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Theoretical Model Predictions for Production of Medically Used Radionuclides on Alpha Induced with Cobalt-59 At Energies of 25 - 172 MeV

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KEYWORDS:

COMPLET code; Excitation function; Radionuclide production; Reaction cross section; TALYS-1.95 code

ABSTRACT

This study used the theoretical nuclear model codes COMPLET and TALYS-1.95 to make theoretical predictions of the medically important production cross-sections for Chromium-51, Manganese-54, Iron-59, Cobalt-59 and Cobalt-60 radionuclides produced in the interaction of alpha- projectile with Cobalt-59 target ≈ 25 – 172 MeV alpha-energies. The results were compared with the measured values in the EXFOR data library. Pearson's correlation coefficient indicates a strong and positive correlation between the predicted and the previously measured medically important production cross-sections for Chromium-51, Manganese-54, Iron-59, Cobalt-59, and Cobalt-60 radionuclides. Further, the results showed that except for Chromium-51, the COMPLET code predicts more successful outcomes than the TALYS-1.95.

INTRODUCTION

Nuclear reaction cross-section data are very important to the field of medical radiobiology in both diagnostic imaging and targeted therapy (Kebede, 2021), which is crucial for the optimized production of radionuclides. In nuclear medicine, radionuclides are used for various useful applications, such as diagnosis, therapy, prevention of many serious ailments, and research to evaluate metabolic, physiologic, and pathologic conditions of the human body (Aydin *et al*., 2007). The successful production and usage of these radionuclides extends to oncology, cardiology, and even psychiatry through imaging procedures where information about the function of every major organ and tissue of the human body can be generated. Many radionuclides used in nuclear medicine are produced in cyclotrons, accelerators, or nuclear reactors, and production is an important and constantly evolving issue. In addition to this, different radionuclides play significant roles in technological applications of importance to our daily lives and scientific research (Aydin *et al*., 2007; Kilinç *et al*.,2016).

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The Positron Emission Tomography (PET) imaging technique is widely used for planning, early diagnosis of cancer, and evaluation of the treatment response in patients with cancer. This imaging technique is also used to study diseases of the heart, brain, thyroid, etc. (Noori *et al*., 2017) for example, Cobalt-57($T_{1/2}$ =272 d) is used as a marker to estimate organ size and for in vitro diagnostic kits. Similarly, Chromium-51 radionuclide ($T_{1/2}$=28 d) is used to label red blood cells and quantify gastro-intestinal protein loss (Aydin *et al*., 2007; Kilinç *et al*., 2016).
Production cross sections for charged particles, especially nuclear reactions on metals that are induced by alpha, are important in medical radioisotope production (Mohamed, 2006; Demir *et al*., 2017). Accordingly, reasonable comparative theoretical reaction model studies with an experiment using light-charged projectiles (proton, deuteron, and alpha) are beneficial (Qaim *et al*., 2016; Tárkányi *et al*., 2019; Amanuel, 2023) because of the non-availability of experimental cross-section data for the production of medical radionuclides, particularly in alpha-induced reactions, which are limited and still need further investigations.
To optimize the production routes, charged particle-induced cross-sections are desired. To optimize the radioisotope produced, a full knowledge of the excitation function is necessary, which helps maximize the yield of the desired product and minimize the radioactive impurities (Qaim *et al*., 2002).

In radionuclide production, accurate reaction cross-section data are required for well-controlled and maximized production routes (Mohamed, 2006; Qaim, 2010). Nuclear reaction model-based computer codes can be essential in predicting production cross-sections, particularly for radionuclides whose experimental data are either unavailable or have significant discrepancies. In addition, theoretical model predictions have played a crucial role in creating optimized reference cross-section data, particularly in producing medically useful radionuclides (Koning *et al*., 2013) that were calculated using the Monte Carlo nuclear reaction simulation codes TALYS 1.95 and COMPLETE.

Despite all efforts, one of the crucial aspects of the reaction mechanisms study is finding optimized production routes for radionuclides, particularly for medically used radionuclides. Moreover, it is evident that the non-availability of experimental cross-section data for producing medically useful radionuclides, particularly in alpha-induced reaction, are very limited and need further investigation. Therefore, the present work focuses on finding optimized production routes for medically useful radionuclides produced in the reaction of α-projectile with ^{59}Co-target, more specifically, to evaluate the nuclear data for the production of Chromium-51, Manganese-54, Iron-59, Cobalt-59, and Cobalt-60 on alpha-induced Cobalt-59 at alpha energy of positron-emitting radionuclides.

MATERIALS AND METHODS

Several theoretical nuclear reaction model-based computer codes have been used to predict radionuclide production cross-sections (Koning *et al*., 2013; Amanuel, 2023). In this work, predictions of production cross sections for ^{51}Cr, ^{54}Mn, ^{59}Fe, and ^{57,60}Co radionuclides produced in the interaction of alpha-projectile with Cobalt-59 target via (a, x) channel were carried out using the computer codes TALYS-

1.95 and COMPLETE. The results were compared with the experimental data (Michel *et al*., 1980).

These codes were selected because they have been successful and widely used for predicting production cross-sections and evaluating reaction data (Amanuel, 2023). The present work also used the default values of the level density nuclear model TALYS-1.95 computer codes.

TALYS-1.95 Code

TALYS-1.95 code is an advanced version of the TALYS code family with additional features. TALYS was initially developed in 1998 when it was decided to implement the combined knowledge of nuclear reactions into one single software package which integrates the pre-equilibrium, direct, optical model, statistical, and fission nuclear reaction models and for all the open reaction channels it gives prediction in one calculation scheme (Qaim *et al*., 2016). A Monte Carlo reaction code that simulates all types of nuclear reactions; it runs on a Linux operation system and is written in the FORTRAN programming language. One of the possible outcomes of using a Monte Carlo method for nuclear data evaluation is that a series of correlations can be extracted from the previous results. Therefore, the objective and vision of its construction were to provide a complete and accurate simulation of nuclear reactions that involve neutrons, photons, protons, deuterons, tritons, ^{3}He, and alpha particles in the 1 keV-200 MeV energy range, with some exceptions. The code's data was based on the reference input parameter library through an optimal combination of reliable nuclear models, resilience, and ease of use (Koning *et al*., 2013).

The theory of excitation functions for the production of medical radioisotopes are obtained with alpha-induced reactions (a,x) for some radioisotopes used in medicine that are important for the development of improved nuclear reaction theory and for many medical applications were calculated by using TALYS 1.95 code. In this code, the reaction cross section for entrance channel α and exit channel β can be expressed, in general, using Hauser-Feshback (Hauser-Feshback, 1952) formalism as follows:

$$\sigma_{\alpha\beta} = \frac{\pi}{k^2}\sum_J \frac{(2J+1)}{(2i_\alpha+1)(2I_\alpha+1)} \frac{\sum_{s,\ell} T_\ell(\alpha)\sum_{s',\ell'} T_{\ell'}(\beta)}{\sum_\alpha \sum_{s,\ell} T_\ell(\alpha)} \quad (1)$$

Where s is the channel spin, T_ℓ represents the transmission coefficients, and l is the orbital angular momentum. The Hauser-Feshback formula is simplest for the energy-average angle integrated cross-section of statistical reactions (reaction cross-section leading to a single final state).

COMPLETE code

Computer code COMPLETE is a modified and advanced version of the Alice code family with additional physics, corrections, and capabilities and has been used to predict production cross-sections (Asres *et al*., 2018). This code has successfully predicted numerous nuclear data sets, particularly for the production of medical radionuclides (Yiğit and Tel, 2013; Asres *et al*., 2019). This code employs the Weisskopf-Ewing (Weisskopf and Ewing, 1940) formulation for compound nucleus (CN) emission and the hybrid, as well as geometry-dependent hybrid, model of Blann for PE emission of particles

(Blann and Vonach, 1983). In the complete code, level densities of residual nuclei play an important role in deciding the shapes and absolute values of excitation functions (Akkoyun *et al*., 2015). This code uses the Weisskopf-Ewing formulation to predict reaction cross-sections as follows:

$$\sigma_{\alpha}\ d\varepsilon_{\beta} = \sigma_{C}\ (\alpha)\frac{(2i_{\beta}+1)u_{\beta}\varepsilon_{\beta}\sigma_{C}\ (\beta)\omega(u_{\beta})d\ \beta}{\sum_{\alpha}\int_{0}^{E_{\alpha}^{m\ x}}(2i_{\alpha}+1)u_{\alpha}\varepsilon_{\alpha}\sigma_{C}\ (\alpha)\omega(u_{\alpha})d\ \alpha} \quad (2)$$

Where u_{α} is the reduced mass of the ejectile α, and σ_C (α) is the cross-section for the formation of the CN.

Comparison between experimental and Theoretical results

The theoretical and experimental reaction cross-sections are plotted against the projectile energies and shown in Figs. 1–5. Pearson's correlation coefficient quantifies the level of mutual statistical dependence between two variables (Baak *et al*., 2020). Typically, their values range from -1 to +1 or 0 to +1, where 0 means no statistical association, +1 means the strongest possible association, and −1 means the strongest negative relation.

In general, the data of this study have been analyzed after the theoretical data have been generated using the computer codes TALYS-1.95 and COMPLETE. The theoretical and experimental total cross-section results are compared using Pearson's correlation coefficient. Correlation is a measure of association between two variables. The mathematical description is given by Tárkányi *et al*. (2019).

$$R=\frac{\sum_{i=1}^{N}(X_{T_i}-<X_T>)(X_{E_i}-<X_E>)}{(N-1)(S_{XT})(S_{XE})} \quad (3)$$

Where;

$$<X_T>=\frac{1}{N}\sum_{i}^{N}(X_{T_i}) \quad (4)$$

$$S_X=\sqrt{\frac{1}{N-1}\sum_{i-1}^{N}(X_{T_i}-<X_T>)} \quad (5)$$

$$<X_E>=\frac{1}{N}\sum_{i-1}^{N}(X_{E_i}) \quad (6)$$

$$S_X=\sqrt{\frac{1}{N-1}\sum_{i-1}^{N}(X_{E_i}-<X_E>)} \quad (7)$$

Where R is the correlation coefficient and unit less, ⟨X_T⟩ and ⟨X_E⟩ are the mean theoretical and experimental reaction cross-sections, respectively, X_{T_i} and X_{E_i} are the theoretical and experimental total cross-sections of the i^{th} value, respectively, N is the number of the theoretical and experimental data, S_{XT} and S_{XE} are the standard deviations of the theoretical and experimental total cross-sections respectively. If $0 \leq R \leq 0.3$, the correlation is weak and positive, $0.3 \leq R \leq 0.7$ describes a moderate correlation, and $0.7 \leq R \leq 1$, the correlation is strong (Baak *et al*., 2020).

RESULTS AND DISCUSSION

The present work investigated the excitation functions of medically important Chromium-51, Mangenes-54, Iron-59, Cobalt-59, and Cobalt-60 radionuclides produced in the interaction of alpha-projectile with Cobalt-59 target at 25–172 MeV alpha-energies. In addition, the experimentally measured excitation functions available in the literature (Michel *et al*., 1980) were compared using the nuclear reaction-model codes TALYS-1.95 and COMPLET.

In COMPLETE code, the level density parameter a, which predominantly affects the equilibrium state components of a cross-section, is calculated from the expression:

$$a = \frac{A_C}{K} \quad (8)$$

Where *A* is the nucleon number of *a* compound system, and *K* is an adjustable constant, which may be varied to match the experimental data. For the present system, a representative ^{59}Co (α, x) ^{60}Co reaction, the value of K was varied (K = values of 8, 10, and 12 were used) to match the experimental data. A value of K = 10 in general reproduced satisfactorily experimentally measured EFs for Cobalt-60 residue. This value is consistently used for other residues populated in the interaction of the alpha-projectile with the target Cobalt-59. For the same representative Cobelt-60 residue, the initial exciton numbers n_o = 4 (2,2,0), 5 (2,2,1) were varied, and it was found that a value of n_o = 4 (2,2,0) better reproduced the measured excitation function (Michel and Brinkmann, 1980). For COMPLETE code prediction, K = 10 and n_o = 4 are consistently used for all residues populated in the interaction of the alpha-projectile with the target Cobalt-59.

Production of Chromium-51 radionuclide

The theoretically predicted and experimentally measured excitation functions for Crominum-51 radionuclide produced via the (α, x) channel in the interaction of alpha-projectile with the Cobalt-59 target are shown in Figure 1. By using the TALYS-1.95 code, predicted cross-section values, except at alpha energies of 90–120 MeV, are in very good agreement with the measurements of Michel and Brinkmann (1980). It may further be seen that COMPLETE code predicted cross-section values that were generally in satisfactory agreement with the measured values of Michel and Brinkmann (1980).

It may be observed from Table 1 that Pearson's correlation coefficient values for TALYS-1.95 and COMPLETE code predicted cross-section values confirm strong positive correlations between the theoretically predicted and experimentally measured production cross-sections (Michel and Brinkmann, 1980).

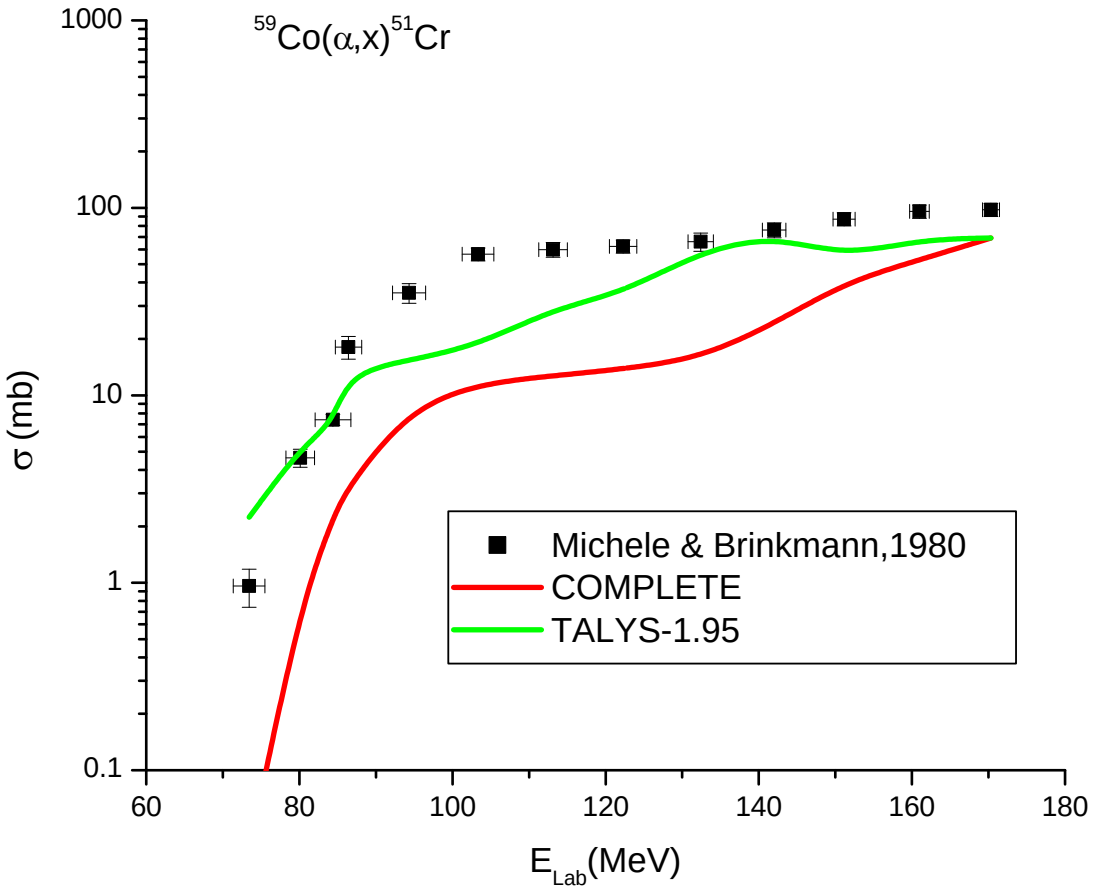

Figure 1. The experimentally measured and theoretically predicted excitation functions for medically used Crominum-51.

Production of Manganese-54 Radionuclide

The experimentally measured production cross-sections for Manganese-54 radionuclide from the literature are compared with the theoretical predictions obtained using the COMPLET and TALYS-1.95 codes. Fig. 2 displays the measured excitation functions along with theoretical predictions for Manganese-54radionuclide produced via the (α, x) channel in the interaction of alpha-projectile with a Cobalt-59 target at 25 MeV–172 MeV. Using COMPLETE code, predicted cross-section values except for 60 MeV–90 MeV are in very good agreement with the cross-section values measured by Michel and Brinkmann (1980). Figure 2 shows that the predicted cross-section values using the TALYS-1.95 code generally underestimate the measured values of Michel and Brinkmann (1980). The COMPLETE code predicted production cross sections for Manigenes-54 radionuclide to have a peak value of 111 mb at ≈170 MeV.

In addition, it may be observed from Table 1 that, for Manganese-54 radionuclide, Pearson's correlation coefficient values between theoretically predicted on COMPLETE and experimentally measured production cross sections confirmed moderately positive correlations. Pearson's correlation coefficient values between theoretically predicted using TALYS-1.95 and experimentally measured production cross-sections confirmed strong positive correlations.

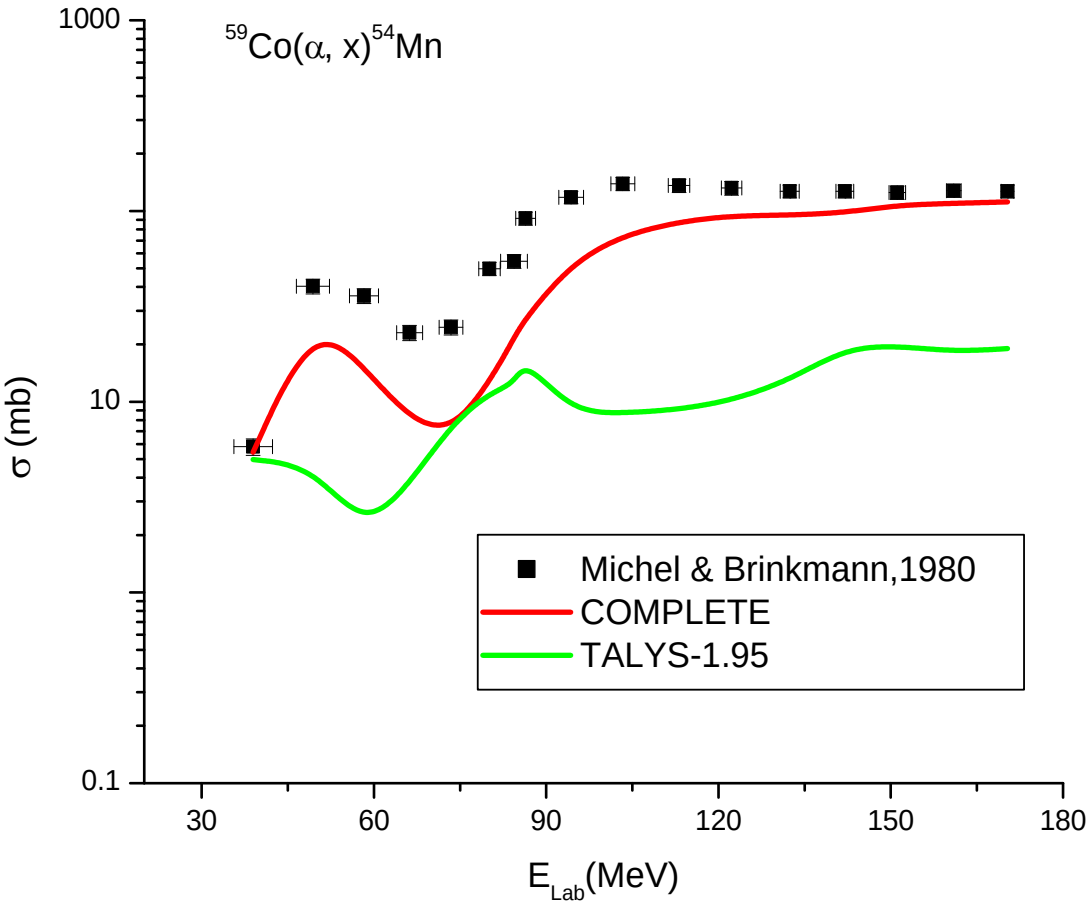

Figure 2. The experimentally measured and theoretically predicted excitation functions for medically used Manganese-54.

Production of Iron-59 radionuclide

Figure 3 displays the experimentally measured excitation functions along with theoretical predictions obtained using TALYS-1.95 and COMPLETE codes for Iron-59 radionuclide produced via (α, x) channel in the interaction of alpha-projectile with the Cobalt-59 target. Using COMPLETE code, the predicted cross-section values in the energy range 55–172 MeV generally agree with the experimental measurements of Michel and Brinkmann (1980). However, the prediction of COMPLETE code

below 55 MeV underestimates the cross-section values measured by Michel and Brinkmann (1980). It may further be observed from Figure 3 that the predicted cross-section values at low energy using the TALYS-1.95 code are in satisfactory agreement with the measured cross-section values of Michel and Brinkmann (1980). On the contrary, the predicted cross-section values at a high energy range using the TALYS-1.95 code underestimate the measured cross-section values of Michel and Brinkmann (1980).

In addition, it may further be observed from Table 1 that Pearson's correlation coefficient values for Iron-59 radionuclide confirmed strong and positive correlations between the cross-section values predicted using the COMPLETE code and the values measured by Michel and Brinkmann (1980).

