Evaluation of Treatment Efficiency of Waste Stabilization Pond and Its Effluent Toxicity in Hawassa University, Southern Ethiopia

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Abstract

Background: Waste stabilization pond is sanitation technology designed for treatment of wastewater in order to reduce the carbon-containing organic matter and remove pathogens from wastewater and its effluent is expected to be used for fish production and irrigation. Hawassa University waste stabilization pond is frequently blocked with solid waste and discharging it's effluent to nearby households and the environment. However, its efficiency and effluent toxicity were not clearly evaluated. The aim of this study was to evaluate the efficiency of waste stabilization pond and its effluent toxicity in Hawassa University, Southern Ethiopia.

Methods: An experiment study was conducted at Hawassa University waste stabilization pond from March 1st and May 30th, 2017. Samples were collected by pre-cleaned plastic bottles using grab sampling techniques. Non conservative parameters were performed on site while conservative's parameters were performed after transported to the Hawassa University chemistry laboratory. Three repeated experiments were performed. The mean value and removal percentage of each parameter of waste stabilization pond was calculated. For acute toxicity, forty-two fry Tilapia young fishes were imported into six aquaria. The mortality and behavioral changes of the fishes were recorded according to toxicity protocol. Statistical Package for Social Sciences Version 21 was used for data analysis.

Results: There is variables efficiency removal of waste water treatment as indicated for total dissolved substance (14%), temperature (19%), manganese (20%), cadmium (96.90%), nitrate (73.77%) and copper (72.00%). The mean difference in effluent removals for total suspended solid, biological oxygen demand, nitrate, cadmium, chromium and copper have significant value between the inlet and outlet of the pond ($p < 0.05$). The lethal concentration of fifty percent of the acute fish toxicity within 24 hours was 76%. All fishes in 100% effluent aquarium died within 24 hours. The lower observable adverse effect and non-observable adverse effect of the concentration were 60% and 40% of the effluent respectively.

Conclusion: There is a significantly higher removal efficiency of the treatment pond on the inlet and outlet for nitrate, cadmium and copper. While, the lower removal efficiency was obtained for total dissolved substance, temperature and manganese. The pure effluent discharged from the pond caused hundred percent fish mortality within a day. Therefore, the pond needs to redesign and upgrade to prevent the ecological health risk and to endure aquatic life like fish.

Keywords: *Bioassay; Fish toxicity; Heavy metals; Physicochemical*

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Background

Waste stabilization ponds (WSPs) are sanitation technologies designed for wastewater treatment to reduce the carbon-containing organic matter at anaerobic, facultative and aerated ponds. It is designed to remove bacteria, viruses, fungi, and protozoan pathogens from wastewater at maturation ponds. It is also provides proper balance of organics, light, dissolved oxygen, nutrients, algal presence and temperature (Amengual-Morro *et al*., 2012; Tilley, *et al*., 2014). The system may consist of a single pond or several ponds in a series where each pond playing a different role in the removal of pollutants (Tilley *et al*., 2014).

In the real world, after efficient treatment of wastewater through WSPs, the effluent designed to provide services as surface water, reused for irrigation or fish production. These prompted the alternatives of reusing wastewater since there scarcity of water resources

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(Ramadan and Ponce, 2016). Even though, WSPs required standards, which has been a serious challenge, especially in developing countries (Li *et al.*, 2018). Some of the reported reasons were being lack of proper operation and maintenance (Li *et al*., 2018); improper design, institutional problems and personnel skill (Boyd, 2005; Stickney, 2002; Omofunmi *et al*., 2018; Tucker, 2000; Tomasso, 2002; Li *et al.*, 2018).

The impact of pond effluents on the surrounding surface water highlighted earlier by different scholars, (Boyd, 2005; Stickney, 2002; Omofunmi *et al*., 2018; Tucker, 2000; Tomasso, 2002). It could accumulate large amounts of sludge which reduce the microbial activity within the pond, breeding sites for mosquitoes and other insects, bad odor problem and difficult to control or predict ammonia levels in the effluent (USEPA, 2015). It also produces toxic and harmful matters that have a potential impacts on the aquatic environment, aqueous ecosystems, social and human health (Kurniawan *et al*., 2006). The other negative impact of improper WSP is that it produces offensive odors leads to negatively impact on the aesthetic value of adjacent rivers and elevates the concentration of the waste properties of receiving water bodies.

More than recommended discharge of heavy metals and others which has non-biodegradable, bio-accumulate, bio-concentrate, bio-magnify characteristics (El-Shafai *et al*., 2006; Sekomo *et al*., 2012; US_EPA, 2002b; Omofunmi *et al*., 2018; Rachna and Disha, 2016) have potential lethal effects on aquatic living things (Rachna and Disha, 2016). Acute fish toxicity or fish bioassay technique is significant to determine the potency or any dose of any physiologically active substances; like chemicals, wastewater, or unknown substances (FOA_USA, 2017).

