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Large Scale Nationwide Screening of Bioavailable Tetracyclines and Arsenic Using Whole-cell Bioreporter from Pangasius and Tilapia Aquaculture System in Bangladesh

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Abstract

In this study, a large-scale screening of bioavailable tetracycline (TC) and arsenic (As) was carried out using Whole-Cell biosensor bacteria from the pond surface waters of selected pangasius and tilapia farms across Bangladesh. Bioavailability of Tetracyclines and Arsenic was detected using biosensor bacteria *E. coli* K12 pTetLux1 and *E. coli* DH5R (pJAMA-arsR), respectively. 62 samples were within the tetracycline detection limit; others were below the detection limit. The highest concentration 31.27 µg/l was found in a pond of Jashore and the lowest concentration 2.57 µg/l, was measured from Trishal, Mymensingh. Tetracyclines were detected in 19.57 % of all analyzed samples and varied from below the detection limit to a maximum of 31.27 µg/l. Khulna showed the maximum percentage of detected samples (90%), followed by Mymensingh (52%), Cumilla (27.5%), Jashore (14%), Bogura (14%), and Sathkhira (10%). Bioavailable As were detected in only 5.7% of all analyzed samples. Detectable as samples were higher in number among the collected samples at Madaripur and higher in percentage at Sathkhira (30%) followed by Madaripur (20%), Cumilla (5%), and Gopalgonj (3.2%). The pick level of Arsenic was found in Madaripur, which was 9.45 µg/l and the lowest figure was 2.35 µg/l, found in Cumilla. Though the concentrations of arsenic in groundwater (tube well water) were high in all studied regions, the concentrations of arsenic in pond water were low in every region. The concentration of as in the experimental ponds was neither very high nor in the danger limit. Use of shallow tube well water, fertilizers, drugs, pesticides, and herbicides possessed with as during fish culture might be the source of as contamination. However, extensive use of antibiotics results in the emergence and spread of antibiotic resistance in the aquatic environment.

Keywords: biosensor, bioavailability, tetracyclines, arsenic, aquaculture, Bangladesh

1. Introduction

Aquaculture is the largest sector of food production in the world, which has the potential to contribute to maintainable food production in the upcoming days (Hameed et al., 2022). In the most recent two decades, Bangladeshi aquaculture has improved expanded, and progressed innovatively with a growing trend to intensify cultural techniques in some regions in Bangladesh (Belton and Azad, 2012; Ali et al., 2013). With the extension and escalation of the aquaculture sector, there has been an increasing interest in using synthetic compounds and organic products (Faruk et al., 2008). Different natural and synthetic substances significantly contribute to treating and inhibiting various diseases, recovering water quality, upturning pond natural production, and growth promoters (Rico et al., 2013). Tetracycline antibiotics are the most important among them. Chlortetracycline and oxytetracycline, the naturally occurring antimicrobial agents were first discovered in the last part of the 1940s.

To improve growth rate and feed efficiency in animals, antibiotics are broadly used to treat and prevent infectious diseases in humans and livestock, and they are likewise utilized in agriculture and aquafarming (Cabello, 2006; FDA, 2009; McEwen and Fedorka-Cray, 2002; McManus et al., 2002). After applying antimicrobials, a substantial portion is discharged into various ecological sections (Zhou et al., 2013). About 30%

to 90% of all antimicrobials utilized in humans and livestock are released unaltered into the environment via domestic sewage (Jjemba, 2006; Lienert et al., 2007).

Groundwater has been utilized comprehensively as the principal wellspring of drinking and irrigation water availability in Bangladesh since the 1960s. It assumes a crucial part in making the nation independent in food production. Groundwater supplies about 90% of irrigation and 99% of drinking water in Bangladesh. However, this water asset is confronting issues remembering quality risk for some regions where the disclosure of contamination from cultivation and arsenic contamination in shallow aquifers makes the water unsafe for public use (Anwar et al., 2008). Groundwater and surface water tainting by arsenic happened normally or from human activities. Surface and groundwater are fundamentally utilized for aquaculture in Bangladesh. Some potential human health dangers are identified with domesticated animals and freshwater fisheries as these can be exposed to As through drinking water, lake water, and feeds.

Arsenic contamination and antimicrobial agents' utilization in aquaculture can be controlled by regular observation of arsenic and antibiotic deposits in various samples. In the present study, 325 farms were surveyed from different regions of Bangladesh where pangasius and tilapia are being cultured commercially to monitor or detect the bioavailable concentration of tetracyclines and arsenic using a whole-cell bacterial biosensor. One of the most important features of whole cell bioreporters (WCBs) is their capability to enumerate bioavailability that gives information associated with the effects of contaminants on living organisms and cannot be assessed by traditional instrumental analysis. The use of WCBs is more beneficial than any other chemical analysis since it is a stable, low-cost, high-throughput process and ideal for a large number of samples to be rapidly screened. This study aimed to provide information on the quantities of bioavailable arsenic and tetracyclines using whole cell bioreporters in pangasius and tilapia farms across Bangladesh and their interaction. To our knowledge, this is the first-ever large-scale monitoring of bioavailable tetracyclines and arsenic using whole-cell bioreporters of environmental samples across Bangladesh.

