PRENSIP

JOURNAL OF AGRICULTURAL PRODUCTION

Year: 2022 | Volume: 3 | Issue: 2 | e-ISSN 2757-6620 | agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

2022 ● VOLUME 3 ● ISSUE 2

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JOURNAL OF AGRICULTURAL PRODUCTION

2022 • VOLUME 3 • ISSUE 2

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JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
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RESEARCH ARTICLE

Microstructure of *Eobania vermiculata* (Müller, 1774): SEM, F-TIR and XRD Methods

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ARTICLE INFO

Article History
Received: 08.06.2022
Accepted: 23.07.2022
First Published: 13.12.2022

Keywords
Characterization
Chocolate band snail
Land snail
Microstructure
SEM

ABSTRACT

In this study, Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analyses are used for the microstructure characterisation of *Eobania vermiculata* samples collected from Iskenderun region. The shells of land snails are discarded as waste; however, they are qualified materials with multiple use areas. To substantiate this proposition, an attempt was made to elucidate the physical and chemical properties of the shells of chocolate band snail, *E. vermiculata*. SEM observations indicated that nacre crystals are always laminated aragonite, usually presenting sharp edges. Nacre crystallites which pile up into columns vertically abreast aligned observed. The crystals are about 390-155 nm thick, and they form stacks along a fixed spacing, filled with biological matter. The XRD and FTIR observations revealed the dominance of the aragonite form of the calcium carbonate crystal in the microstructures of each snail shell with the occurrence of different shell surface functional groups. Thus, further exploration of the shell inclusive of the organic components is required to promote its possible use as a biocomposite. Nonetheless, the present study provides an overview of physical and chemical characteristics of the land snail shells and inlight their potential use in different areas in the perspective of sustainability.

Please cite this paper as follows:

Öksüz, K. E., & Şereflişan, H. (2022). Microstructure of *Eobania vermiculata* (Müller, 1774): SEM, F-TIR and XRD methods. *Journal of Agricultural Production*, *3*(2), 42-47. https://doi.org/10.56430/japro.1128026

Introduction

Eobania vermiculata (Müller, 1774) or chocolate band snail is native to the Mediterranean region but today has a worldwide distribution via anthropogenic activities (Godan, 1979). *E. vermiculata* is registered in Italy, Bulgaria (Dedov, 1998), Greece (Welter-Schultes & Williams, 1999), Turkey (Örstan et al., 2005) and Croatia (Rađa et al., 2012). It has become widespread to evaluate mollusk shells in different areas in the living world. In particular, the shell structures of Gastropoda species seem worth investigating (de Paula & Silveira, 2009).

Mollusk shells are mineralized tissues each with a unique mineral composition. In all three main mollusk classes (Cephalopoda, Gastropoda and Bivalvia) the shell consists of stratified layers (Santana & Aldana Aranda, 2021). Mollusk shells are composed of calcium carbonate and a small amount of organic matrices (Lowenstam & Weiner, 1989). Calcium carbonate has amorphs form and consist of calcite, aragonite and vaterite (Suzuki & Nagasawa, 2013). Vaterite is a mineral that is a polymorph of calcium carbonate, and it is the most unstable one; whereas, calcite is the most stable and aragonite is metastable. Accordingly, most mollusk shells consist of aragonite and/or calcite; yet, vaterite is rarely found (Spann et

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al., 2010). The periostracum is formed as a first layer in the shell formation, and the calcified layer subsequently forms on the periostracum which is not mineralized (Checa, 2000). The first calcification in the mollusk shell begins in the shell gland cells (Nielsen, 2004). The mantle, which supplies the periostracum and calcified layers with inorganic ions and organic matrices through the extrapallial fluid creates mollusk shell (Waller, 1980). The periostracum consists of three layers: inner, middle and outer. Among them inner layer is secreted by the mantle epithelium (de Paula & Silveira, 2009). Nacre which is widely distributed in mollusks is the most studied aragonitic and has stratified microstructure. Stratified microstructure which make to nacre luster occurring it one of the most studied hard tissues and has been of great interest to the pearl industry (Wang et al., 2013). In the gastropods, the aragonite nanocrystals of the nacre which exhibits specific growth pattern and mechanism are stacked in the form of towers (Romana et al., 2013). Nacre is a biomineral consisting (by weight) of 95% aragonite ($CaCO_3$) with the remaining 1-5% being organic matrix (Zhang & Li, 2012). Its microstructure is one of layered “brick” (aragonite tablets) and “mortar” (protein-polysaccharide matrix) (Machado et al., 1991; Santana & Aldana Aranda, 2021).

The microstructure features of the mollusk shell thanks to scanning electron microscopy is in use to the determine the phylogenetic evolution of the mollusk (Machado et al., 1991; Hedegaard, 1997; Lopes-Lima et al., 2010). Infrared spectroscopy which investigated composition of both inorganic and organic materials provides the identification of characteristic functional groups in molecules that correspond to specific molecular vibrations in the molluscan shells (Dauphin, 1999; Wang et al., 2013; Dauphin et al., 2018). The X-ray diffraction method (de Paula & Silveira, 2009) provided us the information about the complex microstructure of different types of deposited calcium carbonate crystals (Lowenstam & Weiner, 1989).

The objective of the present study was to analyze the micro and nanostructure of *E. vermiculata* (Müller, 1774) which is commonly found in Turkey. Analysis was conducted by scanning electron microscopy, its chemical composition was identified by X-ray diffraction Fourier Transform Infrared Spectrometry. The aim of this research will be provides theoretical data for the use of *E. vermiculata* in the biomaterials area and represented useful criteria for studies of the phylogeny of mollusca.

Materials and Methods

In this study, chocolate band snail *Eobania vermiculata*, a pulmonate stylommatophoran gastropod of the *Helicidae* was investigated. The snails were collected at the beginning of March 2018 in the Iskenderun region. Collected individuals were brought to the laboratory and the soft tissue was carefully separated from the shell. Shells were washed with tap water followed by distilled water to remove the mud, sand and other impurities. The shells were kept under the sun for 3 days to dry (Singh & Purohit, 2011).

Fourier Transform Infrared Spectroscopy (F-TIR) Analysis

The samples of raw materials from the chocolate band snail shell powders (*E. vermiculata*) were analyzed at 4000-650 cm^{-1} using a model Jasco/FT/IR-6700 equipped with ATR (Attenuated Total Reflection) Spectrometer.

X-Ray Diffraction Analysis (XRD)

X-ray diffraction analysis (XRD) was conducted to detect the crystallinity of the raw materials from shell powder. The XRD measurements on powder samples were done at 5°-40° with XRD (Malvern Panalytical EMPYREAN 3rd generation, United Kingdom) equipped with Ni-filtered Cu Kα radiation (λ = 1.5406 Å). It was operated with 1°/min deviating and receiving slit at 40 kV and 30 mA and continuous scan was carried out with step size of 0.015° and step time of 0.2 s.

Scanning Electron Microscopy (SEM) Analysis

The samples were mounted on stubs with conductive double-sided carbon tape and coated with gold/palladium in a sputter coater (Polaron SC7620, UK) for 90 s at 9 mA. The samples were examined and photographed using a JEOL JSM 5500 scanning electron microscope (SEM) at an accelerating voltage of 5 kV.

Results and Discussion

Fourier Transform Infrared Spectroscopy (F-TIR)

Infrared characterization was carried out for the sample to study the spectral characteristics indicative of the chemical bonding in the snail shell powder. The infrared spectra of *Eobania vermiculata* shells powder is shown in Figure 1.

The peak around 2969 cm^{-1} appeared due to the CH_2 stretching bonds of aliphatic chains. The F-TIR spectrum revealed lower intensity organic bands; the band at 1786.52 cm^{-1} was attributed to the carboxylate (carbonyl) groups of the acidic proteins in the organic matrix. Four bands characteristic of aragonite, corresponding to the CO_3^{-2} ions, were identified: ν1 at 1082.83 cm^{-1}; ν2 at 855.19 cm^{-1}; ν3 at 1448.49 cm^{-1}; and ν4 at 699.91-712.63 cm^{-1} (Figure 1). The ν1 band to the symmetric stretch mode and the ν4 band corresponds to the planar flexion mode of carbonate vibration.

Also, the characteristic carbonate ν4 bands of aragonite were at 712.63 and 699.91 cm^{-1} and the characteristic carbonate ν2 band of aragonite at 855.19 cm^{-1} revealed the availability of aragonite form of calcium carbonate in the shell powders from snails (Anjaneyulu et al., 2015; Hossain et al., 2015). This was also supported by the study of Weir and Lippincott (1961), Focher et al. (1992), Marxen et al. (2003), Cárdenas et al. (2004), Agbaje et al. (2017), and Parveen et al. (2020).

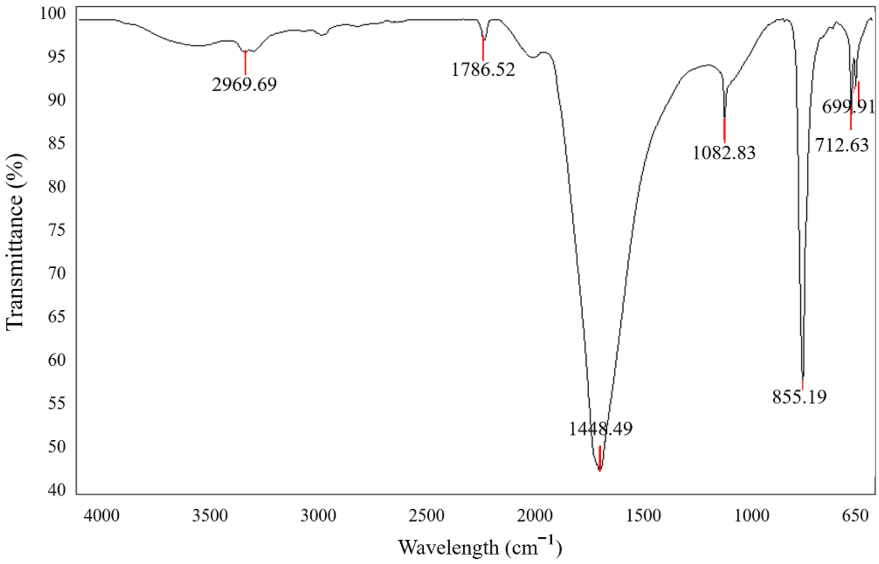

Figure 1. F-TIR spectra of *Eobania vermiculata* shells powder.

X-Ray Diffraction Analysis (XRD)

In order to understand the phase components of chocolate band snail, XRD patterns were conducted. The XRD patterns of shells of the chocolate band snail revealed similarities in crystalline peaks, confirming the existence of the aragonite form of calcium carbonate (Figure 2). The X-ray diffraction data were acquired in steps of 0.015° at scattering angels (2θ) ranging from 5° to 80°. The XRD phase analysis of aragonite, shown in Figure 2, carry high intensity peaks at 2θ = 26.43°, 27.09°, 31.12°, 33.81°, 36.23°, 38.17°, 46.32°, 52.29°, 53.07°, 66.31° with monochromatic Cu-Kα radiation (λ = 1.5406 Å). The XRD pattern (Figure 2) reflected the intense peaks at (26.43°) and (31.12°) planes, providing evidence of the existence of orthorhombic aragonite phase. The XRD parameters of biological aragonite due to the intracrystalline biopolymers, has been confirmed as a widespread phenomenon in chocolate band snail shell (Ren et al., 2009; Li & Zeng, 2012; Parveen et al., 2020).

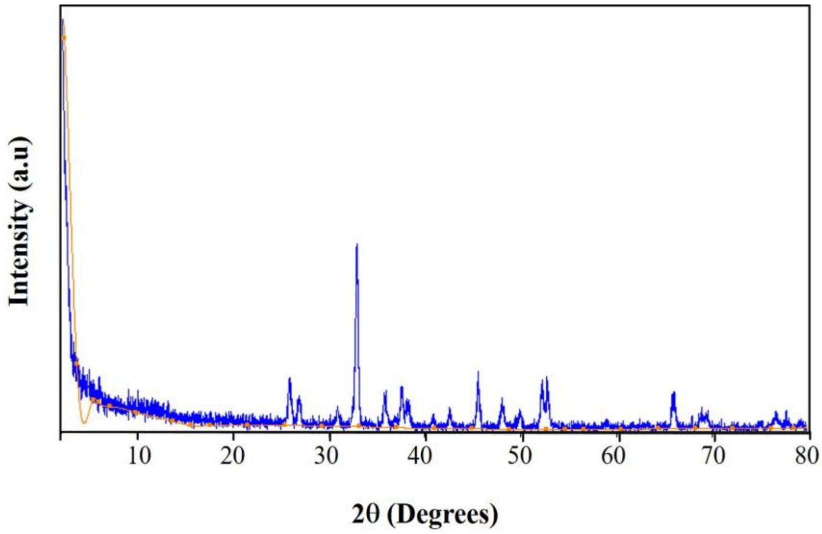

Figure 2. XRD patterns of *Eobania vermiculata* shells powder.

