The acute toxicity of the metaldehyde on the climbing perch

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Abstract. In Asia, Climbing perch (*Anabas testudineus*) is commonly found in paddy fields and irrigation systems. Due to its habitat, Climbing perch is exposed to toxic pesticides used in paddy fields such as metaldehyde which is one of the most widely used molluscicide. This study aims to determine the acute toxicity Lethal Concentration$_{50}$ (LC_{50}) of metaldehyde and its effect on the behaviour and physical changes of the Climbing perch. The fish mortality responses to six different metaldehyde concentrations ranging from 180 to 330 mg/L were investigated. The 96-h LC_{50} values were determined and analysed using three different analysis methods which is arithmetic, logarithmic and probit graphic. The LC_{50} values obtained in this study were 239, 234 and 232 mg/L, respectively. After 96-h of exposure to metaldehyde, the fish showed a series of abnormal behavioural response in all cases: imbalance position, and restlessness of movement. The LC_{50} values show that metaldehyde is moderately toxic to the Climbing perch indicating that metaldehyde is not destructive to Climbing perch. However, long term exposure of aquatic organisms to the metaldehyde means a continuous health risk for the fish population as they are more vulnerable and it is on high risk for human to consume this toxicated fishes.

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1 Introduction

The excess use of pesticides do not only brought adverse influence on agro ecosystems but also cause alteration in physiological processes of non-target organisms as it can cause serious health or environmental damage [1]. There is growing concern that pesticides applications do not only reach its target organism but also reach non-target organisms. Among aquatic organism, fish are very sensitive to wide range of pesticides, which can cause deleterious effects through bioaccumulation [2]. Accumulation of pesticides in tissues of organism can cause physiological and biochemical changes to the tissues, thus influencing the activities of several enzymes and disturb the metabolic process [3]. The risks of pesticides pose to the surrounding environments depend on the pesticide toxicity level and the duration of exposure to organisms. The effect of pesticides toxicity on fish is alarming and has raised awareness among scientist since the matter is highly related to food and water security issues. In addition, the effect of pesticides toxicity on fish also raised questions about health hazards of fish to consumers as fish is one of the source of protein consumed by human [4].

Pesticides can be categorized based on their specific functional target which is insecticides, herbicides, molluscicide, and rodenticides. This study focuses on metaldehyde which is one of the molluscicide. Metaldehyde (2, 4, 6, 8-tetramethyl-1, 3, 5, 7-tetroxacyclo-octane) is a cyclic tetramer of acetaldehyde. It is the active compound in many snail and slug baits [5].

This molluscicide is used in many regions around the world. In East Asia especially in Thailand, Indonesia, Vietnam and Malaysia, metaldehyde is mainly used to prevent crop damage from golden apple snail (*Pomacea canaliculata*). Golden apple snail is a pest causing major problem in paddy fields [6-7]. According to "Global Invasive Species Database", golden apple snail is known as one of the 100 world's worst invasive species and poses serious threat to many agricultural sectors in the world [8]. Metaldehyde kill snails by causing dehydration and paralysis, it is directly absorbed from the gastrointestinal tract and crosses the blood-brain barrier [9-10]. The toxicity of metaldehyde causes central nervous system (CNS) depression to the organism. According to a study conducted on mice, metaldehyde has been detected in brain, blood, and liver. The study proved that metaldehyde reduced brain concentration of neurotransmitters noradrenaline, serotonin, and gamma amino butyric acid leading to involuntary muscle contraction properties to the mice [11].

This study aims to determine the acute toxicity Lethal Concentration$_{50}$ (LC_{50}) of metaldehyde and behavioural changes of Climbing perch (*Anabas testudineus*) due to the exposure from different metaldehyde concentration. The Climbing perch has been selected for the experiments because Climbing perch is the largest freshwater fish that inhabit paddy fields and irrigation systems and has important commercial value. Moreover, it is known for its high tolerance and survival in extreme environmental changes in paddy field and irrigation system. To date, according to the authors' knowledge, there is no study conducted to investigate the toxicity of metaldehyde to Climbing perch. Therefore, it is important to use Climbing perch as test organism for the toxicity evaluation of metaldehyde.