2. Materials and Methods

2.1 Sampling Sites and Collection of Samples

There are some distinct regions of Bangladesh where pangasius and tilapia aquaculture have been carried out at a large scale and dominated by other finfish. The major regions are Mymensingh, Cumilla, Bogura, and Jashore. Besides these areas, pangasius and tilapia are also being cultured in some other regions of Bangladesh on a medium scale (Figure 1). Arsenic contamination is dominant in some particular regions of Bangladesh. Jashore, Comilla, Feni, Chandpur, Madaripur, Shariatpur, and Sathkhira regions are highly affected by arsenic. 325 pond samples were collected for analysis of bioavailable tetracycline, and 212 samples were selected for the analysis of bioavailable arsenic from these regions. Besides these, 45 tube-well water samples from some arsenic sampling areas were collected to compare underground water arsenic concentration with pond water. A 100ml plastic water bottle was used to collect samples. High-resolution GPS coordinates were recorded for each pond. Water bottles were kept in the icebox with coolants to maintain the water temperature around 10-15˚C during transportation and to prevent direct exposure to light. After arrival in the lab, samples were stored in a freezer at -20˚C.

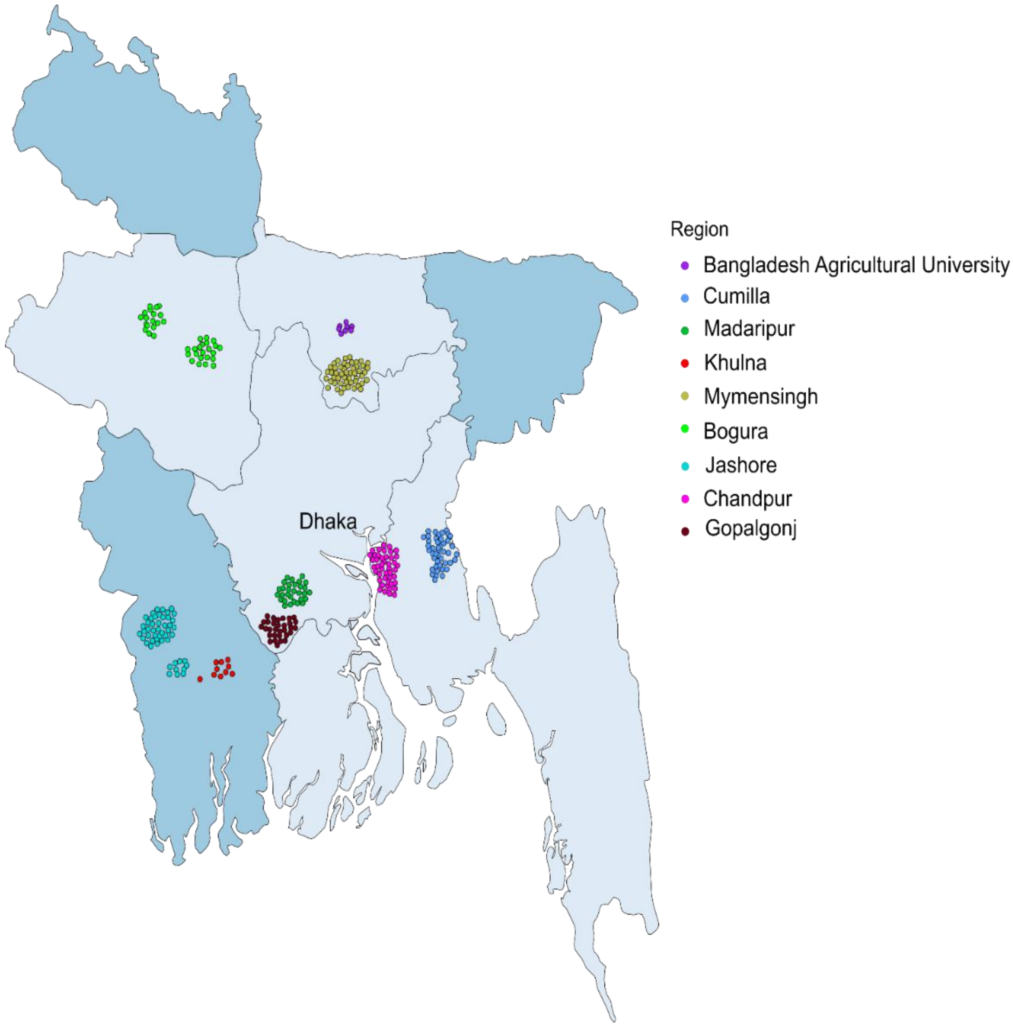

Figure 1. Map of Bangladesh showing all sampling locations of this study. The sampling ponds are colored according to the region in which they are located