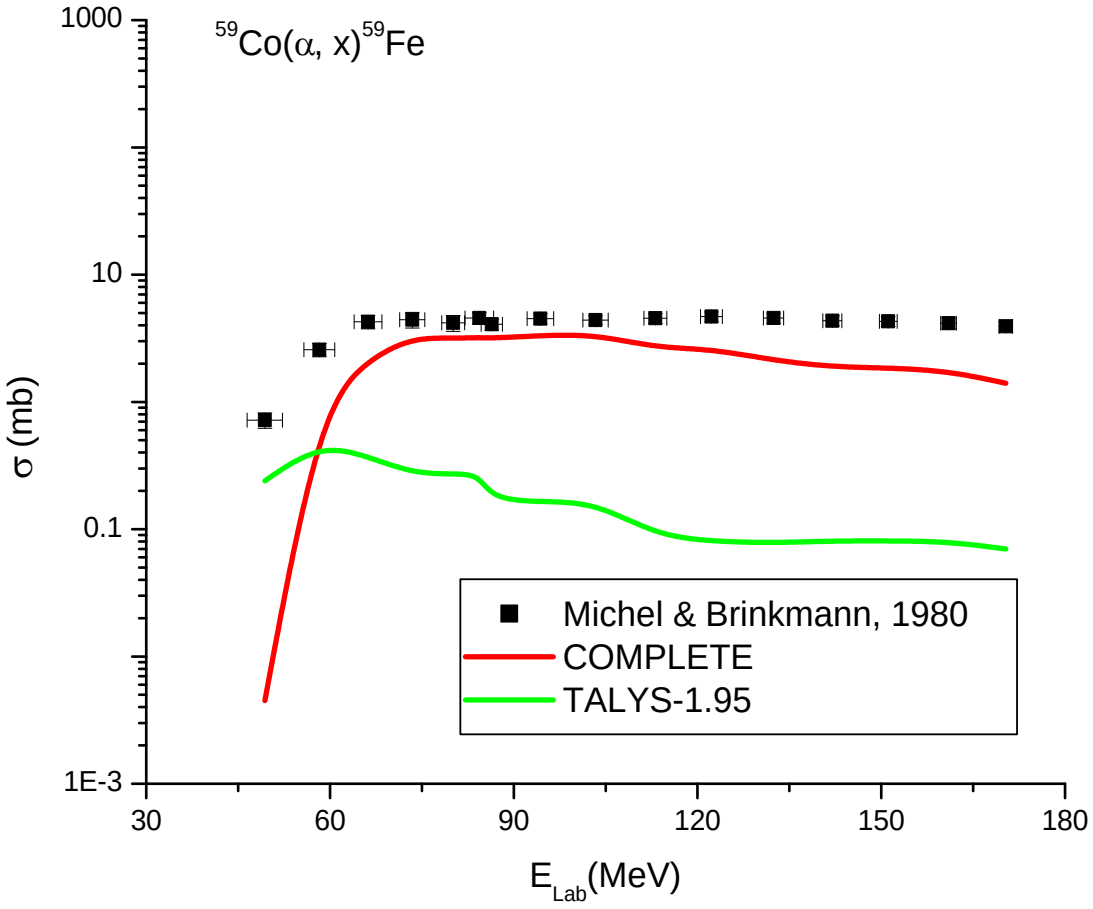

Figure 3. The experimentally measured and theoretically predicted excitation functions for medically used Iron-59.

Production of Cobalt-57 radionuclide

In Figure 4, the measured excitation functions for the medically used Cobalt-57 radionuclide produced via the complex (α, x) channel in the interaction of alpha-projectile with Cobalt-59 target are displayed together with the theoretically predicted excitation functions by using the TALYS-1.95 and COMPLETE codes. Using COMPLETE code, predicted production

cross-sections are in very good agreement with the measured values of Michel and Brinkmann (1980). Using the TALYS-1.95 code, predicted production cross sections in the energy range 30 MeV–90 MeV are in very good agreement with the measured values (Michel and Brinkmann, 1980). However, predicted production cross sections using the TALYS-1.95 code at energies above 90 MeV are in satisfactory agreement with the measured values of Michel and Brinkmann (1980). The COMPLETE predicted production cross sections for Cobalt-57 radionuclide have a maximum value of ≈207 mb at ≈39 MeV. In addition, it may further be observed from Table 1 that Pearson's correlation coefficient values for Cobalt-57 radionuclide confirmed strong and positive correlations between the cross-section values predicted and the values measured by Michel and Brinkmann (1980).

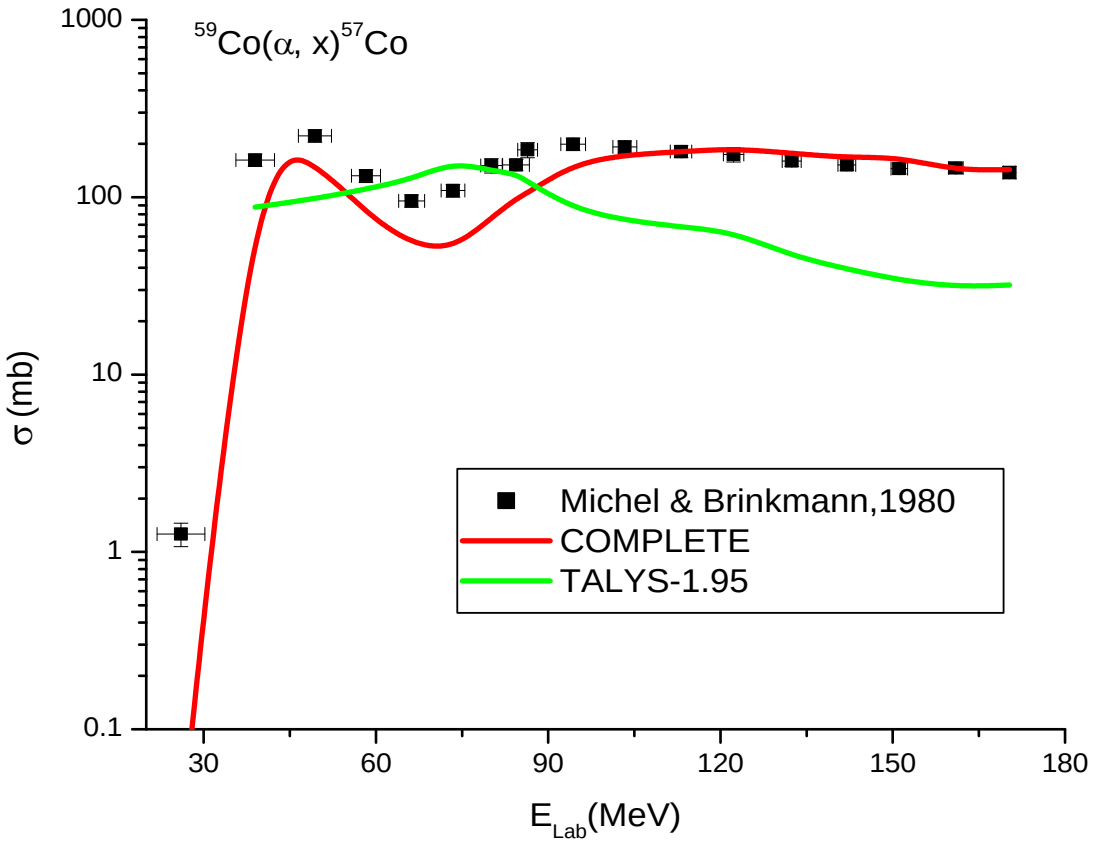

Figure 4. The experimentally measured and theoretically predicted excitation functions for medically used Cobalt-57

Production of Cobalt-60 Radionuclide

Figure 5 displays the experimentally measured and theoretically predicted excitation functions for the medically used Cobalt-60 radionuclide produced in alpha-projectile interaction with the Cobalt-59 target via complex (α, x) channel. The cross-section values predicted using COMPLETE codes overestimate the cross-section values measured by Michel and

Brinkmann (1980). However, predicted production cross sections in the energy range 30 MeV – 45 MeV are in very good agreement with the measured values of Michel and Brinkmann (1980). The prediction of the TALYS-1.95 code underestimates the cross-section values of the measurement of Michel and Brinkmann (1980). In addition, the maximum value of the production cross section for Cobalt-60 radionuclide obtained using the COMPLETE code is about ≈343 mb at ≈39 MeV. It may further be observed from Table 1 that Pearson's correlation coefficient values between theoretically predicted, and experimentally measured production cross-sections confirmed strong and positive associations.

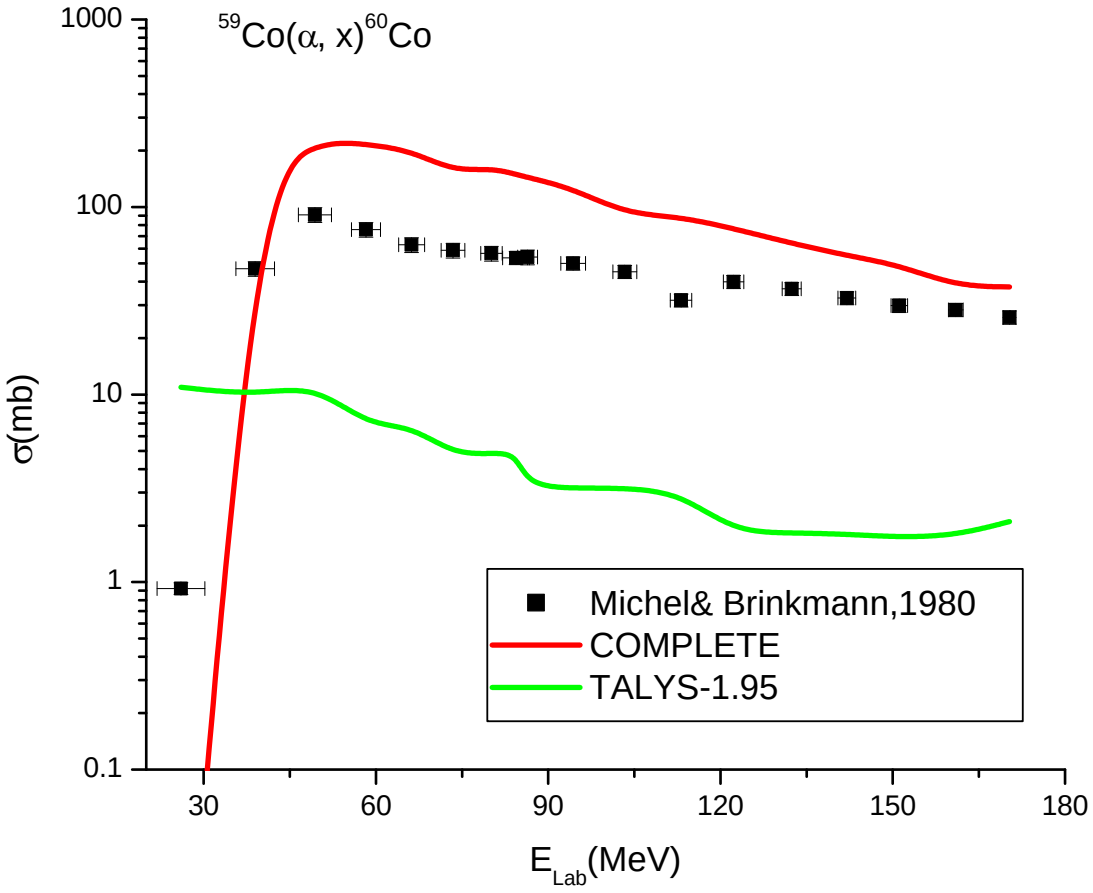

Figure 5. The experimentally measured and theoretically predicted excitation functions for medically used Cobalt-60.

Table 1. Pearson's correlation coefficient, R, between experimental measurements by Michel and Brinkmann (1980) and theoretical predictions

Radionuclide	TALYS-1.95 [COMPLETE]
^{51}Cr	0.921 [0.86]
^{54}Mn	0.65 [0.92]
^{59}Fe	- [0.84]
^{57}Co	0.5 [0.76]
^{60}Co	0.4 [0.96]

CONCLUSION

Excitation functions for the production of radionuclides from the alpha-bombardment of Cobalt-59 were studied for alpha-energies from 25 to 172.5 MeV. The theoretical nuclear reaction model codes COMPLET and TALYS-1.95 were used to make predictions of the medically important production cross-sections for Chromium-51, Mangenes-54, Iron-59, Cobalt-59, and Cobalt-60 radionuclides produced in the interaction of alpha-projectile with Cobalt-59 target ≈ 25 - 172 MeV alpha-energies. The results were compared with the measured values in the EXFOR data library. Pearson's correlation coefficient indicates a strong and positive correlation between the predicted and previously measured production cross-sections for medically important Chromium-51, Mangenes-54, Iron-59, Cobalt-59, and Cobalt-60 radionuclides. Further, the results show that except for Chromium-51, the COMPLETE code predicts more successful outcomes than the TALYS-1.95.

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Contribution of the informal market of village chickens to sustainable livelihoods in KwaZulu-Natal, South Africa

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Training;

Vending

ABSTRACT

The study aimed to determine the contribution of the informal market of village chickens to sustainable livelihoods. The study was conducted in two purposively selected cities namely Durban and Pietermaritzburg, KwaZulu-Natal, South Africa, Central Business District (CBD). A questionnaire was administered to village chicken vendors in the CBD. A total of 50 village chicken vendors which were limited in the CBD, were found and interviewed. In both Durban (100%) and Pietermaritzburg (77%), the majority of village chicken vendors were women ($P<0.05$). Village chicken vendors depended on selling chickens and vending other commodities in both cities ($P>0.05$) to generate income (100%). None (0 %) of the village chicken vendors were exposed to chicken farming training on village chickens in Durban and 11% in Pietermaritzburg have been exposed to training ($P<0.05$). Over 85% were interested in attending chicken farming training in both areas. There was a significant difference ($P<0.05$) between the main source of income and the uses of chickens. The use of village chickens influenced the main source of income, which was not limited to income generation or leisure. It was concluded that the informal market for village chickens contributes to sustaining livelihoods through income, consumption and culturally driven. It is recommended that access to training and resources can grow the informal market.

INTRODUCTION

In 2050, the world population is expected to increase by at least 2.5 billion (Galimova *et al*., 2022) and the demand for animal protein will also increase drastically. This suggests creating traditional markets using untapped animal resources such as village chickens for protein alternatives. Using underutilized animal resources through informal village chicken vending may prevent insufficient conventional protein sources in developing countries that may come with population increase. There has been a

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growing interest in investigating informal market village chicken in response to the agenda of Sustainable Development Goals. Informal village chicken vending refers to producing and selling legal goods and services in urban public spaces in temporal structures (Recchi, 2021). There is a great potential for informally vending village chicken, especially for vulnerable groups in resource-poor settings. Since the informal sectors are mostly ignored or unsupported by the government, individuals are discouraged to participate in this sector (Brown, 2006). The informal market of village chicken has the potential to contribute immensely to achieving specific Sustainable Development Goals (SDGs), such as No Poverty (SDG1) and Zero Hunger (SDG2), particularly for the vulnerable group if the correct measures are taken (Wilson *et al*., 2021). Achieving SDG2 in Sub-Saharan Africa is challenging as the population is rapidly growing, which demands a large amount of animal proteins.

Village chicken (Gallus domesticus) production in Africa is practiced in rural communities (Boudali *et al*., 2022). In South Africa, individuals mostly own village chickens based in resource-poor settings commonly known as rural areas. Their major role in these areas is to provide animal protein through eggs and meat which are crucial for income generation through sales (Elsiddik, 2022). Village chickens are also crucial for traditions and rituals of different ethnicities. However, the production of village chickens is hampered by various challenges, including a high mortality rate, disease prevalence, poor technical support, predation, theft, and poverty. They are reared under an extensive production system with a flock size of less than 100 that depends on scavenging for feed resources (Chowdhury, 2013). This system is characterized by low input and low output as there are no or minimal inputs such as housing, feeding, and health control, resulting in a high mortality rate that reduces the flock size. Village chickens are primarily bred and reared for meat, eggs, cultural practices and income to contribute to family consumption (Mujyambere *et al*., 2022). Considering the poor socio-economic status and food and nutrition insecurity in resource-poor settings of KwaZulu-Natal, South Africa, solutions and recommendations are needed by communities. Village chicken vendors are selling village chickens in the Central Business District for income and they are very scarce. Understanding the dynamics of the village chicken informal market is important to identify gaps and challenges to provide appropriate interventions. This study aimed to assess how the informal market of village chickens contributes to sustainable livelihoods in KwaZulu-Natal. It was hypothesized that village chickens do not contribute to the informal market for sustainable livelihoods.

MATERIALS AND METHODS

Study area

The study was conducted in Pietermaritzburg as shown in Figure 1, which is the capital city of uMsunduzi Municipality within the Province of KwaZulu-Natal. It is located on the tropical eastern coast of South Africa (Nicolson, 2010).

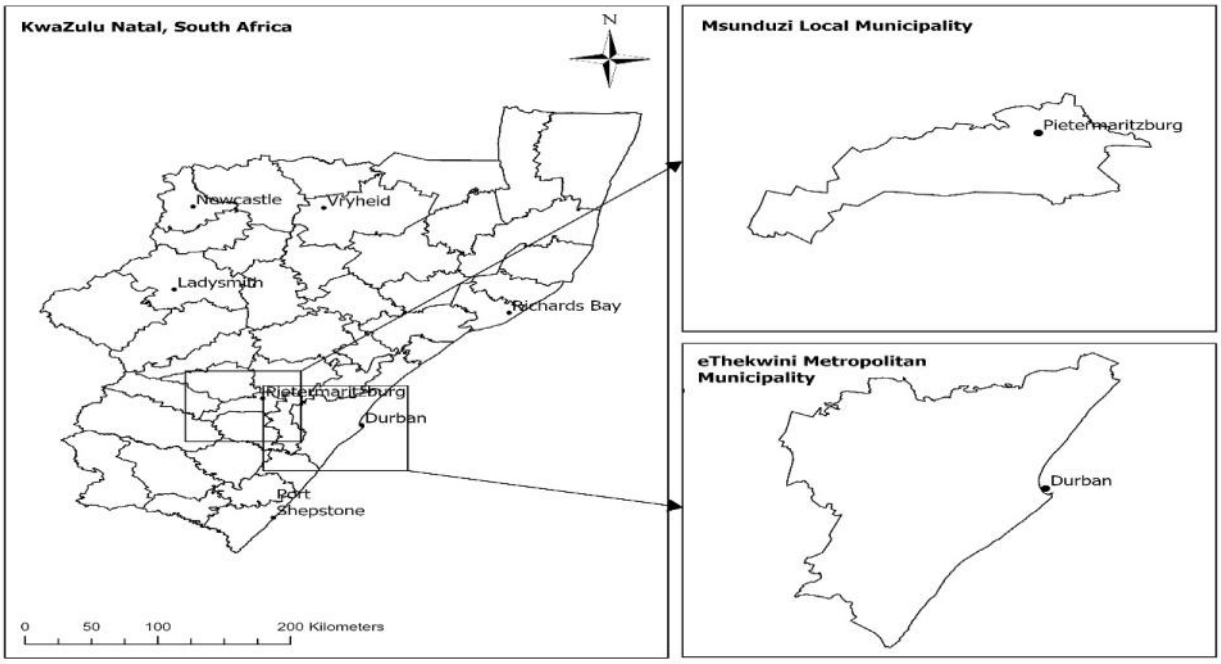

Figure 1:Geographical map of KwaZulu-Natal the study sites in Durban and Pietermaritzburg cities

Pietermaritzburg (29°37′S 30°23′E) experiences summer rainfall and a warm and temperate climate. The average annual rainfall is 865.3 mm, with average maximum temperatures ranging from 22.6°C to 24.5°C and minimum temperatures ranging from 9.9°C to 16°C. The current population is estimated to be over 600,000 residents in Pietermaritzburg.

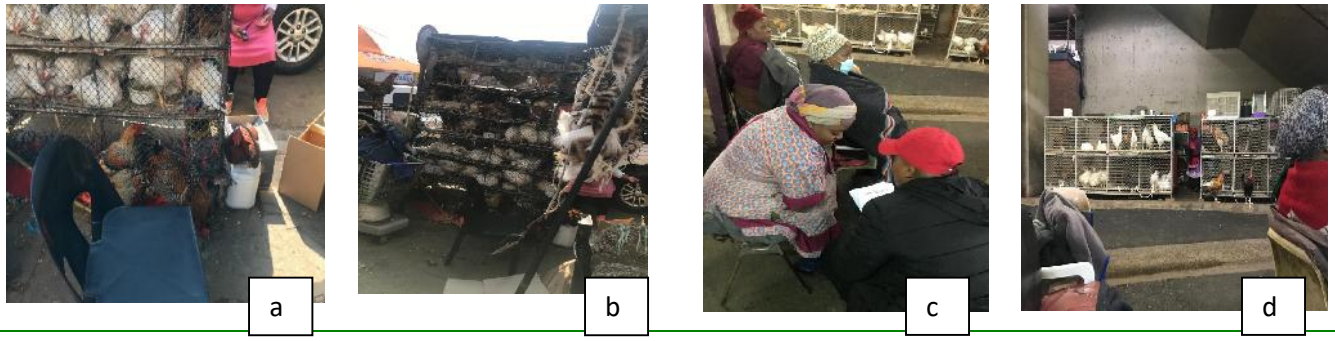

Figure 2: Village chicken vendors with cages (a and b) in Pietermaritzburg Central Business District. Interviewing village chicken vendors (c) chicken cages in Durban Central Business District (d)

Durban is a major coastal city on the eastern coast under eThekwini Metropolitan Municipality in South Africa. The city also known as a tourist venue (Koopman, 2012). The central business district is located adjacent to the harbor which is the most important contributing factor to the city's economy (Timm, 2011). Durban (29°53′S 31°03′E) has an annual

rainfall of 1,009 millimeters and the average temperature in summer is around 24 °C, while in winter the average temperature is 17 °C. The Durban suburbs and neighboring towns have a population of about 3.44 million.

