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RESEARCH ARTICLE

Nile tilapia (*Oreochromis niloticus*) stress response induced by Heavy metals contaminated municipal waste landfill reservoir

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Abstract

Objective: This study aims to investigate Nile tilapia (*Oreochromis niloticus*) stress responses i.e. concentration of C-reactive protein (CRP), cortisol and the alteration of non-specific immunological response (leukocytic differential counts) after cage culture in heavy metal contaminated landfill leachates aquaculture compared with those cultured in unpolluted area.

Material and Methods: The samples were collected from Khon Kaen municipal waste dump reservoir Kambon village, Khon Kaen. Water quality parameters were evaluated monthly. Concentrations of cadmium (Cd), chromium (Cr), and Lead (Pb) in the water, sediment and *O. niloticus*'s muscle were determined at the end of the study using induction coupled plasma-optical emission spectrometry (ICP-OES; Optima 8300) Both water and fish muscle heavy metal concentrations were compared to Thailand's standard limits.

Results: The water quality parameters did not exceed standard values while the concentrations of Cr in the water, sediment did exceed Thailand's standards as well as concentration of Cr in *O. niloticus*'s muscle were higher than FAO limits, whereas Cd and Pb were undetected. Concentration of CRP, cortisol and neutrophil differential count were significantly higher than normal ranges ($P < 0.05$).

Conclusion: We can conclude that heavy metal contamination in landfill leachate were negatively effect on stress and fish health conditions

Keywords: Heavy metals, *Oreochromis niloticus*, stress response

Introduction

The aquatic ecological pollution is recognized worldwide as an important problem to both humans and animal populations which related on the aquatic environment (Yang and Xu, 2022). The most toxic pollutants in landfill leachate are heavy metals which several current biomonitoring efforts rely on samples taken from aquatic organisms surrounding landfill related to possible human consumption risks (Cooper et al., 2017). Therefore, heavy metal residue evaluation in consuming fishes would be benefit to human health. Cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb) were the most common found heavy metals in landfill leachate (Wachirawongsakorn and Sangyoka, 2013, Abu-Daabes et al., 2013, Siyum et al, 2015). In Khon Kaen province, there are approximately 800,000 tons of waste overloads, quantitatively rank 8th in whole country and 1st among the northeastern. Furthermore, 1224 tons of household waste which can be classified as 4.12 tons of infected waste and 300 kg of toxic waste are still need to be handled daily which the highest waste production area was Khon Kaen municipality (9th Region public relation authority, Khon Kaen, 2018). Many of *O. niloticus* aquaculture can be found around Khon Kaen municipal landfill, Kambon village which is located on 10 km north from Khon Kaen city. Those aquaculture ponds around landfill are served as landfill leachate accumulation site which may cause gastrointestinal bacterial overgrowth, clinical and pathological effects on *O. niloticus* primarily and subsequently to human health.

Many reports are available on heavy metal contamination in different aquatic species (El-Moselhy et al., 2014, Kulawik et al., 2016 and Velusamy et al., 2014). The amount of cadmium, chromium and lead residues in *O.* niloticus and other aquatic animal ranged from 0.005 to 62.50 mg/kg which depended on area of study, contaminated cause, water quality, feeding and type of tissue sample etc. (Oost et al., 2003, Bosch et al., 2016, Kwok et al., 2014, Bonsignore et al., 2018, Rajeshkumar and Li, 2018 and Varol et al., 2019) Recently, heavy metals bioaccumulation in water, sediment, edible plants and aquatic animals around Khon Kaen municipal landfill site has been reported. Most of detected heavy metals were arsenic (As), lead (Pb), cadmium (Cd), and chromium (Cr) (Ruchuwararak et al., 2018 and Neerattanaphan, 2017), but their effects on aquaculture fish health condition in stress response e.g. CRP, cortisol and leucocytic differential count still unclear. Examination of these heavy metal's effects on fish stress parameters will lead us to provide better aquaculture management as well as understand its effects to human health as fish consumer. Furthermore, none of them has been done on-site heavy metals contaminated landfill leachate. Therefore, effects of those heavy metals on fish's health condition.