Hawassa University main Campus waste stabilization pond was constructed to treat wastewater discharging from a student cafeteria, dormitories, and teaching laboratories. The final wastewater (effluent) was desired for production of fish and agricultural purposes. However, it is poorly designed and operated as compared to the standard waste stabilization pond (EEPA, 2003). The pond is impaired due to frequent blockages by solid waste. This resulted in overflow and discharging to the nearby environment adjacent to the residential areas. Despite this reality, there is no document about the efficiency of Hawassa University main campus WSP. Therefore, this study evaluated the treatment efficiency of Hawassa University main campus waste stabilization pond, and effluent toxicity.

Materials and Methods

Study Setting and Area

Hawassa city is located in the southern region of Ethiopia, 275km from Addis Ababa. It has a tropical savanna climate, though it borders on a subtropical highland climate. There are two seasons: a lengthy non intense wet season from March to October and a short dry season from November to February. The extra cloudiness of the wet season is sufficient to make it substantially cooler than the dry season despite a higher sun angle. However, the cooler morning temperatures, often close to freezing, occur during the dry season (WMO, 2016). The city has one Government University (i.e. Hawassa University) and six private colleges. Hawassa University **(**HU**)** is the only Government University, which has four colleges and two campuses. This study was conducted on the main campus of the Hawassa University waste stabilization pond. According to data obtained from the main campus construction office, the inlet discharging rate of waste stabilization pond is about 14000 liters per day. The pond divided into different level of compartments for waste treatment: Aerobic I (surface area of 775m^2, depth of 6m and volume of 4650m^3); maturation I (surface area 11525m^2, depth of 8m and volume of 92200m^3); facultative I (surface area of 1426m^2, depth of 6m and volume of 8556m^3); facultative II (surface area of 1426 m^2, depth of 6m and volume of 8556m^3); facultative pond III (surface area of 1426m^2, depth of 6m and volume of 8556m^3) and fish pond (surface area of 1426m^2, depth of 6m and volume of 8556m^3) (HUMC_CO, 2017).

Study design and period

Experimental design was performed to evaluate efficiency of waste stabilization pond and its effluent toxicity using fish bioassay from March 1st and May 30th, 2017.

Waste water sample collection and examination

A. Efficiency of waste water stabilization pond

Three wastewater samples were collected in the 1L polypropylene bottle from the effluent of HU WSP, transported and the following examinations were conducted to determine the efficiency of WSP.

Physicochemical parameters: Thirteen physico-chemical parameters, namely: pH, electrical conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Temperature (Temp.), Dissolved Oxygen (DO), Ammonia (NH3), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD5), Nitrate (NO_3^-), Phosphate (PO_4^{-3}), Nitrite (NO_2-) and turbidity were selected for analysis. Non-conservable parameters such as temperature using (Thermometer), DO (determined by using Winkler's titration, pH using (PTC pH meter), and electrical conductivity (electro conductivity) were measured immediately at site during waste water sample collection. The waste water sample was transported and refrigerated at 4^0C (APHA, 2005) to Hawassa University by chemistry laboratory. The following conservative parameters were conducted in the Hawassa University chemistry laboratory by chemistry technical assistant. The Chemical Oxygen Demand (COD) was measured by DR/2010 HACH (Love land, USA), according to HACH instructions. Biochemical Oxygen Demand (BOD5) test was done based on a measurable depletion of oxygen over five days by diluting the sample with dilution water according to the 5210A method of (APHA, 2005) instructions. Total dissolved solids and TSS were measured by weighting the dried material on the filter; turbidity by turbid-meter and NH_3, PO_4^{3-}, NO_3^- and NO_2- were measured by digital photometer according to the instructions and procedures of (APHA, 2005).

Heavy metals analysis: The selected heavy metals including: lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), copper (Cu) and manganese (Mn) were analyzed using flame atomic absorption spectrophotometer (buck scientific model 210 VGP) at chemistry laboratory, Hawassa University by technical assistant of chemistry department. Finally, mathematical formula and conversion factor were used to calculate percent removal efficiency of physicochemical parameters and heavy metals between the inlet and outlet of HU-WSP (APHA, 2005; EEPA, 2003) were calculated using the following formula**:**

$$\textbf{Efficiency of WSP} = \frac{\text{Inlet} - \text{Outlet}}{\text{Inlet}} \text{x}100\%$$

B. Determination effluent toxicity by fish toxicity bioassay method

It was performed after preparation of aquaria and forty two young Tilapia fishes as follows:

Preparation of different concentrations of aquaria

Concentration of HU-WSP effluent was prepared for toxicity tests based on acute methods, manual of clean water act's prohibition on specific fish's ability to survive, grow and reproduce in whole effluent (NPDES, 2002). The dilution factor was performed based on method recommended by US Environmental Protection Agency (USEPA, 2019). This method recommends a control (i.e. 100% of fish water in this experiment) and a minimum of five effluent concentrations of dilution factor which must be equals or greater than 0.5% (i.e. 20%) to be mixed based on the effluent type (Kurniawan, *et al.,* 2006).Therefore, dilution factor equals or greater 20% (6L) was used in the test. To perform this, 300 Liter of fish water (FW) (Manmade Lake, fishes already living in, found at Hawassa University) was brought into chemistry laboratory. Of this volume of FW, 90L and 210L was used for toxicity dilution and acclimatization purpose, respectively. Also, 90L of effluent (discharged from waste stabilization pond of Hawassa University) was brought into laboratory which used for preparation of aquaria. Finally, six aquaria were prepared and coded as A, B, C, D, E and F as follow. A 90L of FW was distributed as A=30L, B=24L, C=18L, D=12L, E=6L, F=0L. Proportionally, 90L of effluent (WSP) was added to fish water as A=0L, B=6L, C=12L, D=18L, E=24L, F=30L.The amount of FW added also indicated in Table 1.

