Int. J. Environ. Res., 9(3):897-906, Summer 2015
ISSN: 1735-6865

Chromosomal Aberration of Snakehead Fish (*Channa striata*) in Affected Reservoir by Leachate with Lead and Mercury Contamination

Promsid, P.[1,4], Neeratanaphan, L.[1,4*], Supiwong, W.[2,4], Sriuttha, M.[3] and Tanomtong, A.[2,4]

[1] Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

[2] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

[3] Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai 43000, Thailand

[4] Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University

Received 5 Oct. 2014; Revised 5 Dec. 2014; Accepted 5 Dec. 2014

ABSTRACT: The objectives of this study are to investigate chromosomal aberrations of snakehead fish in a leachate-affected reservoir located 100 meters from a municipal landfill which compared to non-affected reservoir. Three snakehead fish were collected and chromosomal aberrations were studied using kidney tissue. Lead and mercury were measured in water, sediment and snakehead fish from the affected reservoir at three sampling sites. The results showed that the average concentrations of lead and mercury in water were 0.0012±0.0003 and 0.0053±0.0036 mg/L, respectively. The average concentrations of lead and mercury in sediment were 3.3650±2.1930 and 0.0890±0.0272 mg/kg, respectively. These values did not exceed the standard for water and soil quality except for the mercury in the water, which was higher than acceptable. Lead was not found in snakehead fish from either reservoir. The average concentrations of mercury in snakehead fish from both reservoirs were 0.1330±0.0792 and 0.1180±0.0350 mg/kg, respectively, which were higher than the standard for mercury contamination in food. This study showed that the accumulation of mercury in snakehead fish was higher than in sediment because it accumulates in organisms through the consumption hierarchy. The diploid chromosome number of snakehead fish in both reservoirs was $2n = 42$ and the percentage of chromosomal breakages of snakehead fish in the affected reservoir was higher than the non-affected reservoir. There were four types of chromosomal breakages: single chromatid gap, isochromatid gap, single chromatid breaks and isochromatid breaks. The difference in percentage of chromosomal breakages in snakehead fish from both reservoirs was statistically significant ($p<0.05$).

Key words: Chromosomal aberration, Municipal landfill, Leachate, Heavy metal, *Channa striata*

INTRODUCTION

The increasing human population affects the consumption of natural resources, which has led to environmental problems such as soil and water pollution. Human consumption is increasing solid waste, and municipal landfills have contamination from leachate, which greatly impacts aquatic ecosystems. Solid waste constitutes an important and emerging environmental problem: it is estimated that waste production varies from 0.5 to 4.5 kg per person per day in different regions of the world (Bakare *et al*., 2005). Landfills are considered the most common methods for disposing municipal solid waste. Municipal landfills in Thailand have a problem caused by leachate; for example, a municipal landfill located in the Muang district, Khon Kaen province, which has been used for the last 46 years, is contaminated. Its use exceeds the maximal use that it was designed to carry. The main toxic compound in municipal landfills is leachate, characterized by its high concentrations of numerous toxic and carcinogenic chemicals (Halim *et al*., 2005; Li *et al*., 2004). It

*Corresponding author E-mail: hlamya@kku.ac.th

also causes mutagenic and carcinogenic diseases in living beings. The contamination of soil, ground and surface water by municipal landfill leachates has become a serious problem due to environmental and human health risks (Slack *et al*., 2005). Leachate is generated by the infiltration and percolation of rainfall, groundwater, run-off or flood water into disposal landfill areas and through the waste layers of an existing or abandoned landfill site (Kjeldsen *et al*., 2002; Renou *et al*., 2008). This type of liquid effluent consists of a huge number of pollutants, such as dissolved organic matter, inorganic salts, heavy metals and xenobiotic organic compounds, which could be toxic and carcinogenic (Christensen *et al*., 2001; Slack *et al*., 2005; Kalcíková *et al*., 2011) and able to induce potential risk for biota and humans. It has been reported that a small amount of landfill leachate can pollute large volume of groundwater, which can contaminate and affect the biodiversity of aquatic ecosystems and subsequently contaminate food chains (Bakare *et al*., 2000; Garaj-Vrhovac *et al*., 2009). Heavy metals have received considerable attention due to their toxicity and potential to bioaccumulate in aquatic biota. Lead (Pb) and mercury (Hg) are two heavy metals found in nature. Their distribution in the environment is governed by natural and anthropogenic activities (Florea *et al*., 2004). They represent a significant ecological and public health concern due to their toxicities and abilities to accumulate in living organisms. Exposure to high concentrations of Pb and Hg damages the nervous system, immune system, kidney and liver in human beings. The genotoxicity of Pb and Hg compounds have been investigated with a variety of genetic endpoints in prokaryotic and eukaryotic cells (WHO, 1991; WHO, 1989). Several studies have confirmed the genotoxic potential of leachates, reporting a significant increase in the frequencies of micronuclei, sister chromatid exchanges, chromosomal aberrations, DNA disturbances and cut-downs of mitotic indexes in different cell types and model systems (Amahdar *et al*., 2009; Bakare *et al*., 2005; Chandra *et al*., 2005; Feng *et al*., 2007; Gajski *et al*., 2011; Li *et al*., 2008; Monarca *et al*., 2002; Sang & Li, 2004; Sang *et al*., 2006; Tewari *et al*., 2005). The cytogenetic abnormalities and DNA damage induced by landfill leachate indicate that consumption of leachate-contaminated water could increase the risk of developing adverse health consequences. As a result, it is important to monitor the potential toxicity by leachate of sanitary landfills (Gajski *et al*., 2012). The measurement of genotoxicity caused by heavy metals in living things, including aquatic animals, is mainly related to sensitivity and a short response time (Gupta & Sarin, 2009). Studies on aquatic organisms exposed to pollutant waters or sediment containing heavy metals have reported DNA strand breakage. Fish are used as sensitive indicators for their genotoxic and mutagenic effects (Yadav & Trivedi, 2006). Chromosomal aberration tests provide a quick method to screen genotoxic effects of chemical substances that are present in the environment (Leme & Marin-Morales, 2008; Leme *et al*., 2008; Hoshina *et al*., 2008; Hoshina & Marin-Morales, 2009). Investigations of the toxic effects of metal pollutants at the cellular level demonstrate cytogenetic aberrations, which warrant environmental monitoring and risk assessment.

