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Chronic Toxicity of Detoxified Kraft Pulp and Paper Mill Wastewater using Photocatalytic with Cu-TiO_2 Foils/Sunlight on *Xenopus levies* (daudin) Tadpoles

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Abstract

Wastewater from pulp and paper mills contain refractory toxic substances with deleterious effects to aquatic organisms when discharged before proper treatment. Most of the effluent treatment technologies used by the industry are predominantly inefficient, resulting in inadequate detoxification. The use of photocatalytic techniques has shown higher effluent treatment potential. However, there is need to evaluate their chronic toxicity reduction. The current study evaluated the chronic effects of detoxified Kraft pulp and paper effluent using photocatalytic on African clawed frogs (Xenopus levies) tadpoles. Chorionic toxicity was carried out according to the standard Amphibian Metamorphosis Assay (AMA) test for 21 days. Chronic toxicity of photocatalytic combined with photovoltaic (PHCPVC); photocatalytic combined with wood ash leachate as an electrolyte (PHCASH); photocatalytic alone (PHCALON); and photo-treatment without Cu-TiO_2 foils (PHOALON) were studied. No Effect Concentration from the Control (NOEC) and Lowest Concentration with an Effect from the Control (LOEC), obtained in the FETAX study were used to evaluate chronic toxicity of the various photocatalytic treatment methods. The study revealed that the most effective photocatalytic treatment was PHCPVC followed by PHCALON, then PHCASH and lastly PHOALON treatment for both Kraft pulp mill effluent (PME) and biologically treated pulp and paper mill effluent (BPPME). PHCPVC reduced average mortality and malformation of tadpoles by 26% and 22% respectively. PHCPVC treatment reduced growth retardation of tadpoles by an average of 18%. Therefore, the study recommends the treatment of Kraft pulp mill effluent by PHCPVC.

Keywords: Chronic Toxicity, Kraft Pulp and Paper, Photovoltaic, Photocatalytic, Xenopus Levies, Effluent Detoxification

INTRODUCTION

Wastewater emanating from pulp and paper processing mills consist of very complex and recalcitrant compounds, for instance lignin derivatives, resin acids, dioxin, furan, hydroxides, synthetic dyes, countless wood extractives, and numerous degradation products of wood (Lindholm-Lehto *et al*., 2015; Oliveira *et al*., 2018; Peitz & Xavier, 2017; Singh & Chandra, 2019). These compounds become xenobiotic substances to several aquatic organisms, if the wastewater is discharged without proper treatment (Dey *et al*. 2013). The industry contributes about hundred million kilograms of the toxicants annually into the

environment *(*Chandra *et al*., 2018; Haq & Raj, 2020; Waye *et al*., 2014)

The pulp and paper mill wastewater has been reported to be toxic to a variety of aquatic animals (Mishra *et al*., 2011; Siphiwe, 2007). The wastewater from industry induce a variety of effects, with some organisms being more susceptible as opposed to others (Malik *et al*., 2009). Sublethal exposures of the effluent to aquatic animals has been reported to cause retarded growth (Singh & Chandra, 2019), diverse reproduction effects (Basu *et al*., 2009; Chiang *et al*., 2011; Mishra *et al*., 2011; Orrego *et al*., 2019; van den Heuvel, 2010), and developmental anomalies (Tyor *et al*., 2012a). Animals exposed to pulp and paper mill effluent, have been observed to suffer from head, tail, eyes, gill, facial, yolk and gut malformations and edematous (Siphiwe, 2007; Tyor *et al*., 2012b). There is consensus therefore to minimize the pulp and paper mill toxicants into receiving waters, subsequently lowering their toxic effects on aquatic ecosystems.

Attempts to reduce toxicants emanating from the pulp and paper mills have relied primarily on conventional mechanical and biological treatments (Lindholm-Lehto *et al*., 2015). There also have been sequential developments in the use of physical and chemical technologies including flocculation, adsorption, coagulation, sedimentation or floatation, aerobic activated sludge, trickling filters and membrane filtration (Crini & Lichtfouse, 2019; Zainith *et al*., 2019). Rigorous studies have been undertaken to identify priority chemicals in the wastewater and applying some advanced methods to either remove them individually or collectively. However, removing individual or mixtures of the substances from the wastewater is a complicated process and require sufficient knowledge of the original compounds in the wastewater (Kamali *et al*., 2019). A majority of these technologies have shown no significant impact on the refractory high molecular compounds in the wastewater (Crini & Lichtfouse, 2019). The technologies currently cited for the treatment of wastewater in the pulp and paper industry, only a few are commonly employed owing to economic and technological reasons, which are most likely inefficient (Chandra *et al*., 2018; Chandra & Singh, 2012; Crini & Lichtfouse, 2019; Haq *et al*., 2017).

