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Sero-epidemiology of Foot and Mouth Disease and Farmers Perception on Vaccinating Cattle against the Disease in Sidama Region, Southern Ethiopia

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FMD;

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Research article

ABSTRACT

Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock that has a significant economic impact. A cross-sectional study was conducted from September 2019 to June2020 in three selected districts of Sidama region, Southern Ethiopia with the objectives of determining the sero-prevalence of cattle against foot and mouth disease virus (FMDV), identifying potential risk factors and assessing farmers' perception on vaccination against FMD. Purposive and systematic random sampling techniques were employed to select the districts and study animals, respectively. A total of 510 cattle were tested for FMDV antibodies using 3ABC-ELISA. The overall cattle and herd level sero-prevalence were 15.5% and 24.7%, respectively. Among the considered risk factors, age of the animal, herd size and season were significantly associated with the sero-positivity of FMDV ($P<0.05$). Out of 120 farmers interviewed 84.2% had never vaccinated their cattle against FMDV. Inaccessibility (83.7%) and unaffordable cost (72.1%) of the vaccine were mentioned as leading causes for the low vaccination practice in the current study areas. Majority of the respondents (68.3%) don't perceive vaccinating cattle against FMDV as one of the preventive measures. In districts with lower perception of farmers on vaccinating their cattle against FMDV, higher sero-prevalence of the disease were recorded. The present serological and questionnaire survey indicated that the presence of FMD sero-positive animals in the current study areas. Therefore, an integrated strategy for disease control has to be designed and implemented which could include enhancing farmers' perception about use of vaccination in preventing FMD and government provision of vaccines at affordable cost to the farmers.

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INTRODUCTION

Ethiopia is one of the countries that possess a huge number of livestock populations in the Africa continent estimated to be 56.5 million cattle, 30.7 million sheep and 30.2 million goats were found in the country (CSA, 2017). The livestock sector contributes about 40% of the agricultural Gross Domestic Product (GDP) and nearly 20% of total GDP, and 20% of national

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foreign exchange earnings in 2017 (World Bank, 2017).Within the cattle population, FMD occurs endemically resulting in several outbreaks every year (Ayelet *et al*., 2012). The causative agent, FMD virus (FMDV), has seven recognized serotypes (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1), with discrete immunologic, antigenic and genetic properties. They also differ in distribution across the globe (FAO, 2007).

Five of the seven serotypes of FMD (O, A, C, SAT 2, SAT 1) were identified in Ethiopia (Rufael *et al*., 2008; Ayelet *et al.,* 2009; Negusssie *et al*., 2010). Serotype C was not identified after 1983; however, a serotype C-specific antibody in cattle was reported (Rufael *et al*., 2008). Morbidity has been reported to reach as high as 100% in susceptible animal populations but it is rarely fatal except in young animals (Kahn and Scottline, 2005). Infected animals show a spectrum of responses to FMD ranging from unapparent infection to severe disease and death (OIE, 2008).

Foot and mouth disease is endemic with high prevalence in Africa, the Middle East, and Asia and is also present in parts of South America (Rweyemamu and Astudillo, 2002). The disease is endemic in Ethiopia and remains largely uncontrolled due to the absence of prophylactic vaccination except for a few dairy herds containing imported breeds (Sahle, 2004;Megersa *et al*., 2009). Serological surveys reported a sero-prevalence that ranges from 5% to 72.1% at the animal level in different parts of the country (Bayissa *et al*., 2011, Sulayeman *et al.,* 2018; Shazali *et al.,* 2021).

In terms of livestock exports from Africa, FMD is often perceived as a major hindrance to international trade (Thomson *et al*., 2004). In part, this perception is based on the assumption that disease freedom is required before export is possible, and has resulted in costly and an elaborated FMD control measures such as disease-free zones in Southern Africa and elsewhere (Bruckner, 2004). Commodity based approaches can provide an acceptable level of risk for exported livestock or livestock products according to international standards(Thomson *et al*., 2004), but in the case of FMD, they still require an understanding of FMD status in cattle entering the market chain.

Recommended control measures for FMD include animal movement restrictions, a vaccination programme, animal quarantine, environmental sanitary controls, outbreak investigation, serological surveillance and slaughtering of sick animals (Chaosuancharoen, 2012). However, it is a global problem since the result of the increasing movement of human and livestock and livestock products (Perry, 2007). This is mainly due to lack of vaccination, free livestock movement among different regions in the countries and across international borders, the existence of multiple FMD virus serotypes, and involvement of wildlife (Sahle, 2004; Rufael *et al.,* 2008).

Studies undertaken in Ethiopia revealed that the disease is still endemic and occurs in different parts of the country (Sulayeman *et al.,* 2018; Shazali *et al.,* 2021). There is neither a nationwide control strategy nor a legislation for making FMD notifiable to the veterinary authorities or for animal movement restrictions to be imposed. Therefore, livestock is at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries (Sahle, 2004). There is a difference in the epidemiology and economic impacts of FMD in the livestock

production systems (Jemberu *et al*., 2014) in different parts of the country. Unidentified farmers' perceptions about risk of the disease, lack of pragmatic vaccination schemes and presence of unrestricted animal movement regardless of certification are the major reasons that could intensify the distribution of FMD alongside the cattle market chain.

Despite this fact, there is no published information regarding the status of FMD and farmers' perceptions and practices on vaccinating their cattle against the disease in Sidama region. Therefore, this study was aimed to generate current information on the sero-prevalence status of FMD and predisposing risk factors and assesses farmers' perception on vaccination against FMDV in selected districts of Sidama region, Southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was carried out in three purposively selected districts of Sidama region, namely Hawassa zuria, Boricha and Wondo Genet. Sidama region is located northeast of Lake Abaya at an altitude of l500 to 2500 m.a.s.l. The region has geographic coordinates of latitude, north,5′ 45″to 6′ 45″ and longitude, east, 38′ to 39′. Mean annual rainfall of this area varies between 1200 mm and 1599 mm, with 15°C-19.9°C average annual temperature (CSA, 2015) (Figure 1).

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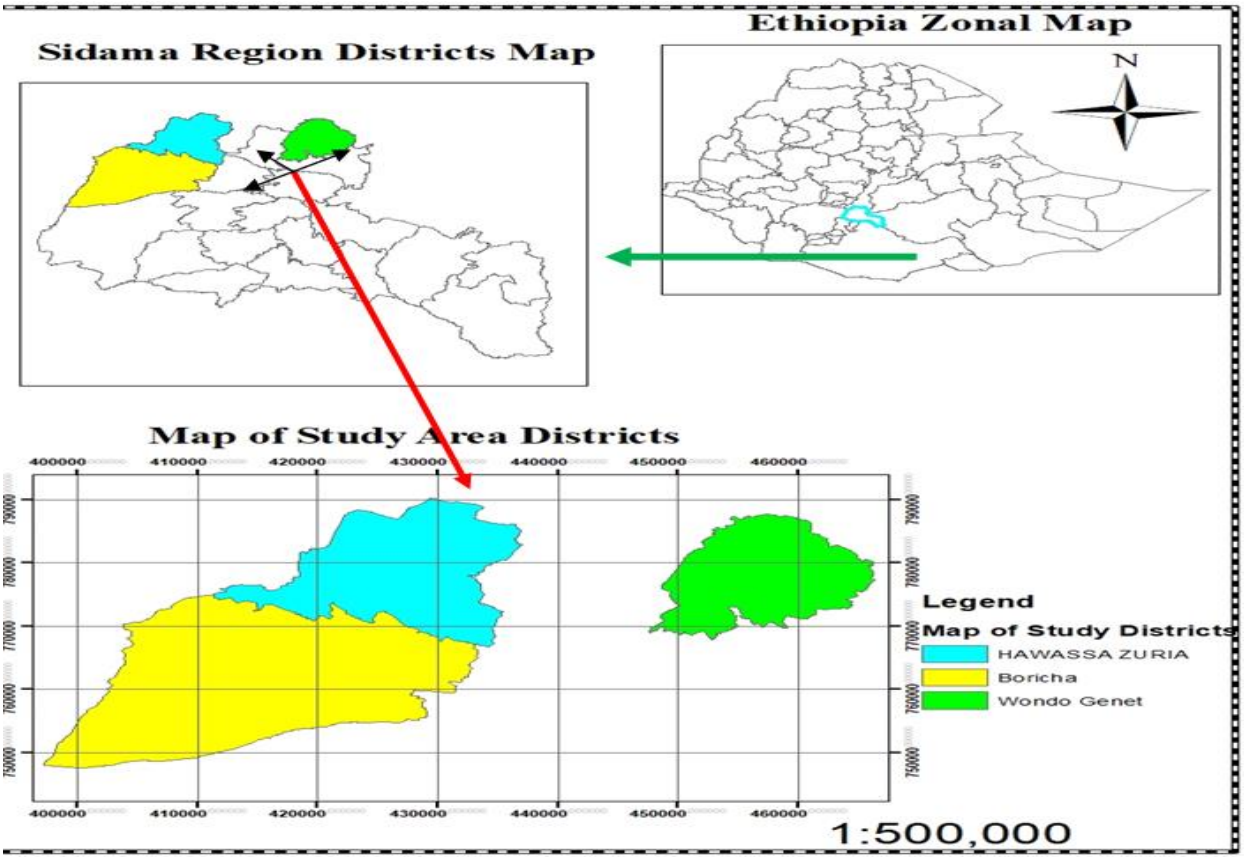

Figure 1: Map showing the study areas

Study design and sampling strategy

A cross-sectional study design was implemented for sero-prevalence study of antibodies against FMDV in the study areas. Hawassa Zuria, Boricha and Wondo Genet districts were selected purposively based on their transport accessibility, geographical location and presence of large cattle population. From each district 30% of kebeles (the smallest administrative units in Ethiopia) were selected using simple random sampling. From each kebeles, 20% of privately owned herds were randomly selected. From each herd, the study animals (cattle) were then selected using simple random sampling method to achieve the required sample size. The alleged potential risk factors for the occurrence of the disease such as age, breed, sex, districts, herd size and composition, season, vaccination history and management were also recorded.

Sampled animals were categorized based on their breed (local and cross), sex (female and male), herd composition (cattle only and cattle mixed with small ruminants), vaccination history (previously vaccinated and non-vaccinated) and management types (intensive and semi-intensive). Ages (young, adult and old) were categorized based on their dental eruption status (Berecha *et al*., 2011) and herd sizes where also classified as large farms size, with more than 50 animals, medium (20 to 50 animals) and small (< 20 animals) (Edao *et al.,* 2018).

Study animal population

Local and cross breeds of cattle kept under intensive and semi-intensive system were included. According to Pace and Wakeman (2003), the age groups of cattle were categorized as (≤3.5years) Young, (3.5years-5.5years) Adult and (> 5.5years) Old.

Sample size determination

The sample size required for the study was calculated based on the following formula (Thrusfield, 2005).

$$n = \frac{Z^2 * Pexp\ (1- Pexp)}{d^2}$$

Where, n= required sample size, Z= statistic for level of confidence = 1.96, Pexp = expected prevalence, 95% confidence level and d^2 = absolute desired precision of 0.05.

Accordingly, based on the above formula and 9.5% expected prevalence (Megersa *et al*., 2009), the sample size computed for animal level prevalence was 132. To increase the precision, calculated sample size was made four fold to 528, but due to shortage of sample collection materials only 510 cattle were considered. This number was allocated proportionally to the respective districts based on the total cattle population in each districts.

Serum sample collection

From each cattle, about l0 ml of blood sample was collected from the jugular vein and kept overnight on a table at room temperature. Then serum was aspirated with pasture pipette and transferred into cryovial and transported to Hawassa University Veterinary microbiology laboratory for storage at −20 °C. All the sera were transported with an ice box containing ice packs to the National animal health diagnostic and investigation center (NAHDIC) for serological test.

Serological diagnostic tests

Sera collected from bovine species was tested by FMDV 3ABC-Ab ELISA (ID Screen®) for the detection of antibody to poly protein called 3ABC which is a useful indicator of FMD virus infection regardless of the serotype involved (Haas, 1997; Mackay *et al.*, 1998). Antibody to 3ABC (nonstructural protein) is found only in virus infected cattles but not in vaccinated animals (De Diego *et al.*, 1997).

Briefly, the test was carried out stepwise as per the manufacturer's manual. First, all reagents were kept at room temperature and homogenized by vortex. The test was carried out in 96 well micro plates. Then 50µl of dilution buffer18 were added in to each well, 30µl of positive control were added in to wells A1 and B1, and the same volume of negative control were also added to wells C1 and D1, the rest wells were filled by 30µl of test sera. Then incubated at 37^{o}C for 2 hours, after incubation the wells were emptied with washing 5 times with 300µl of wash solution along with paying great attention to avoid drying of wells between washing. After washing l00µl of the conjugate lX were added in to each wells and incubated for 30 min at 21^{o}C. The wells were then emptied and washed 5 times with 300µl of wash solution, then l00µl of the substrate solution was added in to each wells and incubated at 2l^{o}C for l5 minutes in the dark. After adding a 100µl of Stop Solution to each well, the optical density (OD) reading was noted using a photometer at wavelength of 450 nm within 2 hours after the addition of the stop solution.

Questionnaire survey

Data concerning farmers' perception towards vaccinating their cattle was collected by using a semi-structured pre-tested questionnaire. It was administered by interviewing individuals selected by systematic random sampling. Before the interview, the objective of the survey was properly explained and verbal consent was obtained from the respondents. The interviews were conducted in local languages (Sidaamu Afoo or Amharic). A total of 120 farmers, 40 farmers from each three districts were interviewed for the questionnaire survey.

Data management and statistical analysis

Data generated from the laboratory investigations and survey was recorded and coded using a Microsoft excel spread sheet (Microsoft Corporation) and analyzed using STATA version 13.0 for Windows (Stata Corp. College Station, TX, USA). The association between dependent and independent variables was analyzed at individual cattle level by using univariable and multivariable logistic regression. Multivariable logistic model was used for variables with a p-value ≤ 0.05 on univariable analysis model. Further selection of variables in the final model was based on stepwise backward elimination procedure. Accordingly, Odds ratio (OR) was used to assess the strength of association between the putative risk factors and sero-positivity of the disease.

RESULTS

FMD sero-prevalence and risk factors

Based on the total 510 sampled cattle, overall sero-prevalence of FMD was 15.5% and 24.7% at the individual animals and herd levels, respectively. Comparatively higher sero-prevalence (32.4%) was recorded in Hawassa

zuria district (p=0.02; OR=2.67%; 95%CI=1.14-5.33) (Table 1).

Table 1: Individual cattle level and herd level sero-prevalence of FMD

Districts	Farmers associations	**Individual cattle**		**Herds**	
		Tested	**Positive (%)**	**Tested**	**Positive (%)**
HawassaZuria	Labu-koromo	68	9(13.2)	27	8(29.6)
	Udo-wotate	65	16(24.6)	22	7(31.8)
	Galo-argisa	64	12(18.7)	22	8(36.4)
Sub total		**197**	**37(18.8)**	**71**	**23(32.4)**
Boricha	Konser-fulasa	50	16(32)	31	11(35.5)
	Fulasa-aldada	48	5(10.4)	24	5(20.8)
	Hanja-chefa	52	7(13.5)	36	7(19.4)
	Aldada-dela	58	5(8.6)	27	3(11.1)
Sub total		**208**	**33(15.8)**	**118**	**26(22)**
Wondo Genet	Watara-qachama	56	5(8.9)	13	2(15.4)
	Abayye	49	4(8.2)	20	4(20)
Sub total		**105**	**9(8.6)**	**33**	**6(18.2)**
Total		**510**	**79(15.5)**	**222**	**55(24.7)**

Risk factors

Breed of the cattle, sex, age, districts, herd composition and size, season, vaccination history and management types were the major exposure or predictor variables considered to predict the response of the outcome variable.

Table 2:Logistic regression analysis of FMD and its putative risk factors for sero-positivity of cattle.

Risk factors	Category	No examined	Prevalence	Univariable			Multivariable		
			No (%) positive	OR	95% CI	P-value	OR	95% CI	P-value
Districts	Wondo genet	105	9(8.6)	Ref.	-	-	-	-	-
	Hawassa zuria	197	37(18.8)	2.67	1.14 – 5.33	0.02	0.63	0.37 – 5.23	0.63
	Boricha	208	33(15.8)	2.01	0.92 – 4.37	0.04	1.38	0.16 – 2.48	0.51
Age	Young	58	3(5.2)	Ref.	-	-	-	-	-
	Adult	67	5(7.5)	1.47	0.33 – 6.47	0.03	1.38	0.31 – 6.23	0.67
	Old	385	71(18.4)	4.14	1.26 – 13.6	0.02	3.60	1.04 – 12. 47	0.04
Herd size	Small	262	31(11.8)	Ref.	-	-	-	-	-
	Medium	229	46(20.1)	1.87	0.14 – 3.07	0.01	2.18	1.27 – 3.76	0.005
	Large	19	2(10.5)	0.87	0.19 – 3.97	0.46	1.03	0.21 – 5.03	0.97
Managt. type	Intensive	78	6(7.7)	Ref.	-	-	-	-	-
	Semi-intensive	432	73(16.9)	2.44	1.02 – 5.82	0.04	2.65	0.34 – 20.97	0.35
Season	Wet	38	14(36.8)	Ref.	-	-	-	-	-
	Dry	472	65(13.7)	0.27	0.13 – 0.56	0.000	0.18	0.07 – 0.42	0.000

OR= odds ratio; CI= confidence interval

Most of the documented variables revealed a high degree of association with FMDV infection or sero-positivity. The final multivariable logistic regression model (Table 2) revealed that age, herd size and season were significantly associated with the sero-prevalence of the disease (P<0.05). Old cattle were 3.6 times at a higher risk of FMD than young cattle.

Farmers' perception and practices related to FMD

Out of 120 respondents 64(53.3%) and 56 (46.7%) of them responded that their production is dairy cattle and mixed production type (Table 3). Bovine pasteurellosis, blackleg, lumpy skin disease, anthrax and FMD were listed in order of vaccination practice by the respondents (Figure 2).

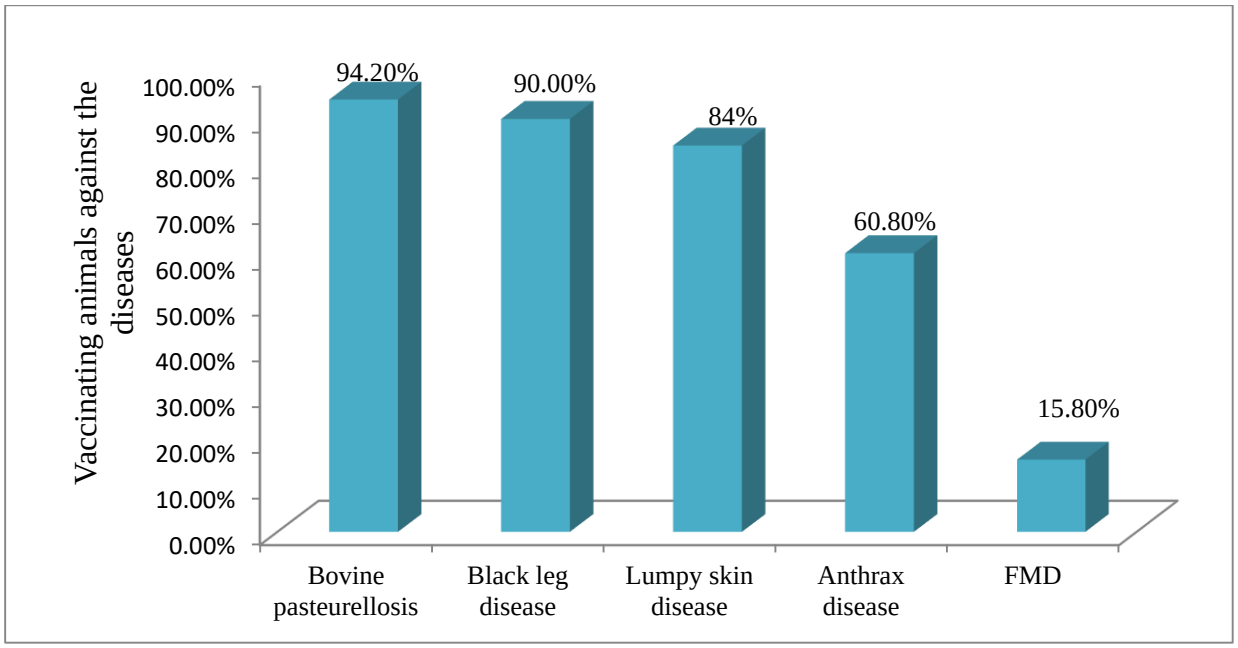

Figure 2: List of diseases and farmers practice to vaccinate their cattle against different animal disease.

Among the major cause for low vaccination practices against FMD in the study area, inaccessibility and unaffordable cost of the vaccine were mentioned by 83.7% (36/43) and 72.1% of the respondents, respectively. Moreover, 68.3% (82/120) of the farmers interviewed don't perceive vaccination as a preventive measure for the disease (Table 4).

Table 4: Farmer's perception on vaccinating their cattle

Variables	Response	Frequency (%)
Dairy cattle production type	Yes	64(53.3)
Mixed production type	Yes	56(46.7)
Vaccinated their cattle against disease	Yes	120(100)
Perceive as vaccination is better than treatment	Yes	104(86.7)
Perceive vaccination as preventive measure against FMD	Yes	38 (31.7)
Vaccinated their cattle against FMD	Yes	19(15.8)
FMD is a common disease	Yes	77(64.2)
Pervious occurrence of FMD in the farm	Yes	66(55%)
Information about FMD	Yes	108(90)
Information about FMD vaccination	Yes	43(35.8)

Sero-prevalence study of FMD

Overall sero-prevalence of FMD recorded in this study (15.5%)was in agreement with the previous findings of15.4% (Mohamoud *et al*., 2011) and 14.05% (Zerabruk *et al*., 2014)from Jijiga zone and Tigray respectively. In contrast, it is higher than previous reports made from different parts of Ethiopia which range from 4.8% - 12.08% (Negussie *et al*., 2011; Abunna *et al*., 2013; Beyene *et al*., 2015; Gelana *et al*., 2016; Belina *et al*., 2016).

Compared to the present finding relatively higher sero-prevalence in bovine was reported as, 24.22%, 38.9% and 21.4% from central Ethiopia (Sulayeman *et al*., 2018), Borena (Melkamsew, 2018) and West Ethiopia (Desissa *et al.,* 2014) respectively. Similarly, higher sero-prevalence of the disease was also reported from the neighboring countries of Africa, 52.5% in Kenya (Kibore *et al*., 2013), 61% in Uganda (Miaron *et al*., 2004) and 72.62% in Nigeria (Lazarus *et al*., 2012). These differences in the prevalence of the disease among the studies could be partly explained by the variation in agro-ecology; epidemiology of the disease and variations in the production or herding systems, vaccination coverage against FMD vaccine, immune status, interaction with cattle with other animals like small ruminants and management type of different study areas.