This crystal conformance of aragonite in nacre is frequently build in biological mineralization. This is due to the presence of fibrils and the polypeptide chains that control crystal improvement (Feng et al., 2000).

Scanning Electron Microscope (SEM)

The arrangement of the crystal layers in mollusk shells has two types. The sheet nacre model of Bivalvia and Cephalopoda mimick so called “brick-and-mortar wall”; on the other hand, Gastropoda mimick “columnar stacking” model (Watabe, 1988). Nacre crystallites pile up into columns vertically abreast aligned in Gastropoda. Accordingly, the vertical piling-up of crystals in these shells promotes the formation of columns or pyramids. These shell calcification sequences will represent useful criteria for studies of the phylogeny of Mollusca. In this study, the crystallized structure of *E. vermiculata* is in the form of vertical columns as stated in the literature. Nacre crystals are always aragonitic, laminated, usually presenting sharp edges (Figure 3).

According to the SEM results, the crystal columns are between about 390-155 nm and their edges are sharp looking. Nacre is one of the leading mechanical properties of shell components of mollusks (Currey, 1988). Addadi et al. (2006) reported that the crystals form a stacks along about 500 nm thick, and they form stacks along a fixed spacing of some 30 nm, filled with biological matter. Placing the crystals at regular intervals with the organic matrix creates a strong material with high tensile and bending strength.

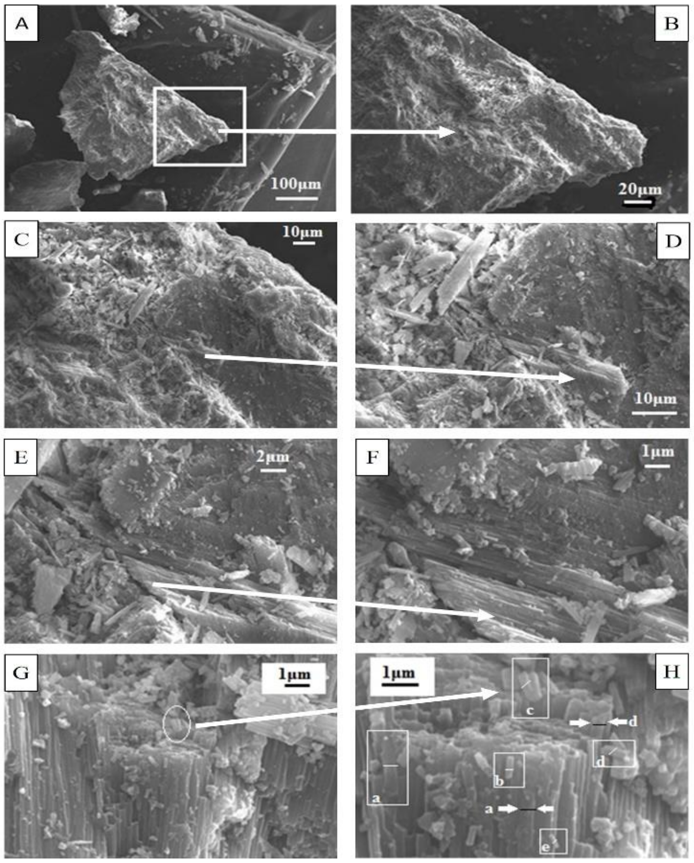

Figure 3. SEM images of crossed lamellar layers occurring in shell of *Eobania vermiculata.* A, B, C, D, E and F the point determined on the *E. vermiculata* shell fragment at 100, 20, 10, 2, 1 micron, respectively. G: Thin prisms from lamellar of *E. vermiculata* at 1 µm (Mag: 20.00 KX). H: Elongate, lath like, thin prisms from acrossed-lamellar layers of *E. vermiculata* at 1 µm (Mag: 40.00 KX): Cross-layered layers are 370 nm, 160 nm, 360 nm, 355 nm, 155 nm thick, respectively.

Conclusion

The results of SEM showed that land snail has species-specific arrangement patterns of calcium carbonate crystals in the diverse layers of shells. Characteristic of Gastropoda shell is the “columnar stacking” model. Accordingly, the vertical piling-up of crystals inside shells promotes the formation of columns. The XRD and FTIR observations revealed the dominance of the aragonite form of the calcium carbonate crystal in the microstructures of each snail shell with the occurrence of different shell surface functional groups.

Species-specific variations in the shell morphology, microstructure, and shell content were prominent for the land snails considered for the study. Nonetheless, the shells of *E. vermiculata* are qualified biological materials that could be used in many fields like bioremediation, biocatalyst, biomedical applications, and a source of lime. Since the shells of the land snails are discarded as waste, subsequent use as a biological material will support the “waste made useful” paradigm in sustainability, both from ecologic and economic perspectives.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSIP

https://prensip.gen.tr

RESEARCH ARTICLE

Mortality in Galla Goat Production System in Southern Rangelands of Kenya: Levels and Predictors

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ARTICLE INFO

Article History
Received: 10.06.2022
Accepted: 06.10.2022
First Published: 15.12.2022

Keywords
Galla goat
Infectious disease
Kaplan-Meier method
Kenya
Mortality rate
Non-infectious diseases

ABSTRACT

Herd health and adaptability are of concern in animal production in the tropics because of the persistent exposure to multiple stresses of low quality and quantity feeds, heat stress, high disease, and parasite incidences, poor husbandry, and breeding practices; the combined effects of these factors is high livestock mortality. High health-related mortality has been frequently reported as the major impediment to livestock production and thus the aim of this article is to investigate the vital infectious diseases and non-infectious factors that account for the majority of deaths which is crucial in determining mortality control strategies. The study applies a descriptive, Kaplan-Meier method, and truncated regression analysis using an eight-year retrospective data spanning from 2014 to 2021 was applied for this analysis. The results indicate infectious diseases as the most important cause of Galla goat mortality. The mean monthly and annual mortality rates are higher and the pre-weaning mortality of Galla goat appeared to be one of the major constraints hampering the development of replacement stock. The risk factors considered for high mortality were the age and sex of the kids. Among the infectious diseases analyzed, bacterial, parasitic, and non-specific infectious diseases were identified as the important causes of Galla goat mortality, while the non-infectious conditions included malnutrition and thermal/cold shock. The analysis provided an improved insight into animal-health-related factors which once addressed could reduce mortality and hence optimize animal husbandry performance in Galla goat production systems. Interventions in Galla goat health and husbandry are recommended to control kids' mortality.

Please cite this paper as follows:

Manyeki, J. K., Kidake, B., Mulei, B., & Kuria, S. (2022). Mortality in Galla goat production system in southern rangelands of Kenya: Levels and predictors. *Journal of Agricultural Production*, *3*(2), 48-57. https://doi.org/10.56430/japro.1128747

Introduction

The development of the livestock sector in Kenya has a considerable prospective opportunity for smallholder farming and income generation and may contribute significantly to poverty alleviation and food and nutrition security. This is because Kenya holds a huge potential for livestock development as about two-third of the total landmass is arid and semi-arid lands (ASALs) suitable for livestock rearing, while only one-third is agriculturally productive and includes the Kenyan highlands, coastal plains, and the lake regions. Disappointingly, only 50% of the ASALs carrying capacity of the land is currently being exploited (Odhiambo, 2013). Similarly, livestock production and productivity are also low and this can partly be attributed to the poor genetic potential of indigenous breeds, frequent seasonal drought, feed shortage in quantity and quality, high prevalence of animal diseases, poor infrastructure, and access to animal health services. Among the highlighted contributing parameters to low productivity, animal disease mortality data are largely lacking and are said to influence substantially the high livestock losses experienced (Mulei et al., 1995; Nkedianye et al., 2011).

Livestock mortality is considered one of the major constraints to herd expansion and genetic improvement. Specifically, as reported by many scholars, in any livestock

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production enterprise, the survival of female young ones is required for herd expansion and breed improvement, while that of male young ones is used as a source of income from sales or as draught animals (*e.g.*, Carles & Schwartz, 1982; Gitau et al., 1994; Thumbi et al., 2013). Since mortality is inevitable for all livestock production systems, understanding its extent and causes at the herd or sector level is a challenging phenomenon (Mayer et al., 2012). Interventions aimed at reducing livestock mortality require specific data on the important causes of mortality (Mayer et al., 2012). For these reasons, an accurate quantitative livestock-health-related mortality within and across different livestock production systems (Aganga et al., 2005; Ershaduzzaman et al., 2007; Fentie, 2016) provides a foundation for understanding, extension, and further research.

Since high livestock-health-related mortality has been the major impediment to various livestock production and thus an indicator of low production and productivity, understanding the vital infectious and non-infectious diseases that account for the majority of deaths is crucial in determining mortality mitigation strategies. Under the maintained hypothesis that production behaviour is driven by a firm's objective of maximizing the profits it enjoys, one cannot ignore the effect of mortality on livestock production. Retrospective studies addressing the causes of death in Galla goats in Kenya are scarcely making it hard in the allocation of limited funds available for such control strategies, and this formed the basis of this analysis. Neither assumption made in the previous studies under similar terrain (Aganga et al., 2005; Ershaduzzaman et al., 2007; Fentie, 2016) can be expected to hold in the more tropical regions of Kenyan ASALs, where livestock are routinely subjected to long periods of nutritional stress and high disease incidences. We, therefore, hypothesized an econometric relationship would be required for these areas. Specifically, this article aimed at addressing this gap of information by estimating the Galla goat mortality rates as well as identifying the infectious and non-infectious factors associated with mortality which would be a target for programs aimed at reducing mortality in rangelands of Kenya employing an econometric technique.

Materials and Methods

Study Area and Animals

The study was carried out at the Kenya Agricultural and Livestock Research Organization (KALRO), Kiboko research station (KALRO-Kiboko, henceforth) of the Arid and Range lands research institute. KALRO-Kiboko was established in 1969 as a research station charged with the mandate of undertaking a national range of research which entailed undertaking work on applied research on specific constraints affecting rangeland productivity and eventually developing appropriate technologies, recommendations, techniques, and knowledge systems that would solve/mitigate production set-backs. The station is an establishment under KALRO and its suitability for livestock production is typical because it is located in ecological zone IV-V which, generally, is not suitable for arable agriculture. The station is located at Kiboko in Makindu Sub-County of Makueni County, about 160 km SE of Nairobi, along Mombasa-Nairobi Highway, and lies between latitude 2° 10' and 2° South and longitude 37° 40' and 37° 55' East.

KALRO-Kiboko research station has the potential to deal with the production of cattle, goats, sheep, and camels, but currently, livestock kept consists of cattle (Boran, Small East African Short Horne Zebu, and their crosses) and Galla goat targeted mainly for red-meat production and quality breeding stock. In the context of this article, the study animals consisted of Galla goats which were collected across Kenyan rangelands with an annual mean population of 534 (with a standard deviation of 71.2) during the study period (2014-2021). Thus, the data were deemed be a representative of the many Galla goat production zones not only in Kenya but also in the East Africa countries. The high standard division implies high volatility of livestock production across years and seasons. Females are more than males with a percent proportion of 66.2% and 33.8%, respectively, for herd build-up for breed selection and income generation, respectively.

Data Type

The analysis aims to assess the various causes of death of Galla goat as recorded by the department of livestock in the KALRO-Kiboko research station from 2014 to 2021 (8 years). Animal health officers frequently visit goat *bomas* for disease diagnosis, treatment, and health-related data capture. Mortality is in cases of death and their causes, and all the cases are recorded and uploaded in the MS Excel data sheet. Nonetheless, the results can, however, be difficult to interpret in the absence of information on the typical pattern of losses in the enterprise, therefore, the first step was to retrospectively compile monthly records of animal stock, type of diseases treated, death and any causes for animal disappearances during the period of study. The second step was to categorize the different infectious and non-infectious conditions that cause mortality and analyze to predict the probability of the occurrences.

Infectious factors were categorized as protozoan (anaplasmosis, babesiosis, ECF, trypanosomosis), bacterial (pneumonia, enteritis, septicemia/toxemia, enterotoxaemia, scours, anemia, CCPP, metritis, pyometra, sinusitis, myocarditis, etc.), parasitic (represented by haemonchosis and haemonchus), Rickettsial diseases (heartwater/cowdriosis) and other conditions (such as bloat, calculi, uremia, hardware disease, hepatitis, senescence, percadisise, trauma, and viral conditions like rabies), and non-specified disease causes. Non-infectious causes included shock (hypothermic and cardiogenic), traumatic injuries, predation, plant poisoning, malnutrition, dystocia, premature birth (abortions and stillbirth), and postnatal/congenital defects. The sample comprises 96 observations, with minimal outliers which were omitted during analysis.