Materials and Methods

Experimental sites

Sampling was conducted in the Municipal landfill site, Kambon village, Muang District of Khon Kean Province, Thailand (Figure 1.). The water, sediment, and fish samples were collected from landfill leachate water reservoir and compared to samples from a non-polluted reference area as the fisheries research and development center, Khon Kaen Province, which has no domestic wastewater contamination as well as agriculture and industrial activities (Figure 2.).

Fish maintenance and experimental challenge

Total of 360 fingerling *O. niloticus* were randomly divided to 2 groups as 180 control (CN) and 180 Treatment *O. niloticus* (TN). Each group was triplicity cultured in 2.5 x 4 m. cages (60 fingerlings per cage). The CN groups were kept in unpolluted reservoir in Fisheries Research and Developing Center, Khon Kaen province. The TN groups were cultured in landfill leachate contaminated reservoir, Kambon village, Khon Kaen province. Both groups were cultured for 90 days

Water sampling

Water temperature, pH, dissolved oxygen (DO) and total ammonia nitrogen (TAN) were evaluated monthly by using simple test kits as described inTable 1. Triplicate of 5 water samples were taken with a water sampler from

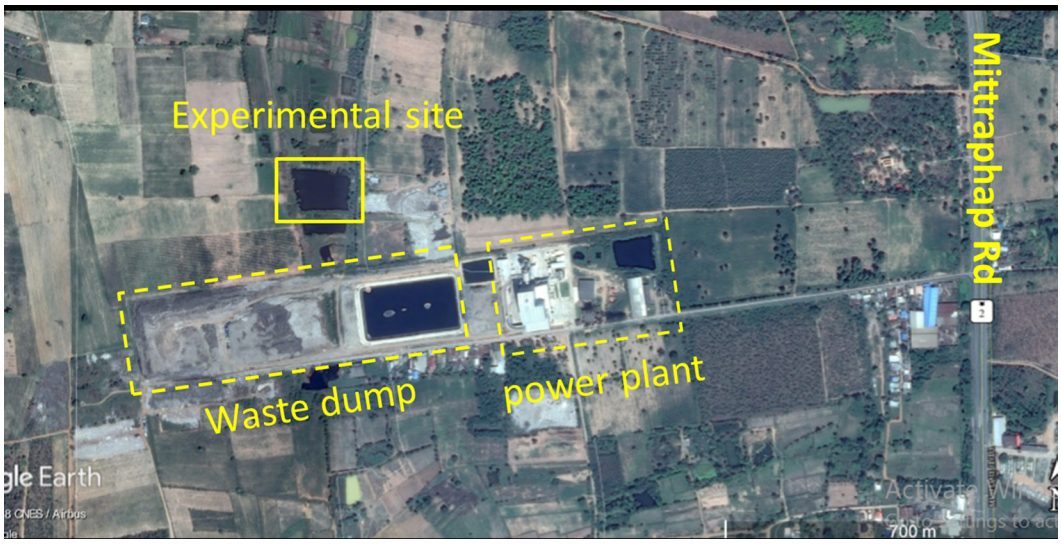


Figure 1. Municipal landfill site, Kambon village, Muang District which conducts the Nile Tilapia's treatment groups



Figure 2. Thapra fish farm, located on the upstream bank of river Chi in Muang District of Khon Kean Province, Thailand which conducts the Nile tilapia's control groups

Table 1. Water quality parameters (temperature, pH, DO, TAN) and analytical methods used for measurement of water quality parameters

Water quality parameters	Analytical methods
Temperature	Eutech thermometer
pH	pH meter, Model EcoScan, pH 5
Dissolved oxygen (DO)	DO meter model 966, Mettler, Toledo
TAN	Titration method

5 locations around reservoir, between 09.00 – 11.00 a.m. at a depth of 30 cm below the water surface with extra pure sulfuric acid and stored at 4°C in clean 1000 mL plastic sampling bottles according to APHA (2012).

Sediment sampling

Triplicate of 5 sediment samples around reservoir up to 20 cm in length were taken from the sites using a grab sampler as described in Neerattanaphan et al. (2017), then transferred to the laboratory and left to dry at room temperature.

