Cytotoxic Assessment of Nile Tilapia (*Oreochromis niloticus*) from a Domestic Wastewater Canal with Heavy Metal Contamination

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Received December 1, 2015; accepted March 3, 2016

Summary The study aimed to assess water quality parameters and heavy metal contamination in water, sediment and Nile tilapia (*Oreochromis niloticus*). Heavy metal concentrations in water were compared to Thailand's standard limits, and chromosomal aberrations in *O. niloticus* from contaminated water were compared with *O. niloticus* from an unaffected area. The samples were collected from a domestic wastewater canal, water quality parameters were assessed, and heavy metal concentrations (Pb, Cd, Cr, Cu, Fe, Mn, Ni, and Zn) in the water, sediment and fish were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The water quality parameters did not exceed standard values. The concentrations of heavy metals in the water and sediment did not exceed Thailand's standards, but the Ni concentration in the water was higher than the standard limits. Similarly, heavy metal concentrations in *O. niloticus* were also below the standard limits. Only the Cr concentration in *O. niloticus* tissues exceeded the standard limits. The diploid chromosome number of *O. niloticus* from both areas was $2n=44$ and the chromosomal damage of *O. niloticus* in the affected area was higher than that in the unaffected area. There were six types of chromosomal aberrations: single chromatid break, single chromatid gap, centromeric gap, fragmentation, deletion and dicentric chromosomes. The most common chromosomal abnormality in the fish samples from the affected area was single chromatid gap, and the difference in chromosomal breakages in *O. niloticus* between the areas was significant ($p<0.05$).

Key words Water quality, Heavy metals, Cytotoxic assessment, *Oreochromis niloticus*.

Pollution of the aquatic ecosystem is globally recognized as a potential threat to both humans and animal populations dependent on the aquatic environment (Biney *et al.* 1987). In Thailand, domestic wastewater is considered to be highly polluted and a serious environmental concern due to pollution by hazardous household waste and domestic non-treated discharges. Common pollutants include organic compounds, inorganic compounds, nitrogen, phosphorus and especially heavy metals such as arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn), according to Mara (2004). Heavy metals in aquatic systems are due to both natural sources and anthropogenic causes. Heavy met-

als affect aquatic organisms and pose considerable environmental concerns (Amisah *et al.* 2009). They have been shown to accumulate in organisms, and some may become biomagnified in food chains (Singh *et al.* 2010). These pollutants are contaminants in wastewater, are harmful to human and aquatic animals and can damage the ecosystem. The Thailand Pollution Control Department (1995) reported that the main source of domestic wastewater (80%) was discharge from households and other buildings at a rate of 291–306L per person a day in 2012; this rate is estimated to rise to 318–322L per person a day in 2017 in northeast Thailand. In Maha Sarakham city, the centrally located Somthawil Canal receives wastewater from the city to reduce the pollution in the water before drainage to the Chi River; however, the water is not treated (Maha Sarakham Administration Office 2012). Thus, the Somthawil Canal is considered

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DOI: 10.1508/cytologia.82.41

Fig. 1. Overview of the Somthawil Canal and locations of the three sites, labeled 1 through 3.

to be highly polluted because it receives wastewater from anthropogenic sources as well as rainfall, and the water is untreated. Nakinchar and Sattayarat (2002) reported that the concentrations of Zn, Pb and Cu in sediments of the Somthawil and Kudnangyai Canals were highest in winter, with a range of $113.55\text{--}834.44\text{ mg kg}^{-1}$, $14.48\text{--}250.70\text{ mg kg}^{-1}$ and $12.96\text{--}267.41\text{ mg kg}^{-1}$, respectively. Priprem *et al.* (2007) studied heavy metals in freshwater fish along the Pong and Chi Rivers. Cd, Co, Cr, Cu, Ni, Pb and Zn levels were determined in muscle and adipose tissues of fish. The results showed that the levels of heavy metal in the adipose tissue were higher than those found in the muscle tissue of fish in both rivers, but the levels did not exceed the standard limits.