Data Analysis

Descriptive statistics, Kaplan-Meier method, and an econometric model approach were employed to analyze data using MS Excel and STATA statistical software (StataCorp, 2016). Under descriptive statistics, causes of animal mortality were compiled and the percentage contribution (proportion) of each cause for mortality was calculated and presented. Additionally, the study also involved computing the probabilities of Galla goat mortality at a certain point of time using the widely used Kaplan-Meier nonparametric method (Kaplan & Meier, 1958; Adelöf et al., 2021). The survival probability of Galla goat at any particular period, S_p was calculated by the formula given below;

$$S_p = \frac{N_S - N_D}{N_S} \quad (1)$$

where, N_S: Number of Galla goats lining at the start and N_D: Number of Galla goats that died.

Since the flock size is dynamic and usually births are experienced at any time, this means that they become a part of the study later. To some cases, there is often a shorter observation period and those goats may or may not experience death in that short stipulated time. However, we cannot exclude those goats since otherwise sample size of the study may become small. The Kaplan-Meier method allow us to compute the survival over time in spite of such difficulties associated with subjects or situations (Goel et al., 2010; Grzesiak et al., 2022). Therefore, for each time interval, survival probability was calculated as the number of Galla goats surviving divided by the number of Galla goats at risk. The total probability of survival till that time period was calculated by multiplying all the probabilities of survival at all-time intervals preceding that time (by applying law of multiplication of probability to calculate cumulative probability) [Kaplan & Meier, 1958; Grzesiak et al., 2022]. Then, the probability of Galla goat dying (cumulative mortality) is 1 (one) minus the probability of Galla goat survival (cumulative survival). Galla goats who have died or sold out are not counted as "at risk", hence are considered "censored" and are not counted in the denominator.

The other phase of analysis involved the determination of the effect the various causes have on mortality. Since econometric models offer estimates of actual values for forecasted variables and indicate both the direction and magnitude of change, then it was found appropriate to determine the effect of infectious and non-infectious factors on Galla goat mortality for uncensored data. In this paper, the outcome measure of interest was mortality, defined as any death of a study animal occurring during the period of observation and attributed to an infectious and non-infectious disease cause. The independent variables included infectious factors being the protozoan, bacterial, parasitic, Rickettsial diseases and other conditions, and non-infectious factors categorized as a shock, traumatic injury, predation, plant poisoning, malnutrition, dystocia, premature birth, congenital/postnatal defects, and non-specified causes. Truncated regression of the normal distribution was performed to identify risk factors for Galla goat mortality. The truncated model was selected because sample truncation is a pervasive issue in quantitative animal sciences, particularly when using observational data such as the data employed in this study (Abot, 2020; Ouédraogo et al., 2022). Truncation reduces the variance compared with the variance in the untruncated (Heckman, 1979; Dongfang et al., 2017). A truncated regression is one in which the values of the explanatory variables are observed only if the value of the dependent variable is observed and, thus following Greene (2010) and Reinhammar (2019), a truncated regression (at zero) was specified as;

$$y_i = X_i'\beta + \varepsilon_i, i = 1, \ldots, n \quad (2)$$

$$\varepsilon_i \sim iidN\ (0, \sigma^2) \quad (3)$$

where, y_i and x_i are observed number of livestock death and risk factors causing mortality respectively, the truncated form below for $y_i > 0$. The factors related to infectious and non-infectious diseases were considered independent variables (x_i) and livestock death as dependent/outcome variable (y_i). Term ε_i is the random error associated with random shocks, not under the control of economic agent *i*, and in this case capture weather changes or any economic adversity. The X-vector parameter estimate for mortality level ($\hat{y}_i$), is expected to have a positive sign, which implies the corresponding variable would increase the level of Galla goat mortality rate. The results of the analyses were final truncated models including all variables (risk factors) significantly associated with Galla goat mortality and the estimation was carried out using the maximum likelihood estimation (MLE) procedure. The use of the MLE technique makes sense because the error terms nested on these equations are assumed to follow a certain distribution, and our goal is to obtain the "most likely" estimate rather than one which minimizes the sum of squares as is the case with ordinary least square.

Results and Discussion

Descriptive Statistic

Descriptive and analytical statistics were used and the causes of Galla goat mortality were compiled, and the percentage contribution (proportion) of each cause for mortality was calculated and presented in Table 1. Mean annual mortality was determined by dividing the number of deaths by the number of alive Galla goats within a particular study period and was expressed as;

$$OMR\ (\%) = \frac{N_{DP}}{N_{SP}}\ x\ 100 \quad (4)$$

where, *OMR*: Overall mortality rates, N_{DP}: Number of deaths per period, and N_{SP}: Number of stock within each period.

As indicated in the Table 1, the Galla goat is characterized by high mortality. The estimated mean monthly and annual mortality are high ranging between 8.86-52.4% (with an annual average of 25.99%). This high mortality rate is comparable to findings by ILCA (1990) that ranges between 25-35% for African sheep and goats, and Otte and Chilonda (2002) mortality risks in all age groups of lamb and kids of around 27-28 percent. Although comparable under the African setup, these mortality rates are more than five times (*i.e.*, 25.99/5) what is observed in most well-managed dairy systems in the developed countries where the all-cause mortality rates are frequently reported to be below 5% (Svensson et al., 2006; Gulliksen et al., 2009). Even though Galla goats are considered well adapted to survive in environments of high disease pressure (Ngila et al., 2016; PACIDA, 2020), mortality rates as observed in this study suggest significant losses.

Table 1. Estimated mean monthly and annual mortality for Galla goat during the 2014-2021 study period.

Years	Mean Monthly Mortality	Annual Mortality Rate
2014	16.9	42.8
2015	9.31	25.9
2016	5.23	20.3
2017	2.62	8.86
2018	5.77	15.1
2019	13.8	34.9
2020	8.4	24.5
2021	18.17	52.4
Overall average	8.88	25.99

The statistical analysis of mortality rates recorded in the Galla goat production system for the period of 2014-2021 was skewed and showed a clear influence of age and sex as shown in Table 2. A higher mortality rate was reported in the young ones (kids below four months) and decreased with increasing age. This result concurred with a recent finding by Alemnew et al. (2020) who observed that as the age of kids increased, the number of kids who died decreased. Early mortality for kids during the first four-month of life accounted for 44.6% of the total mortalities and was particularly high in female kids than in the males. The results contradict the finding of Perez-Razo et al. (1998), Aganga et al. (2005), and Hailu et al. (2006) who recorded higher mortality for male kids compared to females. Comparably, the same pre-weaning kid mortality of about 46.8% was observed by Petros et al. (2014) among the goat kids in Ethiopia, and age in months had a significant effect. The result also concurred with Girma et al. (2011), where higher mortality rates were recorded in females than in males in Arsi-Bale kids kept in a similar environment.

Table 2. Estimated Galla goat mortality distribution by age category.

Age category	Percent Proportion of the Deaths		
	Percentage Proportion of the Total	Male (%)	Female (%)
Kids (<4 months)	44.6	48.5	51.5
Weaners (5-9 months)	27.1	46.2	53.8
Yearlings (10-12 months)	7.61	41.2	58.8
Mature (1-2 years)	4.32	41.4	58.6
Mature (3-4 years)	3.87	3.85	96.2
Mature (5-6 years)	5.51	0	100
Mature (7-8 years)	6.11	2.44	97.6
Mature (>8 years)	0.894	0	100
Total proportion (in %)	100	22.9	77.1

The percentage proportion for the birth-to-weaning contributed to the highest mortality (about 44.6%) in the KALRO-Kiboko Galla goat production system. Similar high mortality of about 67% for calves within a week of their birth (Bunter et al., 2013), with the cause of death most frequently recorded as unspecific. However, there was no significant difference in the number of deaths after one year of goat life. The high mortality of 44.6% of the goat during the first four-month reported in the present study means the ranch cannot raise enough stock to replace the loss, let alone expand the herds and, therefore, more attention to kids' management in the first months of life is a critical prerequisite. This is because a reduction in kids' mortality would translate into an increase in flock size and consequently the increase in male and culled offtake.

Overall, the descriptive statistics show high female mortality which is also inversely related to age. Since the herd composition differed somewhat between the sexes (with the female being two-third of the total population), the high female mortality rates could thus reflect differences in herd composition. The low mortality observed in males might also be because the males are frequently culled upon attaining one year of age to keep the limited number of males for the breeding purpose for a limited period. A similar result was observed in the Mlimbe et al. (2020) study.

Kaplan-Meier Estimates

The Kaplan-Meier estimates for mortality are displayed in Table 3. Looking at the table, the year was divided into twelve intervals, corresponding to the times of death of the 119 Galla goats. The average number of Galla goats was about 410, while a total of 194 goats exited the flock through death or sale. This translate to about 47.3% of the total number of observations that were censored. Based on the Kaplan-Meier functions, the cumulative survival decline could be observed and this translate to an increase in the Cumulative mortality for the entire period of about 29%. The proportion surviving Galla goat on this day seems to be relatively high and ranges from 0.922 to 0.991. comparable survival probability for cows culled for different reasons was observed in Grzesiak et al. (2022) study.

Table 3. Kaplan-Meier estimate for Galla goat.

Time	Total Flock	Births	Sales	Deaths	Proportion surviving on this day	Cumulative survival	Cumulative mortality (CM)
January	458	8	1	8	0.983	0.983	0.017
February	457	16	0	7	0.985	0.967	0.033
March	466	11	9	9	0.981	0.949	0.051
April	459	3	10	6	0.987	0.936	0.064
May	446	17	4	5	0.989	0.926	0.074
June	454	7	3	4	0.991	0.918	0.082
July	454	5	15	6	0.987	0.906	0.094
August	369	20	13	12	0.967	0.876	0.124
September	363	3	2	17	0.953	0.835	0.165
October	347	8	1	27	0.922	0.770	0.230
November	327	14	0	17	0.948	0.730	0.270
December	324	43	9	9	0.972	0.710	0.290

CM = 1-cumulative survival.

The causes of mortality in Galla goat and their relative contribution to the mortalities based on the data collected during the 2014-2021 study period are presented in Table 4. A more in-depth analysis of individual causes of mortality in the Galla goat production system showed that infectious diseases contributing 65.69% of the total flock loss is the largest single factor to the immense flock mortality, while non-infectious conditions contributed only 34.3%. Among the infectious causes of Galla goat mortality identified, bacterial (mainly represented by Contagious Caprine Pleuro-Pneumonia/CCPP, enteritis, enterotoxaemia) was the major problem, followed by the parasite (mainly represented by haemonchosis). The findings of this study are in agreement with Tibbo (2000)'s findings for goats in Awassa Zuria woreda in south western parts of Ethiopia where diseases and infectious parasites were found to be the main causes of high mortality and morbidity. Similarly, Tembely (1998) observed that parasitic and bacteria diseases (such as CCPP and respiratory disease syndrome) were major causes of morbidity and mortality of small ruminates in Ethiopia. In the current study, considerable mortality was also reported related to general disease syndromes (non-specific) such as tumors, anorexia, and sudden deaths. Sudden deaths were deaths with unrecognized syndromes. Rickettsial infections specifically heart water/cowdrosis and dystocia cases were few during the study period. The limited capacity of the existing institutional veterinary officers to serve the vast livestock population in the KALRO-Kiboko station throughout the study period may have worsened the situation leading to the higher mortalities recorded.

The contribution of non-infectious conditions to Galla goat mortality was higher for malnutrition (15.9%) and shock (6.13%). Similar high mortality losses associated with malnutrition were reported by Ocaido et al. (2009) and Mlimbe et al. (2020) in Uganda and Tanzania, respectively. For Galla goat production, predators such as hyenas, cheetahs, and jackals were implicated as important causes of loss which concurred with the finding by Fentie (2016) among the small ruminants in Ethiopia.

Overall, the results show that females are more susceptible to infections and non-infectious factors than males which were the general observation in Kocho (2007)'s study. However, males are highly prone to death from protozoa and parasitic infectious diseases than females.

Table 4. Main causes of Galla goat mortality.