2 Materials and methods

2.1 Test organism and chemical

Healthy adult fish species *Anabas testudineus* weighing 18 ± 4.4 g and total length 11 ± 0.8 cm were obtained from a commercial hatchery in Arau, Perlis and brought to the laboratory in plastic bags with sufficient air. Fish were acclimated to laboratory conditions for 14 days in 30 litre tank of aquarium with continuous aeration using an electric air pump compressor before being used in the experiment. During this period, the fish were fed with commercial fish food and water quality was regularly maintained at 25˚C, pH 7.0 and dissolved oxygen above 5 mg/L. The water was periodically changed to minimize food waste, urine and faeces contamination based on EPA method [12].

The fish were fed with fish feed pellet during acclimating period but they were not fed during the last 48 h prior to the experiment. This is to allow sufficient time for digestive abstinence. The metaldehyde 99% (CAS no.: 108-62-3) used in this study was purchased from Acros Organics.

2.2 Experimental set up

Before starting the test, all aquaria were cleaned with Decon 90 and filled with 30 L of decholorinated tap water. The experimental water was kept in the tank for 24 h in order to decrease the chorine level before the addition of the metaldehyde. Preliminary screening test was done to determine the appropriate concentration range for the metaldehyde. Seventy fish were randomly transferred to seven aquaria tanks where each tanks consist of ten fish. Once fish were transferred into each aquaria, they were exposed to different concentrations (180, 210, 240, 270, 300 and 300 mg/L) of metaldehyde for 96 hours under semi-static conditions. The experimental tests were conducted as in the following where each group represent different metaldehyde concentration:

Group 1: as a control group with no exposure to metaldehyde
Group 2: received 180 mg/L of metaldehyde
Group 3: received 210 mg/L of metaldehyde
Group 4: received 240 mg/L of metaldehyde
Group 5: received 270 mg/L of metaldehyde
Group 6: received 300 mg/L of metaldehyde
Group 7: received 330 mg/L of metaldehyde

Each concentration was examined by triplicate with 10 individual per test group. A stock solution of metaldehyde was prepared by dissolving it in acetonitrile; the necessary volume of stock solution was mixed to the water in aquaria to achieve the desired final concentrations (solvent = 0.005% per aquaria) [13]. During the experiment, fish were not fed. The number of dead fish were counted every 24 hours and removed from the aquarium as soon as possible. Fish mortality was recorded at the end of the 96 hours. The fish behaviour including their activities, loss of equilibrium, abnormal swimming and colour changes in the skin of the fish were observed. Median lethal concentration (LC_{50}) of metaldehyde was calculated based on arithmetic, logarithmic and Probit transformation of the mortality dose curve analysis by referring to the Karber [14], Bliss [15] and Finney [16] method respectively. Arithmetic and logarithmic analyses were done by using Microsoft Excel software whereas probit analysis was done using Biostat professional version 9. Fig. 1 shows the experimental setup for the toxicity test of metaldehyde.

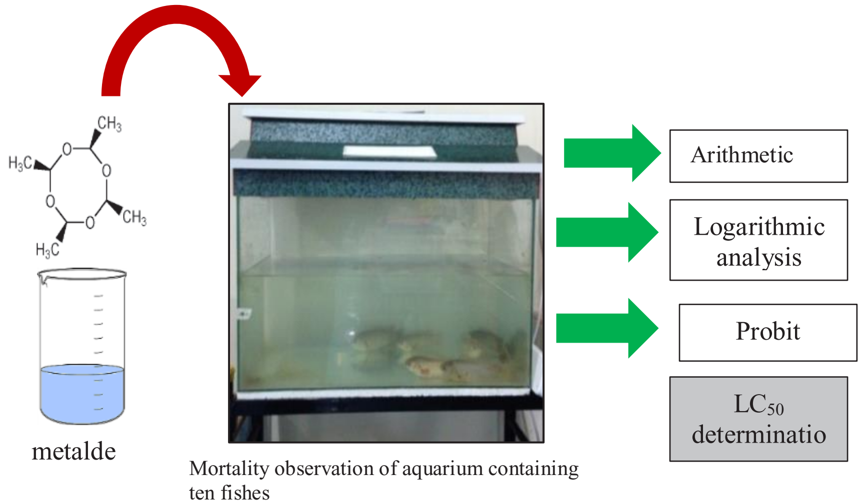

Fig. 1. Experimental setup for toxicity test of metaldehyde and LC_{50} determination methods

3 Results and discussions

The data obtained from the acute toxicity test of metaldehyde for climbing perch revealed that metaldehyde toxicity increased with increasing concentration (Figure 2 and Figure 3). No deaths were recorded after 96 hours in control group (no exposure to metaldehyde) and the lowest metaldehyde concentration of 180 mg/L. On the other hand, the 100% mortality rate was achieved only at 330 mg/L (Fig. 2).

