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PREVALENCE AND HAEMOLYTIC ACTIVITY OF *AEROMONAS* SPECIES IN LAKE EDKU AND CONNECTED DRAINS

MOHAMED A. EL-SHENAWY

National Institute of Oceanography & Fisheries, Kayet Bay, Alexandria, Egypt.

Keyword: Aeromonas secies, fecal indicators, Lake Edku, Fishes, invertebrates.

ABSTRACT

During a year-long survey 2004, 80 water samples from Lake Edku and connected drains as well as 44 fishes and invertebrate samples from Edku fish market were analyzed for the prevalence of *Aeromonas* spp. (*A*. spp.) (qualitatively and quantitatively) as well as for positive hemolytic activity. Fecal pollution indicators including *Escherichia coli* (EC) and fecal streptococci (FS) and some environmental parameters including temperature (T), salinity ‰ (S), pH and dissolved oxygen (DO) were also determined. All water samples (100%) tested were positive for the prevalence of *Aeromonas* spp. in cfu counts ranged between 0.6 x 10^2 and 28.0 x10^3 for water samples and between 0.5 x 10^2 and 8.0 x 10^3 for fish and invertebrate samples with maximum in summer and minimum in winter. Strong positive correlations were noted between *Aeromonas* spp. and both of *E.coli* (r = 0.85) and fecal streptococci (r = 0.90). Also the same positive correlations between *E. coli* and fecal streptococci (r = 0.81 to 0.97), were noted in all water samples. However a negative correlation recorded in water samples of Lake Edku between salinity and each of *A*eromonas spp. (r = - 0.77), *E. coli* (r = - 0.79) and fecal streptococci (r = - 0.75). The incidence of *A.eromonas* spp. were recorded in all water samples (100%) followed by fishes (75%) and invertebrates (37.5%), however the percentage of haemolysin producing *Aeromonas* spp. were found to be 65-70% in lake and drains water samples, and decreased to be 57% and 31% in fishes and invertebrate samples respectively, which may pose a potential health problem.

INTRODUCTION

Aeromonas species are bacteria belonging to *Aeromonadaceae* family and characterized as gram-negative, oxidase-possitive, facultatively anaerobic, glucose fermenting bacterium, which colonize aquatic systems throughout the world (Hazen *et al.,* 1978; Abbott *et al.,* 2003).It is found in lakes, rivers, estuarine and marine waters, sewage effluent, chlorinated and unchlorinated water supplies and in mineral waters (Harnisz and Zmyslowska, 2004; Sen and Rodgers, 2004). It has been also isolated from fish (Santos *el al*., 1999; Vivekanandhan *et al*., 2005), seafood markets (Ullmann *et al.,* 2005; Vivekanandhan *et al.,* 2005), and humans (Janda and Abbott, 1998; Sechi *et al.,* 2002).

Members of genus *Aeromonas* have long been considered as human and fish pathogens. It had expanded from four species (*A*. hydrophila, *A*. sobria, *A.* cavia and *A.* salmonicida) to at least 16 recognized hybridization groups. Nine of these species had been reported to cause a wide variety of human infections including bacteraemia, gastroenteritis, cellulites, meningitis, soft-tissue infections, peritonitis, bronchopulmonary and respiratory tract infection (Barghouthi *et al.,* 1989; Alavandi and Ananthan, 2003,).

The mechanism of the pathogenicty had been related to production of exotoxins (i.e

E-mail: dr_elshenawy@hotmail.com

enterotoxins, cytotoxins, haemolysin) (Thornley *et al.,* 1997). The primary haemolytic toxin seems to be related to enterotoxigenicity (Brenden & Janda, 1987). However *Aeromonas* strains could produce more than one haemolytic toxin with virulence properties (Bizani and Brandlli, 2001; Ghenghesh *et al.,* 2001; Ullmann *et al*., 2005) not only at optimal growth temperature but also at refrigerator temperature. Therefore it could be emerging as food-borne pathogen (Merino *et al.,* 1995; Maalej *el.al.,* 2004) science the common routes of infection suggested for *Aeromonas* are the ingestion of contaminated water or food or contact of the organism with a break in the skin (Schubert, 1991).

The aims of this study were to determine the prevalence and haemolytic activity of *Aeromonas* species in surface waters of Lake Edku and connected drains as well as in Lake Edku fishes.