Fish sampling

All *O. niloticus* were observed monthly in each cage for measuring wet weight and body length. External pathologic examination will be done, respectively. The blood and internal organs will be collected at 1 month at the beginning and at the end of the experiment. Triplicate blood sample of 5 *O. niloticus* in each group will be collect via the caudal vasculature for hematological analysis, then euthanized by an overdose of clove oil (Sigma®, USA). Approximately 100 g of fish epaxial muscle will be collected and preserved in -20°C for heavy metal analysis. Mortality rate data are also recorded until the end of the experiment.

Analysis of heavy metals in water, sediment and fish samples

A total of 2.5 g of each sample were predigested with 3 mL of concentrated nitric acid overnight at 40°C. After cooling the samples, add 2 mL of 30% hydrogen peroxide. Cover the container, place in a high-pressure stainless-steel bomb and incubate in an oven at 160°C for 4 h. After cooling the samples, dilute solution with Milli-Q water, and transfer 50 g into a PET bottle. The heavy metal concentrations in each sample are 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 are taken at 220.353, 226.502, 267.716, 324.752, 259.939, 259.372, 231.604 and 213.857 nm respectively. Evaluate the accuracy of the heavy metal results with certified reference material (CRM) via the 3111C method (APHA 2012). 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, 2012). The heavy metal recoveries were in the 96–100% range, which determined to be acceptable (USEPA, 1994).

C-reactive protein (CRP)

5 µL of *O. niloticus* plasma were dilute to R1 dilution liquid by micropipette, then put the sample to microtiter plate and mixed with 1 drop of R2 conjugate and 1 drop of washing solution. The samples in microtiter plate were analyzed by NycoCard™ Reader II (Abbott, Green Oaks, Illinois, United States) (Sanjak et al., 2019)

Cortisol

Plasma cortisol concentrations were detected by DetectX Cortisol Enzyme Immunoassay Kit (Arbor Assays, Ann Arbor, Miami, United States), according to the manufacturer's recommendations. Briefly, plasma samples were

pre-treated with dissociation reagent then diluted in assay buffer (1:50final dilution) before cortisol measurement. Fecal samples were suspended in 100 µL ethanol, vortexed for 30 min, centrifuged (5 min, 5000 × g), then the supernatant was then diluted in assay buffer (1:20) before cortisol measurement. Skin-mucus samples were suspended in 100 µL 1M Tris–HCl, vortexed for 30 min and centrifuged (5 min, 5000 × g), then the supernatant was used directly in the assay without dilution (webster et al., 2020).

Leukocytic differential count

Triplicate blood sample of 5 *O. niloticus* in each group were collect in day 0, day 30 and day 90 of the experiment via the caudal vasculature for both hematological and antibody analysis, then euthanized by an overdose of clove oil (Sigma®, USA). Thin blood smears were air dried and fixed in absolute methyl alcohol. Slides were stained with Wright-Giemsa and microscopically examined under a 100x. as described by Shahi et al., 2013. The examination of leukocytic differential counts were counted for 100 leukocytes and classified as percentage of monocyte, lymphocyte, eosinophil, neutrophil and basophil (Pollard et al., 2022).

Data analysis

Sample sizes were evaluated by G-Power statistical analysis program by calculating the effect size from mean value of heavy metal which has been reported from on-site sampling of contaminated heavy metals in landfill leachate around Kambon village, Khon Kaen, Thailand comparing to unpolluted site (Neeratanaphan et Al., 2020) which were 1.41 and 0.72 mg/kg. respectively, the result were 5 *O. niloticus* per group (n = 5). *O. niloticus*, sediments and water heavy metal concentrations, mortality rate, growth performance and hematological data; hematocrit and leucocyte differential count from both control and treatment group were analyzed using a one-way ANOVA. Statements of statistical significance were based on $P < 0.05$. Histopathological data were reported descriptively.

Ethical Consideration

This study was approved by the Animal Ethics Committee of Khon Kaen University, with the number No. KKU 107/63.