Channa striata is a species of snakehead fish that is important in the food chain within the aquatic ecosystem. Aquatic ecosystem contamination by heavy metals from leachate has been gaining increasing attention. Chronic exposure and accumulation of these chemicals by aquatic biota may result in tissue damage that produces adverse effects not only in the exposed organisms, but also in organisms including human beings. This study aims to determine the concentration of Pb and Hg in *C. striata* samples from an affected reservoir and a non-affected reservoir which was assumed to have no leachate contamination, as well as to obtain information on chromosomal aberrations.

MATERIALS & METHODS

The three sampling sites are located at the reservoir in municipal landfill in the Muang district of the Khon Kaen, Thailand (Fig. 1). The distance between the affected reservoir and the municipal landfill is 100 meters. Most of the land near the municipal landfill was used for farming and cropping plants such as rice, bananas, cassavas and sugarcane. The reference site was defined as the reservoir, where there was no leachate contamination.

The water, sediment and *C. striata* samples were collected from three sampling sites at the affected reservoir in municipal landfill (Fig. 1) and the non-affected reservoir, which was randomly selected. Each sample was analyzed for Pb and Hg concentrations and chromosome aberrations. The water samples were fixed by nitric acid and the sediment samples were dried by air before analysis of Pb and Hg concentrations.

A total of 2.5 g of each sample was predigested with 3 ml of concentrated nitric acid overnight at 40°C. After cooling, 2 ml of 30% hydrogen peroxide was added. The container was covered, placed in a high-pressure stainless steel bomb and then placed in an oven at 160°C for 4 h. After cooling, the solution

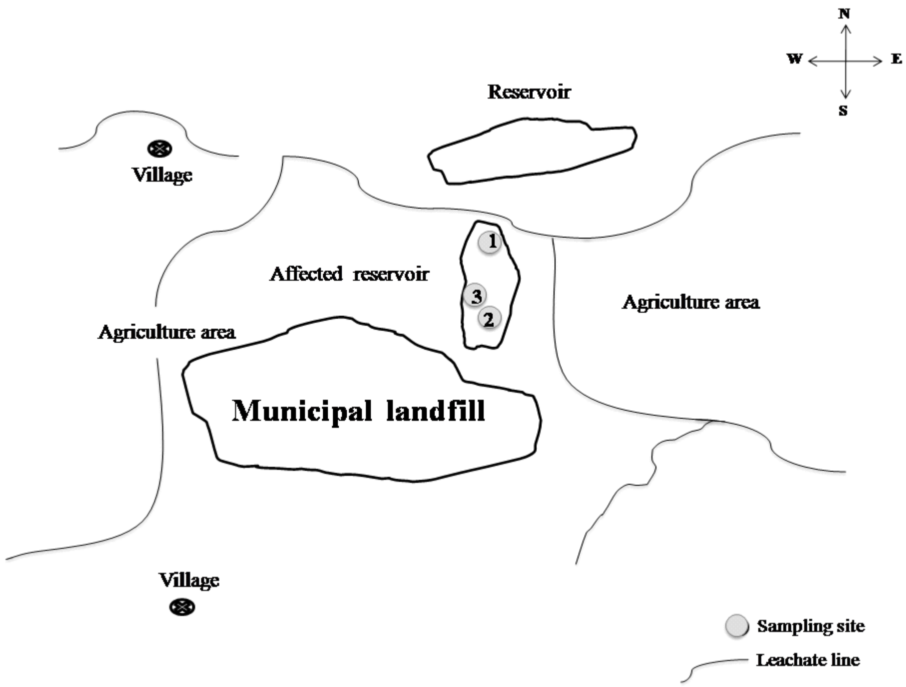

Fig. 1. Overview of the municipal landfill area and locations of the three studied sites, as shown by sites 1 through 3

was diluted with Milli-Q water and transferred into a PET bottle to 50 g. The Pb and Hg concentrations in each sample were determined using induction coupled plasma-mass spectrometry (ICP-MS; model 7500C) (Bailey *et al*., 2003). The wavelength analyses of ICP-MS for Pb and Hg were set to 283.3, 228.8 nm, respectively. The accuracy of the metal concentrations 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 metal spike standard. One spike was analyzed according to the 3111C method, and the other was analyzed with the 3111B method (APHA, 2005). The metal recoveries were in the 96-100% range, which is considered acceptable(USEPA, 1994).