MATERIALS AND METHODS

Study area

Lake Edku is shallow brackish water at North Delta lakes in Egypt. It is situated at latitude 30° 15` N and longitude 31° 15` E east of Alexandria City and extending a bout 19 km to the east of Abu Qir Bay with an average width of 6 km and average depth of 1 m. Lake Edku is connected to the Mediterranean at El-Maadia city at the North-West and receive huge amounts of drainage water (38-280 x 10^6 m^2/day) from the eastern kom Belag drains (Kom Belag, El-Boseily, El-Khayry and Edku drains) and Bersik drain from the south. This initiated an east south to west-north flow, causes a slight elevation of its water above the sea level, decreasing the entrance of sea water to the lake. The last record of fish production (10,230 tons (GAFRD, 2003)) is greatly polluted by the drainage water which carries different agriculture and domestic pollutants (El-Shenawy *et al.,* 2000). Recently a lot of fish cultures surround the lake, receive their water from above drains and discharging it into the lake, which might increase pollution hazards to the water body of the lake.

Sampling

A total of 80 duplicate water samples were collected from 40 sites during four sampling cruises in spring, summer, autumn, and winter 2003. These sites are illustrated in Fig. 1. The bacteriological sampling technique was carried out according to the international standard ISO 5667/9 (1992) using 500-ml sterile bottles and a special sampler, at 20 cm below water surface. Fresh fish (28 samples) and invertebrates (16 samples), harvested from Lake Edku, were purchased from fishermen or markets in Edku city. All samples were kept in icebox and analyzed within max. 6 hours.

Microbiological analysis

Water samples in 1, 10, 100 ml volumes were pumped through 0.45µm-pore-size mixed-ester membrane filter (Millipore). For *Aeromonas* species, the membranes were placed directly on culture plates of m-*Aeromonas* selective agar (Biolife, Italiana) supplemented with 10 mg ampicillin / liter, incubated aerobically for 24-48 h at 30°C (Ormen and Ostensvik, 2001). Ten presumptive colonies of typical *aeromonas* colonies from each sample were sub-cultured on Tryptic Soy Agar (Oxoid) for 24 h, 30°C for purification and subsequently identified to the genus level by Gram stain, oxidase test, oxidation/fermentation test, resistance to the vibriostatic agent 0/129 and API 20E systems (Bio-Merieux Italia). For total coliforms (TC), thermotolerant *Escherichia coli* (EC) and fecal streptococci (FS) the membranes were enumerated on m-Endo-les agar, mFC agar and m-Enterococcus agar (Difco), respectively. Ten random characteristics colonies from each sample were subcultured, confirmed according to ISO 3908/1 (1990) and ISO 789 9/2 (1984). Fish and invertebrates samples were analyzed by suspending of 25 gm of minced whole fish / flesh in 225 ml saline solution followed by filtration. The same media and identification

procedures were done as previously described. The final counts of all bacteria were calculated as cfu/100 ml for water and as cfu/100 gm for fish samples.