Table 1.The description of the data source of village chicken vendors

Variable	Description	Reason for the information request
Demographic data	Owners of the chickens, Gender of the owners and age distribution	Identifying participants in the traditional market of village chickens
Income sources	Village chicken keepers	Evidence on the level of dependency on village chickens
Reason for selling village chickens	Options such as income and food	Indicate the objectives of village chicken vendors which influence the willingness to participate in this system.
The duration of selling village chickens	Years in the Central Business District	The number of years to indicate the time in years spent participating in the market
Targeted population group	Race that should buy the chickens	The type of population group targeted for this informal market
The population group that frequently buy	The race that buys the most	The population group that responds to the informal market of village chickens
Type of feed for village chickens	Feed type	To understand what they are feeding chickens in cages
The duration of village chickens in the cage	How many hours do village chickens spend in cages	To understand and identify the animal welfare of village chickens in the informal market
The type of knowledge	The type of knowledge systems used	The type of knowledge used is important to understand the rearing system
The uses of different breeds	Type of breeds and functions	The function of different types of breeds

Statistical analysis

All data were analyzed using SAS (2011). All demographic characteristics in Durban and Pietermaritzburg were analyzed. The association was measured using Chi-square tests between demographic parameters. The PROC FREQ /CHISQ test was used.

$$V = \sqrt{\frac{X^2}{n.d^*}}$$

Cramer's V was used to measure and examine the strength of the association between two variables using the following model:

V= X^2 = is the chi-square value df*=min (r-1, c-1) and r= the number of rows and c=the number of columns in the contingency table and n=the total sample size.

RESULTS

Description of village chicken vendor's demographics

The participants (i.e. village chicken vendors in both Pietermaritzburg and Durban) interviewed were women and men over 18 years of age. The gender of village chicken vendors had a significant difference (P<0.05) in both cities as

more females were participating in vending village chickens compared to men. In Durban, all (100%) village chicken vendors were female, and 77% of vendors in Pietermaritzburg were female. The age of village chicken vendors ranged from 40 to 50 years (62%) and 50 to 60 years (26%) were common (P>0.05). In Durban, 32% of village chicken vendors have participated in the informal market for over 30 years (P<0.05) in Pietermaritzburg. Their source of income included vending of chickens, vegetables and pension, as shown in (Table 2).

Table 2.The demographic of the informal market village chicken vendors in Durban and Pietermaritzburg Central Business District

Demographic characteristics	Durban%	Pietermaritzburg%	P Value
Gender of the trader			
Female	100	77	*
Male	0	23	
Age of the trader (years)			
20 to 30	16	0	NS
30 to 40	21	15	
40 to 50	21	62	
50 to 60	26	15	
60 to 70	16	8	
Duration of trading (years)			
Less than 5	21	13	*
More than 5	5	41	
More than 10	21	28	
More than 20	21	15	
More than 30	32	3	
The major source of income			
Vending other commodities	47	54	NS
Pensioner	0	0	
Vegetables	0	8	
Chickens	53	38	
Purpose of selling			
Income	100	100	NS
Barter exchange	0	0	
Leisure	0	0	
Training of village chicken vendors			
Training attended	0	11	
Not attended training	100	89	*
Interest to attend	85	95	
No interest in attending	15	5	*
Access to extension officer	8	5	
No access to extension officer	92	95	*

NS = Not significant (P > 0.05), *significant(P < 0.05)

In Pietermaritzburg, more than half of the participants (54%) depended on vending other commodities; in Durban, 53% relied on vending village chickens. A total of 100% of village chicken vendors relied on vending village chickens for income generation in both areas while none was selling for barter exchange and leisure.

The importance of knowledge to village chickens

Figure 4below indicates different types of knowledge used in rearing village chickens for income generation. Village chicken vendors depended greatly on indigenous knowledge (89%), followed by the combination of indigenous and scientific knowledge (10%) and lastly, scientific knowledge (1%). The results (Table 2) indicated that only 11% of village chicken vendors received training with 95% willingness to attend in Pietermaritzburg. Less than 10% of extension officers were available for village chicken vendors to provide knowledge in Durban and Pietermaritzburg.

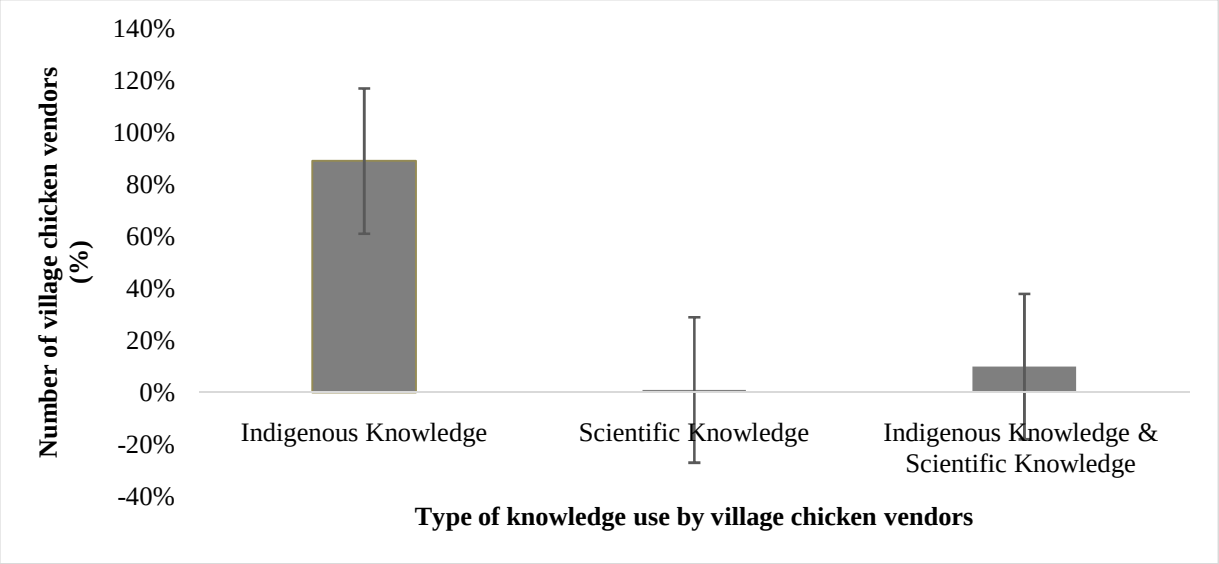

Figure 4: The knowledge used by village chicken vendors in the village chicken markets

Consumers of village chickens by population group

Figure 5 below indicates the population group that purchases village chickens largely. All population groups were targeted to buy village chickens. Results suggest that Africans (82%), followed by Asians (2%),were the predominant consumers of village chickens. Village chicken vendors showed a significant difference ($P<0.05$) in the preferred chickens to sell in both Durban and Pietermaritzburg. Live chickens (98%) were preferred over cooked meat (2%).

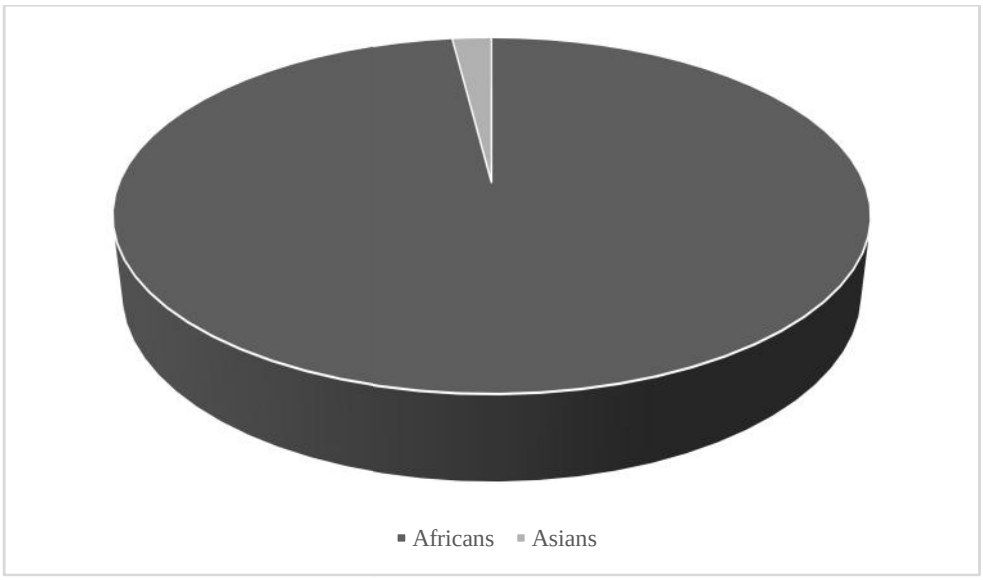

Figure 5.The consumers of village chickens in both Durban and Pietermaritzburg

Type of village chicken breeds for cultural practices.

Figure 6 below indicates the type of village chicken breeds sold by village chicken vendors and breeds in demand in the market. Potchefstroom Koekoek (*Impangela*) is the most sold breed (97%), followed by Boschveld (*Ezibomvu*) (94%), Black Australorp (*Ezimunyama*) (75%) and Broilers (*Ezimhlophezesingisi*) (72%). Results in figure 6 showed that there were breeds demanded in the market such as Potchefstroom Koekoek (*Impangela*) (69%) and Boschveld (*Ezibovu*) (69%). Durban and Pietermaritzburg village chicken breeds have various purposes in the informal market regarding cultural practices. Each breed has its specific function in communicating with ancestors in these areas.

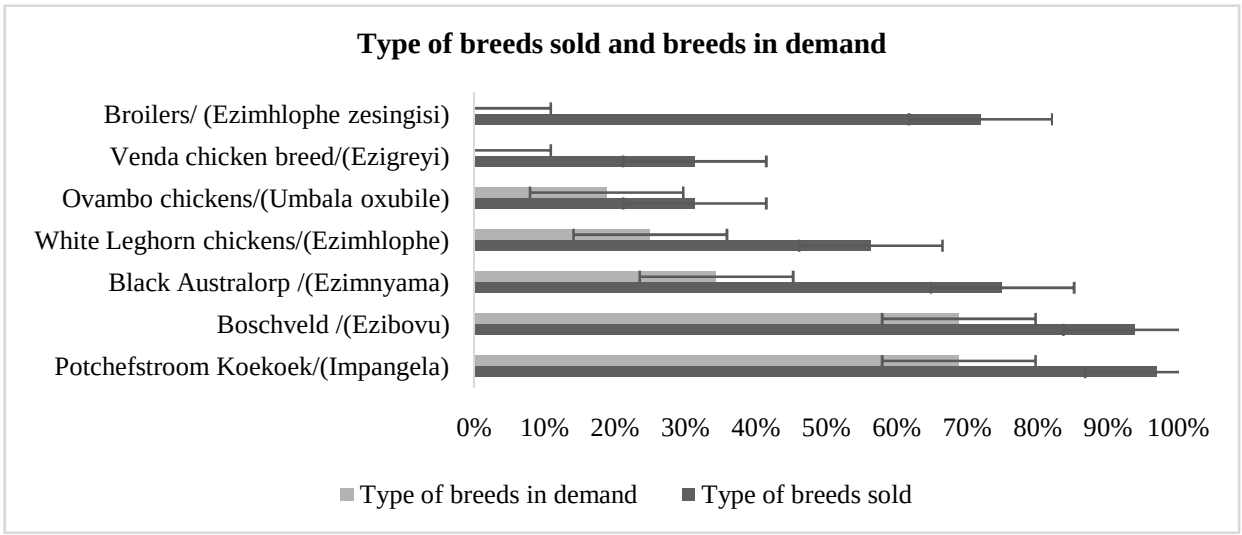

Figure 6. Breeds sold and in demand in the village chicken market

Table 3 shows the level of association in two variables of village chicken vendors in both Durban and Pietermaritzburg. The results indicated a strongest (0.64) insignificant association between type of breed in demand and the reason for breeds in demand.. In addition, the association between gender and age was the weakest (0.28). There was a significant difference ($P<0.05$) between the major source of income and the uses of chickens. The lowest association compared to the breed type in demand and reasons for breeds in demand was insignificant ($P>0.05$).

Table 3. The strength of associations on village chicken vendors in Durban and Pietermaritzburg

Associations	Cramer's V	P value
Gender * Age	0.28	NS
Duration of selling * Major source income	0.30	NS
The major source of income * Uses of chickens	0.60	*
Type of chicken preference * Reasons for preference	0.33	NS
Type of breed in demand * Reasons breeds in demand	0.64	NS

Cramer's V is $-1 \leq V \leq 1$ where -1 is the weakest and 1 is the strongest association.

Important information, recommendations and questions from village chicken vendors

Village chicken vendors rent for R250 to the municipality monthly to sell village chickens in the Central Business District. A total of 100% of village chicken vendors feed yellow maize grain to village chickens daily in cages with 70% buying and 30% planting their maize. Village chickens in cages were housed for 24 hours daily until one is sold to the customer, with the flock size ranging from 10 to 20 chickens per cage. Village chicken vendors only sourced chickens from village chicken producers if funds were available. Results indicated that a cock costs an average of R100 and a hen is R70 depending on the breed. Village chicken vendors sell village chickens in conjunction with crop seeds, sweet potatoes and herbal plants. They were interested in receiving training in poultry management. Concerns about how the government can provide a market for village chickens were observed. Questions on who to consult regarding village chickens were populated predominantly in Durban compared to Pietermaritzburg.

DISCUSSION

In the current study, the majority of village chicken vendors were women compared to men. This is in line with (Chawala, 2022), who reported that the value chain of village chickens is mainly dominated by women and is regarded as a women's value chain. Similar results of Oguttu (2015) indicated that the majority of vendors of chickens are females between the age of 25-50. On the other hand, it is proven that in South Africa, village chickens play a significant role socially, traditionally and economically but this niche is underutilized and poorly managed. Simbizi *et al*. (2022) stated that village chickens were of benefit in South Africa but the sector is underdeveloped. It is evident in the study areas that village chicken vendors depend on indigenous knowledge when rearing village chickens. Gunya *et al*. (2020) indicated that there is less or no information available on village chicken production and their contribution to South Africa. The vending of village chickens in the Central Business

District of the two largest cities is an indication that there is a market for village chickens that the policymakers do not recognize in KwaZulu-Natal. In contrast, in the Eastern Cape, South Africa, Gunya *et al*. (2020) showed that farmers are not selling village chickens because there is no market for these types of chickens. Meanwhile, Assefa (2019) suggested that the domestic market of village chickens through sales of both chickens and eggs creates the opportunity to generate income in Ethiopia.

In South Africa, the country faces challenges such as high unemployment rate and low-skilled communities. As shown in the study, the major source of income of these farmers is vending various commodities in the cities. Street vendors have the potential to build a livelihood and contribute to the economy (Hlengwa, 2016). The findings demonstrated that individuals in resource-poor communities resort to the vending of village chickens for more than 30 years of their lives as the main source of income. As indicated by Reinecke and White (2004), street vending is growing rapidly in developing countries as a response to poverty. This concurs with the current study that the purpose of vending village chickens was for income generation which were used in the homestead for needs such as school fees, groceries and transport. Musa (2022) argued that religion and cultural practices are the other reasons for rearing village chickens. At the same time, village chickens are regarded as the primary source of investment for both women and children (Mujyambere *et al*., 2022). Idamokoro and Hosu (2022) suggested that sales are the only way to generate income through village chickens.

The present study stated that there were no extension services from the government and other institutions to assist with scientific knowledge on how to rear village chickens correctly. Lee *et al*. (2022) revealed that gender inequalities concerning less access to extension services for women have been corrected. The finding that most village chicken vendors depend largely on indigenous knowledge to rear and grow village chickens was expected because formal information or scientific knowledge is not available and accessible. Indigenous knowledge is referred to as philosophies created by societies with long histories of interaction with their natural surroundings (Rogelj *et al*., 2018).

The findings of the current study revealed a strong connection between gender and age of village chicken vendors, as women were shown to be the most populated group in this market. In Sub-Saharan Africa, over 70% of village chicken owners are female and assisted mainly by children and other women (Guèye, 2000). On the other hand, the duration of selling village chickens and the major source of income were less connected since there were different ways of creating income rather than selling chickens such as vending other commodities. In developing countries, street food vending has become one of the sources of income (Mwangi *et al*., 2002). Hence, the current study suggests selling village chickens can be more significant as a major source of income if correct measures such as management and training were provided to village chicken vendors.

The study found that village chicken vendors were interested in selling uncooked and cooked meat, but most preferred selling live chickens. However, Manickavasagam (2018) indicated that various products such as fruits, vegetables, shoes, newspapers and magazines were also sold by vendors. But regarding the current study, vending of village chickens had its challenges as cages, slaughtering rooms, stoves to cook, load shedding and low demand for these products. However, village chicken vendors were open to the idea that assistance could be provided.

In the study, village chickens were accessible to every population group but Africans and Pakistanis living in South Africa were the frequent consumers of this product. Africans use village chickens primarily for cultural practices and Pakistanis use them for consumption. Unathi *et al*. (2017) argued that the demand and consumption of village chickens in South Africa is unknown. Village chicken vendors suggested that village chickens were sold according to breed and breeds in demand for cultural purposes beneficial to the business. For instance, Potchefstroom Koekoek (Impangela) is essential for ancestral ceremonies while Black Australorp (Ezimunyama) is used for traditional cleansing. Most village chicken vendors made high profits from these breeds and there was a strong connection between breeds sold and breeds in demand.

In the current study, village chicken vendors were invested in selling village chickens and interested in producing for themselves while learning more about poultry. But queries and worries such as seeking government assistance were indicated. The biggest challenge was there is no formal market and relevant stakeholders are required in addressing issues and interventions to formalise the existing market. These challenges also include storage as village chickens are stored and confined in cages. for 24 hours daily with a high stocking density. This is a welfare issue as village chickens are scavengers by nature they need to roam around and look for feed resources. This indicates a lack of relevant knowledge and a violation of the five freedoms of animal husbandry but this scientific knowledge is not available to individuals in resource-poor communities. High stocking density in a small cage for so many hours results from a lack of knowledge. Increasing stocking density can encroach on chicken's freedom to express natural behaviour (Tallentire *et al*., 2019). They must exercise their natural behaviour like to roam around and scavenge for feed. Village chickens obtain most of their diet by scavenging for both food and water (Gunya *et al*., 2020). Tenza *et al*. (2023) suggested the development of programs focusing on village chickens for livelihood transformation and women empowerment in resource-poor communities.

In the study, village chickens were provided with maize once or twice per day depending on the sales and profit because buying maize was a challenge. The vendor complained about the price and resorted to restricting feeding chickens to avoid buying more frequently. Depending on one nutrient source may affect the quality of the meat as other nutrients were limited since village chickens were caged for 24 hours. Gondwe and Wollny (2007) showed that village chickens scavenge naturally, and most farmers used maize as a feed supplement.

The market price for chickens was discussed among each other to avoid losing customers and contradictions. Therefore, prices were uniform but depended greatly on the size and type of breed sold. However, no digital scale was available to measure the size of the chicken, so visual observations were used. This is in line with Tilahun *et al*. (2022) who argued that the price of village chickens was influenced by body size and plumage. Similarly, village chickens are considered a high-quality product sold at a higher price (Selamat *et al*., 2022). Other village chicken vendors sold both broilers and village chickens, and the demand was the same in the market. Broilers were significantly added as substitutes if sales for village chickens were low.

In the current study, the vendor's preference for village chickens was due to low inputs such as terms of feed, compared to broilers. This is similar to Alam *et al*. (2020) who revealed that village chickens are always preferred due to their low production cost as they are reared through scavenging of feed resources. Vending chickens in conjunction with other indigenous

herbal plants was significantly suggested for income generation in case there were no chicken sales. The study revealed the eagerness of vendors to attend training on village chickens to improve productivity and disease control, improve knowledge, and expand the business.

CONCLUSION & RECOMMENDATIONS

The study concludes that village chicken vendors use village chickens to sustain livelihoods in KwaZulu-Natal, South Africa. Even though the market is populated by women from youth, adults and old age. The market has existed for over three decades and plays a role for vulnerable groups in resource-poor settings. Village chickens are mainly sold for cultural practices as certain breeds are in demand compared to others. The considerable challenge is the lack of support from different stakeholders to allow the informal market in the main scream value chain as it benefits individuals who lack skills, are unemployed and suffer from hunger. It is recommended that organizations are needed to offer training and provide resources to grow the informal market of village chickens that are located in big cities. This has the potential for a more significant impact and influence on policy. It is also recommended to use underutilized products such as village chickens in achieving the 2030 vision such as SDG 1 and 2, in resource-poor communities as they lack skills and opportunities.

Conflict of Interest

Authors declare no conflict of interest.

Ethics statement

The study was conducted based on the standards required by the Human Social Science Ethics Committee of the University of KwaZulu-Natal (HSSREC/00004846/2022).

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Isolation and Characterization of Antibiotic Producing Actinomycetes from Soils of Hawassa, Southern Ethiopia

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ABSTRACT

Antimicrobial resistance has increased drastically in recent years in the developing countries, and it has leading public health concern. With almost all organisms becoming multidrug resistant to the commonly used antibiotics, there is a need to search for more drugs that are novel in order to address this challenge. *Actinomycetes* are considered as one of the most diverse groups of filamentous bacteria capable of thriving into different types of ecological niches due to their bioactive potential. Therefore, this study was aimed at isolation and characterization of *Actinomycetes* from 20 soil samples that were collected from different sites of Hawassa city, Southern Ethiopia. The *Actinomycetes* were isolated using serial dilution followed by spread plate techniques and antimicrobial activity screening done using modified agar disc diffusion method. *Actinomycete* Isolation Agar (AIA) was used to isolate *Actinomycetes*. A total of twenty nine different *Actinomycetes*, identified as AB1-AB29, were isolated.They were differentiated based on the difference in appearance of the colony morphology and mycelial structure. Their metabolites were tested for antibiotic activities through the primary screening using modified agar disk diffusion methods. Test bacteria were; *E.coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Shigella boydii* and *Salmonella typhi*. Actinoycete isolates with broad spectrum activity were further tested against Methicillin resistant *Staphylococcus aureus*(MRSA) using modified agar disk diffusion methods. Out of 29 isolates, 19(65.5%) *Actinomycetes* showed antimicrobial activity against selected bacterial pathogens. Most of the isolates (84.2%) showed good antimicrobial activity against *Salmonella typhi* though significantly lower than the control drug Ciprofloxacin. Maximum zone of inhibition was 29.2mm observed against *S.typhi*. As the result indicates the *Actinomycetes* isolates showed higher inhibition zone against Gram negative bacteria than Gram positive bacteria. The study indicated that soils of Hawassa may have potential group of *Actinomycetes* with broad spectrum antimicrobial activity. It is therefore suggested that a combination of several molecular analysis methods such as DNA re-association and PCR-based fingerprinting techniques may extremely help to provide broader information about the total genetic diversity of soil *Actinomycetes* obtained in this study.