The snakehead fish samples were transferred to the laboratory. Chromosomes were directly prepared *in vivo* (Chen & Ebeling, 1968; Nanda *et al*., 1995) as follows: colchicine was injected to fish's abdominal cavity and left for 1 h. The kidney was cut into small pieces then mixed with 0.075 M KCl. After discarding all large pieces of tissue, 7 ml of cell sediments were transferred to a centrifuge tube and incubated for 25-30 min. KCl was discarded from the supernatant after centrifugation again at 1,200 rpm for 8 min. The cells were fixed in a fresh, cool fixative (3 methanol: 1 glacial acetic acid) gradually increased to 7 ml before centrifugation again at 1,200 rpm for 8 min; then the supernatant was discarded. The fixation was repeated until the supernatant was clear; then the pellet was mixed with 1 ml fixative. The mixture was dropped onto a clean and cold slide by a micropipette followed by an air-dry technique.

Conventional staining was prepared by using 20% Giemsa's solution for 30 min (Rooney, 2001). Ag-NOR banding (Howell & Black, 1980) 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. After that, the slides were soaked in distilled water until the cover glasses were separated.

Chromosome counting and recording of abnormal chromosomes were performed on mitotic metaphase cells under a light microscope. Twenty clearly observable and well-cells spread chromosome plates were selected and photographed. The fundamental number (NF, number of chromosome arms) was obtained by assigning a value of two arms to metacentric, submetacentric and acrocentric chromosomes and one to a telocentric chromosome. All parameters were used in karyotyping. For the estimation of chromosomal aberration frequency per one specimen, 300 cells were selected out for a 150-well spread after sacrifice. The chromosomal aberrations were estimated by studying the percentage of chromosome breakages on 150 metaphase cells per individual sample under a light microscope.

The concentrations of Pb and Hg and the percentage of chromosomal breakage in snakehead fish from affected and non-affected reservoirs were analyzed using one-way ANOVA. All of the statistical tests were conducted at a 95% confidence level.

RESULTS & DISCUSSIONS

The Pb and Hg concentrations in water and sediment samples of the affected reservoir from the three studied sites are shown in Table 1. The Pb and Hg concentrations in *C. striata* from both reservoirs are shown in Table 2. The Pb and Hg concentrations at all sites ranged from 0.0010-0.0015 and 0.0019-0.0090 mg/L in water, and 1.793-5.871 and 0.0580-0.1090 mg/kg in sediment. The average concentration values of Hg in the water were still higher than is allowed for the water quality standards (0.002 mg/L), (Pollution Control Department of Thailand, 2001). The concentrations of Pb and Hg in water and sediment samples did not exceed the standard of water and soil quality, with the exception of Hg concentration in the water, which was higher than acceptable. Pb was not found in snakehead fish from either reservoir. The average concentration of Hg in snakehead fish from affected and non-affected reservoirs were 0.133±0.079 and 0.118±0.035 mg/kg, respectively, which were both higher than the standard of Hg contamination in food (≤ 0.02 mg/kg), (Pollution Control Department of Thailand, 2001). Statistical analysis indicates that there are no significant differences between the concentrations of Hg in snakehead fish from affected and non-affected reservoirs.

This study reveals that the Pb and Hg concentrations in water, sediment and snakehead fish (*C. striata*) samples from the reservoir affected by leachate correlated with chromosomal aberrations. The Pb and Hg concentrations in the sediment are higher than the water at all measured sites; after being deposited into the sediments, they accumulate in *C. striata*. All Pb and Hg levels meet the standard of water and soil quality, except for Hg in the water, and fortunately, the concentration of Pb was not found in snakehead fish of affected and non-affected reservoirs. However, the average concentrations of Hg in snakehead fish from both reservoirs were higher than Thailand's food quality standard level. This comparative study showed that the accumulation of mercury in snakehead fish was higher than in sediment

Table 1. The concentrations of Pb and Hg in water and sediment samples from affected reservoir

Sampling sites	Water samples		Sediment samples	
	Pb concentrations (mg/L)	Hg concentrations (mg/L)	Pb concentrations (mg/kg)	Hg concentrations (mg/kg)
Site 1	0.0011	0.0090	5.871	0.1090
Site 2	0.0015	0.0050	2.431	0.1000
Site 3	0.0010	0.0019	1.793	0.0580
Average concentration	0.0012±0.0003	0.0053±0.0036	3.3650±2.1930	0.0890±0.0272
Thailand Standard	≤0.05	≤0.002	≤400	≤23