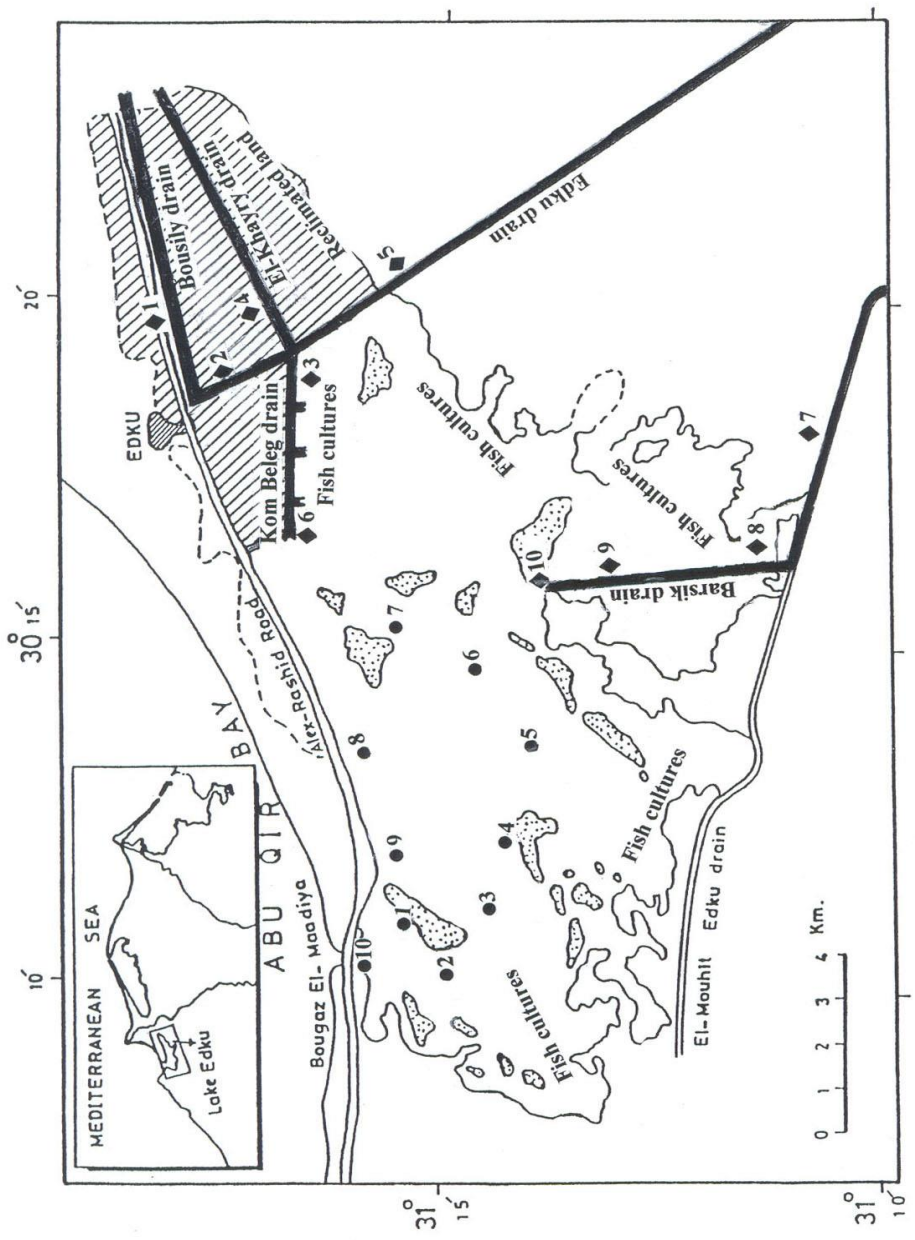

Fig.1. Sampling sites in Lake Edku (●) and connected drains (◆).

Haemolysis assays

Beta-haemolysis activity was assayed by culturing *Aeromonas* spp isolates from each sample (over night cultures in nutrient agar at 37 ^{o}C for 24 h) on brain heart infusion agar (Difco) supplemented with 5% cheep erythrocytes at 35 ^{o}C for 24-48 h (Imziln *et al.,* 1998). Hemolysis was observed by visual clear colorless zones around the colonies. If any *Aeromonas* species isolate from any sample gave positive hemolytic activity, the sample considered as positive. Controls were done with each assay.

Hydro-chemical analysis

Hydro-chemical parameters including pH, salinity, and dissolved oxygen were determined in Marine Chemistry Department, National Institute of Oceanography and Fisheries, Alexandria.

RESULTS AND DISCUSSION

The water quality parameters including temperature (^{o}C), salinity (S‰), pH and dissolved oxygen (mg/l) (DO) were measured in all samples during the course of study. (Table 1 and Fig. 2). Water temperature showed seasonal variation represented high records in summer (27.0 ^{o}C) and minimum (16.9 ^{o}C) in winter in Lake Edku, however in the drains it ranged between 16.5 ^{o}C and 26.5 ^{o}C. Salinity in Lake Edku showed high level in autumn (4.8 ‰) near the connection to the sea and minimum (1.3 ‰) in spring at the center of the lake, while in the drains, it fluctuated between 1.1 ‰ and 2.8 ‰. The pH values showed variations between 7.5 and 8.5 in all water bodies. Dissolved oxygen levels never close to being depleted throughout the study, with high records in Lake Edku (6.5 - 11.0 mg/l) comparing to drains (4.5 - 8.3 mg/l) which might due to the photosynthesis of heavy submerged plants and phytoplankton in the shallow lake.