2.2 Bacterial Strains, Plasmids, and Media

2.2.1 Tetracycline Biosensor Strain

The construction of the tetracycline biosensor strain *E. coli* K12 (pTetLux1) is described by Korpela et al. (1998). In brief, the luciferase operon luxCDABE of *P. luminescens* bacteria from pCGLS-11 was embedded as an EcoRI piece leveled out of the tetracycline-inducible tetA promoter/operator in pASK75. The subsequent plasmid build, pTetLux1, was changed by electroporation into *E. coli* K12 strain M72 to get a bioluminescent antibiotic-responsive biosensor strain. The bacteria were refined at 37 ℃ with air circulation (200 rpm) in Luria-Bertani stock (LB; 1% (w/v), tryptone, 0.5% (w/v) yeast, 0.5% (w/v) NaCl, pH 7.0) enhanced with 100 μg mL-1 ampicillin (LBAmp).

2.2.2 Arsenic Biosensor Strain

The construction of an arsenic biosensor, *E. coli* DH5R (pJAMA-arsR), is described by Stocker et al. (2003). Briefly, arsenic detection by the bacterial biosensor is based on bioluminescence created by the cells due to the presence of arsenic. Under the expression regulation of the ArsR repressor enzyme, bioreporter cells bear a plasmid with genes for bacterial luciferase (luxAB). Cell passage of arsenite causes the arrival of transcriptional constraint and the resulting amalgamation of luciferase by the cells. Arsenate is impulsively decreased by the cells to arsenite and can indirectly induce derepression and luciferase synthesis.

2.3 Preparation of Standard Solution

2.3.1 Tetracycline Standard Solution

On the analysis day, different concentrations of tetracycline standard solutions were prepared from tetracycline stock solution (10mg/ml in 70% ethanol). The concentration of standard solutions were: 150, 100, 75, 50, 25, 12.5, 6.25, 3, 2, 1.5 and 0 µg/l.

2.3.2 Arsenic Standard Solution

Standard solutions were freshly prepared on the day of analysis. For arsenate: Disodium Hydrogen Arsenate ($Na_2HAsO_4 \times 7H_2O$) in the 0- 3000 µg/l concentration range. For arsenite: Sodium arsenite ($NaAsO_2$) in the 0-300 µg/l concentration range.

2.4 Plate Assay

2.4.1 Tetracycline Plate Assay

A tetracycline plate assay was performed using cells cultured in LB media. Cells were cultured in 25ml LB media with 100µg/ml ampicillin in 100ml glass flask directly inoculated from an LB agar plate with ampicillin (100µg/ml) and incubated at 20˚C for 14-16 hours to obtain an OD_{600} around 1.5. The cultured cell was diluted in LB media without ampicillin to get OD_{600}=0.02. Cultured cells were divided onto white 96-well flat-bottom microtiter plates (NUNC™, ThermoFisher Scientific Inc., Massachusetts, USA) as 100 µL aliquots. Beforehand, the wells had been dispensed with 100 µL of samples and standard solutions. The plates were covered by a lid and incubated for 3hrs at 37˚C and 200rpm in darkness. Bioluminescence was read from the plates by a multi-detection microplate reader, the luminometer (FLUOStarOptima, BMG Labtech, Ortenberg, Germany).

2.4.1 Arsenic Plate Assay

Arsenic plate assay was performed by reviving the cell on LB-agar plates with 50 µg/L ampicillin at 37 ℃ overnight. One colony was transferred to 25 ml LB + 50 µg/L ampicillin the next day and grown horizontally at 37 ℃ at 200 rpm. The overnight culture was harvested at 5000 g for 10 minutes and suspended in LB to obtain an absolute OD600 of 0.02. 100 µL of standard or sample solution was mixed with 100 µL cell suspension in white 96-well microtiter plates and incubated at 28℃ for 2 hours. Luminescence was measured with a plate reader where decanal (35 µL decanal + 10 mL 50 % ethanol) was used as an external substrate for the cells.

2.5 Calculation

2.5.1 Tetracycline Concentration

From the measurements of the standard solution rows, standard curves were derived, fitted by third-order polynomial regression.

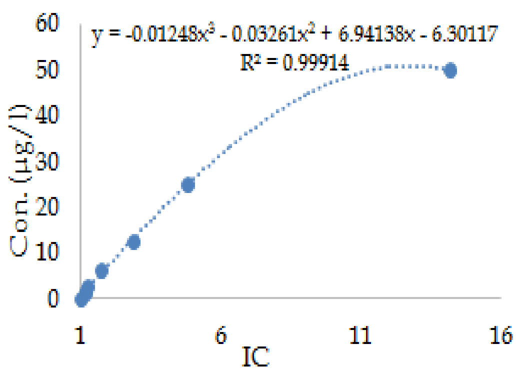

Figure 2. Standard curve for the biosensor assay with *E. coli* K12 (pTetLux1) for Tetracycline, respectively, showing the dependency of the batch for the analysis (IC = induction Coefficient; Con = Tetracycline concentration in µg/L)

The calibration curves were based on an induction Coefficient (IC) which relates the measured bioluminescence in the sample to the bioluminescence in the blanks to correct background noise and account for drift. It was calculated as follows:

$$\text{Induction coefficient (IC)} = \frac{\text{Luminescence in sample solution (LM)}}{\text{Background luminescence in blank (LW)}}$$

2.5.2 Arsenic Concentration

From the measurements of the standard solution rows for both As(V) and As(III), calibration curves were derived and fitted by third-order polynomial regression (examples for two different batches, respectively, are shown in Figure 3). As discussed earlier, pJAMA arsR primarily responds to As(III), and only after enzymatic reduction of As(V) to As(III) also to As(V), which is why As(III) induces the biosensor much stronger.