Table 2. The concentrations of Pb and Hg in snakehead fish (*C. striata*) samples from affected and non-affected reservoirs

Snakehead fish (*C. striata*)	Pb concentrations (mg/kg)		Hg concentrations (mg/kg)	
	Affected reservoir	Non-affected reservoir	Affected reservoir	Non-affected reservoir
Individual 1	Not Detected	Not Detected	0.224	0.086
Individual 2	Not Detected	Not Detected	0.092	0.113
Individual 3	Not Detected	Not Detected	0.083	0.155
Average concentration	Not Detected	Not Detected	0.133±0.079^{ns}	0.118±0.035 ^{ns}
Thailand Standard	≤ 1		≤ 0.02	

Remark: Limit of Detection (LOD) for Pb = 0.02 mg/kg, ns= no significant because it accumulates in organisms through the consumption hierarchy. The sampling sites have running water that leaches Hg from the municipal landfill into the affected reservoir, which is then diluted in water, deposited into the sediments, and accumulated in *C. striata*. This process likely accounts for the increased metal concentration during the rainy season. As this study is a field investigation, there are other possible environmental measures of toxicity in addition to Pb and Hg contamination (Ansari *et al*., 2004).

The diploid chromosome number (2n) of *C. striata* from affected and non-affected reservoirs was 2n=42. The karyotype of *C. striata* from both reservoirs was composed of 6 metacentric, 2 acrocentric and 34 telocentric chromosomes (Fig. 2, 3). The karyotype of *C. striata* from the affected reservoir showed chromosome aberrations. *C. striata* from both reservoirs displayed terminal Ag-NOR on chromosome pair 11 (Fig. 4). The 2n of the mitotic metaphase cells and the karyotypes of *C. striata,* conventionally stained with Ag-NOR are not different in both reservoirs.

Staining chromosomes with Ag-NOR does not detect the chromosomal aberrations of *C. striata,* while conventional staining techniques detect only chromosomal aberrations. The different types of aberrations in the metaphase spread of *C. striata* samples from the affected reservoir are shown in Fig. 5. This study showed that the different types of chromosomal aberrations were single chromatid gap (SG), isochromatid gap (ISCG), single chromatid breaks (SB) and isochromatid breaks (ISCB). The most common chromosomal aberration in the samples from the affected reservoir was SB. The number and percentage of chromosomal aberrations of *C. striata* from the affected and non-affected reservoirs are shown in Table 3. The average Hg concentration values and the average percentage of chromosomal aberrations in the *C. striata* samples from the affected and non-affected reservoirs were 0.133±0.079 mg/kg and 19.333 %, and 0.118±0.035 mg/kg and 0.889%, respectively. This data indicates that the average Hg concentration values and the average percentage of chromosomal aberrations of the *C. striata* samples from the affected reservoir are higher than the samples from the non-affected reservoir. Statistical analysis indicates that there are significant differences between chromosomal aberrations of *C. striata* samples from the affected and non-affected reservoirs with regards to Hg ($p < 0.05$).

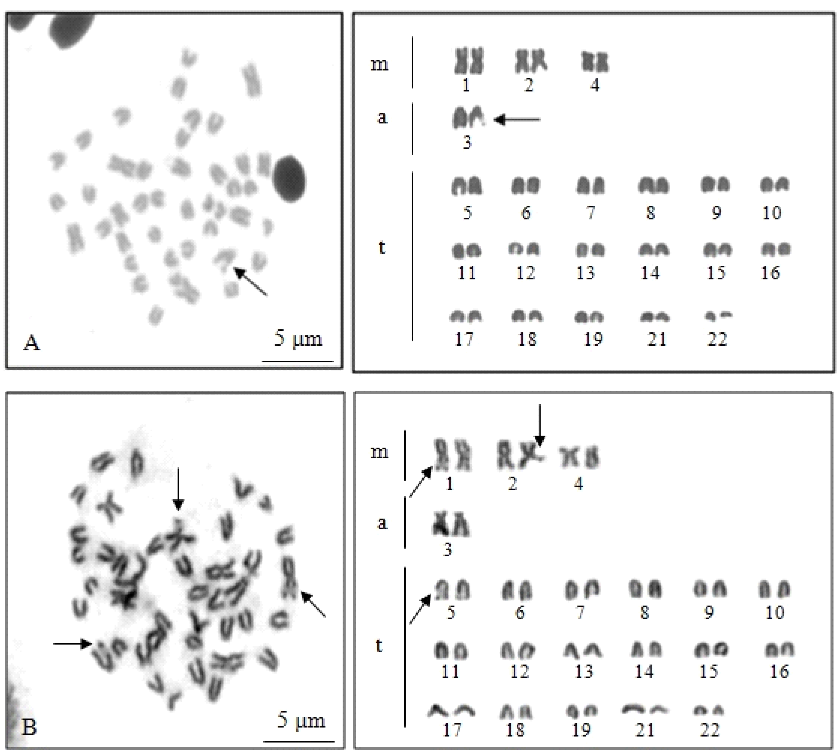

Fig. 2. Metaphase chromosome plates and karyotypes of individual (A, B) snakehead fish (*C. striata*, 2n=42) from the affected reservoir using a conventional staining technique. The arrows indicate the chromosome aberrations