Causes	% Proportion	Male	Female
Infectious factors			
Protozoa	1.31	0.729	0.584
Bacteria	37.8	14.6	23.2
Parasite	9.49	5.11	4.38
Rickettsial diseases	3.65	0.729	2.92
Non specific	8.17	2.04	6.13
Other diseases	5.26	1.75	3.51
Non-infectious factors			
Dehydration	0.876	0.146	0.729
Injury	0.876	0.438	0.438
Predation	4.23	1.31	2.92
Plant poisoning	5.41	2.48	2.92
Malnutrition	15.9	7.59	8.32
Shock	6.13	2.92	3.21
Dystocia	0.146	-	0.146
Congenital defects	0.729	0.292	0.438

Econometric Estimation of the Causes of Livestock Mortality

For a better understanding of the underlying causes of mortality, a truncated model was fitted to investigate the direction and magnitude of the various infectious and non-infectious factors that cause Galla goat mortality. The truncated regression analyses examined all possible interactions among variables and death as the outcome variable. However, before estimating a truncated regression model, one has to address the problem associated with multicollinearity. The test of multicollinearity was done through the computation of a collinearity diagnostic measure referred to as variance inflation factors (VIF). As presented in Table 5, all the independent variables exhibited $VIF_i < 5_i$ (with an average VIF of 1.27), it was concluded that there was no multicollinearity and therefore all the variables were eligible for inclusion in the model estimation. The same conclusion of no multicollinearity can be arrived by checking the coefficient for the collinearity diagnosis. All independent variables are very not highly correlated to one another (r>0.5) with the Eigen values & condition index computed being less than 10, an indication of stability (Table 6). The estimates of sigma square (σ^2) are significantly different from zero at a 1% level of significance, implying a good fit of the specified distribution assumptions of the error term and the Wald Chi-square value showed that statistical tests are highly significant (p<0.000), suggesting that the model had strong explanatory power.

Regarding the various covariant variables included in the model and displayed in Table 5, all variables assumed the expected positive sign. The disease was identified as the major cause of mortality in the Galla goat production system of the study area. In the present study, the magnitude of goat mortality attributed to diseases is 0.768 which is statistically significant (at a 1% level), and the contribution to the overall mortality is about 65.69%. Based on the magnitude of the coefficient on the disease variable, while holding other variables constant, a unit improvement in animal health care would reduce Galla goat mortality by 18.94% (*i.e.*, 24.7×0.768). The high proportion of kid mortality (of 44.6%) due to diseases was also reported in Botswana (Aganga et al., 2005) and a similarly higher percentage contribution of diseases to kid mortality (of about 63%) was reported in Black Bengal Kids in Bangladesh (Ershaduzzaman et al., 2007). The high-risk factor associated with diseases indicates that the most important area of intervention in reducing Galla goat mortality should be health management.

Among the non-infectious conditions associated with Galla goat mortality, malnutrition though highly significant accompanied by a relatively high percent contribution, the magnitude was lower than that of congenital defects and dehydration. This finding concurred with that of Fentie (2016), who found malnutrition (which was presented as feed shortage) as one of the major problems causing mortality in young stock across all species. Malnutrition can be a result of insufficient milk/colostrum in the first 24 hours of birth which is critical for passive transfer of immunity to the kids, this might be due to poor mothering ability by the dams and the seasonal shortage of feed during dry periods. Galla goats mainly depend on browsing, and the provision of supplementary feed is very limited. Accordingly, the high disease-related mortality rate observed can be aggravated by the effect of malnutrition in terms of feed and milk shortages that could compromise the immunity of young stock and expose them to diseases. The effects of mean dystocia were however lost when infection data was included, indicating that this factor may be related to the infectious diseases. Exposure to predators and plant poisoning had only a marginal effect on Galla goat mortality and traumatic injuries showed no statistically significant effect on mortality although the percentage contribution was higher than that of congenital defects. Similar to Fentie (2016)'s findings, in the case of kids, the contribution of predators (hyena, cheetah, etc.) and injury (physical damage) in herds where young and adult animals share the same barns were important causes of mortality.

Table 5. Causes of Galla goat mortality and percentage contribution (proportion).

Variables	Coefficient	Standard Deviation	Proportion	Variance Inflation Factor (VIF)
Diseases	0.768***	0.0259	65.74	1.20
Congenital defects	0.285***	0.0449	0.729	1.06
Dehydration	0.185**	0.0768	0.876	1.04
Dystocia	0.000	(omitted)	0.146	-
Injury	0.113	0.0839	0.876	1.04
Predation	0.0536*	0.0342	4.234	1.84
Plant poisoning	0.0504***	0.0185	5.41	1.15
Malnutrition	0.0787***	0.00702	15.9	1.39
Shock	0.0501***	0.00528	6.13	1.46
Constant	0.527***	0.0549	-	-
sigma (σ^2)	0.173***	0.0173	-	-
Wald chi2	1476.06***	-	-	-
Log-likelihood	16.7	-	-	-
Mean VIF	-	-	-	1.27

***, **, * implies significance at 1%, 5%, 10%, respectively.

Table 6. Collinearity diagnostics.

	Eigen Value	Condition Index
1	2.9090	1.0000
2	1.3512	1.4672
3	1.0450	1.6684
4	1.0036	1.7025
5	0.9684	1.7332
6	0.8842	1.8138
7	0.6564	2.1052
8	0.5353	2.3310
9	0.3829	2.7563
10	0.2640	3.3192
Condition Number		3.3192

Eigen values and condition index computed from scaled raw sscp (w/ intercept) Det (correlation matrix) = 0.4408.

Having confirmed that disease-related syndromes were the main cause of mortality, an attempt was made to identify which particular disease conditions significantly contribute to Galla goat deaths. The results of this analysis is displayed in Table 7. Again all the non-infectious conditions were subjected to a multicollinearity test and all variables exhibited $VIF_i < 5_i$ (with an average VIF of 1.24), implying there was no evidence of multicollinearity. The collinearity diagnosis test (Table 8) indicated that all independent variables are very not highly correlated to one another (r>0.5) with the Eigen values and condition index computed being less than 10, an indication of stability. The estimates of sigma square (σ^2) was significantly different from zero at a 1% level of significance, indicating a good fit of the specified distribution assumptions of the error term and the Wald Chi-square value showed that statistical tests are significant (p<0.000) suggesting that the model had strong explanatory power.

With regards to infectious diseases included in the model, the analysis shows robust results as all were statistically significant at 1 and 10% levels. Among the infectious diseases investigated, bacteria majorly represented by pneumonia, enteritis, septicemia, enterotoxaemia, and CCPP were noted as the first major disease problem of goats, followed by parasites mainly haemonchosis. The percentage proportion mortality as a result of bacteria was 57.56%, and 14.44% was related to parasite problems. Parasites causing mortality in this study are in agreement with Tibbo (2000)’s finding for goats in Awassa Zuria woreda in south western parts of Ethiopia and Aganga et al. (2005) in Botswana. Equally, an in-depth study by Kocho (2007) also observed parasites across the different sites of Southern Ethiopia were not significantly different (p>0.05) which depict that it is a major cross-cutting impediment to the goat production in the region. Among the diagnosed diseases, rickettsia infections majorly represented by heartwater (or cowdrosis) disease had the highest magnitude, hence, its effects should be reduced to the bare minimum. Heartwater has been reported throughout sub-Saharan Africa (Uilenberg, 1983) and in Mozambique, Asselbergs et al. (1993) observed the disease to occur throughout the country and mainly during the rainy season.

Table 7. Disease syndromes related to Galla goat mortality.

Variables	Coefficient	Standard Deviation	Proportion	Variance Inflation Factor (VIF)
Protozoa	0.109*	0.0795	2.00	1.19
Bacteria	0.106***	0.00883	57.6	1.35
Parasite	0.0633***	0.0185	14.4	1.52
Non specific	0.121***	0.0295	12.4	1.23
Other diseases	0.152***	0.0261	8.01	1.12
Rickettsia diseases	0.196***	0.0409	5.56	1.07
Constant	0.882***	0.0739	-	-
Sigma (σ^2)	0.268***	0.0289	-	-
Wald chi2	375.38***	-	-	-
Log-likelihood	-4.4217	-	-	-
Mean VIF	-	-	-	1.24

***, **, * implies significance at 1%, 5%, 10%, respectively.

Table 8. Collinearity diagnostics.

	Eigen Value	Condition Index
1	3.3195	1.0000
2	1.0363	1.7898
3	0.7178	2.1505
4	0.6518	2.2568
5	0.5437	2.4708
6	0.4208	2.8085
7	0.3101	3.2720
Condition Number		3.2720

Eigen values and condition index computed from scaled raw sscp (w/ intercept) Det (correlation matrix) = 0.4114.

Conclusion

This study has investigated mortality in Galla goat and identified the main causes of death and the risk factors associated with infectious and non-infectious disease mortality. The all-cause mortality rate was estimated at 25.99% per 100 animal risk years with an annual cumulative mortality of 29%. The mortality contribution to infectious diseases was estimated at 65.69% risk years. This study has also indicated that mortality in kids born alive is likely to be above 44 percent and may represent the high loss in Galla goats due to husbandry practices of the KALRO-Kiboko research station. The risk factors considered for high mortality were the age and sex of the kids. The pre-weaning mortality of Galla goat appeared to be one of the major constraints in the KALRO-Kiboko farm, hampering the development of replacement stock. The critical time for higher goat mortality in this production system was during the first four months of life-extending up to the ninth month of age. Therefore, for an efficient Galla goat production system, the survival of female kids is required for herd expansion and breed improvement, while that of male kids is used as a source of income from sales; so the mortality of kids, in general, should be reduced to the bare minimum.

Concerning infectious and non-infectious conditions influencing mortality, disease and malnutrition appeared to be the most important causes of Galla goat mortality. Among infectious diseases, bacterial diseases, parasitic conditions, and non-specific diseases were the most common challenges of raising Galla goat on the KALRO-Kiboko farm. Overall, infectious diseases contribute to high mortalities in Galla goats, and they reduce animal performance. However, many of the health problems of Galla goat can be controlled by excellent early nutrition and management. For instance, as observed in this study, kids were at higher risk of dying if they were not separated from adult animals because the separation of sick animals from the flock contributes to kid survival as it minimizes the risk of transmission of contagious diseases. Modest interventions on the significant flock impediments, for example, minimizing flock loss through disease control and protection against predators and proper feeding to curb the effect of malnutrition could potentially boost the flock performances. The veterinary service needs to provide strategic disease prevention, control, and treatment measures. Future studies should investigate the effects of farm management practices on Galla goat mortality which together with the insight provided by this study will help in constructing a conceptual comprehensive stock-and-flow model of a representative Galla goat production system for Kenya. The idea is to develop a holistic systems model for Galla goat production in an extensive Kenyan rangeland environment as it is in the other part of the world.

Acknowledgment

The authors are grateful to the researchers who collated the bare results and provided further data, specifically Patricia Chege, Robert Langat, and David Musangi. We also extend our gratitude to the animal health and production staff of the respective livestock *boma* for their support in data collection.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Journal of Agricultural Production (2022), *3*(2), 58-68 https://doi.org/10.56430/japro.1148305

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Heavy Metal Evaluation of Overused Commercial Fertilizers and Their Interactions with Soil Properties

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ARTICLE INFO

Article History
Received: 25.07.2022
Accepted: 23.10.2022
First Published: 19.12.2022

Keywords
Accumulation
Fertilizers
Harran plain
Heavy metal
Soil

ABSTRACT

Şanlıurfa province has 36% of the agricultural areas in Turkey and 64.1% of the agricultural areas in the GAP Region. With approximately 600 000 tons of chemical fertilizer consumption in 2021, it is the province with the most fertilizer consumption in Turkey. This causes some negative and high environmental risks such as salinization in the soil, heavy metal (HM) accumulation, deterioration of nutrient balance, damage to microorganism activity, and formation of eutrophication in the region. The objective of this study was to determine the HMs (Zn, Ni, Mn, Cu, Mo, Pb, Cd) concentrations of the soils and some commercial fertilizers overused, and evaluate their interactions with soil properties. The average values of HM concentration of the soil are as follows; 32.65 and 46.88 mg kg^{-1} Zn; 649.03 and 730.58 mg kg^{-1} Mn; 79.86 and 95.54 mg kg^{-1} Ni; 0.15 to 0.27 mg kg^{-1} Cd; 0.26 and 0.97 mg kg^{-1} Mo; 8.54 and 18.67 mg kg^{-1} Pb; 19.45-25.37 mg kg^{-1} Cu. HM contents of some fertilizers were found to be very high in this study. This causes an increase in the HM concentration in the soil. HMs concentrations of several soil samples exceeded the threshold level of Europe standards except for Ni, Mo, and Mn. Study results can help the authorities to develop effective fertilizer management strategies for the Harran Plain, which has once again revealed the necessity of applying agricultural activities such as fertilization with a fertilization program prepared under expert control according to the results of soil analysis.