Application of photocatalysis in treating pulp and paper mill effluent has been under evaluations by several studies and is potentially viable, cost-effective and incontestably efficient (Amor *et al*., 2019; Kamali *et al*., 2019). During photocatalysis, a photon of light strikes the catalyst surface, and raise an electron from the valence band to the conduction band leaving behind a positive charge, which oxidizes the adsorbed pollutants or generates hydroxyl radical (Schneider *et al*., 2016). The electron is adsorbed onto oxygen molecule would produce a superoxide ion that participates in contaminants degradation reactions. More efficient forms of photocatalytic systems use titanium dioxide, which is relatively cheap, non-toxic, photolytically and chemically stable and it can be reused (Pérez *et al*., 2015). Doping agents increase the efficiency of degradation positively because they can additionally form hydroxyl radicals either by direct radiation absorption or by accepting conduction band electrons (Schneider *et al*., 2016). Nonetheless, there is the formation of numerous toxic byproducts in the process of photocatalytic treatment. Therefore, toxicity tests are crucial to endorse the effectiveness of such processes (Oller *et al*., 2011).

Photocatalytic techniques have proved to reduce the physicochemical characteristics efficiently and can potentially mineralize persistent organic toxicants in the wastewater. Considering the challenges as mentioned earlier facing the pulp and paper industry, the application of photocatalytic technology to treat the wastewater from this sector is of no doubt (Del Moro *et al*.,

2013). It is, therefore, of significant concern, to evaluate the efficiency of chronic toxicity reduction of photocatalytic techniques on aquatic organism. In the present study, we investigated chronic toxicity of detoxified Kraft pulp and paper mill effluent by photocatalytic with copper-doped titanium dioxide foils on *X. lavies* embryos. We also examined the impact of the detoxification on the growth and development of the tadpoles.

MATERIALS AND METHODS

Effluent Samples and Detoxification

Samples of wastewater were obtained from Mufindi Paper Mills (MPM) in the United Republic of Tanzania. The paper mill manufactures Kraft pulp and paper from *Pinus patula*, *Pinus elliottii*, and *Eucalyptus saliga* (Massawe *et al*., 2016). Effluent from Kraft mill and papermaking section is combined, treated through the mechanical and biological system before it is released into the environment (Sutton & Olomi, 2012). A photocatalytic unit with the same width and length of 20 cm and 24 cm, depth of 4 mm clear transparent glass was fabricated, as shown in figure 1. Twenty-four anodized, copper-doped and annealed titanium foils (30×80 mm) were carefully stuck on a 4-mm-thick glass. One edge of the stuck titanium foils was held 2 cm above the effluent surface with a gentle slope, gradually ending in the effluent on the opposite end. During photovoltaic detoxification, two of 5-mm-thick aluminum electrodes (22 cm by 19 cm) were connected to an 80-watt photovoltaic solar panel, with an anodic current density of 120 A/m^2. The cathode and anode were firmly secured in the effluent at a distance of 2 cm from each other. Three liters of the effluent was used for each photocatalytic detoxification. An electric pump (magi-200) continuously circulated a thin layer of effluent over the foils as sunlight impinged it for 8 h.