Cattle managed semi-intensively were shown higher sero-prevalence than those kept under intensive management. Similarly, higher sero-prevalence was previously recorded in cattle kept under semi-intensive managements (Bedru, 2006). Free movement of animals for watering point and grazing areas, and relatively larger herd holding capacity were the possible causes for the disease prevalence difference in different management system. This is supported by the work of previous studies report that the movement of animals in search of feeds from one area to another and interaction of small ruminants is a significant risk factor for the occurrence of FMD (Gelaye *et al*., 2005;Fevre *et al*., 2006; Habiela *et al*., 2010).

In the current study, sero-prevalence of FMD was significantly higher in old animals than in young groups. Similar findings were also previously reported from central Ethiopia (Sulayeman *et al*., 2018) and Awbere and Babille districts of Jijiga zone (Mohamoud *et al*., 2011). Aged caatle are more probably to have been exposed to FMDV during their lifetime and have developed immunity to the virus. Additionally, old animals are driven freely in grazing and watering points where infection could increase by contact (Jenbere *et al*., 2011).

Higher sero-prevalence was recorded during the dry seasons, which might be associated with herd movement to grazing area after crops were collected. This finding is supported by previous study as dry season increase the risk of FMD occurrence (Sarker *et al*., 2011)and also described as FMD is a seasonal disease mostly seen during the dry season (Jibat *et al*., 2013). Because during the dry season, cattle may experience physiological stress due to the factors such as high temperatures, low humidity, and limited availability of fresh forage and water. This can deteriorate their immune response, making them more susceptible to FMD infection and increasing the sero-prevalence.

Nearly 87% of the respondents' perceived vaccination is better than treatment, but only

15.8% of them had vaccinated animals against the disease. Similarly Megersa *et al* (2009) reported that vaccine as prophylactic measures against FMD was accepted by most farmers, but very few of them regularly vaccinate their animals. On other hand, some farmers did not consider vaccination of FMD as significant prevention methods due to self-limiting disease and low mortality among affected animals. In a district, Wondo genet, where farmers perceive and practice vaccine as a preventive measure, lower FMD sero-prevalence was recorded than the other districts.

The study further revealed that 90% of respondents had information about FMD in the selected districts. Similarly 92.5% awareness level was also previously reported from Bale zone (Misgana *et al*., 2013). From the respondents only15.8% vaccinate their cattle against FMD. Lower vaccination practice against FMD was also reported from Nigeria (Olabode *et al*., 2014). However, comparable higher vaccination practices against the disease were reported from Tanzania (Miaron *et al*., 2004, Moenga *et al*., 2013). Inaccessibility and unaffordable cost of the vaccine were mentioned as a leading cause for the low vaccination practice in the current study areas. Moenga *et al*. (2013) and Soko *et al*. (2018) were also stated that aforementioned causes were the major reason for lower vaccination practices of the farmers.

Most of the respondents from the selected districts had experienced FMD outbreak in their farm at least once before the interview. In line with this investigation previous work reported that FMD is endemic, widely distributed and frequently noted in different farming systems and agro-ecological zones of the country (Asfaw and Sintaro, 2000; Sahle, 2004; Leforban, 2005). Despite this fact 82(68.3%) of the farmers interviewed don't perceive vaccination as preventive measure for the disease. In line with this finding most livestock owners don't perceive vaccinating animals against FMDas one of the important preventive measures (Moenga *et al*., 2013; Soko *et al*., 2018).

CONCLUSIONS & RECOMMENDATIONS

The present serological study indicates that the presence of FMD sero-positive animals in the current study areas. Semi-structured questionnaire based surveys indicated that farmers' awareness about FMD vaccine is very low. Even some farmers' having awareness on FMD vaccine, their perception on vaccinating cattle against FMD is significantly low due to inaccessibility and unaffordability of the vaccine. The current research work explored the complex epidemiological situation of FMD and farmer's perception on vaccine against the disease; thus needs more detailed investigation for vaccine-based control methods and improved veterinary extension services.

Authors' contribution

All authors included in this article are directly or indirectly participated in the planning, execution & analysis of this study. MS and TD participated in data gathering, statistical analysis and writing up of the final manuscript. GH andSA participated in editing of the manuscript. AM assisted the laboratory test. All authors read and accepted the final manuscript.

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Ethics approval and consent to participate

The study was approved by college of natural and computational science research proposal review committee, Hawassa University. Oral informed consent was obtained for both questionnaires interview and blood sample collection to keep the privacy of specific farmers at the time of sample collection. All methods employed for this research were carried out in accordance with pertinent guidelines and regulations.

Competing interests

Authors declare no conflict of interest.

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Assessment of Healthcare Solid Waste Composition, Generation and its Management: the case of Two Hospitals of Shashemene Town, Oromia Regional State, Ethiopia

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KEYWORDS:

Health care waste;
General Waste;
Hazardous Waste;
HCW Management;
Solid Waste Generation

Research article

ABSTRACT

Hazardous wastes from Hospitals could pose threat to the health of healthcare workers, the general public and the environment unless managed properly. The study aimed to appraise the healthcare waste (HCW) composition, generation rate and the prevailing management practices in two Hospitals (a Private and a Government owned) of Shashemene Town, Ethiopia. A cross-sectional study involving Direct Observation, Key Informant Interview, Questionnaire survey and Weighting Scale was conducted to evaluate the current HCW management practices and to quantify the HCW generation rate. Data was analyzed using SPSS version 20. The mean generation rates of HCW were 45.2 ± 5.8kg day–1 (0.20kg bed–1day–1) and 20 ± 2.4 kg day–1 (0.19kg bed–1day–1) from Government Hospital (GH) and Private Hospital (PH), respectively. Of the total solid waste generated, over half (GH: 53.3%; PH: 57.1%) constituted general waste (GW), and the remaining (GH: 46.7%; PH: 42.9%) comprised hazardous waste (HW), which exceeded the WHO threshold (10–25%) intimates the lack of poor waste segregation. There were significant variations between the hospital wards regarding GW (GH: $\chi2 = 31$; $P < 0.001$; PH:$\chi2 = 13$; $P < 0.01$), HW (GH: $\chi2 = 25$; $P < 0.001$; PH: $\chi2 = 10$; $P < 0.01$), and total HCW (GH: $\chi2 = 46$; $P < 0.01$; PH: $\chi2 = 22$; $P < 0.01$). Besides, significant differences were observed between the mean total HCW ($\chi2 = 9.016$;$P < 0.01$), GW ($\chi2 = 9.8$; $P < 0.01$) and the HW ($\chi2 = 5.011$, $P < 0.05$) of the hospitals. Segregation of wastes and pre-treatment of infectious wastes were not properly practiced, and single chamber incinerators was the most utilized treatment method indicating poor management of the HCW. The study establishes that the little attention is given to medical waste management which primarily proceeds from a lack of due implementation of the national healthcare wastes management guideline/directive at the healthcare facility level. If the poor healthcare solid waste management is not properly addressed at the study hospitals, human (healthcare workers, waste handlers, patients, and nearby community) and environmental health risk will be within the bounds of possibility.

INTRODUCTION

Healthcare activities are means of protecting health, curing patients, and saving lives (Debere *et al*., 2013). Hospitals are among the complex institutions which generate a broad range of hazardous waste materials in the course of healthcare activities (Farzadkia *et al*., 2009). Healthcare waste is a major problem in most developing countries of the world due to its growing and endless

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generation coupled with poor management (Abd El-Salam, 2010). Healthcare waste contains a large component (75–90%) of non-risk or general healthcare waste, comparable to Municipal Solid Waste (MSW) and a smaller component (10–25%) of hazardous waste may pose a variety of health risks (WHO, 2014).

The process of collecting, storing, transporting, treating and disposing waste material is known as waste management (Al-Khatib and Sato, 2009). Improper waste management in which the infectious waste is mixed with the general waste can lead to the entire bulk of the wastes becoming potentially infectious. It is well known that inappropriate hospital waste management is pressing both health hazards and environmental pollution, facing many healthcare centers of this developing world (Bdour *et al*., 2007). Diseases like Cholera, Dysentery, Skin Infection, and Infectious Hepatitis can spread epidemic way due to the mismanagement of hospital solid waste (Coker *et al*., 2009). Therefore, it is urgent to determine appropriate methods for the safe management of hospital solid waste.

Uncontrolled combustion of medical waste accounted for 26% of the annual total Dioxins / Furans release in 2003 in Ethiopia (EEPA, 2006). Recently, considerable gap exists with regard to the assessment of healthcare solid waste management practices in Ethiopia. Unfortunately, relevant information on this important aspect of healthcare management is inadequate and research on the public health implications of inadequate management of healthcare solid wastes are few in number and limited in scope (Habtetsion *et al*., 2009). Ethiopia is signatory to the Stockholm Convention on Persistent Organic Pollutants (POPs), which is a global convention with the aim of eliminating some of the most long-lived anthropogenic pollutants (UNEP, 2009). While studies illustrate the solid waste menace in the Ethiopian towns and cities, the data on health care solid waste remains in huge paucity both at regional and national level. The present study, therefore, attempted to determine the healthcare solid waste composition, generation rate as well as evaluate its management systems in two selected hospitals of Shashemene town, Oromia region

MATERIALS AND METHODS

Description of the Study Area

Shashemene town is the capital of Shashemene Woreda (District) in West Arsi Zone of Oromia Regional state, Ethiopia. It lies on the Trans-African highway of Cairo-Cape Town, about 250 km from the capital of Addis Ababa. The town is located at Latitude of 7° 12' North and a Longitude of 38° 36' East. In Shashemene Woreda, there are Teaching and Referral (Shashemene Referral Hospital), and Private (Feya General Hospital) Hospitals providing services for more than 122,046 people. Shashemene Referral Hospital has a total of 240 beds with an average patient's flow of 282 patients/day providing Cafeteria, Emergency, General Medicine, Family Planning, Laboratory, Leprosy, TB and Malnutrition, Epilepsy and Psychiatry, Ophthalmic, Pediatrics, Surgical, Pharmacy, and Inpatient Services. Feya General Hospital is private owned hospital and the hospital is engaged in providing

diagnostic and medical treatment in addition to providing other routine services, such as Out-Patient, Laboratory Services, Pharmacy Services, Emergency, Delivery, Family Planning, and Voluntary Counseling and Testing services etc. The Feya General Hospital accommodates a total of 150 beds with average patients/flow of 98 patients/day.

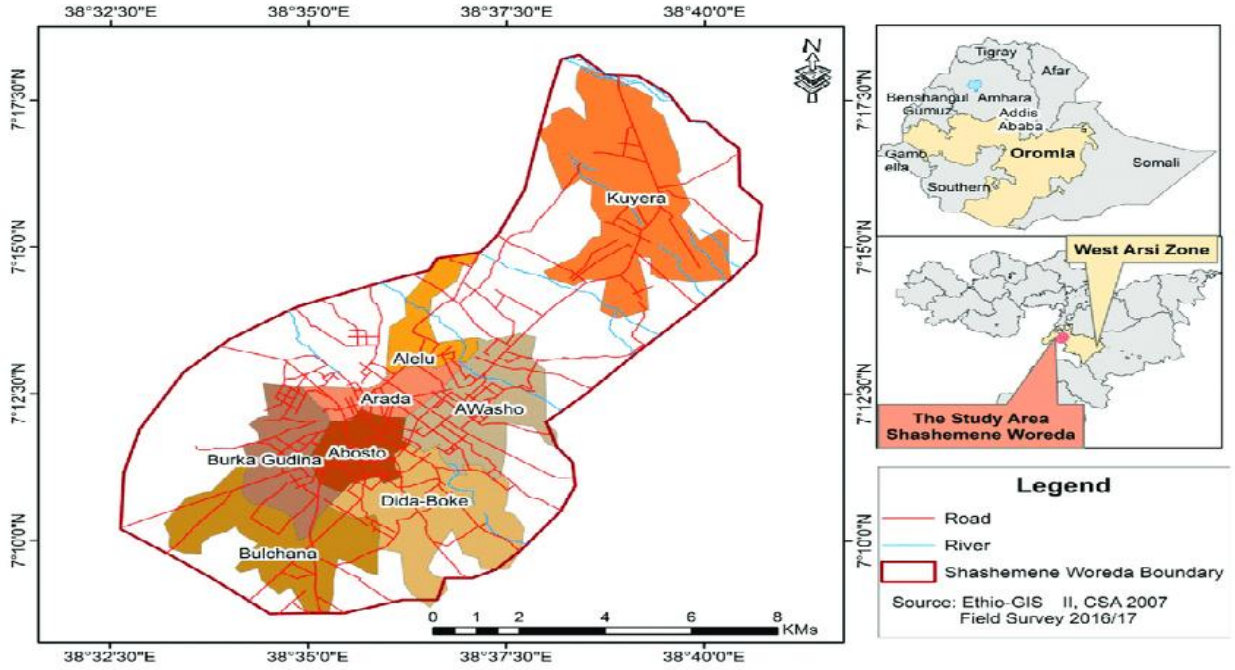

Figure. 1 Geographical map of Ethiopia and Oromia region showing map of the study area (Shashemene town)

The Study Design

It entailed a hospital-based cross-sectional study to evaluate the waste generation rate and its management system in the two hospitals of Shashemene town. The study was conducted in three phases (3 weeks of study). During the first week, a trial collection exercise was conducted before initiation of the regular collection program. The purpose of the first phase (i.e., first week of the study) was to identify the main waste types and characteristics, learn the skills of determining the respective quantities generated from the different departments/Case Teams and plan the daily collection and segregation of waste during the study period. The center of the 2nd phase of study was the management of hospital solid waste while the nub of the 3rd phase was determining the amount of waste generated. In the latter two phases (phase 2 and 3), waste collection and measurement took place for seven consecutive working days. The proposed indicator for the evaluation of hospitals waste management was a daily generated amount of healthcare waste to one bed (general waste per bed per day and hazardous waste per bed per day).

Determination of Sample Size

A stratified random sampling method was used to select the required categories of health

professionals. A random sample is one in which every element in the population has an equal and independent chance of being selected from the sample (Crowther and Lancaster, 2009). The total population size (N) of the two Hospitals was 130, which comprise 75 nursing staffs, 33 medical staffs, and 22 other health and support staffs. This was pooled from government hospital (with 18 medical staff, 50 nursing staff and 12 other health staff) and private hospital (15 medical staff, 25 nursing staffs and 10 other health staff).

The sample size (n) was determined using the Slovin Formula $\frac{N}{1+NE^2}$

where n = Number of samples, N = Total population and E = Error tolerance (level).

Accordingly, the sample size required for the study was calculated to be 98respondents from both hospitals. When fractionated based on proportional allocation, the sample size is constituted of 25medical staffs (15 from Government, and 10 from Private), 56 nursing staffs (35 from Government, and 21 from Private), and 17other health staffs (10 from Government, and 7 from Private).

Data Collection

The method adopted for this study follows the procedure used by Longe and Williams (2006). Accordingly, data collection tools involved questionnaire survey, site visitation (personal observation) and Key Informant Interview. The key informants were purposively selected and includes heath care directors, experts and policy developers in the zone. Both the key informant interview and the Direct Observations were conducted by principal investigators while the questionnaires were administered subsequent to the translation (from English) to the local language of the study area (Afan Oromo). The site visit was conducted by using checklist to review the segregation, handling, collection and storage practices at the various case teams of the study hospitals.

Plastic polyethylene bags and labeled color coded waste containers were used for collection of solid waste from ward, laboratory and departments of the hospitals. The solid waste was manually separated (following appropriate safety precautions) into two categories such as Hazardous and Non-Hazardous as designated in WHO guideline (WHO, 1999). Electronic balance, calculator and recording forms were used for solid waste measurement and recording. Waste generation per day was determined by taking the fraction of the Total Waste produced over the study duration by the length of the study period (7 days) in kg day^{-1}. The solid waste generation can be computed by dividing the total weight of waste (in kg) generated per day with the number of beds in the hospital (i.e., the vacant beds were not considered) expressed as kg/bed/day or dividing the total weight of waste (in kg) generated per day with number of inpatients attended daily in the hospital expressed as kg/patient/day (Kagonji and Manyele, 2011). Likewise, Alagha *et al*. (2018) stated that a universal indicator of Medical Waste (MW) generation is the weight of Healthcare Waste generated per bed per day (kg bed^{-1} day^{-1}) for a given medical facility. Accordingly, waste generation per (occupied) bed per day was calculated as given below:

W_{bd} = [MW weight (kg)]/[(day) × (Bed)] where W_{bd}, is defined as the total weight of MW (in kg) generated per occupied bed per day.

Data Analysis

The data was entered into spread sheet of Microsoft Excel and exported to Statistical Package for Social Sciences (SPSS version 20) for analysis. For testing the Bi-Variate association between Hazardous Waste generated, Patient Flow, and Occupied Beds in study Hospitals, Spearman's Rank Correlation (rs) was computed following Gerald *et al*. (2004). Healthcare Waste (HCW) generation rate and categories of HCW among the different Case Teams in each Hospital were compared using Kruskal-Wallis-test as indicated in Gerald *et al*. (2004). Data from Key Informant Interviews and Direct Observation were analyzed by theme and the content analysis was made manually, by sorting the organized information according to thematic similarities and differences. P-value and rs was reported to present the extent of strength in terms of significant variation and association between two variables, in that order. In all the analysis, level of significance was set at $P< 0.05$.

RESULTS AND DISCUSSION

Healthcare Solid Waste Management Practice

Healthcare Solid Waste Segregation

In general, there is poor segregation of hazardous solid wastes even though the hospitals employ separate receptacles/bins. Besides, as reported by Katusiime (2018), Non-Hazardous Wastes (NHWs)/General Wastes (GWs) were often mixed with infectious wastes in the Government Hospital (GH). Notwithstanding this, both hospitals employed specific/separate bins for the collection of infectious wastes, and sharp wastes were collected in puncture proof safety boxes. Conversely, the hospital solid waste segregation practiced in Private Hospital (PH) was relatively better than the same for the GH. In similar vein, lack of proper HCW segregation in Ethiopia was reported by Tesfahun (2015), Hayleeyesus and Cherinet (2016), Meleko and Adane (2018), and Yazie *et al*. (2019). In Urban Referral Hospital in Uganda, Katusiime (2018) also reported that though waste was generally discarded in large waste bins and sharps in separate sharps containers, the notion of waste segregation was non-existent. On the other hand, as with Tesfahun (2015), the reuse and recycling practice of the NHWs were almost absent in both hospitals; in the GH, however, it was observed that there was some reuse of drug containers (e.g., cans, plastic and bottles) without any precautions. Similarly, Meleko and Adane (2018) found that there were no any observed activities performed by health professionals or other staff to reuse or recycle materials.

In the PH, healthcare solid wastes were merely segregated into infectious, sharps and pathological wastes whereas in GH the segregation was almost absent except the segregation of sharp waste using the safety box. On the other hand, placentas and blood stained cotton pads were kept in separate containers in PH. In the GH, in contrast, anatomical wastes are collected with wound

dressings, placentas and blood/fluid-stained pads in a receptacle outside the wards (Figure. 2d). On the other hand, the use of waste containers with a color code and labelling at the point of generation was implemented in both hospitals. However, it was observed in GH that some receptacles with different color-codes were observed and, hence a color code-label mismatch was practiced (e.g., Blue and Green plastic bins were employed for Infectious Wastes and Non-Infectious Wastes, respectively) (Figure. 3). Yazie *et al*. (2019) in their review of studies conducted on Ethiopian Hospitals indicated that there was no use of proper color-coded bins for waste segregation. This may result in hazardous wastes not only being disposed inappropriately, but also with members of the community gaining access to such wastes. Similar non-compliance had been reported in primary healthcare centers assessments conducted in several developing countries such as Laos, Turkey, Mongolia, among others (Yong *et al*., 2009; Sanida *et al*., 2010). Consequently, as to Katusiime (2018), this will inevitably increase health risks to health workers, waste disposal workers, and the public. generation.

Figure 2. Waste collection and storage systems near the beds (a and b), in corridors (c), outside wards (d) in the Government Hospital, Shashemene, Ethiopia

Figure 3. Cases where there were Color Code–Label Mismatches in the Government Hospital

On the other hand, only 25% of the respondents from GH acknowledged that the wastes were segregated at the point of generation while 50% of the study participants from PH expressed recognition of the practice of segregation at the place of generation

(Table 1). Waste is segregated depending on the quantity, composition, and the disposal method of the waste stream (Shareefdeen, 2012). The present finding was similar with a finding obtained in Addis Ababa (Ethiopia) where almost all of assessed hospitals reported that there was no segregation of wastes and, had no separate bins for the collection infectious waste (Debere *et al*., 2013). As to WHO (2014), segregation of solid wastes should be performed by the producer of the waste as close as possible to its place of generation.

Figure 4. Waste collection and storage systems near the beds (a and b), outside the bed rooms (i.e., in corridors) (c), outside the wards (d) in the Private Hospital, Shashemene, Ethiopia

Healthcare Solid Waste Collection, Interim-Storage and Transport

The arrangements of hospital solid waste collections and storages adjacent to the bed, in the corridor, and outside the wards are presented in Figures 2 and 4. In GH, solid waste generated was usually deposited into the small open plastic bins and open drug cartons close to the bedside, which ultimately produce unhygienic condition near the bed (Figures 2a and b). Conversely, in PH, the solid waste generated in similar location is collected in partially closed receptacles/bins placed away from each bed (Figure 4a and b).

All wards of the GH use substandard dustbins storages, such as trash bags, plastic buckets and drug cartons (that can be easily damaged) to store HCW temporarily in open for about 12–24 hours (Figure 2). In Government Hospitals, Tayework (2016) reported that all hospital solid wastes were temporarily stored in open and substandard dust bins for about unlimited time. The data presented in Table 1 shows that 75% of the respondents from GH indicated that the wastes were collected once per day. Conversely, in PH, colored plastic bins were placed in the designated place (Figure 4a) in each room while leak-proof containers made from stainless steel were placed outside the rooms (Figure 4d). Besides, in PH, healthcare waste was collected on daily basis by cleaning personnel and transported to the on-site handling area. As to the information displayed in the Table 1, 40% and 60% of the respondents from PH laid out that the wastes were collected once and twice a day, in that order. In all studied healthcare facilities, Hayleeyesus and Cherinet (2016) reported that HCW is collected on a daily basis by cleaning personnel and transported to an on-site handling area. On the other hand, in both hospitals, only Pathological and Sharp Wastes were collected, stored and transported in closed plastic buckets/containers within an hour. On the contrary to other Hazardous Wastes (HWs) like potentially infectious and

sharp wastes were collected in puncture-proof containers (Safety Boxes) in both hospitals (Figures 2 and 3a). As to WHO (2014), collection times should be fixed and appropriate to the quantity of waste produced in each area of the healthcare facility and recommends that collection should be carried out daily for most wastes, with collection timed to match the pattern of waste generation during the day.

