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Evaluation of Abnormal Chromosomes in Rice Field Frogs *(Fejervarya limnocharis)* from Reservoirs Affected by Leachate with Cadmium, Chromium and Lead Contamination

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Abstract

The objectives of this study were to investigate abnormal chromosomes in rice field frogs (*Fejervarya limnocharis*) in reservoirs affected by leachate compared with a non-affected area. Nine individual of *F. limnocharis* were collected, and abnormal chromosomes were studied using bone marrow. The level of heavy metal concentrations (cadmium (Cd), chromium (Cr) and lead (Pb)) were measured in water, sediment and *F. limnocharis* samples. The average concentrations of Cd, Cr and Pb in the water and sediment samples from the municipal landfill and non-affected areas were 0.002±0.000, 0.545±0.876 and 0.021±0.009 and not detected, 0.046±0.032 and 0.009±0.002 mg/l in water as well as 0.472±0.060, 18.652±6.791 and 5.369±0.645 and 0.234±0.019, 4.769±0.142 and 2.176±0.783 mg/kg in sediment, respectively. The municipal landfill values were lower than the permissible limit of the water and soil quality standards, while Cr exceeded the water standard. The average Cd, Cr and Pb concentrations in the *F. limnocharis* samples from the municipal landfill and non-affected areas were 0.023±0.007, 1.857±0.498 and 0.393±0.128 and 0.007±0.000, 1.349±0.083 and 0.183±0.005 mg/kg, respectively, with the Cd and Cr levels both lower than the standards, but not the Pb levels. The diploid chromosome number of *F. limnocharis* in both areas was $2n$=26, and the percentage of chromosome abnormalities of *F. limnocharis* in the municipal landfill area were higher than the non-affected area. There were eleven types of chromosome abnormalities, including a single chromatid gap, isochromatid gap, single chromatid break, isochromatid break, centric fragmentation, deletion, fragmentation, translocation, centromere gap, iso-arm fragmentation and single chromatid decompose. The most common chromosome abnormality in the samples from the municipal landfill area was fragmentation. The difference in the percentage of chromosome abnormality in *F. limnocharis* from both areas was statistically significant ($p<0.05$).

Keywords: abnormal chromosomes; landfill leachate; heavy metals; frog; *Fejervarya limnocharis*

1. Introduction

Human consumption increases solid waste, which effects the degradation of natural resources and the environment (Eggen *et al*., 2010). The use of municipal solid waste landfills is the most widely used method of solid waste disposal around the world (Erses and Onay, 2003; Nagendran *et al*., 2006; Giusti, 2009). The landfill system is designed for protective effects that may occur during final disposal, however, if conducted improperly, it adversely affects air, soil, and water quality through gas emissions and leachate. Municipal landfills in Thailand have a problem caused by leachate; for instance, 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 high concentrations of numerous toxic and carcinogenic chemicals (Li *et al*., 2004; Halim *et al*., 2005), and management of leachate is not good enough to reduce environmental risk (Vrhovac *et al*., 2013). When it rains, leachate spreads many toxic compounds into the environment (Slack *et al*., 2005;

Papadopoulon *et al*., 2007; Öman and Junestedt, 2008), such as heavy metals (Al-Yaqout and Hamoda, 2003; Magda *et al*., 2015), including Zn, Cu, Pb, Ni As, Cd, Co, Cr (Xie *et al*., 2015). These heavy metals pollute surface and ground water that bioaccumulates through the food chain to organisms, increases in trophic levels and has deleterious effects on vertebrates, ranging from fish and amphibians to humans (Öman and Junestedt, 2008; Foo and Hameed, 2009; Wang *et al*., 2012; Nannoni *et al*., 2015). The highly toxic heavy metals effect organism morphology, change the balance of essential elements in body tissue and DNA damages (Bertin and Averbeck, 2006; Burger, 2007; Cuypers *et al*., 2010; Simoncelli *et al*., 2015). Cd, Cr and Pb are nonessential elements and potentially highly toxic to human, animals and plants, even at low doses (Gupta *et al*., 2003; Benavidies *et al*., 2005; Notten *et al*., 2005), and in high concentrations they can damages the nervous system, immune system, kidney and liver in humans. The genotoxicity of Cd, Cr and Pb compounds have been investigated with a variety of genetic endpoints in prokaryotic and eukaryotic cells (WHO, 1989; WHO, 1992; WHO, 1995). Measurement of heavy metal genotoxicity in living things, including aquatic animals, is primarily concerned with sensitivity and a short response time (Gupta and Sarin, 2009).