It is wildly recognized that the normal habitat of *Aeromonas* exist in the aquatic environments. The counts of *Aeromonas* species as well as EC and FS are presented in Table 1 and Figs. 3 and 4. All water samples exhibited positive result for *Aeromonas* species and fecal contamination indicators with highest cfu counts in summer and lowest in winter. The same pattern of seasonal trend had been reported by Gavriel *et al.,* (1998) and Maalej *et al.,* (2004). The densities of *Aeromonas* species fluctuated between 4 x 10^3 and 2.8 x 10^4 cfu/100ml in Lake Edku. In Kom Belag drains and Bersik drain they were ($10x10^3$ - $19x10^3$ cfu/100ml) and ($0.6x10^3$ – $3.3x10^3$ cfu/100ml) respectively. Generally *Aeromonas* species counts fluctuated from one to two logs unites higher than EC and FS in all water samples (Table 1 and Figs 3 and 4). These results are in a parallel with findings of Marcel *et al*. (2002). They isolated the highest frequencies in the samples with the highest counts of fecal indicators. The counts of *Aeromonas* species in Lake Edku were in the same rang ($2x10^3$ - $5x10^4$) as reported by Pettibone (1998) in Buffallo River, USA and were lower than those ($2x10^4$ - $3.5x10^5$ cfu/100ml) reported by Vellari *et al.* (2003) in natural mineral waters in Italy. In Kom Belag and Bersik drains the levels of pathogen were in the range ($1x10^3$ - $1x10^4$ cfu/100ml) that was found in Virginia fresh water lakes, USA (Rhodes and Kator 1994) or ($1.3x10^3$ - $3.3x10^3$ cfu/100ml) of Narew River, Poland (Harnisz and Zmyslowska 2004).

Aeromonas spp. density in Lake Water was generally higher than in the connected drains that receive a lot of sewage especially Kom Belag drains. Lake Edku is now surrounding with more than four thousands feddans of fish cultures (Abbas - Personal communication) that receives water from these drains and discharging it into the lake (Fig. 1). This well increase the organic load in lake water beside increases the Aeromonads that are known to occur in the intestinal tract of different fish species (Hanninen *et al.,* 1997; Harnisz and Zmyslowska, 2004). Pianetti *et al.* (1998) regarded *A. hydrophyla* as an indicator of

organic pollution, whereas Spanggaard *et al*. (2000) identified 86% of trout fish digestive tract bacteria as Enterobacteriaceae. This means that the high intensity of the studied bacterium in Lake Edku is probably coming from the fish farms wastewater discharging into the lake.

Correlation matrix between investigated bacteria and selected hydrographical parameters in the studied waters during 2004 are presented in Table 2. Although the density of *Aeromonas* species were higher than fecal indicator parameters in all studied waters, a strong positive correlations were shown between *Aeromonas* species and both of EC (r = 0.85) and FS (r = 0.90) in all water samples. However EC and FS were the most highly correlated during the course of study (r = 0.81-0.97). Araujo *et al.* (1991) and Pettibone (1998) described the same positive correlation. In Lake Edku, a negative correlations were found between salinity and each of *Aeromonas* species (r = 0.77), EC (r = 0.79) and FS (r = 0.75). The same holds true with the findings of Marcel *et al.* (2002) who found higher isolation frequency of *Aeromonas* species and EC when water salinity lower 10‰.

Aeromonads are common contaminants in fresh foods including fish and other seafood (Hanninen *et al*., 1997). The incidence of *Aeromonas* species and fecal indicators in fresh fish and invertebrates from Lake Edku during 2004 were recorded in Table 3. All Fish and invertebrates samples gave positive results for *Aeromonas* species at concentrations ranged from $0.5x10^2$ to $8x10^3$ cfu/100g. Also all samples were positive for EC ($0.2x10^2$ - $2x10^3$ cfu/100g) and FS ($0.5x10^2$ - $8.0x10^3$ cfu/100g). These counts were lower than those ($2x10^2$ - $1x10^6$ cfu/100g) reported in fish and shrimp samples by Neyts *et al*. (2000) in Belgium. The prevalence of Aeromonads in fishes showed seasonal variation with maximum cfu counts occurring in summer and autumn and minimum in winter and spring (Table 3). Holmes and Nicollas (1995) stated that the growth of Aeromonads is temperature dependent, while Gavriel *et al*. (1998) and Sautour *et al.* (2003) reported that the pathogen is often more recovered during the warm months.

