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Intestinal Bacteria in the University of Basrah fish ponds, Iraq

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ABSTRACT

Total bacterial load, total coliforms and faecal coliforms in ponds water and sediments, intestine of common carp Cyprinus carpio and birds faeces were investigated monthly over a period of one year from May 2007 to April 2008. Fish were collected randomly by a cast net from ponds of University of Basra. Total viable bacterial counts were ranging from 2.8±1.9 x 101 to 7.0±2.2 x 103 cfu ml -1 in ponds water, 4.2±2.2 x 104 to 3.8±2.5 x 106 cfu g -1 in ponds sediments, 9.2±2.6 x 104 to 10.9±2.5 x 106 cfu g -1 fish intestine and 2.0±1.4x 106 to 10.7±1.2 x 108 cfu g-l in birds faeces. The most probable number (MPN) of faecal coliforms ranged from 94±5 to ≥920±0 100 ml-1 in ponds water; while MPN ranges in sediment, common carp intestine and birds faeces were 63±18 to ≥540±0 g-l, 43±5 to ≥540±0 g-l and 240±22 to ≥540±0 g-l respectively. The abundance of normal bacteria coliforms was greater in the warm months than in the cold months. There were no sources of human faecal matter in the ponds. So, it is clear that faecal coliforms from water birds faeces significantly contaminated (P< 0.05) the ponds and common carp intestines.

Introduction

Fish take a large number of bacteria into their gut from water, sediment and food (١) suggested that the bacterial flora on fish reflects the aquatic environment. Thus, determining the bacterial contents of the water in ponds, the bacterial level in fish can be predicted (٢). The latter affects the quality and storage life of the fishery products, the quality of fish refers to the degree of contamination with coliform bacteria(٣). Contamination results mainly from rupturing of the fish intestine during poor processing or inadequate washing. Various studies have suggested that intestinal microflora or contamination of fish as a result of enteric bacteria of human or animal origin were responsible for various food spoilages (٤). In addition, it is well known that freshwater fish and their environment harbor human pathogenic bacteria, particularly members of the coliform group (٥).

Faecal coliforms such as *Escherichia coli* usually originate from faeces of warm-blooded animals, to evaluate the sanitary condition of ponds, indicator pathogenic bacteria are considered (٢). Faecal coliforms are sometimes introduced into the pond, these enteric bacilli include *Escherichia coli, Klebsiella, Enterobacter, Serratia, Edwardsiella* and *Citrobacter* (٦). Faecal coliforms in fish reflect the level of pollution of the environment, as the normal flora of fish do not include coliforms (٧) observed that faecal coliforms in pond waters were equivalent to 10 000 L $^{-1}$ and 0.100.15 g $^{-1}$ fish biomass with no more than 10% of samples exceeding 0.50 g $^{-1}$ fish flesh . In this study; water birds were present along the ponds sides, and a preliminary study indicated a possible involvement of water birds faeces in faecal contamination of the ponds. So, the present study was undertaken with the objectives of determining the numbers of viable coliforms in pond water, sediment, intestine of the cyprinid fish *Cyprinus*

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carpio and water birds faeces samples and clarifying the relation between the bacterial load of fish and the pond environment.

Materials and methods

Description of the ponds

The study was conducted in five artificial earthen ponds.Four fish species were cultured viz. *Cyprinus carpio*, *Ctenopharyngodon idella*. *Hypophthalmichthys molitrix* and *Carassius carassius*. Birds, especially *Ceryle rudis*, *Hacyon smyrnensis* and *Egretta alba* dominated in the ponds. The size of ponds is ranged from 0.8 to 1.2 denim

Samples collection

Samples were collected monthly from May 2007 to April 2008. Sterilized glass sample bottles (250 ml) were left capped until used for the ponds water and sediment samples from five different sites within the pond to make composite samples. Water samples were collected 15-20 cm below the water surface to avoid surface contamination, bottom sediment samples were collected by uncapping sterile glass bottles in the sediment layer (٤).Three to five fish samples (20-25g.) were collected by cast net, and fresh faeces of water birds (*Ceryle rudis*, *Hacyon smyrnensis* and *Egretta alba*) along the pond sides were collected in triplicate. All samples were transported to the laboratory within 15-30 min, and microbial examinations were carried out immediately.