Acclimatization of the Fish

Forty two young Tilapia fishes (*Oriochromisniloticus*) were selected for the acute toxicity test. Fishes were brought from center of Fish production (i.e. Manmade Lake) found in biology department of Hawassa University.

Table 1: Preparation of aquaria for fish bioassay toxicity test, 2017

Code of Aquaria	Volume of Effluent from WSP in L	Volume of Fish Water in L	Total Volume aquaria in L (v/v concentration effluent in %)
"A" Aquarium	30	0 (Control)	30 (100)
"B" Aquarium	24	6	30L (80)
"C" Aquarium	18	12	30L (60)
"D" Aquarium	12	18	30L (40)
"E" Aquarium	6	24	30L (20)
"F" Aquarium	0	30	30L (0)
Total	90	90	180

Before toxicity test was performed in each aquaria, all the forty-two young tilapia fishes (*Oriochromisnilot-icus*) were exposed or acclimatized in the large aquarium (210 Liter) using fish water/FW/ for one day (24 hrs.) for adaptation of the fish in the experimental room according to the principle and guideline of Organization for Economic Cooperation and Development (OECD) (OECD, 1992). Finally, seven fishes were imported to each concentration aquarium labeled with code A to F (from 100% to 0% effluent concentration) to start toxicity testing in four consecutive days (96 hrs.).

Behavioral change, mortality, and adverse effect of effluent

After imported into six aquaria, behavioral changes and mortality of Tilapia fish were recorded at 24hrs. 48hrs, 72hrs, and 96 hours in each concentration aquarium according to OECD guideline (OECD, 1992). Fishes were observed every 8 hours using observational fish toxicity checklist, followed by Woolley methods (Woolley, 2008) to identify behavioral change like loss equilibrium, erratic swim and fast respiratory function. Concentration of effluent that causes death of fish was explained in terms of "Lethal Concentration Fifty Percent (LC50), Lowest Observable Adverse Effect of Concentrations (LOAEC) and Non Observable Adverse Effect of Concentrations (NOAEC), respectively.

Quality Control

Before sampling, 1 liter capacity polyethylene sampling bottles were first cleaned by 10% Nitric acid in a hot water bath to remove contaminants from the bottle by autoclaving and incubating them for 24 hours. Then, the bottles were washed and rinsed with de-ionized water. Wastewater samples were collected according to American Public Health Association (APHA, 2005) methods. The first sample was collected from influent and the second sample was collected from effluent of WSP. Here, during sample collection from two sites, bottles were immersed and then the cover of bottles were opened into influent and effluent of WSP in order to avoid air entrance that may contain microbes. The mean value of the three records were obtained from two sites to reach accurate and precise result of all measured parameters and heavy metals for three months. For toxicity test, young fishes were selected according to (OECD, 1992) principle and guideline. The length and weight of each fish was measured in the "Fish Research Center" found at Hawassa University. Accordingly, the length and weight of fish were ranged between 1.5-2.0 inches and 15-25g, respectively.

Data Analysis

Statistical Package for Social Sciences (SPSS) software Version 21 was used to analyze the mean and standard deviation (SD). One tail t-test for two mean sample value was assumed for an equal variance with 95% confidence interval (CI) to determine significance difference of each physicochemical parameters and heavy metals between the two sites. For the fish toxicity test, Scatter (X, Y) bubble chart of Excel was used to determine the cut point of LC50, LOAEC and NOAEC of the effluent. Moreover, R square was used to determine the association between effluent concentration and mortality of the fish.

Operational Definition

***Lethal dose fifty Percent/LC50/*:** The concentration or dose which kills half percent of fish' populations, which is used to estimate the general toxicity of the chemical agents (Agrawal & Paridhavi, 2007).

Lowest Observable Adverse Effect of Concentrations/LOAEC/: It is the highest efficacy (y-axis, i.e. high mortality response) and low potency of effluent compared to Non Observable Adverse Effect of Concentrations (NOAEC) (Duffus *et al.,* 2007), in which could causes adverse alteration of morphology, function, capacity, growth, development, or lifespan of the tested fish (Dorato and Engelhardt, 2005).

Loss of equilibrium: is defined as the inability of fish to maintain an upright position within the water column and immobility which is defined as the inability of fish to move or swim unless prodded.

Non Observable Adverse Effect of Concentrations/NOAEC/: It is the lowest efficacy (y-axis, i.e. low mortality response) and high potency of effluent as compared to Lowest Observable Adverse Effect of Concentrations (LOAEC) (Deshpande *et al*., 2017), which couldn't causes adverse effect (alteration) on morphology, functional capacity, growth, development, or life span of the tested fish (Dorato and Engelhardt, 2005).