Results

Water quality

Water quality parameters (temperature, pH, DO, ammonia) in landfill leachate reservoir (TN) and control (CN) sites are reported in Table 2. The temperature range was 29.04-31.06 °C, the pH range was 7.40-8.84, DO values were 7.576-8.546 mg /L and ammonia was 0.01-0.02 mg/L in both control and treatment sites (Table 2).

Heavy metal in water

Heavy metal average concentration in both CN and TN water sites are shown in Table 3, which were found to have significantly difference ($P < 0.05$) in following ranges; Cd and Pb were undetected but Cr was found in both CN and TN sites as 0.013 and 0.078 mg/kg respectively, which higher than Thailand surface water quality standard (Thailand Pollution control department, 1994) for TN site.

Heavy metal in sediment

Heavy metal average concentration in both CN and TN sediment are shown in Table 4, which were found to have following ranges Cd and Pb were not found but Cr was found in both CN and TN sites 0.023 and 0.344 mg/kg respectively, only soil of TN site was over the standard of Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department, 2004).

Heavy metal in O. niloticus muscle

Heavy metal average concentration in both *O. niloticus* muscle of CN and TN are shown in Table 5., which were significantly difference ($P < 0.05$) in both groups as follows; Cd and Pb were not found but Cr was found in both CN and TN sites 0.023 and 0.344 mg/kg respectively, only soil of TN site was over the standard of Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department, 2004). Cr concentrations were higher than others in muscle of TN group and exceeded standard

Table 2. Water quality parameters (temperature, pH, DO, ammonia) in landfill leachate reservoir (TN) and control (CN) sites

	Control (CN)				Treatment (TN)				Standard*
	Day0	Day30	Day60	Day90	Day0	Day30	Day60	Day90	
Mean temperature (°C)	30.32±0.46	29.66±0.72	29.56±0.64	29.04±0.99	31.06±0.59	30.34±0.56	29.4±0.63	29.14±0.31	23-32
Mean pH	8.84±0.11*	7.96 ±0.40	7.62 ±0.19	7.52 ±0.23	8.22 ±0.26	7.52 ±0.29	7.56 ±0.21	7.4±0.27	6.5-8.5
Mean DO (mg/L)	7.576±0.28	8.034±0.15	8.542±0.31	8.366±0.21	8.496±0.27	8.186±0.15	8.4±0.16	8.546±0.26	≥4
Mean ammonia (mg/L)	0.01±0.04	0.014±0.03	0.02±0.02	0.01±0.01	0.02±0.02	0.012±0.01	0.02±0.01	0.01±0.02	≤0.02

Remarks: Good aquaculture practice for Tilapia farm (National Bureau of Agricultural Commodity and Food Standards, 2010)

Table 3. Heavy metal in water of landfill leachate reservoir (TN) and control (CN) sites

	Mean Cd (mg/kg)	SD	Mean Cr (mg/kg)	SD	Mean Pb (mg/kg)	SD
Control (CN)	0	0	0.003	±0.004	0	0
Treatment (TN)	0	0	0.048	±0.027	0	0
Standard	0.05		0.05		0.05	
P-value	0.00		0.001		0.00	

Remarks: Thailand's surface water quality standards (Thailand Pollution Control Department 1994)

Table 4. Heavy metal in sediment of landfill leachate reservoir (TN) and control (CN) sites

	Mean Cd (mg/kg)	SD	Mean Cr (mg/kg)	SD	Mean Pb (mg/kg)	SD
Control (CN)	0	0	0.124	±0.075	0	0
Treatment (TN)	0	0	4.572	±1.266	0	0
Standard	37		300		400	
P-value	0		0.01		0	

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

limits, but other metal concentrations were not found, thus, under the standard limits.

C-reactive protein (CRP)

CRP of both CN and TN groups are shown in Table 6 and Fgure 3. Our report shows CRP concentration in TN plasma significantly higher than CN which was unpolluted 2.765 mg/dL and 1.6 mg/dL, respectively (P <0.05).

Cortisol

In treated group (TN), the plasma cortisol level significantly higher than those found in CN group 1.60 mg/dL and 2.77 mg/dL respectively at day 90 after cultured in leachate (P =0.05) (Table 6 and Figure 3).