Fish mortality due to exposure of metaldehyde mainly depends on its sensitivity to the toxicants and concentration. At low concentration of metaldehyde approximately less than 220 mg/L, there is high percent of survival (more than 50% survival) or little mortality of Climbing perch. However, as the concentration of Metaldehyde increased, the percentage of survival also decreases. This is due to the toxicant causing severe damage to different systems which leads to the mortality of the fish [17]. The evaluation of LC_{50} value is important step in ecotoxicological studies. There are several methods of calculating the LC_{50}'s of toxicants. In this study the following methods have been used: arithmetic graphic, logarithmic and probit analysis method. From the graph in Figure 2, LC_{50} values using arithmetic graphic method is 239 mg/L, whereas LC_{50} values using logarithmic method is 234 mg/L (Fig. 3).

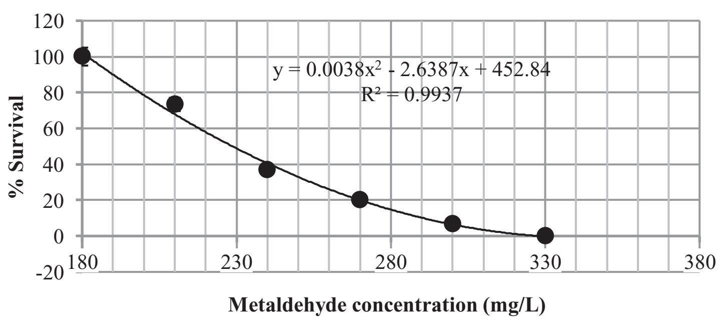

Fig. 2. Arithmetic graphic analysis on percentage (%) survival of Climbing perch (*Anabas testudineus*) after 96 hours incubation with various metaldehyde concentrations

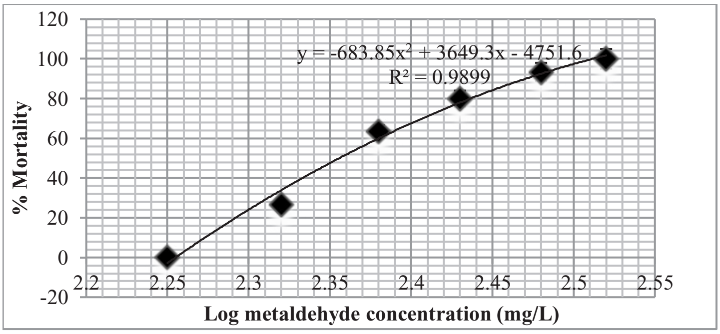

Fig. 3. Logarithmic analysis on percentage (%) Climbing perch (*Anabas testudineus*) 96 hours incubation with various metaldehyde concentrations in logarithmic scale

In additions, the data were also assessed according to probit analysis using Biostat professional version 9. This software was designed for analysis of mortality data from the acute toxicity tests with fish and other aquatic life [18]. Probit analysis minimizes the extremes in the results and maximizes the middle percentage survivals. The graph produced through probit analysis is obtained by excluding the zero and 100% point of survival [19]. Figure 4(a) and 4(b) show the screenshots of probit analysis using Finney method [Lognormal Distribution] and least squares [Normal Distribution] method generated by Biostat Version 9 Software. In Fig. 4(a), the percentage of response is plotted against the log dose whereas Fig. 4(b) shows the percentage of responds plotted against dose in mg/L. Both figures demonstrate an S-shaped idealized concentration response graph, which is the typical of most types of exposure. This "S" shaped curve illustrates that at low doses there is little or no response while at high dose all individuals respond to the effect.