Ethical Considerations

Ethical clearance was obtained from postgraduate and approved by Hawassa University Research Ethical Review committee (HU-RERC). Formal letter was written to Hawassa University construction directorates for waste stabilization pond and to Hawassa University communication and foreign relation to facilitate the research. Support letter was also written for biology and chemistry departments in order to provide Fish and chemical reagents, required for this study, respectively. Moreover, unethically acceptable dose for fish toxicity was not applied.

Results

Efficiency of WSP for physicochemical parameter

The mean of EC were 40.72±2.74 at inlet and 11.56+2.33 at outlet; TSS were 1.07± 0.13 at inlet and 0.34± 0.19 at outlet; COD were 290.67±17.2 at inlet and 178±10.54 at outlet; BOD were 198.70±32.2 and 89.50±10.83 at outlet; NO_3 were 1.22 ± 0.16 at inlet and 0.32± 0.11 at outlet; turbidity were 77.50±8.65 at inlet and 52.90 ±3.50 at outlet of Hawassa University waste stabilization pond. The mean difference was statistical significant for the above physicochemical parameters ($P<0.05$). The pond has high removal efficiency of 72%, 68%, 55%, 74% and 91% for EC, TSS, BOD, NO_3 and NO_2 respectively (Table 2).

Efficiency of WSP for heavy metals

The mean concentration of cadmium were 0.32±0.09mg/L at inlet and 0.01±0.01mg/L at outlet; chromium were 0.22±0.09mg/L at inlet and 0.16±0.07 mg/L at outlet; copper were 0.25±0.11 mg/L at inlet and 0.07±0.04mg/L at outlet of Hawassa University waste stabilization pond. The difference was statistically significant for the above heavy metals ($P<0.05$). The removal efficiency of waste stabilization pond for heavy metals in increasing order were 20%, 27%, 72% and 97%, for manganese, chromium, copper and cadmium respectively (Table 3).

Toxicity testing and fish mortality

Behavioral change

Loss of equilibrium, erratic swimming behavior and fast respiratory function of all fishes were observed in aquarium containing 100% (30 L) of effluent. All the above behaviors were observed to some extent in aquarium containing 80% (24L) effluent of WSP. However, fishes in control groups (i.e. aquarium that contained pure fish water) were showed normal behavior (Table 4).

Fish mortality

During 24 hours experimental period, 0, 0,0,2,4 and 7 fish mortality were recorded in aquaria contained 0% (0L), 20% (6L), 40% (12L), 60% (18L), 80% (2Ll) and 100% (30l) of effluent concentration, respectively. At the 4^{th} day, 1, 3, 4 7, 7 and 7 fish morality were recorded at 0%, 20%, 40%, 80% and 100% effluent concentration, respectively (Table 5).

Adverse Effect of Effluent

There were positive relationships and strong association (R^2=0.84) between the concentrations of effluent and mortality of the fishes at 24 hour. The Lethal Concentration (LC50) that killed half population of fish was 76% of the effluent dose. While, the Lower Observable Adverse Effect Concentration (LOAEC) and Non-observable Adverse Effect Concentration (NOAEC) were 60% and 40%, respectively (Figure1).

Table 2: Physicochemical parameters at the inlet and outlet of Hawassa University main campus waste stabilization pond, Southern Ethiopia, 2017

Parameters	Inlet (Mean ± SD)	Outlet (Mean ±SD)	Efficiency (%)	Df=2, (α=0.05)	EEPA**	WHO***	EPA****
pH	5.80 ± 0.79	6.20 ±0.66	-6.90	0.27	6-9	7.5	8.5
EC(μmh/cm)	40.72±2.74	11.56+2.33	71.61	0.00*	100	400	400
TDS (mg/l)	10.43±2.87	8.96±1.60	14.09	0.32	80	250	-
TSS (mg/l)	1.07± 0.13	0.34± 0.19	68.22	0.02*	30	50	50
Temp(0C)	28.00±3.61	22.67±1.53	19.04	0.07	40	20	30
DO(mg/L)	3.99 ±0.2	4.86±0.42	-21.80	0.08	>5	-	>4
NH_3(mg/l)	0.19±0.09	0.12±0.10	39.06	0.40	30	50	10
COD(mg/l)	290.67±17.2	178±10.54	38.76	0.03*	150	120	100
BOD (mg/l)	198.70±32.2	89.50±10.83	55.15	0.02*	50	50	50
NO_3^-(mg/l)	1.22 ± 0.16	0.32± 0.11	73.77	0.00*	50	10	20
PO_4^{-3} (mg/l)	0.36 ± 0.18	0.20±0.11	44.44	0.08	-	6.5	6.5/5
NO_2^-(mg/l)	0.88 ± 0.10	0.08 ± 1.30	90.91	0.17	-	1	2
Turbidity (NTU)	77.50±8.65	52.90 ±3.50	31.74	0.00*	-	5	-

Significant at α=0.05 level; EEPA: Ethiopian Environmental Protection Agency Permissible Limit of Effluents Discharge Regulations (2003); WHO***: World Health Organization (2004);EPA****: Environmental Protection Agency for Permissible Limit of Effluents Discharge Regulations (2004); EC: electrical conductivity; TDS: Total Dissolved Solids; TSS: Total Suspended Solid; Temp: Temperature; DO: Dissolved Oxygen; NH_3: Ammonia; COD: Chemical Oxygen Demand; BOD: Biochemical Oxygen Demand; NO_3^-: Nitrate; PO_4^{-3}: Phosphate ; NO_2^- :Nitrite*