Leukocytic differential count

Differential count (%) of leukocyte of *O. niloticus* in CN and TN group at the beginning of the study (day 0), day 30 and at the end of the study (day 90) (Table 7.) were described as follows 1) neutrophil of CN; 37.92, 39.92 and 48.75% and TN; 38.75, 46.92 and 54.67% 2) eosinophil of CN; 4.25, 3.58 and 3.50% and TN; 4.00, 2.75 and 2.17% respectively 3) monocyte of CN; 4.33, 4.50 and 4.00% and TN; 4.33, 4.58 and 4.50% 4) lymphocyte of CN; 54.33, 52.83 and 44.00% and TN; 53.75, 45.75 and 38.92%, which determined as stress leukogram.

Discussion

Pollutant such as Heavy metals considered as one of major hazardous pollutants which affect both human and animal health. They also effect fish health by acting as stressors leading to elevation of reactive oxygen species (ROS) production (Lemos et al., 2023). The ROS primarily

Table 5. Heavy metal in *O. niloticus* muscle in landfill leachate reservoir (TN) and control (CN) groups

	Mean Cd (mg/kg)	SD	Mean Cr (mg/kg)	SD	Mean Pb (mg/kg)	SD
Control (CN)	0	0	0.023	±0.101	0	0
Treatment (TN)	0	0	3.44	±1.207	0	0
Standard	1*		2**		0.3*	
P-value	0		0.01		0	

Remarks *: Ministry of Public Health Thailand, 2020
**: Ministry of Public Health, Thailand, 1986

Table 6. C-reactive protein (CRP) of *O. niloticus* and cortisol concentration in control (CN) and Treatment (TN) groups at Day 90

CRP	Day 90 (mg/dL)	SD
Control	5.7	1.763
Treatment	10.575	4.750
P-value	0.001	
Cortisol	**Day 90 (mg/dL)**	**SD**
Control	1.60	0.563
Treatment	2.77	0.380
P-value	0.05	

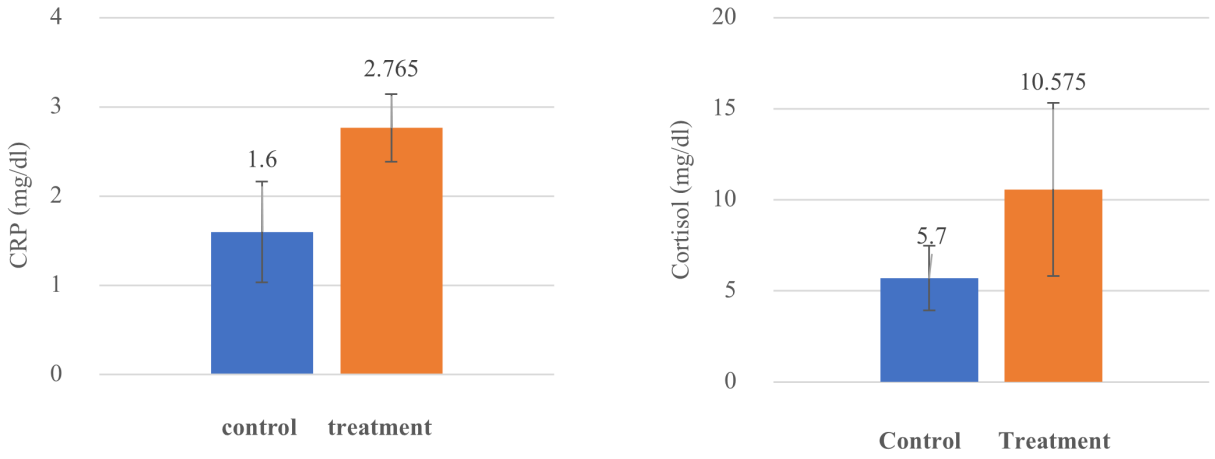


Figure 3. Graphs show C-reactive protein (CRP) concentration of *O. niloticus* in control (CN) and Treatment (TN) groups at Day 90 (mg/dL)

affect the fish gill, which is the main absorption site of many heavy metals (Van der Putte et al., 1981). Gill damage due to heavy metals begins with edema, then hyperplasia, lamellar fusion, epithelial lifting, and necrosis. Gills are also reported to act as storehouse of Cd in experimental studies (Yu et al., 2012). Secondly, heavy metals also affect liver and kidney, which are the prime locations for heavy metal accumulation, metabolism, elimination and excretion (Reynders et al., 2006 and Vesey, 2010).