In communities of aquatic animals, some populations are more sensitive than others and community structure may be adversely affected by heavy metals contamination. However, populations of aquatic animals from polluted areas can show more tolerance to heavy metals compared with those from non-polluted areas (Krenkle, 1973; Suttichaiya *et al*., 2016). Amphibians, which are experiencing a rapid population decline on a global scale (Stuart *et al*., 2004), are excellent bioindicators of environmental contamination due to because of their high sensitivity to contamination and environmental changes related to stress (Hopkins, 2007; Shane *et al*., 2011). Frogs have considerable potential for biomagnification (Richter and Nagel, 2007; Burlibasa and Gavrila, 2011). The development of techniques allowing the analysis of the effects of heavy metals on frogs may lead to monitoring pollutant transfer in municipal landfill areas. Several studies have confirmed the genotoxic potential of heavy metals from landfill leachate, 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 (Monarca *et al*., 2002; Sang and Li, 2005; Feng *et al*., 2007; Salem *et al*., 2014; Promsid *et al*., 2015). The cytogenetic abnormalities induced by landfillleachate indicated that consumption of heavy metal-contaminated water could increase the risk of adverse health consequences. As a result, it is important to monitor the potential toxicity of heavy metals from municipal landfill areas.

Rice field frogs (*Fejervarya limnocharis*) are an important species of frog in the aquatic food chain within the aquatic ecosystem of Thailand, being popular for consumption by Asians. Chronic exposure and accumulation of heavy metals may result in tissue damage in rice field frogs and produces adverse effects not only in the exposed frogs but also in the organisms that consume them, such as humans. This study determined the concentrations of Cd, Cr and Pb in the water, sediment and *F. limnocharis* samples from a municipal landfill area and a non-affected area assumed to have no heavy metal contamination and evaluated chromosomal abnormalities in the associated samples.

2. Materials and Methods

2.1 Sampling sites

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

2.2 Sample collections

The samples of water, sediment and *F. limnocharis* were collected from the sampling area at the affected reservoirs in the municipal landfill. The non-affected area was randomly selected. Each sample was analyzed for Cd, Cr and Pb concentrations and chromosome abnormalities. The water samples were fixed by nitric acid and the sediment samples were dried by air before analysis for the heavy metals concentrations.

2.3 Analysis of heavy metal concentrations in water, sediment and rice field frogs

A total of 2.5 g of each sample was predigested with 3 mg/l of concentrated nitric acid overnight at 40°C; after cooling, 2 mg/l of 30% hydrogen peroxide was added. The container was covered, placed in a high-pressure stainless steel bomb and then transferred

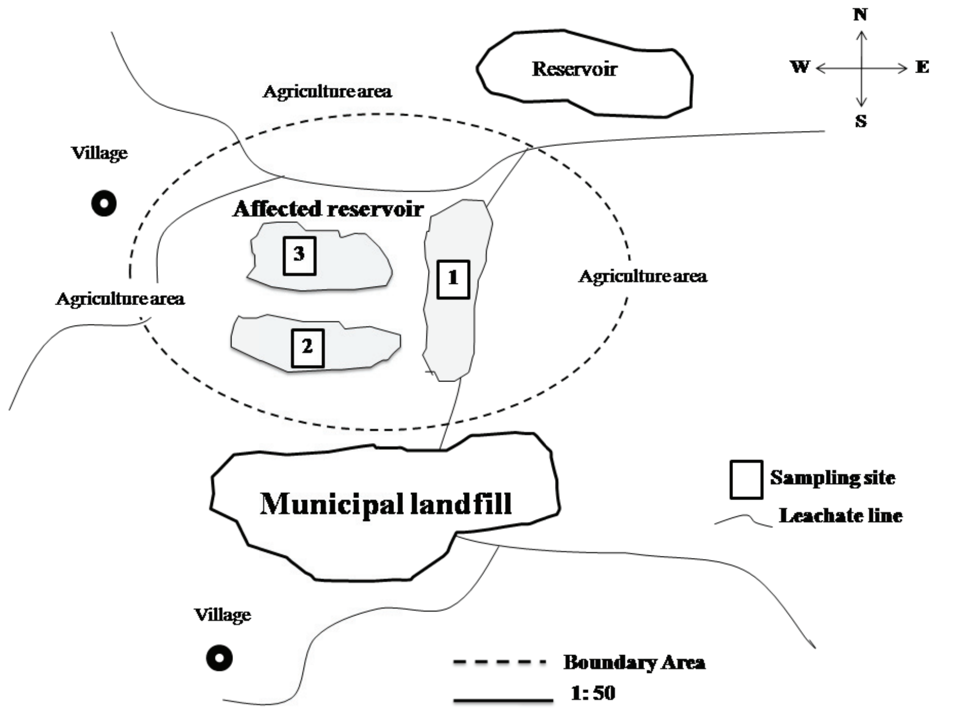

Figure 1. Overview of the municipal landfill area and the location of the three study sites, as shown by sites 1 through 3 in the boundary area