Table 3: Concentration of heavy metals in waste water of inlet and outlet of Hawassa University main campus wastewater stabilization pond, Southern Ethiopia, 2017

Heavy metals (mg/l)	Inlet (Mean ± SD)	Outlet (Mean ± SD)	Df=2 (α=0.05)	Efficiency (%)	EEPA **	WHO ***	EPA ****
Cadmium	0.32±0.09	0.01±0.01	0.00*	96.90	0.01	0.05	-
Chromium	0.22±0.09	0.16±0.07	0.01*	27.27	0.10	-	0.1
Copper	0.25±0.11	0.07±0.04	0.03*	72.00	0.20	0.005	-
Manganese	0.05±0.03	0.04±0.02	0.33	20.00	0.20	0.5	-
Nickel	ND*****	ND	-	-	0.20	-	0.1
Lead	ND	ND	-	-	1.00	-	0

Significant at α=0.05 level; EEPA: Ethiopian Environmental Protection Agency Permissible Limit of Effluents Discharge Regulations (2003); WHO***: World Health Organization (2004) and EPA****: and Environmental Protection Agency for Permissible Limit of Effluents Discharge Regulations (2004); ND*****: stands for "Not detected" by flame atomic absorption spectrophotometer*

Table 4: Behavioral change of Tilapia fish from 24-96 hours acute toxicity using effluent of WSP, Hawassa University, Southern Ethiopia, 2017

Code	% of effluent/ fish water	Number of fishes with behavioral change		
		Loss of equilibrium	Erratic swimming	Fast respiratory function
A	100	7	7	7
B	80	1	1	1
C	60	0	1	0
D	40	1	1	0
E	20	1	1	0
F	0	1	0	0

Table 5: Toxicity test using fish bioassay from effluent of waste stabilization pond at Hawassa University, Southern Ethiopia, 2017

Time (hrs.)	Fish in 6 aquaria (n=42)	Effluent/ fish water (v/v) (%)					
		0	20	40	60	80	100
24	Exposed	**7**	**7**	**7**	**7**	**7**	**7**
	Dead	0	0	0	2	4	7
	Survived	7	7	7	5	3	0
48	Exposed	**7**	**7**	**7**	**7**	**7**	**7**
	Dead	0	0	1	3	5	7
	Survived	7	7	6	4	2	0
72	Exposed	**7**	**7**	**7**	**7**	**7**	**7**
	Dead	0	1	2	3	7	7
	Survived	7	6	5	4	0	0
96	Exposed	**7**	**7**	**7**	**7**	**7**	**7**
	Dead	1	3	4	7	7	7
	Survived	6	4	3	0	0	0

There was positive relationship and very strong association (R^2=0.88) between the concentration of effluent and mortality of the fish. In addition, the concentration of effluent that killed half population of tilapia fish (LC50) was 32%; the lowest concentration of effluent which showed an adverse effect (LOAEC) was 20%, while non-observable adverse effect concentration of effluent (NOAEC) was 0% within 96 hours (Figure 2).

