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Chemical Contaminants and Water Quality in Shrimp Farms of Edavanakkadu Area, Cochin, India

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Abstract

Aquaculture is gaining importance these days as catch from wild cannot keep up with the demand of the growing human population. But the culture system often encounters problems which are potentially harmful to consumers. This study was carried out in October to December 2012 in three randomly selected tiger shrimp (*Penaeus monodon*) farms of Edavanakkadu area, Cochin to evaluate the level of chemical contaminants and water quality. Shrimp samples were analysed for the presence of antibiotics such as chloramphenicol and nitrofuran metabolites. The content of heavy metals such as Cd, Co, Cu, Cr, Mn, Ni, Pb and Zn was evaluated in the samples of shrimp, water and sediment. The water quality parameters (temperature, pH, salinity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, total alkalinity and total hardness) were also evaluated. Shrimp samples were found free of chloramphenicol, but 1-aminohydantoin (AHD), metabolite of the prohibited antibiotic nitrofurantoin was detected in shrimp samples from all three farms in the range of 6.39±0.56, 0.595±0.00 and 10.3±0.00 ppb respectively. This points to the serious situation of usage of the prohibited carcinogenic antibiotics in aquaculture. The levels of heavy metals in the shrimp samples were found to be negligible making them safe for human consumption. Comparatively higher levels of heavy metals were present in the sediment and lower levels in water samples. Most of the water quality parameters were within a normal range prescribed for shrimp culture.

Keywords: Farmed shrimp, chloramphenicol, nitrofuran metabolites, heavy metals, water quality

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Introduction

Aquaculture is currently one of the fastest growing food production sectors in the world. Its increasing global importance is directly related to its contribution to reducing the gap between supply and demand for fish products. Fish and crustaceans are generally regarded as safe and nutritious food, but products from aquaculture have sometimes been associated with certain food safety issues, as the risk of contamination by chemical and biological agents is greater in freshwater and coastal ecosystems than in open seas. The specificity of chemical contamination for aquaculture products as compared to wild aquatic products is related to the origin of the contaminants. Environment, feeding and rearing practices could lead to the accumulation of specific compounds in the flesh of fish. Apart from environmental issues arising from aquaculture, the presence of chemical residues and pathogenic organisms in cultured organisms may render aquaculture products unsafe for human consumption and fail to meet international standards, guidelines and recommendations as aquaculture makes a transition to a major food-producing sector; proper assessment and control of any food safety concerns are becoming increasingly important.

Accumulation of antibiotic residues in the edible tissues of shrimp may alter human intestinal flora and cause problems of food poisoning or allergy (Ma et al., 2006) besides contributing to increased antimicrobial resistance to this "critically important" class of antibiotics to human pathogens. The use of chloramphenicol (CAP) in farm animals intended for human consumption has been restricted (Munns et al., 1994; Nicolich et al., 2006). In 1993, the European Union decided to ban the use of nitrofurans (furaltadone, nitrofurazone, nitrofurantoin and furazolidone) because of the potential mutagenic and carcinogenic effects of their metabolites (Anon, 1993; Van Koten et al., 1993).

High degree of industrialization and urbanization has led to a strong risk of heavy metal contamination in coastal ecosystems in tropical and subtropical countries. Little is known about the flux and fate of metals from the estuaries into these culture areas. Water quality determines the success or failure of an aquaculture operation. Urbanization, industrialization and chemical usage in agriculture makes it extremely difficult to find pollution-free waters in many coastal areas. Given the sensitivity of shrimps (particularly young stages) to pollutants, shrimp culture is highly risky in areas of intense agricultural, urban and industrial development.

In the shrimp farms of Edavanakkadu area of Vypin, Vembanadu Lake is the main source of water for aquaculture activity. Chemical hazard profile of the farm environment *viz.*, water and farm as well as the aquaculture produce from the farm was evaluated in this study.

Materials and Methods

The study was carried out in October-November of 2012. Three shrimp farms were selected randomly in the Edavanakkadu village of Vypin Island where traditional farming *viz.*, prawn filtration improvised by selective multiple stocking of *Penaeus monodon* is carried out. Samples of shrimp, water and sediment were used for the study and stored at –20°C. Shrimp samples of average size of 55 g were collected and transferred in ice boxes. Water samples were collected randomly from different locations of the farm in clean plastic cans and stored for analysis. Water samples for dissolved oxygen (DO) and biological oxygen demand (BOD) were collected in DO bottles and fixed by adding reagents at the farm site. Sediment samples were collected randomly from different locations of the pond bottom and were transferred to the laboratory. Shrimp samples were peeled and the edible tissue was homogenized. Sediment samples were dried under sunlight, powdered and packed in polyethylene bags for analysis.