Water temperature

Temperature was measured on every sampling day between 09.00 and 09.30 am.

Sample preparations

Pond water

Appropriate sample dilutions were made (10^{1}-10^{4}) with sterile physiological saline (0.85%) in deionized water.

Sediment Sediment samples were centrifuged at 3000 c\min for five min. One gram of sediments was diluted in 10 ml of sterile physiological saline. One ml. of the homogenate was serially diluted (10^{-1}-10^{-7}).

Fish intestine

Fish were killed by physical destruction of the brain. The body surface was wiped with 70% ethanol, and the intestines were removed. Intestines were homogenized in a mortar and approximately one gram of wet homogenate was diluted in 10 ml. of sterile physiological saline. One ml. of the homogenate was serially diluted (10^{-1}-10^{-7}).

Water birds faeces

Fresh faeces were collected and homogenized in a mortar aseptically. Approximately one gram of wet homogenate was taken in a tube containing 10 ml of sterile saline. One ml. of the homogenate was then serially diluted (10^{-1}-10^{-8}) (7).

The aerobic plate count (APC as cfu "colony forming unit" per unit of samples), total coliforms (TC) and faecal coliforms (FC) of pond water, sediment, fish intestine, ground water and water birds faeces samples were determined. Samples were analysed for coliforms using the multiple-tube fermentation technique.

Aerobic plate count (APC)

To determine the APC of the pond water, sediment, intestine of Common Carp, ground water and water birds faeces, 0.1 ml of each dilution was spread on the surface of tryptone soy agar (TSA; Oxoid) plates in duplicate and incubated at 30°C for 48 hs. The numbers of viable colonies were counted using a Leica Quebec dark field colony counter, and cfu per unit of sample was calculated.

Coliform

Total coliforms and faecal coliforms in pond water samples were enumerated by the five-tube, most probable number (MPN) procedure (APHA 1998), whereas pond sediment, fish intestine and water birds faeces samples were enumerated by the three-tube MPN procedure (1).

Statistical analyses

Means, standard deviations (SD), correlations and regressions were calculated. With faecal coliforms, the minimum values were used by SPSS (V. 11.0).

Results

Temperature parameter

The water temperature ranged from 15°C to 32°C during the study period (Fig. 1).

Pond water

Table (1) shows the bacterial load and faecal coliforms in water. The monthly measured bacterial load and faecal coliforms were significantly correlated ($P < 0.05$) with temperature of the investigation period (Tables 5 and 6). The total bacterial load varied from $2.8 \pm 1.9 \times 10^{1}$ to $7.0 \pm 2.2 \times 10^{3}$ cfu ml $^{-1}$. The highest bacterial load was recorded in August , and the lowest in January. The MPN values for total coliforms and faecal coliforms were the same in water and ranged from ٨٦±5 to ≥1400±0 100 ml $^{-1}$. Therefore, only faecal coliforms are given in Table (1). Higher numbers of faecal coliforms were observed in summer.

Pond sediments

Table (2) shows the bacterial load and faecal coliforms in sediment. Bacterial load and faecal coliforms were significantly correlated ($P < 0.01$) with the temperatures over time (Tables 5 and 6). The total bacterial load ranged from $4.2 \pm 2.2 \times 10^{4}$ to $3.8 \pm 2.5 \times 10^{6}$ cfu g $^{-1}$ in sediment. The MPN of both total coliforms and faecal coliforms were the same and ranged from ٥٧±18 to ≥900±0 g $^{-1}$. Therefore, only faecal coliforms are given in Table (2). Values for aerobic plate count and faecal coliforms were higher during summer.

