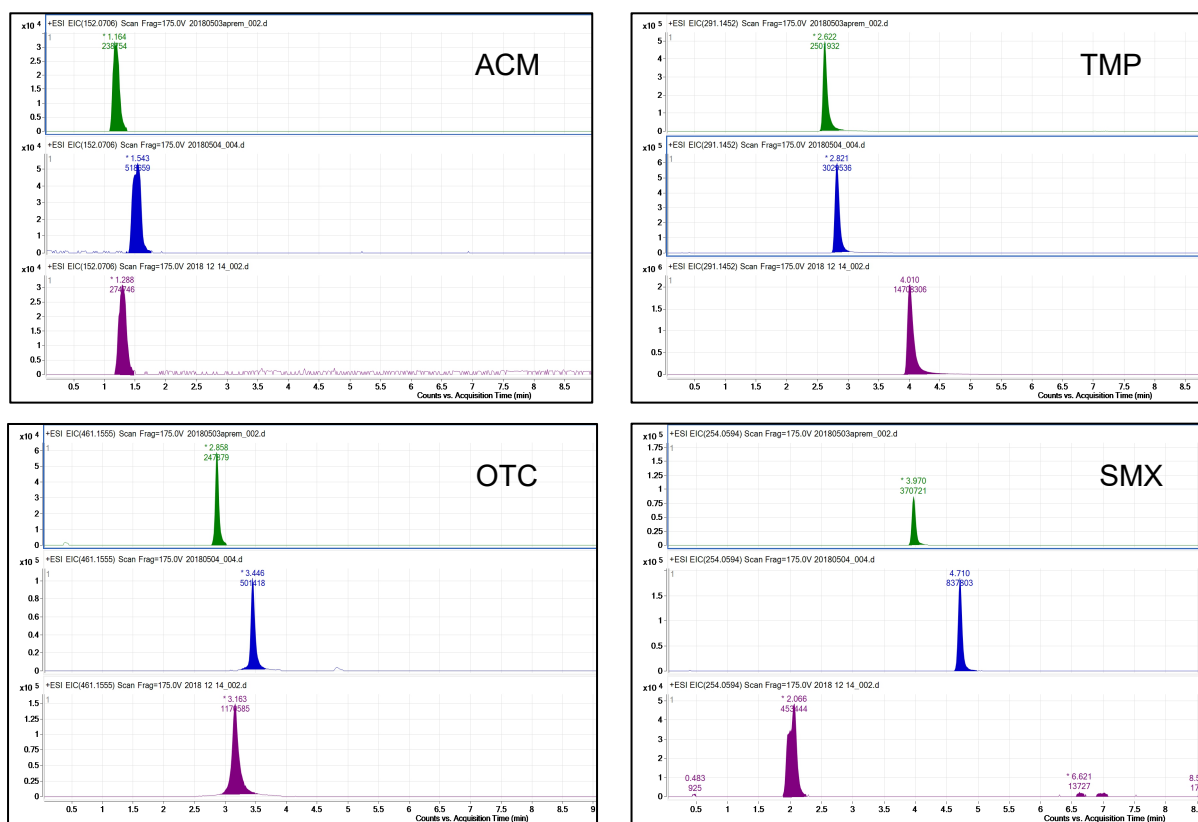
Target Compounds	Retention time (min)	The extracted ions $[\text{M}+\text{H}]^+$ (m/z)	Fragmentor voltage (V)	Polarity
Acetaminophen	1.1	152.0706	175	Positive
Trimethoprim	2.5	291.1452	175	Positive
Oxytetracycline	2.5	461.1555	175	Positive
Sulfamethoxazole	3.9	254.0594	175	Positive
Erythromycin	7.1	734.4685	175	Positive