The results of this study indicated that water quality parameters did not exceed standard values. Temperature, pH, DO, ammonia in landfill leachate reservoir (TN) and control (CN) sites are reported in Table 2. The temperature range was 29.04-31.06°C, the pH range was 7.4-8.84, which are not exceed the maximum recommendation for Nile tilapia aquaculture (FAO, 2020). The DO values were 7.576-8.546 mg/L and ammonia was 0.01-0.02 mg/L in both control and treatment sites, which are acceptable for aquatic life. The pH of both sites did not exceed the standard defined by the Thailand Pollution Control Department (2010).

Popma and Lovshin (1996) and Ebeling et al. (2006) reported that DO is important factor for production for intensive aquaculture systems. Therefore, DO is one of essential needs for *O. niloticus* aquaculture, and should be monitored daily or twice per day. Oxygen solubility is depended on water temperature which higher soluble in cooler water (Swann, 1997). El-Sayed (2006) indicated that *O. niloticus* are able to tolerance lower DO levels than other cultured species and also withstand DO levels lower than 0.5 mg O_2/L.

pH is also fundamental factor for aquaculture. *O. niloticus* can be cultured in pH ranged from 4-11 (Mustapha and Atolagbe, 2018). The best growth in tilapia is seen at pH levels which are neutral or slightly alkaline which are 7-8 (Popma and Lovshin 1996, Mohamed et al., 2009). Acidic aquaculture water effect growth performance and productivities of *O. niloticus* (Popma and Lovshin, 1996). Some heavy metal e.g., Cr toxicity depends on the pH of the water in which the fish resides. Rainbow trout demonstrating variable susceptibility to Cr at pH values of 7.8 and 6.5 (Van der Putte et al., 1981). Although, further investigations by the same authors revealed histologic changes at both pH values, the predominant changes occurred in the gills at pH 6.5 (Van der Putte et al., 1981). In another study in young rainbow trout, Cr toxicity was 50-200 times higher at pH 6.4 to 7.4 than at pH 7.8 to 8.0 (Hogendoorn-roozemond et al., 1977).

Table 7. Graphs show Differential count (%) of leukocytes (neutrophils, eosinophils, lymphocytes and monocytes) of *O. niloticus* in CN and TN group at the beginning of the study (day 0), day 30 and at the end of the study (day 90)

neutrophil (%)						
Day	0		30		90	
Group	CN	TN	CN	TN	CN	TN
Mean (%)	37.91	38.75	39.91	46.91	48.75	54.67
SD	9.14	7.17	4.94	8.59	7.15	5.07
P-value	0.96		0.007*		0.04*	
eosinophil (%)						
Day	0		30		90	
Group	CN	TN	CN	TN	CN	TN
Mean (%)	4.25	4	3.58	2.75	3.5	2.17
SD	1.14	1.13	1.08	0.97	1.57	1.53
P-value	0.72		0.122		0.11	
lymphocyte (%)						
Day	0		30		90	
Group	CN	TN	CN	TN	CN	TN
Mean (%)	54.33	53.75	52.83	45.75	44	38.91
SD	9.87	8.29	6.59	8.90	6.61	5.16
P-value	0.91		0.01*		0.05	
monocyte (%)						
Day	0		30		90	
Group	CN	TN	CN	TN	CN	TN
Mean (%)	4.33	4.33	4.5	4.58	4	4.5
SD	1.07	1.07	1.45	1.51	1.95	1.57
P-value	0.84		0.78		0.38	