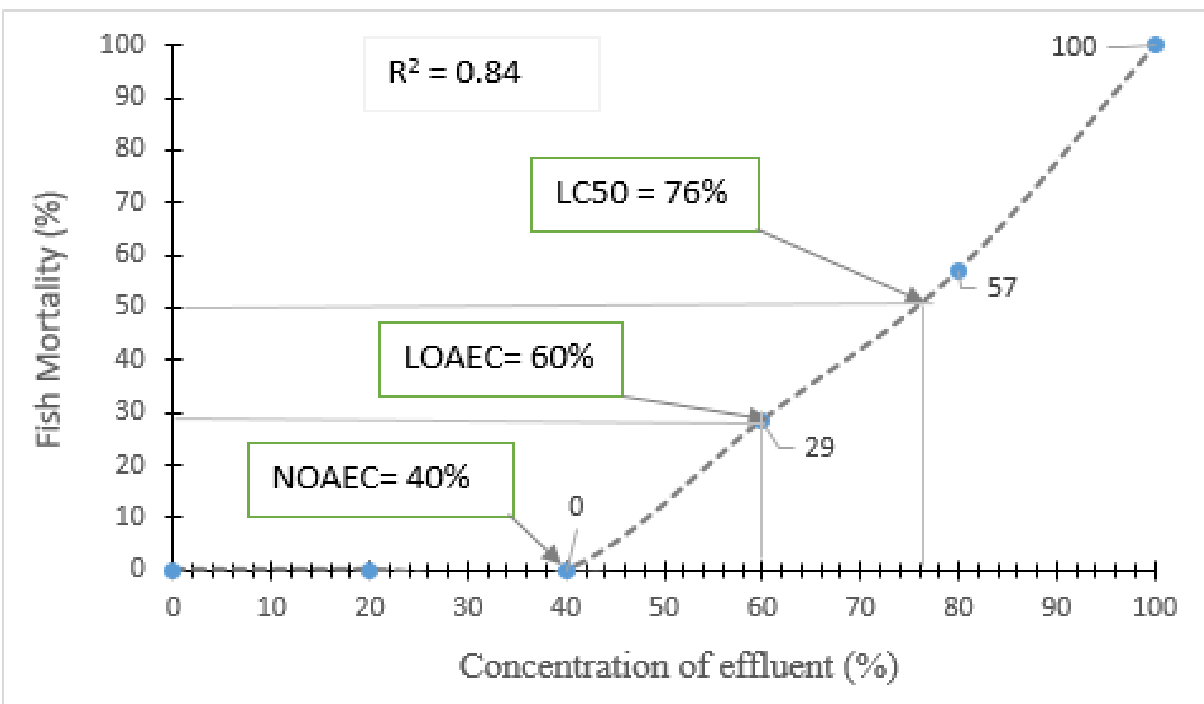

Figure 1: Mortality of fish within 24 hours due to effluent discharged from WSP, 2017

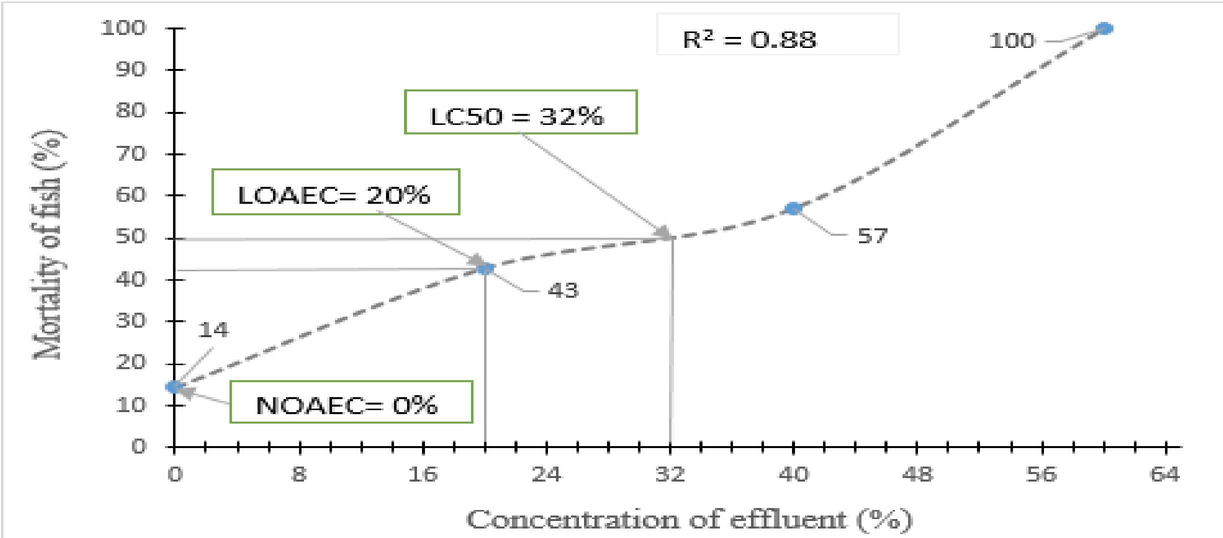

Figure 2: Mortality of fish within 96 hours and concentration of effluent discharged from WSP, 2017

Discussion

There is high removal of efficiency, 72%, 68%, 55%, 74% and 91% 72% and 97% for EC, TSS, BOD, NO_3 and NO_2, copper and cadmium at Hawassa University main campus stabilization pond, respectively. The mean difference was statistically significant for the above physicochemical parameters ($P<0.05$). The mean of EC were 40.72±2.74 at inlet and 11.56±2.33 at outlet; TSS were 1.07± 0.13 at inlet and 0.34± 0.19 at outlet; COD were 290.67±17.2 at inlet and 178±10.54 at outlet; BOD were 198.70±32.2 and 89.50±10.83 at outlet; NO_3 were 1.22 ± 0.16 at inlet and 0.32± 0.11 at outlet; turbidity were 77.50±8.65 at inlet and 52.90 ±3.50 at outlet.

The mean difference of Chemical Oxygen Demand/COD/, which was statistically significant between the inlet and outlet with the lower removal efficiency of 38.76%. In the current study, the mean value COD from influent and effluent is exceeded the national standard (EEPA, 2003); permissible limit of effluent discharge regulations recommended by WHO, EPA (WHO, 2004; EPA, 2004). The current efficacy report COD is greater than reported from Sebeta waste stabilization pond (8%) (Dejene & Prasada, 2011). However, it was less than efficiency reported at Kality waste stabilization pond/KWSP/ (78%) found in Addis Ababa (Dagne, 2010) and Botswana WSP (72%) (Gopolang and Letshwenyo, 2018). The discrepancy of the results among different studies might be due to amount of organic compounds in wastewater required to oxidize the polluted organic matter as Sadek *et al.* (2016) reported.