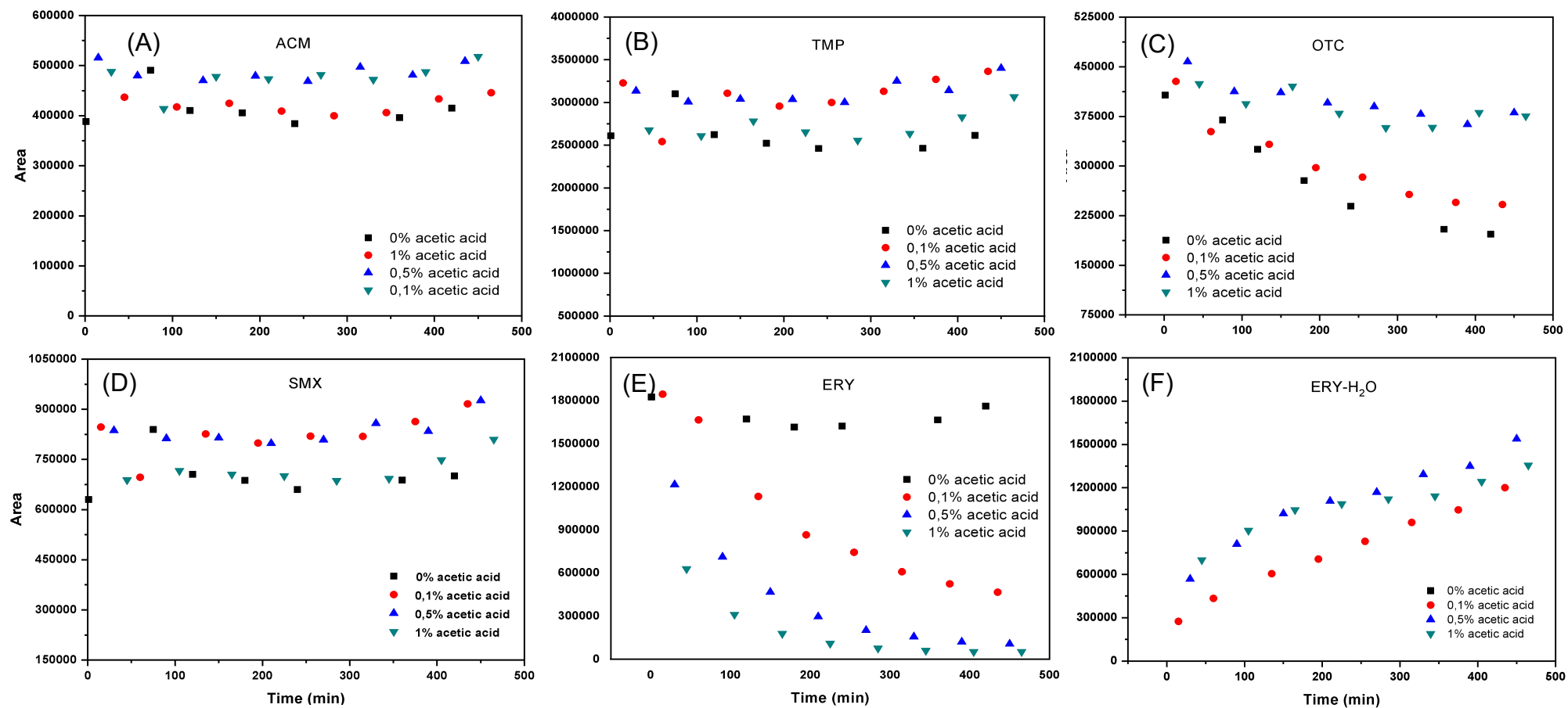
**Table S3.** Half maximal effective concentrations (EC<sub>50</sub>) of selected pharmaceuticals (µg L<sup>-1</sup>) for algae, invertebrate and fish for each detected compound.

Compounds	Algae			Invertebrate			Fish		
	EC <sub>50</sub> (µg L <sup>-1</sup> )	Species	Reference	EC <sub>50</sub> (µg L <sup>-1</sup> )	Species	Reference	EC <sub>50</sub> (µg L <sup>-1</sup> )	Species	Reference
Acetaminophen	134 000	<i>Scenedesmus subspicatus</i>	Henschel et al., 1997	30 100	<i>Daphnia magna</i>	Kim et al., 2007	>160 000	<i>Oryzias latipes</i>	Kim et al., 2007
Trimethoprim	110 000	<i>Selenastrum capricornutum</i>	Halling-Sørensen et al., 2000	120 700	<i>Daphnia magna</i>	Kim et al., 2007	>100 000	<i>Oryzias latipes</i>	Kim et al., 2007
Oxytetracycline	342	<i>Selenastrum capricornutum</i>	Eguchi et al., 2004	22 640	<i>Daphnia magna</i>	Isidori et al., 2005	>1 000 000	<i>Brachydanio rerio</i>	Isidori et al., 2005
Sulfamethoxazole	1540	<i>Scenedesmus vacuolatus</i>	Białk-Bielińska et al., 2011	189 200	<i>Daphnia magna</i>	Kim et al., 2007	>750 000	<i>Oryzias latipes</i>	Kim et al., 2007