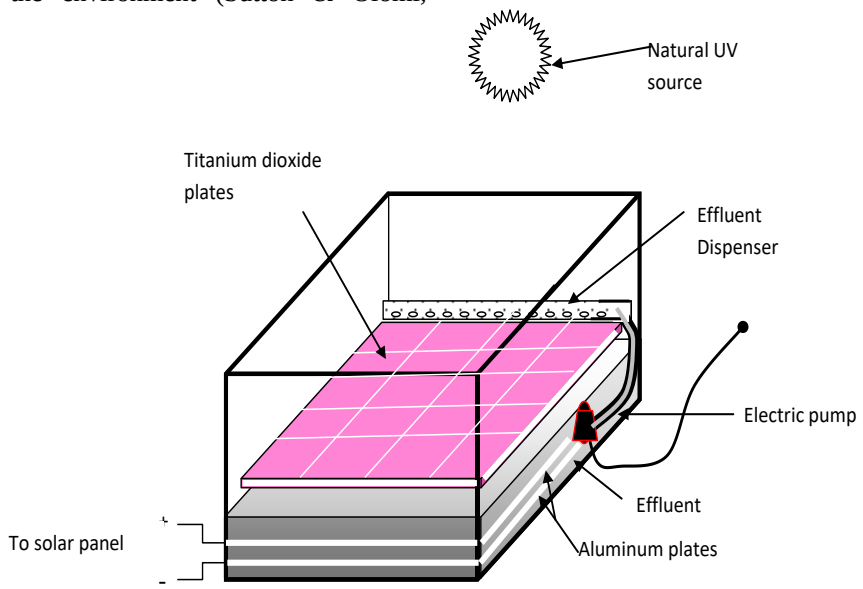

Figure 1: A Sketch of Fabricated Sunlight Photocatalytic Wastewater Treatment Reactor.

The effluent from Kraft pulping mill (PME) and effluent discharged into the environment after the mechanical and biological treatment (BPPME) were treated using four photocatalytic methods in the presence of sunlight. The detoxification combinations were: photovoltaic coupled with photocatalytic (PHCPVC), photocatalytic alone (PHCALON), photocatalytic with wood ash leachate as an

aiding electrolyte (PHCASH) and the effluent was circulated over a plain transparent glass in the presence of sunlight (PHOALON).

Assay Tests

African clawed frogs (*X. laevis)* were selected as sentinel organisms for the bioassays. The adult frogs were netted from Lake Victoria and identified using standard dichotomous guidelines (DeVito, 2003). Standard guidelines for Frog Embryo Teratogenesis Assay Xenopus (FETAX) test was employed to determine the highest concentration with no difference from the control (NOEC) and lowest concentration with a difference (effects) from control (Bantle, 1995).

Chronic toxicity was carried out as per the standard Amphibian Metamorphosis Assay (AMA) test described in a Detailed Review Paper (DRP) on Amphibian Metamorphosis Assay for Organisation for Economic Cooperation and Development (OECD) in conjunction with FETAX test (Bantle & Sabourin, 1991; OECD, 2004). After breeding, tadpoles were fed with t-bites during pre-exposure (after stage 45/ 46) and study period. The study was initiated on stage 49/50 and continued to stage 56. Frog embryo staging was done using Nieuwkoop and Faber standard staging table (Nieuwkoop & Faber, 1994). After 21 days (stage 56), surviving embryos were deeply anesthetized with 3% (w/v) methyl succinic acid diethyl ester and preserved in 3% formalin.

Chronic toxicity study was laid down in completely randomized block design (CRBD). Two concentrations from photocatalytic treated and untreated PME and BPPME were used in the chronic toxicity study. These were, highest concentration with no difference from control (NOEC) and lowest concentration with a difference from control (LOEC), which was determined in the FETAX study. To each aquarium, four liters of filtered pond water was added with iodine at a concentration of 7 μg/L to aid proper functioning of thyroid glands. Twenty five (25) healthy tadpoles (after stage 51) were put in each aquarium. To each aquarium, NOEC and LOEC supernatant PME and BPPME of various photocatalytic were dosed. The pond water and effluent dose were changed after every three days to avoid any side effects from accumulated dirt.

Feeding regime was adjusted according to AMA test requirements. The test specimens were exposed to test samples for 21 days. Hatched tadpoles were fed once in two days and water was changed after every two days. Adequate quantity of feed was administered to maintain good water quality and prevent clogging of gill filters with food particles and detritus. Proper feeding was maintained by increasing the feed with increasing growth of tadpoles. At initiation, 30 mg of feed per animal per day was administered. Malformations of the tadpoles were scored as per the AMA test score sheet and any malformation was scored regardless of its level of severity (Osano, 2002).