Please cite this paper as follows:

Büyükkılıç Yanardağ, A. (2022). Heavy metal evaluation of overused commercial fertilizers and their interactions with soil properties. *Journal of Agricultural Production*, *3*(2), 58-68. https://doi.org/10.56430/japro.1148305

Introduction

The capital of the Southeastern Anatolia Project (GAP), one of the largest projects in the world, is Şanlıurfa. Şanlıurfa and Harran Plains is a large region covering an area of approximately 1,500 km². Within the scope of the GAP project, irrigated agriculture started in 1995 with the transmission of water to the plain through the Atatürk Dam Şanlıurfa Tunnels. In this way, plants such as cotton and corn, which need more water, have begun to be grown. Plant nutrient needs of these plants with high yield potential, which are grown in direct proportion to the increasing water need, have also increased and fertilizer usage habits have started to meet this need. Agricultural production and yield increased significantly in the plain. Since the fertilizer preference of the producers, for whom the fertilizer usage habits of the region are not directly dependent on the fertilizer type, were determined only based on price, the producer using a single type of fertilizer in the region could not be determined. Di-ammonium phosphate (DAP-18N:46P_2O_5), composite (20N:20P_2O_5:20K_2O, 15N:15P_2O_5:15K_2O, and 15N:15P_2O_5:15K_2O+Zn), ammonium nitrate (26%N, 33%N), ammonium sulfate (21%N), calcium ammonium nitrate (CAN-26%N) and urea (46%N) fertilizers are used extensively in the Harran Plain.

Irrigation of these plains, which constitute the largest arable and arable land of the GAP Region, is one of the most important components of the GAP in terms of regional development and

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high agricultural production potential (Güven & Taşlıgil, 2013). Irrigation in the region is in the form of surface irrigation. Due to this irrigation habit, the soils of the region faced the problem of salinization with the effect of clay texture. The main clay mineral of the Harran Plain soil is smectite, and the remaining clay minerals are palygorskite, chlorite, illite, and kaolinite. The high smectite content of the soil indicates that the soils are difficult to cultivate and will become alkaline with increasing Na^+ ions over time. Unplanned and unconscious irrigation, fertilization, and salinization in the region pose a great risk to sustainable agriculture (Seyrek et al., 2005).

Harran Plain has intensive agricultural production, increasing population and increasing food consumption depending on this population. For this reason, excessive commercial fertilizers are used in agricultural activities in this region. According to the province-based fertilizer consumption results of the Ministry of Agriculture, while 319,962 tons of fertilizer was consumed in Şanlıurfa in 2010, it was reported as 406,604 tons in 2015, 591,326 tons in 2020 and 597,376 tons in 2021. As a result, fertilizer consumption is increasing day by day. The increase in the chemical fertilizers used in agriculture has many negative effects on the environment and soil. Some of those; salinization in soil, deterioration of nutrient balance, damage to microorganism activity, nitrate accumulation in water and formation of eutrophication cause soil erosion, chemical fertilizers mix with water and increase the amount of phosphate in rivers, streams and lakes (İ. Sönmez et al., 2008; Liu & Lal, 2015).

The population growth rate in developed countries is 5 times lower than in developing countries. To meet the needs of the growing population in developing countries, little attention is paid to events such as environmental pollution and security that may have an impact after a long time, and they become quite commonplace (Atılgan et al., 2007). One of them is the damage to the environment of increased uncontrolled fertilizer consumption. Soil pollution must be prevented to hinder the deterioration of public health owing to the food and product chain in the future. This situation may create a serious food safety problem years later. The increasing use of chemical fertilizers (especially the use of high amounts) has come to endanger public health. It is also known that the useful living communities in the soil are naturally adversely affected by the chemicals used, as the quality of life of the plants decreases, and even the life of the plants.

The application time and amount of fertilizer applied to the soil affect plant yield and quality. The chemical fertilizers used in increased doses causes the accumulation of harmful and toxic substances in plants (Kara, 2005; Arora et al., 2008; Derin, 2019). The effects of HM accumulation on living organisms and soil can be short-term or long-term, so it is necessary to follow the accumulation closely, and to do the fertilizer dose amount and timing well (Sabiha-Javied et al., 2009; Derin, 2019).

Recently, commercial fertilizers used for agricultural purposes combine with some compounds and elements and accumulate in the soil so much that it adversely affects plant production. Agricultural activities carried out to increase the quality to provide the continuity of plant production and the product are very closely related. Some activities and practices can change soil properties (Kadıoğlu & Canbolat, 2014).

Micronutrients such as iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) are essential metals for plant yield and, most importantly, growth. But plants can accumulate heavy metals (HMs) or non-essential metals such as lead (Pb), nickel (Ni) and chromium (Cr) in the soil and can cause serious environmental problems (Mitsios et al., 2005). The HM concentration in the soil solution is important and plays a critical role for check the availability of the ions to plants (Lorenz et al., 1994). The bioavailability and solubility of HM ions vary greatly as many factors influence their concentration in the soil solution. The metal solubility or availability is affected by some factors such as soil pH (Sanders et al., 1987), organic matter content and clay content (Mitsios et al., 2005).

Recently, commercial fertilizers used for agricultural purposes combine with some compounds and elements and accumulate in the soil so much that it adversely affects plant production. Agricultural activities carried out to increase the quality to provide the continuity of plant production and the product are very closely related. Some activities and practices such as fertilization of the soil can change the soil properties (Kadıoğlu & Canbolat, 2014). The objective of this study were to (1): determine HMs (Zn, Ni, Mn, Cu, Mo, Pb, Cd) concentrations of the soils and some commercial fertilizers overused in the plain and (2): evaluate their interactions with soil properties in the plain.

Materials and Methods

Site Descriptions

The location of the study area (Harran Plain) was given in Figure 1. Harran Plain is in the southeastern province of Şanlıurfa, which is at the center of Turkey's largest irrigation and development project (GAP). It is located between 38°39'-39°30' East longitudes and 36°43'-37°11’ North latitudes (mean elevation: 415 m) and covers an area of 225,000 ha.

Although 25 soil series were determined in the Harran plain, 6 of them are common in the study area. 21 of these series are clayey, two are clayey silt and the other is clayey loam texture. The predominant silicate clay mineral is smectite group clays, and palygorskite clay mineral is also found to be important. Although the lime content of its soils with an A-B-C horizon is high, the organic matter content is generally around 1% (DSİ, 2003; Büyükkılıç, 2009).

According to climate data, the annual average temperature in the Harran Plain is 16.1 ºC, the annual average precipitation is 365 mm and evaporation is 1 848 mm (MGM, 2021). It is known that its climate is arid and semi-arid, and the lack of water is excessive during most of the year (Akış et al., 2005; M. E. Sönmez, 2012).

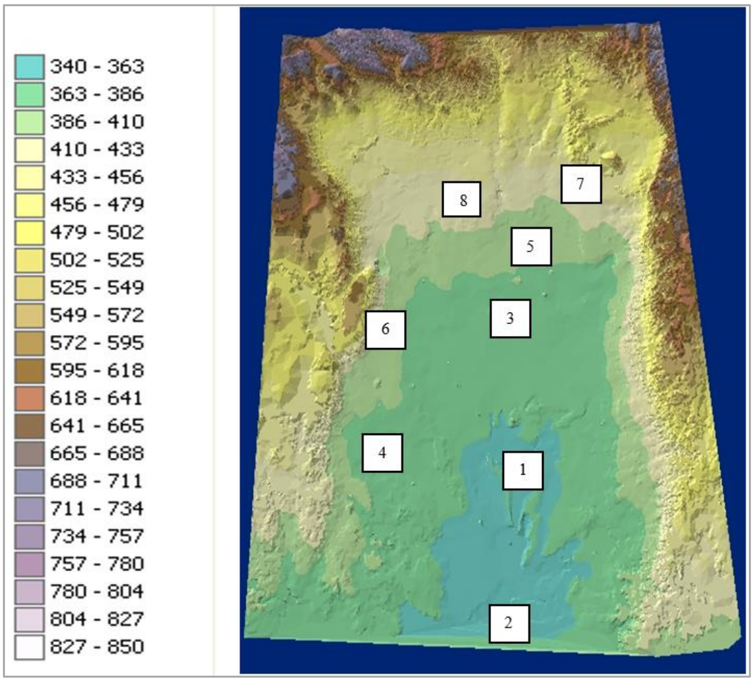

Figure 1. The location points of the studied soil.

Soil and Commercial Fertilizers Sampling

Total samples from soil profiles were taken related to soil series from the genetic horizon (Table 1). Soil profiles (8 soil samples) were selected from different locations of the Harran Plain, which were grouped into 4 zone (Z) related to the elevation in the south, middle and north zones in the studied area. Zones identification and soil series in the Harran Plain were given in Table 1. Soil samples were taken from the soil surface horizon (approximately 0-30 cm) and subsurface horizon (approximately 30-60 cm) with three replicates, which were air-dried, ground, sieved to pass a 2-mm mesh and then stored in plastic bags for laboratory analyses.

Table 1. Zones identification and soil series in the study area.

Zone Code	Profile Code	Series name	Coordinates
Z1	1.	Çekçek	36º56'49.9"N 38º54'05.8"E
	2.	Kısas 2	36º58'05.6"N 39º01'09.5"E
Z2	3.	Bellitaş	37º01'09.6"N 39º09'13.2"E
	4.	İkizce	37º01'50.1"N 39º07'29.9"E
Z3	5.	Sırrın	37º05'46.1"N 39º02'14.4"E
	6.	Beğdeş	36º50'49.1"N 38º54'16.9"E
Z4	7.	Gürgelen 2	36º47'13.6"N 39º05'25.8"E
	8.	Akçakale	36º45'37.0"N 39º58'53.8"E

Universal Transverse Mercator (UTM): 37.

Some commercial fertilizer samples were purchased from various markets in Şanlıurfa city of Turkey, which are overused in the plain. 16 overused commercial fertilizers including 5 samples of diammonium phosphate (DAP, 18% N: 46% P_2O_5); 4 samples of composite 20:20 (20% N: 20% P_2O_5), one sample of composite 5 (15% N: 15% P_2O_5: 15% K_2O), one sample of composite 6 (15% N: 15% P_2O_5 + Zn); one sample of ammonium nitrate 1 (26% N), one sample of ammonium nitrate 2 (33% N), one sample of calcium ammonium nitrate (CAN; 26% N), one sample of ammonium sulfate (21% N), and one sample of urea (46% N) were selected according to the most consumed varieties in the plain. The collected samples were ground for analysis, and kept in plastic bags before laboratory analyses.

Chemical and Statistical Analysis

The analysis was determined as follows: pH measured in a 1:1 water soil ratio solution according to Peech (1965)'s method; soluble salts according to Bower and Wilcox (1965); $CaCO_3$ according to Schribler calcimetric method; organic carbon according to Duchaufour (1970), cation exchange capacity following the method of Chapman (1965). Soil and fertilizers samples were digested by microwave and concentrations of HMs (Zn, Ni, Mn, Cu, Mo, Pb, Cd) were determined using an Agilent 7500a model of ICP-MS (Inductively Coupled Plasma Mass Spectrometer) and also atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer).

All data were analyzed by parametric multifactor analysis of variance (ANOVAs) and if need nonparametric test using the software package "SPSS Version 19.0" since the interaction between factors and processes was significant differences between soils/plants for selected subsets of data. The separation of means was made according to Tukey's verified significant difference at $p<0.05$. Relationships among properties were studied using Pearson correlations. Descriptive statistics (mean, median, standard deviation and range) of soil and properties were performed applying the Excel for Windows software package.

Results and Discussion

Soil Properties and Total Heavy Metal Contents of Soil

Soil characteristics of the studied area were given separately (surface and subsurface horizon) in Table 2. The clay contents were predominant, which varied between 28.5 and 57.8% in the surface, 27.1 and 65.7% in the subsurface horizons. The pH values ranged from 7.14 and 8.52 in the surface and 7.28 and 8.45 in the subsurface horizons. The soil salinity was high in some profiles, especially in Z1 due to excessive irrigation and high clay content. Soil organic matter contents were between 0.97 and 2.76% in the surface, and between 0.70 and 2.02% in the subsurface horizons. Lime contents were changed between 15.59 and 37.41% in the surface, and between 14.03 and 38.19% in the subsurface horizons. Cation exchangeable capacities were very high in some profiles (Table 2).

Table 2. Selected properties of the surface soils and subsurface horizons in the soil studied (n= 8 profiles).

Parameter	Unit	Surface Horizon			Subsurface Horizon		
		Max.	*Min.*	*Mean*	*Max.*	*Min.*	*Mean*
pH	-	8.52	7.14	7.98	8.45	7.28	7.98
EC	dS m^{-1}	15.92	0.19	2.88	15.62	0.44	1.94
$CaCO_3$	%	37.41	15.59	26.35	38.19	14.03	26.84
OM	%	2.76	0.97	1.45	2.02	0.70	1.36
CEC	$cmol^{(+)}$ kg^{-1}	49.22	28.78	38.89	48.76	28.44	37.28
Clay	%	57.80	28.50	46.96	65.70	27.10	51.16

EC: Electrical conductivity, OM: Organic matter, $CaCO_3$: Lime contents, CEC: Cation exchangable capacity.