The other parameter is BOD which determine the oxygen requirement in the pond. In the current study, the mean concentration obtained from effluent site is higher than the national level (EEPA, 2003; WHO, 2004; EPA, 2004). On the other hand, the current mean ± SD (89.50 ± 10.83mg/L) values of BOD obtained from effluent is higher than the mean ± SD (1.82±0.12mg/L) obtained from WSP found in Ikorodu, Southwest, Nigeria (Omofunmi, *et al.*, 2018).

The efficiency of Hawassa University WSP for BOD was 55%; which is less than the reported from Hawassa referral hospital WSP (94%) (Hunachew and Getachew, 2011); and higher than finding reported from Sebeta WSP (16%) (Dejene & Prasada, 2011). This differences might be due to the type of waste water treated and amount of microbe found in waste water. Moreover, in the current findings, the mean concentration of BOD found at effluent site has a potential to cause the death of fish and other aquatic organisms due to higher than permissible limits; such type of explanation was also given by Rachna and Disha (2016). On the other hand, some organic materials found in the wastewater has enough to resistant microbial oxidation (Ram *et al.*, 2011).

Moreover, the current ratio for BOD5/COD is 0.5, which is between 0.3 and 0.6, is significant to know the biodegradability index of the raw influent wastewater, as this choice would considerably affect the effluent quality. If BOD/COD < 0.3, biodegradation will not proceed, thus it cannot be treated biologically (Khaled and Gina 2014). Therefore, the higher the BOD/COD the more oxygen stripping capacity the discharged effluent has when discharged into receiving waters and the more potential for damage to biological life in those waters as researcher indicated. Oxygen is used biologically/chemically to break down the organic matter (Ahmed *et al*., 2013).

The dissolved oxygen (DO) was the other an important parameter to determine either sufficient or insufficient oxygen content to remove organic matter found in waste water of the pond. In the current study, the presence of oxygen was found higher in the outlet than inlet as indicated by negative efficiency. The current amount oxygen in effluent (4.86±0.42) is lower than reported from WSP in Ikorodu, Nigeria (7.50 ± 1.10) (Omofunmi, *et al*., 2018), slightly lower than the National Agency recommendation (5mg/L) (EEPA, 2003). But, the current study reported is slightly higher than EPA's permissible limit of effluent discharge (EPA, 2004). Therefore, current amount of dissolved oxygen is less suitable survival and metabolic activities of fish as national recommendation (EEPA, 2003).

Phosphate (PO_4^{3-}) and Nitrate (NO_3^-) are responsible for eutrophication of water bodies if exceeds than recommendation value. In this study, the level of both parameters decreased from inlet to outlet. There were a decreased of concentration phosphate ion from 0.36 ± 0.18mg/L in inlet / influent to 0.20±0.11mg/L outlet/ effluent, with 44.44% removal efficiency. The decrease in magnitude was not significant between effluent and influent sites. The amount Phosphate in the current study is lower than the national recommendation value of permissible discharge limit. A variable removal efficacy of phosphate between 15% and 50% was reported in study conducted on WSP (Powell *et al.,* 2008). The high amount of phosphate in effluent can cause algal blooms in the ponds which can prevent sunlight from penetrating down to the underwater in the system and hindering the treatment process (Powell *et al*., 2008). However, the low amount of phosphate was found in the current study not enough to cause algae bloom in the pond. Due to lower amount concentration as compared to national recommendation value of permissible discharge limit.

In the present study, the mean concentration of NO_3^- between the inlet and outlet was statistically significant with removal efficiency of 74%. It is higher than the efficiency reported from Hawassa Referal -WSP (59.64%) (Hunachew and Getachew, 2011) and Sebeta Teaching-WSP (68.58 %) (Dejene and Prasada, 2011) in Ethiopia. Similarly, in the current study the highest efficiency (90.91%) was found for $N0_2^-$.This is higher than the efficiency obtained from Hawassa Referral-WSP (54.81%) (Hunachew and Getachew, 2011). The other important parameter was NH_3; its mean concentration decreased from influent to effluent. The efficiency of Hawassa university waste stabilization pond for NH_3 removal was 39.06%; which is less than the efficiency of ST-WSP (41.83 %) (Dejene and Prasada, 2011).

The alkalinity and acidity of Hawassa University-WSP was evaluated. According, the mean pH value of waste water at inlet and at outlet of HU- WSP and was 5.80 ± 0.79 and 6.20 ±0.66, respectively. The pH of water in the study area was within the recommended World Health Organization value for domestic uses (WHO, 2004). The removal efficiency of the pond for pH was negative. The negative result indicates that the difference value of pH at both inlet (acidic) and outlet (acidic, slightly acidic). This study is inconsistent the finding obtained from Botswana WSP, which was decreased from Inlet (7.03 ± 0.5) to outlet (6.87 ± 0.4) of WSP (Gopolang and Letshwenyo, 2018). As implication of this parameter, the pH values of water examined were higher than 7, indicating that it is alkaline which is good for fish rearing.