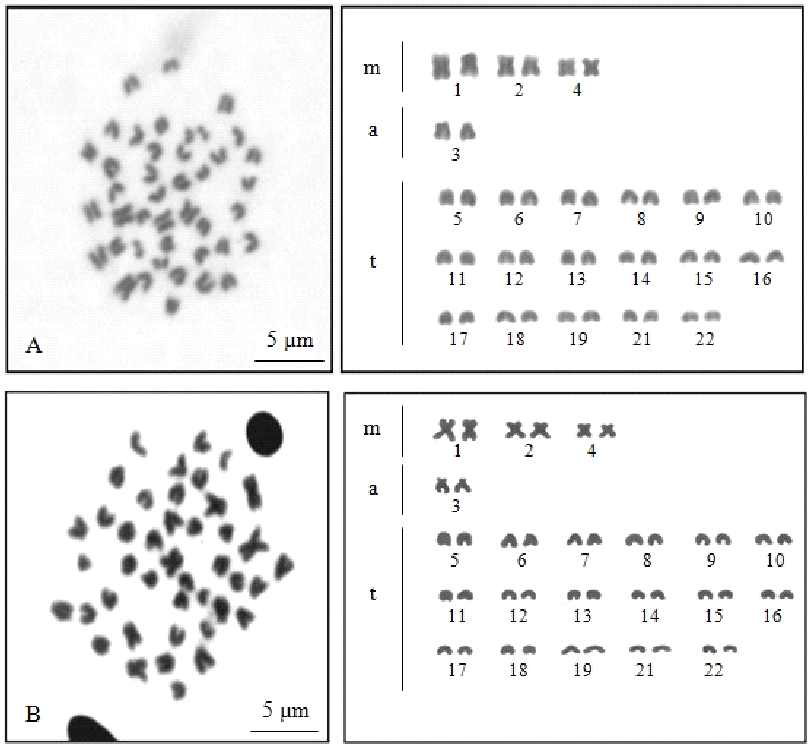

Fig. 3. Metaphase chromosome plates and karyotypes of individual (A, B) snakehead fish (*C. striata*, 2n=42) from the non-affected reservoir using a conventional staining technique

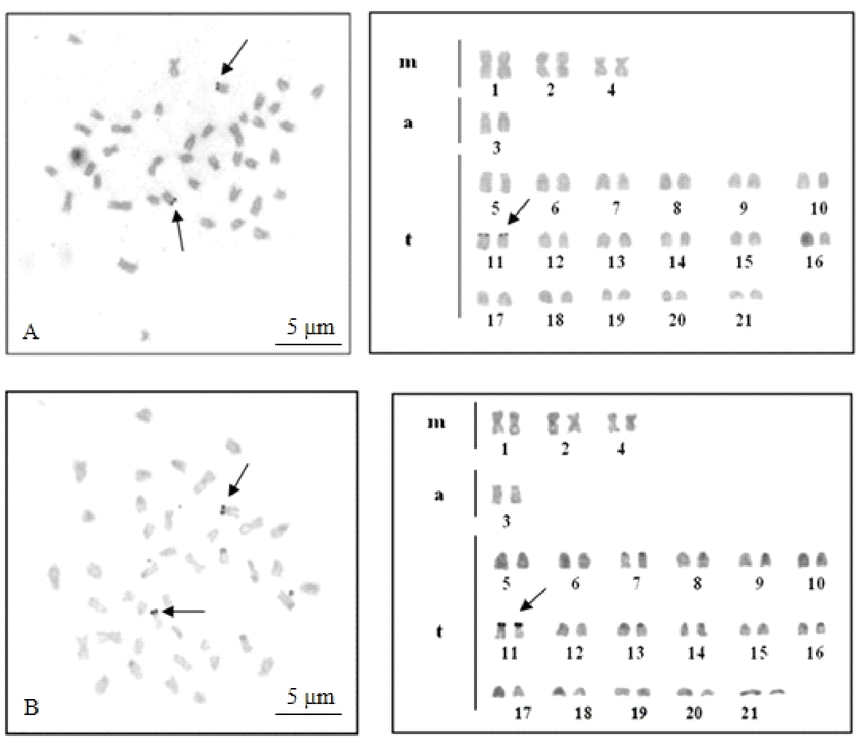

Fig. 4. Metaphase chromosome plates of snakehead fish (*C. striata*, 2n=42) of the non-affected reservoir (A) and the affected reservoir (B) using an Ag-NOR straining technique. The arrows indicate NOR-bearing chromosomes pair 11