Fish

Table (3) represents the bacterial load and faecal coliforms in common carp intestine. The bacterial load and faecal coliforms were significantly correlated ($P < 0.01$ and $P < 0.05$) with the temperatures (Tables 5 and 6). The total bacterial load varied from $9.2 \pm 2.6 \times 10^{4}$ to $10.9 \pm 2.5 \times 10^{6}$ cfu g $^{-1}$. The MPN for both total coliforms and faecal coliforms in fish intestine were the same and ranged from 37±5 to ≥900±0 g $^{-1}$. Therefore, only faecal coliforms are given in Table (3). The aerobic plate count and MPN of faecal coliforms were higher during summer.

Water birds faeces

The faecal coliforms of water birds faeces significantly contaminated ($P < 0.05$) the ponds and common carp intestine (Table 7). Table (4) shows the bacterial load and faecal coliforms in water birds faeces. The total bacterial load varied from $2.0 \pm 1.7 \times 10^{7}$ to $10.7 \pm 1.2 \times 10^{8}$ cfu g $^{-1}$. The highest bacterial load was observed in August, and the lowest in January. The MPN values for both total coliforms and faecal coliforms were the same in the range 203±22 to ≥900±0 g $^{-1}$. Therefore, only faecal coliforms are given in Table (4).

Discussion

Results showed variations in total bacterial load of pond water, sediment, common carp intestine and water birds faeces over time. The bacterial load in the samples was highest in August. This may result from the high water temperature (32°C). Low temperature (15°C) may be the major factor in decreasing bacterial loads in the pond water, sediment and common carp intestine during January. It has been reported that bacterial load might be increased with an increase in water temperature (8). The higher bacterial load in the fish pond sediment than in the water has also been observed by (9). (10); (11) reported that the bacterial flora on fish reflected the biology of aquatic environment. The present findings support this statement (Table 5). In this study; the significantly higher ($P < 0.01$) bacterial load in fish intestine during the summer period might result from high metabolic activities of the fish related to larger quantities of food consumed during that period. (12) observed similar results in the intestinal bacterial load of common carp. Bacterial load associated with common carp intestine was correlated ($P < 0.0001$) with the bacterial levels in the pond environment, especially the pond sediment (Table5). Water birds faeces contained faecal coliforms, and the present study showed significant correlations (Table 6) and regressions (Table 7) of faecal coliforms in water birdsfaeces and coliforms in pond water, sediment and fish intestine(13) observed similar results in the faecal coliforms in pond water,sediment and fish visceral samples from four different catfish pond.(14) counted a large number of *E coli* (faecal coliform) in cultured channel catfish during the warmer periods (summer and autumn) and a very low number in the colder periods (winter and spring) and Tilapia grown in a recirculating system had a lower faecal coliform count than tilapia cultured in a non-circulating system (15). In the present study; faecal coliform was detected in larger numbers in pond water, sediment and fish intestines at 22- 32°C, decreasing in number at temperatures of l5- 19°C. And in the present findings, faecal coliforms in pond water and common carp intestine ranged from ٨٦±5 to ≥1400±0 100 ml^{-1} and 37±5 to ≥900±0 to g^{-1} respectively. The World Health Organization (1989) gave a tentative guideline for pond aquaculture levels of coliforms of a geometric mean of $\leq 10^3$ faecal coliforms per 100 ml.

There were no inputs for sources of human or other animal faeces directly to the ponds. So, it is clear that faecal coliforms are imported to the present ponds via water birds and, obviously; this faecal coliform significantly contaminated ($P < 0.05$) the ponds and common carp (Table 7). (16) (17) mentioned that pond environments receive faecal coliform contamination via warm-blooded animal faeces. Faecal coliforms represent a potential problem in pond effluent management. Fish and fish products have long been considered a vehicle of food-borne bacterial parasitic infections leading to human illness (18).