INTRODUCTION

Antibiotics are substances normally of low molecular weight capable of inhibiting or slowing the growth of pathogenic microorganisms. They are often secondary metabolite produced by microorganisms and seem to have no definite role in the growth of the cell source. Microorganisms produce antibiotics normally during their late log phase of growth until their stationary phase. One of their key benefits to the source organism is said to be their ability to inhibit the growth of other microorganisms growing in the same

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environment in nature hence providing the source with a competitive advantage. Antibiotic producing microorganisms can then compete with others and survive in nature for a long time (Onlamoon, 2008).

Antimicrobial compounds are produced by various living organisms such as bacteria, fungi, and plants. Among the various groups of organisms that have the capacity to produce antimicrobial agents, the *Actinomycetes* are the most capable candidate (Gebreselema *et al.*, 2013). *Actinomycetes* are slow growing, Gram-positive bacteria, having high G+C content from 55-75 % (Ningthoujam *et al*., 2009). They resemble fungi because of their filamentous appearance and spore production property and bacteria because of the presence of peptidoglycan in their cell wall and possession of flagella (Mythili and Das, 2011). *Actinomycetes* are inexhaustible producers of antimicrobial agents (Atta *et al*., 2011). Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by *Actinomycetes*, representing 45% of all bioactive microbial metabolites discovered (Berdy, 2005). The secondary metabolites obtained from the class *Actinomycetes* are of special interest because of their diverse biological activities such as antibacterial, antifungal, antioxidant, antitumor and antiviral. Among *Actinomycetes*, *Streptomyces* species produce around 7,600 compounds. Many of these secondary metabolites are potent antibiotics, which has made *Streptomycetes* the primary antibiotic-producing organisms exploited by pharmaceutical industry and responsible for the formation of more than 60 % of known antibiotics. Further 15 % are made from a number of related *Actinomycetes*, *Micromonospora*, *Actinomadura*, *Streptoverticillium*, and *Thermo Actinomycetes* (Jensen *et al*., 2007; Ramesh *et al*., 2009).

The emergence of resistance to the commercially available antibiotics and multidrug-resistant pathogenic bacteria are issues of extreme concern in present time for the whole human community. Due to these issues, there is rapid spread of infectious diseases leading to morbidity and mortality especially among the elderly and immune-compromised patients (Hong *et al*., 2009). To overcome this situation the discovery of novel drugs with lesser side effects is need of present time.

Choice of natural materials like soils in researches is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore hopefully, novel metabolites are exploited as a result of the geographical variation. Besides, the important approaches helpful in discovering new microbial species or unknown bioactive substances include isolation and characterization of microorganisms from relatively unknown or unstudied areas (Moncheva *et al*., 2002).

In Ethiopia, a few studies showed the existence of antibiotic producing microorganisms from different ecosystems. Biniam (2008) isolated antimicrobial producing *Actinomycetes* from southern part of Ethiopian Rift Valley alkaline lakes like Hora and Chitu. The potential of a mushroom compost as a good source for antibiotic producing thermophlic *Actinomycete* was also reported by Moges (2009). Atsede and Fassil (2018) also isolated and screened antibiotic producing *Actinomycetes* from soil

collected from the rhizosphere of plants and agricultural soils of Ethiopia. However, there is no such scientific report on antibiotic producing microorganisms from soil samples collected in Hawassa city. Therefore, the present study aimed to isolate and characterize antibiotic-producing *Actinomycetes* from soil samples of Hawassa, Southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Hawassa City. It is located 275 km away from Addis Ababa, the capital city of Ethiopia. It is located at 70' 03'' latitude and 80'29" east and lies at an altitude of 1708 m above sea level. The city has a total area of 15,720 hectares and the city Administration of Hawassa consists of 8 sub cities and 32 kebelles. The city experiences a sub humid type of climate having an average annual temperature of about 20.3^{o}Cand mean annual precipitation of about 933.4 mm (Hawassa city Administration, 2007)

Collection of Soil Samples

Twenty (20) soil samples were collected from four different sites of Hawassa city namely: Main campus (5), Monopol (5), Mount Tabor (5) and Mount Alamura (5). Two hundred grams of soil samples were taken from a depth of 11-16 cm from the soil surface using sterile spoon (Chaudhary *et al*., 2013). The soil samples were collected and placed in dry, clean, sterile polyethylene bags and transported aseptically to Microbiology Laboratory of the Department of Biology, Hawassa University where the entire research work was carried out. The collected samples were labeled with details such as: name of collection site, date of collection and pH of soil. The pH of soil was measured before collecting the soil samples. The collected soil samples were air dried at room temperature for a week to reduce gram negative bacteria (Oskay *et al*., 2004). The soil samples from sterile plastic bag were grinded using sterile mortar and pestle and sieved aseptically using 250 µm pore size mesh to remove small pieces of stones and organic matter. The samples were then placed in polyethylene bags to avoid external contamination and kept in refrigerator at 4^{o}C until used.

Isolation and Cultivation of *Actinomycetes*

From each sample, 1g of soil sample was added in the test tube containing 10 ml distilled sterile water and shaken well using vortex mixer for 3 minutes and serially diluted by using serial dilution method up to 10^{-7}. These test tubes were considered as stock cultures for different soil sample sites. From the stock culture, 1 ml was used to prepare the final volume of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} by serial dilution method. Thereafter 0.1 ml of the suspension from 10^{-3}, 10^{-5}, and 10^{-7} was taken and aseptically spread onto *Actinomycete* Isolation Agar (AIA) medium (Millipore, and Sigma, Germany) by applying spread plate technique and incubated at 30°C for 7 d. The colonies were picked and sub cultured for purity by streaking on nutrient agar. The pure colonies were isolated and identified by the color of hyphae, colony morphology and the presence or absence of aerial and substrate mycelium (Reddy *et al*., 2011). After incubation, the slants containing pure *Actinomycetes* isolates were preserved at 4°C for the further studies.

Screening of *Actinomycetes* for Antimicrobial Activity

Test Microorganisms

Antimicrobial properties against selected microorganisms acquired from the Ethiopian Health and Nutrition Research Institute (EHNRI) and Hawassa University Referral Comprehensive Specialized Hospital were investigated *in vitro*. The test bacteria used for primary screening were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella boydii* and *Salmonella typhi*. Methicillin-resistant *Staphylococcus aureus* (MRSA) (clinical isolate) which was provided by Hawassa University Referral Comprehensive Specialized Hospital was also used for secondary screening.

Primary Screening of the Isolates by Disc Diffusion Method

Antimicrobial screening was done using disc diffusion method as described by Kirby Bauer (1979). The stocked *Actinomycetes* isolates were revived by sub-culturing on Nutrient Agar plates. The colony was then picked and inoculated into 5ml nutrient broth and incubated at 30^{o}C for 10 days. Thereafter, the prepared culture was standardized to 0.5 McFarland turgidity standard using the spectrophotometer (optical density of 1.0 at 625 nm) by adding sterile distilled water to obtain the desired cell density of 1.5 X 10^{8} (cell/ml) (CLSI, 2012).

Paper discs (6 mm in diameter) were prepared from Whatman No 1 filter papers and sterilized by autoclaving at 121^{o}C, 15psi for 15 minutes (Ngeny *et al*., 2013). The disc (6 mm in diameter) was impregnated with 15µl of the 7d old culture broth and placed on Mueller Hinton Agar inoculated with the test isolates. Standard antibiotic (Ciprofloxacin) was used as a positive control and filter paper disc soaked with sterile distilled water was used as a negative control. They were then incubated at 37^{o}C for 24 hours. The isolates with antimicrobial activities were identified by measuring the inhibition zone in millimeters (mm) using a ruler. The absence of growth or a less dense growth of test bacteria near the disc was considered as positive for production and secretion of antibacterial metabolite by the isolates (Kekuda *et al*., 2010).

Secondary Screening of the Isolates

Based on the zone of inhibition in primary screening, *Actinomycete* isolates that have broad spectrum antimicrobial activity were further assessed against Methicillin-resistant *Staphylococcus aureus* (clinical isolate). Antimicrobial activity of the secondary metabolites that had broad spectrum activity in primary screening was determined by use of Kirby Bauer disk diffusion method (Brown, 2009). One week old broth cultures of the *Actinomycetes* were used with sterile paper discs soaked in the cultures for 30 minutes were used to inoculate *Actinomycetes* on Muller Hinton Agar (MHA) media seeded with methicillin resistant *Staphylococcus aureus* (MRSA) (clinical isolate). The petri dishes were incubated at 37°C for 24 hours. Standard antibiotic (Ciprofloxacin) disc was used as a positive control and filter paper disc soaked with sterile distilled water was used as a negative control. The antibacterial activity was determined by measuring the diameter of the inhibitory zones with a ruler (CLSI, 2012).

Morphological Characterization

A loop full of the isolates were streaked on each medium and incubated at 30^{o}C for 7 to 10 days. The color of aerial mycelium, reverse color, and nature of the colony was observed and recorded.

Microscopic Characterization

The arrangement of spores and sporulating properties of the selected isolates were examined microscopically by using cover slip culture method by inserting sterile cover slip at an angle of 45°C in the *Actinomycete* isolation agar medium. A loop full of each isolate was taken separately from 7 d old culture, inoculated at the insertion of cover slip and incubated at 30°C for 7 d. Then, the cover slip was removed by using sterile forceps and placed upward on a clean glass slide. Finally, the cover slip was observed for the morphology of isolates under the microscope at 100x magnifications (Cappuccino and Sherman, 2002).

Gram Stain

A thin smear of the 7 d old *Actinomycetes* cultures were inoculated into grease free slides. Thereafter, they were heat fixed and placed in a staining rack. The slides then flooded with crystal violet for one minute and then rinsed with distilled water gently. Gram's iodine was then gently flooded on the smears and allowed to stand for one minute before gently rinsing with distilled water. This was then decolorized using 95% ethyl alcohol for 20 seconds and immediately rinsed with water to avoid over decolonization. Finally Safranin was gently flooded on the smears and let to stand for one minute before rinsing with distilled water. The slides were then blot dried using absorbent paper and then viewed using a light-microscope under oil-immersion (100x) (Cappuccino and Sherman, 2002).

Physiological Characterization

Temperature on Growth

The identified isolates were streaked on *Actinomycetes* isolation agar and incubated at 25°C, 30°C, 37°C and 40°Cat pH of 7 and after 7 d their growth was observed. The optimum temperature for maximum growth was determined by visual examination of the growth.

Biochemical Characterization

Urea hydrolysis

For this, isolates were inoculated into sterile urea agar slants and incubated at 30°C for 7 d and a change in color was observed (Betson., 1994; Collee *et al.*, 1996).

Catalase test

Catalase enzyme present in some microorganisms breaks down hydrogen peroxide to water and oxygen and this helps them in survival since hydrogen peroxide is lethal to cells. A modified version of the method described by Cappuccino and Sherman, (2002) was used in which isolates were grown on starch casein agar plates at 30^{o}C for 7 d and thereafter a colony was picked with a sterile stick and placed on a sterile glass slide containing a drop of hydrogen peroxide. Production of bubbles was indicative of positive results hence the production of free oxygen (Collee *et al.*, 1996).

Starch Hydrolysis

The isolates were streaked on starch agar plates and incubated at 30°C for 7 d. After incubation, iodine solution was poured on the agar and examined for hydrolysis of starch by the production of clear zone around the microbial growth and representing a positive result. Starch in the presence of iodine imparts a blue-black color to the medium indicating the absence of starch-splitting enzymes and representing a negative result (Benson, 1994;Collee *et al*., 1996).

Citrate test

Simmons citrate agar slant were prepared and a single colony of the isolates were streaked on the surface of the slant culture. Then the slants were incubated at 30ºC for 7d. After 7d of incubation the citrate utilizing bacteria were produced a blue color in slant surface of the media as a result of alkaline end products. This indicates the tested bacteria were citrate positive (Collee *et al*., 1996)

Oxidase test

A small amount of *Actinomycete* isolates were obtained from 7 day old culture and put on sterile filter paper. Then 1-2 drops of tetramethyl phenylenediamine dihydrochloride was added to the culture and the reaction was observed. A positive reaction was indicated by a color change to dark blue or purple and a negative test will result in the absence of color (Betson., 1994; Collee *et al.,* 1996)**.**

Statistical Analysis

The collected and recorded data was analyzed using SPSS (version 20.0)software. The different inhibition zone measurements in triplicate were compared by performing One-way ANOVA ranked with Duncan's multiple range tests with descriptive analysis type on different isolates against different test pathogens. All statistical results with $P<0.05$ were considered to be statistically significant.

RESULTS

Isolation of *Actinomycetes*

Twenty nine (29) isolates of *Actinomycetes* were isolated from 20 different soil samples of which 12(41.38%) from Mount Tabor, 11(37.93%) from Main campus, 4(13.79%) from Mount Alamura and 2(6.89%) from Monopol (Table 1). All the 29 isolates grown on *Actinomycetes* isolation agar showed morphology typical of *Actinomycetes*. The colonies were slow growing, aerobic, folded and with aerial and substrate mycelia of different colors.

Table 1: *Actinomycetes* isolates from soil samples collected from different sites of Hawassa city

Collection sites	No. of soil samples	No. of isolates	Codes
Mount Tabor	5	12	AB1-AB12
Main campus	5	11	AB13-AB23
Mount Alamura	5	4	AB24-AB27
Monopol	5	2	AB28-AB29

Antimicrobial activity screening of the isolated *Actinomycetes*

From the 29 isolated *Actinomycetes*, 19 (65.5%) showed antimicrobial activity against at least one of five test bacteria isolates. Sixteen (16) *Actinomycetes* isolates showed antimicrobial activity against *S. typhi*, thirteen (13) showed antimicrobial activity against *S. aureus*, nine (9) showed antimicrobial activity against *K. pneumonia*, seven (7) *Actinomycetes* isolates showed antimicrobial activity against *S. boydii* and two (2) showed activity against *E. coli* (Fig.1).

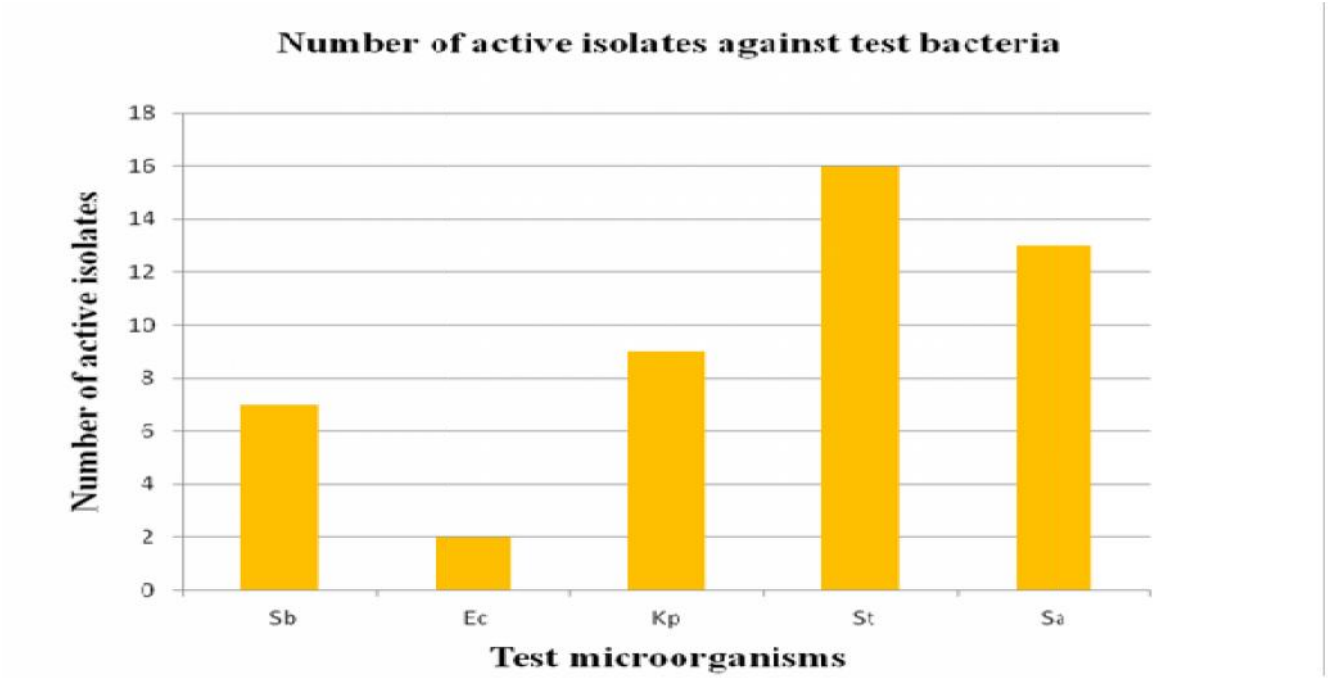

Figure 1: Number of active *Actinomycetes*that inhibited tested microorganisms

(Sb-*Shigella boydii*, **Ec**-*Escherichia coli*, **Kp**-*Klebsiella pneumonia*, **St**-*Salmonella typhi*, **Sa**- *Staphylococcus aureus*)

Antimicrobial Screening

Total number of isolates which showed positive result in antibacterial activity (at least against one test bacteria) was 19 (65.5%)**.** *Salmonella typhi* was susceptible to all the isolates apart from isolates AB2, AB10 and AB14. On the other hand, *E. coli* was resistant to most of the isolates apart from isolates AB9 and AB25. *Shigella boydii* was also resistant to most of the isolates apart from isolate AB1, AB2, AB6, AB13, AB19, AB22 and AB25. Among the tested isolates AB1, AB4, AB5, AB6, AB9, AB13, AB22, AB25 and AB27 proved to be a broader antibiotic spectrum as the acted against most of the test isolates. Among broader antibiotic spectrum isolates AB13 and AB25 were proven to inhibit four of five test pathogens. Isolates AB16 and AB20 showed poor activity. They were only active for *Salmonella typhi*. Isolates AB2, AB3, AB7, AB10, AB14, AB19, AB24 and AB28 showed dual inhibition. Among active isolates tested against human pathogens, six (6) isolates were active against Gram negative bacteria and only

thirteen (13) isolates were active against both Gram positive and Gram negative bacteria, and no isolate was found active against only Gram positive bacteria (Table 2).

Table 2: Sensitivity of selected test microorganisms for *Actinomycetes* isolated from different sampling sites

Actinomycetes	**Test microorganisms**						**Spectrum**
Isolates	Sampling site	*E.coli*	*K.pneumonia*	*S.boydii*	*S.typhi*	*S.aureus*	**activity**
AB1*	Tabor	-	-	+	+	+	3
AB2	Tabor	-	-	+	-	+	2
AB3	Tabor	-	-	-	+	+	2
AB4*	Tabor	-	+	-	+	+	3
AB5*	Tabor	-	+	-	+	+	3
AB6*	Tabor	-	-	+	+	+	3
AB7	Tabor	-	+	-	+	-	2
AB8	Tabor	-	-	-	-	-	0
AB9*	Tabor	+	+	-	+	-	3
AB10	Tabor	-	+	-	-	+	2
AB11	Tabor	-	-	-	-	-	0
AB12	M.C	-	-	-	-	-	0
AB13*	M.C	-	+	+	+	+	4
AB14	M.C	-	+	-	-	+	2
AB15	M.C	-	-	-	-	-	0
AB16	M.C	-	-	-	+	-	1
AB17	M.C	-	-	-	-	-	0
AB18	M.C	-	-	-	-	-	0
AB19	M.C	-	-	+	+	-	2
AB20	M.C	-	-	-	+	-	1
AB21	M.C	-	-	-	-	-	0
AB22*	M.C	-	+	+	+	-	3
AB23	M.C	-	-	-	-	-	0
AB24	Alamura	-	-	-	+	-	1
AB25*	Alamura	+	-	+	+	+	4
AB26	Alamura	-	-	-	-	-	0
AB27*	Alamura	-	+	-	+	+	3
AB28	Monopol	-	-	-	+	+	2
AB29	Monopol	-	-	-	-	-	0

Legend: + = active against test organism; - = inactive against test organism, *= Show broad spectrum activity, M.C=Main campus

From a total of 29 isolates of *Actinomycetes* tested for antimicrobial activity against human pathogenic bacteria: *Escherichia coli*, *Klebsiella pneumonia*, *S. boydii*, *Salmonella typhi* and *Staphylococcus aureus*, 19 (65.5%) isolates showed antimicrobial activity against at least one test microorganism. All of the inhibition zones produced by isolates showed significant differences when compared with control ciprofloxacin tested against test organisms ($P<0.05$). The antimicrobial activity of all the isolates tested against *K. pneumonia* were statistically significant ($P<0.05$) from ciprofloxacin (25.16 mm) which was the control drug (Table 3). *Escherichia coli* was resistant to all of the isolates except isolates AB9 and AB25. The two isolates that showed antimicrobial activity against *E. coli* were

significant as compared with control drug ciprofloxacin (18.33 mm). When compared with control drug ciprofloxacin (18.33 mm), isolate AB25 showed good antimicrobial activity (15.8mm) as opposed to isolate AB9 (9.45mm). The zones of inhibition of active isolates against *S. boydii* were significant (P<0.05). Isolate AB6 showed good activity (21.90mm) when compared with control ciprofloxacin (26.25mm) as opposed to the rest which were active against *S. boydii*.