into an oven at 160°C for 4 h was used. After cooling, the solution was diluted with Milli-Q water and transferred into a PET bottle to 50 g. The Cd, Cr and Pb concentrations in each sample were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; model Optima 8300) (Chand and Prasad, 2013). The wavelength analyses of ICP-OES for Cd, Cr and Pb were set to 226.502, 267.716 and 220.353 nm, respectively. The accuracy of the heavy metal concentration results was evaluated with certified reference material (CRM) via the 3111C method (APHA, 2005). Two aliquots of the CRM were spiked with a known level of a metal spike standard. One spike was analyzed according to the 3111C method, and the other spike was analyzed with the 3111B method (APHA, 2005). The metal recoveries were in the 96-100% range, which is considered acceptable (USEPA, 1994).

2.4 Chromosome preparation and conventional staining

The *F. limnocharis* individuals were transferred to the laboratory. Chromosomes were directly prepared in vivo (Chen and Ebeling, 1968; Nanda *et al*., 1995) as follows. Colchicine was injected into the *F. limnocharis*'s abdominal cavity and left for 8 h. The bone marrow was cut into small pieces and 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 30 min. KCl was discarded from the supernatant after centrifugation at 2,000-2,500 rpm for 10 min. The cells were fixed in a fresh, cool fixative (3 methanol: 1 glacial acetic acid) that was gradually increased to 7 ml before centrifugation at 2,000-2,500 rpm for 10 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 using a micropipette, which was followed by an air-dry technique.

Conventional staining was prepared using 20% Giemsa's solution for 30 min (Rooney, 2001). Ag-NOR banding (Howell and Black, 1980) was performed by adding four drops of 50% silver nitrate and 2% gelatin on the slides. 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 were separated.

2.5 Chromosome counting and recording of abnormal chromosomes

Chromosome counting and recording of the abnormal chromosomes were performed on mitotic metaphase cells under a light microscope. Three hundred clearly observable and healthy cells that were spread across the 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. All parameters were used in karyotyping. The cytotoxicity was evaluated from chromosome abnormalities by studying the percentage of chromosomes abnormalities on 200 metaphase cells per individual sample under a light microscope.

2.6 Statistical analysis

The concentrations of Cd, Cr and Pb in the environment, including rice field frogs (*F. limnocharis*), and the percentage of chromosome abnormalities in *F. limnocharis* from the municipal landfill and non-affected areas were analyzed using a t-test. All statistical tests were conducted at a 95% confidence level.

3. Results and Discussion

3.1 Heavy metal concentrations in water, sediment and rice field frog

The average concentrations of Cd, Cr and Pb in the water and sediment samples from the municipal landfill and non-affected areas were 0.002±0.000, 0.545±0.876 and 0.021±0.009 and not detected, 0.046±0.032 and 0.009±0.002 mg/l in water, as well as 0.472±0.060, 18.652±6.791 and 5.369±0.645 and 0.234±0.019, 4.769±0.142 and 2.176±0.783 mg/kg in sediment, respectively (Table 1). The average concentrations of Cr in the water from the municipal landfill area were still higher than the levels allowed by the Pollution Control Department of Thailand (2001) for water quality standards (0.05 mg/l), however, Cd and Pb did not exceed the standards. The average concentrations of Cd, Cr and Pb concentrations did not exceed the standards in water

Table 1. Heavy metal concentrations in the water and sediment from the municipal landfill area and the non-affected area

Samples	Water (mg/1)			Sediment (mg/kg)		
	Cd	Cr	Pb	Cd	Cr	Pb
Samples of the municipal landfill area						
Sample 1	0.002	1.557	0.027	0.535	25.601	6.043
Sample 2	ND	0.070	0.026	0.416	18.322	4.757
Sample 3	ND	0.009	0.011	0.466	12.032	5.307
Mean± SD	0.002±0.000	0.545±0.876[ns]	0.021±0.009[ns]	0.472±0.060[a]	18.652±6.791[a]	5.369±0.645[a]
Samples of the non-affected area						
Sample 1	ND	0.010	0.007	0.212	4.893	1.275
Sample 2	ND	0.070	0.008	0.239	4.799	2.549
Sample 3	ND	0.057	0.011	0.250	4.614	2.703
Mean± SD	-	0.046±0.032 [ns]	0.009±0.002 [ns]	0.234±0.019[a]	4.769±0.142[a]	2.176±0.783[a]
P-value	-	0.379	0.074	0.003	0.024	0.006
Thailand standard*	0.005	0.05	0.05	≤ 37	≤ 300	≤ 400