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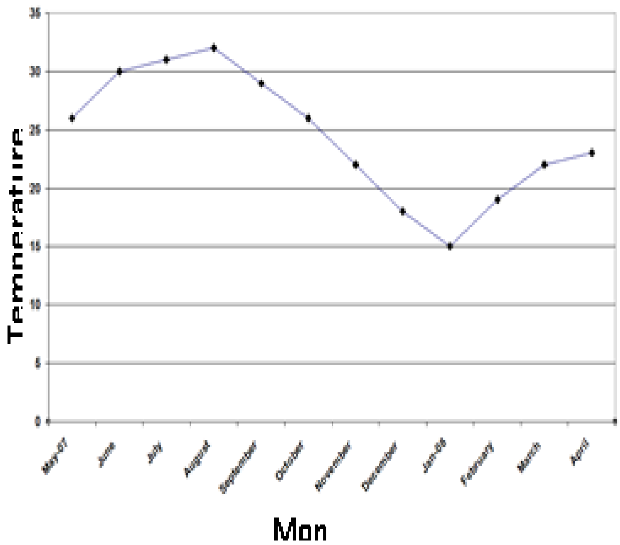

Figure (1): Temperature of pond's water

Table (1): Bacterial load and most probable number index of faecal coliforms in pond's water

Month	APC (cfu ml $^{-1}$)	Faecal coliforms (MPN 100 ml $^{-1}$)
May 2007	2.7±1.8 x 10^2	≥920±0
June	3.1±2.1 x 10^3	≥920±0
July	5.0±2.8 x 10^3	≥920±0
August	7.0±2.2 x 10^3	≥920±0
September	2.5 ±1.3 x 10^3	≥920±0
October	3.2±1.3 x 10^2	≥920±0
November	6.0±3.7 x 10^2	≥920±0
December	7.0±4.2 x 10^1	350±60
January 2008	2.8±1.9 x 10^1	94±5
February	3.1±2.2x 10^1	94±5
March	3.4±2.6 x 10^2	٢٨٠±30
April	-	-

APC: Aerobic Plate Count (mean±SD),
MPN: Most Probable Number (mean±SD).
-No sample

Table (2): Bacterial load and most probable number of fecal coliforms in the pond's sediments

Month	APC (cfu g $^{-1}$)	Faecal coliforms (MPN g $^{-1}$)
May 2007	9.4±3.3x 10^5	≥540±0
June	2.9±2.0 x 10^6	≥540±0
July	3.5±2.9 x 10^6	≥540±0
August	3.8±2.5 x 10^6	≥540±0
September	10.7 ±1.3 x 10^5	≥540±0
October	2.1±2.2 x 10^5	≥540±0
November	2.7±1.6 x 10^5	≥540±0
December	5.8±3.4x 10^4	240±22
January 2008	4.2±2.2 x 10^4	٧9±18
February	6.1±2.8x 10^4	63±18
March	3.7±2.6 x 10^5	240±22
April	-	-

Table (3): Bacterial load and most probable number of faecal coliforms in the intestine of *C. carpio*

Month	APC (cfu g $^{-1}$)	Faecal coliforms (MPN g $^{-1}$)
May 2007	2.7±3.3x 10^6	≥540±0
June	7.6±3.7 x 10^6	≥540±0
July	9.1±3.2 x 10^6	≥540±0
August	10.9±2.5 x 10^6	≥540±0
September	3.8 ±2.8 x 10^6	≥540±0
October	8.4±3.9 x 10^5	≥540±0
November	3.9±3.0 x 10^5	≥540±0
December	2.0±1.8x 10^5	240±22
January 2008	9.2±2.6 x 10^4	79±9
February	2.3±2.0x 10^5	43±5
March	7.4±3.7 x 10^5	240±22
April	-	-

Table (4): Bacterial load and most probable number of faecal coliforms in Water birds faeces