Table 1. Participants' response on the waste collection, treatment and disposal practices at the surveyed hospitals

Question	Response	Hospital	
		Government	Private
Do you segregate waste at the point of generation?	Yes	3(25%)	5(50%)
	No	7(58.3%)	4(40%)
	I don't know	2(16.7%)	1(10%)
Do you use gloves, boots, masks & caps when handling HCW?	Yes	3(25%)	4(40%)
	No	9(75%)	6(60%)
On-site handling (patient's bed to storage place)	Once per day	7(58.3%)	5(50%)
	Twice per day	1(8.4%)	3(30%)
	Irregular	4(33.3%)	2(20%)
On-site handling (storage place to final disposal)	Once per day	9(75%)	4(40%)
	Twice per day	1(8.3%)	6(60%)
	Irregular	2(16.7%)	0(0%)
Bags were filled with more than ¾ (75%)	Yes	7(58.3%)	6(60%)
	No	3(25%)	4(40%)
	I don't know	2(16.7%)	0(0%)
The filled bags were closed tightly before transferred	Yes	6(50%)	5(50%)
	No	2(16.7%)	2(20%)
	I don't know	4(33.3%)	3(30%)
The filled bags were replaced with empty one at the same time of discharge	Yes	5(41.7%)	7(70%)
	No	7(58.3%)	3(30%)
Vessel used for the transport of a sharp waste was perforated	Yes	8(66.7%)	2(20%)
	No	4(33.3%)	8(80%)
Waste treatment method used	Incineration	9(75%)	6(60%)
	Open burning	2(16.7%)	2(20%)
	Burial	1(8.3%)	2(20%)
Do you treat hazardous HCW differently from general waste?	Yes	5(41.7%)	7(70%)
	No	3(25%)	2(20%)
	I don't know	4(33.3%)	1(10%)

From PH and GH involved in the present study, only 40% and 25% of the respondents, respectively, indicated that waste bags and sharp containers were filled to ¾ full (Table 1). Waste bags and sharps containers should

be filled to no more than three quarters full and once this level is reached, they should be sealed ready for collection (WHO, 2014). On the other hand, 70% and 41.7% of the participants from PH and GH, respectively, made known that the filled bags were replaced with empty one during waste collection (Table 1). Replacement bags or containers should be obtainable at each waste-collection location so that full ones can immediately be replaced (WHO, 2014).

As with Tesfahun (2015), Tayework (2016) and Meleko and Adane (2018), both hospitals lack of proper and purpose-built waste storage rooms; however, there was a temporary waste storage room for pharmaceutical wastes in the PH. Conversely, similar to the findings reported by Meleko and Adane (2018), both hospitals employ interim waste storage (plastic bucket) outside the wards; wastes were stored for 24–36 and 12–24 hours in the GH (Figure 2d) and PH (Figure 4d) Hospitals, respectively. Debere *et al*. (2013) observed that hospital solid wastes were stored in temporary storage area from 2 weeks up to one month before final disposal.

On the other hand, in both hospitals, the Janitors handle the HWs without wearing Personal Protective Equipment (PPE) and empty (discard) the smaller containers into the larger ones which are placed in the corridors and outside the door. Accordingly, 75% and 60% of the respondents from GH and PH, in that order, revealed that the waste handlers did not employ PPE while managing of the wastes. In a similar study by Meleko and Adane (2018), only about 24% of the waste collectors/waste handlers had worn glove and boots during waste collection and transportation of healthcare wastes. In their study on the East and West Kumbo health districts of Cameroon, Dzekashu *et al*. (2017) found that the use of PPE like Gloves (100%) were the most common practice by waste handlers, followed by Aprons (85.2%) and Boots (55.6%).

In GH, HWs and NHWs were mixed outside each room (Figure 2d), and transported to incineration area using wheeled trolleys/handcart (Figure 5a), waste bags (carrying the same by their hands, on their shoulders, or using pushcarts) (Figures 5a and b). Although the transport of HCWs in the PH was comparatively better and principally involved wheeled trolleys/carts (Figure. 5c and d), the same appeared not to be appropriately sized according to the volumes of waste generated at a health-care facility. Moreover, during the transport of the solid wastes from the interim storage receptacles to the treatment area within the hospital premises could entail the possibility of infectious waste droppings on the walkways (Figure 5). Awodele *et al*. (2016) indicated that wheel barrows and trolleys comprised the major means of evacuating the waste whereas Dzekashu *et al*. (2017) observed that transportation of medical waste was virtually (96.7%) executed by lifting, and only 3.3% of the health facilities employed trolleys for transporting wastes.0.05).

Figure 5. Healthcare solid waste transport in the Government (a, b) and Private (c, d) Hospitals

Healthcare Solid Waste Treatment and Disposal Practice

In both hospitals, waste storage and transporting plastic buckets were not treated with disinfectants as suggested by WHO (2014). Besides, as with Tesfahun (2015), none of the hospitals practiced pre-treatment of highly infectious waste. However, the Laboratory Department of the PH treated infectious waste (including Cultures and Stocks, Sharps, materials contaminated with Blood, etc.) by autoclave machine. Conversely, 41.7% and 70% of the respondents from GH and PH, respectively, maintained the belief that HWs should be treated differently from GWs (Table 1). The existing methods employed to treat solid wastes by both hospitals, in their order of importance, were Incineration (brick-made to burn sharp wastes), Open Burning, and Burial (Table 1). As a rule, the choice of treatment system involves consideration of waste characteristics, technology capabilities and requirements, environmental and safety factors, and costs – many of which depend on local conditions (WHO, 2014).

In GH, there was construction of new incinerator for treatment at the time of study because the former one became out of use due to unavailability of gas connection, old and filled with ash (Figure 6a). Consequently, the partially burned healthcare solid waste was further burnt and disposed of in an open pit within the GH (Figure 6b and c). In the PH, conversely, the incinerator was not only properly functioning as depicted by incomplete burning of waste (Figure 7a and b), but also was located close by the residential area of the medical staff within the hospital premises. Consequently, apart from failure to significantly reduce the volume of treated waste, incinerators of both hospitals generated a plume of smoke to their immediate environments. The use of low combustion single-chamber incinerators for the treatment of healthcare waste was against the Stockholm Convection on Persistent Organic Pollutants (POPs) (UNEP, 2009) since such incinerators release air pollutants to the environment (Diaz *et al*., 2005).

On the other hand, both hospitals had an open hand dug pit in their backyard that was used for the open burning and direct dumping of GWs. While the GH had an open placental pit for disposal of pathological and anatomical

waste generated from the delivery and operation rooms (Figure. 6d), the PH employed one closed placental pit (Figure. 7c) and two closed ash pits. As with the case reported by Meleko and Adane (2018), HCWs in both hospitals were not disposed of in appropriate sealed and labeled containers. Such malpractices were also reported in Jos Metropolis, Nigeria (Ndidi *et al*., 2009). Generally, each component of HCW management practices in both surveyed hospitals did not conform to the Ethiopian National Healthcare Waste Management Guideline. Such poor HCW management were also reported in similar studies conducted in Nigeria (Di Bella *et al*., 2012), South Africa (Nemathaga *et al*., 2008), and Iran (De Titto *et al*., 2012).

Figure 6. Solid waste treatment methods: (a) Incineration, (b and c) open burning at waste dump site and (d) Anatomical, Pathological and Placental burial pits in the Government Hospital of Shashemene town.

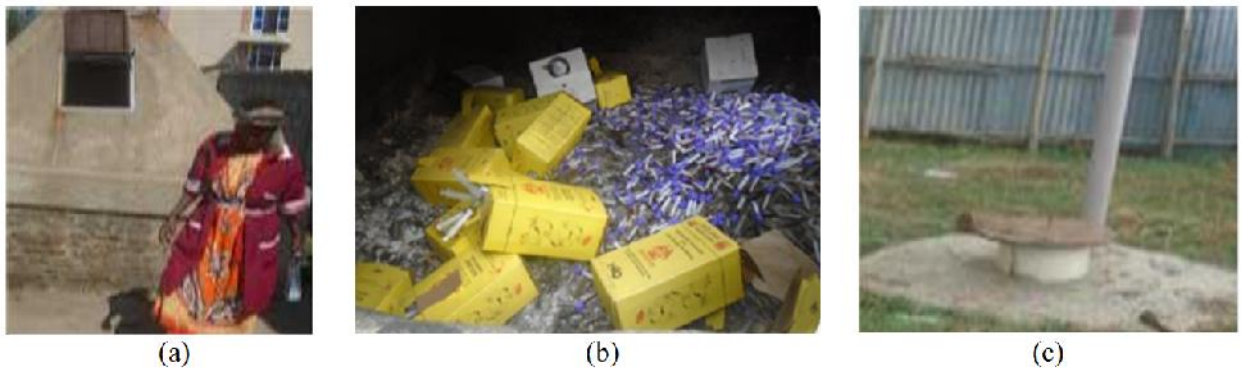

Figure 7. Solid waste treatment methods: Incineration (a and b), and (c) Anatomical, Pathological and Placental pit employed in the Private Hospital

Waste Generation and Characterization

The total weight of HCW generated in GH and PH in Shashemene town were 316.5 kgweek^{-1} and 140 kg week^{-1}, respectively. Conversely, an average total of 45.2 ± 5.8kgday^{-1}(0.20kg bed^{-1}day^{-1}) of HCW was generated from GH whereas only below half

as much (20 ± 2.4kg day^{-1} or 0.19kg bed^{-1}day^{-1}) of the same was produced from PH (Tables 2, 3, and 4). Although the above generation rate was somehow comparable to 0.164 kg bed^{-1}day^{-1} reported by Meleko and Adane (2018), the same was lower than a result obtained in Ethiopia (1.5kgbed^{-1}day^{-1}: Tesfahun, 2015), Bangladesh (1.24kgbed^{-1}day^{-1}: Hassan *et al*., 2008), Egypt (1.03kgbed^{-1}day^{-1}: Shouman *et al*., 2013) and France (3.3kgbed^{-1}day^{-1}: Windfeld and Brooks, 2015). Variations in waste generation hinges on the type or level of healthcare facility (WHO, 1999; Bdour *et al*., 2007; Haylamicheal et al., 2011; WHO, 2014), hospital specializations (WHO, 1999), location (Rural or Urban) (WHO, 2014), established waste management methods, proportion of reusable items employed in healthcare facilities (WHO, 1999; Bdour *et al*., 2007; Haylamicheal *et al*., 2011), level of activity (number of occupied beds, number of patients per day, and/or number of staff), type of department, temporal variations (e.g. weekday versus weekend, seasonal), and level of infrastructure development of the country (Bdour *et al*., 2007; Haylamicheal *et al*., 2011; WHO, 2014).

Table 2. Distribution and healthcare solid waste generation rates by point source and type in Government Hospital of Shashemene

Government Hospital	**Non-Hazardous Waste (Kg week^{-1})**	**Hazardous Waste (Kg week^{-1})**				**Total Hospital Waste (Kg Week^{-1})**	**Percentage of Total Hospital Waste (%)**	**Average Daily Hospital Waste (Kg Day^{-1})**
		IW	**PhW**	**SW***	**PaW**			
OPD	30.3	5.9	14	0.6	7.0	57.8	18	8.3
Surgical	27.9	4.7	3.9	1.5	-	38	12	5.4
Pediatrics	20.4	7.3	3.5	4.7	8.1	44	14	6.3
Obs/Gyn.	16.5	9	9	5.2	10.2	49.9	16	7.1
Medical	27.6	10.5	2.4	1.6	3.1	45.2	14	6.5
Laboratory	15.9	5.5	8.9	2.7	9.1	42.1	13	6.0
Emergency	30	4.2	-	3.2	2.1	39.5	13	5.7
Total (Kg week^{-1})	***168***	***47.1***	***41.7***	***19.5***	***39.6***	***316.5***	***100***	
Mean ± SD (Kg Day^{-1})	**24.1 ± 6.3**	**6.7 ± 2.3**	**6.0 ± 4.8**	**2.8 ± 1.7**	**5.7 ± 3.9**	**45.2 ± 5.8**	**-**	**45.2 ± 5.8**
% wt. by Type	53.3	14.8	13.2	6.2	12.5	100	-	

IW = Infectious Waste; PhW: Pharmaceutical Waste; SW: Sharp Waste; PaW: Pathological Waste
**Includes Needles, Blades, Lancet Needles, Syringes, and Scalpel Blades*

Of the total waste generated in GH, 53.3% (24.1 ± 6.3 kg day^{-}1 or 0.1 kg bed^{-1}day^{-1}) was GW while 46.7% (21.1 ±3.2 kg day^{-1} or 0.091kg bed^{-1}day^{-1}) was HW (Tables 2 and 4). Equally, of the total waste generated in PH, 57.1% (11.4 ± 2 kg day^{-1} or 0.11 kg bed^{-1}day^{-1}) was GW whereas 42.9% (8.6 ± 1.3 kg day^{-1} or 0.079 kg bed^{-1}day^{-1}) was HW (Tables 3 and 4). A little over half of the total HCW generated from the study hospitals (GH:

53.3%; PH: 57.1%) constituted GW. Meleko and Adane (2018) reported that 0.091 kg /bed/ day (55.5%) was GW and the remaining 0.073 kg/bed/day (44.5%) was HW. Likewise, in similar studies conducted in Ethiopia, Azage and Kumie (2010), Debere *et al*. (2013), and Hayleeyesus and Cherinete (2016) the GW accounted for the 52, 58.69, and 65.1% of the total HCW. Moreover, the result of the present study is comparable with a results obtained from healthcare facilities in Ethiopia where 48% (Azage and Kumie, 2010), 41.31% (Debere *et al*., 2013), and 42.1% (Meleko and Adane, 2018) of HCW were HW. Conversely, the present finding was much bigger than a result identified in Sudan where only 20% of the total HCW generated was HW (Ahmed *et al*., 2014).

On the other hand, between 75% and 90%of the waste produced by health-care providers are actually NHWs or GWs, and the remaining 10–25% is HWs in nature (WHO, 2014). It is apparent that the proportions accounted by HWs from the present study were higher than the same reported by the WHO (2014). Yazie *et al*. (2019) pointed out that the fractions of HW generated from healthcare facilities were intolerably high with a range stretching from 21–70% of the total solid waste. As indicated by Hayleeyesus and Cherinete (2016), the higher proportion of HWs in the present study (as well as in most healthcare facilities in Ethiopia) could be ascribed to the lack of segregation of waste at the point of generation. In a study done on private and government hospitals, Debere *et al*. (2013) found that the HW and non-HWs were mixed in the hospital's temporary storage areas.

Table 3. Distribution and healthcare solid waste generation rates by point source and type in Private Hospital of Shashemene

Private Hospital	**Non-Hazardous Waste (Kg week^{-1})**	**Hazardous Waste (Kg week^{-1})**				**Total Hospital Waste (Kg Week^{-1})**	**Percentage of Total Hospital Waste (%)**	**Average Daily Hospital Waste (Kg Day^{-1})**
		IW	**PhW**	**SW ***	**PaW**			
OPD	11.5	0.5	4.1	1.5	3.0	20.6	14	2.9
Surgical	12.1	2.3	2.5	2.5	2.2	21.6	15	3.1
Pediatrics	13.3	3.1	2.4	0.8	3.2	22.8	17	3.3
Obstetrics/Gyn.	11.9	2.0	0.7	1.6	4.1	20.3	15	2.9
Medical	8.2	1.9	4.2	2.2	1.5	18	13	2.6
Laboratory	9.4	4.3	-	2.0	-	15.7	11	2.2
Emergency	13.6	1.2	4.6	0.4	1.2	21	15	3.0
Total (Kg week^{-1})	***80***	***15.3***	***18.5***	***11***	***15.2***	***140***	***100***	
Mean ± SD (Kg day^{-1})	**11.4 ± 2.0**	**2.2 ± 1.2**	**2.6 ± 1.8**	**1.6 ± 0.8**	**2.2 ± 1.4**	**20 ± 2.4**	**-**	**20 ± 2.4**
% wt. by Type	57.1	10.9	13.2	7.9	10.9	100	-	

IW = Infectious Waste; PhW: Pharmaceutical Waste; SW: Sharp Waste; PaW: Pathological Waste
**Includes Needles, Blades, Lancet Needles, Syringes, and Scalpel Blades.*

The types of hazardous wastes generated from the study hospitals were infectious, pharmaceutical, pathological, and sharps. In both hospitals, the infectious [GH: 14.8%; PH: 10.9%] and pharmaceutical [GH: 13.2%; PH: 13.2%] wastes dominate the HWs category while sharp wastes [GH: 6.2%; PH: 7.9%] contribute for the lowest fractions to the total as well as to the selfsame category in either hospital (Tables 2 and 3). In a similar vein, Hayleeyesus and Cherinete (2016) found that infectious waste (21.1% of the total HCW) dominated the HW fraction while the sharp waste contributed the least (1.5%) to the total solid waste from the healthcare facilities.

Table 4. Average daily healthcare solid waste generation by types of wastes in Shashemene hospitals

Types of Waste	Type of Hospital			
	Government		Private	
	Kg Day^{-1}	**Kg Bed^{-1} Day^{-1}**	**Kg Day^{-1}**	**Kg Bed^{-1} Day^{-1}**
General Waste	24.1	0.1	11.4	0.11
Infectious Waste	6.7	0.03	2.2	0.02
Pharmaceuticals	5.9	0.025	2.6	0.024
Sharps	2.8	0.012	1.6	0.015
Pathological Wastes	5.7	0.024	2.2	0.02
Total HCSW	**45.2**	**0.20**	**20**	**0.19**

HCSW stands for Healthcare Solid Waste

In the GH (Table 2) the highest percentage of the total HCW was generated from OPD (18%) followed by Obstetrics/Gynecology (16%) while the lowest proportion of total waste was generated from Surgical Ward (12%). On the other hand, the Pediatric Ward (17%) followed by Obstetrics/Gynecology, Emergency, and Surgical wards (15% each) contributed for the highest fractions of total waste generated from PH whereas the waste proceeding from the Laboratory (11%) was the lowest proportion of the total HCW (Table 3). Meleko and Adane (2018) reported that the largest portions of total waste were contributed by Gynecological Ward followed by Medical Ward while the lowest proportion was generated in Office.

As to the data presented in Table 5, there were significant differences in the different wards of the Government Hospital with respect to general waste (χ^2 = 31; P <0.001), hazardous waste (χ^2 = 25; P <0.001), and total healthcare waste (χ^2 = 46; P <0.01). Likewise, significant variations were observed in the Private Hospital wards regarding the general waste (χ^2= 13; P <0.01), hazardous waste (χ^2 = 10; P <0.01), and total healthcare waste (χ^2 = 22; P <0.01). Similarly, Meleko and Adane (2018) reported that there were significant variations among the different wards in relation to general waste (χ2=41.815;P< 0.01), hazardous waste (χ2=44.324;P<0.01), and total healthcare waste (χ2 = 44.604;P< 0.01).

Table 5. Comparison of mean generation rate of healthcare solid waste and categories of HCSW by departments within the government and private hospitals using Kruskal Wallis H test

Wards	Type of Hospital					
	Government			Private		
	GW	HW	THCW	GW	HW	THCW
OPD	30.3	27.5	57.8	11.5	9.1	20.6
Surgical	27.9	10.1	38	12.1	9.5	21.6
Pediatrics	20.4	23.6	44	13.3	9.5	22.8
Obs/Gyn.	16.5	33.4	49.9	11.9	8.4	20.3
Medical	27.6	17.6	45.2	8.2	9.8	18
Laboratory	15.9	26.2	42.1	9.4	6.3	15.7
Emergency	30	9.5	39.5	13.6	7.4	21
Chi-Square	31	25	46	13	10	22
P-Value	0.0001	0.0001	0.001	0.001	0.001	0.001

GW: General waste; HCW: Healthcare Waste; HW: Hazardous Waste; Obs/Gyn: Obstetrics/Gynecology; OPD: Outpatient Department

According to Table 6, there were significant differences between the mean total HCW ($\chi2$ = 9.016;*P*< 0.01), general waste ($\chi2$ = 9.8;*P*< 0.01) and the hazardous waste ($\chi2$ = 5.011;*P*< 0.05) of the study hospitals. Similar to the findings of the present study, Debere *et al*. (2013) reported that there was statistically significant difference for total amount of HCW ($\chi2$ = 30.65;*P*< 0.001),HW ($\chi2$ = 20.431;*P* < 0.01) and NHW ($\chi2$ = 29.011;*P*< 0.001) among the surveyed hospitals.

Table 6. Comparison of healthcare waste generation rates and categories of HCW using Kruskal-Wallis test among the surveyed hospitals

Type of Hospital	Mean Rank		
	Total HCW*	GW**	HW***
Government	10.86	11.00	10.00
Private	4.14	4.00	5.00
Chi-square	9.016	9.800	5.011
P- Value	0.003	0.002	0.025

** Healthcare Waste, ** General Waste, *** Hazardous Waste*

The Spearman's rank correlation coefficient (r_s) (Table 7) showed that there was a positive linear relationship between number of patients and quantities of hazardous waste generation rates in both PH (r = 0.954, P< 0.05) and GH (r = 0.847, P< 0.01). Similarly, there was positive correlation coefficients among HW generation rates and occupied beds in both PH (r = 0.964, P< 0.05) and GH (r = 0.821, P< 0.001) (Table 7). Moreover, positive associations were observed between patient flow and occupied beds in PH (r = 0.991, P< 0.01) and GH (r = 0.955, P< 0.05). In their study on HCWs, Tadesse and Kumie (2014) found that there was a positive linear relationship between number of patients and

the HCWs generated in all government facilities studied. Conversely, Issam *et al*. (2009), Haylamicheal *et al*. (2011), and Komilis *et al*. (2011) reported that there was a positive correlation between the total HCW generation rates and the number of beds.

Table 7. Spearman's Correlation Matrix (r_s) between Hazardous Wastes (HW) generated, Patient Flow and Occupied Beds in Private and Government Hospitals

Government Hospital		HW	Patient Flow	Occupied Beds
Spearman's rho	HW	1.00	0.954**	0.964***
	Patient Flow		1.00	0.955**
	Occupied Beds			1.00
Private Hospital		HW	Patient Flow	Occupied Beds
Spearman's rho	HW	1.00	0.847*	0.821*
	Patient Flow		1.00	0.991**
	Occupied Beds			1.00

, **, and * represent P < 0.05, P < 0.01, and P < 0.001, in that order*

CONCLUSION

Although the HCW generations rates were relatively low, the standard HCW segregation was lacking in both hospitals. Consequently, except the sharp materials, all other HCWs were mixed with the GWs (mainly in GH), and hence, the proportion of HWs generated in the study hospitals surpassed the corresponding threshold measure indicated by the WHO. Besides, the waste collectors handle the HCWs without employing PPE, exposing themselves to potential health risk. Both hospitals principally treated HCW using low combustion single-chamber incinerators which could potentially contribute to the release of huge amounts of air pollutants to the environment.