Several studies have linked increases in cytogenetic abnormalities in fish and shellfish to polluted environments. Mohamed *et al.* (2008) measured the cytogenetic changes by observing the frequency of chromosomal aberrations in the gill cells of *O. niloticus* treated with copper sulfate and lead acetate, which revealed chromatid breaks, gaps, deletion, "stickiness" and fragmentation. Mahrous and Abdou (2001) determined that environmental water pollution (agricultural or industrial wastewater) has significant effects on *O. niloticus* and *Clarias lazera*, observing chromosome breaks, deletion and centromeric attenuation in somatic cells. Parveen and Shadab (2012) studied *C. punctatus* exposed to cadmium chloride for three days. The results showed that *C. punctatus* exposed to 5.0 mg L^{-1} have chromosomal abnormalities such as chromosomal breaks, gaps, translocations and dicentric and ring chromosomes. One study showed that Cd produced genotoxic effects in fish. Nile tilapia is one of the most widely farmed freshwater fish (Martins *et al.* 2004) and is a key species in aquatic ecosystems as a community food source and an economically important fish species. Various species of Nile tilapia have been favored by many earlier researchers as test models for both cytogenetic and molecular studies

(Martins *et al.* 2000, 2002). Therefore, this study was carried out to assess the heavy metal contamination and investigate the chromosomal aberrations of Nile tilapia affected by domestic wastewater compared with fish from a non-polluted reference area as well as to provide insight into environmental management in the future.

Materials and methods

Sampling sites

Sampling was conducted in the Muang District of Maha Sarakham Province, Thailand (Fig. 1). The water, sediment, and fish samples were collected from three sampling sites at Somthawil Canal during the winter season and compared to samples from a non-polluted reference area, the reservoir in the Khon Kaen Province, which has no domestic wastewater contamination.

Water sample

Water was tested for temperature, pH, dissolved oxygen (DO) and biological oxygen demand (BOD). Duplicate water samples were taken with a water sampler from three locations, sites 1, 2 and 3, between 09:00–11:00 a.m. at a depth of 30 cm below the water surface with sulfuric acid and stored at 4°C in clean 1000-mL plastic sampling bottles according to APHA (2005).

Sediment sample

Duplicates of three sediment samples (sites 1, 2 and 3) up to 20 cm in length were taken from the sites using a grab sampler as described in Boyd and Tucker (1992), then transferred to the laboratory and left to dry at room temperature.

Fish sample

Six *O. niloticus* specimens were collected from the domestic wastewater canal and the reference site. The wet weight and total body length of the fish were mea-

sured. Then, kidney cell preparations were performed, and the fish were frozen for further investigation.

Analysis of heavy metals in water, sediment and fish samples

A total of 2.5 g of each sample was predigested with 3 mL of concentrated nitric acid overnight at 40°C. After cooling the samples, 2 mL of 30% hydrogen peroxide was added. The container was covered, placed in a high-pressure stainless steel bomb and incubated in an oven at 160°C for 4 h. After cooling the samples, the solution was diluted with Milli-Q water, and 50 g was transferred into a PET bottle. The heavy metal concentrations in each sample were determined using induction coupled plasma-optical emission spectrometry (ICP-OES; Optima 8300) (Chand and Prasad 2013). The ICP-OES measurements for Pb, Cd, Cr, Cu, Fe, Mn, Ni, and Zn were taken at 220.353, 226.502, 267.716, 324.752, 259.939, 259.372, 231.604, and 213.857 nm, respectively. The accuracy of the heavy metal results was evaluated with certified reference material (CRM) via the 3111C method (APHA 2005). Two aliquots of the CRM were spiked with a known amount of heavy metal standard. One spike was analyzed according to the 3111C method, and the other was analyzed with the 3111B method (APHA 2005). The heavy metal recoveries were in the 96–100% range, which was determined to be acceptable (USEPA 1994).