Sample extraction for analysis of nitrofuran metabolites and chloramphenicol was done as per the method of USFDA (2004) and Barbara et al. (2002). Analysis was done using LC MSMS (API 4000 Q Trap of AB Sciex, Canada). UPLC BEH C_{18}-150×2.1 mm column was used with gradient elution at a flow rate of 0.6 ml min^{-1} consisting of a buffer system of acetonitrile and 0.5% formic acid in water for nitrofuran metabolites and buffer systems of methanol and 0.5 mM ammonium acetate in water for chloramphenicol.

For the analysis of heavy metals, the shrimp homogenate was digested with conc. HNO_3 in a microwave (Multiwave 3000, Microwave reaction system, Anton Par), diluted to 50 ml with milli Q water and filtered in 0.2 μ filter. The dried sediment powder was digested by adding 9:4 mixture of conc. nitric acid and perchloricacid and then diluted to 50 ml and filtered. Water samples were directly filtered for analysis. The analysis was carried out in inductively coupled plasma (ICP) photometer (ICAD 6300 Duo view, Thermofisher, USA).

Water temperature was measured using a calibrated mercury thermometer and pHusing pHmeter (pH Tutor, Eutech instruments). Parameters such as salinity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, total alkalinity and total hardness were determined as per APHA (2005). Nitrate and nitrite levels were measured using spectroquont photometer SQ 118 (Merck).

Statistical analysis

Data obtained from the experiment was subjected to one way analysis of variance (ANOVA) using the statistical package for social sciences, SPSS 16 (SPSS Inc., Chicago). Post hoc analysis was carried out using Duncan's test. Pearson correlation matrix was used to test the significant relationship between the heavy metals in the sediment.

Results and Discussion

Chloramphenicol and nitrofuran metabolites

Chloramphenicol was found to be absent in all the samples from three farms. Nitrofuran metabolite, 1-Aminohydantoine (AHD) was present in shrimp samples collected from the three farms. Other nitrofuran metabolites like semi carbazide (SC), 3-amino-2-oxazolidinone and 3-amino-4-morpholinomethyl-2-oxalidinone were absent in the samples. AHD has structural similarity to the carcinogenic 5-nitrofuran compounds. There is no toxicological data available for AHD, but toxicological data from a study of nitrofurantoin led to the conclusion that there was some evidence of carcinogenic activity (kidney tubular cell neoplasm) of the parent drug in male rats (Sujittra, 2004).

The source of contamination in this area is not clear and it is assumed that it may be from hatchery, feed, local sun dried fish or organic fertilizer. It is suspected that the broodstock was treated self destructively with nitrofurans in the hatchery or in the farms, the shrimps were fed with feed containing protein sources from treated animals or the organic manure from treated animals was used in the farm during preparation for plankton growth. Eventhough the use of nitrofuran drugs (furazolidone, furaltadone, nitrofurazone, nitrofurantoin) was banned in EU for food animal production, (Council regulation (EEC) No 2901/93 1993; Commission Regulation (EC) No 1442/95 1995) the drugs are readily available for veterinary and human therapy (Vasheghani et al., 2008). Nitrofurans were commonly employed as feed additives for growth promotion, and mainly used for livestock (poultry, swine and cattle), aquaculture (fish and shrimp) and bee colonies for prophylactic and therapeutic treatment of bacterial and protozoan infections such as gastrointestinal enteritis caused by *Escherichia coli* and *Salmonella* spp. (Draisci et al., 1997), fowl cholera and coccidiosis black heads (Mccalla, 1983; Draisci et al., 1997). Studies examining the bioavailability of nitrofuran metabolites have demonstrated the possibility of residual transfer to secondary species. It was reported that 41 % of the total amount of radio-labelled (^{14}C) furazolidone consumed was bioavailable to rats (Vroomen et al., 1990) and stability of metabolites during storage and cooking of meat was also demonstrated recently (Cooper et al., 2007).