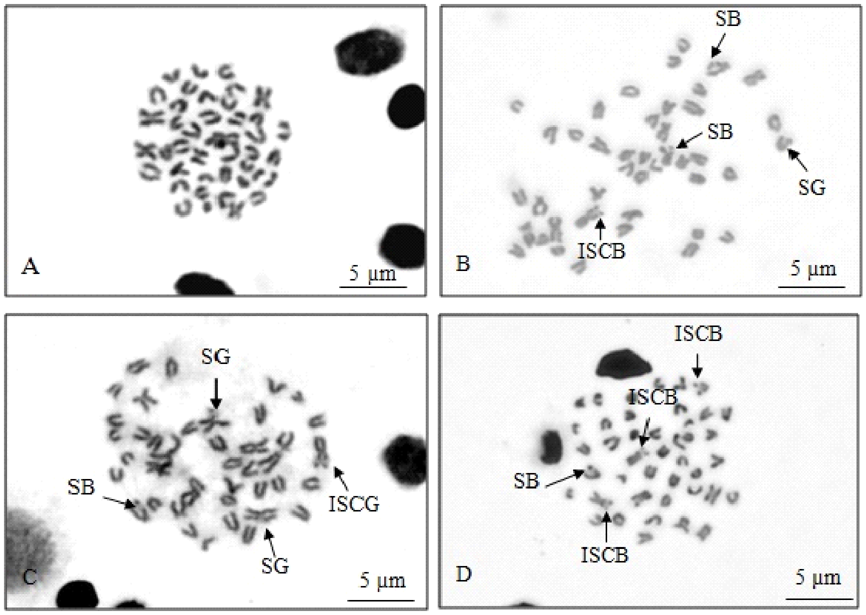

Fig. 5. Different types of aberrations in metaphase spread of *C. striata* (2n=42) showing single chromatid gap (SG), isochromatid gap (ISCG), single chromatid breaks (SB) and isochromatid breaks (ISCB) affected by leachate. (A: Non-affected reservoir, B-D: Affected reservoir)

Table 3. The number and percentage of chromosomal aberrations of *C. striata* from affected and non-affected reservoirs

Snakehead fish (*C. striata*)		Pb concentration (mg/kg)	Hg concentration (mg/kg)	The number of chromosomal aberrations, Replication 1	2	3	The percentage of chromosomal aberrations
Affected reservoir	Individual 1	Not Detected	0.224	4	4	6	10.667
	Individual 2	Not Detected	0.092	4	5	5	9.333
	Individual 3	Not Detected	0.083	10	9	9	18.667
Average		Not Detected	0.133±0.079 [ns]	6[a]	6.666 [a]	6.666 [a]	19.333±5.048 [a]
Non-affected reservoir	Individual 1	Not Detected	0.086	2	0	1	2
	Individual 2	Not Detected	0.113	0	1	0	0.667
	Individual 3	Not Detected	0.155	0	0	0	0
Average		Not Detected	0.118±0.035 [ns]	0.666 [b]	0.333 [b]	0.333 [b]	0.889±1.018 [b]

Remark: Limit of Detection (LOD) for Pb = 0.02 mg/kg, ns= no significant, a&b=significant

Results obtained with the chromosome checks of *C. striata* from the leachate-affected and non-affected reservoirs indicate the diploid chromosome numbers are not different. The NOR location can describe the chromosome evolution. The samples of *C. striata* from both reservoirs displayed terminal Ag-NOR on chromosome pair 11 while Supiwong et al. (2009) reported that Ag-NOR was located on region adjacent to the centromere of chromosome pair 14. The data reports basic knowledge about *C. striata* and its cytogenetics will be applied to studies of breeding, conservation and chromosome evolution in this fish. In addition, the snakehead fish Hg samples from the affected and non-affected reservoirs were not significantly different, but there is a significant difference in chromosomal aberrations between the affected and non-affected samples. It appears that organisms received less toxic pollutants and were then able to build up resistance. These data indicate that the chromosomal aberrations are not only caused by Hg but also affected by other heavy metals, their combination and duration of exposure. However, the snakehead fish living in Hg-contaminated sites need to develop some degree of tolerance to Hg toxicity to survive. The snakehead fish from the affected reservoir adapt to their environment. They can endure

Hg contamination and survive in the aquatic ecosystem near the municipal landfill, which is a highly contaminated area. The snakehead fish are affected by Hg from leachate in the area of the municipal landfill, and this can also occur in any eukaryotic organism. Humans, for example, can be affected like the snakehead fish by the food chain or food web. Exposure to high concentrations of Hg is known to cause damage to the nervous system, kidney and liver in human beings. The cytotoxicity confers chromosome abnormalities in developing fetuses and is a major cause of early spontaneous abortions in humans. Aneuploidy accounts for over 90% of fetal loss (Hassold, 1986). Fortunately, this study detected only a small number of chromosomal aberrations in snakehead fish from the non-affected reservoir, which was selected at random. These data indicate that people consume snakehead fish from this reservoir.

In addition to Pb and Hg, the reservoirs are affected by several other pollutants, such as fertilizers, chemicals and insecticides. These pollutants may contaminate and affect the aquatic ecosystem and environment of *C. striata*. This study was carried out with a small number of snakehead fish, but the detection of chromosomal aberrations suggests that municipal landfills must be managed better. In order to assess the real impact of the leachate from municipal landfills on the ecosystem, it is necessary to continuously biomonitor this area with more collections and tests.