Remarks: *Limit of Detection (LOD) for Cd=0.001 mg/l, Cr=0.001 mg/l, Pb=0.005 mg/l, ns=not significant, a=significant, ND=not detected*
** Thailand standard (Pollution Control Department of Thailand, 2001)*

Table 2. Heavy metal concentrations in *F. limnocharis* from the municipal landfill area and the non-affected area

Samples	Heavy metal (mg/kg)		
	Cd	Cr	Pb
Samples of the municipal landfill area			
Sample 1	0.032	2.115	0.423
Sample 2	0.013	1.912	0.229
Sample 3	0.036	1.938	0.444
Sample 4	0.018	1.806	0.426
Sample 5	0.022	1.945	0.298
Sample 6	0.025	1.591	0.459
Sample 7	0.025	1.817	0.654
Sample 8	0.017	1.870	0.312
Sample 9	0.021	1.717	0.288
Mean±SD	0.023±0.007	1.857±0.498[a]	0.393±0.128 [ns]
Samples of the non-affected area			
Sample 1	ND	1.275	0.186
Sample 2	ND	1.333	0.177
Sample 3	0.007	1.438	0.186
Mean±SD	0.007±0.000	1.349±0.083[a]	0.183±0.005 [ns]
P-value	-	<0.001	0.076
Thailand standard*	0.05	2	0.2

Remarks: *Limit of Detection (LOD) for Cd=0.001 mg/l, Cr 0.001 mg/l, Pb=0.005 mg/l, ns=not significant, a=significant, ND=not detected*

** Thailand's food quality standards (Pollution Control Department of Thailand, 1986)*

from the non-affected area. The concentrations of Cd, Cr and Pb in the sediment samples of the municipal landfill and non-affected areas did not exceed the standards for soil quality. Statistical analysis indicated that there were significant differences between the Cd, Cr and Pb concentrations in the sediment samples from the municipal landfill and non-affected areas (p=0.003, 0.024 and 0.006, respectively).

The average Cd, Cr and Pb concentrations in *F. limnocharis* samples obtained from the municipal landfill were 0.023±0.007, 1.857±0.498 and 0.393±0.128 mg/kg, respectively. Average Cd, Cr and Pb concentrations obtained from non-affected area were 0.007±0.000, 1.349±0.083 and 0.183±0.005 mg/kg, respectively (Table 2). These values were lower than the standard for the Pollution Control Department of Thailand (1986). In contrast, the average Pb concentrations in *F. limnocharis* samples from the municipal landfill exceeded the standard (0.2 mg/kg). Statistical analysis indicated that there were significant differences between the Cr in the *F. limnocharis* samples from the municipal landfill and non-affected area ($p<0.001$).

This study revealed that the Cd, Cr and Pb concentrations in the water, sediment and *F. limnocharis* samples from the municipal landfill area correlated with chromosome abnormalities. These heavy metal concentrations in the sediment are higher than those in the water from both areas; after being deposited into the sediment, heavy metal accumulates in *F. limnocharis.* This comparative study showed that the accumulation of this heavy metal in *F. limnocharis* was higher than in the water but lower than in the sediment because it accumulates in organisms through the consumption hierarchy. In addition, *F. limnocharis* are found in the water and sediment, they lay large clutches of pigmented eggs in standing bodies of water. The tadpoles are mottled with brown and grow to approximately 2.5 cm, living at the bottom of shallow puddles and ditches (Lim and Lim, 2002; Das, 2007; Baker and Lim, 2008).

Thus, this study showed that individuals of *F. limnocharis* have a higher accumulation of Cd, Cr and Pb compared to that of water, but is lower compared with the sediment, and a life cycle of this species consists of living in both the water and sediment. Therefore, *F. limnocharis* is a creature that is expected to adapt well to the environment. However, the average concentration of Cd, Cr and Pb in the water and sediment samples from the municipal landfill area was higher than in the non-affected area. Statistical analysis indicated that there were significant differences between the concentrations of these heavy metals in the samples from contaminated and non-affected areas. These data indicated that the municipal landfill area has running water and heavy metals from the municipal landfill accumulating in the affected area, which is then diluted in water, deposited into sediment, and accumulated in *F. limnocharis*. This process likely accounted for increased metal concentrations during the rainy season. The flow of landfill leached increases in rainy seasons and spreads into the environment (Slack *et al*., 2005; Papadopoulon *et al*., 2007; Öman and Junestedt, 2008). This study was a field investigation; there are other possible environmental measures of toxicity in addition to heavy metal contamination (Ansari *et al*., 2004; Neeratanaphan *et al*., 2014; Promsid *et al*., 2015). Landfill leached is a dangerous pollutant that produces many risks for the environment. The government should be concerned with leach management before it spreads into the environment (He *et al*., 2006; Vrhovac *et al*., 2013; Fernandes *et al*., 2015).