As the HCW management in both hospitals was poor, sufficient resource allocation, periodic training, and strict supervision and proper implementation of HCW management is pivotal. Additionally, heath care facilities in Oromia region, should acquaint HCW management guideline for standardized waste categorization and safer handling. Besides, the key stakeholders of the region involving heath care directors, experts and policy developers should join hands in structuring the HCW management system in a way that appraises human and environmental health. For this, further researches need to be conducted in health care facilities of the region and the wider nation with consideration of human health risk assessment.

Ethical Consideration

Ethical clearance was obtained from Zonal Health Office whereas an approval to conduct the study was secured from the Managers/Directors of the respective Hospitals. The experimental procedures were explained to the individual participants and thereafter their consent to participate in the study was obtained. The participants were free either to participate or not. Besides, the right

of respondents to interrupt or withdraw the interview when they deem it necessary was duly respected. Data collectors were trained touse protective materials when handling healthcare wastes. Privacy and confidentiality were assured by way of employing anonymity.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Effect of 17α-Methyl Testosterone on Sex Reversal and Growth Performance of Nile tilapia, *Oreochromis niloticus*

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KEYWORDS:

Sex-reversal;

Methyl testosterone;

Mono-sex production;

Hapa;

Nile tilapia

Research article

ABSTRACT

The present study aimed at developing all-male Nile tilapia using 17α-methyl testosterone (17α-MT) along with its growth and feed utilization performances. Three days old Nile tilapia fry were stocked in plastic jars with 5 L capacity installed in four fiber glass tanks. The fry were fed with 0, 30, 60, and 100 mg MT/kg diets for 30 days. Later, the fry were shifted to hapas installed in a pond and then, reared for four months separately. The fish were fed with the control diet. The results showed that the highest male population (93.6%) was observed in the fish treated with a 60 mg MT/kg diet, while the lowest (82.6%) was observed in the fish treated with a 30 mg MT/kg diet. The results also showed that the fish fed with a 60 mg MT/kg diet had significantly higher mean body weight (24.1±1.40 g), specific growth rate (2.5±0.10%), feed conversion ratio (1.3±0.10), and protein efficiency ratio (0.63±0.11) than the untreated fish treated (control) group. In conclusion, a 60 mg MT/kg diet can be considered as an optimal and economically viable dose for Nile tilapia sex reversal along with its optimum growth and feed utilization performances.

INTRODUCTION

Among several cultivable fish species, tilapia has been identified as the second most important aquaculture fish species in the world, particularly in the tropical and sub-tropical countries next to carp (El-Sayed, 2006; Dagne *et al*., 2013). It is also considered as one of the most important traded fish in the world (Kyule *et al*., 2014; Magbanua and Ragaza, 2022). Farmed tilapia production increased significantly from 383,654 tons in the 1990s to 4,514,615 tons of production in 2020 (FAO, 2022). Basically, for optimal production performance of the semi-intensive fish culture, high quality fish feed are the most preferred due to their good palatability and digestibility required for body maintenance, growth, reproduction and health (Howlader *et al.*, 2023).

Although Nile tilapia has such good characteristics, its precocious and prolific reproduction and early sexual maturation of female has become one of the main challenges

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for considering this species for commercial aquaculture (Chakraborty and Benerjee, 2012; Munguti *et al.,* 2014). This resulted in the reduction of growth rate at the onset of sexual maturation and the production of a large number of fry/fingerlings (Munguti *et al.,* 2014). Considering the differential growth patterns of males and females, all-male Nile tilapia production has been given better consideration as males are capable of showing a better growth rate and food conversion efficiency (Chakraborty and Banerjee, 2012). It is in this context, production of all-male populations of Nile tilapia is vital for increasing fish production under low management practices (El-Sayed, 2006). Among the various techniques employed, hormonal sex-reversal has been considered as an effective method (Ferdous and Ali, 2011; Jamila *et al.,* 2017). Even though it is the most important method, reports of different authors are not consistent (Wahby and Shalaby, 2010; Celik *et al.,* 2011; Lakshmi, 2015). Celik *et al.* (2011) reported that the efficiency of such a method can be affected by different environments, feeding rates, and feeding management, and overall production management. Hence, the evaluation of such method in a specific production environment is crucial. Thus, the main objective of this study was to find out an optimum dose of 17α-methyl testosterone on the proportion of Nile tilapia male population along with growth performance and feed utilization efficiency of Nile tilapia under a semi-intensive production system.

MATERIALS AND METHODS

Experimental setup

The experiment was carried out at Ziway Fishery and Other Aquatic Life Research Centre, Ziway. The research Centre is situated at 163 km Southeast direction of Addis Ababa, the capital city of Ethiopia. It is located at $7^{o}52'$ to $8^{o}8'$ N latitude and $38^{o}40'$ to $38^{o}56'$ E longitude, at an altitude of 1636 m above sea level. For this study, indoor and outdoor experimental setups were used. For the indoor experiment, four fibre glass tanks each with a size of 2000 ML were prepared. Within each tank, three plastic circular shape jars with 5 L capacity were installed for hormone-treated feed trial experiments. For the outdoor experiment, 12 hapas with 1.5 m x 2 m x 1 m size were installed in a concrete pond. Before installation of the hapas, the pond was dried for one week and then, refilled with water at depth of 90 cm.

Source of experimental fish and feed formulation

Sexually matured Nile tilapia broodstock that measured 200 to 250 g body weight were selected from the holding tank of the Ziway research centre and immediately transferred into hapas with 1.5 m x 2 m x 1 m size installed in a pond at a stocking density of 4 fish/hapas with a sex ratio of 1 male to 3 females for mating (ref.). From these hapas, newly hatched Nile tilapia fry were collected and transferred in plastic bottles having 5 L water holding capacity for feed trial experiments. The experiment was conducted in triplicates with four treatments (i.e., 0, 30, 60, and 100 mg MT/kg of diets).

Fish diet was formulated by mixing 43% of fishmeal, 36% of Niger cake, 10% of wheat bran, 7% of white corn flour, 2% of vitamins and mineral premix, and 2% of sunflower oil, having 38% of crude protein. Later, the mixed ingredients was divided into four groups, in which the first one was not treated with 17α-

methyl testosterone (0 mg MT/kg of diets) was used as a control diet, while the remaining three groups were treated with 30, 60 and 100 mg MT dissolved in 95% ethanol per kilogram diet and were used as tested diets. The control diet was also mixed with the same amount of ethanol without hormone. Then, the diets were dried at room temperature for 24 hours and were stored in a refrigerator (Celik *et al*., 2011).

Indoor and outdoor experiments

Immediately after hatching, 300 newly hatched fry collected from the hapas were transferred into 12 plastic bottles installed in 4 fiberglass rearing tanks for three days of acclimatization. Later, after hatching 240 fry, 20 fry per plastic bottle, with an average body weight of 0.05 g were distributed in 12 plastic bottles having 5 L water holding capacity installed in four fiberglass rearing tanks in triplicates and reared for one month. To facilitate water circulation, the bottles were provided with small holes which were less than the size of the fry. The fry were, then, fed four times at 8:00, 12:00 and 16:00 and 18:00 hours a day with 0 mg MT, 30 mg MT, 60 mg MT and 100 mg MT/kg diets having 38% crude protein for 30 days at 20% of their body weight (Shamsuddin *et al*. (2012). Early morning, uneaten food and faecal matter were removed daily using siphoning. Oxygen was provided to each tank using aerators. After 30 days of treatment, total body weight of the all fry were measured and then transferred and stocked in hapas with a size of 1.5 m x 2 m x 1 m installed in the grow-out pond at an average stocking density of 18 fish per hapa and reared for four months. During this time, the fish were fed three times at 9:00, 13:00 and 17:00 hours a day with a control diet at 10% of their body weight (Mugo-Bundi (2013). The overall research procedure was approved by the research committee of Hawassa University and thus, all applicable international and national guidelines for the care of animals and use of animals were followed by the authors.

Data collection and sex determination

Every 15 days (2 weeks) of interval, individual body weight and body length of hormone-treated fish stocked in plastic bottles were recorded early morning. The mortality of the fry was recorded daily. Similarly, every 15 days of interval, individual body weight and body length of the experimental fish stocked in hapa were recorded. At the end of the experiment (on week 16, i.e. harvesting week), the final body weight and body length of all the fish were recorded. In addition, the sex of each fish was identified based on external and internal observation of the sex organs of the fish. Iodion solution as dye was used to differentiating the sex of the fish when the secondary sexual characters were found difficult to differentiate. Ten fish per treatment were dissected and the morphology of the gonads was examined and recorded. Water quality parameters such as water temperature, pH, dissolved oxygen, conductivity, and total dissolved solid were measured using Potable Multi-Parameter Kit.

Following final body weight and length measurements, calculation of growth parameters were performed using the following formula described by Eyo *et al.* (2013).

I. Calculation of growth and feed utilization efficiency:
   - Body weight gain (BWG) = Final body weight (FBW) – Initial body weight (IBW)

- Daily growth rate (DGR) = Weight gain/Number of experimental days
- Specific growth rate (SGR % per day) = ((LnFBW- LnIBW)/ Number of days) *100

II. Calculation of feed utilization efficiency:
- ✓ Food conversion ratio (FCR) = Amount of dry food intake/Weight gain
- ✓ Protein efficiency ratio (PER) = Weight gain/amount of crude protein

III. Calculation of survival rate, condition factors and fish yield (put Ref. for each formula):
- ✓ Survival rate (SR%) = Number of harvested fish/Number of stocked fish *100
- ✓ Condition factor (CF) = Final body weight/ (Length3) x 100
- ✓ Total production = (No. of fish harvested × FBW/Area of rearing place) x10000 m2 x production cycle

Statistical analysis

Based on the data recorded and calculated values basic statistics were computed using SPSS 20 version after the data were tested for normality and equal variance. The statistical significance among growth parameters and sex ratio of fish fed with different test diets were computed using one-way ANOVA (Analysis of Variance) in the SPSS. Significance was assigned at a 5% level of probability. For between mean treatments significant variation, was performed using Tukey HSD standardized range test α = 0.05 level of significance as described according to El Greisy and El-Gamal (2012).

RESULTS

Indoor growth and survival rate of Fry

The mean body length and weight of fry at stocking and after 30 days of hormone treatment of all groups are presented in Table 1. The initial mean body length and body weight of fry were the same (1.3 cm and 0.05 g). After one month of rearing, the length of fry ranged from 3.3±0.12 cm (30 mg MT/kg diet) to 3.7±0.30 cm (60 mg MT/kg diet) while the mean body weight of the fry ranged from 0.81±0.18 g (60 mg MT/kg diet) to 0.84±0.13 g (control diet). Additionally, the survival rate of the fish in different treatments was high, ranging from 88.2±2.1 to 92.2±1.6%. In all cases, there was no significant (P > 0.05) difference in survival rates among the treatments (Table 1).

Table 1: Mean body size parameters with standard error (Mean + SE) of fry at stoking and after 30 days of oral administration with different doses of 17α-methyl testosterone (17α-MT).

Parameters	Treatments			
	0 mg MT/kg diet	30 mg MT/kg diet	60 mg MT/kg diet	100 mg MT/kg diet
Initial body length (cm)	1.3±0.37	1.3±0.37	1.3±0.37	1.3±0.37
Initial body length (g)	0.05±0.02	0.05±0.02	0.05±0.02	0.05±0.02
Body length (cm) after 30 days of treatment	3.4±0.20[a]	3.3±0.12[a]	3.7±0.30[a]	3.4±0.20[a]
Body weight (g) after 30 days of treatment	0.84±0.13[a]	0.82±0.20[a]	0.81±0.18[a]	0.82±0.15[a]
Survival rate (SR%)	90.2±1.4[a]	90.2±1.2[a]	92.2±1.6[a]	88.2±2.1[a]

Note: Values with the same letter across a row are not significantly different (P > 0.05)

Effects of MT on sex ration

The sex ratio of Nile tilapia fed with hormone-treated diets is presented in Table 2. The gonad differentiation of fish fed with the MT-treated diets gave a higher male proportion than fish fed with the control diet. The maximum male population (93.6%) was observed for the fish fed with a 60 mg MT/kg diet followed by a 100 mg MT/kg diet (86.7%), while the lowest male population was observed for the fish fed with the control diet (54.3%). The results showed that the fish fed with a 60 mg MT/kg diet produced significantly ($P < 0.05$) higher male population than the other groups while the fish fed with the control diet produced the least significant.

Table 2: Percentage of male population of Nile tilapia produced after oral administration with different doses of 17α-methyl testosterone (17α-MT).

Dose of MT	Male %	P value
0 mg MT/kg (control) diet	54.3^{a}	0.35
30 mg MT/kg diet	82.6^{b}	< 0.001
60 mg MT/kg diet	93.6^{c}	< 0.001
100 mg MT/kg diet	86.7^{b}	0.005

Note: Values with the same letter across column are not significantly different ($P > 0.05$)

Outdoor growth performance

The different growth parameters such as mean body weight gain, daily growth rate, and specific growth rate, and feed utilization efficiency such as feed conversion ratio and protein efficiency ratio of Nile tilapia fed with different doses of MT-treated diets are presented in Table 3. The results showed that the highest mean body weight (24.1±1.4 g) and body weight gain (24.0±1.2 g) were recorded in the fish fed with 60 mg MT/kg diet followed by 100 mg MT/kg diet (19.1±0.8 g and 19.0±0.2 g), while the lowest (14.3±1.8 g) and 14.2±0.1 g) was observed for the fish fed with a control diet, respectively. Similarly, the highest mean body length (11.1±1.2 cm) was observed for the fish fed with 60 mg MT/kg diet, followed by 10.2±1.2 cm mean body length of fish fed with100 mg MT/kg diet, while the lowest (9.1±0.79 cm) was observed for the fish fed with control diet (Table 3). The results also showed that the fish fed with hormone treated diets had significantly ($P < 0.05$) higher final mean body weight, in which the fish fed with 60 mg MT/kg diet had significantly ($P < 0.05$) higher final mean body weight, and mean weight gain than at least from the fish fed with the control diet. Figure 1 also showed the growth trend of body weight of the fish, in which fish body weight increased steadily for the first six weeks, and then, the rate of growth slightly increased.

Table 3: Mean growth parameters with standard error (Mean + SE) of Nile tilapia treated with different doses of 17α-methyl testosterone (17α-MT).

Parameters	Treatments			
	0 mg MT/kg diet	30 mg MT/kg diet	60 mg MT/kg diet	100 mg MT/kg diet
Initial body length (cm/fish)	1.3±0.37[a]	1.3±0.37[a]	1.3±0.37[a]	1.3±0.37[a]
Initial body weight (g/fish)	0.05±0.02[a]	0.05±0.02[a]	0.05±0.02[a]	0.05±0.02[a]
Final body length (cm/fish)	9.1±0.79[a]	9.6±1.03[a]	11.1±1.19[a]	10.2±1.17[a]
Final body weight (g/fish)	14.3±1.84[a]	18.3±2.46[b]	24.1±1.38[b]	19.1 ±0.79[b]
Body weight gain (g/fish)	14.2±0.02[a]	18.2±0.02[b]	24.0±0.21[c]	19.0±0.21[b]
Daily growth rate (g/fish/day)	0.12±0.01[a]	0.15±0.01[ab]	0.20±0.1[b]	0.18±0.01[ab]
Specific growth rate (%/fish/day)	2.2±0.2[a]	2.3±0.01[ab]	2.5±0.1[b]	2.4±0.1[ab]

Note: Values with the same letter across rows are not significantly different (P > 0.05)

The results also showed that the fish fed with hormone treated diets had significantly (P < 0.05) higher final mean body weight, in which the fish fed with 60 mg MT/kg diet had significantly (P < 0.05) higher final mean body weight, and mean weight gain than at least from the fish fed with the control diet. Figure 1 also showed the growth trend of body weight of the fish, in which fish body weight increased steadily for the first six weeks, and then, the rate of growth slightly increased.

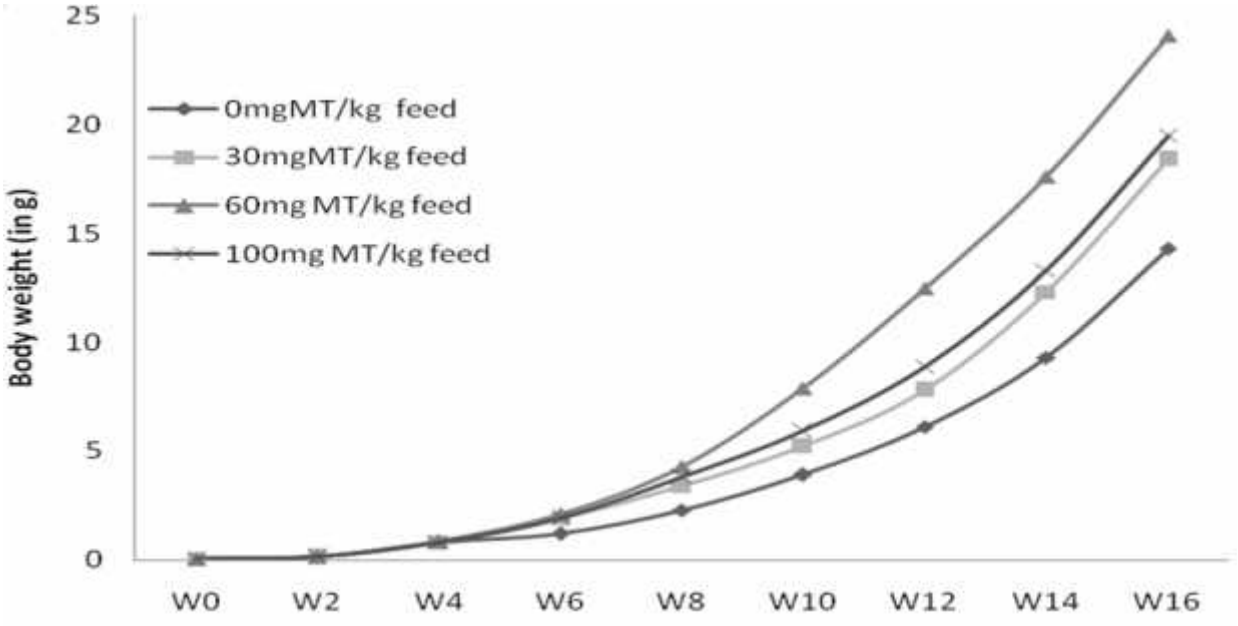

Figure 1: The trend of mean weight of different group of Nile tilapia reared in hapa installed in pond, where W0 to W16 are rearing times in weeks at two weeks interval, W0 is for initial week, i.e. week zero, while W16- week sixteen, i.e. harvesting week

Similarly, the results revealed that the highest mean daily growth rate (0.20±0.1 g/day) and specific growth rate (2.5±0.01%/day) were recorded for the fish fed with 60 mg MT/kg treated diet than the other groups of fish fed with different level of MT. They were significantly different ($P < 0.05$) at least from the lowest mean daily growth rate (0.12±0.01 g/day) and specific growth rate (2.2±0.02%/day) of the fish fed with the control diet. The trend of daily growth rate and specific growth rate fluctuated as the rearing period increased (Figures 2 and 3).

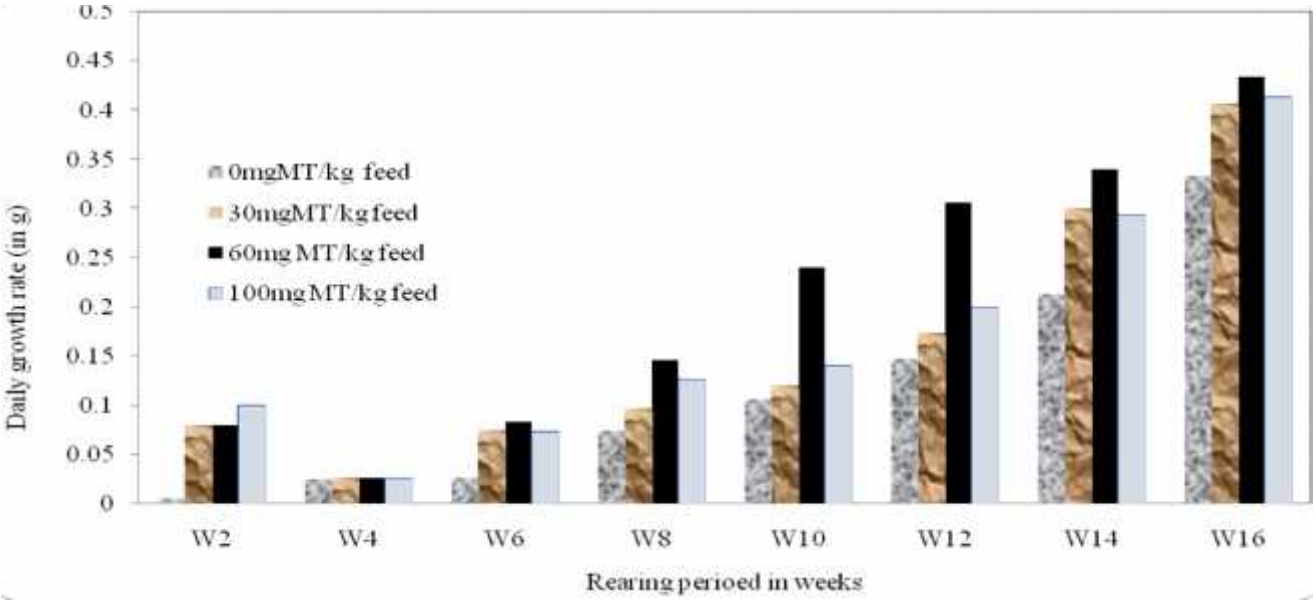

Figure 2: The trend of daily growth rate of different group of Nile tilapia reared in hapa installed in pond

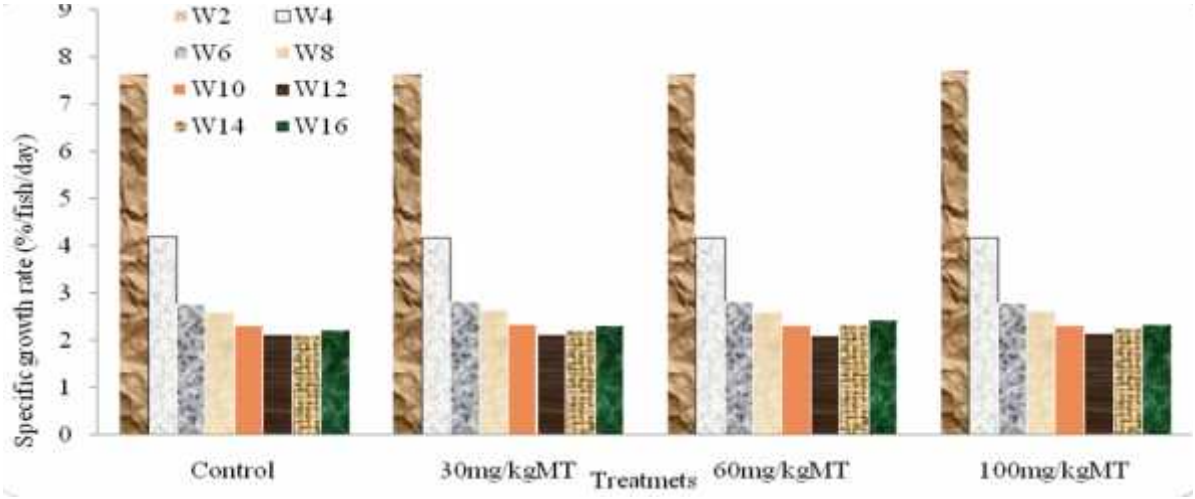

Figure 3: The trend of specific growth rate of different group of Nile tilapia reared in hapa installed in pond, where W2 to W16 are rearing times in weeks, in which fish sampling wars taken at two weeks interval, W2 was the first sampling times in which fish were sampled after two weeks rearing, while W16- week sixteen, i.e. harvesting week.