Aeromonas species were isolated in 100% of water samples and in 75% and 37.5% of fish and invertebrates samples (Table 4).The same was true with 100% incidence of *Aeromonas* species in freshwater samples reported by Hanninen *et al*., (1997) in Finland and 93-100% in Rivers in California, USA (Ardi and Olson, 2002), whereas it was up to 96% in lakes and reservoirs in Spain (Borrell *et al.,* 1998) and 82% in lagoon water in Abidjan, South Africa (Marcel *et al.,* 2002). The Aeromonads were isolated in 75% of studied fish samples. It was higher (32%) than those reported by (Ullman *et al*., 2005) in Germany, (34%) in New Zealand (Bremer *et al.,* 2003), (37%) in India (Vivekanandhan *et al.,* 2005), (42%) in Sweden (Krovace *et al.,* 1996) and (50%) in Taiwan (Tsai and Chen 1996) and lower (93%) than those in Finland (Hanninen *et al.,* 1997). In the current study, the incidence of pathogen in invertebrates (Shrimp and Crap) (37%) were much lower than those recorded in fish (75%). This is in agreement with the findings of Tsai and Chen (1996) and Vivekanandhan *et al.* (2005). The chitinous shell of the shrimp and crap might not be the conducive for proliferation of the *Aeromonas*, as the moisture rich body of fish (Vivekanandhan *et al.,* (2005).

The production of haemolytic toxins is regarded as strong evidence of pathogenic potential in aeromonads (Santos *et al.,* 1999). The incidence of haemolysin producing Aeromonades was detected in 65% to 70% of studied water samples and in 57% and 31% of fish and invertebrates samples respectively (Table 4). The same percentage (70%) of isolated *Aeromonas* strains was recorded in Virginia fresh water lakes (Rhodes and Kator, 1994), while a slightly higher percentage (63% - 88%) was found in environmental samples in India (Alavandi & Ananthan, 2003). In fish, the current study showed that 57% of samples harbor haemolysin producing

Aeromonads. The same percentage (57%) of isolated Aeromonads strain was reported in retail fish in Malaysia (Heng *et al.,* 2005). However higher percentage 79%, 88% and 98% were reported in seafood in Taiwan, Mexico and Germany respectively (Tsai and Chen, 1996; Castro-Escarpulli *et al.,* 2003; Ullman *et al.,* 2005).

Since the micro-organism is considered as food-borne pathogen and can grow and produce haemolysin not only at optimal growth temperature but also at refrigeration temperature (Maalej *et al*., 2004), the high prevalence *Aeromonas* specially haemolysin species in Lake Edku waters as well as in fishes and invertebrates, intended for human consumption, is of great importance from both epidemiological and ecological point of view. Unless the ecology and exotoxicology of this pathogen is fully understood, the control and prevention of infection might be possible through (1) Avoiding direct contact of people specially fishermen with lake water (2) Heat treating fishes and seafood before consumption, since the pathogen is heat sensitive (D_{55} = 1.17 min) (Buckle, 1989). (3) Proper handling, transportation, storage and marketing of fishes at refrigeration temperature, since the pathogen tolerates freezing temperature (Al-Harbi and Uddin, 2005). (4) Avoid cross contamination after cooking and refrigerate at temperature below 0ºC.

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Table 1. Annual mean values of ivestigated bacterial and hydrographical parameters recorded in surface waters of Lake Edku, Kom Belag drains and Bersik drain during 2004.

