# Microbiological quality of fish grown in wastewater-fed and non-wastewater-fed fishponds in Hanoi, Vietnam: influence of hygiene practices in local retail markets

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## ABSTRACT

Mean water quality in two wastewater-fed ponds and one non-wastewater-fed pond in Hanoi, Vietnam was $\sim 10^6$ and $\sim 10^4$ presumptive thermotolerant coliforms (pThC) per 100 ml, respectively. Fish (common carp, silver carp and Nile tilapia) grown in these ponds were sampled at harvest and in local retail markets. Bacteriological examination of the fish sampled at harvest from both types of pond showed that they were of very good quality ($2 - 3$ pThC $g^{-1}$ fresh muscle weight), despite the skin and gut contents being very contaminated ($10^2 - 10^3$ pThC $g^{-1}$ fresh weight and $10^4 - 10^6$ pThC $g^{-1}$ fresh weight, respectively). These results indicate that the WHO guideline quality of $\leq 1000$ faecal coliforms per 100 ml of pond water in wastewater-fed aquaculture is quite restrictive and represents a safety factor of $\sim 3$ orders of magnitude. However, when the fish from both types of pond were sampled at the point of retail sale, quality deteriorated to $10^2 - 10^5$ pThC $g^{-1}$ of chopped fresh fish (mainly flesh and skin contaminated with gut contents); this was due to the practice of the local fishmongers in descaling and chopping up the fish from both types of pond with the same knife and on the same chopping block. Fishmonger education is required to improve their hygienic practices; this should be followed by regular hygiene inspections.

**Key words** | coliforms, fishculture, hygiene, retail markets, Vietnam, wastewater

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## INTRODUCTION

Fish production in excreta-fertilized fishponds is a very ancient practice, especially in the Far East and notably China where the practice is believed to have been initiated over 3,000 years ago (Zhiwen 1999). In Vietnam wastewaters are used for aquaculture as a source of both water and nutrients (Vo 2001). The nutrients supports the growth of plankton and other micro-organisms which are consumed by the fish with little additional feeding taking place. In periurban Hanoi there are ~2,500 ha of aquaculture ponds, over 99 percent of which are used for fish culture, mainly carp and tilapia, with a small area (<1 percent) for shrimp production (Mai *et al.* 2004). Most of the wastewater-fed fishponds are located in Thanh Tri district in the south of the city, where there are ~330 ha of wastewater-fed fishponds (Vo & Edwards 2005); there is also widespread wastewater use for rice culture which is often alternated with fish production (Tran 2001).

In order to assure the microbiological safety of fish raised in wastewater-fed fishponds the World Health Organization's guideline is that the fishpond water should have a faecal coliform count of ≤1000 per 100 ml (WHO 1989); this guideline value is expected to be retained in the new guidelines which are currently being prepared (WHO 2006). Various bodies have made recommendations for the microbiological quality of fish rather than the fishpond water. For example, the International Commission on Microbiological Specifications for Foods (1986) recommended an '*m*' value of 11 *E. coli* $g^{-1}$ and an '*M*' value

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of 500 *E. coli* $g^{-1}$ of uncooked fresh and frozen fish flesh, where *m* and *M* are defined as follows: if the *E. coli* count is $<m$ the quality is 'satisfactory'; if it is $>M$ it is 'unsatisfactory'; and, if no more than three out of five fish samples have values between *m* and *M*, it is 'acceptable'. In Vietnam the national standards are $\leq 100$ *E. coli* $g^{-1}$ of uncooked fresh and frozen fish flesh and $\leq 3$ *E. coli* $g^{-1}$ of cooked fish flesh (Ministry of Health 1998). A comprehensive review of, and the corresponding rationales for, microbiological criteria for safe fish are given in Institute of Medicine (2003).

Studies on the microbiological quality of fish raised in wastewater-fed fishponds are few with some studies indicating that faecal bacteria may penetrate the fish flesh when fish is grown in highly polluted water (Buras *et al.* 1985, 1987; Buras 1990), whereas other studies found no or little penetration of micro-organisms in aquaculture environments in which the fish were not stressed (Edwards 1992). Furthermore, the level of microbiological cross-contamination and quality of wastewater-fed fish sold to consumers at retail markets are unknown. In this paper we report the results of an investigation into the microbiological quality of fish from wastewater-fed and non-wastewater-fed fishponds in Thanh Tri district of Hanoi, both at harvest and at the point of sale in local retail markets.