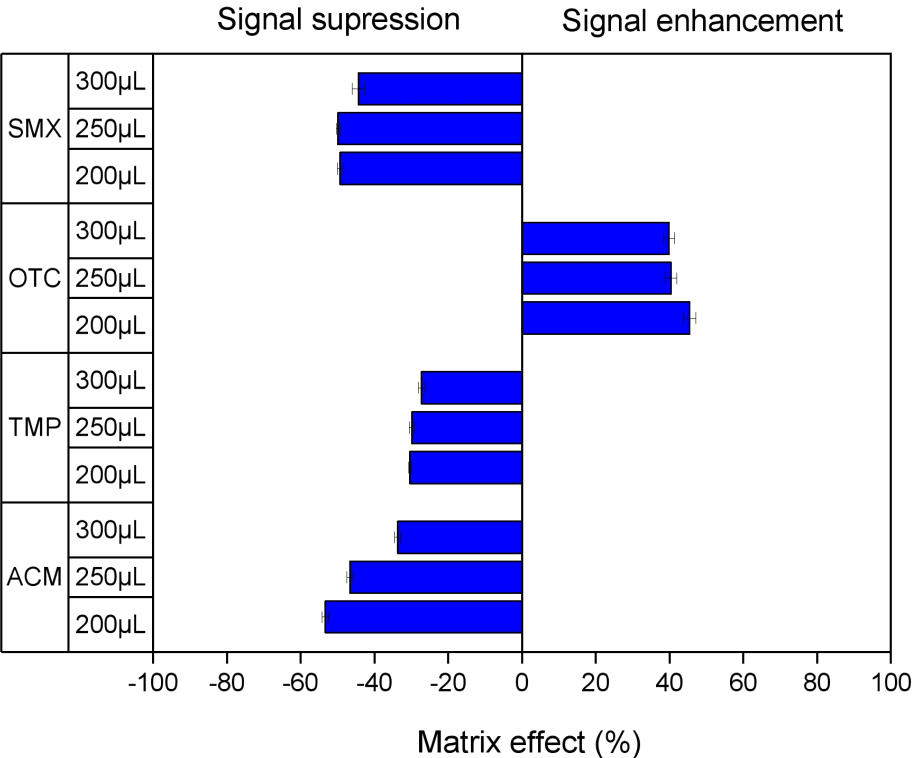
**Fig. S4.** Chromatographic resolution and peak sensitivity of target compounds with various ionic mobile phase additives (*green: formic acid; blue: acetic acid; purple: ammonium acetate*)



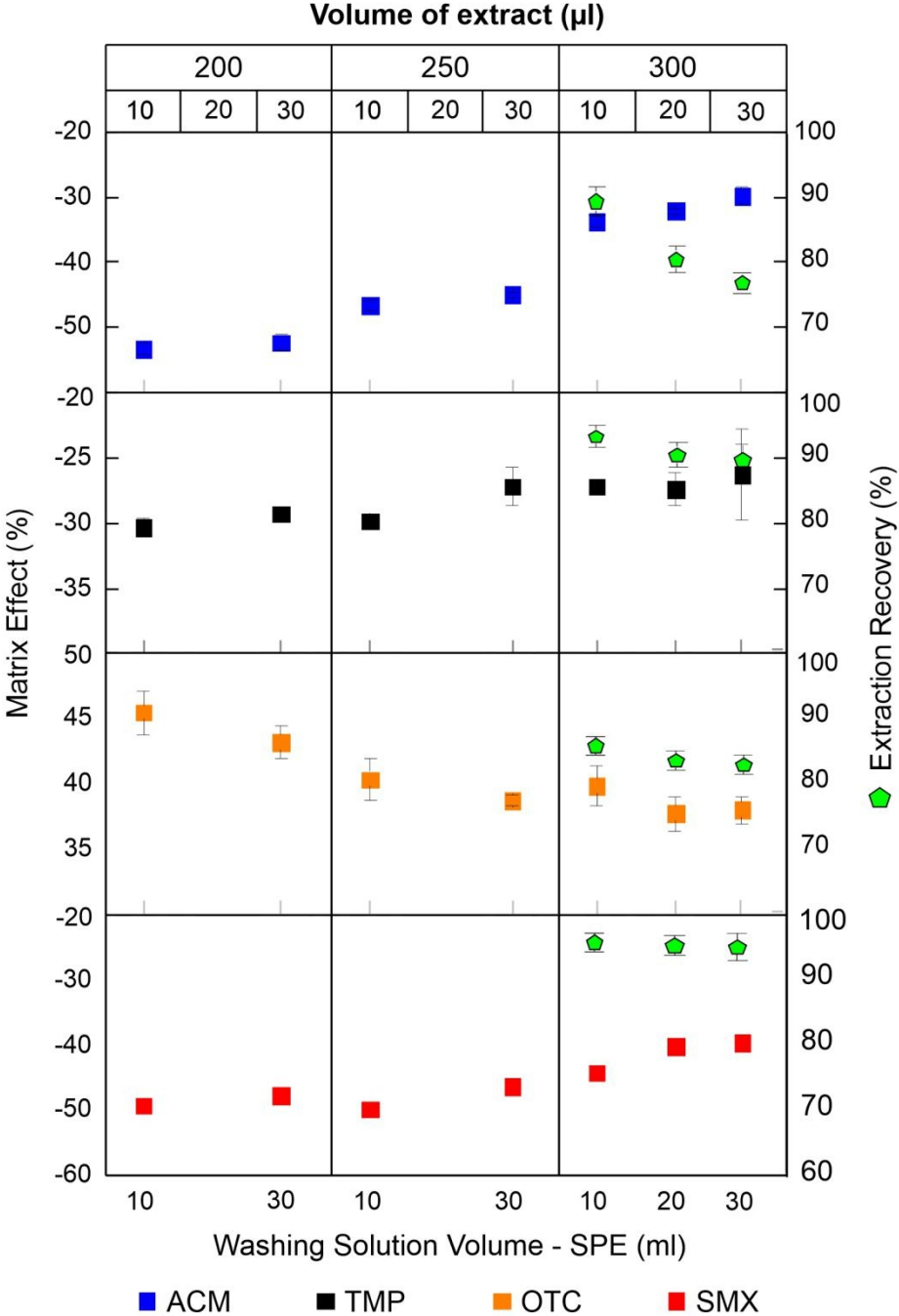
**Fig. S5.** Stability of pharmaceuticals in various acetic acid percentage in mobile phase