Areas	Stations	*A*.spp	EC	FS	T (^{o}C)	S (S‰)	pH	DO (mg/l)
Lake Edku	1	$7.1x10^3$	$3.4x10^2$	$2.0x10^2$	21.63	2.60	8.20	8.75
	2	$8.5x10^3$	$12.0x10^2$	$4.0x10^2$	21.40	2.30	7.89	8.58
	3	$11.6x10^3$	$15.0x10^2$	$6.0x10^2$	22.08	1.85	8.23	9.00
	4	$8.8x10^3$	$18.0x10^2$	$5.0x10^2$	21.83	1.90	8.43	9.65
	5	$15.2x10^3$	$24.0x10^2$	$6.5x10^2$	22.13	1.72	8.30	8.13
	6	$15.0x10^3$	$34.0x10^2$	$7.5x10^2$	22.00	1.60	7.95	8.03
	7	$12.0x10^3$	$29.0x10^2$	$5.0x10^2$	22.68	1.42	8.08	8.65
	8	$9.4x103^3$	$21.0x10^2$	$3.0x10^2$	22.05	1.94	8.50	9.00
	9	$10.3x10^3$	$15.0x10^2$	$1.2x10^2$	22.53	1.82	8.23	9.18
	10	$6.3x10^3$	$5.0x10^2$	$1.2x10^2$	22.50	3.44	8.43	9.00
	mean	$10.4x10^3$	$17.x10^2$	$4.3x10^2$	22.08	2.06	8.22	8.80
Kom Belag drain	1	$1.8x10^3$	$5.5x10^2$	$1.9x10^2$	21.13	1.70	8.12	6.38
	2	$7.5x10^3$	$25.0x10^2$	$1.2x10^2$	20.78	1.91	7.78	5.61
	3	$11.3x10^3$	$29.0x10^2$	$1.2x10^2$	21.98	1.30	7.85	5.30
	4	$2.9x10^3$	$7.5x10^2$	$3.0x10^2$	21.90	1.53	8.82	6.15
	5	$3.4x10^3$	$8.0x10^2$	$4.0x10^2$	22.15	1.90	7.93	6.30
	6	$5.6x10^3$	$34.0x10^2$	$12.0x10^2$	21.93	1.67	7.90	6.15
	Mean	$5.6x10^3$	$30.0x10^2$	$7.5x10^2$	21.64	1.67	8.07	5.98
Bersik drain	7	$2.8x10^3$	$6.0x10^2$	$1.9x10^2$	21.65	2.85	7.45	5.63
	8	$2.9x10^3$	$9.0x10^2$	$2.7x10^2$	22.80	2.46	8.13	6.13
	9	$3.5x10^3$	$6.0x10^2$	$3.0x10^2$	21.10	1.81	7.65	6.18
	10	$3.5x10^3$	$7.0x10^2$	$4.0x10^2$	22.65	1.64	7.80	6.00
	Mean	$3.3x10^3$	$7.0x10^2$	$2.9x10^2$	22.05	2.19	7.76	5.98

***A.* spp: *Aeromonas* spp EC: *E.coli* FS: Fecal streptococci T: Temperature S: Salinity DO: Dissolved oxygen**

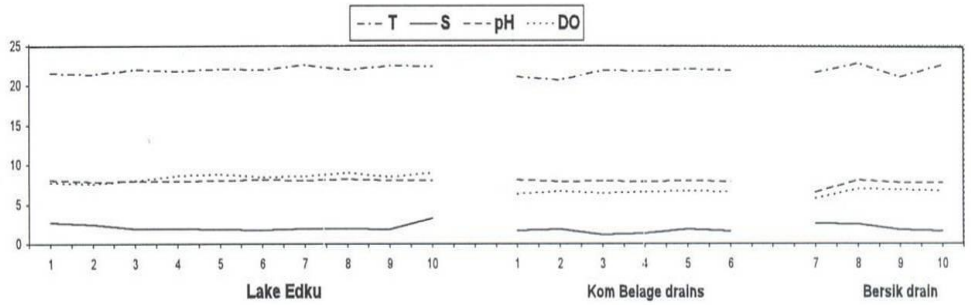

Fig. 2. Annual meam values of temperature °C (T), salinity ‰ (S),pH and dissolved oxygen mg/l (DO) recorded in surface waters of Lake Edku, Kom Belage drains and Bersik drain during 2004.

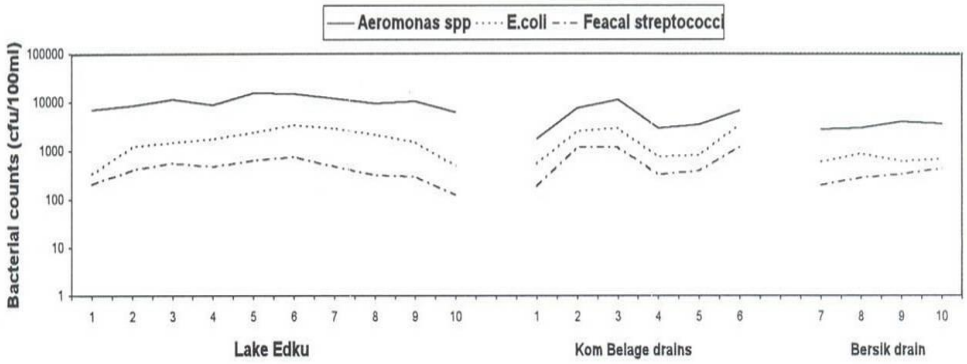

Fig. 4. Annual means of bacterial counts (cfu/100ml) recorded in surface waters of Lake Edku, Kom Belage drains and Bersik drain during 2004.