## METHODS

### Study locations and sampling

#### Fishponds

The study was carried out in two wastewater-fed ponds and one nominally non-wastewater-fed (control) pond in Yen So commune, Thanh Tri district. The areas of the wastewater-fed ponds were ~3 and ~15 ha and their liquid depths were ~1.5 − 2 m. Both ponds were fed with raw wastewater directly from the Kim Nguu River through a pumping station located in the commune; ponds also received direct discharges of domestic wastewater from households around the ponds. The Kim Nguu river is essentially a wastewater canal: CEETIA (1997) found it to be heavily polluted, with biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations some 3 − 7 times higher than the Vietnamese permitted standard levels ($\leq$50 mg BOD $l^{-1}$ and $\leq$100 mg COD $l^{-1}$ for wastewaters discharged into water bodies used for aquaculture and crop irrigation). Toan (2004) found thermotolerant coliform (ThC) numbers of $3 \times 10^7$ per 100 ml in the inlet of a fishpond in Yen So commune fed with water from the Kim Nguu river.

The control pond, with an area of ~14 ha and a depth of ~1.5 − 2 m, was located on the alluvial plain adjacent to the west bank of the Red River beyond a flood-control dyke. Red River water was used to feed the control pond as wastewater could not be economically pumped across the dyke. Before the control pond was selected for the study, samples of its contents were analysed for ThC numbers (details in Results section below).

In Yen So commune the most commonly cultured fish are common carp (*Cyprinus carpio*), silver carp (*Hypophthalamichthys molitrix*), and Nile tilapia (*Oreochromis niloticus*). The growing season is ~10 months and at harvest common carp weigh ~500 − 600 g, silver carp ~200 − 300 g, and tilapia ~150 − 200 g. In this study, five individual fish of each of these three species were collected at ~7 a.m. immediately after they had been harvested from the wastewater-fed and non-wastewater-fed ponds. Each fish was placed in a sterile plastic bag. At the same time the fish samples were collected, grab samples of the fishpond water were collected from 15 − 20 cm below the surface in sterile 500-ml glass sampling bottles. The fish and fishpond water samples were then protected against heat and sunlight and transported to the laboratory within 30 minutes. Samples were kept at 4 − 5°C upon arrival at the laboratory and analyzed within six hours of collection.

#### Local retail fish markets

There are several retail markets within 4 km of Yen So commune to which fish are transported in bamboo baskets on bicycles or motorbikes early in the morning. At the market the fish are kept alive in small aerated basins filled with tap water (Figure 1); the same basin is used for fish from both wastewater-fed and non-wastewater-fed ponds. The fishmongers, who are usually women, generally sit on small wooden chairs close to the ground. They gut and clean the fish on small wooden chopping boards placed on the ground (Figure 2). Normally the scales are removed and

**Figure 1** | A local retail fish market in Yen So commune.

the gut removed through a cut in the side of the fish. Carp are then chopped into pieces, placed in a polythene bag and sold. Tilapia are de-gutted and sold as whole fish after the scales have been removed. The same knife is used for all stages of fish processing. The fishmongers clean the chopping board only twice a day, generally at the end of the morning and afternoon trading sessions.

**Figure 2** | Processing of a carp at a local retail market in Yen So commune.

The fish sampled at the markets were 'tracked' from the fishpond at harvesting and accompanied to the market, so that it was known which fish came from the wastewater-fed ponds or the non-wastewater pond. At the market whole fish were purchased and the fishmonger asked to process each fish in the normal way (i.e., to remove the scales and gut the fish, then chop it into pieces). Each fish processed in this way was then placed in a sterile plastic bag and taken immediately to the laboratory for analysis.

## Microbiological examination

### Fish sampled at harvest

Samples of the skin, muscle and intestinal tract of the whole fish samples were collected separately under aseptic conditions, as follows:

(a) skin samples were taken from a 10-$cm^2$ ($2 \times 5$ cm) central area of the fish by marking out, using a sterile template and scalpel, the outline of the desired area and then removing, with sterile scalpel and forceps, as thin a layer of the skin as possible ($1 - 2$ mm); the skin sample was then placed in a sterile Petri dish.
(b) flesh (muscle) samples were taken by first sterilizing the surface with a red-hot knife blade and then removing, with sterile scalpel and forceps, the flesh immediately below the singed surface so that a sample could be taken of the raw flesh below; each sample collected in this way weighed $\sim 5$ g.
(c) the whole intestinal tract of each fish was removed aseptically with sterile scalpel and forceps.