Chromosome preparation and assessment

Chromosomal preparations from kidney cells were obtained based on the techniques by Chen and Ebeling (1968) and Nanda *et al.* (1995) with some modifications. The fish samples were transferred to the laboratory, 0.2% phytohemagglutinin (PHA) solution was injected into the fish abdominal cavity, and then 0.05% colchicine solution was injected into the fish abdominal cavity (1 mL per 100 g body weight) after 24 h. The treated samples were left for 1–2 h before incubation on ice. Fish kidneys were removed and cut into small pieces before being mixed with 0.075 M KCl, and large pieces were discarded. The 8-mL cell pellet was transferred to a 15-mL centrifuge tube and incubated for 25–35 min at room temperature. KCl was discarded following supernatant centrifugation at 1200–1500 rpm for 10 min, and cells were fixed in cold, freshly made fixative solution (3 methanol:1 acetic acid) to stop KCl. The fixative was gradually added up to 8-mL before centrifuging again at 1200–1500 rpm for 10 min, and the supernatant was discarded. The fixation process was repeated three to four times until the supernatant was clear, and the pellet was mixed with 1 mL fixative. The mixture was spotted onto a clean slide by a micropipette followed by air drying (Kasiroek *et al.* 2017).

Conventional staining was carried out using 20% Giemsa's solution for 30 min (Rooney 2001, Chooseang-

jaew *et al.* 2017). Ag-NOR banding was performed by adding four drops of 50% silver nitrate and 2% gelatin on slides, respectively. The slides were sealed with cover glasses and incubated at 60°C for 5 min. Then, the slides were soaked in distilled water until the cover glasses separated (Howell and Black 1980, Sangpakdee *et al.* 2017).

Chromosome counting and the recording of abnormal chromosomes were performed on mitotic metaphase cells under a light microscope. Three hundred clearly observable and well-spread chromosome plates were selected and photographed. The fundamental number (NF, number of chromosome arms) was obtained by assigning a value of two to metacentric, submetacentric and acrocentric chromosomes and one to a telocentric chromosome. All parameters were used in karyotyping (Chooseangjaew *et al.* 2017). The cytotoxicity was evaluated from chromosomal aberrations by determining the percentage of chromosome breakages per 100 metaphase cells per individual sample with a light microscope.

Statistical analysis

Heavy metal concentrations in the environment, including *O. niloticus* samples from the domestic wastewater canal, and the percentage of chromosomal abnormalities in *O. niloticus* from the domestic wastewater canal and reference site were analyzed using a *t*-test. All of the statistical tests were conducted at a 95% confidence level.

Results and discussion

Water quality

Water quality parameters (temp, pH, DO and BOD) in the domestic wastewater canal are shown in Table 1. The temperature range was 30.46–32.40°C, the pH range was 7.28–10.56, DO values were 7.36–8.76 mg L⁻¹ and BOD was 7.00–8.03 mg L⁻¹ in the study site. The results indicated that the water quality parameters did not exceed standard values, and DO and BOD values are acceptable for aquatic life. The pH of site 2 exceeded the standard defined by the Thailand Pollution Control Department (2001), but the average pH did not exceed the Thailand standard. Alabaster and Lloyd (1980) identified the pH range that is not directly lethal to freshwater fish as 5.0–9.0. pH; values between 6.5 and 9.0 are satisfactory on a long-term basis for fish and other freshwater aquatic life. Inland freshwater with fish generally has pH values ranging from approximately 6.0 to 9.0 (Ellis 1937), with most water, particularly those with healthy, diverse, and productive fish and macroinvertebrates communities, having a pH between approximately 6.5 and 8.5. While some organisms can tolerate environments with higher or lower pH values, they are rare, and their extreme tolerances are not reflective of the pH tolerated by the majority of organisms occurring in a given aquatic eco-

Table 1. Water quality parameters (temperature, pH, DO and BOD) of the domestic wastewater canal.

Domestic wastewater canal	Parameters			
	Temperature (°C)	pH	DO (mg L ⁻¹)	BOD (mg L ⁻¹)
Site 1	30.46	8.56	8.56	8.03
Site 2	32.40	10.56	7.36	7.00
Site 3	32.10	7.28	8.76	7.93
Mean±SD	31.65±1.04	8.80±1.65	8.23±0.76	7.65±0.57
Standard	—	5–9	4.00	20

Remarks: Thailand's wastewater quality standards (Thailand Pollution Control Department 2001)

Table 2. Heavy metal concentrations in the water of the domestic wastewater canal.