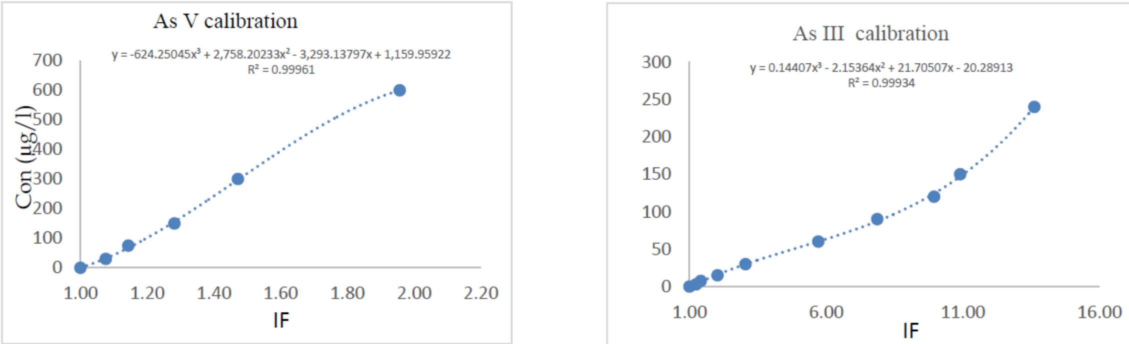

Figure 3. Two example calibration curves for the biosensor assay with *E.coli* pJAMA arsR for As(V) and As(III), respectively, showing the dependency of the batch for the analysis (IF = induction factor; Con = As concentration in µg/L)

The calibration curves were based on an induction factor (IF) that relates the measured bioluminescence in the sample to the bioluminescence in the blanks to correct background noise and account for drift. It was calculated as follows:

$$\mathrm{IF} = \frac{\text{bioluminescence in the sample}}{\text{mean bioluminesence in blanks before and after the sample}}$$

2.6 Statistical Analysis

Statistical analysis was done using Microsoft Excel Software 2010 and SPSS 23 (Statistical Package for Social Science). Collected data were coded and entered into a database system and analyzed.

3. Results

3.1 Bioavailable Tetracycline Concentration in Different Ponds

Bioavailable tetracycline concentrations of 325 ponds were analyzed in laboratory conditions using a whole-cell bacterial biosensor named *E.coli* K12 pTetlux1. A total of 62 samples were in the range of the tetracycline detection limit others were below the detection limit (Table S1). Biosensors can usually detect the bioavailability of any sample if the Induction coefficient is 50% higher than the neutral IC (IC 1). For this reason, the Induction coefficient of 1.5 and more than 1.5 is considered to measure the bioavailability of tetracyclines. The tetracycline concentrations were higher in some Jashore, Cumilla, and Mymensingh ponds and very low in some Chandpur, Madaripur, and Satkhira ponds. The highest concentration 31.27 µg/l was found in a pond of Jashore and the lowest concentration 2.57 µg/l was measured from Trishal, Mymensingh. Most samples in the Khulna region showed tetracycline concentration at the detection limit. All samples of Chandpur and Madaripur showed TC Concentration below the detection limit.

A comparative study of bioavailable tetracycline in different regions of Bangladesh was done and the results are shown in table 3.1. Samples were categorized into 9 sections. These were below detection, >0 to ≤3, >3.0 to ≤4.0, >4.0 to ≤6.0, >6.0 to ≤8.0, >8.0 to ≤10.0, >10.0 to ≤12.0, >12.0 to ≤15.0, >15.0 to ≤20.0 and >20 µg/l. Among these, the highest number of samples (258) were below the detection limit. Among the samples at the TC detection limit, the >4.0 to ≤6.0 section showed the highest number of samples and the number was 24. All the samples of Chandpur and Madaripur were below the detection limit. Only two samples with more than 20 µg/l concentrations were detected, from Bogura and Jashore. A comparative study of measured bioavailable tetracycline in different regions of Bangladesh is shown in Figure 4.