Remarks*: Significantly different between CN and TN

Several types of nitrogenous waste, ammonia (NH_3) is most dangerous for aquaculture, which are one of the most important factors for aquaculture and can be harmful to most tropical organisms (Crab et al., 2007, Effendi et al., 2015, Wang and Leung, 2015) which can cause several pathological lesions on *O. niloticus*'s organs, risen FCR and can be lethal (LD_{50}) when NH_3-N reached to 7.1 mg/L (El-Sherif and El-Feky, 2008). Nitrogen species are final products of protein metabolism which majorly produced from cultured species, uneaten feed etc. Ionized ammonium (NH_4^+ or NH_4^+-N or IA-N) and unionized ammonia (NH_3 or NH_3-N or UIA-N) levels are in equilibrium, being reliant on the pH and temperature of the water (Timmons et al. 2002) which is more toxic to aquatic organisms when decreasing of pH and rising of temperature. These two forms together make up total ammonia nitrogen (TAN).

Although, the results of this study indicated that the water quality parameters did not exceed standard values, and DO and TAN values are acceptable for aquatic life. The pH of both sites did not exceed the standard defined by the Thailand Pollution Control Department (2010).

Heavy metal average concentration in both CN and TN water sites are shown in Table 2., which were found to have significantly difference (P <0.05) in following ranges; Cd and Pb were undetected but Cr was found in both CN and TN sites as 0.013 and 0.078 mg/kg respectively, which higher than Thailand surface water quality standard (Thailand Pollution control department, 2010) for TN site. This result favoring the findings of present study of other waste dump leachate evaluation e.g.: Neeratanapan et al., 2017, the heavy metals were found in water sample near electronic waste dump site in Kalasin Province in Thailand which were Mn, Ni, Pb, Cr, Mn, Pb, Ni and Cr, respectively. The majority of heavy metals found in municipal landfills are cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) (Krystofova et al., 2009, Madu et al., 2011 and Carolin et al., 2017). In Belfort, France, the Etueffont municipal solid waste landfill had record with high and very high levels of Cd, Cu, Zn and Ni concentrations in leachates (Salem et al., 2014).

Heavy metal average concentration in both CN and TN sediment are shown in Table 3., which were found to have following ranges; Cd and Pb were not found but Cr was found in both CN and TN sites 0.023 and 0.344 mg/kg respectively, only soil of TN site was over the standard of Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department, 2010). This is because sediments act as accumulation site for all contaminants and organic matter from landfill leachate.

Heavy metal average concentration in both *O. niloticus* muscle of CN and TN are shown in Table 4., which were significantly difference (P <0.05) in both groups as follows; Cd and Pb were not found but Cr was found in both CN and TN sites 0.023 and 0.344 mg/kg respectively, only soil of TN site was over the standard of Thailand's soil quality standards for habitat and agriculture (Thailand Pollution Control Department, 2010). Cr concentrations were higher than others in muscle of TN group and exceeded standard limits, but other metal concentrations were not found, thus, under the standard limits. Consistent with the current findings, Sriuttha et al. (2017) reported that *O. niloticus* from in wastewater canal, Maha Sarakham Province, Thailand showed higher Cr concentrations than those of the other metals (Pb, Cr, Cu, Fe, Mn, Ni and Zn) and also over standard limits. This may be due to TN experimental site is being used as the household waste dump including metals, leather, electronic and electric wares etc., which is correspond with report of chromium industrial usage trends (National academy of science, 1974). Other two reasons were 1) Effects of rain, also be a major cause of Cr accumulation in TN site due to the experiment was conducted in rainy season from September to December. And 2) Chromium, especially Hexavalent Cr (CrVI) is high water solubility which lead to more bioavailable and toxic in lower concentrations than other type of Cr (Grubeša et al., 2016), which caused more Cr accumulation in *O. niloticus* compared to other metals. Cr is taken up both through gastrointestinal and respiratory tract. The amount varies depend on form of chromium and the media (Bakshi et al., 2018). The toxicity of Cr on fish is highly related to water pH. Van der Putte et al., (2002) reported that *Oncorhynchus mykiss* showed susceptibility to Cr at pH over 7.8 and under 6.5. The results of the latter investigation indicated that at pH 7.8, a considerable amount of Cr accumulates in the internal organs rather than in the gills, whereas the gills retain a greater amount of Cr at pH 6.5 than do other organs. Previous study indicates that Cr levels in gills are higher than in other organs (Van Hoof et al., 1981). Gheorghe et al. (2017) revealed that heavy metals are significant to moderate risk considering the measured environmental concentrations, acute and long-term effects on ecosystem and aquatic species. Therefore, *O. niloticus* cultured in leachate reservoir may be harmful for human health as top consumers.