3.2 Chromosome evaluation of rice field frogs (F. limnocharis)

The diploid chromosome number (2*n*) of *F. limnocharis* from the municipal landfill and non-affected areas was 2*n*=26. According, Joshy and Kuramoto (2008) also reported 2*n*=26 chromosomes to five species of the genus *Fejervaya* (Anura: Ranidae) from South India. The karyotype of *F. limnocharis* from both areas consisted of 18 metacentric, 6 submetacentric and 2 acrocentric regions, and displayed terminal Ag-NOR on chromosome pair 6 (Figures 2-4). Additionally, in municipal landfill area, the karyotype of *F. limnocharis* showed many types of chromosomal abnormalities (Figure 2).

Staining chromosomes with Ag-NOR banding technique did not detect abnormal chromosomes in *F. limnocharis*. However, conventional staining techniques only detected abnormal chromosomes. The different types of abnormalities in metaphase spread cells from *F. limnocharis* samples from the municipal landfill area are shown in Figure 5. Eleven types of chromosome abnormalities were observed including single chromatid gap (SCG), isochromatid gap (ISCG), single chromatid break (SCB), isochromatid break (ISCB), centric fragmentation (CF), deletion (D), fragmentation (F), translocation (T), centromere gap (CG), iso-arm fragmentation (ISAF) and single chromatid decompose (SCD). The most common abnormal chromosomes in the samples from the municipal landfill area were F. The numbers and percentages of abnormal chromosomes of *F. limnocharis* samples from the municipal landfill and non-affected areas are shown in Table 3. The number of abnormal chromosomes of the *F. limnocharis* samples of SCG, ISCG, SCB, ISCB, CF, D, F, T, GC, ISAF and SCD were 63, 17, 21, 7, 31, 73, 161, 4, 66, 11 and 21 abnormally from the municipal landfill area and 1, 0, 4, 0, 0, 4, 6, 0, 1, 0 and 0 abnormally from the non-affected area, respectively. Two hundred clearly observable chromosomes were used for this study; the total number of abnormal chromosomes found in the *F. limnocharis* samples of the municipal landfill and non-affected areas were 475 and 16, respectively. In addition, the cell numbers of abnormal chromosomes in the *F. limnocharis* samples of the municipal landfill and non-affected areas were 350 and 16, respectively. The average Cd, Cr and Pb concentrations and average percentages of abnormal chromosomes in the

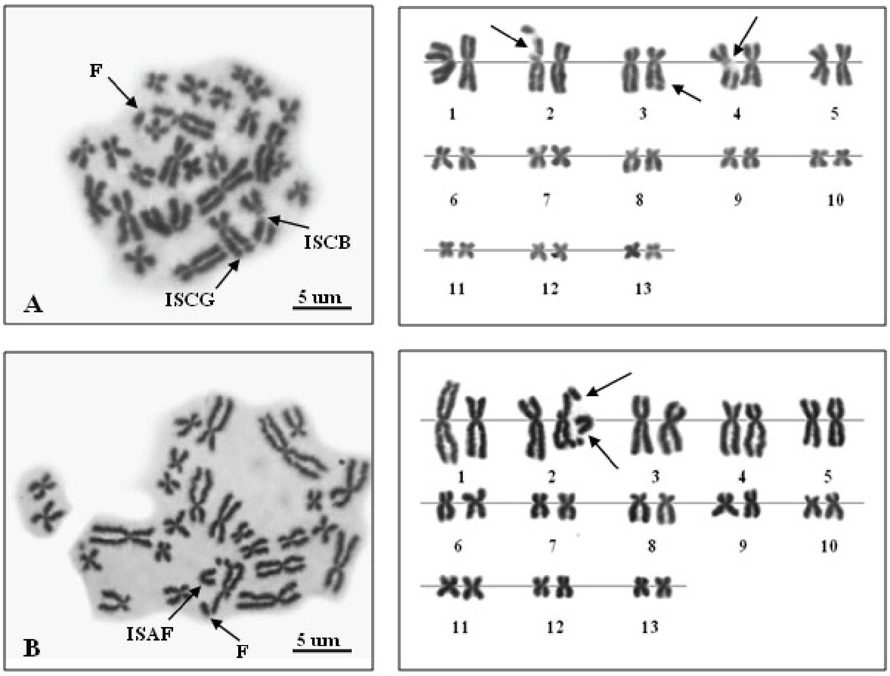

Figure 2. Metaphase chromosome plates and karyotypes of individual (A, B) rice field frogs (*F. limnocharis*, 2*n*=26) from the municipal landfill area using a conventional staining technique. The arrows indicate chromosome abnormalities (fragmentation; F, isochromatid gap; ISCG, isochromatid break; ISCB, iso-arm fragmentation; ISAF)