Table 1. Comparative study of bioavailable tetracycline in different regions of Bangladesh

Region	No. of sample	Below detection	>0-≤3	>3.0-≤4.0	>4.0-≤6.0	>6.0-≤8.0	>8.0-≤10.0	>10.0-≤12.0	>12.0-≤15.0	>15.0-≤20.0	>20
Bogura	50	43	0	0	1	1	3	1	0	0	1
Cumilla	50	39	0	1	2	1	3	1	1	2	0
Jashore	40	33	0	0	0	3	1	0	1	1	1
Khulna	10	1	0	0	3	2	2	0	0	2	0
Mymensingh	53	26	1	2	17	7	0	0	0	0	0
Chandpur	51	50	0	0	0	0	0	0	0	0	0
Gopalganj	31	27	0	2	1	0	1	0	0	0	0
Madaripur	30	30	0	0	0	0	0	0	0	0	0
Satkhira	10	9	0	0	0	1	0	0	0	0	0
Total	325	258	1	5	24	15	10	2	2	5	2

Percentages of TC detected samples within the total experimented sample within every region were calculated (Figure 3.6). Tetracyclines were detected in 19.57 % of all analyzed samples and varied from below the detection limit to a maximum of 31.27 μg/l. Khulna showed the maximum percentage of detected samples (90%), followed by Mymensingh (52%), Cumilla (27.5%), Jashore (14%), Bogura (14%) and Sathkhira (10%). The lowest value was found in the samples of Chandpur and Madaripur (0%).

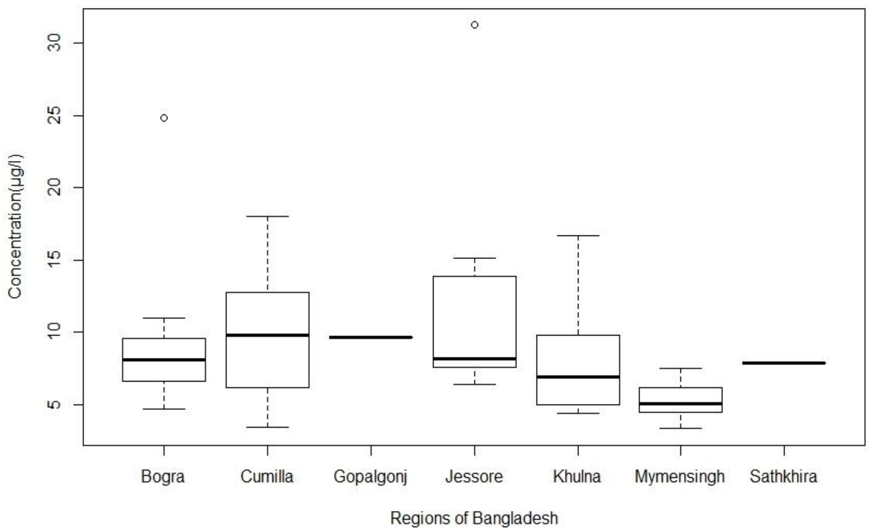

Figure 4. Measured bioavailable concentration of tetracycline (only detected values) in ponds water samples. The median, the 25th and 75th percentiles, the lowest datum within 1.5 times the interquartile range of the lower quartile, and the highest datum within 1.5 times the interquartile range of the upper quartile are shown in each box. Dots reflect outliers

3.2 Bioavailable Arsenic (As) Concentration of Ponds in the Study Area

Only 12 samples among 212 showed as presence (Table S2) as concentrations of the pond's water sample varied with the geographical location of the ponds. Some ponds showed a statable amount of as and some were null. In this study, the pick level of Arsenic was found in Madaripur, which was 9.45 µg/l. On the other hand, among the detected samples lowest figure was 2.35 µg/l, found in Cumilla. Concentrations were zero or below the detection limit in all ponds in Jashore, Chandpur, and Mymensingh regions.

Availability of as in water samples among the collected samples from selected regions was calculated in percentage value. Bioavailable As were detected in only 5.7% of all analyzed samples. Detectable as samples were higher in number among the collected samples at Madaripur and higher in percentage at Sathkhira (30%) followed by Madaripur (20%), Cumilla (5%), and Gopalgonj (3.2%). On the contrary, all the samples from Jashore, Chandpur, and Mymensingh were found below the detection limit and the percentage of arsenic was Zero (0%). Availability of as based on percentage value among samples from different regions of Bangladesh is shown in Figure 5.