Data for several biological endpoints were taken. Hind limb length (HLL), Whole Body Length (WBL) and Snout to Vent Length (SVL) measurements were taken using a digital camera fitted with Motic software of accuracy. Other measurements collected include wet weight, presence or absence of mandibles and daily mortality. Malformations of tadpoles were determined at the 21st day by comparing morphological development of tadpoles with a book of abnormalities (Bantle, 1991).

Statistical Analyses

The data obtained including tadpoles' mortality, wet weight, length (SVL, WBL and HLL) and malformations of tadpoles was analysed by two-way ANOVA (SPSS version 21.0) for any significant difference based on concentration, treatment method and effluent type. Tukey post-hoc tests were employed to separate means wherever significant differences were discerned. Malformations of tadpoles were scored regardless of the severity level.

RESULTS AND DISCUSSION

Mortality of Tadpoles

Percent mortality of tadpoles exposed for 21 days to LOEC and NOEC of the various photocatalytic treated and untreated PME and BPPME is presented in figure 2. Percent mortality of tadpoles was highest in LOEC compared to NOEC for all photocatalytic treated and untreated PME and BPPME. PHCPVC treatment and the control recorded the least mortality for both NOEC and LOEC for the two effluent types. Analyses of variance revealed no significant difference ($P<0.05$) in mortality among PHCPVC, PHCALON and the control for both NOEC and LOEC for the two effluent types. However, there was a significant difference ($P<0.05$) in mortality of tadpoles among PHOALON and PHCASH treated and untreated PME and BPPME. Highest reduction in mortality of tadpoles was recorded in PHCPVC followed by PHCALON then PHOALON and lastly PHCASH for both effluent types and concentrations. PHCPVC and PHCALON were the most efficient in reducing mortality because of precipitation of toxic high molecular to low molecular compounds photo catalytically (Rasalingam *et al*., 2014). However, some studies have also reported that incomplete catalysis of high molecular compounds may result in increased toxicity (Micheletto *et al*., 2019), which may have caused tadpoles' mortality in both PHCALON and PHCPVC. Additionally, mortality of tadpoles in PHCALON and PHCPVC might have been caused by low molecular compounds, which has also been reported to be toxic to some aquatic organisms (Kamali *et al*., 2016). In the absence of catalytic process, for instance in PHOALON, the toxic high-molecular compounds remained stable even after treatment as reported by Chandra and Singh (2012). These findings corroborate those of a previous study, which reported high mortality of *Rana sylvatica* tadpoles at low concentrations of PCBs contaminated sediments (Savage *et al*., 2002). PCBs have also been identified to be part of the pulp and paper wastewater effluents.

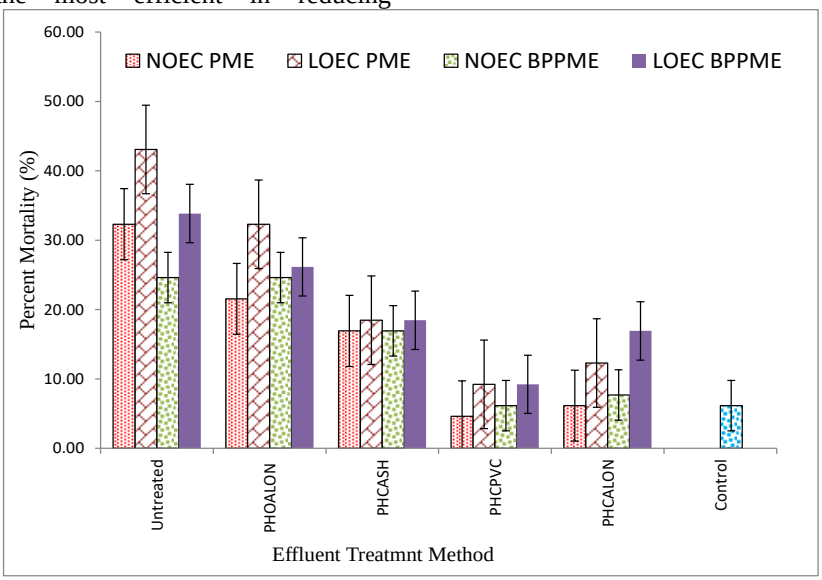

Figure 2: Mortality of Tadpoles after 21 Days Exposure to LOEC and NOEC of the Various Photocatalytic Treated and Untreated PME and BPPME.