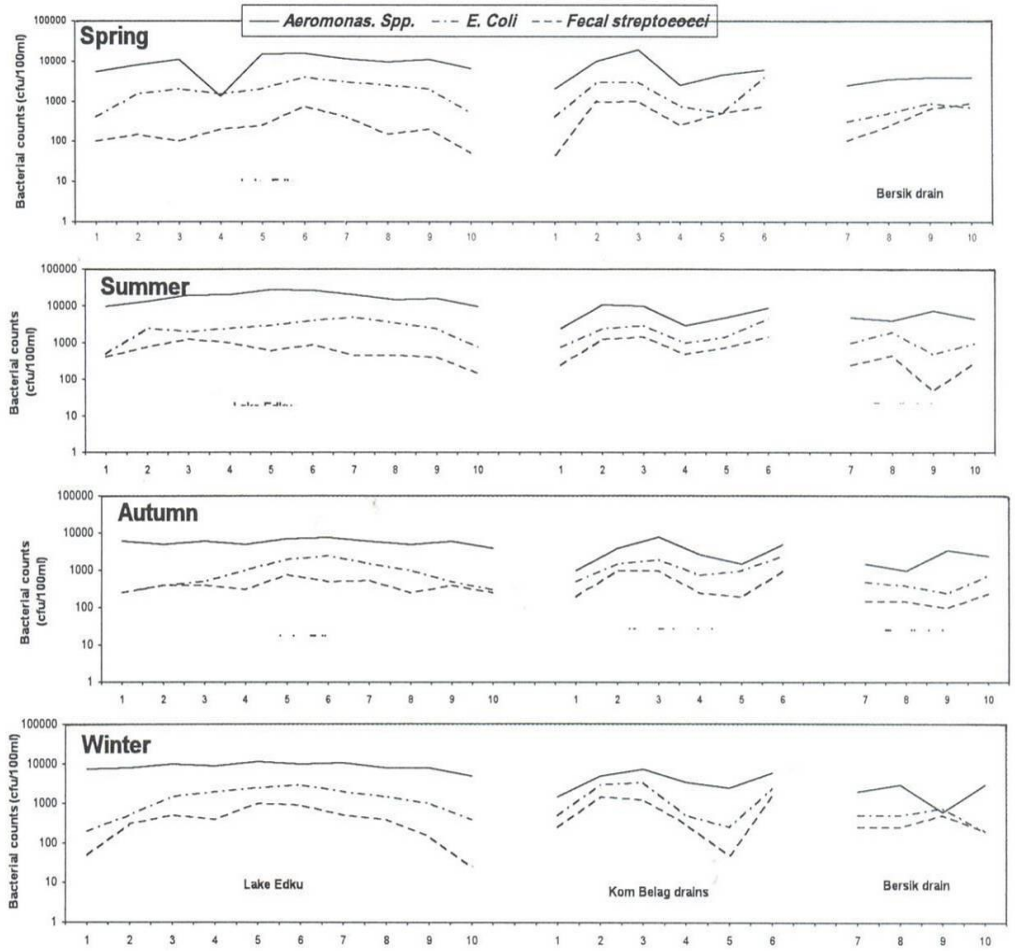

Fig.3. Bacterial counts (cfu/100ml) recorded in surface waters of Lake Edku, Kom Belag dranis and Bersik drain during 2004.

Table 2. Correlation matrix between investigated water parameters in Lake Edku, Kom Belag and Bersik drains during 2004.

Parameters	A.SPP	EC	FS	T	$S‰_0$	pH	DO
A.SPP	1						
EC	**0.85**	1			**Lake Edku waters**		
FS	**0.90**	**0.81**	1		**n = 40**		
T	0.20	0.26	-0.09	1			
$S‰_0$	**-0.77**	**-0.79**	**-0.75**	-0.08	1		
pH	-0.09	0.08	-0.32	0.28	0.10	1	
DO	0.16	0.32	-0.02	**0.64**	-0.13	0.38	1

Parameters	A.SPP	EC	FS	T	$S‰_0$	pH	DO
A.SPP	1						
EC	**0.86**	1			**Kom Belag and Bersik drains**		
FS	**0.91**	**0.97**	1		**n = 40**		
T	-0.12	-0.12	-0.18	1			
$S‰_0$	-0.48	-0.38	-0.45	0.10	1		
pH	0.22	0.28	0.30	0.09	**-0.57**	1	
DO	0.03	0.08	0.13	0.23	-0.25	**0.76**	1

Marked correlations are significant at p < .05000

A.spp: ***Aeromonas*** **spp** **EC***: E.coli* **FS: Fecal streptococci** **T: Temperature (^oC)**

$S‰_o$: Salinity **DO: Dissolved oxygen**

Table 3. Counts of investigated bacteria in fishes and invertrates (cfu/100g).