Heavymetal residues

The level of heavymetals in the shrimp, sediment and water is given in Table 1. The shrimp samples were found to be safe for consumption in terms of heavymetals. In the case of shrimp, the content of Cu, Mn and Zn were found comparatively higher than other metals while in sediment and water level of Zn was highest among the metals tested. Presence of cadmium and lead was observed in the shrimp samples from all three farms. The content of heavymetals was found very low in the case of water samples compared to shrimp and sediment. It was difficult to establish a general pattern of progressive relationship between heavy metals in water and shrimp tissue. The presence of Cr, Mn and Zn were higher in the sediment compared to other metals. Yet muscle of shrimp accumulated higher concentration of Zn and Mn indicating higher bio availability of Zn and Mn.

In the present study, the content of Cd in the farm water ranges from 0.29 – 1.61 ng ml^{-1} and in the sediment from 1.35 to 4.5 ng mg^{-1}. The range obtained for the water is in agreement with US EPA (USEPA, 1983) tolerance level of <0.01 mg L^{-1} for waste water as well as 0.05 mg L^{-1} maximum

Table 1. Heavy metal content in shrimp, sediment and water of selected farms

	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Shrimp (ng mg^{-1})								
Farm 1	0.13±0.07	0.03±0.004	2.63±0.30	10.18±0.71	8.65±0.32	1.19±0.04	0.18±0.08	30.63±1.46
Farm 2	0.03±0.004	0.04±0.008	2.41±0.14	8.67±0.60	8.58±0.26	1.17±0.088	0.136±0	27.02±0.32
Farm 3	0.024±0.002	0.04±0.006	2.40±0.0173	9.73±0.23	7.39±0.22	0.994±0.02	0.13±0.021	23.34±0.61
Sediment (ng mg^{-1})								
Farm 1	4.5±0.098	11.78±0.24	178.8±8.4	26.48±1.41	104.58±12.40	53.98±1.43	15.66±0.41	111.9±10.9
Farm 2	1.35±0.08	5.75±0.31	61.75±11.03	6.43±0.45	73.51±8.19	31.58±12.01	6.09±0.75	75.07±12.52
Farm 3	2.94±0.064	8.28±0.78	103.75±7.53	16.17±0.84	127.7±1.43	35.24±3.06	10.39±062	106.2±3.64
Water (ng ml^{-1})								
Farm 1	1.61±0.00	0.86±0.13	0.76±0.17	3.91±0.03	0.62±0.18	2.14±0.09	3.92±0.48	0
Farm 2	0.29±0.06	0.369±0.00	1.11±0.02	4.32±0.48	0.67±0.12	1.53±0.03	3.45±1.16	4.66±1.99
Farm 3	0.32±0.01	1.094±0.00	0.21±0.02	3.49±0.04	0.88±0.10	2.07±0.18	2.63±0.28	10.24±1.98

Values are means ± standard error, n=3

contaminant level (MCL) (USEPA, 1986) for natural waters. Cadmium used to be an important factor in aquatic monitoring studies, because it has been found to be toxic to fish and other aquatic organisms (Woodworth & Pascoe, 1982). Cd could be harmful to fishes even in low concentrations. Cd was found in the range from 0.02 to 0.13 ng mg^{-1} in the shrimp samples. It is below the permissible limit of 3 ng mg^{-1} for crustaceanns (FDA, 2001).

The concentration of Cobalt in the farm water ranged from 0.86 to 1.094 ng ml^{-1}, while in the sediment, it varied from 5.75 to 11.78 ng mg^{-1}. However, the water could be said to be environmentally sound as the value obtained is comparable to the USEPA (1995). Co was found in the range of 0.03 to 0.04 ng mg^{-1} in the shrimp samples.

Chromium concentration in the farm water varied from 0.21 to 1.11 ng ml^{-1} and in the sediment varied from 61.75 to 178.8 ng mg^{-1}. Katz & Salem (1994) have reported that Cr contamination is common in soils and in both ground and surface waters in industrial areas. In the shrimp sample Cr was found in the range of 2.41 to 2.63 ng mg^{-1}. It is below the permissible limit of 12 ng mg^{-1} for crustaceanns (FDA, 2001).

Copper concentration in the farm water varied from 3.49 to 4.32 ng ml^{-1} and in the sediment varied from 6.43 to 26.48 ng mg^{-1}. In the shrimp sample Cu was found in the range of 8.67 to 10.18 ng mg^{-1}. Oysters and crustaceans are the richest sources of copper (Bukola et al. 2011). The high Cu level may be due to elevated metal-binding protein synthesis as recorded by Yacoub (2007). Copper can become acutely or chronically toxic to aquatic lives at elevated levels. The toxicity of copper to aquatic lives varies with the physical and chemical conditions of water. Hardness, alkalinity, pH, DO and temperature can affect the toxicity of copper (Olaifa et al., 2004)

Manganese concentration in the farm water varied from 0.62 to 0.88 ng ml^{-1} and in the sediment 73.51 to 127.7 ng mg^{-1}. In the shrimp sample Mn was found in the range of 7.39 to 8.65 ng mg^{-1}.