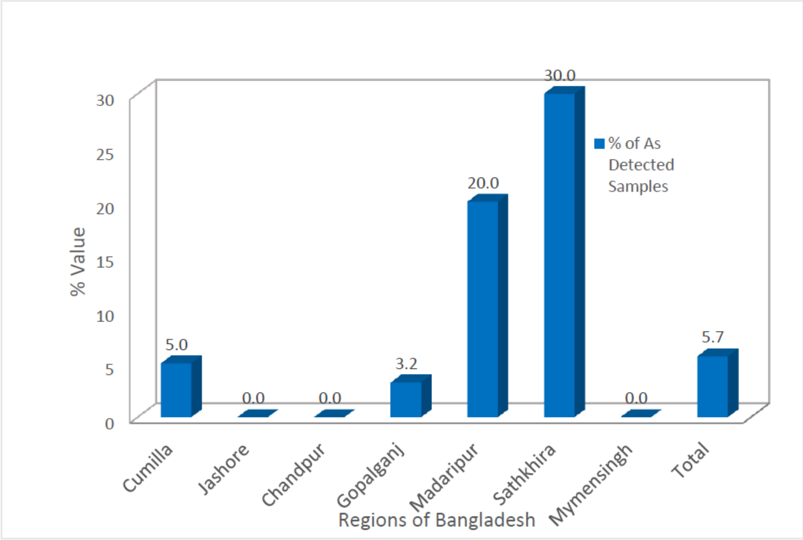

Figure 5. Percentages of samples at bioavailable arsenic detection limit

3.3 Bioavailable Arsenic (As) Concentration of Tube Wells in the Study Area

Individual water samples were collected from the tube wells during the study period to analyze the as concentration of tube well (underground) water from the same regions (Table S3). A total of 45 samples were culled from all these regions where five from each region. Among the supplied samples, twelve were found to be below the detection level, and their concentration was assumed to be zero. The highest value of as concentration was found in Madaripur which was 37.96 µg/l. Near all water samples of Gopalgonj, Jashore, Chandpur and Madaripur were found to be affected with arsenic but in variable concentrations.

3.4 Comparative Study on Available Percentages between Tube Well and Pond Water Samples

A comparative study was performed on the percentages of available As between tube wells and pond water. All of the tube well's sample of Gopalgonj was above the detection limit and the availability was considered 100%. But the percentage of availability of as in pond water samples was only 3.2%. The percentages detected in pond water were 5 and 0 in Cumilla and Chandpur, respectively, but had higher levels in tube well (70%). A relatively higher percentage of detected As was found both in pond and tube well water in Madaripur (20% and 80%) and Sathkhira (30% and 60%). Overall, the percentage of detectable bioavailable as in pond and tube well water were 5.7 and 71.1, respectively. A comparative study on available as percentages between tube wells and pond water samples has been given in Figure 6.

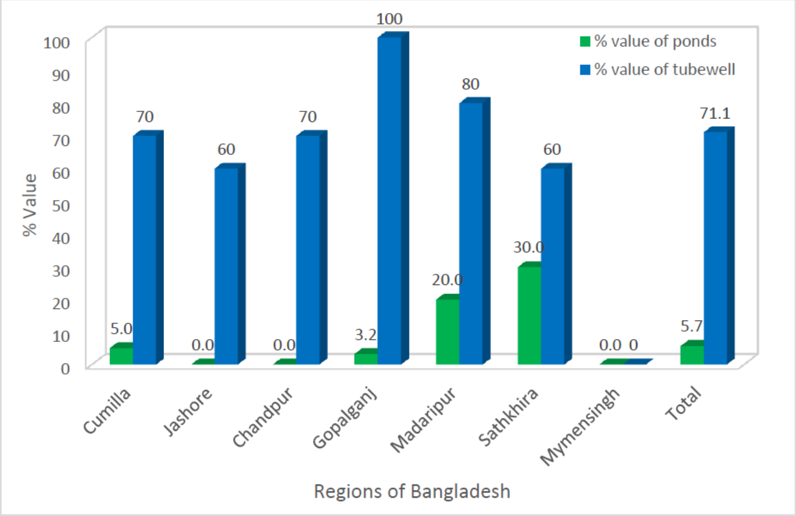

Figure 6. Percentage of As between tube well and pond water samples

4. Discussion

4.1 Tetracycline Concentration

Anti-microbial treatment has been applied in aquaculture for more than 60 years. Among the most used drugs in veterinary medicine, antibiotics are dominant (Sanders, 2005). The main motive behind the management of contagious diseases in hatcheries is to avoid production losses; stop microbes from accessing new facilities; stop the transmission of disease; and inhibit the intensification of pathogens already widespread in the water body (Phillips et al., 2004; Winton, 2001; Lupin, 2009). Utilization of antibiotics and other synesthetic compounds have contributed to the higher production and development of the aquaculture sector but has also been criticized due to probable harmful impacts on human being and ecology (Holmström et al., 2003; Heuer et al., 2009; Sapkota et al., 2008; Rico et al., 2013). Deposits of conceivably harmful substances of antibiotics can amass in the treated animal, bringing about a potential danger for buyers and to market and export of cultured products. (Heuer et al., 2009; Sapkota et al., 2008). The broad utilization of antimicrobials in aquafarming can prompt the opportunity of antibiotic-resistant bacteria both inside and outside the aquaculture systems (Le et al., 2005; Sørum, 1999; Inglis, 2000).

In this study, the range of tetracycline value in detection level was 3.3 to 31.27 µg/l, which is more or less similar to Murat and Emine (2016), who detected the highest TC concentration was 50.0±2.5 µgL^{-1} at 1st week, and the lowest was 8.2±0.41 µgL^{-1} at 7th week. The average value of tetracycline was 1.82±3.99 µgl^{-1}. Karthikeyan & Meyer (2006) stated the Tetracycline concentration in the influent was 48±3.21 µgL^{-1} and in the effluent, the TC concentration was 3.6±0.31 µgL^{-1} which is more than the present study.