CONCLUSIONS

Leachate from municipal landfills has heavy metal contamination that impacts organisms in aquatic ecosystems. Heavy metals contaminate water, sediment and organisms further along in the food chain, which causes biomagnifications to occur (i.e., when the concentration of heavy metals in an organism exceeds the background concentration of heavy metals in its diet). While the Pb concentrations for both water and sediment and the Hg concentration in sediment met the water and soil quality standards, Hg in the water exceeded quality standards for surface water. Fortunately, the concentration of Pb was not found in snakehead fish from either reservoir, but the average concentrations of Hg in snakehead fish from both reservoirs were higher than Thailand's food quality standard level. Exposure to high concentrations of Hg is known to cause structural aberrations of chromosomes but it does not affect diploid chromosome number. Although the average concentrations of Hg in snakehead fish from the non-affected reservoir was higher than Thailand's food quality standard level, chromosomal aberrations were found at extremely low levels. This snakehead fish can endure Hg contamination and survive in the aquatic ecosystem. Thus, the accumulation of Hg in fish species should be a concern because of its potential effects on human health. The government should be informed about municipal landfill management to protect humans from harm.

ACKNOWLEDGEMENT

This research was funded by the Genetics and Environmental Toxicology Research Group and Integrated Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, Thailand.

REFERENCES

Amahdar, L., Anouar, A., Ababou, B., Verschaeve, L. and Hilali, A. (2009). In vitro genotoxicity of Settat town landfill leachate, Morocco. Arh. Hig. Rada. Toksikol, **60,** 179-184.

Ansari, T., Marr, I. and Triq, N. (2004). Heavy metals in environments in marine pollution perspective-a mini review. J. Appl. Sci, **4,** 1-20.

APHA, (2005). Standard Methods for the Examination of Water and Wastewater, American Public Health Association, 21 st edition, Washington DC.

Bakare, A. A., Mosuro, A. A. and Osibanjo, O. (2000). Effect of simulated leachate on chromosomes and mitosis in roots of Allium cepa(L.). J. Environ. Biol, **21,** 251-260.

Bakare, A. A., Mosuro, A. A. and Osibanjo, O. (2005). Anin vivo evaluation of induction of abnormal sperm morphology in mice by landfill leachates. Mutat. Res, **582,** 28-34.

Bailey, R. M., Stokes, S., and Bray, H. (2003). Inductively Coupled Plasma Mass Spectrometry (ICPMS) for Dose Rate Determination: some Guidelines for Sample Preparation and Analysis, Oxford, UK:Oxford Luminescence Research Group, School of Geography and the Environment, University of Oxford.

Chandra, S., Chauhan, L. K. S., Murthy, R. C., Saxena, P. N., Pande, P. N. and Gupta, S. K. (2005). Comparative biomonitoring of leachaste from hazardous solid waste of two industries using Allium test. Sci. Total. Environ, **347,** 46-52.

Chen, T. R. and Ebeling, A. W. (1968). Karyological evidence of female heterogamety in the mosquito fish, *Gambusiaaffinis*. Copeia, **1,** 70-75.

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., Albechtsen, H. J. and Heron, G. (2001). Biochemistry of landfill leachate plumes. Appl. Geochem, **16,** 659-718.

Florea, A. M., Dopp, E., Obe, G. and Rettenmeier, A. W. (2004). Genotoxicity of organometallic species, in: Hirner, A.V., Emons, H. (Eds.), Organic Metal and Metalloid Species in the Environment: Analysis, Distribution, Processes and Toxicological Evaluation, Springer-Verlag, Heidelberg, 2004, pp. 205-219.

Feng, S., Wang, X., Wie, G., Peng, P., Yang, Y. and Cao, Z. (2007). Leachates of municipal solid waste incineration bottom ash from Macao: heavy metal concentrations and genotoxicity. Chemosphere, **67,** 1133-1137.

Garaj-Vrhovac, V., Oreˇ scˇanin, V., Ruk, D. and Gajski, G. (2009). In vitro assessment of genotoxic effects of electric arc furnace dust on human lymphocytes using the alkaline comet assay. J. Environ. Sci. Health A. Tox. Hazard. Subst. Environ. Eng, **44,** 279-287.

Gajski, G., Oreˇ scˇanin, V. and Garaj-Vrhovac, V. (2011). Cytogenotoxicity of sewage sludge leachate before and after calcium oxide-based solidification in human lymphocytes. Ecotoxicol. Environ. Safe, **74,** 1408-1415.

Gajski, G., Oreˇ scˇanin, V. and Garaj-Vrhovac, V. (2012). Chemical composition and genotoxicity assessment of sanitary landfill leachate from Rovinj, Croatia. Ecotoxicol. Environ. Safe, **78,** 253-259.

Gupta, M. and Sarin, N. B. (2009). Heavy metal induced DNA changes in aquatic macrophytes: Random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker. J. Environ. Sci, **21,** 686-690.