Heavy metal concentrations were given in Table 3. The average values of HM concentration of the soil are as follows: 32.65 and 46.88 mg kg^{-1} Zn; 649.03 and 730.58 mg kg^{-1} Mn; 79.86 and 95.54 mg kg^{-1} Ni; 0.15 to 0.27 mg kg^{-1} Cd; 0.26 and 0.97 mg kg^{-1} Mo; 8.54 and 18.67 mg kg^{-1} Pb; 19.45-25.37 mg kg^{-1} Cu. The heavy metal concentrations in zones were Z4>Z3>Z1>Z2, respectively (Table 3). There were statistically significant relations in the surface horizon between Zn and Ni, Cd; Ni and Cd in Z1, between Zn and Ni in the Z2, between Ni and Cd in the Z3, between Mo and Cd in Z4. In the subsurface horizon, statistically, the correlation was shown in the Z1, between Mo and Cd in the Z2, Zn and Mo in the Z3, and Ni and Cu in Z4. As a result of the analysis of variance (Two-way ANOVA), it was seen that the interaction of soil depth, zone and depth x zone of all heavy metals except Mo was not significant. On the other hand, statistical differences were observed with Mo in the interaction of zone and zone x depth ($p<0.05$).

The international threshold level of some HMs in the soil were given in Table 4. Concentrations of several soil samples exceeded the permissible limits of Europe standards except for Ni, Mo, and Mn contents of the soil. Zinc is an active biochemical process and is also known to be involved in various biological and chemical interactions with various elements in the soil. The mean average of Zn for world soils is 64 ppm and Zn concentration changes between 10 and 300 mg kg^{-1} in agricultural soils (Kabata-Pendias & Pendias, 2001). In our study, Zn concentrations are lower than the maximum permissible limit; similar results are found by Kabata-Pendias and Pendias (2001) in agricultural soils. The mean average of Mn for world soils is 437 ppm, while for the U.S. soils is 495 ppm. Mn contents of the studied soil were higher than permissible limits. In this study, the Mn content of the soil was higher than the standard concentration of other authors (Kabata-Pendias & Pendias, 1992), one reason for this result can be the high HM contents of some applied fertilizers to plain.

Table 3. Heavy metals concentrations in the soil.

Depth	Zone	Zn	Mn	Ni	Cd	Mo	Pb	Cu
Surface	**1**	32.65 ± 4.91	730.58 ± 120.12	84.27 ± 12.85	0.15 ± 0.10	0.30 ± 0.35	11.98 ± 2.95	19.45 ± 2.64
	2	33.09 ± 10.04	651.82 ± 235.91	79.86 ± 15.90	0.17 ± 0.08	0.26 ± 0.15	14.34 ± 1.37	19.69 ± 2.74
	3	43.70 ± 11.68	649.03 ± 212.85	90.33 ± 5.80	0.20 ± 0.15	0.57 ± 0.11	11.73 ± 5.66	23.90 ± 1.75
	4	46.88 ± 17.51	670.99 ± 94.91	91.31 ± 2.75	0.27 ± 0.21	0.98 ± 0.73	8.54 ± 3.30	20.78 ± 1.10
Subsurface	**1**	41.74 ± 17.86	620.18 ± 132.13	87.27 ± 15.99	0.22 ± 0.20	0.53 ± 0.08	11.44 ± 2.36	20.32 ± 3.30
	2	40.62 ± 1.26	691.51 ± 159.40	89.91 ± 7.58	0.20 ± 0.07	0.33 ± 0.07	14.62 ± 0.32	21.46 ± 0.61
	3	41.86 ± 5.37	661.65 ± 125.82	95.54 ± 5.22	0.24 ± 0.10	0.40 ± 0.16	18.67 ± 3.54	25.37 ± 1.74
	4	39.00 ± 9.21	698.19 ± 166.19	88.66 ± 9.91	0.27 ± 0.12	0.49 ± 0.04	11.80 ± 4.43	20.79 ± 2.84
Two-way ANOVA Analysis								
Depth		0.42 ns	0.13 ns	0.95 ns	0.69 ns	0.74 ns	1.56 ns	0.91 ns
Zone		1.25 ns	0.04 ns	1.13 ns	1.28 ns	2.18 *	0.55 ns	1.34 ns
Depth x Zone		1.62 ns	0.72 ns	0.60 ns	0.44 ns	2.29 *	1.27 ns	0.28 ns

*p<0.05, **p<0.01, ns: Not significant.

Table 4. The international threshold level of some HMs in the soil (mg kg^{-1}).

Heavy Metals	EU	USA	Canada	UK	Turkey*
Zn	300	200-300	1400	200	47
Cu	140	80-200	170	63	20
Pb	300	300	150	70	10
Cd	3.0	3.0	79.5	1.4	0.2
Ni	75	50-110	210	50	90
Mo	0.5				0.6
Mn	437**				670

Source: CCME (2001), *Turkey (This study, 2022), **Kabata-Pendias (2001).

Likely, the soils of arid and semi-arid regions often have high Ni content. The grand mean for world soils is calculated to be 22 ppm, and 19 ppm was reported for U.S. soils. Likely that the soils of arid and semi-arid regions often have high Ni content. (Schacklette & Boerngen, 1984). Ni content changes between 1 and 100 mg kg^{-1} (Kabata-Pendias & Pendias, 2001) in agricultural soil. Some samples were higher than the standards in the study areas. The Cu content of agricultural soils change between 5 and 50 mg kg^{-1}, which the concentrations below 8 mg kg^{-1} may indicate a deficiency for some crops as Cu is an essential micronutrient (Mermut et al., 1996). The results of the study showed that all samples had normal range of Cu concentration in the agricultural soils of Harran Plain.

Soils in arid and semiarid regions, generally have higher Mo contents (Kabata-Pendias & Pendias, 1992). In this study, some results of Mo were slightly high the permissible limit. Evaluation of Pb levels in soils that are toxic to plants is not easy; however, several authors have produced results for very similar concentrations ranging from 100 to 500 ppm (Kabata-Pendias & Pendias, 2001). The results of Pb in soils were less than the permissible limit in this study. Recently, Pb concentrations in soils have increased day by day, and this situation can seriously inhibit microbial processes. All these effects should be expected mainly in soils with low CEC. Whereas, in the long run, they can also occur in other soils with higher CECs (McBride & Spiers, 2001).

Relationship between Soil Heavy Metal Concentrations and Some Soil Properties

The trace element migration rates in the soil profiles are influenced by physical, chemical, and biological soil properties of which the most important are the Eh-pH system, CEC, salt content, amount and quality of organic matter, plant species, temperature and water, soil conditions such as pH and texture play a very important role in the availability of HMs in the soil (Öborn et al., 1995; Mermut et al., 1996; Puschenreiter & Horak, 2000). Correlation analysis was used to set up relationships between total concentrations of HMs and soil properties, which are given in Table 5. The CECs reflected the organic C and clay contents of the soils studied. Heavy metals (HMs) contents were affected by pH, CEC, Ca, $CaCO_3$, and clay contents in the surface horizon and by $CaCO_3$, CEC, and CEC in the subsurface horizons. The correlation coefficients analyses showed that there were positively relations between Zn and pH; Mn and CEC; Ca and Cu; Mo and $CaCO_3$; Pb and Clay in the surface horizons. On the contrary, total Zn, Ni, and Mo contents were negatively correlated with $CaCO_3$, CEC, and CEC in the subsurface horizons. Salinity, pH, organic matter, lime and clay contents were influenced the uptake of HMs in the soil (Grant et al., 2002).

Table 5. Correlation coefficients between soil parameters and total concentration of heavy metals.

Soil Properties		Heavy Metal Concentration						
		Zn	**Mn**	**Ni**	**Cu**	**Mo**	**Pb**	**Cd**
Surface Horizon	**pH**	0.506*	-0.206	0.445	0.288	0.201	-0.09	0.138
	EC	-0.323	0.457	-0.418	-0.495	-0.287	0.212	-0.319
	$CaCO_3$	0.110	-0.398	-0.353	-0.197	0.546*	-0.227	0.408
	SOC	-0.238	-0.081	-0.427	-0.404	-0.172	-0.168	-0.205
	Ca	0.158	0.334	0.185	0.517*	-0.249	0.219	0.045
	K	-0.267	0.434	0.269	0.218	-0.437	0.326	-0.020
	Mg	0.238	-0.189	0.406	0.473	-0.016	-0.274	-0.078
	Na	-0.153	0.483	-0.218	-0.343	-0.054	-0.012	-0.172
	CEC	0.123	0.550*	0.090	0.236	-0.348	0.288	-0.065
	Clay	-0.336	-0.262	0.080	-0.016	-0.425	0.514*	-0.188
Next to Surface Horizon	**pH**	-0.157	-0.219	-0.007	-0.015	0.051	-0.154	0.143
	EC	-0.017	0.073	-0.357	-0.262	-0.183	-0.118	-0.217
	$CaCO_3$	-0.530*	-0.384	-0.439	-0.242	-0.263	-0.341	-0.146
	SOC	-0.085	0.104	-0.071	-0.016	-0.043	0.320	-0.218
	Ca	-0.268	0.079	-0.217	0.036	-0.389	0.295	-0.114
	K	0.104	-0.046	0.201	0.099	0.244	-0.212	0.334
	Mg	0.018	-0.156	0.243	0.308	0.001	0.244	0.019
	Na	-0.190	0.374	-0.357	-0.488	-0.148	-0.359	0.123
	CEC	-0.432	0.148	0,529*	-0.368	-0.547*	-0.051	-0.183
	Clay	0.269	-0.079	0.216	0.118	0.333	0.151	0.015

* Correlation is significant at the 0.05 level (p), ** Correlation is significant at the 0.01 level (p).

Zone 2 (Z2) had high pH, low salinity, low organic matter, high calcium carbonate, and also high clay contents; this is the reason for the low HM concentration in Z2. The concentrations of HMs were in the following order Z4>Z3>Z1>Z2 in the studied area. Interactions between clays and metals in soil regulate plant availability of metals required for the growth of crops, while also soils contain a complex mix of clay, organic matter, coarse particles, water, air, and biological entities with properties that change both in space and over time. Therefore, it is not easy to accurately predict the chemical reactions and long-term travel of metals in a given soil (Hesterberg, 2006). All concentrations of several soil samples exceeded the permissible limits of Europe standard except for Ni, Mo, and Mn.

Soil pH is controlled by the availability of toxic elements to plants in the soil. The bioavailability of Zn and Cu is mainly controlled by pH and organic carbon content (Bhogal et al., 2003) Low Mn solubility was found at neutral to alkaline pH. CEC played an important role in the sorption of Cd in soils. Schuman (1979) also found that HMs in soil were associated primarily with clay fraction. Similarly, positive correlations between total HM contents and clay contents of soils were found in Oklahoma Benchmark soils (Lee et al., 1997). McBride and Spiers (2001) indicated that soils with HMs contamination are dependent on the soil location, source of fertilizer source, and soil organic matter.

Total Heavy Metal Contents in Commercial Fertilizers

The heavy metal (HM) contents of some commercial di-ammonium-phosphate (DAP) fertilizers were given in Table 6. Accordingly, Cu content was 28.29 mg kg^{-1} in DAP 4, Zn content was highest 183.84 mg kg^{-1} in DAP 2 and 305.84 mg kg^{-1} in DAP 4, Pb and Mo content was high 9.12 mg kg^{-1}, 7.94 mg kg^{-1} in DAP 1, Ni content was high 13.20 mg kg^{-1} in DAP 1 and 27.62 mg kg^{-1} in DAP 4, Mn content was high 193.99 mg kg^{-1} in DAP 1 and 67.95 mg kg^{-1} in DAP 4, and Cd content was high 17.16 mg kg^{-1} in DAP 2 and 18.10 mg kg^{-1} in DAP 3.

Table 6. Heavy metal contents of various di-ammonium-phosphate (DAP) fertilizers used in Harran Plain.

Fertilizers	Contents	Cu	Zn	Pb	Mo	Ni	Mn	Cd
		mg kg^{-1}						
DAP 1	18N:46P_2O_5	0.01	45.34	9.12	7.94	13.20	193.99	3.52
DAP 2	18N:46P_2O_5	0.00	183.84	1.75	3.41	10.37	24.56	17.16
DAP 3	18N:46P_2O_5	0.37	109.57	2.90	5.42	9.16	24.84	18.10
DAP 4	18N:46P_2O_5	28.29	305.84	3.59	4.73	27.62	67.95	7.52
DAP 5	18N:46P_2O_5	0.50	102.40	0.88	4.93	11.35	25.78	10.52

Heavy metal contents in composite fertilizers were given in Table 7. Accordingly, Cu contents was high 15.95 mg kg^{-1} in composite 3 and 15.46 mg kg^{-1} in composite 4, Zn and Pb content was very high 8813.99 mg kg^{-1}, 175.14 mg kg^{-1} in composite 3 and 3021.12 mg kg^{-1}, 130.62 mg kg^{-1} in composite 6, Mo content was high 3.73 mg kg^{-1} in composite 6 and 2.79 mg kg^{-1} in composite 2, Ni content was high 16.93 mg kg^{-1} in composite 1 and 22.03 mg kg^{-1} in composite 3. Mn content was found to be high 54.72 mg kg^{-1} in composite 3 and 119.12 mg kg^{-1} in composite 5, while the Cd content was high 7.75 mg kg^{-1} in composite 2 and 6.37 mg kg^{-1} in composite 4.