Feed utilization efficiency and condition factor and weight-length relationship

The mean values of feed utilization efficiency parameters and condition factors of Nile tilapia fed with a control diet and MT-treated diets are presented in Table 4. The food conversion ratio (FCR) of different fish fed with different levels of MT ranged from 1.3±0.1 to 2.3±0.1, while from 0.37±0.1 to 0.63±0.11 for protein efficiency ratio (PER). The best FCR and PER were obtained for the fish fed with a 60 mg MT/kg diet and were significantly ($P < 0.05$) different at least from the fish fed with the control diet.

The condition factor of Nile tilapia fed with control, 30 mg MT, 60 mg MT and 100 mg MT/kg diets were similar and ranged from 1.8±0.12 to 2.1±0.17. According to cube law, the 'b' values for all groups of fish are in a good condition. The 'b' values ranged from 2.8±0.16 to 2.9±0.14.The results showed that both Futon condition factor and weight-length relationship parameters were no significant differences among groups (Table 4).

Table 4: Mean feed utilization efficiency, condition factor, survival and total production with standard error (Mean + SE) of Nile tilapia treated with different doses of 17α-methyl testosterone (17α-MT)

Parameters	Treatments			
	0 mg MT/kg diet	30 mg MT/kg diet	60 mg MT/kg diet	100 mg MT/kg diet
Food conversion ratio	2.3±0.1[a]	1.8±0.3[b]	1.3±0.12[b]	1.8±0.13[b]
Protein efficiency ratio	0.37±0.1[a]	0.48±0.12[a]	0.63±0.11[a]	0.50±0.20[a]
Fulton condition factor	1.9±0.13[a]	2.1±0.17[a]	1.8±0.14[a]	1.8±0.12[a]
Weight-length relationship	2.8±0.16[a]	2.9±0.21[a]	2.9±0.18[a]	2.9±0.14[a]
Survival rate (SR%)	100±0.00[a]	100±0.00[a]	97.9±0.12[a]	100±0.00[a]
Total production (TP kg/ha/y)	2574.3±52.1[a]	3294.6±42.6[b]	4338.7±73.2[c]	3438.2±63.6[b]

Note: Values with the same letter across row are not significantly different ($P > 0.05$)

Survival rate of fish and fish production

The survival rate and total production of all the Nile tilapia groups are presented in Table 4. The survival rates of different Nile tilapia groups were similar, ranging from 97.9±1.2% to 100±0.0%, while the total productions of different groups ranged between 2574.3±52.1 kg/year/ha and 4338.7±73.2 kg/year/ha. The fish fed with a 60 mg MT/kg diet had significantly higher total production at least from the fish-fed with the control diet. The results revealed that as the amount of MT dose increased from 60 mg MT/kg to 100 mg MT/kg diet the total fish production decreased (Table 4).

Water quality parameters

The different water quality parameters (pH, temperature, dissolved oxygen, total dissolved solid and conductivity) recorded during the experimental periods for both indoor and outdoor experiments are presented in Table 5. The results of the indoor experiment showed that the different water parameters across treatments were similar. The values of temperature ranged from 24.0±0.3 °C to 24.4±0.5 °C, pH ranged from 7.6±0.4 to 7.8±0.6

and dissolved oxygen from 3.6±0.3 mg/l to 3.8±0.7 mg/l. The values of total dissolved solids and conductivity also ranged from 448.6±3.4 mg/l to 451.1±3.2 mg/l and from 592.4±2.6 µs/cm to 594.9±2.7 µs/cm, respectively. However, none of the water quality parameters were significantly different among the treatments. For outdoor experiments, the results showed that the mean values of all water quality parameters recorded during the experimental period were optimal (28.5±1.1 °C for temperature, 8.7±2.0 for pH, 8.7±1.3 mg/l for dissolved oxygen, 543.3±3.5 mg/l for total dissolved solid and 721.4±5.27 µs/cm for conductivity).

Table 5: Mean values of different water quality parameters with standard error (Mean ± SE) recorded during indoor and outdoor experiments.

Water quality parameters	Indoor experiment				Outdoor experiment
	Treatments				
	0 mg MT/kg diet	30 mg MT/kg diet	60 mg MT/kg diet	100 mg MT/kg diet	
Temperature (°C)	24.4±0.5[a]	24.3±0.4[a]	24.3±0.4[a]	24.0±0.3[a]	28.5±1.1
pH	7.7±0.3[a]	7.7±0.4[a]	7.8±0.6[a]	7.6±0.4 [a]	8.7±2.0
Dissolved oxygen (mg/l)	3.6±0.3[a]	3.6±0.8[a]	3.8±0.5[a]	3.8±0.7[a]	8.7±1.3
Total dissolved solid (mg/l)	450.7±3.9[a]	448.6±4.7[a]	451.1±4.1[a]	449.6±5.2[a]	543.3±3.5
Conductivity (µs/cm)	593.6±3.4[a]	594.9±2.7[a]	592.4±2.6[a]	593.9±3.7[a]	721.4±5.2

Note: Values with the same letter across raw are not significantly different (P > 0.05).\

DISCUSSION AND CONCLUSIONS

This study demonstrates that the application of 17α methyl testosterone at 30, 60, and 100 mg per kg diets produced a higher male population and growth performance than fish fed with untreated diet. Among the hormone-treated diets, the fish fed with 60 mg MT/kg diet produced a significantly higher male population and body growth (P < 0.05) than the other groups. This result agreed well with the reports of El-Greisy and Gamal (2012) who reported a significantly higher male population using 60 mg MT/kg diet than 40 and 80 mg MT/kg diets. This result also coincides well with the results of Celik *et al*. (2011) and Shamsuddin *et al*. (2012) who reported 93.7% and 95% male population, using 60 mg MT/kg diet treated for 28 and 21 days, respectively. A similar result was reported by Ferdous and Ali (2011) who observed a maximum (94.3%) male population using a 60 mg MT/kg diet. These results are in line with the findings reported by Marjani *et al.* (2009) in which higher growth performance of fish was obtained after hormone treatment.

The present results also showed a higher male population (93.6%) for 60 mg MT/kg diet than the report of Abdul (2007) (89%) and Asad *et al.* (2010) (68%) for the same MT doses. On the other hand, the results of the present study showed a relatively lower male population as compared with 99-100% for fish treated with 60 mg MT/kg diet (Vera–Cruz and Mair, 1994; Smith and Phelps, 2001). Such differences could be due to differences in management practices. As also indicated by Lakshmi (2015) and Sourav (2016) such differences in male population could be due to factors such as the level of hormone in the diet, feeding and feeding frequency, water quality parameters, treatment duration, size at which the fry is selected for experimentation and stocking density.

Different doses of MT resulted in the different growth rates of Nile tilapia in which fish group treated with a 60 mg MT/kg diet showed significantly higher mean body weight, body weight gain, daily growth rate, and specific growth rate of Nile tilapia than the other groups. This result is in agreement with the report of Pechsiri and Yakupitiyage (2005) and Opiyo *et al*. (2014), who observed optimal growth performance of fish when treated with 60 mg MT/kg diet. Also, the results of the present study showed that the fish treated with MT treated diets had significantly higher feed utilization efficiencies than fish treated with control diet. Statistically, the fish fed with a 60 mg MT/kg diet had the best feed utilization efficiency in terms of feed conversion ratio and protein efficiency ratio. This implies that 60 mg MT/kg diet promotes better growth performance, better feed utilization in fish as also reported by Mugo-Bundi (2013).

The significant growth performance and feed utilization efficiency of fish fed with MT/kg diets agreed well with the findings of Howerton *et al*. (1992) and Varadaraj *et al*. (1994) who reported a higher growth rate in *O. mossambicus* fed with MT treated diet than the fish fed untreated diet. The present results also indicated that the fish treated with 60 mg MT/kg diet for 30 days showed maximum growth performance and feed utilization efficiency than the other groups, and further increase or decrease of hormone dose do not have much influence on growth and feed utilization efficiency in fishes as it seems optimal dose. This result also coincides with the work of Lakshmi (2015) who reported faster growth and better feed utilization of Nile tilapia when treated with a 60 mg MT/kg diet. El-Greisy and El-Gamal (2012) also reported higher growth and feed utilization efficiency in fish treated with 60 mg MT/kg diet than 40 and 80 mg MT/kg diets. This implies that a 60 mg MT/kg diet is an optimum dose of MT hormone for optimal production of male population, better growth and feed utilization efficiency and can be considered as an optimal dose for Nile tilapia sex-reversal under the current production management.

On the other hand, the condition factor of all groups of Nile tilapia were similar implying that condition factors were not influenced by MT doses. As stated by Ayode (2011), the fish from all the treatments showed isometric growth, implies that all the groups were in good condition.. The present work also revealed that Nile tilapia followed the cube law completely in all groups of fish in which their values were close to the theoretical value (b = 3). The length-weight relationship was found to be in a linear form conforming to the general formula expressing the relationship between the length and weight of fishes. This could be due to the fact that the length-weight relationship of fish varies depending upon the condition of life in the aquatic environment (Ighwel *et al*., 2011), but it is an important tool that gives information on the growth pattern of animals. In conclusion, 60 mg MT/kg diet can be considered as an optimal dose for Nile tilapia sex reversal along with its optimum growth and feed utilization performances.

Authorship contribution Statement

Berhanu B, Workagegn KB, Pavanasam N**:** Conceptualization, methodology development, visualization, data analysis and writing up of the manuscript. Workagegn KB, and P Natarajan supervision, validation, formal analysis, writing, reviewing and editing of the manuscripts

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Fuzzy Programming Approach to Solve Multi-Objective Fully Fuzzy Transportation Problem

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Multi-Objective Programming;
Triangular Fuzzy Number;
Fuzzy Transportation Problem;
Fuzzy Decision Variables;
Ranking Function;
Fuzzy Programming Method.

Research article

ABSTRACT

The aim this study is presenting the solution methodology of multi-objective fuzzy transportation problem with fuzzy decision variables, where all the input parameters and decisions variables of the programming problems are assumed to be triangular fuzzy number and triangular fuzzy decision variables respectively. Moreover the objectives under considerations are minimization of cost of transportation and minimization of shipping time under fuzzy environment. The fuzziness of the objective functions and the fuzzy constraints of the programming problem are defuzzified using the ranking function and the equality property between two fuzzy numbers, respectively. The consequent crisp multi-objective fuzzy transportation problem is tackled by employing fuzzy mathematical programming approach. Finally fuzzy decision is made after solving the resultant mathematical programming problem using LINGO(Schrage and LINDO Systems (1997)) software. Illustrative numerical example is presented in support of the proposed methodology.

INTRODUCTION

Transportation problem(TP) is a particular class of linear programming, which is associated with day-to-day activities in our real life and mainly deals with logistics. It helps in solving problems on distribution and transportation of resources from one place to another. The goods are transported from a set of sources (e.g., factory) to a set of destinations (e.g., warehouse) to meet the specific r equirements, Das *et al.* (2016). In other words, transportation problems deal with the transportation of a product manufactured at different p lants (supply origins) to a number of different warehouses (demand destinations). Transportation problems were well known as a basic network problem in its classical category. The formulation and discussion of transportation model was introduced by Hitchcock (1941).

Classical TP models and techniques have been effectively applied to problems with a well-defined or accurately known parameters for many years. The coefficient parameters of the majority of TP models are considered

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to be single real number which is accurately known. However, such assumptions are not suitable for dealing with a number of issues that occur in real life because many of the parameters are imprecise and vague as a result of both natural and anthropogenic effects. This motivates to formulate the TP models in uncertain environment. One of the uncertain environments is fuzzy environ-ment. Moreover in most of the literatures, authors assumed the decision parameters as fuzzy numbers while the decision variables as crisp ones. Since the variables are crisp, the solution obtained as the crisp, which is a real number. The solution is exact value in the fuzzy programming problems with fuzzy parameters. The fuzzy aspect of the decision is partly lost in this case so, it is reasonable and important to consider fuzzy mathematical programming problem with fuzzy decision variables. Fuzzy transportation problems involving fuzzy decision variables are considered in this study. Kaur and Kumar (2012) studied a special type of fuzzy TP by assuming that a decision maker is uncertain about the precise values of transportation cost only, where the transportation cost is represented by generalized trapezoidal fuzzy numbers. In their proposed work all the supply and demand of products are crisp parameters that means there is no uncertainty about the supply and demand of the product. According to the explanation of Kumar and Kaur (2011), there may be factors which imposes the occurrences of fuzziness in TP. Some of these are, the decision maker has not enough information about the unit transportation cost of transportation operation and thus the transportation cost uncertain, there may be some sort of vagueness with respect to the demand of a newly introduced product to the market and there exists uncertainty about the product availability at a source or supplier because of time factor. Many authors introduced tools to solve TP. Since transportation problem (TP) is special case of linear programming (LP) problem, one straightforward approach is to apply the existing LP techniques to the fuzzy TP. These techniques are discussed by many researchers namely; Buckley (1988), Buckley (1990), Mitlif (2016), Ebrahimnejad (2013), Ebrahimnejad (2015), etc. However, some these techniques only give crisp solutions, which represent a compromise in terms of fuzzy data.

The traditional view on TP is mainly concerned with distributing any homogeneous product from a group of supply centers, called sources, to any group of receiving centers, called destinations, in such a way as to minimize the single objective total transportation cost, where the transportation cost per unit product is constant regardless of the amount transported, but most of the time in real-life situation, the TPs are not designed as single objective function. The TP that deals with multiple-objective functions is called a multi-objective transportation problem (MOTP). The MOTP is a special type of multi-objective linear programming problem in which objective functions conflict with each other. Furthermore, objective functions are frequently in conflict, thus there is no one best (global optimum) solution, but rather a group of equally good (non-dominated) alternatives known as pareto optimal (PO) solutions. In the framework of multi-objective programming problems, numerous scholars from a wide variety of academic disciplines discussed their work. Recently, multi-objective problems have been proposed by researchers such as Sayyah *et al.* (2019); Sahih *et al.* (2021); Sosa and Dhodiya (2021); Geshniani *et al.* (2020), and others. Researchers namely; Acharya *et al.* (2014) and Dutta *et al.* (2016) introduced MOT problem in stochastic environment. Chakraborty and Chakraborty (2010) discussed cost-time minimization TP, where the demand, supply and transportation cost per unit of the quantities are fuzzy. Nomani *et al.* (2017) introduced a weighted goal programming to solve multi-objective transportation problems with crisp parameters. They used weighted approach based on goal programming to obtain compromise solutions. Roy *et al.* (2018) pro-

posed multi-objective transportation problem (MOTP) under intuitionistic fuzzy environment. They have assumed transportation cost, the supply and the demand parameters as a intuitionistic fuzzy numbers. Jalil *et al.* (2017) proposed a solution approach for obtaining compromise optimal solution of fully fuzzy(all the parameters and decision variables are fuzzy) multi-objective solid transportation problems. In their proposed problem they used ranking function for the defuzzification of fuzzy objective function and the property of equality between fuzzy numbers for the defuzzification of fuzzy constraints. El Sayed and Abo-Sinna (2021); Moges *et al.* (2023); Malik and Gupta (2022); Niksirat (2022), etc. are the works done under fuzzy environment.

As is mentioned above (Paragraph 2), in most of the literature, authors regarded the decision variables as being crisp while the decision parameters are assumed to be fuzzy. This assumptions leads to crisp decisions which is illogical. A fuzzy decision multi-objective fully fuzzy transportation problem proposed in this study. Ranking function and equality between two triangular fuzzy numbers are employed for defuzzification purpose and finally the equivalent crisp multi-objective model is solved fuzzy programming method.

The paper is organized as follows: following the introduction, basic preliminaries are presented in Sect. 2. The mathematical model of multi-objective fuzzy TP is presented in Sect. 3. Solution procedures are provided in 4. Numerical examples are provided in support of the proposed method in Sect. 5. Finally, Conclusion is provided in Sect. 6 followed by supportive references.

BASIC PRELIMINARIES

Definition: [Roy *et al.* (2018)]: A tri-angular fuzzy number $\tilde{a}$ is denoted by (a^p, a, a^o), where a^p, a, a^o are real numbers. The membership function $(\mu_{\tilde{a}}(x))$ of $\tilde{a}$ is given below:

$$\mu_{\tilde{a}}(x) = \begin{cases} 0, & x \leq a^p \\ \frac{x-a^p}{a-a^p}, & a^p \leq x \leq a \\ \frac{a^o-x}{a^o-a}, & a \leq x \leq a^o \\ 0, & otherwise \end{cases}$$

Note: The point 'a' is the core value of triangular fuzzy number $\tilde{A}$, where $\mu_{\tilde{a}}(a) = 1$ a^p and a^o are the lower and upper bounds of support of triangular fuzzy number $\tilde{A}$ respectively.

Definition : [Roy *et al.* (2018)]: Let $\tilde{a} = (a^p, a, a^o)$ and $\tilde{b} = (b^p, b, b^o)$ be two triangular fuzzy numbers ,then
(i) $(a^p, a, a^o) \oplus (b^p, b, b^o) = (a^p + a^p, a + b, a^o + b^o)$
(ii) $k(a^p, a, a^o) = (ka^p, ka, ka^o)$, $k \geq 0$
(iii) $(a^p, a, a^o) \otimes (b^p, b, b^o) = (a^p b^p, ab, a^o b^o$, if $a^p \geq 0$ *and* $b^p \geq 0$

Definition : [Ebrahimnejad (2017)]: Let $\tilde{a} = (a^p, a, a^o)$ and $\tilde{b} = (b^p, b, b^o)$ be two triangular fuzzy numbers ,then
(i) $\tilde{a} = \tilde{b}$ iff $a^p = b^p$, a=b and $a^o = b^o$
(ii) $\tilde{a} = (a^p, a, a^o) \geq 0$ iff $a^p \geq 0$

Definition :Ebrahimnejad (2017)]: Fuzzy TP is said to be balanced transportation problem when total supply from all the sources is equal to the total demand in all destinations.

Definition : [Kumar *et al.* (2011)]: A ranking function is a function $\mathbb{R}$:$F(R) \to R$, where F(R) is a set of fuzzy numbers defined on set of real numbers, which maps each fuzzy number into the real line, where a natural order exists. Let $\tilde{a} = (a^p, a, a^o)$ be a triangular fuzzy number, then $\mathbb{R}(\tilde{a}) = \frac{a^p + 2a + a^o}{4}$

Definition : [Hasan *et al.* (2015)]: Multi-Objective Optimization Problem (MOOP) involves more than one objective function that are to be minimized or maximized. Answer of MOOP is the set of solutions that define t he b est t radeoff between competing objectives. The following is general mathematical Form of MOOP:

$$\max / \min \quad Z_m(x), m = 1, 2, 3, ..., M \quad (2.1)$$
$$\text{s.t.} \quad g_j(x) \geq 0, j = 1, 2, ..., J \quad (2.2)$$
$$h_k(x) \geq 0, k = 1, 2, ..., K \quad (2.3)$$
$$x^L_{\leq} x_i \leq x^U \quad (2.4)$$

MATHEMATICAL MODEL

The mathematical model for MOFTP with fuzzy decision variables is represented as:

$$\min : \widetilde{Z}_k \approx \sum_{i=1}^{m} \sum_{j=1}^{n} ((c_{ij}^k)^p x_{ij}^p, c_{ij}^k x_{ij}, (c_{ij}^k)^o x_{ij}^o), k \in \{1, 2...K\} \quad (3.1)$$

subject to

$$\sum_{j=1}^{n} (x_{ij}^p, x_{ij}, x_{ij}^o) \approx (a_i^p, a_i, a_i^o), i \in \{1, 2, 3, ..., m\} \quad (3.2)$$

$$\sum_{i=1}^{m} (x_{ij}^p, x_{ij}, x_{ij}^o) \approx (b_j^p, b_j, b_j^o), j \in \{1, 2, 3, ..., n\} \quad (3.3)$$

$$\widetilde{x}_{ij} \succeq 0, i \in \{1, 2, 3, ..., m\}; j \in \{1, 2, 3, ..., n\} \quad (3.4)$$

where,

i. the fuzzy total availability and fuzzy total demand are assumed to be equal(balanced fuzzy transportation problem), $'m'$ is total number of supply points and 'n' is total number of destination points,

ii. $\widetilde{a_i}=(a_i^p, a_i, a_i^o)$ is the fuzzy availability of the commodity at i^{th} origin and assumed to be triangular fuzzy number,

iii. $\widetilde{b_j}=(b_j^p, b_j, b_j^o)$ is the fuzzy requirement of the commodity at j^{th} destination and assumed to be triangular fuzzy number,

iv. $\widetilde{c}_{ij}=(c_i^p j, c_i j, c_i^o j)$ is the fuzzy cost coefficient involved with fuzzy variables in the objective function from i^{th} origin to j^{th} destination, which is also assumed to be triangular fuzzy number,

v. $\widetilde{x}_{ij}=(x_{ij}^p, x_{ij}, x_{ij}^o)$ is the fuzzy quantity that should be transported from i^{th} origin to j^{th} destination and assumed to be triangular fuzzy decision variables.