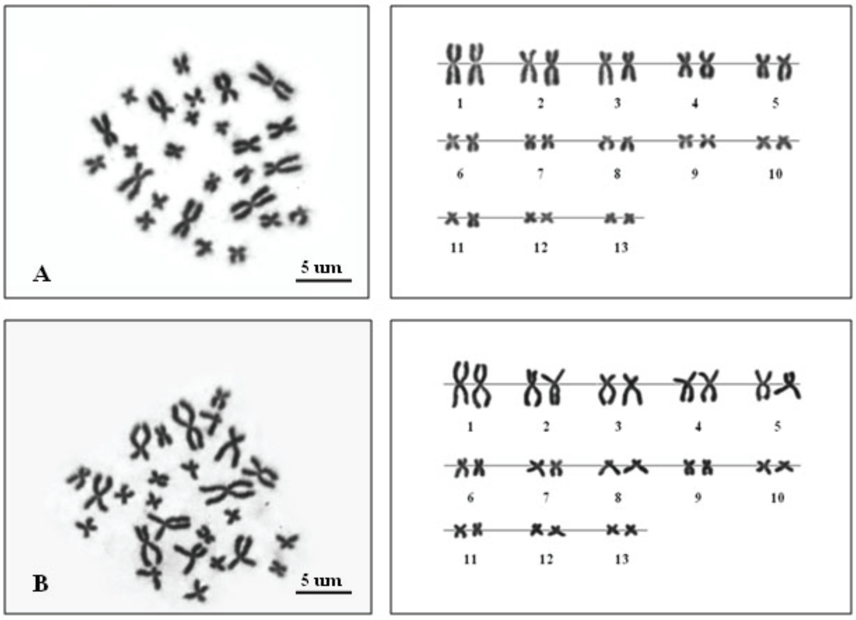

Figure 3. Metaphase chromosome plates and karyotypes of individual (A, B) rice field frogs (*F. limnocharis*, 2*n*=26) from the non-affected area using a conventional staining technique

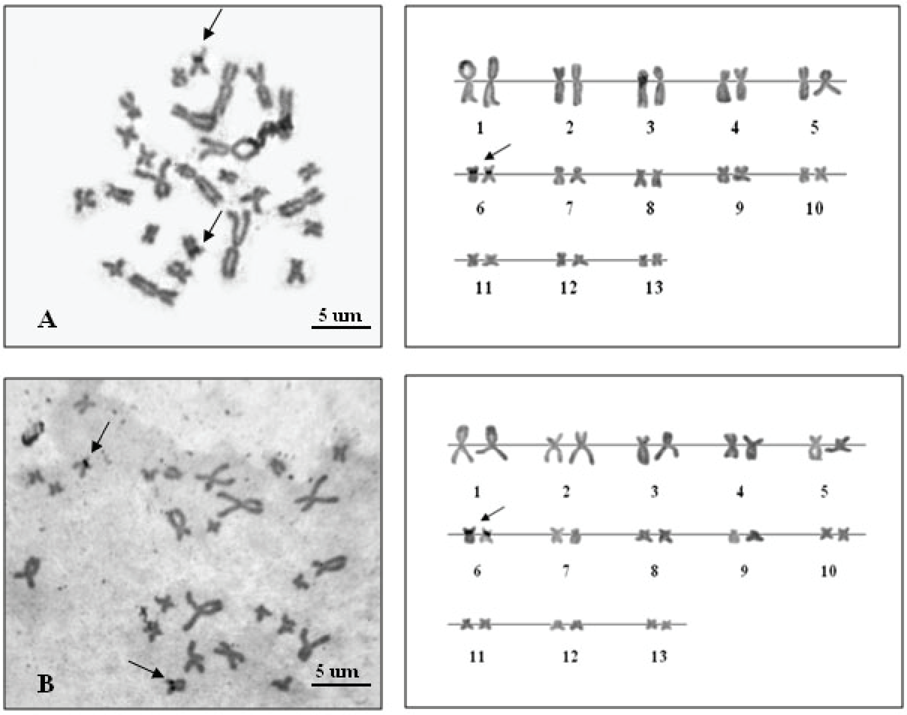

Figure 4. Metaphase chromosome plates of rice field frogs (*F. limnocharis*, 2*n*=26) from the non-affected area (A) and the municipal landfill area (B) using an Ag-NOR straining technique. The arrows indicate NOR-bearing chromosomes pair 6

F. limnocharis samples from the municipal landfill area were 0.023±0.007, 1.857±0.498 and 0.393±0.128 mg/kg and 19.44%, respectively, and from the non-affected area were 0.007±0.000, 1.349±0.083 and 0.183±0.005 mg/kg and 2.67%, respectively.