Table 7. Heavy metal contents of various composite fertilizers used in Harran Plain.

Fertilizers	Contents	Cu	Zn	Pb	Mo	Ni	Mn	Cd
		mg kg^{-1}						
Composite 1	20N:20P_2O_5	4.39	682.73	16.50	0.72	16.93	17.27	3.76
Composite 2	20N:20P_2O_5	0.66	96.94	3.18	2.79	7.29	17.15	7.75
Composite 3	20N:20P_2O_5	15.95	8813.99	175.14	2.65	22.03	54.72	3.85
Composite 4	20N:20P_2O_5	15.46	133.29	1.69	1.41	13.49	7.90	6.37
Composite 5	15N:15P_2O_5:15K_2O	0.17	43.52	8.34	0.00	2.84	119.12	0.02
Composite 6	15N:15P_2O_5:15K_2O+Zn	6.02	3021.12	130.62	3.73	6.21	15.22	8.92

The heavy metal content of some nitrogen fertilizers overused in the Harran Plain was given in Table 8. The results showed that Ni and Mn contents are high in ammonium nitrate 1 and CAN fertilizers. In this study, the HM content of some fertilizers was very high. This was one of the reasons for the high concentration of HMs in the soil (Ajayi et al., 2012; Büyükkılıç Yanardağ et al., 2016). Some commercial fertilizer types such as composite and DAP, which was overused in the plain, have higher HM contents. There was an important relationship between HMs and fertilizers. As it is known, land applications of commercial fertilizers can directly pollute the soil if it has high HM content.

Commercial fertilizers used in agriculture leave significant HMs to soils. The most important of these metals are arsenic, lead, cadmium, copper and nickel. Their entry into the soil is mostly done with phosphorus fertilizers and their raw materials. Research to produce phosphorus fertilizer has shown that the HM contents of phosphate rock are significantly high (especially imported from abroad). It has also been determined that the Cd and As content of phosphate rock is very high compared to other fertilizers (Köleli & Kantar, 2006).

Table 8. Heavy metal contents of some nitrogen fertilizers used in Harran Plain.

Fertilizers	Contents	Cu	Zn	Pb	Mo	Ni	Mn	Cd
		mg kg^{-1}						
Ammonium Nitrate 1	%26 N	0.00	0.00	2.81	0.00	23.97	83.64	0.01
Ammonium Nitrate 2	%33 N	0.00	0.00	0.59	0.00	1.15	1.10	0.02
Ammonium Sulfate	%21 N	0.00	0.00	0.00	0.00	1.92	2.37	0.01
CAN	%26 N	0.00	1.39	3.03	0.00	32.84	97.90	0.00
Urea	%46 N	0.00	0.00	0.00	0.00	2.09	2.70	0.01

Fertilizer-metal standards valid in some countries in the world (Benson et al., 2014) were given in Table 9. Accordingly, fertilizers with high HM content were used in some countries. Although the maximum Pb, As, Cd, and Ni concentrations were determined as 11, 81, 114, and 201 mg L^{-1} P, respectively, according to the volume principle of phosphoric acid in phosphorus fertilizers in fertilizer production, this ratio is the limit for lead concentration in composite fertilizers. It is approximately 5 times the value (100 mg kg^{-1}). Heavy metal, especially Cd content of DAP, TSP, and composite fertilizers used in agriculture to get more efficiency is high (>8 mg kg^{-1} fertilizer) [Köleli & Kantar, 2006].

When we examined the different heavy metal of the same fertilizers; while Cu, Zn, Ni are more in DAP 4 fertilizer, Pb, Mo, Mn are more in DAP 1 fertilizer. Also, Cd content was more in DAP 1 fertilizer. When we examined the composite fertilizers; Cu ratio more in composite 3 and composite 4, Zn and Ni in composite 1 and composite 3, Mo in composite 3, Mn and Cd in composite 4. Ni and Mn are found to be very high in all nitrogen fertilizers except of CAN. Generally, phosphate fertilizers are produced from phosphate rocks by pickling. Sulfuric acid is used for pickling single superphosphate and phosphoric acid is used for pickling triple superphosphate. The results showed that due to the different heavy metal content of phosphate rocks used in fertilizer production, fertilizers with the same N:P content produced may have different heavy metal concentrations. The final product thus obtained contains all the heavy metals found as components in the phosphate rock (Mortvedt, 1996; Dissanayake & Chandrajith, 2009). Commercial inorganic fertilizers and especially phosphate fertilizers can potentially contribute to the global transport of heavy metals (Carnelo et al., 1997).

The increase in the HM ratio in the water and especially in the soil is very effective in soil fertility, aquatic life, and ecosystem activities. Metals enter the plant body and affect many important metabolic activities (Köleli & Kantar, 2006; Asri et al., 2007). In particular, fertilizers containing high sodium and potassium content reduce the deterioration of soil structure, and the soil pH, increased the properties of acidic irrigation or increased the benefits of other agricultural activities (Savci, 2012). Continuous use of acid-forming nitrogen fertilizers causes a decrease in soil pH, calcification, and a decrease in yield if not carried.

The basic use of fertilizers in the soil causes an increase in pH, and increases in the soil and plants, while its harmful effects can cause a sudden decrease in the pH of the seedlings and a decrease in yield and quality. In addition, they accumulate in the soil and cause soil pollution (Savci, 2012). When a large amount of potassium fertilizer is given to the soil, it disrupts the balance of Ca, Fe, and Zn and prevents nutrients from being taken up by plants. However, considering the negative effects on organisms, various earthworms and soil mites have devastating and lethal effects (Savci, 2012).

Table 9. Fertilizer-metal standards valid in some countries in the world (Benson et al., 2014).

Metal	China	Canada			Australia			Japan
	F	MC	PF	PFM	PFC	FM	FNM	FP
Cd	8	20	300	10	50	-	-	8
Cu	-	-	-	-	-	-	-	-
Ni	-	180	-	-	-	-	-	-
Pb	100	500	-	-	-	-	500	100
Zn	-	1850	-	-	-	-	-	-

F: mg kg^{-1} fertilizer, MC: mg kg^{-1} dw max. acceptable metal concentration, PF: mg kg^{-1} P product P fertilizers, PFM: mg kg^{-1} product P-free fertilizers, PFC: mg kg^{-1} product fertilizers composed entirely of micronutrients, FM: mg kg^{-1} all fertilizers and micronutrients, FNM: mg kg^{-1} fertilizers with essential nutrients with micronutrients, FP: mg kg^{-1} by-product phosphate fertilizers.

Soil properties affect the retention of some metals (Cd, Zn and Ni) during fertilizer application. Fertilized soils had significantly lower ($p<0.05$) levels of present and total metals than unfertilized soils, indicating that prolonged fertilization did not increase the metal concentration in the soil (Jones et al., 2002; Franklin et al., 2005). At concentrations of Cd, Mo, and As in high phosphorus fertilizers, agronomic application rates do not significantly increase total soil concentrations above background levels for many years, although their availability by plants for root uptake into tissues may increase (McBride & Spiers, 2001).

It is known that heavy metals occur in soil in various chemical forms - soluble in water, changeable but depending on certain regions of organic and inorganic components and in the structure of primary and secondary minerals with a balance between these forms (Mclaren & Crawford, 1993). This balance may change depending on the changes in physical, chemical and biological properties of the soil. Sims (1986) concluded that fertilizer application can not only provide nutrients to plants but also alter the speciation and bioavailability of HMs in soil.

Conclusion

For the growing human population, increasing food production and improving soil quality has been a very important research topic. Especially with the use of commercial fertilizers with high metal content, serious soil pollution has occurred in agricultural lands. Toxic HM enters the food chain by passing it to the plant through fertilizers and then to the soil. Therefore, maximum tolerable limits for crops were determined by many countries. The soils in the plain are well developed and consist of A-B-C horizons, clay, carbonate contents, and CEC values are high; however, they have about 1% organic matter, low EC (non-saline) and classified as Vertisol. Total Fe contents in the majority of the surface soils are about 3%. The results of the study showed that HMs in soil zones were in the following order Z4>Z3>Z1>Z2. The reasons of those ratios were high pH, low salinity, low organic matter, high calcium carbonate, and also high clay contents. There were positively relations between Zn and pH; Mn and CEC; Ca and Cu; Mo and $CaCO_3$; Pb and Clay in the surface horizons. On the contrary, total Zn, Ni, and Mo contents were negatively correlated with $CaCO_3$, CEC, and CEC in the subsurface horizons. Although HM concentrations of common commercial fertilizers were lower than the standards, HM contents of some fertilizers were found to be very high in this study. This causes an increase in the HM concentration in the soil. HMs concentrations of several soil samples exceeded the threshold level of Europe standard except for Ni, Mo and Mn. Study results can help the authorities to develop effective fertilizer management strategies for the Harran Plain, which has once again revealed the necessity of applying agricultural activities such as fertilization with a fertilization program prepared under expert control according to the results of soil analysis.

Conflict of Interest

The author has no conflict of interest to declare.

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http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSİP

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RESEARCH ARTICLE

Length-Weight Relationship and Condition Factor of Prussian Carp (*Carassius gibelio*, Bloch, 1782) from Asi River

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ARTICLE INFO

Article History
Received: 23.09.2022
Accepted: 04.11.2022
First Published: 15.12.2022

Keywords
Asi River
Fisheries management
Invasive species
LWR
Orontes

ABSTRACT

This study aimed to determine the length-weight relationship and condition factor of Prussian carp (*Carassius gibelio*, Bloch, 1782) from the Asi River. Samples were collected by 12-18 mm mesh sized fyke-nets between November and December 2021. Totally 88 specimens have been collected from the Turkish part of Asi River, Hatay, Türkiye. Lengths and weights ranged from 10.2 to 29.8 cm and 19.47 to 408.59 g, respectively. The b-values were calculated as 3.08 and the LWR equation was estimated as $W = 0.0138*L^{3.08}$. The Fulton's condition factor (K) and the relative condition factor (K_n) values were calculated as 1.76±0.03 and 1.01±0.01, respectively. This study provides the valuable data on the length-weight relationship and condition factors of *C. gibelio* from the Asi River. The findings of the present paper revealed that *C. gibelio* showed an isometric growth and this region is relatively suitable for the growth of this species. Therefore, these data will be a valuable background for further biological studies and local fisheries management strategies.

Please cite this paper as follows:

Şimşek, E., & Kale, S. (2022). Length-weight relationship and condition factor of Prussian carp (*Carassius gibelio*, Bloch, 1782) from Asi River. *Journal of Agricultural Production*, *3*(2), 69-77. https://doi.org/10.56430/japro.1179095

Introduction

Carassius gibelio is a benthopelagic species and mainly feeds on plankton, benthic invertebrates, plant material and detritus. It has a wide distribution from European water to eastern Asia (Kottelat & Freyhof, 2007). The presence of *C. gibelio* from Turkish inland waters was reported from Gala Lake in 1988, for the first time (Baran & Ongan, 1988). Subsequently, many reports have followed from several Anatolian waters (Özcan, 2007; Yerli et al., 2014).

Asi River flows through highly populated cities before pouring its waters into the Mediterranean Sea. Therefore, it suffers from pollutants caused by human induced activities that are common in the basin such as agriculture and animal husbandry. Recent studies showed that low dissolved oxygen, organic pollution, microplastic pollution, and metal toxicity risk are major concerns for aquatic animals in the Asi River (Kılıç & Yücel, 2019; Turan et al., 2020; Kılıç et al., 2022). Since *C. gibelio* species are tolerant to low oxygen concentrations and pollution (Kottelat & Freyhof, 2007), they

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can establish an important fish stock in freshwater systems with known environmental pollution problems like the Asi River.

Several methods are used to estimate fish population parameters, and the length-weight relationship is known to be one of the most important tools for fish populations in a particular geographic region (Tesch, 1971; Seçer et al. 2021; Acarlı et al., 2022). For that reason, studies investigating the length-weight relationship of aquatic organisms are frequently updated depending on the possible spatiotemporal variations (Acarlı et al., 2022).