Domestic wastewater canal	Pb (mg L ⁻¹)	Cd (mg L ⁻¹)	Cr (mg L ⁻¹)	Cu (mg L ⁻¹)	Fe (mg L ⁻¹)	Mn (mg L ⁻¹)	Ni (mg L ⁻¹)	Zn (mg L ⁻¹)
Site 1	0.04	0.003	0.003	0.002	0.95	0.21	0.002	0.13
Site 2	ND	0.003	0.003	ND	0.70	0.67	ND	0.05
Site 3	0.02	0.003	0.003	0.104	0.90	0.78	0.179	0.14
Mean±SD	0.03±0.02	0.003±0.0003	0.003±0.0003	0.053±0.072	0.85±0.13	0.55±0.30	0.90±0.125	0.11±0.05
Standard	0.05	0.05	0.25	0.1	—	1.00	0.1	1.00

Remarks: Thailand's surface water quality standards (Thailand Pollution Control Department 1994), ND=Not detected

Table 3. Heavy metal concentrations in the sediment of the domestic wastewater canal.

Domestic wastewater canal	Pb (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Site 1	6.58	0.49	10.77	4.31	5958.05	178.57	3.86	22.89
Site 2	7.21	0.47	13.11	12.56	5486.02	174.33	3.94	33.60
Site 3	6.02	0.37	9.27	2.75	4079.75	182.36	2.53	41.43
Mean±SD	6.60±0.60	0.44±0.07	11.05±1.93	6.54±5.27	5174.61±977.11	178.42±4.02	3.44±0.79	32.64±9.31
Standard	400	37	300	—	—	1800	1600	—

Remarks: Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department 2004)

system.

Heavy metal concentrations in the water from the domestic wastewater canal

Heavy metal concentrations (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn) in the water are shown in Table 2. Heavy metal concentrations in the water were found to have the following ranges: Pb, not detected to 0.04; Cu, not detected to 0.104; Fe, 0.70–0.95; Mn, 0.21–0.78; Ni, not detected to 0.179 and Zn, 0.05–0.14 mg L⁻¹. Cd and Cr concentrations were 0.003 mg L⁻¹. These ranges and the average concentrations of Pb, Cd, Cr, Cu, Fe, Mn and Zn did not exceed Thailand's surface water quality standards (Thailand Pollution Control Department 1994). However, the Ni concentration at site 3 and the average concentration exceeded the standard. Ni is one of the most common metals in surface water (USEPA 1986). Ni enters surface water naturally from smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion, and waste incineration (NAS 1975, WHO 1991). Ni poisoning symptoms in fish include surfacing, rapid mouth movements, opercula movements, and, prior to death, convulsions and loss of equilibrium (Khangarot and Ray 1990). Other symptoms include

decreased concentrations of glycogen in muscle and liver with simultaneous increases in levels of lactic acid and glucose in the blood (Ghazaly 1992). Heavy metals may not highly accumulate in the surface water of the canal because they settle into the sediment.

Heavy metal concentrations in the sediment

Heavy metal concentrations in the sediment (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn) are shown in Table 3. Heavy metal ranges in the sediment were as follows: Pb, 6.02–7.21; Cd, 0.37–0.49; Cr, 9.27–13.11; Cu, 2.75–12.56; Fe, 4079.75–5958.05; Mn, 174.33–182.36; Ni, 2.53–3.94 and Zn, 22.89–41.43 mg kg⁻¹. The concentrations of Pb, Cd, Cr, Mn and Ni did not exceed Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department 2004), and Cu, Fe and Zn have no standard limits. However, they were higher than those recorded in the water samples. This is because sediments act as reservoir for all contaminants and organic matter deposited by humans and the ecosystem.

Heavy metal concentrations in Oreochromis niloticus from the domestic wastewater canal and reference site

Heavy metal concentrations in the *O. niloticus* tis-

Table 4. Heavy metal concentrations in *Oreochromis niloticus* of the domestic wastewater canal and reference site (wet weight).