Table 3: Primary screening of antimicrobial activity (mm) of Actinomycetes isolates

'-': refers inactive, '-ve control ':filter paper disc soaked with sterile distilled water, '+ve' control: Ciprofloxacin

Isolates	Test microorganisms				
	Gram negative			Gram positive	
	E.coli	*K.pneumonia*	*S.boydii*	*S. typhi*	*S.aureus*
AB1	-	-	9.25±0.97	13.75±0.91	11.88±0.41
AB2	-	-	15.79±0.82	-	11.20±0.75
AB3	-	-	-	17.62±0.42	12.50±0.68
AB4	-	10.88±0.41	-	26.63±0.33	10.74±0.44
AB5	-	11.92±1.10	-	18.55±0.62	13.57±0.72
AB6	-	-	21.90±0.40	26.21±0.55	11.04±0.37
AB7	-	17.95±0.44	-	13.30±0.69	-
AB9	9.45±0.50	14.48±0.48	-	11.24±0.67	-
AB10	-	10.51±0.78	-	-	11.35±0.44
AB13	-	12.43±0.60	7.93±0.68	17.30±0.27	12.00±0.50
AB14	-	10.54±0.77	-	-	15.08±0.54
AB16	-	-	-	26.10±0.38	-
AB19	-	-	11.68±0.85	18.99±0.58	-
AB20	-	-	-	8.32±0.17	-
AB22	-	13.58±0.66	8.00±1.00	10.80±0.38	-
AB24	-	-	-	15.00±1.00	16.86±0.34
AB25	15.80±0.18	-	10.65±0.75	21.80±0.34	6.57±0.25
AB27	-	10.25±0.86	-	20.00±0.00	14.33±0.76
AB28	-	-	-	29.11±0.57	18.69±0.32
+ve control	18.33±0.57	25.16±0.76	26.25±0.66	30.33±0.57	25.00±1.00
-ve control	-	-	-	-	-

Salmonella typhi was sensitive to most of the *Actinomycete* isolates (84.2%). The highest zone of inhibition was also shown for isolate AB28 against *Salmonella typhi* (29.11mm). Inhibition zones produced by AB4, AB6, AB16 and AB28 against *Salmonella typhi* were 26.63mm, 26.21mm, 26.10mm and 29.11mm, respectively, which were strong active when compared with control ciprofloxacin (30.33mm) but all of them were statistically significant (P<0.05). *Salmonella typhi* showed resistant against isolates AB2, AB10 and AB14. Thirteen isolates (68.42%) exhibited antimicrobial activity against *Staphylococcus aureus* which was the highest next to *Salmonella typhi*. The isolate AB28 (18.69 mm) showed the highest inhibitory activity against *Staphylococcus aureus* when compared to others. Isolates that showed the

second, third and fourth antimicrobial activities against *Staphylococcus aureus* were AB24 (16.86mm), AB14 (15.08mm) and AB27 (14.33mm), respectively. All the active isolates tested against *Staphylococcus aureus* also showed statistically significant result ($P<0.05$) as shown in Table 3.

Among active isolates tested against pathogens, six (6) isolates were active against Gram negative bacteria only, thirteen (13) isolates were active against both Gram positive and Gram negative bacteria and none of the isolate was found active against Gram positive bacteria.

Among the 19 active isolates from soil, the highest number was from mount Tabor (47.37%) followed by Main campus (16.58%), Mount Alamura (15.79%) and Monopol site (5.26%). According to the spectrum of 19 active *Actinomycetes*, it was found that most *Actinomycetes* inhibited two tested microorganisms (8 isolates) followed by 7, 2, and 2 isolates that inhibited 3, 4 and 1 tested microorganisms, respectively (Table 4).

Table 4: Antimicrobial activity (mm) of selected isolates against Methicillin resistant *Staphylococcus aureus* (MRSA) in secondary screening

	Isolates									Control
	AB1	AB4	AB5	AB6	AB9	AB13	AB22	AB25	AB27	Cipro
MRSA	-	11±0	14±1	8±1	16±1	-	-	13±1	-	24±1

Cipro: ciprofloxacin, MRSA: methicillin resistant Staphylococcus aureus -: no inhibition zone

Secondary Screening

Based on primary screening, 9 isolates with wider spectrum activity were further tested against Methicillin resistant *Staphylococcus aureus* (MRSA) (clinical isolate) using modified disc diffusion method. Secondary screening of 9 isolates selected from primary screening revealed that 5 isolates showed inhibition against clinical isolate of Methicillin resistant *Staphylococcus aureus* (MRSA), with inhibition zone size above 8mm to 16mm diameter. Isolates AB1, AB13, AB22 and AB27 did not show any antimicrobial activity during secondary screening against MRSA. The antimicrobial activities of isolates were statistically significant ($P<0.05$) when compared to the standard antibiotics of ciprofloxacin. Among those five isolates, 4 of them were from mount Tabor site and 1 from Mount Alamura site. Three isolates (AB5, AB9 and AB25) showed maximum inhibition zone size of diameter against Methicillin resistant *Staphylococcus aureus* (MRSA) (clinical isolate). Two of them were isolated from Mount Tabor site and one was from Mount Alamura (Table 4).

Characterization of selected *Actinomycete* isolates

After taking the pH of all soil samples, it was found that almost all soil samples were neutral to alkaline except three samples from Monopol site which were acidic (less than pH value of 6). Based on secondary screening against Methicillin resistant *Staphylococcus aureus*

(MRSA) (clinical isolate), five (5) active broad spectrum *Actinomycete* isolates namely AB4, AB5, AB6, AB9, and AB25 were used in characterization.

Morphological Characterization

Macroscopic characteristics of the selected isolates were studied by growing the isolates on *Actinomycetes* Isolation Agar (AIA), Nutrient Agar and yeast extract malt agar. The isolates showed different growth patterns on each of the medium. The growth of the *Actinomycete* isolates were highest in *Actinomycetes* Isolation Agar (AIA), moderate growth was observed at yeast extract malt agar, and low growth was seen at Nutrient Agar comparatively.

The nature of colonies was found rough, smooth, chalky, and powdery and it was noted that colonies had different colors ranging from white, whitish, yellow, brown, and pink colonies on *Actinomycetes* Isolation Agar (AIA) plates. Some colonies were very hard to pick from agar surface, which is also a characteristic of *Actinomycetes*. The microscopic observations showed that all the isolates were Gram positive as they retained the primary color (crystal violet) hence appeared blue and this is a characteristic of *Actinomycetes* (Table 5).

Table 5: The morphological characteristics of the isolates on Actinomycetes isolation agar

Isolates	Appearance of colonies	Gram stain
AB4	White powdery	Gram positive
AB5	Brown rough	Gram positive
AB6	Pink	Gram positive
AB9	Yellow smooth	Gram positive
AB25	Whitish yellow	Gram positive

Physiological and Biochemical Characterization

In biochemical tests, all the isolates were able to hydrolyze both starch and urea. Citrate was positive for all the isolates, all the isolates showed positive result for catalase test and oxidase was also positive for all the isolates. Isolates AB4 was grown at a temperature range of 25-30°C, AB9 on a temperature range of 30-37°C while none of the isolates were able to grow at the temperature of 40°C. Optimum temperature for most of the isolates was found at 30°C (Table 6).

Table 6: Physiological and biochemical characteristics of selected isolates

	Characteristics of isolates				
Types of test	AB4	AB5	AB6	AB9	AB25
Starch	+	+	+	+	+
Citrate	+	+	+	+	+
Catalase	+	+	+	+	+
Urea	+	+	+	+	+
Oxidase	+	+	+	+	+
Opt. T^o	25-30°C	30°C	30°C	30-37°C	30°C

Opt. T^o: optimum temperature; +: positive; -: negative

DISCUSSION

Antibiotic resistance is one of the most pressing public health issues worldwide. Presently, antibiotic-resistant organisms are extensively emerging and causing great challenge for a number of infectious diseases and current clinical care. As a result, there has been growing interest in searching valuable antibiotics from soil *Actinomycetes* in diversified ecological niches (Abo-Shadi *et al*., 2010). *Actinomycetes* are the richest sources of bioactive compounds (Suthindhiran and Kannabiran, 2009). Almost 70% of all recognized antibiotics have been isolated from *Actinomycetes* of which 75% and 60% are used in medicine and agriculture respectively (Kumar *et al*., 2012). Isolation of *Actinomycetes* has always been facing difficulties while comparing with other bacteria and fungi (Williams and Cross, 1971). This may be due to their long incubation period. However, *Actinomycetes* isolation ratio has increased by pretreatment of the samples by air drying for a week (Oskay *et al*., 2004).

In this study, out of total 29 *Actinomycete* isolates, 19 (65.5%) showed antimicrobial activity against the test pathogens. Primary screening using the disc diffusion methods revealed 65.5% (n=19/29) of the isolates were effective inhibitors against the test pathogens. This finding is higher than the finding (26.7%) of the previous study done by Abebe*et al*. (2013) from soil samples of Gondar town, North West Ethiopia. However, this result is in agreement with the report of Atsede and Fassil (2018) that 60% of *Actinomycete* isolates showed antimicrobial activity against at least one test pathogen. Sawasdee *et al*. (2011) reported that 80% of the isolates showed antimicrobial activity against at least one test microorganism and this value was higher than the present findings. Khasabuli and Kibera (2014) also reported that all the isolates IS1-IS15 showed positive results against at least one tested pathogen.

Test bacteria showed varied responses to metabolites of *Actinomycete i.e.,* being susceptible to one isolate and resistant to the other isolate. *Salmonella typhi* was susceptible to most of the isolates other than AB2, AB10 and AB14. This was consistent with the report of Khasabuli and Kibera (2014). *Escherichia coli* were resistant to all of the isolates except from AB9 and AB25 isolates. *Shigella boydii* was susceptible to metabolites from isolates such as AB1, AB2, AB6, AB13, AB19, AB22 and AB25 while it was resistant to the other metabolites from the remaining isolates.

The results of this study showed that the inhibition zones were maximum against Gram negative bacteria when compared to Gram positive bacteria. This finding differs from the previous reports of Abebe *et al*. (2013), Gebreselema *et al*. (2013) and Atsede and Fassil (2018) who reported higher inhibitory effect in Gram positive pathogens than in Gram negative. Several other studies conducted elsewhere also showed Gram positive isolates were more susceptible to the antibiotics produced by *Actinomycetes* than Gram negative bacteria (Sawasdee *et al*., 2011; Sheik *et al*., 2017). However, a study done in Chennai, India reported higher inhibitory effect on Gram negative bacteria than Gram positive bacteria (Fatima *et al*., 2017) which is in agreement with the present study. Kamal *et al*. (2018) also reported highest inhibitory effect in Gram negative pathogens than Gram positive bacteria. The reason for higher antibacterial activity of

Actinomycetes towards Gram negative bacteria in comparison to Gram positive bacteria tested might be due to the nature of the cell wall of the Gram negative bacteria which is easier to break than those of the Gram positive bacteria. However, this hypothesis did not hold good with the findings of some researchers, who observed much higher inhibitory reaction against the Gram positive bacteria than the Gram negative bacteria (Basilio *et al*., 2003; Oskay *et al*., 2004; Sacramento *et al*., 2004).

On secondary screening using the disk diffusion methods against Methicillin resistant *Staphylococcus aureus* (MRSA) five out of nine *Actinomycete* isolates showed antimicrobial activity with different inhibition zones. The previous study indicated that, the inhibition zone of isolates against MRSA ranged from 0-15 mm (Yucel and Yemac, 2010). In this study, the inhibition zone of nine isolates against MRSA ranged from 0-16 mm which was found to be good when compared to Yucel and Yemac's results (Yucel and Yemac, 2010). However, the present result is less when compared to the activity reported by Abebe *et al*. (2013) who found zone of inhibition ranged from 0-20mm. Such differences in the results might be due the variation in the strains and biotypes of Actinomycetes from the different habitats. According to the present result, ciprofloxacin had 24±1 mm inhibition zone against MRSA, which had greater inhibition zone when compared to the isolates tested.

The results demonstrate that, the type of culture medium and incubation temperature has a significant effect on the production of antibiotics by the antibiotic producing organisms. The aerial mycelium, substrate mycelium growth and pigmentation showed distinct variation based on the culture media in which the isolates were grown. Among the three culture media used, the preferred medium in this experiment was *Actinomycete* Isolation Agar where the maximum numbers of colonies were isolated and this may be due to the inclusion of sufficient amount of nutrient in this media under 30°C. This is in agreement with the results of Atsede and Fassils (2018) who reported 30°C was optimum temperature for most of the potential *Actinomycetes* isolates. Kumar *et al.* (2012) also reported AIA as the best media for the isolation of *Actinomycetes*. All the potential isolates in this study have the ability to hydrolyze starch and urea. Oxidase, citrate and catalase tests were also positive for all potential isolates. Therefore, after observation of cultural, morphological, physiological and biochemical characteristics it was confirmed that these isolates obtained from soil of Hawassa belong to the species of the genus Streptomyces. Previous studies conducted by Kalyani *et al*. (2012); Sudha and Hemalatha (2015); Midhun and Girijasankar (2016); Ramendra *et al*. (2016); Sreejetha *et al*. (2016); Sujatha and Swethalatha (2016) showed that Streptomyces sp. being producers of useful bioactive metabolite have an antibacterial effect with a broad spectrum of activities. Another study showed that Streptomyces species produced about 7,600 compounds which have antimicrobial properties which are highest among *Actinomycetes* producing antibiotics in the soil (Das *et al*., 2010).

CONCLUSION & RECOMMENDATIONS

Antimicrobial resistance is a global problem which demands for novel antimicrobial structure against pathogenic microbes. Actinomycetes are famous for antibiotic production and continued

to be explored in hope of getting novel antibiotics. The isolates from soil of Hawassa city might be a promising candidate for discovering novel. *Actinomycetes* isolates recovered from mount Tabor samples account largest number of antimicrobial bioactive compounds. It is therefore suggested that a combination of several molecular analysis methods such as DNA re-association and PCR-based fingerprinting techniques may extremely help to provide broader information about the total genetic diversity of soil Actinomycetes community. Perhaps, such methods may lead to the improvement in isolating antibiotic producing strains of soil Actinomycetes obtained in this study.

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Bovine Mastitis: *Staphylococcus aureus* isolation and identification from Small holder Dairy Farms located in and around Hawassa town, Southern Ethiopia

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KEYWORDS:

California Mastitis Test (CMT);

Dairy cows;

Hawassa town;

Mastitis;

Staphylococcus aureus

ABSTRACT

Mastitis is a widely distributed disease of dairy cattle in most countries, including Ethiopia. The most commonly recovered bacterial pathogen during mastitis is *Staphylococcus aureus* in dairy cows worldwide. With this, a cross-sectional study was conducted from March 2021 to August 2021 on dairy farms in and around Hawassa town to isolate and identify *Staphylococcus aureus* from bovine mastitis milk and to determine risk factors associated with the occurrence of mastitis. A total of 250 lactating cows were randomly selected for clinical and subclinical mastitis from 29 small holder dairy farms. Clinical signs and the California Mastitis Test (CMT) were used to identify clinical and subclinical mastitis, respectively. Accordingly, a standard bacteriological study targeting *S. aureus* was conducted with all (n=127) milk samples collected from clinical and subclinical mastitis cows. Data generated from these methods were analyzed using STATA Corp. Version 12. Association between the risk factors and mastitis were determined with $p<0.05$ to be a statistically significant one. During the study period, 50.8% of cows had mastitis, of which 4.8% and 46% showed clinical and subclinical mastitis, respectively. The quarter-level proportion was 27.4%; of which the clinical form was 2.9%, while the subclinical mastitis was 24.5%. Logistic regression analysis showed a significant association among cows of different age groups, lactation stages, and frequency of farm cleaning status per day with the occurrence of mastitis ($p < 0.05$). Bacterial identification targeting *S. aureus* was done, and this agent was identified in 60 (47.2%) milk samples. This pathogen was found to be higher (47.8%) in subclinical than in clinical (41.6%) mastitis. In conclusion, this study showed that mastitis was prevalent in dairy cattle of the study area, with a higher case of *S. aureus* in subclinical mastitis. However, the detection of *S. aureus* in nearly half of the milk sample collected from mastitic cows indicated the possible presence of other pathogens. Therefore, further study to recover other potential pathogens commonly causing mastitis can be a good approach.

INTRODUCTION

Mastitis, characterized by inflammation of the udder and teats, is a prevalent condition in dairy cattle. It can manifest in two primary forms: clinical and subclinical mastitis (Ruegg *et al*., 2017; Taponen *et al*., 2017). The disease is known for its damage to the udder tissue, which

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is reported in numerous species, mainly in domestic dairy animals. This pathology is the most frequent disease of dairy cattle and can be potentially fatal (Gutierrez-Chavez et al., 2019). Bovine mastitis has been reported as the most important disease on dairy farms because of the reduction of farm profitability, decreased milk production, discarded milk, treatment costs, and culling (Julian, 2016).

Subclinical mastitis (SCM) refers to inflammation of the mammary gland in the absence of visible gross lesions in the udder or its secretions, with the presence of pathogenic microorganisms and an increased number of somatic cells in the milk (Smith, 1996; Radostits *et al*., 2007). Even if there is a great loss related to both conditions, clinical mastitis continues to be a problem in many dairy herds (Gezehagn *et al*., 2020). Moreover, mastitis has serious zoonotic potential associated with the shedding of bacteria and their toxins in the milk. Mastitis is caused by a wide spectrum of pathogens and is epidemiologically categorized into contagious and environmental mastitis. Contagious pathogens are those for which the udders of infected cows serve as the major reservoir. They spread from cow to cow, primarily during milking, and tend to result in chronic subclinical infections with flare-ups of clinical episodes (Abebe *et al*., 2016). Environmental bacteria live in the surrounding environment of the cows and are considered opportunistic, causing clinical infections with short duration (Blowey and Edmondson, 2010).

Diagnosis of subclinical diagnosis is done with the indirect tests which usually depend on the cellular interaction between the reagent and certain protein factors in mastitis milk. The most common methods are somatic cell count (SCC) and the California mastitis test (CMT) (Rafik *et al*., 2014). During SCC, the adherence of bacteria stimulates macrophage migration and the migration of neutrophils from blood into the milk, which will lead to a high SCC, swelling of the mammary gland, damage to the host defense system, and epithelial cells (Douaa *et al*., 2016). When milk and CMT reagent are mixed in equal amounts, the CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is now released from the nuclei. DNA will string or gel together to form a stringy mass. As the number of leukocytes increases in a quarter, the amount of gel formation will increase in a linear fashion (Melleneger, 2001).

Several bacterial pathogens are implicated in bovine mastitis. From an epidemiological and pathophysiological standpoint, the pathogens are regarded as contagious, teat skin opportunistic, or environmental (Radostits *et al*., 2007). *Staphylococcus aureus* is the etiological agent more commonly associated with the disease and is normally related to both subclinical and chronic infection, leading to severe economic loss to dairy farms (Kubota *et al*., 2007). According to a livestock agriculture office report, the incidence of mastitis has become popular, and some professionals also recommend digging in detail about the area, as such the study was designed to figure the prevalence value and to identify the factors associated with mastitis infection. In addition, although many studies have been conducted by different researchers on the isolation and identification of *S. aureus* from bovine mastitis milk in Ethiopia, including the present study area, it is necessary to update the information to find out if there was any change in the

epidemiology of the bacteria. Therefore, this study was aimed to estimate the prevalence of clinical and subclinical mastitis, to isolate and identify *S. aureus* from mastitic milk and to determine the associated risk factors of bovine mastitis in small-holder dairy farms in and around Hawassa town, Southern Ethiopia.

MATERIALS AND METHODS

Study Area

The study was conducted in Hawassa City and its surroundings (Fig. below). Hawassa is the capital city of the Sidama Region, which is located 275 km south of Addis Ababa with a total human population of 157,879. Geographically, it lies between 7°03'1.35"N latitude and 38°29'43.81"E longitude at an altitude of 1750 meters above sea level. The area annually receives an average of 800 - 1000 mm of rainfall, of which 67% falls in the long rainy season, which extends from June to September, with an average annual temperature of 22°C and 51.8% mean relative humidity. The area is mainly covered by dry savanna and bush-type vegetation. The total livestock population of the Sidama region (including Hawassa town) is estimated to constitute 2,413,482 cattle, 308,903 goats, 467,858 sheep, 34,709 horses, 16,376 donkeys, 1,824,841 poultry, and 44,364 beehives (CSA, 2020).