Similar sample types (skin, flesh or intestinal tract) from each of five fish of a single species (common carp, silver carp or tilapia) were removed, pooled, placed in a polyethylene bag to give a five-fish composite sample, which was then weighed. Nine times this weight of a solution of 0.1% peptone and 0.85% sodium chloride at pH 7.5 was added and this 1-in-10 dilution was then homogenized in a BagMixer model VW400 stomacher (Interscience, St Nom, France) for 30 seconds. This dilution

was then used for microbiological analyses directly or diluted further, as described below.

### Fish sampled at markets

A ~10-g sample of fish flesh was taken from one of the pieces of fish in each of five plastic bags containing the same fish species (common carp, silver carp or tilapia). These samples were then pooled in a polyethylene bag to give a five-fish composite sample which was then weighed. They were then diluted and homogenized, as described above.

### Bacteriological analyses

Serial 1-in-10 dilutions to $10^{-7}$ were made of each fish or wastewater sample using the peptone-NaCl diluent. Bacteriological analyses for presumptive ThC, enterococci and aerobic standard plate counts were then carried out within 30 minutes using the procedures recommended by the Nordic Committee on Water and Food Analysis (Danish Standards Association 1999, 2001, 2002). Spread plates of membrane lauryl sulphate agar (MM0615 broth with 15 g L0011agar $l^{-1}$; Oxoid Ltd, Basingstoke, Hampshire, England) and Slanetz & Bartley agar (Oxoid CM0377) were used for presumptive ThC and enterococci, respectively, with incubation at 44°C for 24 h (ThC) and 48 h (enterococci). Pour-plates of tryptone yeast extract agar (Oxoid CM1012 water plate count agar) were used for standard plate counts (SPC) following incubation at 37°C for 48 h. After incubation colonies growing on the agar plates were enumerated and the counts of cell-forming units (CFU) per g of fish (fresh weight) and CFU per100 ml of fishpond water determined.

### Statistical analyses

The student *t* test was used to compare the geometric mean results from the wastewater-fed and the non- wastewater-fed ponds, and ANOVA for those from the three fish species. The data were analyzed in Excel 2003 (Microsoft Corp., Seattle, WA).

## RESULTS

### Fishpond water

The two wastewater-fed fishponds had significantly higher mean counts of presumptive ThC ($p < 0.0001$) and enterococci ($p < 0.001$) than the nominally non-wastewater-fed pond: two orders of magnitude higher for presumptive ThC and one order of magnitude higher for enterococci; there was no difference in the standard plate counts (Table 1). The ThC counts in the wastewater-fed ponds were nearly three orders of magnitude higher than the WHO (1989) guideline value of $\leq 1000$ per 100 ml, whereas those in the nominally non-wastewater-fed pond were less than one order of magnitude above this guideline value.

### Fish sampled at harvest

There was no major significant differences (i.e., those important from a public health perspective) in bacteriological

**Table 1** | Numbers of faecal indicator bacteria and standard plate counts in wastewater-fed and non-wastewater-fed fishponds

| | Wastewater-fed ponds[a] | | | Non-wastewater-fed pond | | | |
|---|---|---|---|---|---|---|---|
| **Bacterial group** | **N[c]** | **Mean[d]** | **σ** | **n** | **Mean[c]** | **σ** | ***p* (*t* test)[b]** |
| Presumptive thermotolerant coliforms | 9 | 5.92 | 0.91 | 10 | 3.79 | 0.57 | **0.0001** |
| Enterococci | 7 | 4.41 | 0.68 | 10 | 3.43 | 0.47 | **0.001** |
| Standard plate count | 8 | 7.79 | 1.02 | 10 | 7.57 | 0.79 | 0.183 |

[a]There was no significant difference in the bacterial counts in the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b]Values in bold indicate significant differences.
[c]Number of water samples analysed.
[d]Log geometric mean bacterial numbers per 100 ml.

qualities between the skin, gut contents or flesh for the three fish species when comparing their origin from either wastewater-fed or non-wastewater-fed ponds (Tables 2–4). Comparison of bacterial numbers in skin samples from the three fish species revealed no significant differences, except in one case where skin samples from wastewater-fed silver carp had a higher SPC than non-wastewater-fed silver carp.