Wet Weight of Tadpoles

Wet weight changes of tadpoles exposed to LOEC and NOEC of the various photocatalytic treated and untreated PME for 21 days is presented in figure 3. The control recorded the highest wet weight of

tadpoles during the whole study period. The control recorded the highest final wet weight (NOEC-PME; 78.23 mg), followed by PHCPVC (NOEC-PME; 48.58 mg) and then PHCPVC (LOEC-PME; 43.62 mg). Analyses of variance revealed a significant difference (*P<0.05*) in the final wet weight among LOEC-PME, NOEC-PME and the control. PHCPVC treatment was the most efficient among all photocatalytic treatments with the highest wet weights for both LOEC and NOEC for the entire study period. In the entire study period, wet weights of tadpoles was highest in PHCPVC, followed by PHCALON, then PHCASH and lastly PHOALON. The relatively low retardation of tadpoles' growth (wet weight) by PHCPVC and PHCALON may be due to the photocatalytic mineralisation of toxins. However, PHCPVC and PHCALON were below the control possibly because of either incomplete mineralisation of higher molecular toxins (Chandra & Singh, 2012) or presence of low molecular toxins (Micheletto *et al*., 2019).

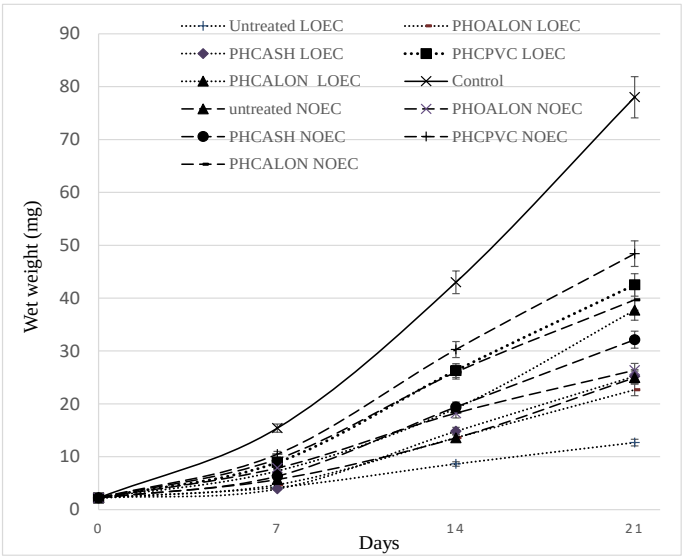

Figure 3: Changes in Wet Weight Mean of Tadpoles Exposed to LOEC and NOEC of Photocatalytic Treated and Untreated PME for 21 Days.

Wet weight changes of tadpoles during exposure to LOEC and NOEC of the various photocatalytic treated and untreated BPPME for a period of 21 days is presented in figure 4. The control recorded the highest wet weight of tadpoles in the whole study period. The control also recorded the highest final wet weight (NOEC-PME; 78.23 mg), followed by PHCPVC (NOEC-PME; 48.58 mg) and then PHCPVC (LOEC-PME; 43.62 mg). Analyses of variance revealed a significant difference (*P<0.05*) in wet weight among LOEC-PME, NOEC-PME and the control. PHCPVC treatment proved the most efficient among the other photocatalytic treatments with the highest wet weights for both LOEC and NOEC during the entire study period. PHCPVC was followed by PHCALON, then PHCASH and lastly by PHOALON with highest wet weight of tadpoles over the entire study period. The low tadpole growth retardation by PHCPVC and PHCALON may possibly arise from mineralisation of toxins. However, PHCPVC and PHCALON recorded lower wet weight than the control presumably due to either incomplete mineralisation higher molecular weight toxins (Chandra & Singh, 2012) or presence

of low molecular weight toxins as reported by Micheletto *et al*. (2019).