Results showed that the tetracycline varied up to a maximum of 31.27µg/L and a minimum of 3.3µg/l. Lalumera et al. (2003) found 246.3 and 578.8 µg/kg d.w of oxytetracycline and flumequine in surface water. Lalumera et al. (2003) also reported that flumequine was found in the highest toxicity in EC50 bioluminescence assay within a range of 12–15 mg/l, whereas the EC50 values for oxytetracycline ranged from 121–139 mg/l. That is more than the value of the present study.

Tetracyclines are significantly steadier in the ecosystem than other antimicrobial agents, permitting TCs to continue for prolonged periods, disperse more, and amass at high concentrations and sully water sources and soils (Ingerslev et al., 2001). Tetracyclines illustrate very low biodegradability in the state of administered tests and are seen as constant in fertilizer and soil, with high sorption potential (Kümmerer et al., 2000; Sassman and Lee, 2005).

4.2 Arsenic Contamination of Pond's Water

Arsenic concentrations are commonly higher in groundwater than in surface water. Food grown in contaminated water is detrimental and attributed threat of arsenicosis. Of collected water samples, 5.7% was detectable by *E.coli* K12 pTetlux1 where concentrations of as were found highest in a pond in Madaripur (Table S2). The highest concentration was found at 9.45 µg/l among the detectable samples and it was the only sample that scored closer to 10 µg/l while the standard arsenic limit in the case of Bangladesh is 50 µg/L (UNICEF, 2008). Arsenic concentrations at or above 50 µg/L are associated with potential human health risks. Ingestion of arsenic via food or water may also seriously affect the human cardiovascular system. Because acute and chronic exposure may lead to heart failure (Fennel et al., 1981; Goldsmith et al., 1986).

Among the total twelve (12) arsenic-detectable samples, nine (9) samples were found above 5 µg/L (Table S2). Within all the samples just 4.25% is over 5 µg/L which is as yet in resistance level both for As-influenced water body and As concentration in water body because any scientists enrolled no calamity in this convergence of As, more explicitly, any aquaculture products. Moreover, aquatic organisms raise above approximately 18 µg/L of As concentration has adverse effects. From a report, adverse effects of arsenicals on aquatic organisms have been found at concentrations of 19 to 48 ug/l in water, 120 mg/kg in diets, and 1.3 to 5 mg/kg fresh weight in tissues (Eisler, 1988). Arsenic is mainly accumulated in the livers of fish. The liver tissue of fish has shown a significant decrease in all enzymatic activity (Humtsoe *et al.*, 2007). Fathead minnow (*Pimephales promelas*) and flagfish (*Jordanella floridae*) to arsenite (4.3 mg/L, and 4.12 mg/L, for 29 and 31 days, respectively) showed a significant reduction in their growth (Lima et al., 1984). All of the calamities above from different sources materialized at high as concentrations, where arsenic concentration in the pond's water found through this study was relatively low and can be stated as a danger-free circumference.

4.3 Arsenic Contamination of Tube Well Water

The World Health Organization (WHO) created Guidelines for Drinking Water Quality (GDWQ) to protect general well-being from drinking water contaminants. The 1993 release of WHO GDWQ built up the arsenic drinking water rule of 10 µg/L (10 ppb). Currently, as far as possible in Bangladesh is set to 50 µg/L (UNICEF, 2008). In this study, most of the tube well water samples from Madaripur and Jashore were higher than 10 µg/L with the highest value of 37.96 (µg/L) in Madaripur which are below the standard limit for Bangladesh but a matter of great concern in case of international standard. Since the mean everyday admission of arsenic from drinking water will mostly be under 10 µg/L.

Though the concentrations of arsenic in groundwater (tube well water) were high in all studied regions, the concentrations of arsenic in pond water were low in every region (Figure 6). This might be the dilution of surface water/runoff water with underground water in the pond. Farmers often use underground water in the pond when the water level goes down, especially in the dry season.

The As concentration in the experimental ponds was neither very high nor within the danger limit. Dittmar et al. (2010), Khan et al. (2009, 2010) reported that as contaminated groundwater used for irrigation in Bangladesh and West Bengal (India) and also indicated that As concentrations in surface waters of these areas would be high. Nowadays, shallow tube well's water is not only used for irrigation but also for fish culture. It would be a reliable and possible source of as contamination of the selected water bodies. Again, other sources such as using fertilizers, drugs, pesticides, and herbicides possessed with as during fish culture might be another source of as contamination.