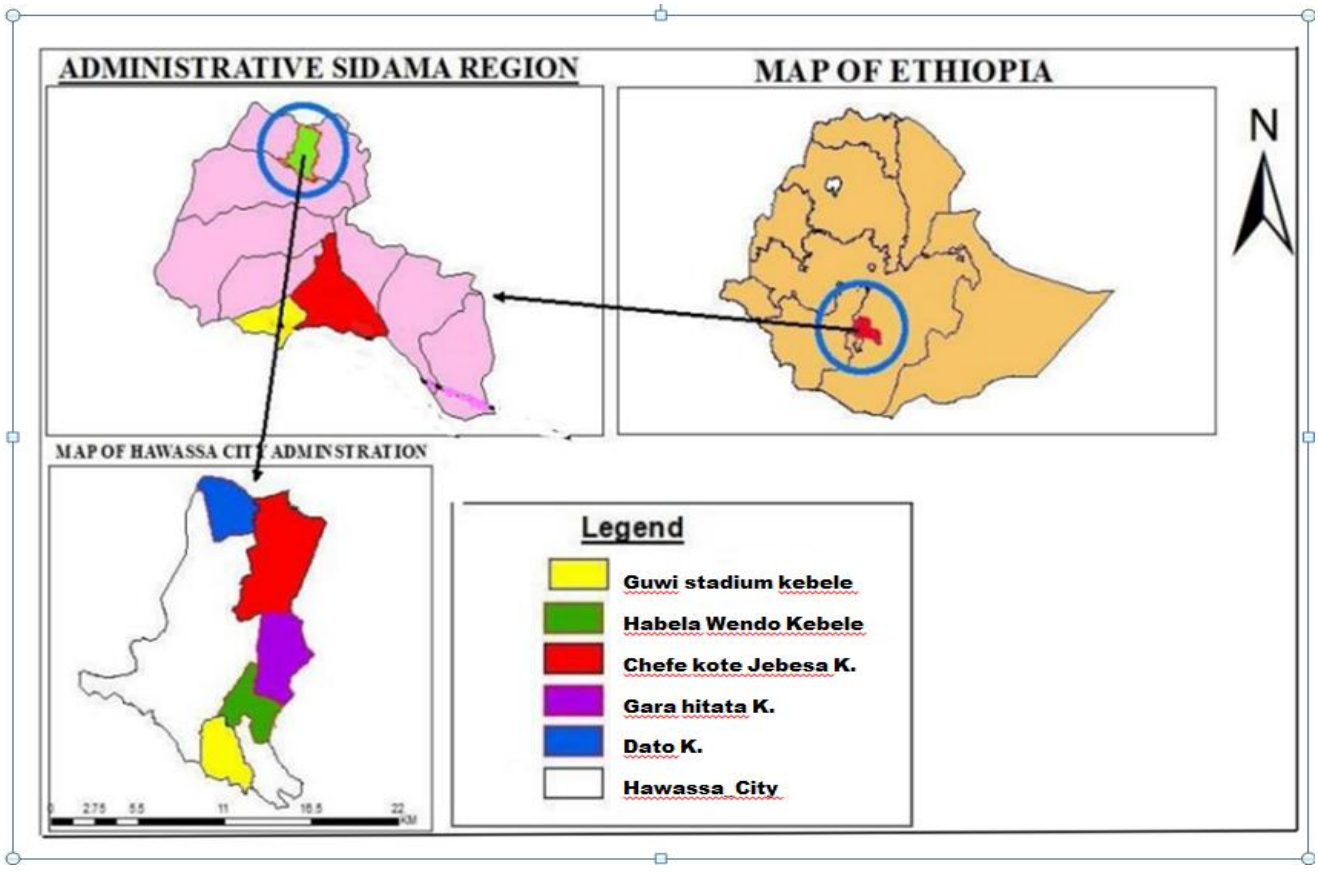

Figure. Study area map

Study Animals

The study was conducted on lactating cross-bred cows selected randomly from 29 small holder dairy farms and the associated risk factors were recorded on the sheet designed for it. Risk factors such as age, body condition score (BCS), parity, lactation stage, and average milk yield per day, were recorded on the sheet designed for sample collection. Age and BCS were determined with observation of dentition as per Johnson (1998) while for that of body condition Sharad *et al*. (2016) classification was used. Later the age classification was made into three as $\leq$3, 4 to 5 and $\geq$ 6 years. The other information (parity, lactation stage, and average milk yield per day) was recorded from the farms recording. After identifying the months of the cow's lactation; classification was made into three as early (for the first 3 months), mid (4 to 6 months) and late (above seven months) lactation stages.

Study Design

A cross-sectional study was conducted from March 2021 to August 2021 on dairy farms in and around Hawassa to isolate and identify *Staphylococcus aureus* from clinical and subclinical mastitis cows.

Sample Size Determination

The sample size was calculated according to the formula given by Thrusfield *et al*. (2017) using expected prevalence of 81.1% (Duguma *et al*., 2014), 95% confidence interval and a significance level of 5%. The minimum number of cattle needed in the study was calculated to be 236, however, we included 250 lactating cows in this study.

Study Methodology

Dairy cows were randomly selected from 29 small holder farms located in and around Hawassa city administration. From these farms, each selected lactating cow was screened for mastitis based on clinical examinations and the California Mastitis Test. Milk samples from cows with mastitis were subjected to bacteriological examination to identify *S. aureus*. Furthermore, information regarding potential risk factors for both clinical and subclinical mastitis, such as husbandry systems, the status of farm hygiene, and previous history of mastitis, was collected from interviews with farm owners. Additionally, animal identification, including age, body condition score (BCS), parity, lactation stage, and average milk yield per day, were recorded on the sheet designed for sample collection. Age and BCS were determined with dentition (Johnson, 1998) and body condition (Sharad *et al*., 2016) observation, respectively.

Clinical Inspection of the Udder

For clinical mastitis, the udders of the study cows were examined visually and by palpation for the presence of any abnormalities, such as hard and swollen quarters, pain (kicking upon touching the udder), heat, and abnormal secretion in the mammary gland (the presence of clots or flakes in milk or watery consistency, and blood-tinged secretions).

California Mastitis Test

Cases of subclinical mastitis were diagnosed based on CMT results (i.e., observation of the nature of gel formation), which show the presence and severity of the infection. From each quarter of the udder, a squirt of milk sample was dropped into each of the strip cups on the CMT paddle, and an equal amount of CMT reagent was added to each cup and mixed gently. The test result was interpreted based on the thickness of the gel formed by the CMT reagent and milk mixture and scored as 0 (negative), T (trace), 1 (weak positive), 2 (distinct positive), and 3 (strong positive). Finally, quarters with a CMT score of 1 or above were judged as positive for subclinical mastitis; otherwise, they were considered negative (Quinn *et al*., 2002).

Milk Sample Collection

Milk samples from mastitis positive cow were collected after cleaning and disinfection with 70% alcohol before milk sampling. Approximately 10 ml of milk was taken from each quarter after discarding the first three milking streams aseptically into sterile bottles for bacteriological investigation and labeled. The sample was placed in an icebox containing ice packs and transported immediately to Hawassa University, Faculty of veterinary Medicine Microbiology laboratory. Upon arrival, the collected samples were immediately stored at +4 °C for a maximum of 24 hours until culturing.

Isolation and Identification

The bacteriological culture was performed following the standard microbiological technique (Quinn et al., 2002). A loopful of milk sample was streaked on sterile 5% sheep blood agar (Himedia, India), and the plates were incubated aerobically at 37 °C and examined after 24–48 h of incubation. The colonies were identified based on morphological characteristics, hemolytic pattern, and Gram's staining reaction. The representative colonies that were positive for Gram's staining and had a typical grape-like structure under a microscope were further sub-cultured on nutrient agar plates (Oxoid, UK) and incubated at 37 °C for 24 hrs followed by biochemical tests, and grow on mannitol salt agar (MSA) and purple agar base media.

A catalase test using 3% hydrogen peroxide (H_2O_2) was performed to identify catalase-positive and catalase-negative bacteria. The colonies that were identified as Gram positive cocci were subjected to catalase test and catalase positive isolates were sub-cultured on MSA and incubated at 37°C. Examination was made after 24–48 hrs for growth and change in the color of the medium. The presence of growth and change in pH of the media (red to yellow color) were regarded as confirmative identification of the salt-tolerant *Staphylococci*. The fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium (Quinn *et al*., 2002).

The tube coagulase test was conducted according to the method of Robertson *et al*. (1999). Accordingly, 0.1 ml of fresh cultures of suspected *staphylococci* grown on Nutrient Broth for 18-24 hours was added to 0.5 ml of 1/10 diluted sterile rabbit plasma (Sigma) in the test tube. The tube was incubated at 37°C and examined every 4-24 hours to see the presence of clotting. The reaction was considered

coagulase-positive if any degree of clotting was visible (Tallent *et al*., 2001). The suspected culture was also inoculated on a purple agar base (PAB) with 1% maltose media and incubated at 37°C for 24 hrs. *S. aureus* isolates showed rapid fermentation of maltose and acid metabolites which turn the culture medium and colonies to yellow. Therefore, isolates that were considered positive for *S. aureus* showed catalase-positive, coagulase-positive, and growth with a yellowish coloration of medias (MSA and PAB) (Quinn *et al*., 2002).

Data Analysis

Collected data were entered into a Microsoft Excel spreadsheet and coded before statistical analysis. The prevalence of mastitis was calculated by dividing the number of mastitis-positive cows (clinical and subclinical) by the total number of animals examined. The degree of association between risk factors and the prevalence of mastitis was analyzed using the odds ratio (OR). Furthermore, logistic regression was used to examine the association of the potential risk factors with the occurrence of mastitis using STATA Corp. version 12.0 statistical software. In all analyses, a 95% confidence level and a p-value<0.05 were used to determine statistical significance.

RESULTS

Prevalence of Mastitis

In this cross-sectional study, from a total of 250 lactating cows examined, 127 (50.8%) were found to be affected with both clinical and subclinical mastitis infection. Of these, 4.8% (12/250) and 46% (115/250) showed clinical and subclinical mastitis, respectively. The quarter-level overall prevalence of mastitis was 27.4% (274/1000), while quarter-level clinical and subclinical mastitis were 2.9% (29/1000) and 24.5% (245/1000), respectively. There was a higher prevalence of subclinical mastitis than clinical mastitis, both at the cow and quarter levels (Table 1).

Table 1. Prevalence of mastitis at cow and quarter level

Forms of mastitis	**Cow level (N= 250)** **No. (%) positive**	**Quarter level (N= 1000)** **No. (%) positive**
Clinical	12 (4.8%)	29 (2.9%)
Subclinical	115 (46%)	245 (24.5%)
Total	**127 (50.8%)**	**274 (27.4%)**

Bacterial Isolation

Out of 127 milk samples collected from mastitis positive cows and subjected for bacteriological examination of *S.aureus*. *S. aureus* was isolated from 41.6% (5/12) of the clinical cases and 47.8% (55/115) of the subclinical cases, respectively. The overall prevalence of *S. aureus* was 47.2% (60/127), as indicated in Table 2.

Table 2. Prevalence of S. aureus in clinical and subclinical mastitis

Form of mastitis	No. of cow examined	No. of isolated *S. aureus* Cases (%)
Clinical	12	5 (41.6)
Subclinical	115	55 (47.8)
Total	**127**	**60 (47.2)**

Risk Factors Associated with Mastitis

Multivariate logistic regression analyses showed risk factors such as parity, milk yield, and husbandry system were found to be not significantly associated with the occurrence of mastitis. However, cows age, late lactation stage and farm hygiene frequency were significantly associated with the occurrence of mastitis (Table 3).

Table 3. Logistic regression analysis of potential risk factors for the occurrence of mastitis in the study area

Risk factors	Categories	No. of cows		Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
		Examined	Positive (proportion)			
Age (years)	≤3	93	21 (22.6)	1	1	
	4-5	98	47 (47.9)	3.16(1.68, 5.92)	3.7 (1.63, 8.43)	0.002
	≥6	59	47 (79.7)	13.43(6.04, 29.85)	17.61 (5.3, 58.44)	0.000
Parity	≤2	134	43 (32.1)	1	1	
	≥3	116	72 (62.1)	3.46 (2.06, 5.83)	1.29 (0.59, 2.79)	0.512
Lactation stage (Months)	Early	85	27 (31.7)	1	1	
	Mid	89	43 (48.3)	2.01 (1.08, 3.72)	1.81 (0.9, 3.62)	0.094
	Late	76	45 (59.2)	3.11 (1.63, 5.95)	2.1 (1.0, 4.32)	0.049
Milk yield per day (lit.)	≤10	86	45 (52.3)	1	1	
	11-15	82	34 (41.5)	1.56 (0.84, 2.80)	1.26 (0.61, 2.63)	0.531
	≥16	82	36 (43.9)	1.41 (0.76, 2.58)	1.39 (0.66, 2.94)	0.384
Husbandry system	Intensive	233	102 (43.7)	1	1	
	Semi-intensive	27	13 (48.1)	1.10 (0.49, 2.45)	1.97 (0.68, 5.88)	0.213
Frequency of farm cleaning per day	≥ 4 times	36	10 (27.7)	1	1	
	3	130	53 (40.7)	2.36 (1.37, 4.15)	2.94 (1.58, 5.81)	0.002
	2	84	52 (61.9)	4.24 (1.80, 9.90)	5.1 (1.85, 13.7)	0.002

OR: odds ratio, lit.: liter, No.: number

DISCUSSION

In the present study, the overall prevalence of mastitis was 50.8% and 27.4% at cow and quarter level, respectively. This result was in line with that of Abera *et al*. (2010) who reported prevalence of 46.7% at cow level and 29% at the quarter level in Adama town and Hundera *et al*. (2005) who reported 52.8% at cow level around Sebeta. In most countries, irrespective of the cause, the prevalence of

mastitis is about 50% in cows and 25% in quarters. However, the present findings are lower than the previous reports of Abebe *et al*. (2016), Zeryehun and Abera (2017), Elemo *et al.* (2017), and Tegegne *et al.* (2020) who reported in Hawassa, South Ethiopia (62.6%); Eastern Harrarghe Zone, Eastern Ethiopia (64.3%); in Asella, Southern Eastern Ethiopia (65.36%); Addis Ababa, central Ethiopia (70%), respectively. However, the finding was higher than the previous reports of Workineh *et al*. (2002) Mungube *et al*. (2004) and Kerro and Tareke (2003) who reported 38.2%, 39.8% and 40% in Adami Tulu (Central Ethiopia), in and around Addis Ababa and Southern Ethiopia; respectively. This variability in the prevalence of mastitis, irrespective of the cause, between different reports could be attributed to differences in farm management practices, or environmental conditions in different parts of the country (Radostits *et al*., 2007).

In the present study the prevalence of subclinical mastitis (46%) is higher than clinical mastitis (4.8%). This finding supports previous studies conducted in various regions of the country, which have consistently concluded that subclinical mastitis is more prevalent than clinical mastitis. For instance, Kerro and Tareke (2003) reported a prevalence of 62.9% and 37.0%, Abebe *et al*. (2016) found a prevalence of 59.2% and 3.4% in southern Ethiopia, Mekbib *et al*. (2010) reported a prevalence of 48.6% and 22.4% in Holeta, Abera *et al*. (2010) observed a prevalence of 36.7% and 10.0% in Adama, and Tassew *et al.* (2017) found a prevalence of 27.86% and 11.45% in and around Assosa town of subclinical and clinical mastitis, respectively. The higher prevalence of subclinical mastitis compared to clinical mastitis can be attributed to the challenges in detecting subclinical cases, as they lack visible symptoms that prompt animal owners to seek treatment. In contrast, clinical cases are more easily detectable due to their observable signs, leading to higher treatment-seeking behavior (Radostits *et al*., 2007).

In the present study, it was observed that the prevalence of mastitis was significantly higher during the late stage of lactation, accounting for 59.2% of cases. This finding is consistent with previous studies conducted by Almaw *et al*. (2008), Getahun *et al.* (2008), and Abera *et al*. (2012), which also reported a higher prevalence of mastitis during this stage. However, this finding contradicts the results of a study by Kerro and Tareke (2003), where a higher prevalence of mastitis was reported during the early stage of lactation. These discrepancies regarding the effect of lactation stages among different studies could potentially be attributed to variations in the age and parity of the sampled cows, as suggested by Isae and Kurtu (2018).

The present study revealed that the prevalence of mastitis was higher in older cows (>6 years of age) (79.7%) than in younger (<3 years) cows (22.6%).In support to this, the findings by Kerro and Tareke (2003), and Busato *et al*. (2000), who found that the risk of clinical and subclinical mastitis increases significantly with the advancing age of the cow. It has been well documented that older cows have larger teats and more relaxed sphincter muscles, which increase the accessibility of infectious agents in the cows' udder (Radostits *et al*., 2007).

The findings of the current study regarding the increased prevalence of mastitis with parity are consistent with previous reports by Zeryehun *et*

al. (2013), Abunna *et al*. (2013), Belayneh *et al*. (2014), and Dabele *et al*. (2021). This association may be attributed to the fact that primiparous cows possess a more effective defense mechanism against mastitis compared to multiparous cows (Erskine, 2001). The likelihood of infection increases over time in multiparous cows, leading to a prolonged duration of infection (Radostits *et al*., 2007).

According to the current findings, there was a higher prevalence of mastitis (61.9%) observed in cows housed in facilities that were cleaned twice per day (less frequently cleaned), compared to cows housed in facilities cleaned four times or more per day (more frequently cleaned). This suggests that the frequency of cleaning the cow houses had an impact on the prevalence of mastitis, with less frequent cleaning associated with a higher prevalence. Interestingly, the study did not find a significant association between the husbandry system and the previous history of mastitis with the prevalence of mastitis in the cows. This implies that factors such as the type of husbandry system (e.g., grazing, confinement) and the presence of a previous history of mastitis did not have a notable effect on the prevalence of mastitis in the specific study. It is important to consider that these findings are specific to the study conducted and may not be universally applicable.

Based on the microbiological analysis conducted in this study, it was found that the overall prevalence of *S. aureus* isolates in cows with both clinical and subclinical mastitis was 47.2%. This finding aligns with the previous studies conducted by Mekibib et al. (2010) (47%) in Holeta town and Legesse *et al*. (2015) (48.3%) in Addis Ababa city. However, it is worth noting that the present prevalence was higher than that reported in other studies (Workineh *et al*., 2002; Tesfaye *et al*., 2013; Zeryehun *et al*., 2013;Yohannis and Molla, 2013). On the other hand, there were studies, namely Abebe *et al*. (2016) and Zenebe *et al*. (2014) that reported a higher prevalence of *S. aureus* isolates compared to the present study. The variations in the occurrence of *S. aureus* among different reports could be attributed to differences in the farm management practices and environmental factors.

In this study, *S.aureus* was more frequently identified in subclinical mastitis than in clinical cases. This is almost similar to previous studies that proved *S.aureus* is the principal causative agent of subclinical mastitis (Tassew *et al*., 2017). This difference might be explained by the already documented evidence that *Staphylococcus* species are adapted to survive in the udder and usually establish chronic subclinical infection of long duration, from which it is shed through milk, serving as a source of infection for other healthy cows and transmitted during the milking process (Radostits *et al*., 2007).

CONCLUSION & RECOMMENDATIONS

Mastitis is one of the most important infectious diseases of dairy cows. The subclinical form is the most prevalent type of mastitis in the study farms, which might indicate that dairy farm owners give more attention to clinical mastitis than to subclinical mastitis, which receives very little attention. The prevalence of mastitis was found to be significantly different among age groups, lactation stages of cows and cleaning frequency of the farm (Hygiene). Based on the above conclusions, regular screening for early

detection and treatment of subclinical mastitis together with creating awareness in the community of the risk of *S. aureus* as a public health concern should be in place.

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Length-based estimates of growth parameters and mortality rates of Nile Tilapia (*Oreochromis niloticus*, L. 1758) in Lake Abaya, Southern Ethiopia

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KEYWORDS:

Exploitation rate;

Growth parameters;

Lake Abaya;

Length at maturity;

Mortality rates;

Recruitment

ABSTRACT

The Nile tilapia, *Oreochromis niloticus*, is one of the most commercially important fish species in Ethiopia. Effective management is essential to sustaining their fisheries and providing benefits for the local communities. The study was aimed at determining the basic population characteristics (growth, mortality rates, and recruitment), size at first maturity, length at first capture, and stock status of *O. niloticus* in Lake Abaya. These basic quantitative population characteristics enable a fisheries manager to identify population changes resulting from fishing. The parameters were determined using length frequency data collected from 4089 samples of *O. niloticus* ranging from 23 to 47 cm in total length. The total length (TL) and total weight (TW) of *O. niloticus* samples were gathered between September 2021 and August 2022. The length-weight relationship parameters were ($TW = 0.0157TL^{3.0192}$, $R^2 = 0.9603$) and the condition factor K=1.69. The population parameters were determined using the ELEFAN I routine in FiSAT software. Estimated von Bertalanffy growth parameters were ($L\infty$) = 49.35 cm, growth curvature (k) = 0.36 yr^{-1}, age at length zero (t_o) = -0.40, and growth performance index (Φ') = 3.0.The estimated values of total natural and fishing mortalities were Z= 1.34 yr^{-1}, M =0.34 yr^{-1},and F= 1.0 yr^{-1}, respectively. The current exploitation rate (E) was0.74, which is higher than the optimal (E = 0.5) and indicates that *O. niloticus* in Lake Abaya was overexploited. In order to maintain the sustainability of the fish population, it is advised that the local authorities establish regulations for the management of *O. noloticus* in Lake Abaya. These regulations should include protecting the use of small fishing gear and safeguarding fish that are caught smaller than their length at first maturity.

INTRODUCTION

The growth parameters of fish populations can be determined through direct readings of hard structures (otoliths, spines, or vertebrae) and indirect estimates based on length distribution data over time (Gayanilo *et al.*, 2002; Panfili *et al*., 2002). Length-based stock assessment tools are relatively more useful in tropical and sub-tropical waters since the seasonal differences in the hard structures of these relatively warm waters are subtle and often present unclear band marks (Sparre and Venema, 1992; Panhwar and Liu, 2013).

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The analysis of fish stock population dynamics in tropical environments was made easier by the introduction of relative growth models and length-based stock assessment approaches (Huxley, 1993; Froese and Binohlan, 2000). These techniques were used to evaluate life-history theories and produce empirical estimates of pertinent biological and fisheries parameters, including longevity and length at first maturity (Stergiou, 2000; Froese and Binohlan, 2000). Additionally, it aids in forecasting fish population exploitation, which could be useful in choosing between different management options (da Costa and Araújo, 2003; Froese, 2006; Garcia and Duarte, 2006; da Costa *et al*., 2014; Sá-Oliveira *et al*., 2015).

Fish population biology and ecology are reflected in growth and mortality factors, which are crucial for modeling fish stock population dynamics. These metrics, which offer important information on the fluctuation of fish size over time and the reduction in population biomass owing to fishing and/or natural causes, are essential inputs for stock assessments (Pauly, 1983; Sparre and Venema, 1998).

Lake Abaya is one of the Rift Valley lakes in Ethiopia. Currently, this lake is the 4th most important in the country in terms of fisheries, contributing about 8% of capture fisheries to national and local markets (Gashaw and Wolff, 2014). About four commercially important fish species are in Lake Abaya: Nile tilapia (*Oreochromis niloticus*), Nile perch (*Lates niloticus*), African catfish (*Clarias gariepinus*), and *Bagrus docmac*.

These fish species are used as a source of income and livelihood for fishing communities in Lake Abaya. *Oreochromis niloticus* is the most important fish species and has a great contribution to the annual catch and yield of total landings. Due to high demand for fish food and market prices, the fishing process takes place throughout the year with heavy fishing pressure and a continual trend of yield reduction. The current state of knowledge regarding the life history and population dynamics of *O. niloticus* stock in Lake Abaya is lacking, despite the lake's considerable ecological and socioeconomic significance. A good understanding of fish population dynamic show mortality, growth, and recruitment interact to affect abundance is required for informed fisheries management.