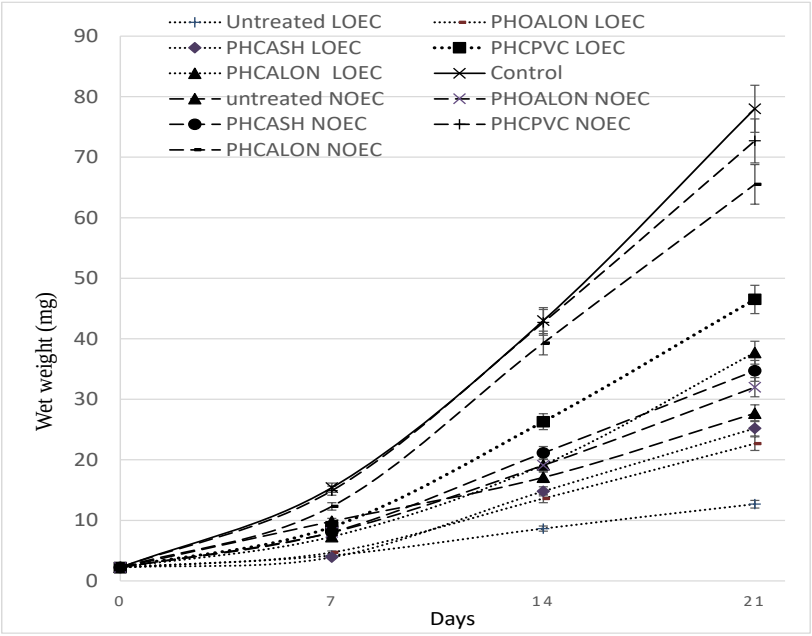

Figure 4: Wet Weight Changes of Tadpoles during 21 Days Exposure to LOEC and NOEC of the Various Photocatalytic Treated and Untreated BPPME.

Length of Tadpoles

Whole Body Length

WBL of tadpoles exposed to LOEC and NOEC of the various photocatalytic treated and untreated BPPME and PME for 21 days is presented in table 1. NOEC recorded the longest WBL compared with LOEC for both effluent types. There was a significant difference ($P<0.05$) in WBL of NOEC and LOEC. This was because of high concentration of toxins in LOEC compared with NOEC thus affecting proper growth. PHCPVC treatment recorded the longest WBL in NOEC for both PME and BPPME, while the shortest WBL was in LOEC for untreated PME and BPPME. The second longest WBL was recorded in PHCALON in both LOEC and NOEC for both PME and BPPME. Among all photocatalytic treatments, PHOALON recorded the shortest WBL in both NOEC and LOEC for PME and BPPME. PHCASH was the third with the longest WBL in LOEC and NOEC for both effluent types. Analysis of variance revealed a significant difference ($P<0.05$) in WBL among all the photocatalytic treatments and untreated effluent.

Table 1: WBL of Tadpoles Exposed to LOEC and NOEC of the Various Photocatalytic Treated and Untreated BPPME and PME for 21 Days

Effluent type	PME		BPPME	
Concentrations	LOEC (mm)	NOEC (mm)	LOEC (mm)	NOEC (mm)
Control	17.26 ± 0.15	17.26 ± 0.15	17.26 ± 0.15	17.26 ± 0.15
PHCPVC	13.78 ± 0.28	15.47 ± 0.28	14.22 ± 0.43	15.68 ± 0.28
PHOALON	11.51 ± 0.29	12.62± 0.25	12.28 ± 0.60	13.00 ± 0.21
PHCALON	12.71 ± 0.35	14.50 ± 0.24	13.49 ± 0.19	15.38 ± 0.24
PHCASH	11.29 ± 0.40	12.79 ± 0.30	12.54 ± 0.44	13.29 ± 0.38
Untreated	10.67 ± 0.35	11.68 ± 0.24	11.35 ± 0.35	12.33 ± 0.26

Snout to Vent Length

SVL of tadpoles exposed for 21 days to LOEC and NOEC of the various photocatalytic treated and untreated BPPME and PME is presented in table 2. NOEC recorded the longest SVL compared with LOEC for both BPPME and PME. There was a significant difference ($P<0.05$) in SVL in both NOEC and LOEC. This was because of high concentration of toxins in LOEC compared with NOEC, resulting in retarded growth. PHCPVC treatment recorded the longest SVL in NOEC for both PME and BPPME, while the shortest SVL was in LOEC for untreated PME and BPPME. The second longest SVL was recorded in PHCALON in LOEC and NOEC for both PME and BPPME. Among all photocatalytic treatments, PHOALON recorded the shortest SVL in both NOEC and LOEC for PME and BPPME. PHCASH was the third with the longest SVL in LOEC and NOEC for both effluent types. Analysis of variance revealed no significant difference ($P<0.05$) in SVL among all the photocatalytic treatments and untreated effluent. On the other hand, PHCASH and untreated were not significantly different in NOEC for both PME and BPPME. This implied that PHCPVC treatment removed most toxins that caused retardation in growth of tadpoles.