Area	Samples	Pb (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Domestic wastewater canal	Sample 1	0.12	0.01	2.46	0.39	32.88	1.55	0.06	17.25
	Sample 2	0.33	0.02	2.25	0.49	67.04	15.16	0.44	20.50
	Sample 3	0.21	0.04	4.50	0.66	40.78	29.82	0.29	26.40
	Mean±SD	0.22±0.10	0.02±0.02	3.07±1.24	0.51±0.14	46.90±17.88	15.51±14.14	0.27±0.19	21.38±4.64
Reference site	Sample 1	0.50	0.02	1.92	0.92	20.72	2.96	0.72	22.21
	Sample 2	0.43	0.10	1.87	1.40	41.50	6.28	0.76	36.91
	Sample 3	0.27	0.03	1.92	0.93	45.29	4.37	0.76	23.08
	Mean±SD	0.40±0.12	0.05±0.05	1.90±0.03	1.08±0.27	35.84±13.23	4.54±1.66	0.75±0.02	27.40±8.25
P-value		0.11 ^{ns}	0.37 ^{ns}	0.17 ^{ns}	0.03 ^a	0.43 ^{ns}	0.25 ^{ns}	0.01 ^a	0.33 ^{ns}
Standard		0.5	0.1	2.0	20	—	—	—	100

Remarks: Thailand's food quality standards (Thailand Pollution Control Department 1986), a=significant, ns=not significant.

sue samples (Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn) are shown in Table 4. The wet weight and total length of *O. niloticus* were 54–102 g and 14–19 cm, respectively. The concentrations of heavy metals in fish tissue are closely associated with the metal concentrations of water and sediment from the domestic wastewater canal. The range of the concentrations are: Pb, 0.12–0.33; Cd, 0.01–0.04; Cr, 2.25–4.50; Cu, 0.39–0.66; Fe, 32.88–67.04; Mn, 1.55–29.82; Ni, 0.06–0.44 and Zn, 17.25–26.40 mg kg⁻¹ wet weight in the domestic wastewater canal and Pb, 0.27–0.50; Cd, 0.02–0.1; Cr, 1.87–1.92; Cu, 0.92–1.40; Fe, 20.72–45.29; Mn, 2.96–6.28; Ni, 0.72–0.76 and Zn, 22.21–36.91 mg kg⁻¹ wet weight in the reference area. These data revealed that concentrations of heavy metals in *O. niloticus* in both areas were low and that Pb, Cd, Cu and Zn levels did not exceed Thailand's food quality standards, while Fe, Mn and Ni have no standards. There were significant differences in Cu and Ni concentrations in the *O. niloticus* from the domestic wastewater canal and reference area, while the other heavy metal concentrations were not significantly different between the fish from the two areas.

Cr concentrations were higher than those of the other metals in *O. niloticus* and exceeded standard limits, but other metal concentrations were lower than the standard limits. This may be due to human activities and the effects of rain, for example, stainless steel and dye or pigments coming into contact with running water, causing Cr to leach into the reservoir. Subsequently, Cr in wastewater will not silt or condense into the sediment. Thus, it can move with the water flow, which likely caused contamination in the water source. Higher levels of Cr in water caused more Cr accumulation in Nile tilapia compared to other metals. Although the other heavy metal concentrations did not exceed standards, essential heavy metals also become toxic when their concentrations exceed those required for correct nutrition. According to Otchere (2003), metal toxicity occurs when an organism is unable to cope with additional metal concentrations by direct usage, storage and excretion. The results obtained

from the laboratory analysis indicate that *O. niloticus* from the domestic wastewater canal is contaminated with heavy metals. There are other possible environmental measures of toxicity in addition to the heavy metal concentrations found in this field study (Ansari *et al.* 2004). Heavy metals are well-known environmental pollutants that cause serious health hazards to humans, but their effects are not immediate and can appear after many years (Liu *et al.* 2007). Heavy metal release into the aquatic environment can damage both aquatic species diversity and ecosystems due to their toxicity and accumulative behavior (Edwards *et al.* 2001, Smith and Windom 1972). Pb, Cd, Cr, Cu, Fe, Mn, Ni and Zn are usually found in wastewater, and these metals are toxic to aquatic life at low concentrations, particularly in fish, and may affect the food chain and the ecosystem (Ellis 1996). Therefore, Nile tilapia caught from the domestic wastewater canal may pose health hazards for consumers.