**Fig. S6.** Matrix effect (%) as the effect of final volume of extract.



**Fig. S7.** Extraction recovery, matrix effect, volume of extract and washing solution volume



**Table S8.** Concentration of targeted compounds in studied area (ngL<sup>-1</sup>)

Region	Concentration (ngL <sup>-1</sup> )							
	Farm area				Reference sites			
	ACM	TMP	OTC	SMX	ACM	TMP	OTC	SMX
<b>Brebes</b>								
Min	0	0	0	0	2.1	0.0	0.0	0.0
Max	7.7	5.0	11.5	2.8	11.3	4.8	7.7	4.4
Mean	3.7	2.0	2.6	0.2	5.3	1.0	2.4	0.9
STD	2.1	2.3	3.7	0.7	3.6	2.2	3.5	2.0
<b>Tegal</b>								
Min	0.0	0.0	0.0	0.0	0	0	0	0
Max	9.2	3.5	9.9	3.7	9.6	1.5	5.8	2.9
Mean	3.1	0.2	1.8	0.6	3.5	0.3	1.2	1.1
STD	2.5	0.9	3.5	1.2	3.6	0.7	2.6	1.5
<b>Pemalang</b>								
Min	0	0	0	0	0	0	0	0
Max	9.5	5.5	5.5	2.7	9.8	6.2	12.9	4.3
Mean	2.4	0.3	0.6	0.2	5.1	1.2	5.1	1.5
STD	3.2	1.2	1.6	0.8	3.7	2.8	4.8	2.1
<b>Pekalongan</b>								
Min	0	0	0	0	0	0	0	0
Max	9.5	5.5	12.3	4.5	10.9	14.3	9.5	5.9
Mean	3.3	0.4	2.7	0.5	5.0	3.8	5.3	1.8
STD	2.5	1.4	4.3	1.3	5.2	6.2	4.0	2.7

**Table S9.** The concentration of OTC (ngL<sup>-1</sup>) detected in farm area (inlet, pond, outlet)

Region	Concentration (ngL <sup>-1</sup> )		
	Inlet	Pond	Outlet
<b>Brebes</b>			
Min	0.0	0.0	5.0
Max	3.4	0.0	11.5
Mean	0.7	0.0	7.2
STD	1.5	0.0	2.7
<b>Tegal</b>			
Min	0	0	0
Max	0.0	2.5	9.9
Mean	0.0	0.5	5.4
STD	0.0	1.1	4.3
<b>Pemalang</b>			
Min	0	0	0
Max	0.0	0.0	5.5
Mean	0.0	0.0	1.9
STD	0.0	0.0	2.5
<b>Pekalongan</b>			
Min	0.0	0.0	4.7
Max	0.0	0.0	12.3
Mean	0.0	0.0	8.0
STD	0.0	0.0	3.3

**Fig. S10.** Design of shrimp farm areas in the studied area (Tegal)





**Fig. S11.** Duck farming area in Brebes Regency located in the surroundings of the aquaculture area.





**Fig. S12.** Spearman correlation coefficients based on antibiotic concentrations in water