5. Conclusion

Only 62 of the 325 samples were within the detection limit. Tetracycline varied up to a maximum of 31.27µg/L and a minimum of 3.3µg/l. In this study, we report that there is a dangerous potential that results from the TC used in aquaculture in Bangladesh. Extensive use of antibiotics results in growing antibiotic resistance to harmful bacterial populations and the spread of antibiotic resistance in aquatic and terrestrial environments. The concentrations of as in shallow groundwater were usually low in the case of Bangladesh standards. Most are below the WHO provisional guideline value for drinking water of 50 µg/L. Most of the previous studies related to aquatic as focused mostly on As distribution, speciation, and bioaccumulation in groundwater and soil in Bangladesh, while the present study mainly focused on the bioavailability of As in freshwater ponds of highly As-affected areas. However, the as concentration in the detected pond's water samples was low. No researcher reported any catastrophe in such a concentration of as, specifically, aquaculture products. So, it can be stated that pangasius and tilapia raised in the studied regions are safe for consumption.

Considering the fast development and significance of aquaculture in various parts of Bangladesh and the widespread, intensive, and frequently uncontrolled utilization of antibiotics for fish culture, further endeavors are expected to stop the development and spread of antibacterial resistance in aquaculture. Again, for better assurance, the study on the bioavailability of As in aquatic biota more specifically in pangasius and tilapia of these selected farms would be performed in the future which will increase the acceptability of our products in the international market with appropriate value and thus enhance our national income.

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Authors Contributions

Dr. MEA, Dr. MAR, Dr. SRI, AA, and Prof. MLA were responsible for the planning and design of the experimental work; Dr. MEA with assistance from Dr. SRI and AA conducted fieldwork and laboratory analysis in Bangladesh; all authors contributed to the interpretation of data; MEA and SRI drafted the MS with input from Dr. MAR, and Prof. MLA.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

Open access

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Appendix

Table S1. Bioavailable tetracycline concentration of different regions

SL. No.	Region	IC	Con. (μg/l)	SL. No.	Region	IC	Con. (μg/l)
1		1.51	8.07	32		2.59	9.52
2		1.52	8.25	33		1.70	4.42
3		1.51	8.08	34		1.80	4.99
4	Bogura	1.64	4.71	35		2.19	7.23
5		1.68	4.97	36		1.54	3.53
6		1.63	24.85	37		1.51	3.33
7		2.63	11.10	38		1.71	4.50
8		2.13	11.38	39		1.88	5.46
9		1.52	7.65	40		1.83	5.18
10		1.55	8.06	41		1.70	4.43
11		2.19	17.52	42		2.02	6.23
12		1.67	9.85	43		1.91	6.29
13	Cumilla	1.73	4.46	44		1.53	4.20
14		1.55	3.45	45		1.64	4.90
15		1.77	4.68	46		1.71	5.26
16		1.66	9.78	47		1.53	4.19
17		2.22	18.05	48	Mymensingh	1.58	4.56
18		1.96	14.23	49		1.62	4.79
19		1.50	7.30	50		1.54	4.27
20		1.54	7.85	51		2.19	7.49
21		3.19	15.13	52		2.14	7.31
22	Jashore	2.12	8.18	53		1.59	4.62
23		2.80	12.60	54		1.69	5.19
24		5.96	31.27	55		1.89	6.21
25		1.86	6.43	56		2.05	6.92
26		2.04	6.39	57		1.60	4.66
27		3.83	16.60	58		2.02	5.97
28		2.14	6.93	59		1.95	5.60
29	Khulna	3.84	16.67	60		1.76	4.65
30		2.65	9.83	61	Gopalgonj	2.06	9.63
31		1.75	4.69	62	Sathkhira	1.51	7.90

IC=Induction Coefficient. IC≥1.5 is only considered to measure bioavailable tetracycline

Table S2. Bioavailable Arsenic concentration of ponds at studied regions

SL. No.	Area	IF	As Concentration (µg/l)
1	Cumilla	1.54	2.35
2		1.66	8.37
3	Gopalgonj	2.78	4.59
4	Madaripur	1.50	5.37
5		1.89	9.45
6		1.69	7.37
7		1.72	7.45
8		1.66	6.79
9		1.53	5.26
10	Sathkhira	1.66	6.26
11		1.69	6.53
12		1.66	6.76

Table S3. Bioavailable arsenic concentration of tube well water in different regions

SL. No.	Area	IF	As Concentration (µg/l)
1		5.59	33.10
2		3.68	21.02
3	**Gopalgonj**	3.33	18.71
4		3.64	20.72
5		2.05	9.46
6		4.88	28.64
7	**Madaripur**	6.34	37.96
8		3.20	17.80
9		2.25	10.99
10		5.59	27.91
11		5.51	27.51
12	**Jashore**	4.23	20.62
13		3.48	16.04
14		2.20	7.78
15		3.77	17.86
16		1.67	4.31
17		1.83	5.35
18		1.83	5.32
19	**Chandpur**	1.96	6.15
20		1.77	4.96
21		2.06	6.86
22		3.45	15.81
23		1.78	4.56
24	**Sathkhira**	3.92	17.05
25		3.43	18.90
26		2.23	7.79
27		2.31	11.10
28		3.03	16.71
29	**Cumilla**	3.43	18.80
30		1.59	4.77
31		3.01	16.71
32		3.81	18.86