Assessment of chromosomal aberrations

This study revealed that heavy metal concentrations in *O. niloticus* samples from both areas are correlated with chromosomal aberrations. The diploid number of chromosomes in *O. niloticus* from the domestic wastewater canal and reference site was $2n=44$. Most chromosomes were rod-shaped, with one pair of long acrocentric 'marker' chromosomes that could be distinguished from others in the complement. This is in accordance with previous studies (Arai and Koike 1980, Vervoort 1980, Chen and Chen 1983, Majumdar and McAndrew 1986, Crosetti *et al.* 1998, Sofy *et al.* 2008), which showed that the diploid number of *O. niloticus* was $2n=44$. The karyotype of *O. niloticus* from both areas was composed of 2 submetacentric, 12 acrocentric and 30 telocentric chromosomes (Fig. 2). This is inconsistent with the report of Sofy *et al.* (2008), which demonstrated that *O. niloticus* has 2 submetacentric, 24 subtelocentric and 18 telocentric chromosomes. The karyotype of *O. niloticus* from the study site showed chromosomal

Fig. 2. Conventional staining of the metaphase chromosome plates and karyotypes of individual (A, B) *O. niloticus* ($2n=44$) samples from the domestic wastewater canal. Arrows indicate chromosomal aberrations.

Fig. 3. Conventional staining of the metaphase chromosome plates and karyotypes of individual (A, B) *O. niloticus* ($2n=44$) samples from the reference site.

aberrations, and *O. niloticus* from both areas displayed terminal Ag-NOR on chromosome pair 1 (Fig. 4). The $2n$ of the mitotic metaphase cells and karyotypes of *O. niloticus*, conventionally stained with Ag-NOR, did not differ between the sample areas. Staining with Ag-NOR did not detect the chromosomal aberrations of *O. niloticus*, while conventional staining techniques could detect chromosomal aberrations. The different types of abnormalities in the metaphase-spread cells of *O. niloticus* samples from the domestic wastewater canal are shown in Fig. 5. The different categories of chromosomal aberrations were single chromatid gap (SCG), single chromatid break (SCB), centromere gap (CG), fragmentation

(F), deletion (D) and dicentric chromosome (DC). The most common chromosomal aberration in the samples from the study site was SCG. The number and percentage of chromosomal aberrations of *O. niloticus* samples from the domestic wastewater canal and reference site are shown in Table 5. The number of types of chromosomal aberrations of the *O. niloticus* are as follows: SCG, SCB, CG, F, D and DC were 20, 66, 20, 6, 5 and 40 aberrations from the domestic wastewater canal and 1, 8, 4, 2, 0 and 2 aberrations from the reference site, respectively. One hundred clearly observable slides were used for this study, and the total number of chromosomal anomalies found in the *O. niloticus* samples from the do-

Fig. 4. Ag-NOR banding of metaphase chromosome plates and karyotypes of *O. niloticus* ($2n=44$) from the reference site (A) and domestic wastewater canal (B). Arrows indicate NOR-bearing chromosome pair 1.

Fig. 5. Different types of chromosomal aberrations in the metaphase spread cells of *O. niloticus* ($2n=44$), showing a single chromatid break (SCB), single chromatid gap (SCG), centromere gap (CG), deletion (D), fragmentation (F) and dicentric chromosome (DC) (A: reference site, B–F: domestic wastewater canal).

mestic wastewater canal and reference site were 157 and 17, respectively.

In addition, the number of cells with chromosomal aberrations in the *O. niloticus* samples from the domestic wastewater canal and reference site was 87 and 14 cells, respectively. The average percentage of chromosomal aberrations in the *O. niloticus* cells from the canal and reference site were 87 and 14%, respectively. Statistical analysis indicates that there are significant differences in the numbers of chromosomal aberrations and the cells with chromosomal aberrations between *O. niloticus* samples from the domestic wastewater canal and refer-

ence site ($p=0.024, 0.006$).

Cytogenetic analysis of chromosomes has been employed as a biological dosimeter to estimate the effect of genotoxic agents (pollutants) on fish and is very useful for direct detection of effects in somatic cells (Yunis 1983, Radwan 1996). Chromosomal analysis of *O. niloticus* samples from the domestic wastewater canal and an unaffected reference site indicated that the diploid chromosome numbers are the same, and the NOR location can provide information about the chromosomal evolution. The *O. niloticus* samples displayed terminal Ag-NOR on chromosome pair 1, which agrees with the

Table 5. The number and percentage of chromosomal aberrations of *O. niloticus* samples from the domestic wastewater canal and reference site.