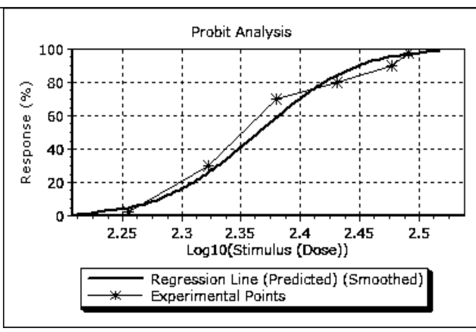

Fig. 4(a). Screenshot of Probit analysis based on Finney method [Lognormal Distribution] generated by Biostat Professional version 9 Software

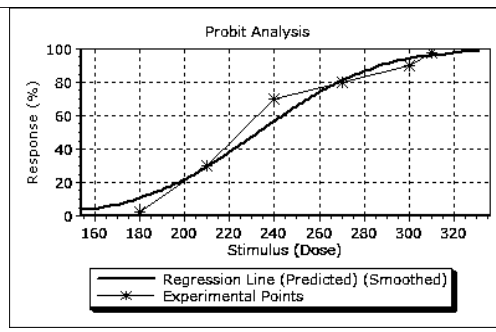

Fig. 4(b). Screenshot of Probit analysis based on least squares [Normal Distribution] method generated by Biostat Professional version 9 Software

Table 1 show the regression statistics of two types of probit analyses generated based on Finney method [Lognormal Distribution] and Least squares [Normal Distribution] method. The two methods of probit analysis gave different values for LC_{50} of metaldehyde. LC_{50} value using Finney method [Lognormal Distribution] is 232 mg/L while LC_{50} value obtained using Least Square [Normal Distribution] method is 233 mg/L. The Alpha value (confidence interval) for both method is 0.05, since p value is greater than 0.05 ($p>0.05$), therefore there is no significant difference.

The determination of LC_{50} value by using the three methods is in good accordance and results revealed that metaldehyde is least toxic to the Climbing perch. According to OECD Test Guidelines scale, the LC_{50} value of the metaldehyde is acute category IV to climbing perch (Table 2) [20].

Table 1. Details of regression statistics for LC_{50} value of metaldehyde concentration determination using Finney method [Lognormal Distribution] and Least Square [Normal Distribution] of probit analysis generated from Biostat Professional version 9 Software

	Finney method	Least square
LC_{50}	**232**	**233**
LC_{50} LCL	214	199
Log_{10} [LC50]	2	0.0236
Beta	15	0.0064
Beta Standard Error	3	178
LC_{50} Standard Error	8	9
LC_{50} UCL	247	266
Intercept	-31	-0.5001

Table 2. The classification of pesticides toxicity on fish.

Toxicity class	Description	LC_{50} (Concentration over 96 hour period for fish) mg/L
Acute I	Extremely high toxicity	≤1 mg/L
Acute II	High toxicity	>1 - ≤10 mg/L
Acute III	Moderate toxicity	>10 - ≤100 mg/L
Acute IV	Least toxicity	≥ 100 mg/L

Different species correspond differently to the same type of pesticide. Although no research has been done on acute effect of metaldehyde on climbing perch but limited work has been done on acute effect of metaldehyde on other fish species. Reported 96-h LC_{50} values for the metaldehyde on rainbow trout *Oreochromis niloticus, Oreochromis mykiss* and bluegill (Lepomis macrochirus) was 62 mg/L, 75 mg/L and 10 mg/L respectively (Table 3) [21-22]. While the EC_{50} value of metaldehyde for two aquatic invertebrate *D. magna* and *P. corneus* was 90 mg/L and 200 mg/L, respectively. The EC_{50} value for algae, *D. subspicatus* was 200 mg/L [22]. In fact, this study found that the 96-h LC_{50} value of the Climbing perch is higher than other aquatic organisms due to it has high endurance under extreme circumstances.

Climbing perch is highly adapted to life in unfavourable conditions because it possesses an accessory air breathing organ [23-24]. In addition, it also can survive in low oxygen concentrated water and resistant against diseases [25]. [23] found that Climbing perch had high resistance toward carbofuran pesticides, with LC_0 value of 560 ppb and the LC_{100} value of 1560 ppb compared to the marine sheepshead minnow (*Cyprinodon variegatus*) with LC_{50} value of 386 ppb [26].