CRP is one of acute phase protein (APP) which response for acute phase response (APR), synthesized in the liver (Gabay and Kushner, 1999). Bayne and Gerwick (2001) identified APPs in mammals and in both teleosts

and elasmobranchs, including CRP, serum amyloid P (SAP), and several components which there are more in teleost included transferrin (TF) and thrombin but only CRP was reported to cause an increase in acute phase plasma. Our report shows CRP concentration in TN plasma significantly higher than CN which was unpolluted 2.765 mg/dL and 1.6 mg/dL, respectively (P <0.05). This is in accordance with previous studies e.g., in human, high level of CRP was examined after heavy metals e.g., Pb and Cd exposure (Khan et al., 2008, Barregard et al, 2016). In contrast, Pollack et al. (2017) reported that Cadmium, lead and mercury were not associated with CRP in women after cadmium, lead, and mercury exposure. In fish, recent reports showed that the level of CRP was significant increase in Cd polluted *Cyprinus Carpio* and *Hypophthalimchthyes molitrix* groups compared with healthy group (Hassan et al., 2017).

One of the major stress indicators of most aquatic species is cortisol, which produced by the adrenal cortex in the adrenal glands in teloasts. This stress indicator act as hormone which is induced by stress environment such as extensive light, temperature, pathogen as well as heavy metal contamination (Lemos et al., 2023). Elevation of cortisol can interfere cardiovascular system, hormonal regulation, osmoregulation, metabolism and productivities (Grosell et al., 2021). The cortisol levels in day 90th in this study between CN and TN were significant difference (P <0.05). These were in accordance with the result of Sajjad et al. (2018) who reported that concentration of cortisol in the *Labeo rohita* gradually risen up within 35 days after exposed to 1.4mg/L of lead(II) acetate. Some of recent studies by Marchi, 2004 and Gagnon et al., 2006 revealed that heavy metal like copper disrupt endocrine regulation in rainbow trout (*Oncorhynchus mykiss*). Furthermore, heavy metals could change secretory pattern and interfere hormone signaling cellular pathways of cortisol which corresponds with present study. Increasing of serum cortisol can finally deteriorate fish's growth performance and productivity by interference of growth hormone mechanism which can be considered as negative effect of heavy metal (Sajjad et al. 2018).

Stress leukograms is induced mainly by glucocorticoids from adrenal cortex of adrenal gland. These steroid hormones function as glucose metabolism regulators. Glucocorticoids reduce immune system function by feedback mechanism which could represent lymphopenia and neutrophilia, with occasional monocytosis and eosinopenia (Grzelak et. al., 2017). This is in accordance with our findings (Table 6.), which showed lymphopenia and neutrophilia in TN group in day 30 and last day of the experiment (P <0.05). and found in both acute and chronic stage. Khangarot et al. (1999) also reported that *Saccobranchus fossilis* exposed for 28 days to 0.1, 1.0, and 3.2 mg/L concentrations of Cr(IV) shows the increasing of neutrophils and thrombocytes and decreasing of lymphocytes to less than 12.9% compared with untreated fishes. In the other hand, Wepner et al. (1992) reported that long term exposure of chromium (0.098 mg/L) on *Tilapia sparrmanii* have no significant changes in leukocytic count.

Conclusion

The water quality parameters did not exceed standard values but the concentrations of Cr in the water, sediment did exceed Thailand's standards also concentration of Cr *O. niloticus*'s muscle were higher than FAO limits whereas Cd and Pb were undetected. Total length, body weight, survival rate were significantly lower in TN (P <0.05). Increasing of cortisol concentration and C-reactive protein (CRP) as well as stress leukogram shown by neutrophilia, eosinophilia, lymphopenia can be determined as stress response in TN. We can conclude that heavy metal contamination in landfill leachate were negatively effect on growth performance, stress and health conditions and also bioaccumulate which could affect to human health as a top consumer.

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