Common heavy metals were analysed in this study by flame atomic absorption spectrophotometer (Buck Scientific Model 210 VGP) instrument. In the current study revealed that the level of Lead (Pb) and Nickel (Ni) was below the detection limit (not detected). However, the mean concentration of Cadmium (Cd), Copper (Cu), Chromium (Cr) and Manganese (Mn) detected at inlet and outlet. These was slightly lower than that of the maximum permissible discharge level recommended at national level EEPA .WHO (EEPA, 2003; EPA, 2004). WHO, 2004). A significant difference was obtained for the above detected heavy metals. This is similar to report from Hawassa Referral Hospital-WSP (Hunachew & Getachew, 2011) except the value for Cu (p-value=0.383). It was reported small concentration of heavy metals have a potential to contaminate water. It can also deteriorate the quality of soil as well as the agricultural produce if they reaches water bodies or irrigation, respectively (Deepak *et al.*, 2016).

The treatment efficiency of the pond for cadmium was the highest among the heavy metals followed by copper. The treatment efficiency for cadmium (96.90%) in this study is higher than the finding from Hawassa, Ethiopia (Hunachew and Getachew, 2011); Australia Waste Stabilization Pond (86%) (Nalenthiran *et al*., 2015). The current efficiency of the pond for Copper (72.00%) lower than report from Hawassa Referral-WSP (86.95%) (Hunachew and Getachew, 2011). The removal efficiency of Hawassa WSP for Chromium was 27.27%, which is less than the finding obtained from Australia Waste Stabilization Pond for Cr (51 %) (Nalenthiran *et al*., A.2015). The difference among heavy metals obtained from the different studies might be due to type of waste water being treated.

Furthermore, bioassay toxicity on young tilapia fish was the other activity to evaluate the HU-WSP performance. Accordingly, all young tilapia fish found in aquarium with effluent concentration of 100% was dead within the first 24 hours. This implies that if the Hawassa university effluent reaches water bodies and neighboring community, it might have a potential to affect aquatic living organism and terrestrial organism. At this time, the concentrations of Lethality (LC50) was 76% of concentration (v/v). This study Lower observable adverse effect concentration (LOAEC) was 60% effluent concentration. This amount of concentration/ dose had a biologically significant effect on survival of fish. Meanwhile, the value of non-observable adverse effect concentration (NOAEC) was 40% (v/v) of the dose, which hadn't a biologically significant effect on survival of fish. In the present study, the concentrations of Lethality is less than the finding obtained in Iran WSP; i.e. the LC50 for effluent after secondary treatment was 85.6% (v/v) with the non-observable adverse effect concentration and Lower observable adverse effect concentration being 94% and 58%, respectively (Movahedian *et al*.,2005).

This study also found that there was strong correlation between the concentration of effluent and mortality of the fish within 24 hours with the correlation coefficient (r=0.84). This figure is greater than the standard correlation (r=0.80) (Cohen, 1988; Evans, 1996). In addition, this study found fish living in aquaria containing 40%, 20% effluent concentration did not induce any sudden behavioral change like loss of equilibrium, decrease swimming and respiratory function and pigmentation (change skin color) as compared to those in 100%, 80% and 60% of effluent concentration within 24 hours. This indicated that the high concentration of effluent in aquaria could kill and show behavioral change on fishes during the experiment.

Moreover, LC50 within four days (96 hrs.) was thirty two percent of effluent. At this time, the lowest concentration of effluent at which an adverse effect is observed (LOAEC) was twenty percent. However, non-observable adverse effect concentration (NOAEC) appeared at zero concentration/dose. This indicates that the addition of small concentration of effluent to the fish water within four days initiates the alteration of morphology, functional capacity, growth and development of the fry tilapia fish. The result of the analysis indicated that there is a strong relationship (R^2=0.88) between fish mortality and effluent concentration; which is greater than the standard correlation (r=0.80) cited previously (Cohen, 1988; Evans, 1996). However, the control group of fry Tilapia fish, which stayed in 100% fish water concentration were showed normal behaviors like normal swimming, breathing, well synchronized and no color change among the tilapia fish during 96 hours.

Limitation of the study

This study focuses only on common physicochemical parameters and heavy metals which might not represent all physicochemical parameters and heavy metals found in the oxidation pond. Moreover, the toxicity test was performed on a small subset of one population of tilapia fish. Thus it may not represent all fish and other aquatic living organisms due to the genetic strain and variability and potential to resistance toxic substance. The adverse effect of effluent on fish may not represent the impact of the effluent on the environment. In addition, all experiment were conducted in lab condition which might not indicate similar result in waste water in environment.

Conclusion

In this study more than half percent of efficiency WSP was indicated for Electric conductivity, total suspended solid, biological oxygen demand, NO_3^- and NO_2^-, copper and cadmium at Hawassa University main campus stabilization pond. However, the lower waste water treatment efficiency were obtained for total dissolved substance, temperature, turbidity, chemical oxygen demand, ammonia, phosphate, chromium and manganese. The pure effluent caused hundred percent of young tilapia fish mortality within a day. Therefore, the authors recommended that pond need to redesign and upgrade to prevent the ecological health risk and to endure aquatic life including fish. Furthermore study should be conducted in vitro rather than in vivo to describe adverse effect of effluent the on the environment

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Author's contribution

SKK involved in develop the methods and conducted data analysis. STT who involved in data collection, analysis and interpretations. EDW who participated in conceiving the idea, developing the study. All authors involved in manuscript writing, reanalyzing the data and re- edited the comments of the manuscript.

Competing of interests

The authors declare that they have no competing interests.

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