Halim, C. E., Amal, R., Beydoun, D., Scott, J. A. and Low, G. (2005). Evaluating the applicability of regulatory leaching tests for assessing the hazards of Pbcontaminated soils. J. Hazard. Mater, **120,** 101-111.

Haiyasut, K. (1989). Cytogenetics and Cytotaxonomy of Genus *Zephyranthes*. Department of Botany, Faculty of science, University of Chulalongkon, Bangkok, Thailand.

Hassold, T. J. (1986). Chromosome abnormalitie in human reproductive wastage. Trendes. Genet, **2,** 105-110.

Hoshina, M. M. and Marin-Morales, M. A. (2009). Micronucleus and chromosome aberrations induced in onion (*Allium cepa*) by a petroleum refinery effluent and by river water that receives this effluent. Ecotox. Environ. Safe, **72,** 2090-2095.

Hoshina, M. M., Angelis, D. F. and Marin-Morales, M. A. (2008). Induction of micronucleus and nuclear alteration in fish (*Oreochromis niloticus*) by a petroleum refinery effluent. Mutat. Res, **656,** 44-48.

Howell, W. M. and Black, D. A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia, **36,** 1014-1015.

Kalcíková, G., Vávrová, M., Zagorc-Koncan, Z. and Gotvajn, A. Z. (2011). Seasonal variations in municipal landfill leachate quality. MEQ. Int. J, **22,** 612-619.

Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A. and Christensen, T. (2002). Present and long-term composition of MSW landfill leachate: a review 32, 297–336 Crit. Rev. Environ. Sci. Technol, **32,** 297-336.

Leme, D. M. and Marin-Morales, M. A. (2008). Chromosome aberration and micronucleus frequencies *Allium cepa* cells exposed to petroleum polluted water-a case study. Mutat. Res, **650,** 80-86.

Leme, D. M., Angelis, D. F. And Marin-Morales, M. A. (2008). Action mechanisms of petroleum hydrocarbons present in waters impacted by na oil spill on the genetic material of *Allium cepa* root cells. Aquatic Toxicol, **88,** 214-219.

Li, G., Sang, N. and Zhao, Y. (2004). Micronuclei induced by municipal landfill leachate in mouse bone marrow cells in vivo. Environ. Res, **95,** 77-81.

Li, G., Yun, Y., Li, H. and Sang, N. (2008). Effect of landfill leachate on cell cycle, micronucleus, and sister chromatid exchange in *Triticum aestivum*. J. Hazard. Mater, **155,** 10-16.

Monarca, S., Feretti, D., Zerbini, I., Alberti, A., Zani, C., Resola, S., Gelatti, U. and Nardi, G. (2002). Soil contamination detected using bacterial and plant mutagenicity tests and chemical analyses. Environ. Res, **88,** 64-69.

Nanda, I., Schsrtl, M., Feichtinger, W., Schlupp, I., Parzefall, J. and Schmid, M. (1995). Chromosomal evidence for laboratory synthesis of triploid hybrid between the gynogenetic teleost *Poecilia formosa* and its host species. J. Fish Biol, **47,** 619-623.

Pollution Control Department of Thailand. (2001). Water Quality Standards. [online]. Available http://www.pcd.go.th/ [2012, August 10].

Rooney, D. E. (2001). Human Cytogenetics: Constutitional Analysis: a Practical Approach. Oxford University Press, London.

Sang, N. and Li, G. (2004). Genotoxicity of municipal landfill leachate on root tips of *Vicia faba*. Mutat. Res, **560,** 159-165.

Sang, N., Li, G. and Xin, X. (2006). Municipal landfill leachate induces cytogenetic damage in root tips of *Hordeum vulgare*. Ecotoxicol. Environ. Safe, **63,** 469-473.

Slack, R. J., Gronow, J. R. And Voulvoulis, N. (2005). Household hazardous waste in municipal landfills: contaminants in leachate. Sci. Total Environ, **337,** 119-137.

Supiwong, W., Jearranaiprepame, P. and Tanomtong, A. (2009). A new report of karyotype in the chevron snakehead fish, *Channa striata* (Channidae, Pisces) from Northeast Thailand. Cytologia, **74,** 317-322.

Tewari, A., Chauhan, L. K. S., Kumar, D. and Gupta, S. K. (2005). Municipal sludge leachate-induced genotoxicity in mice-a subacute study. Mutat. Res, **587,** 9-15.

U.S. Environmental Protection Agency, (1994). Sample Preparation Procedure for Spectrochemical Determination of

Total Recoverable Element, Method 2002. Environmental Monitoring Systems Lab, Cincinnati, Ohio.

WHO, (1989). Environmental Health Criteria 85: Lead-Environmental Aspects. WHO, Geneva, Switzerland.

WHO, (1991). World Health Organization. Environmental Health Criteria, 118: Inorganic Mercury Environmental Aspects. WHO, Geneva, Switzerland.

Yadav, K. K. and Trivedi, S. P. (2006). Evaluation of genotoxic potential of Chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations, Asian Pacific J. Cancer Prev, **7,** 472-476.