Level of nickel in the farm water ranged from 1.53 to 2.14 ng ml^{-1}, whilst sediment concentration of Ni varied from 31.58 to 53.98 ng mg^{-1}. Ni can cause allergic reaction and is a hazardous metal notified by the USFDA (1993) even though not covered by EC regulations for fish and other aquatic products. The maximum residue level (MRL) for nickel is 70 - 80 ng mg^{-1} and the shrimp samples analysed showed concentration levels only in the range of 0.99 to 1.19 ng mg^{-1}.

Lead level in the farm water varied between 2.63 - 3.92 ng ml^{-1} and between 6.09 to 15.66 ng mg^{-1} in sediment. Lead was detected in the shrimp sample in the range of 0.13 to 0.18 ng mg^{-1} which is below the recommended Pb level (2 ng mg^{-1}) in fish and fishery products (FAO, 1983). Lead is a highly toxic metal to man since it causes brain damage, particularly to the young and induces aggressive behaviour (Ramadan, 2003).

Zn concentration in the farm water was found between 4.66 to 10.24 ng ml^{-1} and in the sediment between 75.07 to 111.9 ng mg^{-1}. Mining smelting and sewage disposal are major source of zinc pollution. Zn content in the shrimp sample was ranged between 23.34 to 30.63 ng mg^{-1}. Zinc values recorded in the present study are lower than the FAO recommended standard (1000 mg kg^{-1}) in fish and fishery products (FAO, 1983). Zinc toxicity is rare but, concentrations in water up to 40 mg L^{-1}, may induce toxicity characterized by symptoms of irritability, muscular stiffness and pain, loss of appetite, and nausea (NAS-NRC, 1974). Zinc is unusual in that it has low toxicity to man, but relatively high toxicity to fish (Alabaster & Loyd, 1980). The higher level of heavy metals in sediment relative to the level in water is expected since sediment has been described as the ultimate sink or reservoir for heavy metal and pollutant discharge in water (Louma & bryan, 1981; Samir et al., 2006).

Correlation analysis of heavymetals in the sediment

Pearson's correlation coefficient has been used to evaluate inter elemental association in the sediment and the results are given in Table 2. The elemental pairs of Cadmium with Co, Cr, Cu and Pb, Cobalt with Cr, Cu and Pb, Cr with Cu and Pb, Mn with Zn and Ni with Zn are significantly correlated ($p<0.01$). The significance of elemental association can be the identical source or common sink of paired elements (Singh et al., 2002; Nyangababo et al., 2005). Some of the elements were poorly correlated indicating that they might have different geochemical factors influencing their concentration in the sediment samples (Addo et al., 2011).

Table 2. Pearson correlation matrix showing the correlation coefficients between the heavymetals in the sediment samples

	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Cd	1							
Co	0.97**	1						
Cr	0.96**	0.92**	1					
Cu	0.99**	0.94**	0.97**	1				
Mn	0.51	0.45	0.37	0.53	1			
Ni	0.68*	0.74*	0.78*	0.64	0.02	1		
Pb	0.98**	0.93**	0.98**	0.98**	0.42	0.73*	1	
Zn	0.69*	0.66	0.59	0.73*	0.84**	0.11	0.59	1

** Correlation is significant at 0.01 levels (two tailed)
* Correlation is significant at 0.05 levels (two tailed)

Water quality of shrimp farms

The physico-chemical parameters of water are important for identification of the nature, quality and type of water (fresh, brackish and saline) for any aquatic ecosystem (Abdo, 2005). Water quality of the shrimp farms is given in the Table 3. Temperature was a little above the optimal level for tiger shrimp farming.Temperature is a factor of great importance for aquatic ecosystem, as it affects the organisms, as well as the physical and chemical characteristics of water (Delince, 1992).The optimum temperature for most of the brackish water penaeid shrimp is 28-33°C, 17-33°C is normal range and less than 14°C or more than 35°C is said to be critical (Wood et al., 1992).