Table 2: SVL of Tadpoles Exposed to LOEC and NOEC of the Various Photocatalytic Treated and Untreated BPPME and PME for 21 Days

Effluent type	PME		BPPME	
Concentrations	LOEC (mm)	NOEC (mm)	LOEC (mm)	NOEC (mm)
Control	7.21 ± 0.02	7.21 ± 0.02	7.21 ± 0.02	7.21 ± 0.02
PHCPVC	6.53 ± 0.05	6.95 ± 0.04	6.77 ± 0.04	7.75 ± 0.04
PHOALON	5.57 ± 0.02	5.31 ± 0.08	5.96 ± 0.05	6.79 ± 0.08
PHCALON	5.87 ± 0.04	6.35 ± 0.04	6.10 ± 0.08	6.46 ± 0.07
PHCASH	5.15 ± 0.07	5.95 ± 0.06	5.35 ± 0.04	5.77 ± 0.05
Untreated	5.33 ± 0.05	5.94 ± 0.02	5.36 ± 0.07	5.62 ± 0.04

Hind Limb Development

HLL of tadpoles exposed to LOEC and NOEC of the various photocatalytic treated and untreated BPPME and PME for 21 days is presented in figure 6. There was a significant difference ($P<0.05$) in tadpoles' HLLs between PME and BPPME, indicating higher toxicity in PME compared with BPPME for all photocatalytic treatments. Tadpoles' HLLs were longer for NOEC compared with LOEC for all the photocatalytic treatments for both BPPME and PME. Moreover, tadpoles' HLLs for NOEC were significantly different ($P<0.05$) from LOEC for both effluent types. The control recorded the longest HLL, which was not significantly different ($P<0.05$) from the ones for NOEC of PHCPVC and PHCALON in both BPPME and PME. This implies that PHCPVC and PHCALON had similar toxins removal capabilities. Untreated effluent recorded the shortest HLLs length followed by PHOALON and then PHCASH for LOEC and NOEC in both effluent types. However, there was no significant difference ($P<0.05$) in HLLs between PHOALON and PHCASH for both effluent types. PHCASH treatment (with catalysis) was not better than PHOALON (without catalysis) owing to the presence of some toxic ions introduced to the effluent from the wood ash leachate during photocatalysis. More ions in water may result in a higher salinity, contributing to toxicity in some aquatic organisms (Weber-Scannell & Duffy, 2007).

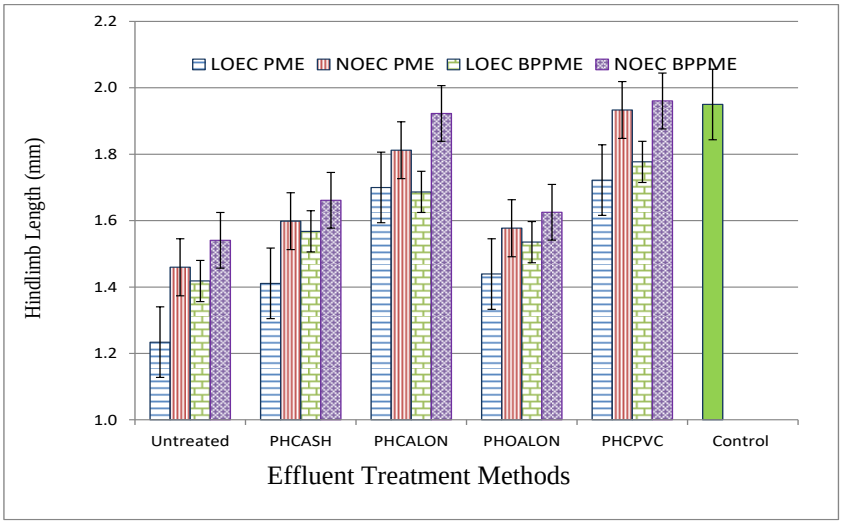

Figure 5: HLL of Tadpoles Exposed to LOEC and NOEC of the Various Photocatalytic Treated and Untreated BPPME and PME for 21 Days.