These data indicated that the average concentration values of heavy metal and the average percentage of abnormal chromosomes of the *F. limnocharis* samples from the municipal landfill area were higher than the samples from the non-affected area. Statistical analysis indicated that there were significant differences between the total number of abnormal chromosomes and the cell number of abnormal chromosomes in *F. limnocharis* samples from the municipal landfill and non-affected areas with respect to Cd, Cr and Pb (p= 0.001, 0.002).

The results of chromosome analysis in *F. limnocharis* samples from the heavy metal affected and non-affected areas indicated that the diploid chromosome numbers were not different. The *F. limnocharis* samples displayed terminal Ag-NOR on chromosome pair 6. In addition, Patawang *et al.* (2014) reported that Ag-NOR was located in the region adjacent to the centromere of chromosome pair 6. The data reporting basic knowledge about *F. limnocharis* and its cytogenetics will be applied to studies on breeding, conservation and chromosome evolution in *F. limnocharis*. The *F. limnocharis* heavy metal samples from the municipal landfill and non-affected areas were significantly different, and there was a significant difference in the abnormal chromosomes between the municipal landfill and non-affected samples.

Thus, our data showed that the average percentage of abnormal chromosomes of the *F. limnocharis* samples from the municipal landfill area was higher than the samples from the non-affected area. In addition, these data indicated that the abnormal chromosomes were more frequent in the *F. limnocharis* that lived in the high heavy metal contamination area. However, the abnormal chromosomes are not only caused by Cd, Cr and Pb; they are also caused by other heavy metals, their combination and duration of exposure. *F. limnocharis* living in the Cd, Cr and Pb contaminated areas need to develop a degree of tolerance to heavy metal toxicity to survive. The *F. limnocharis* from the municipal landfill area adapts to its habitat environment, since they can endure heavy metal contamination and survive in the ecosystem of the municipal landfill, which is a highly contaminated area.

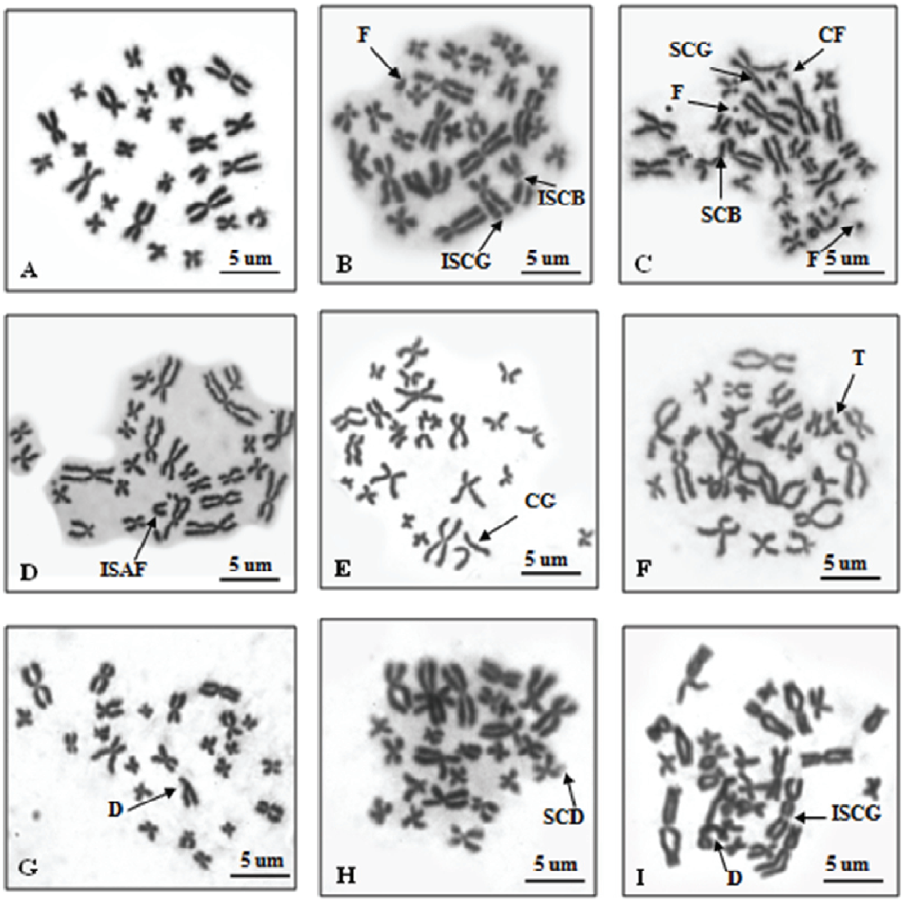

Figure 5. Different types of aberrations in metaphase spread of *F. limnocharis* (2*n*=26), showing single chromatid gap (SCG), isochromatid gap (ISCG), single chromatid break (SCB), isochromatid break (ISCB), centric fragmentation (CF), deletion (D), fragmentation (F), translocation (T), centromere gap (CG), iso-arm fragmentation (ISAF) and single chromatid decompose (SCD) affected by Cd, Cr and Pb contaminations. (A: Non-affected area, B-I: Municipal landfill area)