Type of samples	Season	No.of samples	*Aeromonas* spp		*E.coli*		Fecal streptococci	
			range	mean	range	mean	range	mean
Fishes [1]	Spring	8	$1.0x10^2$ - $6.0x10^3$	$2.0x10^3$	$0.2x10^2$ - $0.6x10^3$	$3.5x10^2$	$0.5x10^2$ - $2.0x10^3$	$1.2x10^2$
	Summer	8	$3.0x10^2$ - $8.0x10^3$	$4.0x10^3$	$0.8x10^2$ - $2.0x10^3$	$10.0x10^2$	$2.0x10^2$ - $8.0x10^3$	$2.0x10^2$
	Autumn	6	$4.0x10^2$ - $8.0x10^3$	$5.0x10^3$	$0.5x10^2$ - $1.2x10^3$	$9.0x10^2$	$5.0x10^2$ - $4.0x10^3$	$2.2x10^2$
	Winter	6	$5.0x10^2$ - $4.0x10^3$	$1.0x10^3$	$2.0x10^2$ - $1.0x10^3$	$6.0x10^2$	$0.5x10^2$ - $3.0x10^3$	$1.8x10^2$
	Whole year	28	$1.0x10^2$ - $8.0x10^3$	$3.0x10^3$	$0.2x10^2$ - $2.0x10^3$	$7.0x10^2$	$0.5x10^2$ - $8.0x10^3$	$1.8x10^2$
Invertebrates [2]	Spring	4	$0.5x10^2$ - $4.0x10^3$	$2.8x10^3$	$1.0x10^2$ - $6.0x10^3$	$3.0x10^2$	$1.0x10^2$ - $2.0x10^3$	$1.0x10^2$
	Summer	4	$8.0x10^2$ - $3.0x10^3$	$2.5x10^3$	$1.8x10^2$ - $2.0x10^3$	$10.0x10^2$	$2.0x10^2$ - $6.0x10^3$	$2.4x10^2$
	Autumn	4	$5.0x10^2$ - $4.0x10^3$	$3.3x10^3$	$2.0x10^2$ - $1.6x10^3$	$12.0x10^2$	$4.0x10^2$ - $7.0x10^3$	$2.0x10^2$
	Winter	4	$3.0x10^2$ - $3.0x10^3$	$2.0x10^3$	$3.0x10^2$ - $2.0x10^3$	$8.0x10^2$	$1.0x10^2$ - $3.0x10^3$	$1.0x10^2$
	Whole year	16	$0.5x10^2$ - $4.0x10^3$	$2.6x10^3$	$1.0x10^2$ - $2.0x10^3$	$8.5x10^2$	$1.0x10^2$ - $3.0x10^3$	$1.8x10^2$

[1] It includes one or more of the following species: More frequent Tilapia, Mugil, Carp, Eels and Clarias.

[2] It includes one or more of the following species: More frequent Shrimp and Crap.

Table 4. Percentage of incidence and haemolysin producing *Aeromonas* spp isolated from Lake Edku, Kom-Belag and Bersik drains, Fish and Invertebrates during 2004

Type of samples	No. of samples	Incidence and (%) of *Aeromonans* spp.		Incidence and (%) of haemolysin producing *Aeromonas* spp.	
Water (Lake Edku)	40	40/40	(100%)	26/40	(70%)
Water (Kom Belag drains)	24	24/24	(100%)	16/24	(66%)
Water (Bersik drain)	16	16/16	(100%)	9/16	(65%)
Fishes[(1)]	28	21/28	(75%)	16/28	(57%)
Invertebrates[(2)]	16	6/16	(37.5%)	5/16	(31%)

[(1)] **It includes one or more of the following species: More frequent Tilapia, Mugil, Carp, Eels and Clarias.**

[(2)] **It includes one or more of the following species: More frequent Shrimp and Crap.**

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