Fish from both wastewater-fed and non-wastewater-fed ponds contained similar bacterial numbers in their gut contents: $10^5$–$10^6$ presumptive ThC $g^{-1}$ and $10^3$–$10^5$ enterococci $g^{-1}$ (Table 3). Amongst the fish from the wastewater-fed ponds common carp contained significantly higher numbers of presumptive ThC and enterococci than silver carp and tilapia. Common carp are primarily bottom feeders and thus will be exposed to high bacterial numbers in pond sediment, whereas silver carp and tilapia primarily feed in the water column where bacterial concentrations are lower.

Fish flesh samples collected by the stringently aseptic technique contained no or very few faecal indicator bacteria, whereas the SPC were $\sim 10^3$ CFU $g^{-1}$ (Table 4). No significant differences were found in bacterial numbers between fish from the wastewater-fed and the non-wastewater fed ponds. Thus the very limited penetration of faecal bacteria into the fish flesh came primarily from the fish gut.

## Fish sampled at point of retail sale

The bacteriological qualities of all three fish species from both types of fishpond were substantially worse after handling, cleaning and purchase in the local retail markets than that at harvest: the geometric mean presumptive ThC and enterococci counts in the fish samples from both the wastewater-fed and non-wastewater-fed ponds were

**Table 2** | Numbers of faecal indicator bacteria and standard plate counts on the skin of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds

| | | Wastewater-fed ponds[a] ($n^b$ = 20) | | | Non-wastewater-fed pond (n = 18) | | | |
|---|---|---|---|---|---|---|---|---|
| **Bacterial group** | **Fish** | **n** | **Mean[d]** | **σ** | **N** | **Mean** | **σ** | ***p* (*t* test)[c]** |
| Presumptive thermotolerant coliforms | Common carp | 6 | 2.53 | 0.83 | 6 | 2.46 | 0.90 | 0.44 |
| | Silver carp | 6 | 2.30 | 0.43 | 6 | 2.19 | 1.71 | 0.44 |
| | Tilapia | 8 | 2.95 | 1.10 | 6 | 3.27 | 1.18 | 0.69 |
| *p* (ANOVA) | | | 0.38 | | | 0.35 | | |
| Enterococci | Common carp | 6 | 1.93 | 1.35 | 6 | 2.51 | 0.84 | 0.80 |
| | Silver carp | 6 | 2.07 | 1.48 | 6 | 1.88 | 1.29 | 0.40 |
| | Tilapia | 8 | 3.10 | 1.10 | 6 | 3.08 | 0.99 | 0.48 |
| *p* (ANOVA) | | | 0.20 | | | 0.18 | | |
| Standard plate counts | Common carp | 6 | 5.35 | 0.82 | 6 | 5.01 | 0.44 | 0.20 |
| | Silver carp | 6 | 5.47 | 0.69 | 5 | 4.29 | 1.14 | **0.03** |
| | Tilapia | 8 | 5.48 | 1.15 | 4 | 5.50 | 0.61 | 0.51 |
| *p* (ANOVA) | | | 0.96 | | | 0.10 | | |

[a]There was no significant difference in the bacterial counts on the skin of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b]Number of skin samples analysed.
[c]Values in bold indicate significant differences.
[d]Log geometric mean bacterial numbers $g^{-1}$.

**Table 3** | Numbers of faecal indicator bacteria and standard plate count in the gut of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds

| | | Wastewater-fed ponds[a] (n[b] = 20) | | | Non-wastewater-fed pond (n = 18) | | | |
|---|---|---|---|---|---|---|---|---|
| **Bacterial group** | **Fish** | **N** | **Mean[d]** | **σ** | **n** | **Mean** | **σ** | ***p* (*t*-test)[c]** |
| Presumptive thermotolerant coliforms | Common carp | 6 | 5.33 | 1.12 | 6 | 6.19 | 0.85 | 0.91 |
| | Silver carp | 6 | 4.67 | 0.91 | 6 | 4.65 | 0.95 | 0.48 |
| | Tilapia | 8 | 5.17 | 1.07 | 6 | 4.62 | 1.47 | 0.21 |
| *p* (ANOVA)[c] | | | 0.53 | | | **0.04** | | |
| Enterococci | Common carp | 6 | 3.75 | 1.01 | 6 | 5.11 | 1.35 | 0.96 |
| | Silver carp | 6 | 3.36 | 0.64 | 6 | 3.25 | 0.62 | 0.39 |
| | Tilapia | 8 | 3.42 | 0.60 | 6 | 2.57 | 0.90 | **0.02** |
| *p* (ANOVA) | | | 0.63 | | | **0.001** | | |
| Standard plate counts | Common carp | 6 | 7.97 | 0.73 | 6 | 8.32 | 0.21 | 0.85 |
| | Silver carp | 6 | 7.42 | 0.48 | 6 | 7.85 | 0.75 | 0.86 |
| | Tilapia | 8 | 7.58 | 0.63 | 6 | 7.60 | 0.85 | 0.52 |
| *p* (ANOVA) | | | 0.30 | | | 0.20 | | |

[a]There was no significant difference in the bacterial counts in the gut of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b]Numbers of individual fish analysed.
[c]Values in bold indicate significant differences.
[d]Log geometric mean bacterial numbers $g^{-1}$.

$10^2$–$10^5$ CFU $g^{-1}$ and the SPC ranged from $10^6$–$10^7$ CFU $g^{-1}$ (Table 5). Numbers of presumptive ThC and enterococci were significant higher in silver carp than in common carp and tilapia. In general, there was no significant difference between the bacteriological qualities of the fish from the wastewater-fed ponds and those from the non-wastewater-fed ponds.

## DISCUSSION

The water in the non-wastewater-fed pond receiving water from the Red River was faecally contaminated at a level of just under $10^4$ presumptive ThC per 100 ml, but the quality of the flesh of fish from this pond at harvest showed little if any faecal contamination (maximum 2–3 presumptive ThC $g^{-1}$). The flesh from fish harvested from the much more contaminated wastewater-fed ponds (just under $10^6$ presumptive ThC per 100 ml) contained similar levels of presumptive ThC and was thus of an equally satisfactory microbial quality. Thus very few faecal indicator bacteria penetrated into the fish flesh even in the highly faecal polluted wastewater-fed fish pond. However the SPC of the fish flesh was $10^2$–$10^4$ CFU $g^{-1}$, indicating that bacterial penetration did occur, but at similar levels in the wastewater-fed and non-wastewater-fed ponds.

A limited number of other studies have investigated the association between microbiological qualities of the fishpond water and the fish in both laboratory environments and functioning waste-fed aquaculture ponds. A few studies, mainly conducted in Israel, have suggested a threshold bacterial concentration in the fishpond water above which

**Table 4** | Numbers of faecal indicator bacteria and standard plate count in the flesh of fish collected immediately after harvest from wastewater-fed and non-wastewater-fed ponds

| | | Wastewater-fed ponds[a,b] (n[c] = 20) | | | Non-wastewater-fed pond (n = 18) | | | |
|---|---|---|---|---|---|---|---|---|
| **Bacterial group** | **Fish** | **n** | **Mean[e]** | **σ** | **n** | **Mean[c]** | **σ** | ***p* (*t*-test)[d]** |
| Presumptive thermotolerant coliforms | Common carp | 6 | 0.41 | 0.28 | 6 | 0.30 | 0 | 0.17 |
| | Silver carp | 6 | 0.30 | 0 | 6 | 0.30 | 0 | – |
| | Tilapia | 8 | 0.30 | 0 | 6 | 0.41 | 0.28 | 0.86 |
| *p* (ANOVA)[d] | | | 0.32 | | | 0.39 | | |
| Enterococci | Common carp | 6 | 0.41 | 0.28 | 6 | 0.30 | 0 | 0.17 |
| | Silver carp | 6 | 0.51 | 0.53 | 6 | 0.30 | 0 | 0.17 |
| | Tilapia | 8 | 0.30 | 0 | 6 | 0.41 | 0.28 | 0.86 |
| *p* (ANOVA) | | | 0.48 | | | 0.39 | | |
| Standard plate count | Common carp | 6 | 3.03 | 0.94 | 6 | 3.13 | 0.97 | 0.57 |
| | Silver carp | 6 | 3.40 | 1.20 | 6 | 2.65 | 0.55 | 0.09 |
| | Tilapia | 8 | 2.72 | 0.46 | 6 | 3.88 | 0.78 | 0.99 |
| *p* (ANOVA) | | | 0.38 | | | **0.03** | | |