Even though significant stock assessment and population dynamics studies of *O. niloticus* stocks have been carried out in Lakes Tana (Workiye *et al*., 2019), Chamo (Buchale *et al*., 2019; Million Tesfaye *et al*., 2021), Hawassa (Yosef *et al*., 2017), and Langeno (Genanaw *et al*., 2022), there is no comparable data regarding economically significant fish species in Lake Abaya. Therefore, the present study was aimed at determining the basic population parameters (growth, mortality rates, and recruitment), size at first maturity, length at first capture, and stock status of *O. niloticus* in Lake Abaya. The results can provide baseline information for fishery managers and scientists to design fishery exploitation and management strategies for further exploration of *O. niloticus* stock in the lake.

MATERIALS AND METHODS

Study area

Lake Abaya is the second-largest lake in Ethiopia after Lake Tana, a highland lake, and

one of the two southernmost Rift Valley lakes. It is situated in South Ethiopia Regional State, between 5°55'9'' and6°35'30'' N latitude and 37°36'90'' and 38°03'45'' E longitude (Fig. 1). The lake is 60 km long and 20 km wide, with a surface area of 1160 square kilometers. It has a maximum depth of 13 m and is found at an elevation of 1268 m, which makes it the largest Rift Valley Lake. This lake contains several islands, the greatest of which is Aruro; the others are Gidicho, Welege, Galmaka, and Alkali. Its southwest shore is home to the village of Arba Minch, while the southern banks are part of Nech Sar National Park. The principal perennial rivers that enter Lake Abaya are the Harre, Hamassa, Bilate, Gidabo, and Galana rivers.

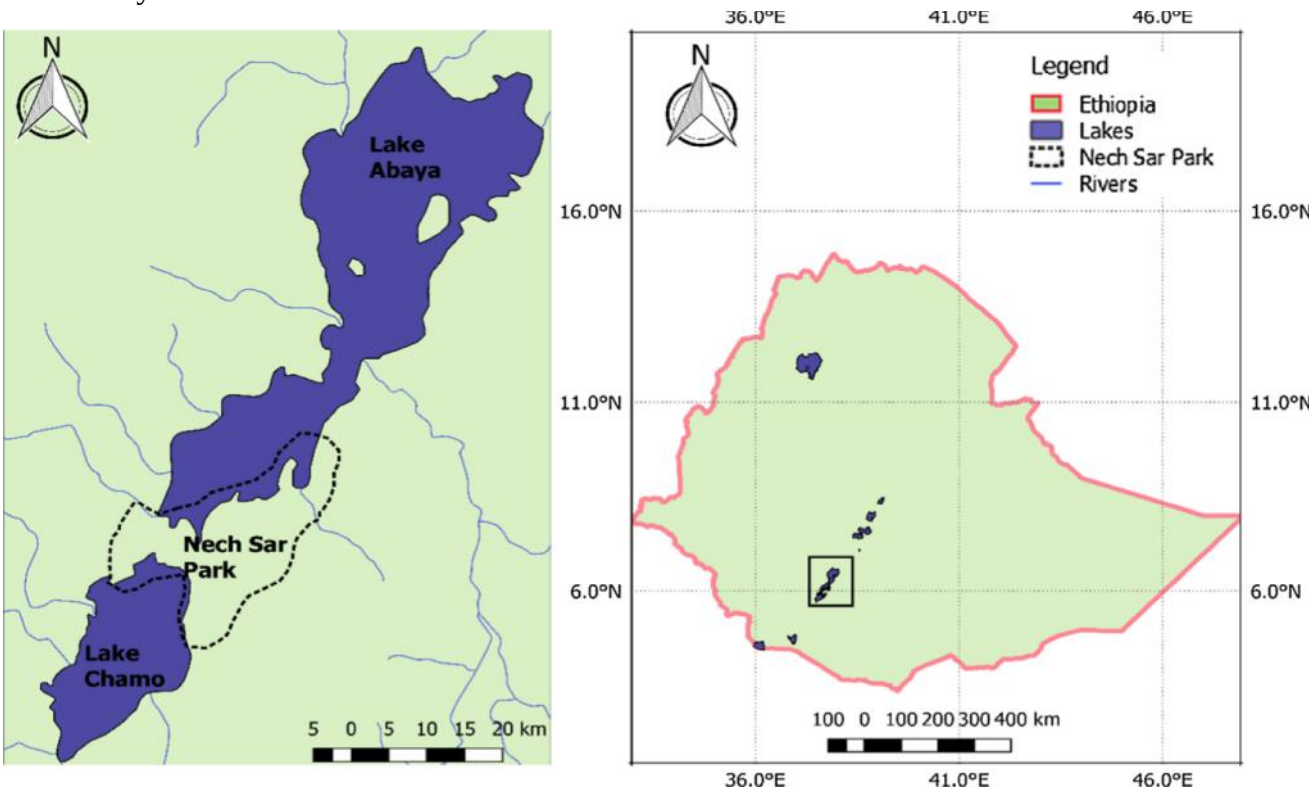

Figure 1. Outline map of Ethiopia with a detailed view on Lake Abaya and Chamo (Shape file downloaded from www.maplibrary.org)

Methods of sampling and data collection

Samples of *O. niloticus* were gathered from three cooperatives engaged in commercial fishing in Lake Abaya. From September 2021 to August 2022, samples of *O. niloticus* were randomly collected for 12 days each month at four commercial fishing landing sites (Ella, Hillo, Langama, and Gubena). Employing a measuring board and a sensitive electronic balance, the total length and total weight of fresh fish samples were determined to the nearest 0.1 cm and 0.1 g, respectively. Apart from the visual identification of sex by the examination of the gonads and abdominal dissection, sex was also identified by external features.

Data analysis

Length-weight relationship and condition factor

The length-weight relationship was calculated using the power function described by Le Cren(1951).

$$TW = a \quad ^{b} \text{---------- [1]}$$

Where,
TW = total weight (g), *a* = the intercept, TL =total length (cm), and *b* = the slope of length-weight regression

The Fulton's condition factor (*K*) is often used to reflect the nutritional status or well-being of an individual fish. It was calculated using the formula described by Fulton (1904), which is indicated below.

$$K = \frac{T}{T^{3}} * 100 \text{ ---------- [2]}$$

Where,
K = Fulton's condition factor
TW = total weight of fish in grams (g)
TL = total length of fish in (cm)

Estimation of growth parameters

The FiSAT II, ELEFAN I software's K-scan technique was employed to evaluate the asymptotic length (L∞) and growth rate (K) based on the length frequency data. Using Pauly's empirical formula (Pauly, 1979), the theoretical age at zero (t_o) was computed.

$$\text{Log} \quad (-t_0) \quad = \quad -0.3922 - 0.2752 * L \quad (\text{L}\infty) - 1.038 * \text{Log(k)} \text{---------- [3]}$$

Where,

t_o= is the theoretical age at which fish would have at zero length.
L∞ = asymptotic length, k= von Bertalanffy growth constant
Growth performance indexes were calculated by Munro and Pauly(1984):

$$\Phi' = L \quad (k) + 2 * L \quad (\text{L}\infty) \text{--------- [4]}$$

Where, Φ′ = growth performance index, k and L∞ are defined above

The length at first maturity (L_{50}) was computed as Froese and Binohlan's (2000) equation:

$$\text{Log}(L_{50}) = 0.8979 * L \quad (\text{L}\infty) - 0.0782 \text{---- [5]}$$

The longevity (A0.95)of the cohort was computed as (Spare and Venema, 1997).

$$\text{A0.95= to} + \frac{2.9}{K} \text{---------- [6]}$$

Where,
A0.95 is the age at which 95% of the cohort would be dead as a result of natural means;

t_o = is the theoretical age at which fish would have at zero length;

k = Von Bertalanffy growth constant

Estimated mortality parameters

Sparre and Venema (1992) state that the total mortality (*Z*) was estimated using a linearized length-converted catch curve. The natural mortality coefficient (*M*) was computed as follows using Taylor's method:

$$\text{M} = \frac{-\ln(1-0.95)}{A0.95} \text{----------[7]}$$

A0.95 indicates the age at which 95% of the population would die from natural causes. The

calculation of the fishing mortality (*F*) was done by Qamar *et al*. (2016).

$$F = Z - M \text{ ---------- [8]}$$

Where, *F* = fishing mortality, *Z* = total mortality, and *M* = natural mortality.
The exploitation rate (*E*) was calculated as (Georgiev and Kolarov, 1962).

$$E = \frac{F}{Z} \text{---------- [9]}$$

The length at first capture (Lc) was estimated from the equation of Beverton and Holt (1957), which applies the growth constants of vBGF, the mean length of the fish catch ($\overline{L}$), and the total mortality parameter (Z):

$$L_C = \overline{L} - k(\frac{L\infty - \overline{L}}{Z}) \text{ ----------[10]}$$

The length at optimum cohort biomass or yield pre-recruitment (L_{opt}) was estimated from L∞, K, and M using the Beverton (1992) formula:

$$L_{opt} = L\infty * (\frac{3}{3+\frac{M}{K}}) \text{---------- [11]}$$

Where, L∞, K, and M are as defined above.

RESULTS AND DISCUSSIONS

Length-weight relationship and Fulton's condition factor

The present study is conducted on a total sample of 4089 specimens (2344 females and 1745 males) of *O. niloticus*. The monthly pooled length-frequency data of *O. niloticus* specimens were grouped into two-centimeter intervals. The obtained fish samples had lengths ranging 23 to 47 cm (mean = 35 cm) and weights ranging from 190 to 1676 g (mean = 933 g). However, 97.6% of the catches were placed in the 25-41 cm range. The remaining 1% and 1.4% were less than 25cm and greater than 41cm, respectively. The most commonly observed value was the mid-length of 28 cm, which was followed by the length groups of 30 and 32 cm (Fig.2).

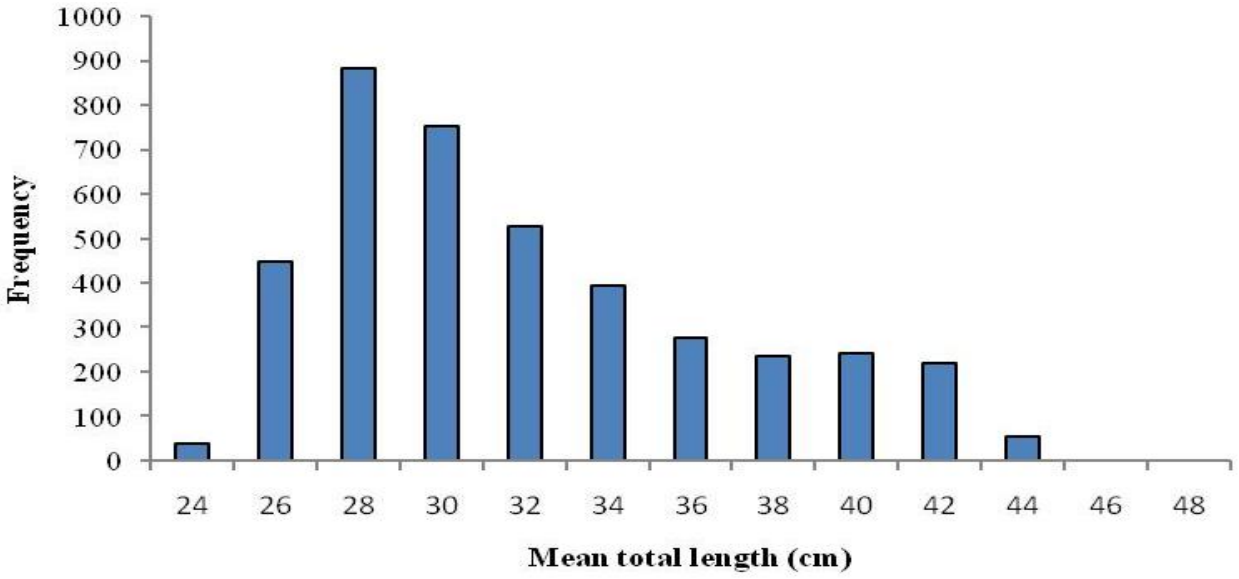

Figure 2: Size structure of *O. niloticus* in Lake Abaya.

When comparing, the maximum total length (47cm) observed in the present study is relatively smaller than those reported for the same fish in Lake Chamo: 57 cmTL (Yirgaw *et al.*, 2000) and 53.4 cm TL (Buchale *et al*., 2019), 48.5 cm TL in Gilgel Gibe I Reservoir (Mulugeta, 2013), and 48 cm TL in Alwero Reservoir (Genanaw *et al.*, 2017). However, the observed maximum length for *O. niloticus* in Lake Abaya is larger than those in Lake Langeno, 35.5 cm TL (Genanaw *et al*., 2022), Lake Beseka (25.0 cm TL), and Lake Hawassa (29.0 cm TL) (Yosef *et al*., 2017). Fishing typically reduces fish size structures due to fishing gear selectivity and leads to fisheries-induced evolution toward smaller sizes and earlier maturity (Borrell, 2013). Fish undergo a reduction in size and early maturation to replenish themselves before being eliminated by fishing when fishing pressure increases dramatically.

The relationship between total length and body of *O. niloticus* was established with the use of a scatter plot diagram and power function (Fig. 3). The relationship was described by the equation $TL = 0.0157TL^{3.0192}$($R^2 = 0.9603$, r = 0.9799). A strong positive correlation was found between the length and weight of the *O. niloticus* population in Lake Abaya, as indicated by the correlation coefficient (r = 0.9799). The regression coefficient "b" was found to be 3.0192 when utilizing the best-fit power function regression to analyze the length-weight relationship. The power of the formula did not show a statistically significant deviation from the 3.0 hypothetical value($P > 0.05$).

This study showed an isometric growth of fish and a strong correlation between length and weight, as evidenced by the exponential value (*b* = 3.0192) and correlation coefficient (r = 0.9799), respectively. Fish can grow in three different ways during their lives: isometric (*b* = 3), negative allometric (*b*< 3), or positive allometric (*b*> 3), depending on the deviation of *b* (Nehemia and Maganira, 2012). When "*b*" is greater than 3, the fish increase in weight more than an increase in length, whereas if it is less than 3, the fish becomes lighter for its weight. However, in an isometric growth scenario, the fish maintains its body form as it grows longer (Riedel *et al*., 2007). The growth pattern of *O. niloticus* in Lake Abaya was isometric, based on the "*b*" value found in this investigation.

The length-weight relationship of *O. niloticus* was found to be similar in some of the earlier studies: 3.03 in Lake Ziway (Zenebe, 1988); 3.04 in Lake Langeno (Gashaw and Zenebe, 2008); 3.017 in the River Nile (Shalloof and El-Far, 2017); 3.034 in the Aulia Dam (Ahmed and Abdel, 2016); and 3.09 in the Lake Victoria cage system (Ngodhe and Owuor, 2019).

On the other hand, some of the previously reported studies showed positive allometric length-weight relationships of *O. niloticus* were 3.18 in Lake Chamo (Buchale, 2020), 3.16 Lake Victoria (Ngodhe and Owuor, 2019), 3.19 in Lake Ziway (Gashaw and Zenebe, 2008), and 3.366 in Lake Naivasha (Keyombe *et al*., 2017) while, 2.33 in Lake Naivasha (Cishahayo *et al*., 2022), 2.89 in Lake Langeno (Genanaw *et al*., 2022), 2.76 in Alwero Reservoir (Genanaw *et al.*, 2017), and 2.934 in Lake Ardibo (Endalk *et al*., 2018) were some of the negative allometric length-weight relationships of *O. niloticus*.

The value of *b* can vary annually due to a variety of factors, such as season, habitat, gonad maturity, sex, nutrition, stomach fullness,

health, preservation techniques, and environmental circumstances (Bagenal and Tesch, 1978; Arslan *et al*., 2004; Froese, 2006; Yilmaz *et al*., 2012; Ali *et al*., 2016). Furthermore, variations in fish growth patterns could also be related to the species' condition, phenotype, environment, and specific geographic region (Tsoumani *et al*., 2006).

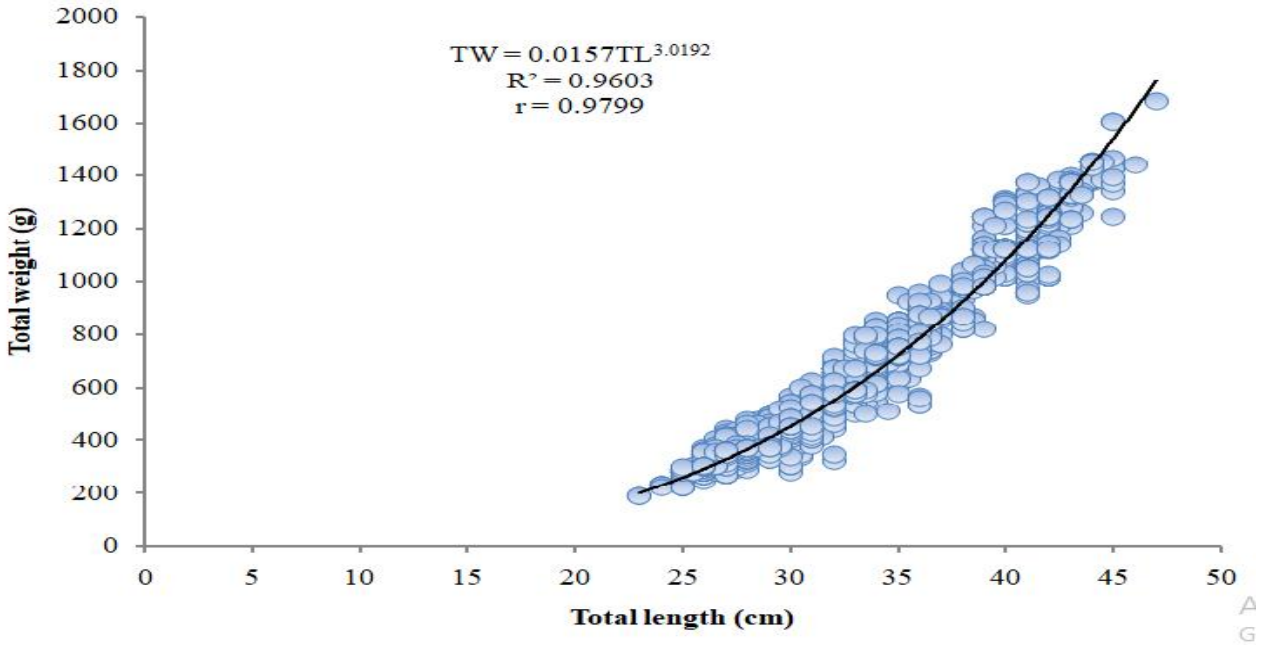

Figure 3: Length-weight relationship of pooled *O. niloticus* in Lake Abaya.

Fulton's condition factor

As shown in Table 1, the monthly mean values of Fulton's condition factor (K) for females, males, and combined sexes ranged from 1.58 to 1.77. For females, males, and combined sexes, the average K value was 1.70, 1.68, and 1.69, respectively. In *O. niloticus* of Lake Abaya, there was statistically no significant variation in K between the sexes or with the month's interaction ($P > 0.05$).

The condition factor is a metric that represents the fish's physiological state with respect to feeding, spawning, and other elements of their overall health. According to Blackwell *et al*. (2000), high condition factor values imply advantageous environmental conditions (such as habitat and prey availability), while low values suggest less favorable environmental conditions. The ecological habitat of fish species can be evaluated using the condition factor, which is also highly influenced by biotic and abiotic environmental factors (Ayoade, 2011; Onimisi and Ogbe, 2015; Abu and Agarin, 2016).Five categories were created by Morton and Routledge (2006) based on the K values: very bad (0.8–1.0), bad (1.0–1.2), balance (1.2–1.4), good (1.4–1.6), and very good (> 1.6). However, according to Ayoade (2011), a fish in good health has a condition factor greater than one.

In the present study, the average value of the condition factor was 1.70, 1.68, and 1.69 for

females, males, and combined sexes, respectively. As previously mentioned, the condition factor in this study was more than 1.6, indicating that *O. niloticus*in Lake Abaya is doing quite well.

Table 1. Mean monthly condition factor of females, males and combined *O. niloticus*

Months	Females	Males	Combined sexes
Sep-21	1.58	1.60	1.58
Oct-21	1.68	1.66	1.67
Nov-21	1.63	1.63	1.63
Dec-21	1.61	1.59	1.60
Jan-22	1.67	1.62	1.65
Feb-22	1.71	1.69	1.70
Mar-22	1.73	1.67	1.70
Apr-22	1.71	1.71	1.71
May-22	1.77	1.75	1.76
Jun-22	1.76	1.72	1.74
Jul-22	1.77	1.77	1.77
Aug-22	1.75	1.75	1.75
Average	1.70	1.68	1.69

Estimated growth parameters

The *O. niloticus* in Lake Abaya was predicted to have von Bertalanffy growth parameters of asymptotic length (L∞) and annual growth constant (k) of 49.35 cm and 0.36 yr^{-1}, respectively. The estimated theoretical age at birth (t_o) was -0.40. The longevity (A0.95), the age at which 95% of the population would be dead as a result of natural means, was 8.72 years. The growth performance index Phi (*Φ'*) was estimated at 3.0 (Fig. 4).

The estimated value of L∞ in this study was lower than the estimates from the studies in Lakes Chamo, 55.0 cm (Buchale *et al*., 2019), and Victoria, 58.8 cm (Njiru *et al*., 2004). However, compared to Lakes Langeno (35.7 cm; Genanaw *et al*., 2022), Tana (44.1 cm; Workiye *et al*., 2019), Victoria (46.24 cm; Yongo *et al*., 2018), Koka (44.5 cm; Gashaw, 2016), and Naivasha (42.0 cm; Waithaka *et al*., 2020), the L∞ in this study was higher.