Besides, Climbing perch as a water-breathing fish takes only less toxicant through gills from the water and it is highly tolerant to toxicants [27]. A study shows that Climbing perch was found to be moderately susceptible to the Epizootic ulcerative syndrome (EUS) infection causes by aquatic oomycete fungus (*Aphanomyces invadans),* compared to EUS susceptible such as Snakehead (*Channa striata*), tropical snakeskin gourami (*Trichopodus pectoralis*), koi carb (*Cyprinus carpio*), and broadhead catfish (*Clarias macrocaphalus*) [28].

Table 3. Acute toxicity of metaldehyde to fish species.

Species	96 hours LC50 (mg/L)
Rainbow trout (*Oreochromis niloticus*)	62
Rainbow trout (*Oreochromis mykiss*)	75
Bluegill (*Lepomis macrochirus*)	10

Behavioural changes could be the first response of an organism to environmental change. Therefore, in this study, it was observed that Climbing perch individuals exhibited a variety of behavioural changes when subjected to different metaldehyde concentrations ranging from 180, 210, 240, 270, 300 to 330 mg/L. When there is no metaldehyde concentration in the aquarium (control group), there were no deaths, and behavioural and physical appearance were normal. However, the behavioural changes and clinical toxic symptoms were observed as the Climbing perch exposed to various metaldehyde concentrations (180,210, 240, 270, 300 and 330 mg/L). After exposure to metaldehyde concentration, Climbing perch only showed preference for the top area of the aquarium due to escape conduct of the fish and for aerial breathing, in order to avoid contact of the pesticide with gills. This behaviour is similarly reported for *J. multidentata* which showed similar alteration after 96 hours of exposure to the insecticides (chlorpyrifos and cypermethrin) [29].

Furthermore, Climbing perch also continuously secreting excess amount of mucus from the body and thus a thick layer of mucus was deposited in the gills and skin. Massive mucus discharge by the fish is due to the metaldehyde's toxic mode of action causing irreversible damage in the mucous cells of the skin, thus leads to excessive mucus production, and destruction of the mucus cells [30-31]. When the fish died, the mouth and the opercula were wide open and the skin colour of the fish changed from dark to pale. The same appearance was observed for *Etropus suratensis* exposed to different concentration of tannery effluent. [32] observed the changes in appearances such as the fish succumbed to tannery effluent with mouth and operculum wide open. The changes in fish body colour was also noted.

According to [33], at the beginning of the toxic effect of a toxicant, several biochemicals upregulated occurs in maintaining the hemeotasis mechanism such as biochemical action, defensive mechanism and cellular alteration, and at the same time affected protein that is downregulated due to inhibition. Prolong or the increasing concentration of toxicant leads to cellular alteration due to imbalance of protein recovery and toxic effect associated with the induction of program cell death. Hence, death of cell is related to the alteration of fish behaviour and physical appearance [33].

4 Conclusions

The LC_{50} value of molluscicide metaldehyde tested on freshwater fish Climbing perch based on arithmetic, logarithmic and probit analyse revealed the susceptibility of the fish to the toxic stress (LC_{50} 213, 234 and 232 mg/L). Comparison using the LC_{50} data indicates that metaldehyde was less toxic compared to other pesticide due to its robustness and high survivability. However, exposure of metaldehyde may cause alteration in physical and behaviour of Climbing perch at higher metaldehyde concentrations. With the resistant of the Climbing perch in paddy field with high concentration of metaldehyde raised concerns since this fish will be consumed by human as this pesticide is most likely to have some health risk to humans who consume it. Thus, there is a need to abide to regulation and education on proper use of pesticides to preserve the environment.