[a]There was no significant difference in the bacterial counts in the flesh of the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b]Only three of the 20 fish examined had measurable numbers of presumptive ThC and enterococci per g of flesh (zero colony formation was recorded as $< 2\,g^{-1}$).
[c]Numbers of individual fish analysed.
[d]Values in bold indicate significant differences.
[e]Log geometric mean bacterial numbers $g^{-1}$.

bacteria enter the edible muscle tissues of fish and thus increase the risk of exposure for consumers of the fish. Buras and co-workers (Buras *et al.* 1985, 1987; Buras 1990) reported such a threshold concentration of total culturable bacteria in fishpond water of $1 - 5 \times 10^6$ per 100 ml, but this seems to have been due to a major malfunction in the wastewater treatment plant which introduced such high organic loadings into the receiving fishpond that the fish were extremely stressed and only just able to survive (P. Edwards, personal communication, 2005). A study in Thailand reported an SPC range in septage-fed fishponds of $1.8 \times 10^5$–$2.0 \times 10^6$ per 100 ml of pond water which produced fish with minimal bacterial penetration into their flesh (Edwards *et al.* 1984). Exposure of 132 healthy tilapia to fishpond *E. coli* concentrations of up to $10^6$ cfu per 100 ml from wastewater sources led to little or no detectable bacterial or bacteriophage penetration into their flesh (Fattal *et al.* 1988, 1993). In the United States Hejkal *et al.* (1983) found a maximum of 25 faecal streptococci per 100 g of fish muscle even though the gut contained $> 10^5$ per 100 g. The fish in the Buras studies were grown under conditions of high stress which is atypical of normal aquaculture ponds; thus the penetration of micro-organisms into the fish flesh in this study may have been an exceptional case. The results of the current study, together with other studies on well-managed 'normal' wastewater-fed fishponds (reviewed by Edwards 1992), suggest that the maximum permissible number of faecal indicator bacteria in wastewater-fed fishponds should be less than that which would lead to significant contamination of the fish flesh. However, further research is needed to assess how many orders of magnitude are needed to provide a realistic (i.e.,

**Table 5** | Numbers of faecal indicator bacteria and standard plate counts in fish samples (flesh, skin, bone) from wastewater-fed and non-wastewater-fed ponds purchased at local retail markets

| | | Wastewater-fed ponds[a] ($n^b$ = 52) | | | Non-wastewater-fed pond ($n$ = 64) | | | |
|---|---|---|---|---|---|---|---|---|
| **Bacterial group** | **Fish** | ***n*** | **Mean[d]** | **σ** | ***n*** | **Mean** | **σ** | ***p* (*t* test)[c]** |
| Presumptive thermotolerant coliforms | Common carp | 10 | 2.89 | 0.69 | 20 | 3.45 | 1.80 | 0.82 |
| | Silver carp | 20 | 4.23 | 1.35 | 20 | 4.28 | 1.28 | 0.55 |
| | Tilapia | 22 | 3.49 | 0.78 | 24 | 3.73 | 0.95 | 0.81 |
| *p* (ANOVA)[c] | | | **0.004** | | | 0.15 | | |
| Enterococci | Common carp | 10 | 2.68 | 0.92 | 20 | 3.23 | 1.29 | 0.87 |
| | Silver carp | 20 | 4.33 | 1.26 | 20 | 3.70 | 0.69 | **0.02** |
| | Tilapia | 22 | 3.68 | 0.63 | 24 | 3.54 | 0.57 | 0.22 |
| *p* (ANOVA) | | | **0.0003** | | | 0.24 | | |
| Standard plate count | Common carp | 10 | 6.49 | 0.54 | 20 | 6.65 | 1.31 | 0.64 |
| | Silver carp | 19 | 6.93 | 0.52 | 19 | 6.91 | 0.93 | 0.46 |
| | Tilapia | 22 | 6.93 | 0.65 | 24 | 6.99 | 0.95 | 0.58 |
| *p* (ANOVA) | | | 0.11 | | | 0.57 | | |

[a]There was no significant difference in the bacterial counts in the fish harvested from the two wastewater-fed ponds (*t* test: $p > 0.05$).
[b]Numbers of individual fish analysed.
[c]Values in bold indicate significant differences.
[d]Log geometric mean bacterial numbers $g^{-1}$.

not over-restrictive) safety factor for faecal bacterial indicator numbers in wastewater-fed fishponds.