It is possible that the various water bodies' varying environmental conditions account for the differences in estimations of the von Bertalanffy growth parameters (L∞ and k) when compared to similar research. The size of the population and the ways in which fish adjust throughout their lives are other elements that influence growth. As noted by Sparre and Venema (1998), this could potentially vary throughout stocks and species and be impacted by various methods of analysis. Similar species can have different growth rates in different habitats (Lowe-McConnell, 1982). The length characteristics (TL_{max} and L∞) may be influenced by genetics, resource availability, and population density. The fishing pressure is also a factor for change in the asymptote length (L∞) of fish in a given water body. If fishing gear is selective and oriented toward harvesting

larger individuals, large individuals may become rare in overexploited fisheries, and the scarcity of these individuals in a given sample will certainly underestimate growth parameters.

In comparison to Lakes Langeno, 2.61 (Genanaw *et al*., 2022), Ziway, 2.76 (Gashaw, 2006), and Naivasha, 2.57 (Waithaka *et al*., 2020), the estimated growth performance index (Φ' = 3.0) in the current study was greater. On the other hand, the index in the present study was lower than in Lakes Chamo, 3.16 (Million *et al*., 2021), and Victoria, 3.14 (Yongo *et al*., 2018). The most effective method for determining the average growth parameters of a particular species is to use the growth performance index, which should show comparable values when comparing several groups within the same species (Gulland, 1983; Sparre and Venema, 1998). The availability of food, environmental factors, and fishing pressures can all have an impact on a fish species' growth performance index, in addition to the genetic composition that dictates the species' potential for growth (Getabu, 1992).

O. niloticus in Lake Abaya had an approximate lifespan of 8.72 years, which is comparable to the lifespan of *O. niliticus* in Lake Langeno, 8.9 years, as indicated in Genanaw *et al*. (2022). Both biological elements and environmental factors can have an impact on lifespan. Fish life spans are significantly influenced by a variety of biological criteria, including sex, genetic makeup, diet, reproduction, age, and maturation, in addition to environmental influences including salinity, temperature, and predation (Das, 1994).

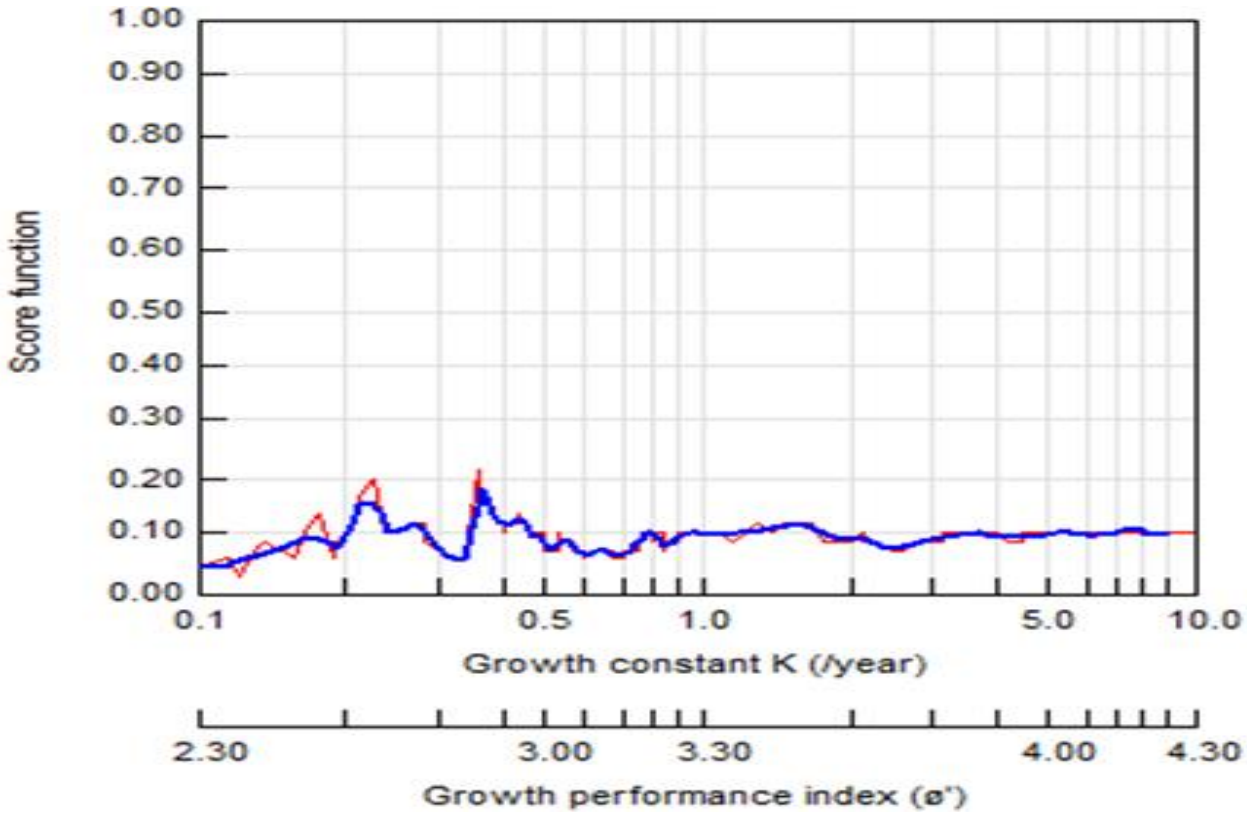

Figure 4: ELEFAN I K-Scan routine FiSAT II output for *O. niloticus* in Lake Abaya.

Estimated mortality parameters

In order to estimate total mortality, a length composition data set was created and prepared for a linear regression analysis between the X and Y variables (Table 2). The mortality parameters were determined using a linearized length-based catch curve analysis. As indicated in figure 5, the slope of the regression line (b) is -1.34, and hence, the estimated total mortality rate (*Z*) was 1.34 yr^{-1}. The natural mortality rate (*M*) and fishing mortality rate (*F*) were 0.34 $yr^{-1}$and 1.0 yr^{-1},respectively. Using these mortality estimates, the exploitation rate (*E*) was computed as 0.74, which indicates that *O. niloticus* in Lake Abaya is overexploited.

Table 2: Parameters for length-based catch curve analysis

Length group (cm)						x	y
	Catch	k	L∞ (cm)	Δt (L1,L2)	(L1+L2)/2	t(L1+L2)/2	Ln(C(L1,L2)/Δt)
23-25	40	0.36	49.35	0.22	24	1.85	5.21
25-27	450	0.36	49.35	0.24	26	2.08	7.54
27-29	884	0.36	49.35	0.26	28	2.33	8.13
29-31	752	0.36	49.35	0.29	30	2.60	7.87
31-33	529	0.36	49.35	0.32	32	2.90	7.41
33-35	394	0.36	49.35	0.36	34	3.24	6.99
35-37	279	0.36	49.35	0.42	36	3.63	6.51
37-39	237	0.36	49.35	0.49	38	4.08	6.18
39-41	243	0.36	49.35	0.60	40	4.62	6.01
41-43	222	0.36	49.35	0.76	42	5.29	5.68
43-45	57	0.36	49.35	1.05	44	6.17	3.99
45-47	2	0.36	49.35	1.71	46	7.47	0.16
Total	4089						

Fish mortality can be attributed to both natural and anthropogenic factors. Most of the natural mortality could be attributed to old age, diseases, and predation factors in the aquatic ecosystem. The natural mortality (*M*) in the present study is lower than the fishing mortality (*F*), and indicating that the primary cause of mortality for *O. niloticus* in Lake Abaya is attributed by fishing factors. When comparing the estimated values for mortality (*F >M*), it is possible to conclude that fishing mortality was a more important source of mortality for *O. niloticus* in Lake Abaya.

A population dominated by mortality was also indicated by the Z/K ratio of 3.72 estimated in this study. The population is growth-dominated if the ratio Z/K is less than 1, mortality-dominated if it is greater than 1, and in equilibrium when growth and mortality are equal if it is equal to 1. If Z/K = 2 in a mortality-dominated population, the population is considered lightly exploited (Beverton and Holt, 1957). According to Beverton and Holt's (1957) general criteria, the Z/K value in the current study indicated that the *O. niloticus* population in Lake Abaya was highly exploited. This is also revealed by the estimated high

exploitation rate (E= 0.74), which indicates a state of overexploitation. As per Gulland's (1971) assumptions, a sustainable yield is considered optimal if F is equal to M or if the rate of exploitation (E) is 0.5. If E>0.5, it is typically assumed that the stock has been overexploited.

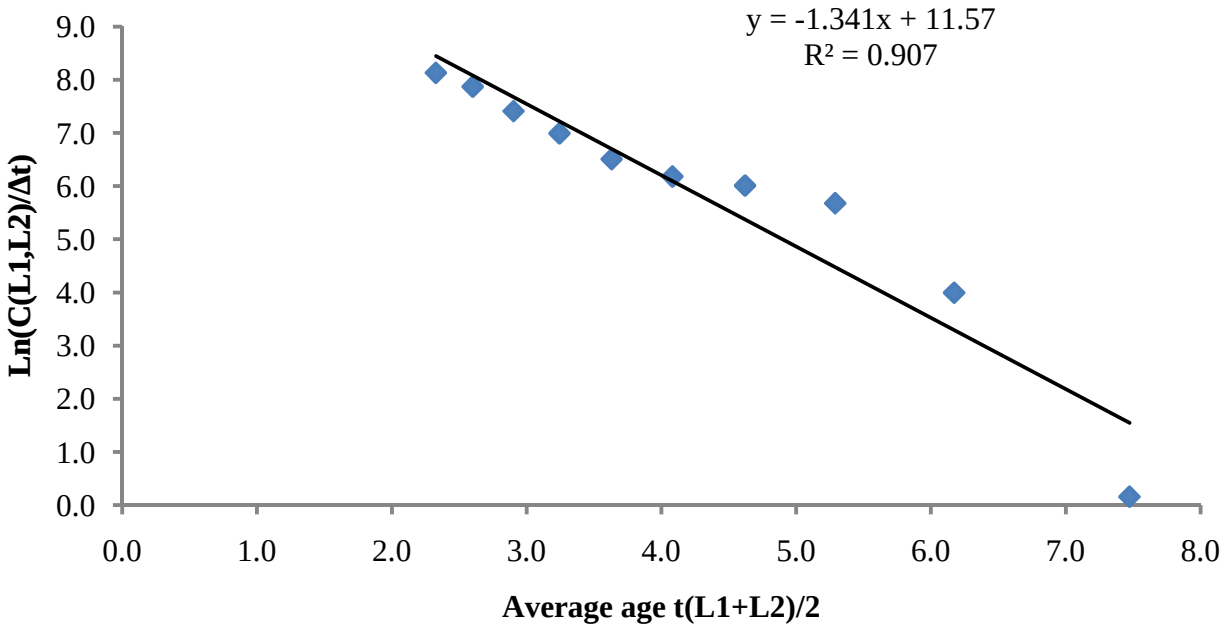

Figure 5: Linearized length-based catch curve of *O. niloticus* in Lake Abaya.

Length at first maturity (L_{50}) and length at first capture (Lc) were 27.68 cm and 27.97 cm, respectively. The optimum length (L_{opt}) of *O. niloticus* in Lake Abaya was also estimated at 37.53 cm. The L_{50} and Lc of *O. niloticus* in Lake Abaya were almost similar and vulnerable for fishing. Based on the evidence shown in this study, *O. niloticus* in Lake Abaya is ready to be removed through fishing at its first spawning stage. Catching fish with total length less than or equal to L_{50} is the main cause of overfishing, and it is recommended that the mesh size of fishing nets used in Lake Abaya should be increased to catch fish above 28 cm for conservation of the stock. When the results of virtual population analysis (VPA) were considered (Fig. 6), fish with total a length of 27-32cm had more exposure to fishing gear, whereas fishing mortality was higher for fish with a total length above 39 cm.

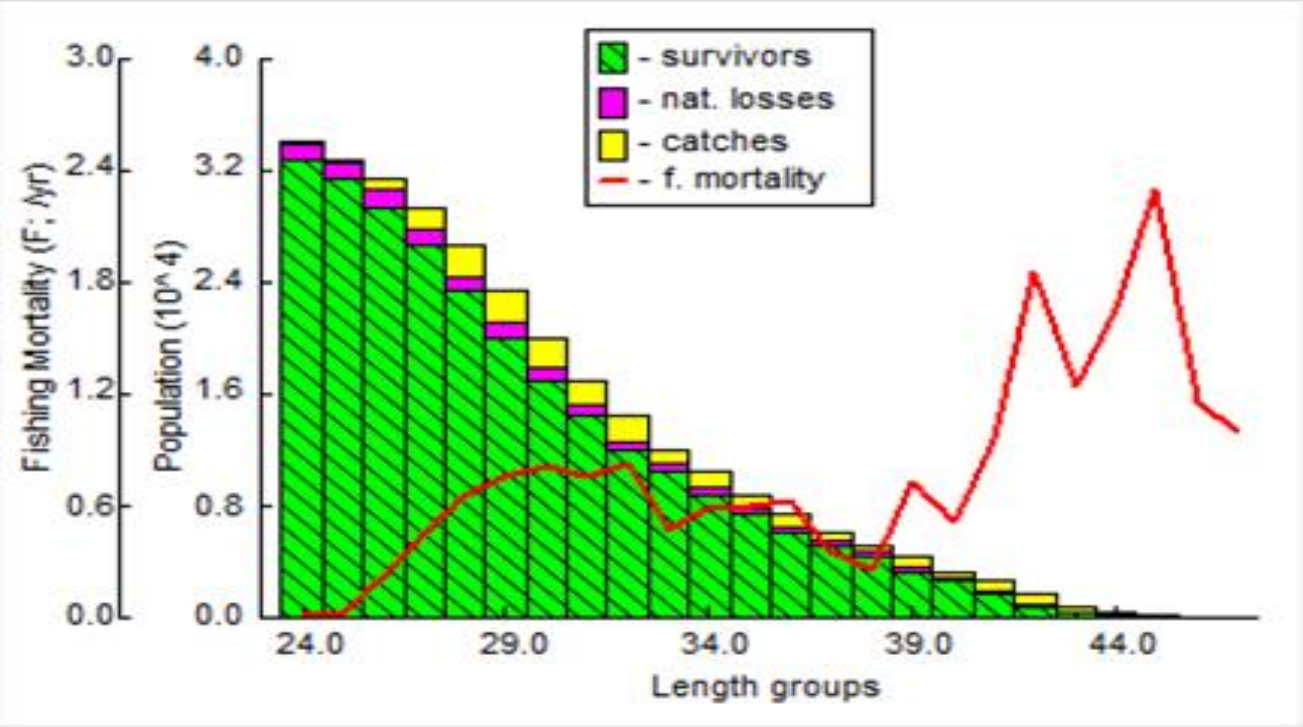

Figure 6: Estimated virtual population of *O. niloticus* in Lake Abaya.

The length at first maturity (L_{50}) of *O. niloticus* in the present study was higher than that of Lakes Chamo 23.6 cm (Buchale *et al*., 2021), Hayq 12.8 cm for females and 12.9 cm for males (Tessema *et al*., 2019), Langeno 16.62 cm (Genanaw *et al*., 2022), and Hawassa 20.8 cm for females and 20.3 cm for males (Muluye *et al*., 2016). According to Fryer and Iles (1972) and Lowe-McConnell (1987), the size of maturation varies depending on demographic conditions and is influenced by both genes and environment. Depending on the fishery's selectivity, fishing pressure can affect population structure, growth, and early maturation (Jorgensen *et al*., 2007). In water bodies with high fishing pressure, the fish devote more resources to reproduction than to somatic body building (Bandara and Amarasinghe, 2018). According to Jonsson *et al*. (2014), fish that live in harsh situations also exhibit early sexual maturity since this is a coping mechanism for maintaining maximal reproduction under stressful conditions.

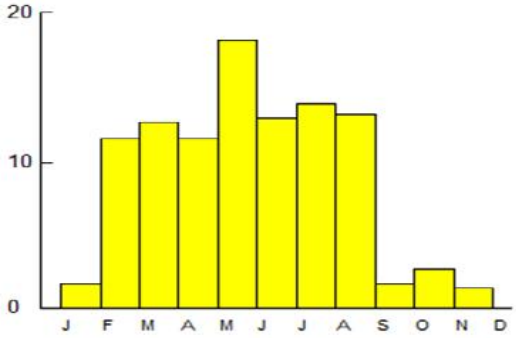

Figure 7: The seasonal recruitment pattern of *O. niloticus* in Lake Abaya.

Estimated seasonal recruitment pattern and relative yield per recruitment

The estimated recruitment pattern of *O. niloticus* in Lake Abaya was year-round, with one peak period (May)in the year (Fig. 7). Recruitment adds younger fish to the fishery

and can vary from year to year by orders of magnitude.

The peak recruitment season of *O. niloticus* in the present study was similar to *O. niloticus* in Lake Langeno, as indicated in Genanaw *et al*. (2022). The biology of tropical freshwater fish reproduction appears to be significantly influenced by patterns of rainfall and variations in water levels (Wootton, 1990). The monthly average rainfall in the study area is higher in April, May, and October, which could be one of the probable reasons for the occurrence of a peak recruitment pattern for *O. niloticus* in May.

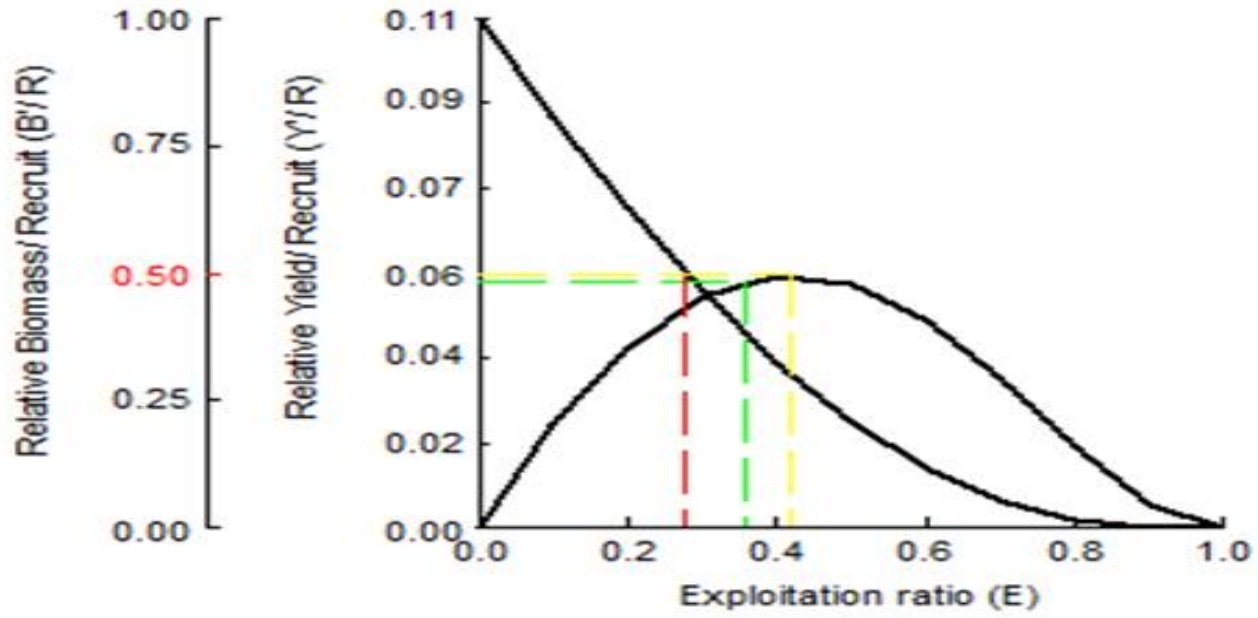

Figure 8: Beverton and Holt's relative yield per recruitment curve for *O. niloticus* in Lake Abaya.

The degree to which the current rate of exploitation is optimal, below optimal, or excessive with respect to the population's capacity for self-renewal was ascertained using the correlation curve between the exploitation rate and yield per recruitment. According to the estimated yield per recruitment curve, the current exploitation rate was 0.74 with a yield per recruitment of 0.029, the optimal exploitation rate (E_{opt}) was 0.5 with a yield per recruitment of 0.05, and the exploitation rate for maximum yield (E_{max}) was 0.421 with a yield per recruitment of 0.053 (Fig. 8). It is evident that the *O. niloticus* population in Lake Abaya was overexploited because the present exploitation rate was higher than the ideal exploitation rate. In order to monitor and set regulations for *O. niloticus* fishing in Lake Abaya, the local authorities need to be aware of this circumstance. The fish that are collected should be the same size at which they have spawned, in order to preserve the sustainability of the fish population.

CONCLUSIONS & RECOMMENDATIONS

The growth pattern of *O. niloticus* in Lake Abaya was isometric, which implied that an increase in body length is proportional to body weight. The groups with mean lengths ranging from 25 cm to 41 cm accounted for

approximately 97.6% of the total capture and significantly influenced the yield of fish. The average value of Fulton's condition factor was 1.70, 1.68, and 1.69 for females, males, and combined sexes, respectively, which implied that the wellbeing of *O. niloticus* in Lake Abaya was in a very good health condition.

This study generated important information on population dynamics parameters (growth, mortality rates, and recruitment) and other crucial life-history characteristics that can serve as basic stock assessment tools for *O. niloticus* management in Lake Abaya. The asymptotic length ($L\infty$) and growth rate (k) were 49.35 cm and 0.36 per year, respectively. The length at first capture (Lc) was estimated at 27.97 cm, while the length at first maturity (L_{50}) was estimated at 27.68 cm. Based on the result, harvesting the fish at a length less than or equal to the length at first maturity may alter the recruitment potential of the stock, which in turn may result in thecollapse of the stock. The long lifespan in which 95% of the population would be dead as a result of natural means was 8.72 years.

Moreover, the current exploitation rate (E= 0.74) is higher than the optimal (E = 0.5) and indicates that *O. niloticus* in Lake Abaya was overexploited. Based on these results, it is recommended that the fisheries management of the lake should include controlling or restricting the usage of small fishing gear in addition to reducing fishing efforts to ensure the sustainability of this commercially important

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