<i>O. niloticus</i> samples	Number of chromosomal aberrations						Total number of chromosomal aberrations	Number of cells with chromosomal aberrations	Percentage of chromosomal aberrations
	SCB	SCG	CG	F	D	DC			
Domestic wastewater canal									
Individual 1	10	30	10	4	3	17	64	36	36
Individual 2	5	12	5	1	1	5	29	21	21
Individual 3	5	24	5	1	1	18	54	30	30
Average/Total	20	66	20	6	5	40	157	87 ^a	87 ^a
Reference site									
Individual 1	1	4	1	1	0	1	8	7	7
Individual 2	0	2	2	0	0	0	4	4	4
Individual 3	0	2	1	1	0	1	5	3	3
Average/Total	1	8	4	2	0	2	17	14	14
<i>P</i> -value								0.024 ^a	0.006 ^a

Remarks: a=significant.

results of Supiwong *et al.* (2012) who reported that Ag-NOR was located on the region adjacent to the centromere of chromosome pair 1. This study provides basic information about *O. niloticus*, and the cytogenetics results can be applied to studies on breeding, conservation and chromosome evolution in *O. niloticus*. The *O. niloticus* samples from the affected and unaffected areas were significantly different in some concentrations of heavy metals and chromosomal aberrations.

Generally, there was a significant difference in the chromosomal aberrations between the domestic wastewater canal and reference site samples. These chromosomal abnormalities may lead to genetic changes in the *O. niloticus* population. Concerns have been expressed about the genetic consequences of pollution to fish populations exposed to low concentrations of heavy metals over prolonged periods (Barker and Rackham 1979). The data from this study indicated that the chromosomal aberration frequency increased with an increase in concentrations of heavy metals (Frag *et al.* 2009).

The results of this study corresponded to those found by Mohamed *et al.* (2008), who reported enhancement in the frequency of centromeric attenuation, chromatid break, chromatid gap, deletion, fragmentation, ring chromosome and end to end association in samples exposed to industrial and agricultural wastes. The higher level of chromosomal aberrations in *O. niloticus* from the affected area may be the result of environmental contamination by domestic wastewater or due to contaminated water with heavy metals and other pollutants such as fertilizers, chemicals and insecticides. Although this study was performed with a small number of *O. niloticus* samples, the detection of chromosomal aberrations suggests that the wastewater canal must be better managed and that the public should consider their management of wastewater discharge.

Conclusion

The water quality of the domestic wastewater canal is suitable for aquatic life. The public should be informed of the results of this study so that they can properly consider the effects of discharging wastewater. Water, sediment and fish obtained from the domestic wastewater canal had concentrations of heavy metals that were lower than the standard limits. Pb, Cd, Cr, Cu, Fe, Mn, and Zn in the water and heavy metals in the sediment of the canal were present at levels below the Thailand standard limits. However, the Ni concentration in individual samples and the average levels exceeded the standard limit in water samples. Pb, Cd, Cu, Fe, Mn, Ni and Zn levels were below the Thailand standard limits in *O. niloticus* taken from the domestic wastewater canal, and only the Cr concentration in *O. niloticus* exceeded the standard, which suggests that fish caught from the domestic wastewater canal can pose health hazards to consumers. The average percentage of chromosomal aberrations of the *O. niloticus* samples from the domestic wastewater canal is higher than the samples from the reference site. The chromosomal abnormalities occurred in fish exposed to water pollution over a long period of time. Exposure to high concentrations of heavy metals is known to cause structural abnormalities of chromosomes, but it does not affect the diploid chromosome number. These fish can endure heavy metal contamination and survive in the contaminated ecosystem.

Acknowledgements

This study was supported by the Research Group on Toxic Substances in Livestock and Aquatic Animals, ASEA-UNINET Thailand scholarship, Faculty of Science and Integrated Water Resource Management Research and Development Center in Northeast Thailand,

Khon Kaen University.

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