Our results indicate that these fish flesh qualities were satisfactory in terms of their faecal bacterial indicator counts and complied with the recommendations of the International Commission on Microbiological Specifications for Foods (1986) and the Vietnamese Ministry of Health (1998). They are in partial agreement with the fish quality classification scheme developed by Buras *et al.* (1987); this proposed "that in the case of fish grown in wastewater, the quality of the fish should be determined by the presence of any bacteria in the muscles" and "that the indicators should be bacteria that grow on nutrient and mFC agar, and the bacteriological quality should be expressed as: 0 – 10 bacteria $ml^{-1}$, very good; 10 – 30 bacteria $ml^{-1}$, medium quality; more than 50 bacteria $ml^{-1}$, not acceptable" [*sic*; it is not clear what quality was to be assigned for 31 – 49 bacteria $ml^{-1}$]. Thus, based on this scheme, the fish flesh qualities at harvest were 'very good' on the basis of their *E. coli* counts but 'not acceptable' on the basis of their SPC (Table 4). It is difficult to imagine a wastewater-fed (or river-water-fed) aquaculture situation in which the "nutrient and mFC agar" counts are the same as this would imply that all (or essentially all) the bacteria present were faecal coliforms/*E. coli*. We thus accept the classification of Buras *et al.* (1987) but only in terms of the *E. coli* counts $g^{-1}$ of fish flesh and not in terms of the SPC $g^{-1}$.

Surprisingly, the fish from both the wastewater-fed and the non-wastewater fed ponds contained similar bacterial concentrations in their gut contents ($\sim 10^5$–$10^6$ presumptive ThC $g^{-1}$ and $\sim 10^3$–$10^5$ enterococci $g^{-1}$). This may be partly explained by the relatively high numbers of presumptive ThC) in the non-wastewater pond ($\sim 10^4$ per 100 ml). Our findings of high numbers of faecal bacteria in the gut content are in agreement with several studies reviewed by Edwards (1992). Fish samples (muscle, skin, bone) purchased at retail markets contained from ~1,000 to ~20,000 presumptive ThC $g^{-1}$, irrespective of whether the fish originated from the wastewater-fed or the non-wastewater fed ponds. This indicates that significant faecal cross-contamination occurred at the markets during handling and processing of the fish for human consumption. As noted by Buras *et al.* (1987), "exposure to pathogens can occur when fish are handled and cleaned. During the digestive tract removal, the content is usually spilled and contaminates the intestinal cavity of the fish and the hands of the handler. Casual rinsing does not prevent contamination". Clearly, local environmental health officers/assistants need to educate local fishmongers so that (a) they are aware of the risks for faecal contamination of the fish products and possible occupational health risks of their unhygienic practices and (b) they are then able to implement and sustain improved hygiene practices; they also need to be regularly inspected by the local environmental health officers/assistants to ensure that their fish-handling and cleaning practices are always hygienic.

Finally, it should be noted that only the bacteriological quality of fish from wastewater-fed and non-wastewater-fed fishponds was investigated by the use of bacterial indicators. Thus, the possible occurrence and food-safety aspects of fishborne zoonotic parasites, in particular trematode parasites, bacterial and viral pathogens, and any bio-accumulation of toxic chemicals in the wastewater, were not assessed.

## CONCLUSIONS

- Fish grown in both wastewater-fed and nominally non-wastewater-fed fishponds with presumptive ThC counts of $\sim 10^6$ and $\sim 10^4$ per 100 ml, respectively, were of very good quality at harvest ($2 - 3$ presumptive ThC $g^{-1}$ of flesh). This indicates that the current WHO guideline value for wastewater-fed aquaculture ($\leq 1000$ *E. coli* per 100 ml of fishpond water) is quite restrictive as it represents a high factor of safety of three orders of magnitude.
- Grossly unhygienic fish handling and cleaning practices at the local retail markets caused significant recontamination ($10^2 - 10^5$ presumptive ThC $g^{-1}$) of the fish grown in both wastewater-fed and nominally non-wastewater-fed ponds.
- The fishmongers in the local retail markets should be informed about their unhygienic fish handling and cleaning practices, and how these can be improved to reduce the faecal cross-contamination of the fish they sell.

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