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References

1. J. H. Chandra, S. Sridhar, J. Chem. Pharm. Sci. **8**(1), 34–39 (2015)
2. H. Y. Zhang, C. Wang, H. Z. Lu, W. B. Guan, Y. Q. Ma, Ecotoxicol. Environ. Saf., **74(**6), 1653–1658 (2011)
3. K. N. Mukesh, African. J. Environ. Sci. Technol. **7(**7), 725–727 (2013)
4. A. Lazartigues, M. Thomas, D. Banas, J. Brun-bellut, C. Cren-olivé, C. Feidt, Chemosphere. **91**, 530–535 (2013)
5. R. C. Gupta, *Veterinary Toxicology Basic and Clinical Principles* (Elsevier, 2007)
6. A. L. Sanico, S. Peng, R. C. Laza, R. M. Visperas, Crop. Prot. **21**, 137–143 (2002)
7. J. Y. Figueroa, M. L. P. Almazan, F. G. Horgan, Crop Prot. **62**, 23–31 (2014)
8. Global invasive species database. Available: http:// www.issg.org/database/welcome/ [Accessed 31 Dec 2015]
9 L. K. Dolder, Vet. Med. **1**, 213–215 (2003)
10. T. F. Booze, F. W. Oehme, Toxicol. Sci. **6**(3), 440–446 (1986)
11. J. D. Mills, S. E. R. Bailey, C. R. McCrohan, M. A. Wedgwood, *Effect of molluscicides on feeding behavior and neuronal activity, in Symposium organized by the British Crop Protection Council in association with the Malacological Society of London*, 77–83 (1989)
12. United States Environmental Protection Agency, *Ecological Effect Test Guidelines OPPTS 850.1075 Fish Acute Toxicity Test, Freshwater and Marine,* (1996)
13. R. H. Da Cuña, G. Rey Vázquez, M. N. Piol, N. V. Guerrero, M. C. Maggese, F. L. Lo Nostro, Ecotoxicol. Environ. Saf. **74**(4), 1065–73 (2011).
14. G. Karber, **162**, 480–483 (1931)
15. C. L. Bliss, Ann. Appl. Biol., **22**(1), 134–167 (1935)
16. D. J. Finney, "Probit Analysis, 3rd ed.," in Cambridge University Press, XV-333 (1971).
17. C. I. Abraham, "Biodegradation of Dyes," Unpublished Ph.D dissertation. Mahatma Gandhi University (2013)
18. P. B. A. Fai, S. O. Fagade, Ecotoxicol. Environ. Saf. **62**(1), 128–131 (2005)
19. D. L. Reish, P. S. Oshida, *Manual of Methods in Aquatic Environment Research, Part 10: Short-term Static Bioassays*. Food & Agriculture Org. (1987)
20. Organisation for Economic Co-operation and Development, "Hazardous to the aquatic environment," International Labour Organization, 2010. [Online]. Available: http://www.ilo.org/legacy/english/protection/safework/ghs/ghsfinal/ghsc14.pdf. [Accessed: 28-Mar-2016].
21. P. L. Pingali, P. A. Roger, *Impact of Pesticides on Farmer Health and the Rice Environment.* Int. Rice Res. Inst. (1995).
22. E. F. S. Authority, EFSA J. **8**(10), 1–71 (2010)
23. G. Hitchcock, *"Climbing perch (Anabas testudineus) (Perciformes: Anabantidae) on*

Saibai island, Northwest Torres Strait: First Australian Record of this exotic pest fish," in Memoir of the Queensland Museum, 207–211 (2008)
24. G. R. Allen, *Field guide to the freshwater fishes of New Guinea* (1991).
25. M. Khatune-Jannat, M. M. Rahman, M. A. Bashar, M. N. Hasan, F. Ahamed, M. Y. Hossain, Sains Malaysiana **41**(10), 1205–1210 (2012)
26. R. Eisler, U.S. Fish Wildl. Serv. **85(**10), 1–63 (1988)
27. S. S. Jacob, N. B. Nair, and N. K. Balasubramanian, "Toxicity of certain mosquito larvicides to the larvivorous fish Aplocheilus lineatus (Cuv. & Val.) and Macropodus cupanus (Cu. & Val.)," Environ. Pollut. **28**, 7–13 (1982)
28. S. F. Afzali, H. H. M. Daud, I. Sharifpour, M. Afsharnasab, S. Shankar, Vet. World. **8**(9), 1038–1044 (2015)
29. R. I. Bonansea, D. A. Wunderlin, M. V. Amé, Ecotoxicol. Environ. Saf. **129**, 311–319 (2016)
30. K. C. Hallett, A. Atfield, S. Comber, T. H. Hutchinson, Sci. Total Environ. **543**, 37–43 (2016)
31. R. Triebskorn, K. Christensen, I. Heim, J. Molluscan Stud **64**, 467–487 (1998)
32. G. Taju et al., Chemosphere. **87**(1), 55–61 (2012)
33. M. K. Sabullah, S. A. Ahmad, J. Hussin, A. J. Gansau, M. R. Sulaiman, J. Environ. Bioremediation Toxicol. **2**(1), 28–32 (2014)