Similarly, other eukaryotic organisms can also be affected by heavy metal, such as humans for example, that are affected through the food chain or food web, and the exposure to high heavy metal concentrations is known to cause damage to the nervous system, kidney and liver. The cytotoxicity confers chromosome abnormalities in developing fetuses and is a major cause of early spontaneous abortions in humans and aneuploidy accounts for over 90% of fetal loss (Hassold, 1986). In the cytotoxicity evaluation of environmental samples, developments that are similar to classical toxicology have been undertaken. Fortunately, this study detected a small number of abnormal chromosomes in *F. limnocharis* in non-affected area, which were randomly selected, indicating that local people can consume *F. limnocharis* from this area.

Cd, Cr and Pb contaminate landfill leachates, however, the areas are affected by several other pollutants, such as fertilizers, chemicals and insecticides. These pollutants may contaminate and affect the ecosystem and environment where *F. limnocharis* resides. Taking into account this study detected abnormal chromosomes in *F. limnocharis* that lived in the municipal landfill, the data suggests that these areas must be better managed. To assess the real impact of the municipal landfill on the ecosystem, it is necessary to continuously monitor this area. The local people who live around the municipal landfill should not consume organisms in this area.

Table 3. Number and percentage of chromosomal abnormalities in *F. limnocharis* samples from the municipal landfill and non-affected areas

F. limnocharis Samples	The number of abnormal chromosomes											Total number of abnormal chromosomes	The cell number of abnormal chromosomes	The percentage of abnormal chromosomes
	SCG	ISCG	SCB	ISCB	CF	D	F	T	CG	ISAF	SCD			
Municipal landfill area														
Individual 1	5	1	0	0	0	13	17	2	33	2	4	77	48	24
Individual 2	7	3	1	5	0	8	37	0	9	2	7	79	67	33.5
Individual 3	8	3	1	0	0	2	13	0	1	3	5	36	31	15.5
Individual 4	3	0	4	0	7	3	21	0	6	0	3	47	28	14
Individual 5	6	1	3	0	1	8	5	2	3	1	0	30	25	12.5
Individual 6	8	2	0	0	9	10	17	0	2	0	1	49	37	18.5
Individual 7	10	0	8	0	3	17	7	0	8	1	0	54	39	19.5
Individual 8	12	5	4	1	3	11	28	0	1	2	0	67	47	23.5
Individual 9	4	2	0	1	8	1	16	0	3	0	1	36	28	14
Average/Total	63	17	21	7	31	73	161	4	66	11	21	475[a]	350[a]	19.44
Non-affected area														
Individual 1	1	0	1	0	0	2	4	0	1	0	0	9	9	4.5
Individual 2	0	0	3	0	0	1	0	0	0	0	0	4	4	2
Individual 3	0	0	0	0	0	1	2	0	0	0	0	3	3	1.5
Average/Total	1	0	4	0	0	4	6	0	1	0	0	16 [a]	16 [a]	2.67
P-value												0.001	0.002	

Remarks: *a=significant*

4. Conclusions

The Cd, Cr and Pb concentrations in *F. limnocharis* from the municipal landfill area were higher than the non-affected area. Heavy metals contamination in the water, sediment and organisms affects animals that are higher up the food chain, causing biomagnification. Fortunately, the Cd, Cr and Pb concentrations in the water and sediment from both areas were lower than the standard level, however, the Cr levels exceeded the standard of water quality from the municipal landfill area. Although the average concentrations of Cd and Cr in *F. limnocharis* from both areas met Thailand's food quality standard level, except for the Pb levels from the municipal landfill area, chromosome abnormalities were found at extremely low levels in the non-affected area. The average percentage of chromosome abnormalities in the *F. limnocharis* samples from the municipal landfill was much higher than the samples from the non-affected area, evidencing the chromosomal abnormalities were more frequent in individuals living in the high heavy metal contaminated area. Exposure to high concentrations of heavy metal causes structurally abnormal chromosomes, without affecting the diploid number. *F. limnocharis* can endure Cd, Cr and Pb contamination and survive in the contaminated ecosystem. Therefore, the accumulation of Cd, Cr and Pb in *F. limnocharis* species should be a concern because of its potential effects on human health.

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