Crisp equivalent of multi-objective fuzzy transportation problem

Since FMP model from (3.1) to (3.4) cannot be solved directly, so ranking function and the property of equality between the fuzzy numbers are respectively applied on the fuzzy objective functions and fuzzy constraints models of MOFTP. The resultant crisp equivalent is obtained as:

$$\min : Z_k = \frac{1}{4} \sum_{i=1}^{m} \sum_{j=1}^{n} ((c_{ij}^p)^k x_{ij}^p + 2c_{ij}^k x_{ij} + (c_{ij}^o)^k x_{ij}^o), k = 1, \quad (3.5)$$

subject to

$$\sum_{j=1}^{n} x_{ij}^p = a_i^p, i \in \{1, 2, 3, ..., m\}; \quad (3.6)$$

$$\sum_{j=1}^{n} x_{ij} = a_i, i \in \{1, 2, 3, ..., m\} \quad (3.7)$$

$$\sum_{j=1}^{n} x_{ij}^{o} = a_i^{o}, i \in \{1,2,3,...,m\} \quad (3.8)$$

$$\sum_{i=1}^{m} x_{ij}^{p} = b_j^{p}, j \in \{1,2,3,...,n\} \quad (3.9)$$

$$\sum_{i=1}^{m} x_{ij} = b_j, j \in \{1,2,3,...,n\} \quad (3.10)$$

$$\sum_{i=1}^{m} x_{ij}^{o} = b_j^{o}, j \in \{1,2,3,...,n\} \quad (3.11)$$

$$x_{ij}^{o} - x_{ij} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.12)$$

$$x_{ij} - x_{ij}^{p} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.13)$$

$$x_{ij}^{p} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.14)$$

Solution procedure

Now we use the fuzzy programming programming technique to solve the crisp multi-objective programming problem of 3.5 to 3.14. The solution procedures based on the fuzzy programming method is detailed below.

Step 1: Find the ideal solutions $x^1, x^2, ..., x^k$ by picking one objective function at a time and leaving the other objective functions.

Step 2: Construct a pay-matrix with the help of individual best solutions found by the above step. Using table 1 estimate the bounds of z_k (k=1,2,3...,K) from the Payoff matrix.

Step 3: For every objective function z_k (k=1,2,3...,K), we formulate membership function using any one of the following techniques of maximization or minimization:

Table 1: Payoff matrix

	$z_1(x)$	$z_2(x)$	. . .	$z_K(x)$
$x^{(1)}$	$z_1(x^{(1)})$	$z_2(x^{(1)})$	. . .	$z_K(x^{(1)})$
$x^{(2)}$	$z_1(x^{(2)})$	$z_2(x^{(2)})$	. . .	$z_K(x^{(2)})$
.	.	.	. . .	.
.	.	.	. . .	.
.	.	.	. . .	.
$x^{(K)}$	$z_1(x^{(K)})$	$z_2(x^{(K)})$	. . .	$.z_K(x^{(K)})$

Step 4

Case 1: Membership function is formulated in the case of maximization problem as:

$$\mu_{z_k}(x) = \begin{cases} 0, & \text{if } z_k \leq lb_k^- \\ \frac{z_k - lb_k^-}{ub^* - lb_k^-}, & \text{if } lb_k^- \leq z_k \leq ub_k^* \\ 1, & \text{if } z_k \geq ub_k^* \end{cases}$$

where lb_k^- denotes the worst lower bound of z_k and ub_k^* denotes the best upper bound of z_k

Case 2: For minimization problem the membership function is formulated as:

$$\mu_{z_k}(x) = \begin{cases} 0, & \text{if } z_k \geq ub_k^- \\ \frac{ub^- - z_k}{ub^- - lb_k^*}, & \text{if } lb_k^* \leq z_k \leq ub_k^- \\ 1, & \text{if } z_k \leq lb_k^* \end{cases}$$

Where ub_k^- denotes the worst upper bound of z_k and lb_k^* denotes best lower bound of z_k.

Case 1: Apply the augmented variable, λ with max-min operator to formulate a crisp single objective mixed integer programming problem as:

$$\max : \lambda \quad (3.15)$$

subject to

$$\mu_{z_k}(x) \geq \lambda, \ k = 1,2,...K \quad (3.16)$$

$$\sum_{j=1}^{n} x_{ij}^{p} = a_i^{p}, i \in \{1,2,3,...,m\} \quad (3.17)$$

$$\sum_{j=1}^{n} x_{ij} = a_i, i \in \{1,2,3,...,m\} \quad (3.18)$$

$$\sum_{j=1}^{n} x_{ij}^{o} = a_i^{o}, i \in \{1,2,3,...,m\} \quad (3.19)$$

$$\sum_{i=1}^{m} x_{ij}^{p} = b_j^{p}, j \in \{1,2,3,...,n\} \quad (3.20)$$

$$\sum_{i=1}^{m} x_{ij} = b_j, j \in \{1,2,3,...,n\} \quad (3.21)$$

$$\sum_{i=1}^{m} x_{ij}^{o} = b_j^{o}, j \in \{1,2,3,...,n\} \quad (3.22)$$

$$x_{ij}^{o} - x_{ij} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.23)$$

$$x_{ij} - x_{ij}^{p} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.24)$$

$$x_{ij}^{p} \geq 0, i \in \{1,2,3,...,m\}, j \in \{1,2,3,...,n\} \quad (3.25)$$

$$0 \leq \lambda \leq 1 \quad (3.26)$$

Case 2: Apply the augmented variable, λ with min-max operator to formulate a crisp single objective mixed integer programming problem as:

$$\min : \lambda \quad (3.27)$$

subject to

$$\mu_{z_k}(x) \leq \lambda, \ k \in \{1,2,3,....K\} \quad (3.28)$$

$$\sum_{j=1}^{n} x_{ij}^{p} = a_i^{p}, i \in \{1,2,3...m\} \quad (3.29)$$

$$\sum_{j=1}^{n} x_{ij} = a_i, i \in \{1,2,3...m\} \quad (3.30)$$

$$\sum_{j=1}^{n} x_{ij}^{o} = a_i^{o}, i \in \{1,2,3...m\} \quad (3.31)$$

$$\sum_{i=1}^{m} x_{ij}^{p} = b_j^{p}, j \in \{1,2,3...n\} \quad (3.32)$$

$$\sum_{i=1}^{m} x_{ij} = b_j, j \in \{1,2,3...n\} \quad (3.33)$$

$$\sum_{i=1}^{m} x_{ij}^{o} = b_j^{o}, j \in \{1,2,3...n\} \quad (3.34)$$

$$x_{ij}^{o} - x_{ij} \geq 0, i \in \{1,2,3...m\}; j \in \{1,2,3...n\} \quad (3.35)$$

$$x_{ij} - x_{ij}^{p} \geq 0, i \in \{1,2,3...m\}; j \in \{1,2,3...n\} \quad (3.36)$$

$$x_{ij}^{p} \geq 0, i \in \{1,2,3...m\}; j \in \{1,2,3...n\} \quad (3.37)$$

$$0 \leq \lambda \leq 1 \quad (3.38)$$

Step 5 At the end, the equivalent single objective MP model is solved by using appropriate techniques or existing software. The obtained PO solutions substituted back to original fuzzy objective function, as the result we can find fuzzy optimal values of each fuzzy objective functions.

Numerical example

In this part, application numerical example is solved using the provided approach, and the conclusions drawn from the results are discussed in further detail.

A firm has two sources O_1 and O_2 and three destinations D_1 ,D_2 and D_3. The fuzzy supply of the commodity from O_1 and O_2 are (75, 95, 125) and (45, 65, 95), respectively. Request of fuzzy demanded product at D_1 ,D_2 and D_3 are (35, 45, 55), (25, 35, 45) and (60, 80, 110), respectively. The company wants to determine the fuzzy quantity of the commodity that should be transported from each origin to each destination so that the total fuzzy transportation cost is minimum with minimum transfer time. For i=1,2, j=1,2,3., let the fuzzy transportation cost for unit quantity of the commodity from i^{th} source to j^{th} destinations be $\tilde{c}_{ij}$, the fuzzy transportation time is also considered as $\tilde{t}_{ij}$ and $\tilde{x}_{ij}$ represents the allocations(or amounts), which is non negative triangular fuzzy real variable. The theoretical data on fuzzy cost of transportation and fuzzy delivery time, is given in the table 2 below.

Table 2: Fuzzy transportation cost per unit(in Rupees) and fuzzy transportation time per unit(in minute)

	D_1	D_2	D_3
$\tilde{c}_{1j}(O_1)$	(15,25,35)	(55, 65,85)	(85,95,105)
$\tilde{c}_{2j}(O_2)$	(65,75,85)	(80,90,110)	(30,40,50)
$\tilde{t}_{1j}(O_1)$	(4, 6, 8)	(6, 8,10)	(7,9,11)
$\tilde{t}_{2j}(O_2)$	(3,5,7)	(5,7,9)	(11,13,15)

From the table 2 mathematical model for MOFTP with fuzzy decision variables becomes:

$$\min : \tilde{z}_1 \approx (15,25,35) \otimes \tilde{x}_{11} \oplus (55,65,85) \otimes \tilde{x}_{12} \oplus (85,95,105) \otimes \tilde{x}_{13} \oplus (65,75,85) \otimes \tilde{x}_{21} \oplus (65,75,85) \otimes \tilde{x}_{21} \oplus (80,90,110) \otimes \tilde{x}_{22} \oplus (30,40,50) \otimes \tilde{x}_{23} \quad (3.39)$$

$$\min : \tilde{z}_2 \approx (4,6,8) \otimes \tilde{x}_{11} \oplus (6,8,10) \otimes \tilde{x}_{12} \oplus (7,9,11) \otimes \tilde{x}_{13} \oplus (3,5,7) \otimes \tilde{x}_{21} \oplus (5,7,9) \otimes \tilde{x}_{22} \oplus (11,13,15) \otimes \tilde{x}_{23} \quad (3.40)$$

Subject to

$$\tilde{x}_{11} \oplus \tilde{x}_{12} \oplus \tilde{x}_{13} \approx (75,95,125) \quad (3.41)$$

$$\tilde{x}_{21} \oplus \tilde{x}_{22} \oplus \tilde{x}_{23} \approx (45,65,95) \quad (3.42)$$

$$\tilde{x}_{11} \oplus \tilde{x}_{21} \approx (35,45,65) \quad (3.43)$$

$$\tilde{x}_{12} \oplus \tilde{x}_{22} \approx (25,35,45) \quad (3.44)$$

$$\tilde{x}_{13} \oplus \tilde{x}_{23} \approx (60,80,110) \quad (3.45)$$

$$x_{11}, \tilde{x}_{12}, \tilde{x}_{13} \succeq 0 \quad (3.46)$$

$$\tilde{x}_{21}, \tilde{x}_{22}, \tilde{x}_{23} \succeq 0 \quad (3.47)$$

defuzzification is done using the concept discussed in the subsection 3.1. Then the case(3.27-3.37) of evaluation of membership functions for the minimization (Max-min operator) is applied on the crisp MOTP along with all the procedures discussed above. The fuzzy programming method is applied on the aforementioned crisp equivalent MOTP to find the ideal solutions as detailed below

$x^{(1)} = (x^p_{11}, x_{11}, x^o_{11}, x^p_{12}, x_{12}, x^o_{12}, x^p_{13}, x_{13}, x^o_{13}, x^p_{21}, x_{21}, x^o_{21}, x^p_{22}, x_{22}, x^o_{22} x^p_{23}, x_{23}, x^o_{23})$= (35,35,55,10,10,10,30,50,60,0,10,10,15,25,35,30,30,50)
$x^{(2)} = (x^p_{11}, x_{11}, x^o_{11}, x^p_{12}, x_{12}, x^o_{12}, x^p_{13}, x_{13}, x^o_{13}, x^p_{21}, x_{21}, x^o_{21}, x^p_{22}, x_{22}, x^o_{22}, x^p_{23}, x_{23}, x^o_{23})$= (0,10,30,25,35,35,50,50,60,35,35,35,0,0,10,10,30,50)

At the corresponding ideal points the values of crisp objective functions are obtained and given as: z_1=10737.5, z_2=1440.
After constructing a pay-of matrix 2, the bounds of the two objective functions are given by:

$$10737.5 \leq z_1(x) \leq 11825$$

$$1440 \leq z_2(x) \leq 1465.$$

The the single objective crisp problem is obtained by formulating membership function. Thus the programming problem becomes:

$$\max : \lambda \quad (3.48)$$

subject to

$$z_1 + 1387.5\lambda \leq 11825 \quad (3.49)$$
$$z_2 + 25\lambda \leq 1465 \quad (3.50)$$
$$x_{11}^p + x_{12}^p + x_{13}^p = 75 \quad (3.51)$$
$$x_{11} + x_{12} + x_{13} = 95 \quad (3.52)$$
$$x_{11}^o + x_{12}^o + x_{13}^o = 125 \quad (3.53)$$
$$x_{21}^p + x_{22}^p + x_{23}^p = 45 \quad (3.54)$$
$$x_{21} + x_{22} + x_{23} = 65 \quad (3.55)$$
$$x_{21}^o + x_{22}^o + x_{23}^o = 95 \quad (3.56)$$
$$x_{11}^p + x_{21}^p = 35 \quad (3.57)$$
$$x_{11} + x_{21} = 45 \quad (3.58)$$
$$x_{11}^o + x_{21}^o = 65 \quad (3.59)$$
$$x_{12}^p + x_{22}^p = 25 \quad (3.60)$$
$$x_{12} + x_{22} = 35 \quad (3.61)$$
$$x_{12}^o + x_{22}^o = 45 \quad (3.62)$$
$$x_{13}^p + x_{23}^p = 60 \quad (3.63)$$
$$x_{13} + x_{23} = 80 \quad (3.64)$$
$$x_{13}^o + x_{23}^o = 110 \quad (3.65)$$
$$x_{ij}^o - x_{ij} \geq 0 \quad (3.66)$$
$$x_{ij} - x_{ij}^p \geq 0 \quad (3.67)$$
$$x_{ij}^p \geq 0 \quad (3.68)$$
$$0 \leq \lambda \leq 1 \quad (3.69)$$

By employing the LINGO software, the resultant equivalent crisp programming problem (3.48)-(3.69) is solved. The PO solutions and the agumated variable λ obtained are given consecutively as: x^*=(31.66375,41.66375,61.66375,0,3.336248,3.33666248,43.33675,50,60, 3.336248 ,3.336248 ,3.336248 ,25,31.66375,41.66375,16.66375,30,50), λ=0.6668124. The compromising fuzzy objective functions values are $\tilde{z}_1$=(6875.31, 10341.77, 16075.04) and $\tilde{z}_2$=(748.32, 1355.13, 2335).
Figurative description of fuzzy optimal triangular cost and triangular transfer time are detailed in the figure below.

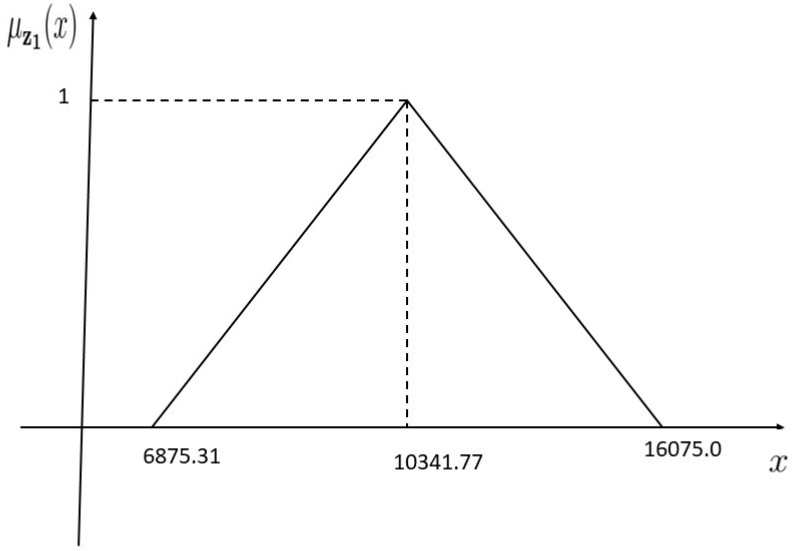

Figure 1: Minimum fuzzy transportation cost

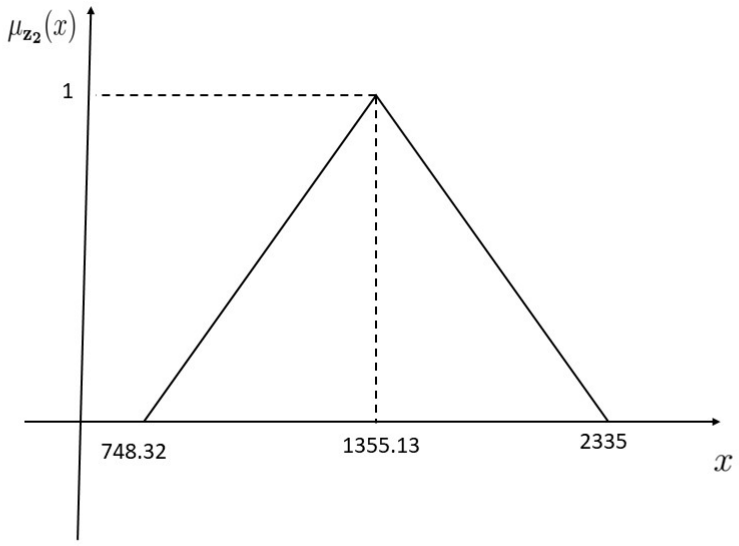

Figure 2: Minimum fuzzy transfer time

DISCUSSION

The defuzzified crisp MOTP is solved by fuzzy programming method. Using Max-min operator, the resultant single objective crisp integer MP problem is coded into LINGO optimization package version 19.0 software and fuzzy PO solutions are obtained. The minimum total fuzzy cost of transportation and the minimum fuzzy transfer time are obtained and interpreted as follows:
Fuzzy transportation cost and fuzzy transfer time $\widetilde{z}_1$=(6875.31, 10341.77, 16075.04) and $\tilde{z}_2$=(748.32, 1355.13, 2335) respectively are calculated by back substitutions of PO solutions into fuzzy objective functions (3.1) of the the programming problem.
It is found that the least amounts of minimum total transportation cost and transfer time are 6875.31 and 748.32 units respectively. The the most possible amounts of minimum total transportation cost and transfer time are 10341.77 and 1355.13 units respectively. Furthermore, the greatest amounts of minimum total transportation cost and total transfer time are 16075.04 and 2335 units respectively.

CONCLUSION

The classical transportation problem (TP) is primarily concerned with distributing any homogeneous product from a group of supply centers, known as sources, to any group of receiving centers, known as destinations, in such a way that the single objective total transportation cost is minimized, where all parameters are crisp (precisely defined). However, in many circumstances, the decision maker lacks precise knowledge of the TP parameters. and the nature of the TPs are not designed as single objective function. If the nature of the information is vague and the decision maker objectives preference are conflicting, t he c orresponding programming problem is fuzzy multi-objective programming problem, and thus fuzzy MOTP arises. In this paper, we have discussed a solution approach for solving TP, with more than one objective function by considering the presence of vagueness in the real life data of transportation problems, where all the parameters and decision variables are considered as triangular fuzzy numbers. Initially, the fuzzy objective functions are defuzzified by applying the ranking function for trian-

gular fuzzy numbers on each individual objective function. The equality constraints involved in the multi-objective TP model with fuzzy parameters are converted to their crisp equivalent by using the property of equality among the fuzzy numbers. Finally fuzzy programming approach is used to find the compromise solution of the crisp multi-objective programming problem. Optimization solver, LINGO Schrage and LINDO Systems (1997), is used to solve one application example by proposed method and solutions and findings are discussed in detail. As the fuzzy technique is often employed to model many real-world situations, including supply chain modeling, information theory problems, inventory operations problems etc., the suggested method may eventually be extended to address such optimization problems.

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Evaluation of Toxicological Risks and Effects of Microplastics on Nile Tilapia (*Oreochromis niloticus*) under in Vitro Laboratory Conditions

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Microplastics;
Toxicological risks;
Oreochromis niloticus;
Aquatic organisms;
Biological effects;
In vitro condition

Research article

ABSTRACT

Microplastics have been reported by many literatures as contaminants of environmental water bodies and are ingested by aquatic organisms due to their small sizes. Knowing the effects of microplastics to fresh water fish which are kept in ponds helps in managing fish keeping practice. The objective of research was to determine the toxicity of microplastics to Nile tilapia (*Oreochromis niloticus)*. The Experiment was done in 80 fish samples. Microplastics which were prepared for the batch experiment were introduced in the aquarium followed with observation for 21 days. The digestion of fish gills and intestines involved 10 mL of 10% (w/v) KOH solution and incubation at 65^{o}C for 24 hours. Engulfed microplastics were determined using stereo microscope and At-IR spectrophotometer for confirmation. Engulfed microplastics were observed to be in mean range of 3.37 x 10^2±4.04 x 10^2 to 2.32 x 10^3 ± 3.57 x 10^3 particles/kg in gills and 4.68 x 10^4 ± 3.02 x 10^4 to4.40 x 10^4 ± 5.34 x 10^4 particles/kg in intestines. The observed responses were loss of equilibrium for 35% of fish, abnormal swimming for 49% of fish, abnormal ventilator behavior for 59% of fish, abnormal appearance for 39% of fish and average growth weight increase in control experiment fish was 6.10±2.62 g compared to 1.7 ± 3.62 g in test fish. There was no mortality of Nile tilapia. The responses of fish to the presence of microplastics in aquarium indicated that microplastics had adverse effects to Nile tilapia (*Oreochromis niloticus*). More researches have to be done on fish physiological changes caused by microplastics.

INTRODUCTION

Plastics are produced in large amount for different applications, but have been accompanied with waste deposition in urban areas (Moore, 2008; Zhang, 2017). About 70 to 80% of plastic contaminants originate from anthropological activities (Mvowiec, 2017). Plastic wastes in environments occur in different sizes, the smallest forms are called microplastics (Sadri and Thompson, 2014; Thakur *et al*., 2022). When large proportions

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of plastic wastes are mismanaged, they enter the environments where they evolve into microplastics via progressive fragmentation through the process of bio-degradation, photo-degradation, thermo-oxidative degradation or mechano-chemical degradation (Mvowiec, 2017; Guilhermino, 2021).

Microplastics have been found in marine biota because of their presence in ecosystems (Mistri, 2022). Animals exposed to microplastics under laboratory setting have shown several adverse effects like histological alterations, lesions in the gastrointestinal tract, intestinal inflammation, neurotoxicity, oxidative stress, damage, immuno-regulation, feeding behavioral change, and developmental alterations (Jovanovic, 2017). Oxidative stress and inflammation are caused by generated reactive oxygen species (ROS) (Subaramaniyam et al. 2023;Yao *et al*., 2023).