Table 3. Water quality parameters of shrimp farms

Parameters	Farm1	Farm2	Farm3
Temperature (°C)	33.33±0.67	33.66±0.88	33.33±0.88
pH	7.33±0.17	7.33±0.17	7.16±1.17
Salinity (ppt)	3.65±0.06	3.74±0	3.59±0
DO (ppm)	9.63±0	11.02±0.23	8.75±0.40
BOD (ppm)	1.12±0	1.69±0.49	2.57±0.08
COD (ppm)	12.04±0.80	30.49±1.60	29.96±1.07
Total alkalinity (ppm)	76.66±0.67	86.33±1.45	91.5±1.5
Total hardness (ppm)	533.33±3.33	560±0	506.66±3.33
Nitrate (ppm)	0.6±0.1	0.6±0	0.9±0
Nitrite (ppm)	0.015±0.004	Nd	Nd

Values are means ± standard error, N= 3

pH is an index of the presence of metabolites, photosynthetic activity and the fertility of the pond water. Low pH is reported to be harmful to crustaceans and higher pH can lead to alkaline death. The pH values obtained in the present study is 7.16-7.33. The optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production (Ramanathan et al., 2005). High turbidity can lower pH during monsoon, and in summers, the high temperature enhances microbial activity, causing excessive productivity leads to increase production of CO_2 and reduced pH (Narayani, 1990).

In the present study, salinity was found to be very low (3.59-3.74 ppt) and it may be due to the rain received in the study area prior to days of sample collection. Boyd (1987) illustrated the concept of salinity changes during wet season at four river mouths in Thailand. Salinity plays an important role in shrimp farming as it is responsible for many functions such as metabolism, growth, osmotic behaviour, reproduction etc. Soundarapandian & Gunalan (2008) recommended a salinity range of 10-35 ppt was ideal for P. monodon culture.

Dissolved oxygen should be maintained in the range of 3-10 ppm. The ideal level is 5-6 ppm. DO below 3 ppm is not favourable for the health and growth of shrimp. The higher values obtained in the present study (8.75-11 ppm) can be due to increased photosynthetic activity at the sampling time in the afternoon (Sachidanandamurthy & Yajurvedi, 2006).

Biological oxygen demand (BOD) test was found to be more sensitive test for organic pollution. Greater the BOD values, the more rapidly oxygen is depleted in the water. The BOD level obtained in the present study was in the range of 1.12 to 2.57 (Table 3) indicating lower level of organic pollution. Waters with BOD more than 35 mgl^{-1} are not considered good quality for fish culture (Pande & Sharma, 1999). COD is a measure of oxidizable impurities. High BOD and COD are noticed along with high fish mortality by interfacing with respiratory metabolism (Venkataraman, 1996). Present study showed lower COD levels of 12 to 30 ppm. Chattopadhyay (1988) suggested 10 – 20 ppm BOD load and 80 – 100 ppm of COD as the optimum range for aquaculture.

A total alkalinity of 76 to 91 ppm was found in the ponds. Detrimental effect of less than 50 ppm CaCO3 and beneficial effect of more than 80 ppm CaCO3 in shrimp culture pond has been reported (Law, 1988). In brackish water, alkalinity and hardness are usually high, so these variables are seldom important in management of shrimp farms. Hardness level of water obtained in the present study (506.66 - 560 ppm) was found to be higher than the optimal level of 40 - 400 ppm (Mohanty et al., 2014). Maximum hardness in water can be attributed to low water level and high rate of evaporation due to higher atmospheric temperature thereby increasing the solubility of calcium and magnesium salts (Garg, 2003).

Nitrite and nitrate are products of nitrification processes. In aerobic condition nitrite, a toxic substance for aquatic animals, normally exits at low levels in waters since it is usually converted into nitrate, the least toxic nitrogenous compound for aquatic animals. Nitrite accumulates in waters when the rate of ammonia oxidation exceeds the rate of nitrite oxidation and the denitrification process of heterotrophic bacteria in anaerobic conditions (Boyd &Tucker, 1998). The concentrations of nitrite in the water samples were within the suggested level (<0.055 ppm) of the Asian Working Group on Coastal and Marine Environment (AWGCME, 2009). The content of nitrate were higher than suggested levels (<0.06 ppm) for protecting aquatic life.

The presence of AHD, metabolite of the prohibited antibiotic nitrofurantoin in the shrimp samples from the three farms clearly indicates the serious situation on the use of the prohibited carcinogenic antibiotic in shrimp farms. Although the source of contamination is not clear, the results point to the antibiotic contamination in cultured shrimp meant for export from India. Comparatively higher levels of heavymetals were present in the sediment samples and a lower level in water samples. Most of the water quality parameters were within the normal range prescribed for shrimp culture.

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