Month	APC (cfu g $^{-1}$)	Faecal coliforms (MPN g $^{-1}$)
May 2007	4.8±3.5x 10^7	≥540±0
June	4.7±4.0 x 10^8	≥540±0
July	6.9±3.2 x 10^8	≥540±0
August	10.7±1.2 x 10^8	≥540±0
September	7.2 ±3.2 x 10^8	≥540±0
October	10.2±1.9 x 10^8	≥540±0
November	2.0±1.7 x 10^7	≥540±0
December	8.0±3.5 x 10^6	≥540±0
January 2008	2.0±1.4x 10^6	240±22
February	7.7±2.3x 10^6	240±22
March	5.4±3.2 x 10^7	≥540±0
April	-	-

APC: Aerobic Plate Count (mean±SD), MPN: Most Probable Number (mean±SD).

Table (5): Correlation coefficient for aerobic plate count (APC) in different populations

	Temperature	Water	Sediment	Intestine	Water birds faeces
Temperature	1	0.52	0.67	0.63	0.33
Water	-	1	0.83	0.85	0.60
Sediment	-	-	1	0.88	0.51
Intestine	-	-	-	1	0.56
Water birds faeces	-	-	-	-	1

Table (6): Correlation coefficient for faecal coliforms in different populations

	Temperature	Water	Sediment	Instestine	Water birds faeces
Temperature	1	0.50	0.60	0.5	0.53
Water	-	1	0.80	0.87	0.58
Sediment	-	-	1	0.76	0.61
Intestine	-	-	-	1	0.51
Water birds faeces	-	-	-	-	1

Table (7): Regression on faecal coliforms of Water birds faeces

Variable	Slope	Intercept	*P*
Water	1.13	-3.02	0.01
Sediment	1.0	-1.21	0.01
Intestine	0.61	-1.08	0.03
Water birds faeces	-	-	-

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بكتريا الأمعاء في أحواض أسـماك جامعة البصرة.

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الخلاصة

تم تقصي الحمل البكتيري الكلي وبكتريا القولون الكلية وبكتريا القولون البرازيـة في ماء ورواسب وأمعاء أسماك الكارب الشائع وبراز الطيور في أحواض أسماك جامعة البصرة. أجريت الدراسة على مدى عام كامل اعتبارا من شهر آيار٢٠٠٧ لغاية شهر نيسان ٢٠٠٨ .جمعت الأسماك عشوائيا بأستخدام شباك الرمي (السلية). تراوح الحمل البكتيري الكلي لماء الأحواض بينx 110 2.8±1.9 الى ٧.0 ±2.2 x 310 بكتيريا /مل. كما تراوح الحمل البكتيري الكلي لرواسب الأحواض بين. 2.2±4.2 x 410 الى ٣.٨±٢.٥ x 610 بكتيريا /غم. أما الحمل البكتيري الكلي لأمعاء الأسماك فقد تراوح بين 9.2±2.6.x.410 الى 10.9± ٢.٥.x .106 بكتيريا /غم. وقد كان الحمل البكتيري الكلي لبراز الطيور 2.0 ±1.4.x .610 الى 10.7± 1.2 x 810 بكتيريا /غم. وباستخدام طريقة العد الأكثر احتمالاً MPN، تم تقصي اعداد بكتيريا القولون البرازية، اذ تراوحت بين 94±5 الى ≤920±0 MPN/ 100 مل لماء الأحواض، ٦٣±18 الى ≤540±0 MPN/ غم لرواسب الأحواض و43±٥ الى ≤540±0 MPN/ غم لأمعاء الأسماك و240±22 الى ≤540±0 MPN/ غم لبراز الطيور. كما بينت الدراسة الحالية أن أعلى وفرة لبكتيريا القولون كانت خلال الأشهر الدافئة مقارنة بالأشهر الباردة. ليس هناك مصدر لمشكلة براز الانسان في الاحواض، لذلك من الواضح أن تلوث الأحواض وأمعاء أسماك الكارب الشائع ببكتيريا القولون البرازيـة من براز الطيور المائية (P < 0.05).