In urban areas, there are various anthropogenic activities which lead to microplastic accumulation in water bodies in which fish are found living (Khan *et al*., 2018; Mayoma *et al.*, 2020). Fish can be easily stressed due to exposure to toxic substances in water (Reverter, 2018). The reported clinical signs by literatures on fish responses to abnormal water conditions have not been well documented on fresh water fish commonly known as Nile tilapia (*Oreochromis niloticus*) once exposed to microplastics. Fish keeping is highly encouraged and takes place in ponds in various tropical regions in Africa where Tanzania is among of them. With knowledge that fresh water ponds are susceptible to microplastic pollution in urban regions, therefore there was need of finding out how far the microplastic exposure to fresh water fish, especially Nile tilapia could have effect. The research was conducted to find out how fish commonly known as Nile tilapia were affected by microplastics and to evaluate their effects by *in vitro* observation in laboratory**.**

MATERIALS AND METHODS

Materials

An Aquarium was made of glass (30 cm x 30 cm x 60 cm), with a capacity of 40 L. Two aerators, two air and water filters, a wire mesh covering, and an aquarium fish net from the fish equipment business shop were used to support the aquarium. A laboratory thermometer and a multimeter for pH, water conductivity and Total dissolved solids (TDS) were obtained from the laboratory. Plastic buckets, (each 20 L), and a sieve were bought from the domestic shops. Fish feeds were bought from the animal feed shops. Stereo microscope, hand lens, and 100 mL beakers were supplied by the laboratory. Potassium hydroxide (Analar compound) and Standard microplastics: Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), nylon 6, 6 and polyethylene terephthalate (PET) were supplied by Precur Chem and Equipment Ltd, Tanzania.

Samples of fish for toxicological study

Samples of Nile tilapia for microplastic toxicological study were collected from Kibaha Fish Farming Ponds in Coast Region, Tanzania. The selection of this freshwater fish was based on the fact that it is commonly kept in most fish farmers in tropical regions due to the ability of withstanding extreme conditions like high temperature, change of pH or

changes of total dissolved solids. The fish have also ability to survive during transport and the period of caring and observation. Small fish aged two months old (length 12 mm−14 mm and/ or weight 30 g–50 g) were captured and collected using nets. The fish were captured during the sunset and were kept in ponds within nets until early morning before sunrise where they were collected in two buckets of (20 L) each containing 10 fish. Fish in one bucket was used for test experiment while in another bucket was for the control experiment. All the experiments involving fish samples were performed in batches of 20 fish and a total of 4 batch samples were collected. About 4 L of water from the pond was added in each fish bucket for maintaining fish medium.

Preparation and aquarium maintenance

The test and control aquaria were set near the window where sun rays did not struck directly. Two aerators for each aquarium were connected to the filters, and then placed in the aquarium using aquarium fish net. Clean tap water was half-filled n each aquarium one day before fish introduction. The water in the aquarium was left overnight in order to dechlorinate. In the next day, aeration was performed to increase the amount of oxygen and filtration was done for half an hour to remove some suspended solids. Thereafter, room temperature, pH, conductivity and total dissolved solids (TDS) of the water in the aquaria and the buckets (from the fish ponds) were measured. Each fish was weighed to obtain initial weight (w_1) and length (l_1) prior to placing in the aquarium. The fish were placed in the separate aquaria then were left to acclimatize for 20 min. Then water from fish farm ponds in the buckets was mixed with de-chlorinated aquarium tap water which was already in the aquarium in order to fill the left space. The purpose of retaining the pond water was to keep the association bacteria (symbiosis) used by fish in their life. Lastly, each aquarium was covered with wire mesh in order to ensure fish safety. The fish in both aquaria were starved for one day, then fed with ground fish feed pellets 10% (w/w). The fish were left to acclimatize for nine days before feeding with microplastics. The fish life was maintained by continuous aeration of the aquarium water, and measuring pH, water, room temperature, conductivity and TDS. The nitrate levels were controlled by dilution with 25% of clean water after every two days. The gravel was set as aquarium bed in order to trap suspended solids and feces. The feces were removed after every two days by siphoning.

Exposure of Nile tilapia to microplastics

Microplastics with size of 30 µm for PVC, and 1000 µm for PET, 2000 µm for PE, 2000 µm for PP and 600 µm nylon 6,6 were mixed together in a 250 mL beaker followed with mixing with water to form a suspension. The microplastics were prepared in different concentration dose for exposure to fish; 2.7 x 10^7, 9.0 x 10^8, 1.3 x 10^8 and 6.7 x 10^7 particles/m^3. The prepared micro plastic suspension was introduced in the test aquarium on the tenth day. The aquarium contained fish of average weight 40 g. Then the observation was performed for 21 days according to the Organization for Economic Co-operation and Development (OECD, 2019). The behaviours (responses) of fish in the test aquarium were compared with the fish in the control aquarium. The toxic effects of

micro plastics on fish were observed by noting and recording the number of individuals affected using some established clinical signs (Table 1) as stipulated in the OECD (2019). Furthermore, the physical characteristics of the aquarium media (pH and conductivity), weight of fish, and concentration of microplastics were observed.

Table 1. Clinical signs used in observation of responses of Nile tilapia to microplastics exposure

Observation	Clinical Sign
Loss of Equilibrium	Abnormal horizontal orientation
	Abnormal vertical orientation
	Loss of buoyance control (floating at surface or sinking to the bottom)
Abnormal swimming behavior	Hypo activity (Decrease in spontaneous activity)
	Hyperactivity (Increase in spontaneous activity)
	Corkscrew swimming (Rotation around long axis, erratic movement)
	Abnormal surface distribution (abnormal depth selection, close to water air interface
	Abnormal bottom distribution/behaviour abnormal depth selection, bottom of tank)
	Over-reactive to stimulus (flight, or avoidance response to: visual, tactile touch) or vibration stimulus
	Loss of schooling/shoaling behaviour (individual fish show, loss of aggregation and social interaction)
	Dense schooling/shoaling behaviour (increase in clumped association of fish)
Abnormal ventilator (Respiratory) function	Hyperventilation (increase in frequency of opercula ventilator movements with possible open mouth and extended opercula)
	Hypoventilation (decreased frequency of opercula ventilator movements).
	Coughing (fast reflex expansion of mouth and opercula not at water surface-assume to clear ventilator channels)
	Gulping (mouth movements at water surface resulting in intake of water and air)
	Head shaking (rapid lateral head movements)
Abnormal skin pigmentation	Darkened (malefic markings)
	Lightened (weak pigmentation)
	Mottled (discoloration)
Appearance and Behaviour Abnormalities	Oedema (abnormal swelling due to accumulation of fluids)
	Haemorrhage (sub-mucus bleeding)
	Faecal (anal) casts (string of faeces hanging from anus or on tank floor)

Source: OECD (2019).

Analysis of engulfed microplastics

After 21 days, all fish from test and experiment aquaria were weighed for final length and weight, and then were dissected to collect intestinal parts and gills. The gills and intestines were separately digested using 10% KOH solution (10% (w/v) following with incubation at 65^{o}C for 24 hours. Later, distilled water was added to dilute the unused KOH before filtration. The particles on the filter paper were washed thoroughly with water during filtration. A stereo microscope (10 x magnifications) was used to count the microplastics which was performed first by placing all microplastics in the petri-dish which had four partitions prepared by lines of

pen-ink. Secondly, the petri-dish was placed on the microscope stage for visualization and counting of microplastics in all petri-dish compartments (Masura *et al*., 2015). Further confirmation of the microplastics was done using Attenuated Fourier Transform Infrared Spectrophotometer instrument (At-FT-IR, Bruker, Massachusetts, USA).

Data analysis

Excel descriptive statistics in analysis ToolPak was used to analyse row data for mean, range and standard deviation of microplastics engulfed by Nile tilapia. Pearson correlation was used to determine the relationship between microplastics which were engulfed and responses of Nile tilapia. Student t-test (n = 20)was used to determine the significant difference of effects of engulfed microplastics on Nile tilapia and significant difference in growth among the microplastic fed fish (tested fish) and fish which were not fed with microplastics (non-tested fish).

RESULTS

Water temperature, pH, total dissolved solids (TDS) and electric conductivity

Water temperature, electric conductivity, pH and total dissolved solids were monitored as part of water quality assurance for fish growth in both test and control experiments. Ammonia content in aquarium which was caused by defecation and food remains was controlled by diluting water and siphoning out after every two days of experiments. Dilution and siphoning were also means of regulation of pH, TDS and conductivity. The room temperature was controlled by air circulation using fans and opening windows. The room temperature therefore, ranged from 24.8 to 27^{o}C and water temperature ranged from 24.00 to 26.00 ^{o}C. The temperature range was within the water quality requirement for fish farming, which is 24^{o}C to 30^{o}C (Ezeanya *et al*., 2015). The water conductivity (mean ± standard deviation) in tested fish had changed by 62% (465 ±117.15 µs/cm) from the pond water conductivity (750±254 µs/cm), the pH had changed by 0.9% (6.8 ± 0.4) from the pond pH (6.7 ± 0.2) and TDS had changed by 63.9% (236.5 ±32.4 ppm) from the pond TDS (370.0 ± 127.3 ppm). The water conductivity in non-tested fish had changed by 64% (480 ± 80.1 µs/cm) the pond water measurements were 750±254 µs/cm (electric conductivity), pH had changed by 0.9% (6.8 ± 0.2) from the pond pH, 6.7 ± 0.2, and total dissolved solids had changed by 72% (269.75 ±104.85 ppm) from the pond TDS, 370.0 ± 127.3 ppm. There was no significant difference between water conductivity (t-test, p = 0.7, df = 40), total dissolved solids (t-test, p = 0.09, df = 40), and pH (t-test, p = 0.05, df = 40) in test and control experiments. The recommended pH range for fish growth is 6−9, electric conductivity is 100−2000 µs/cm (Ezeanya *et al*., 2015) while total dissolved solids is 500 ppm−1500 mg/L (Scannell and Jacobs, 2001).

Microplastic abundance and wet weight change of Nile tilapia

Microplastics were found in both intestines and gills of the tested fish in great abundance. Polyvinyl chloride microplastics were the most abundant because of the size (30 µm) which was smaller than other microplastic sizes. Other microplastics with size 600 µm to 2000 µm were not determined in both gills

and intestines. The microplastic concentrations in intestines were in concentration range of 4.68 x 10^3 ± 3.02 x 10^3particles/kg to 4.40 x 10^4±5.34 x 10^4 particles/kg (mean ± standard deviation) and in gills with concentration range of 3.37 x 10^2 ± 4.04 x 10^2particles/kg to 2.32 x 10^3 ± 3.57 x 10^3 particles/kg (Table 3).

The wet weight concentration (mean ± standard deviation) increase for test fish was 1.7± 0.66–3.66±0.74g and for non-test fish was 5.2 ± 1.85–6.54 ± 2.11 g. The results indicated that the non-test fish had great increase in wet weight compared to test fish (Table 2) although the statistical comparison (ONE way-ANOVA) indicated that there was no significant difference in growth changes (df = 79, p = 0.9) between test fish and non-test fish. The change in growth length was high in non-test fish. There was significant difference ($p<0.05$, p = 0.00, t-test) in the effects of microplastics among test fish, also there was medium correlation (r = 0.3, p = 0.00) between microplastic effects on fish weight changes. The change in growth weight of test fish gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relationship between growth weight change and microplastics did not depend on concentration of microplastics, which means even low concentration could affect negatively the fish growth (Figure 1).

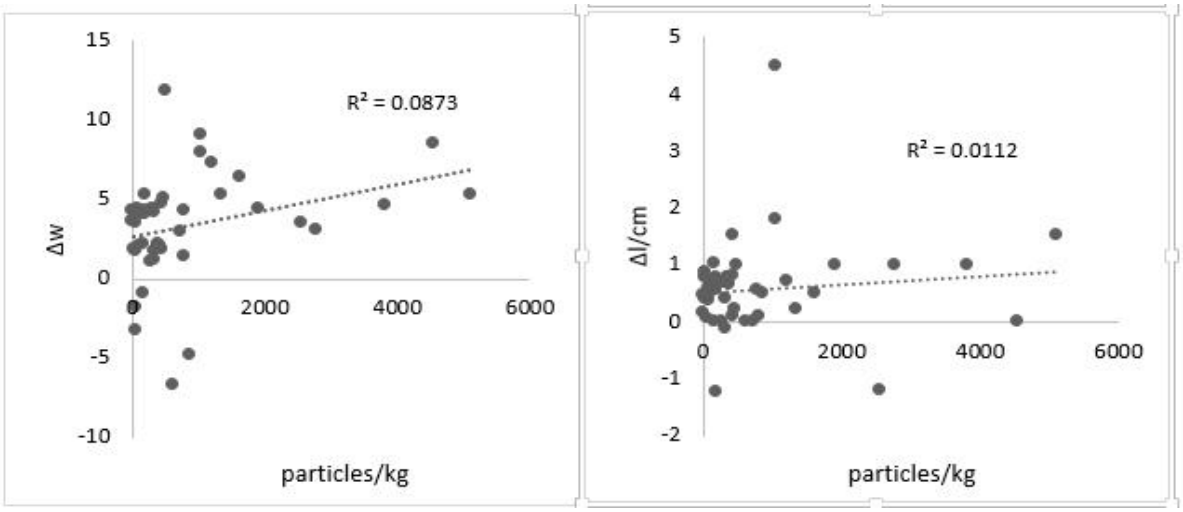


Figure 1. Relationship between (a) Growth change in weight and microplastic concentration (b) Growth length change and microplastic concentration

The change in growth length of test fish had a range of 0.1 ± 0.06 to 1.21 ± 1.16 cm (mean ± standard deviation) compared to the non-test which had range of 0.62±0.12 to 1.7±1.0 cm (Table 2). The mean length growth change in test fish was smaller compared to non-tested fish because of some fish which had fin rot which resulted to loss of tails. The results indicated that there was small correlation (r= 0.1, p = 0.00) between microplastic effects on fish length changes. The change in growth length of tested fish gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relationship between growth length

change and microplastics did not depend on concentration of microplastics.

Table 2. Engulfed microplastics in gills and intestines and fed microplastics and Comparison of mean change in fish weight (g), change in fish length (cm) between tested and non-tested fish in relation to the engulfed MPs (particles/kg)

Engulfed microplastics (mean ± standard deviation) in gills and intestines together with fed microplastics			
Exp	**Exposed Ps/m^3**	**Gills, Particles/kg**	**Intestines**
1	2.7×10^7	$3.37 \times 10^2 \pm 4.04 \times 10^2$	$4.68 \times 10^3 \pm 3.02 \times 10^3$
2	9.0×10^7	$9.95 \times 10^2 \pm 9.70 \times 10^2$	$5.50 \times 10^3 \pm 9.31 \times 10^3$
3	1.3×10^8	$1.79 \times 10^3 \pm 1.88 \times 10^3$	$4.40 \times 10^4 \pm 5.34 \times 10^4$
4	6.7×10^8	$2.32 \times 10^3 \pm 3.57 \times 10^3$	$1.08 \times 10^4 \pm 1.00 \times 10^4$

Comparison of (mean ±standard deviation) change in fish weight (g), change in fish length (cm) between tested and non-tested fish in relation to the (mean ±standard deviation) engulfed microplastics (particles/kg)					
Exp	**Change weight**		**Change in Length**		**Engulfed particles**
	Tested fish	**Non-Test fish**	**Tested fish**	**Non-Test fish**	
1	1.75 ±0.66	5.2 ±1.88	0.46±0.42	0.65±0.34	$5.03 \times 10^3 \pm 3.07 \times 10^3$
2	3.66 ±0.74	6.54 ±2.11	0.6±0.18	0.62±0.12	$3.58 \times 10^3 \pm 9.94 \times 10^3$
3	2.53 ±2.9	6.52 ±2.32	1.21±1.16	0.8±0.5	$4.59 \times 10^4 \pm 5.36 \times 10^4$
4	1.7 ±3.62	6.10 ±2.62	0.1±0.06	1.7±1.0	$2.33 \times 10^4 \pm 3.85 \times 10^4$
Mean	2.41±0.92	6.09±0.54	0.57±0.49	0.95±0.53	$1.94 \times 10^4 \pm 1.71 \times 10^4$

Biological Effects of Microplastics on Nile tilapia

The microplastic effect was found in the fourth day from the test initiation. The fish which were tested with microplastics and indicated loss of equilibrium were 4±1 fish (mean ± standard deviation), abnormal swimming behavior were 4±2 fish, abnormal ventilatory function were 6 ± 4 fish and abnormal external appearance were 3±2 fish (Table 3).

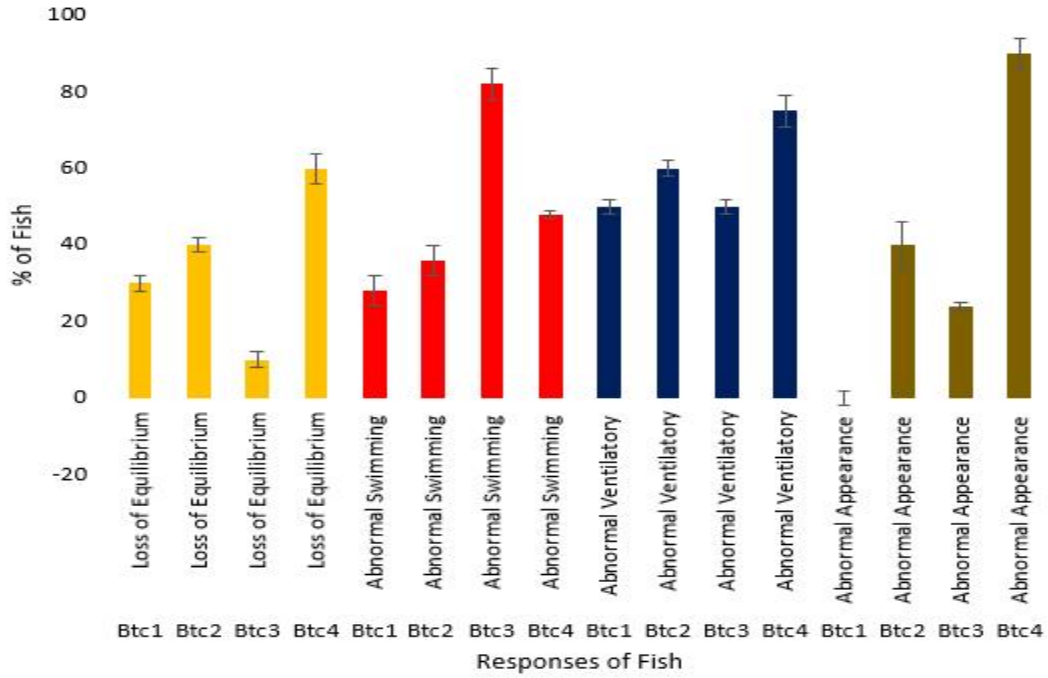
Table 3. The number (mean ± standard deviation) of Nile tilapia which had positive responses on microplastic exposure and the observed clinical signs.

Observation	**Abnormal clinical sign**	**Number of fish**
Loss of Equilibrium	Horizontal Orientation	4 ± 2
	vertical orientation	4 ± 2
	Loss of buoyancy control	4 ± 2
Swimming Behavior	Hypo activity	4 ± 4
	Hyperactivity	5 ± 4
	Surface distribution	6 ± 4
	Bottom distribution	2 ± 4
	Dense schooling	8 ± 1
Ventilatory/Respiration Function	Hyperventilation	9 ± 2
	Hypoventilation	1 ± 2
	Irregular ventilation	6 ± 2
	Gulping	9 ± 4
Abnormal skin pigmentation	Lightened	1 ± 2
External Appearance	Excess mucus secretion	5 ± 6
	Faecal casts (anal)	1 ± 1
	Fin rot	3 ± 4

Some tested fish with difficulty in ventilation by gulping behaviour and selection of space aggregated in one space near the aquarium wall, and some fish had fin rot compared to the non-tested fish without microplastics. However, results did not involve any mortality cases of fish because they were not observed in experiments. The No Observable Effect Concentration (NOEL) was assumed to correspond to the highest concentration tested which was 4.40 x 10^4 particles/kg similar to the report by Hasselerharm (2022).

Evaluation of microplastic toxicity on Nile tilapia

The percentage of fish which had abnormal external appearance was high, followed by abnormal swimming behaviour, abnormal ventilatory function and lastly loss of equilibrium (Figure 2).



Key: Btc is for butch number

Figure 2. Percentage of Fish with their response to Microplastics

The loss of equilibrium was determined by observing fish which had shown clinical signs, namely abnormal horizontal orientation, abnormal vertical orientation and loss of buoyance which was in 35% of fish. The percentage of fish which had shown loss of equilibrium gave scattered cycles which were underlying straight line to show linear relationship between the two variables. The relation to engulfed microplastics had a large negative correlation coefficient, r = -0.7, p = 0.14. Therefore, there was a large relationship between percentage of fish which had loss of equilibrium and the engulfed microplastics (Figure 3).

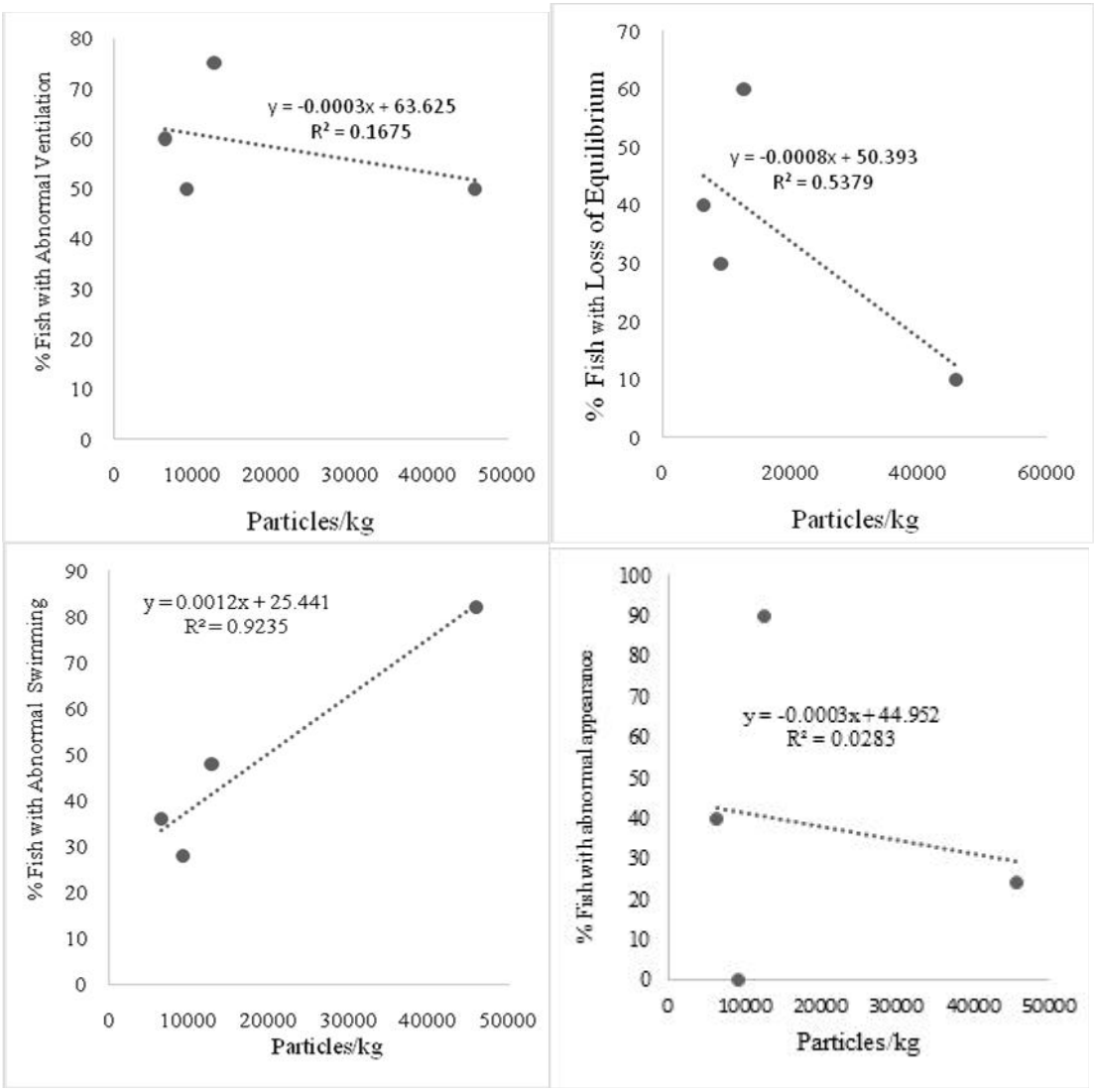


Figure 3. Relationship between fish with abnormal appearance and concentration of engulfed microplastics

The abnormal swimming in fish was observed in 48.1% of fish. The clinical signs which were observed in fish for abnormal swimming were: Hypo activity, abnormal bottom distribution and dense schooling or shoaling. The percentage of fish which had abnormal swimming indicated relationship with engulfed microplastics (Figure 3), where the clustered cycles were close to the straight line. The correlation coefficient, r = 0.9, p = 0.14 indicated presence of large relationship between the percentage of fish with abnormal swimming and engulfed microplastics.