Malformations of Tadpoles

Malformation of tadpoles exposed to LOEC and NOEC of the various photocatalytic treated and untreated BPPME and PME for 21 days is presented in figure 6. Highest percent malformations of tadpoles were scored by untreated LOEC-PME, followed by LOEC-BPPME and then PHOALON treated LOEC-PME and LOEC-BPPME. There was no significant difference ($P<0.05$) in the percent of tadpoles malformed among LOEC-PME and LOEC-BPPME for both untreated and PHOALON treated effluent. There was also no significant difference ($P<0.05$) in percent of tadpoles malformed scored by the control, NOEC-PME, LOEC-PME, NOEC-BPPME, LOEC-BPPME treated by PHCPVC and PHCALON and NOEC-BPPME treated by PHCASH. It implies that PHCPVC and PHCALON reduced most harmful compounds to tadpoles through photocatalysis.

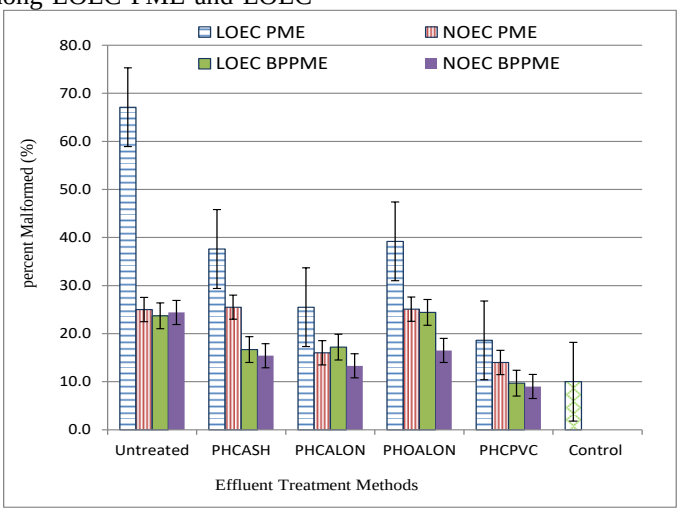

Figure 6: Malformation of Tadpoles Exposed to LOEC and NOEC of the Various Photocatalytic Treated and Untreated BPPME and PME for 21 Days.

Both LOEC-PME and LOEC-BPPME treated PHCASH scored a higher percent malformation as a consequence of the toxicity from hydroxides introduced from the wood ash leachate employed as a supporting electrolyte. Hydroxides of copper, iron, sodium, manganese, iron, zinc, chromium, and cadmium are among the metals that disrupt the proper functioning of the central nervous system (Goyer, 1997; Zahrim *et al*., 2007).

CONCLUSIONS AND RECOMMENDATIONS

The study revealed that the Kraft pulp and paper mill wastewater was toxic even at low concentrations (NOEC). The study also revealed that photocatalytic treatment using titanium dioxide foils as a catalyst, reduces the chronic toxicity of Kraft pulp and paper mill wastewater. Both PHCPVC and PHCALON treatment proved effective in reducing chronic toxicity of the wastewater compared with other photocatalytic treatments. PHCPVC treatment reduced mortality of tadpoles on average by 29.3% (LOEC-PME and LOEC-BPPME) and 23.1% (NOEC-PME and NOEC-BPPME). PHCPVC treatment reduced malformation of tadpoles on average by 31.3% (LOEC-PME and LOEC-BPPME) and 13.2% (NOEC-PME and NOEC-BPPME). The study also revealed that PHCPVC treatment reduced the impacts caused by untreated effluent on tadpoles' WBL, SVL and HLL. PHOALON treatment and the respective untreated wastewater were equally toxic to the tadpoles. PHOALON treatment reduced mortality of tadpoles on average by 5.4% (LOEC-PME and LOEC-BPPME) and 9.2% (NOEC-PME and NOEC-BPPME). The study recommends the treatment of Kraft pulp and paper mill wastewater by PHCPVC; however, a complete study of risk assessment should be conducted for other animals.

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