Abnormal ventilation in test fish was observed in 58.8% of fish. The clinical signs for abnormal ventilation observation were: hyper ventilation and hypoventilation. The percentage of fish which had abnormal ventilation indicated a relationship with number of engulfed microplastics (Figure 3)

where the clustered cycles were close to the straight line. The correlation coefficient, r = -0.4, p = 0.13 indicated the presence of medium correlation between percentage of fish with abnormal ventilation and engulfed microplastics.

Abnormal appearance of test fish was not observed in 38.5%. The clinical signs were; oedema, fin rot, and excessive mucus on epidermal part. The clustered cycles were close to the straight line to show close relationship between percentage of fish with abnormal appearance and engulfed microplastics (Figure 3). The correlation coefficient, r = -0.17, p=0.13 indicated small relationship between percentage fish with abnormal appearance and engulfed microplastics.

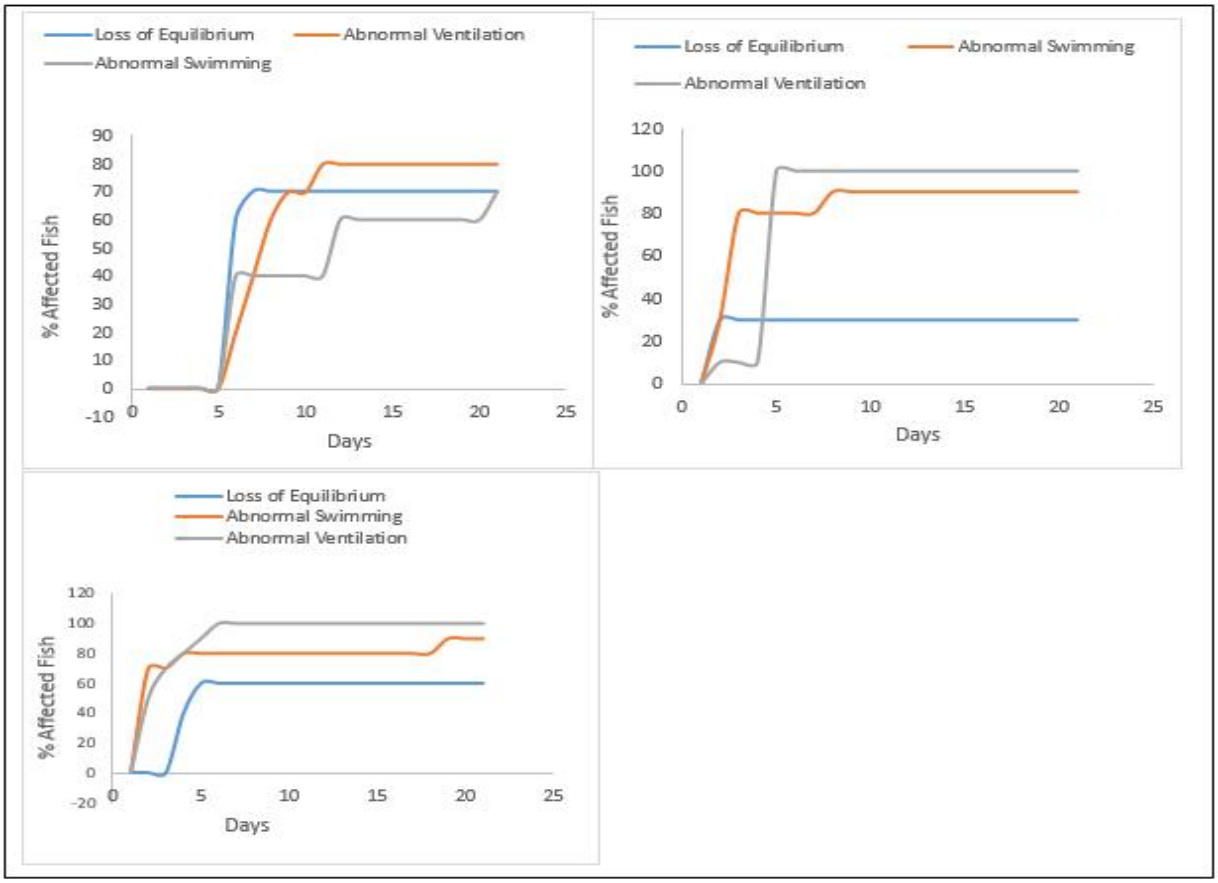


Figure 4. Responses of fish to microplastic exposure in four different experiments

The responses of fish on exposure to microplastics was monitored daily and results indicated that the percentage of tested fish which had positive responses started within day 3 to day 5 from the day microplastics were introduced, then increased to the maximum in 10 days (Figure 4). Among the fish which were exposed to microplastics, 72.5% of them had small weight and 75% had smaller length growth compared to fish which were not exposed to microplastics. The observation was made for daily increase of wet weight in both tested fish and non-tested fish.

The daily wet weight increase in tested fish was low compared to the non-tested fish (Figure 5).

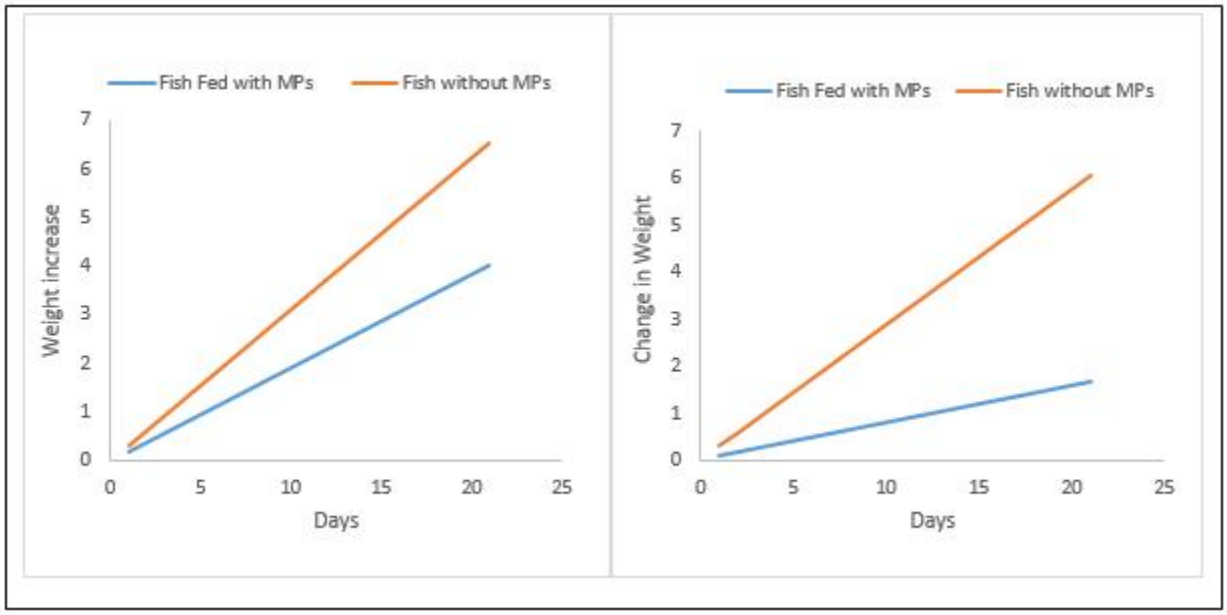


Figure 5. Change in Wet Weight of *Oreochromis niloticus* with days for tested fish and non-tested fish in two different experiments.

DISCUSSION

The temperature conditions which were used in keeping fish for study were in the normal range which is required for fish farming and did not differ much from the temperature 17.7ºC to 27.7ºC used by Chaudhary and Sharma (2018) in the experiment for tilapia in fish tank. Water electric conductivity, total dissolved solids, and pH were within the limit of water quality requirements where pH is 6.0-9.0 for freshwater fish, TDS upper limit is 1500 ppm, although the one that causes effect is 5000−10,000 ppm (Scannel and Jacobs, 2001). One report by Chaudhary and Sharma (2018) for fresh water fish kept in the tank, indicated that water was kept alkaline with pH range of 8.3−8.50 in both control and test tanks and the water electric conductivity was 1730µs/cm–1980 µs/cm, the conditions which were different from what used in this study but both were within the limits of freshwater fish growth. In this study, it was found that microplastics concentrated in gills and intestines the scenario which indicates that the test fish in aquarium could not manage eliminating particles of microplastics during taking in water through gills, and eating food. Events like these have been reported also in other studies for microplastics in marine biota because of presence in ecosystems (Thompson *et al*., 2009).

The responses of Nile tilapia on exposure to microplastics in this study have been reported as fish stress caused by toxic contaminants of water in many literatures (Coyle, 2004; Davidson *et al*., 2011; Reverter, 2018; Sridhar, 2021). Cocci *et al*. (2022) reported that microplastic occurrence in fish was found to be correlated with antioxidant enzymes (catalase and superoxide dismutase) and cytokinases (interleukin 1 ß, 10 and

interferon) levels, causing reactive oxygen species (ROS) generation and immune cell infiltration in the gut. Moreover the study by Alimba and Faggio (2019) indicated that microplastic presence in marine vertebrates produces oxidative stress in proteins, lipids and deoxyribonucleic Acid (DNA) by altering the antioxidant defuse mechanisms, i.e. enzyme catalase (CAT), superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GP_x) and reduced glutathione (GSH) genes at the catalytic and transcriptional levels. They regulate the gene expression that controls oxidative stress by acting as pro-oxidant stimuli activating antioxidant gene expression through nuclear factor erythroid 2–related factor 2(N_rf2)-dependent mechanism. The clinical signs like abnormal swimming which was observed in Nile tilapia in this study has been reported also by Wright *et al*. (2020) who had observed that the polyvinyl chloride which were exposed to brown trout had reduced swimming activity and there was change in their circadian rhythms. Furthermore, the report by Tang *et al*. (2018) indicated that microplastic exposure in sea bream heads altered the JNK (c-Jun N-terminal kinase) and ERK (extracellular signal-regulated kinase) signalling pathways which are involved in the detoxification process of fish. Even other abnormal behaviours observed in Nile tilapia in this study might be attributed to the similar situation as Yao *et al*. (2023) reported for the exposed high concentration of polystyrene microplastics in golden pompano (*Trachinotus ovatus*) which had caused oxidative damage and up-regulation of genes (GrPn78, X6p-1, and Elf-2α), resulting in endoplasmic reticulum stress (ERS) and severe oxidative stress which raised the BAX/BcL-2 ratios and induced death. More studies report similar cases of microplastic exposure effects although they do not mension directly the clinical signs which have been considered in our observation in Nile tilapia but the oxidative stress must be the reason. For instance; the study by Cao *et al*. (2023) reported the effects of polyethylene microplastics in carp and found that they elevated the expression of P53, NF-KB, P6S, 1KKα, 1KKß, caspase-3 genes in the gills. Also the extended exposure to polystyrene microplastics in coach juveniles (*Paramisgurnus dabbyanus*) was studied by Wang *et al*. (2022) and found to inhibit the expression of keep 1-Nrf2 signalling pathway genes inducing aptosis by upgrading proteins (P53, gadd 45 ba and caspase 3b) expressions, also were found to up-regulate TNF-α and PTGS2A which are gene markers in the inflammatory mechanism in zebra fish (Umamaheswari *et al*., 2021). Lastly, the study by Chen *et al*. (2021) on tilapia had found that polyethylene microplastics caused oxidative stress in the liver by damaging cell membrane increasing lipid peroxidation (PLO) levels, in the brain, dorsal muscles and gills, higher brain activity where 32% of the fish had microplastics in the dorsal muscle (Barboza *et al*., 2020). In addition poor quality of water that was contributed by presence of microplastic contamination might have lead also to diseases that rise from bacteria and fungi. Because fouling organisms like fungi and bacteria can easily attach to microplastics causing them to be agents of diseases (Dekiff *et al*., 2014). Furthermore, responses indicated by clinical signs in this study have also been observed in other studies for effects of toxin

contamination where 30% to 35% of individual fish responded positively for example some had frequent surface movement with gulping of air (Islam *et al*., 2021), the abnormal alterations were associated with physiological responses which were inductive of stress.

CONCLUSION AND RECOMMENDATION

The test fish engulfed microplastics which were found in intestines and gills. The engulfed microplastics resulted to stress in Nile tilapia which was observed from different responses according to clinical signs and the lag in proper growth. There were no mortality of Nile tilapia but the responses to presence of microplastics in aquarium indicated that microplastics had adverse effects to Nile tilapia. The evaluation of toxicity of microplastics to Nile tilapia indicated that fish in contaminated ponds have high susceptibility to the effect of microplastics which might lead to death if the conditions have not been controlled. Although there have been reports on the effects of microplastics as a cause of oxidative stress to fish, more studies need to be done to give clear description for some observations like fin rot and abnormal mucus secretion responses which might be associated to microplastic harbouring microorganism in large amount.

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To ensure reproducibility, the methodology section should be detailed and transparent. The following information must be included: use precise and recognized scientific nomenclature for all materials and indicate their source; specify all equipment used, including the manufacturer's name, model number, and relevant technical specifications in parentheses (e.g., "centrifuge (Eppendorf, Model 5424)"); describe your procedures step-by-step, ensuring all experimental parameters are specified; provide a comprehensive description for any novel method, including rationale and limitations; reference the original source for established methods, clearly stating and justifying any deviations; and explicitly confirm that procedures involving human subjects or animals were conducted in adherence to ethical standards set by relevant national governing bodies, including providing approval details and measures taken to minimize harm.

The statistical analysis done and statistical significance of the findings, when appropriate, should be mentioned. Unless absolutely necessary for a clear understanding of the article, a detailed description of statistical treatment/analysis may be avoided. Articles based heavily on statistical considerations, however, need to give details particularly when new or uncommon methods are employed. Standard and routine statistical methods employed need to give only authentic references.

Results

The purpose of your data presentation (i.e. the result section) is to support your discussion and conclusions. Therefore, only include data that is crucial to understand your key findings. Structure the data in a unified and logical sequence, allowing the reader to follow the remaining sections easily. Avoid unnecessary repetition: if data is presented in a table or figure, do not repeat it in the text. Instead, use the text to emphasize or summarize noteworthy findings. Choose either tables or figures to present the same data – avoid presenting it both ways. Critically

analyze and interpret the implications of your data within the discussion section, providing a thoughtful and comprehensive interpretation.

Discussion

The discussion section should provide an insightful analysis or interpretation of your results, moving beyond a mere restatement of your findings. Interpret your results and explain their relevance to the wider scientific community, drawing clear connections to established knowledge and identifying new insights. Avoid directly repeating the results and rather analyze them with logical deductions and scientific justification. Acknowledge and discuss any study limitations or potential biases. The conclusions should answer your research questions, but avoid unsupported statements and refrain from claiming priority on ongoing work. Make sure hypotheses are clearly identified, and include recommendations only when they directly address your findings and are of critical importance.

Acknowledgment

The acknowledgment section should be brief and reserved for recognizing specific and substantial scientific, technical, or financial contributions to the research. Avoid acknowledging routine departmental facilities, general encouragement, or assistance with manuscript preparation, such as typing or secretarial support. Focus only on acknowledging individuals and institutions that directly contributed to the intellectual or material aspects of the research.

Tables

Tables should be prepared in MS Word's Table Editor, using (as far as possible) "Simple1" as the model:

(Table … Insert … Table … Auto format …Simple 1). Tables taken directly from Microsoft Excel are not generally acceptable for publication.

Use Arabic (1, 2, 3 …), not Roman (I, II, III …), numerals for tables. Footnotes in tables should be indicated by superscript letters beginning with "a" in each table. Descriptive material not designated as a footnote maybe placed under a table as a Note.

Illustrations

Preparation: Similar figures should be arranged into plates whenever possible; leave very little space between adjoining illustrations or separate them with a thin white line. Line art should be scanned at 900 dpi, photographs (halftone or color) at 300 dpi, and figures with line art and halftones at 600 dpi. Crop the illustrations to remove non-printing borders. Make lines thick enough and text large enough to compensate for the reduction. Dimensions of the original artwork should not exceed 28 cm x 21.5 cm; the printed area of the journal page measures 20.3 x 14 cm. Submission: TIFF or JPG files of figures should be of high quality and readable in Adobe Photoshop. Do not embed figures in the manuscript document.

The figures will be evaluated during the Editorial reading of the article, and if necessary, instructions will be provided for the submission of adequate illustrations.

Insert … Symbol … Special characters

All data should be given in the metric system, using SI units of measurement.

Use "." (point) as the decimal symbol. Thousands are shown spaced, thus: 1 000 000. Use a leading zero with all numbers <1, including probability values (*e.g.* $p < 0.001$).

Numbers from one to nine should be written out in the text, except when used with units or in percentages (*e.g.* two occasions, 10 samples, Five seconds, 3.5%). At the beginning of a sentence, always spell out numbers (*e.g.* "Twenty-one trees were sampled... ").

Use the 24-hour time format, with a colon ":" as separator (*e.g.* 12:15 h). Use day/month/year as the full date format (*e.g.* 12 August 2001, or 12/08/01 for brevity in tables or figures). Give years in full (*e.g.* "1994–2001", never "94–01"). Use the form "1990s", not "1990"s" or "1990ies".

Use the en-dash – for ranges, as in "1994–2001" (Insert … Symbol … Special characters En dash).

In stating temperatures, use the degree symbol " º ", thus " ºC ", not a super script zero " 0 ". (Insert … Symbol … Normal text),

Define all symbols, abbreviations and acronyms the first time they are used, *e.g.* diameter at breast height (DBH), meters above sea-level (masl). In the text, use negative exponents, *e.g.* $g\ m^{-2}$, $g\ m^{-2}\ sec^{-1}$, $m^3\ ha^{-1}$ as appropriate. Use "h" for hours; do not abbreviate "day".

If possible, format mathematical expressions in their final version (*e.g.* by means of Equation Editor in MS Word or its equivalent in Word Perfect or Open Office); otherwise, make them understandable enough to be formatted during typesetting (*e.g.* use underlining for fractions and type the numerator and denominator on different lines).

MS word equations can be used for all mathematical equations and formulae (Insert….Equations).

References

All literatures referred to in the text should be cited as exemplified below.

Please inspect the examples below carefully, and adhere to the styles and punctuation shown.

Capitalize only proper names ("Miocene", "Afar", "The Netherlands") and the initial letter of the title of papers and books, *e.g.* write "Principles and procedures of statistics", not "Principles and Procedures of Statistics".

Do not italicize Latin abbreviations: write “et al.”, not “*et al.*”

References in the text should use the ‘author-year’ (Harvard) format:

(Darwin and Morgan, 1993) or, if more than two authors, (Anderson et al., 1993). Arrange multiple citations chronologically (Hartman and Kester, 1975; Anderson et al., 1993; Darwin and Morgan, 1994).

References in the list should be in alphabetical order, in the following formats:

Journal article

Kalb J.E. 1978. Miocene to Pleistocene deposits in the Afar depression, Ethiopia. SINET: *Ethiop. J. Sci.* 1: 87-98.

Books

Whitmore T.C. 1996. An introduction to tropical rain forests. Clarendon Press, Oxford, 226 pp.

Steel R.G.D. and Torrie J.H. 1980.Principles and procedures of statistics.2nd ed. McGraw-Hill Book Co., New York.633 pp.

Book chapter

Dubin H.J. and Grinkel M. 1991.The status of wheat disease and disease research in warmer areas. In: Lange L.O., Nose1 P.S. and Zeigler H. (Eds.) Encyclopedia of plant physiology. Vol. 2 A Physiological plant ecology. Springer-Verlag, Berlin. pp. 57-107.

Conference /workshop/seminar proceedings

Demel Teketay. 2001. Ecological effects of eucalyptus: ground for making wise and informed decision. Proceedings of a national workshop on the Eucalyptus dilemma, 15November 2000, Part II: 1-45, Addis Ababa.

Daniel L.E. and Stubbs R.W. 1992.Virulenceof yellow rust races and types of resistance in wheat cultivars in Kenya. In: Tanner D.G. and Mwangi W. (eds.). Seventh regional wheat workshop for eastern, central and southern Africa. September 16-19, 1991. Nakuru, Kenya: CIMMYT. pp. 165-175.

Publications of organizations

WHO (World Health Organization) 2005. Make every mother and child count: The 2005World Health Report. WHO, Geneva, Switzerland.

CSA (Central Statistical Authority) 1991.Agricultural Statistics. 1991. Addis Ababa, CTA Publications. 250 pp.

Dissertation or Thesis

Roumen E.C.1991. Partial resistance to blast and how to select for it. Ph.D. Thesis. Agricultural University, Wageningen. The Netherlands.108 pp.

Gatluak Gatkuoth 2008. Agroforestry potentials of under-exploited multipurpose trees and shrubs (MPTS) in Lare district of Gambella region. MSc. Thesis, College of Agriculture, Hawassa University, Hawassa.92 pp.

Publications from websites (URLs)

FAO 2000.Crop and Food Supply Assessment Mission to Ethiopia. FAOIWFP. Rome. (http://www. fao. or~/GIE WS). (Accessed on 21 July 2000).

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