To date, there are various species, both native and invasive, belonging to different families in the Asi River (Demirci & Demirci, 2009; Bayçelebi, 2020). Some biological parameters of *Barbus luteus* (Gökçek & Akyurt, 2008), *Capoeta capoeta* (Demirci & Yalçın Özdilek, 2010), *Alburnus orontis*, *Chondrostoma kinzelbachi* (Özcan & Altun, 2015), *Capoeta barroisi* (Demirci & Yalçın Özdilek, 2015), *Garra rufa* (Demirci et al., 2016), *Capoeta angorae* (Alagöz Ergüden & Turan, 2017), *Anguilla anguilla* (Demirci et al., 2020), *Barbus lorteti* (Demirci & Yalçın Özdilek, 2021), *Salaria fluviatilis* (Alagöz Ergüden, 2021a), *Garra turcica* (Alagöz Ergüden, 2021b), *Clarias gariepinus* (Şimşek et al., 2022) have been assessed in previous studies. Since Cyprinidae family carries commercial importance in the Asi River (Demirci & Demirci, 2009), information regarding its population characteristics is important for the establishment of proper management strategies. Therefore, this study aimed to determine the length-weight relationship and condition factor of *C. gibelio* in the Asi River Basin.

Materials and Methods

The study was conducted in Turkish part of Asi River. The surface waters of the Asi River are shared between Lebanon, Syria, and Türkiye. Once the Asi River reaches the Turkish territory, it merges with Burç and Karasu streams and pourers to the Mediterranean Sea (Figure 1). The Mediterranean climate zone is known for hot summers and low precipitation (Kılıç & Can, 2017; Kılıç, 2018). Annual precipitation and mean temperature of basin 816 mm and 16.8 °C, respectively. The annual streamflow of the river is 1.17 km^3/year (Şimşek et al., 2022). Basin suffered from many anthropogenic influences mainly agriculture, husbandry, and urbanization (Kılıç et al., 2018; Kılıç & Yücel, 2019).

Figure 1. The location of the Asi River.

Samples were collected between November and December 2021 with 12-18 mm mesh sized fyke-nets. Total lengths (TL) and wet weights (W) were measured to the nearest 1.0 mm and 0.01 g, respectively. The LWR of *C. gibelio* was estimated with Eq. (1) suggested by Ricker (1975). The parameters were calculated by the log-transformation equation: $\log(W) = \log(a)$

+ b log(TL) where W is the weight, TL is the total length, and a and b are constants.

$$W = aL^b \tag{1}$$

Fulton (1904)'s coefficient of condition factor (K) was calculated by Eq. (2).

$$K = \frac{W}{L^3} \times 100 \tag{2}$$

In this equation, K is the condition factor, W is the total weight (g), and L is the total length (cm). Besides, the relative condition factor (K_n) was also presented using Eq. (3) [Le Cren, 1951].

$$K_n = \frac{W}{aL^b} \tag{3}$$

where K_n is the relative condition factor, a is the intercept and b is the slope derived from the LWR estimation, W is the total weight (g), L is the length (cm).

Results

This study provides LWR and condition factor of *C. gibelio* from the Turkish part of the Asi River collected between November and December 2021. A total of 88 specimens of *C. gibelio* was collected from the Asi River. The fish size ranged from 10.2 to 29.8 cm (TL) and weighed between 19.47 and 408.59 g (Figure 2). The mean TL and mean TW values of samples caught from the Asi River were calculated as 16.55±0.41 cm and 94.35±8.48 g (Table 1).

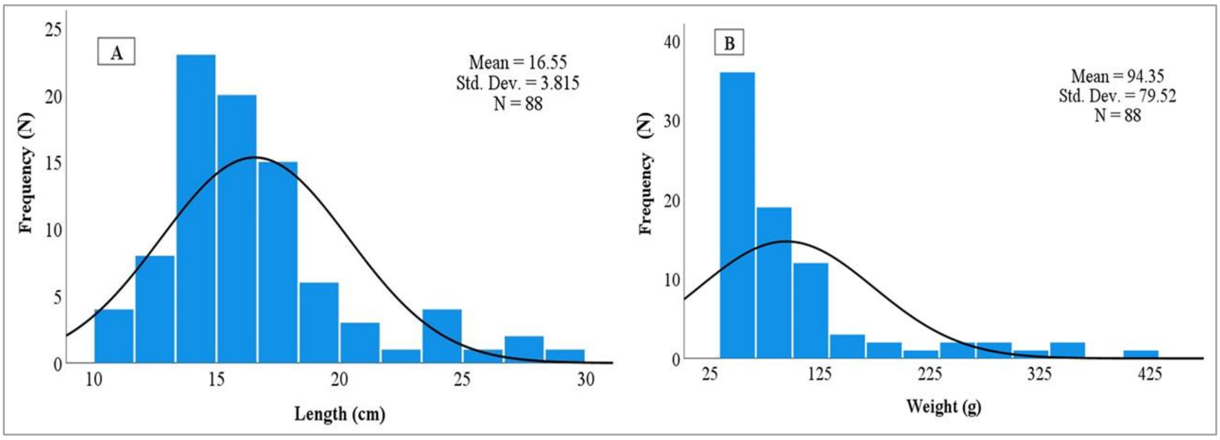

Figure 2. Frequency of lengths (A) and weights (B) of *C. gibelio* from Asi River.

The LWR of C. *gibelio* was estimated as $W= 0.0138*L^{3.08}$. The exponent of the length-weight relationship, b, showed an isometric growth (Figure 3).

Fulton's condition factor (K) and relative condition factor (K_n) values varied from 1.247 to 2.331 and from 0.717 to 1.348, respectively. The estimated mean K and K_n values were 1.759±0.025 and 1.012±0.014, respectively (Table 1).

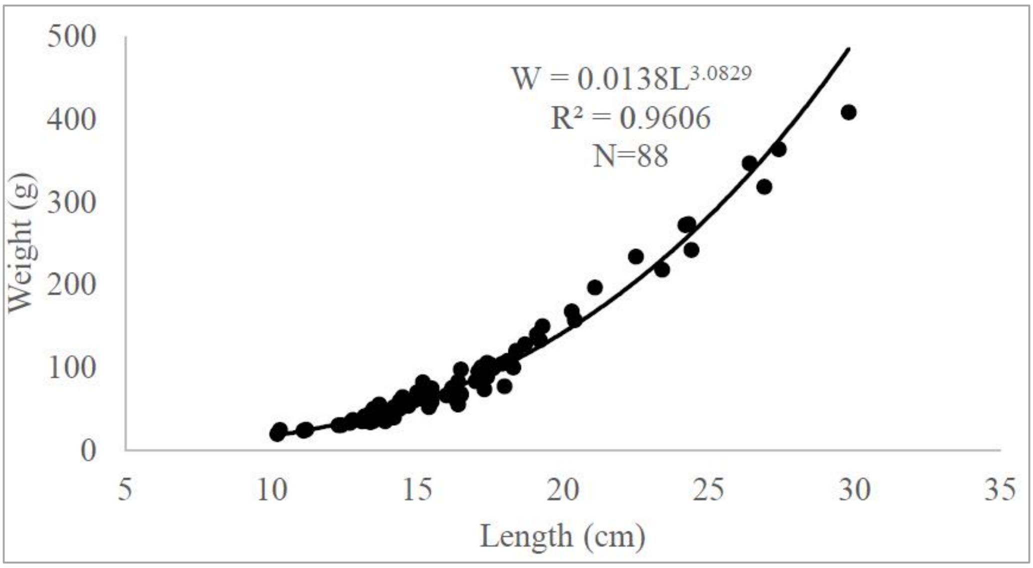

Figure 3. The length-weight relationship of *C. gibelio* collected from the Asi River during the study period.

Table 1. Some biological parameters of *C. gibelio* from Asi River.

N	Total Length (cm)		Weight (g)		a	b	r^2	SE of b	Growth Type	K		K_n	
	Range	Mean±SE	Range	Mean±SE						Range	Mean±SE	Range	Mean±SE
88	10.2-29.8	16.55±0.41	19.47-408.59	94.35±8.48	0.0138	3.0829	0.9606	0.067	I	1.25-2.33	1.76±0.03	0.72-1.35	1.01±0.01

N: Sample size, a and b: Parameters of length-weight relationships equation, r^2: Coefficient of correlation, SE: Standard error, K: Fulton's condition factor, K_n. Relative condition factor, I: Isometric growth.

Discussion

Although the Asi River is an important area in terms of species diversity, this region has important fish stock problems due to seasonal water shortages and overfishing (Demirci & Yalçın Özdilek, 2010, 2015, 2021; Demirci et al., 2020). Therefore, monitoring and protection of fish populations in this region is essential for a sustainable ecosystem and fisheries. The present study investigated the basic biological information (LWR, *K*, K_n) of *C. gibelio* in the region. Furthermore, the length-weight relationships for *C. gibelio* were compared with available literature from different regions (Table 2).

The "b" value of LWR is reported to be within the range of 2.5 to 3.5 for fish (Carlander, 1977). The "b" value estimated in this study was within the standardized range. In addition, previous studies examining LWR of *C. gibelio* from different environments also reported coherent "b" values to the previously described standards (Table 2). The "b" value is also a practical tool to determine the growth type of fish stock. In this study, the growth of *C. gibelio* was evaluated as an isometric growth, meaning that the weight gain with length was isometric. Our results are similar to those previously found by Çiçek et al. (2022) in the Asi River. Also, the growth type observed in this study is similar to previous studies in different regions of Türkiye (Table 2).

Table 2. Length-weight relationship parameters ($W=aTL^b$) of *C. gibelio* reported worldwide (G: Gender, F: Female, M: Male, A: All, N: Sample size, a: Intercept of the relationship, b: Slope of the relationship, r^2: Coefficient of correlation).

Location	G	N	a	b	r^2	Reference
Volvi Lake, Greece	A	102	0.0142	3.110	0.980	Kleanthidis et al. (1999)
Ömerli Dam Lake, Türkiye	A	730	0.0099	3.180	0.991	Tarkan et al. (2006)
İznik Lake, Türkiye	A	363	0.0084	3.250	0.989	
Lysimachia Lake, Greece	F	267	0.0660	2.580	0.800	Tsoumani et al. (2006)
Pamvotis Lake, Greece	F	494	0.0190	3.060	0.940	
	M	13	0.0440	2.780	0.970	
Chimaditis Lake, Greece	F	205	0.0600	2.740	0.880	
Kerkini Lake, Greece	F	51	0.0490	2.720	0.850	
Volvi Lake, Greece	F	50	0.0210	2.960	0.990	
Kastoria Lake, Greece	F	52	0.0260	2.890	0.970	
Vegoritis Lake, Greece	F	49	0.0090	3.250	0.960	
Trichonis Lake, Greece	F	12	0.0040	3.380	0.950	
Mikri Prespa Lake, Greece	F	18	0.2200	2.330	0.730	
Doirani Lake, Greece	F	51	0.1000	2.400	0.820	
Koronia Lake, Greece	F	51	0.1400	2.360	0.720	
Zazari Lake, Greece	F	20	0.0340	2.810	0.830	
Buldan Dam Lake, Türkiye	A	2325	0.0310	2.870	0.985	Sarı et al. (2008)
Chimaditis Lake, Greece	A	600	0.0336	2.810	0.920	Leonardos et al. (2008)
Doirani Lake, Greece	A	205	0.0137	3.059	0.989	Bobori et al. (2010)
Mikri Prespa Lake, Greece	A	49	0.0094	3.187	0.994	
Volvi Lake, Greece	A	143	0.0214	2.945	0.972	
Flanders, Belgium	A	10364	0.0107	3.183	0.990	Verreycken et al. (2011)
Danube River, Romania	A	314	0.0298	2.866	0.902	Gheorghe et al. (2012)
Anzali wetland, Iran	A	95	0.0224	2.879	0.900	Moradinasab et al. (2012)
Aksu River, Türkiye	A	128	0.0138	3.114	0.976	İnnal (2012)

Table 2. (continued).

Location	G	N	a	b	r^2	Reference
Seyitler Reservoir, Türkiye	F	123	0.0700	2.130	0.838	Bulut et al. (2013)
	M	24	0.2940	2.640	0.784	
	A	149	0.0270	2.940	0.813	
Ikizcetepeler Dam Lake, Türkiye	F	374	0.0240	2.890	0.609	Erdoğan et al. (2014)
	M	106	0.0160	2.980	0.700	
Ain Zada Reservoir, Algeria	A	93	-1.790	3.040	0.940	Mimeche and Biche (2015)
Cişmigiu Lake, Romania	A	94	0.0055	3.630	0.913	Stavrescu-Bedivan et al. (2015)
Brăneşti Lake, Romania	A	62	0.0523	2.657	0.879	
Sâi River, Romania	A	106	0.0087	3.249	0.990	
Marmara Lake, Türkiye	A	2213	0.0173	2.974	0.976	İlhan and Sarı (2015)
Seyhan River, Türkiye	F	299	0.0600	2.610	0.912	Alagöz Ergüden (2015a)
	M	18	0.1090	2.400	0.961	
	A	317	0.0670	2.570	0.927	
Seyhan Dam Lake, Türkiye	A	160	0.0519	2.650	0.933	Alagöz Ergüden (2015b)
Kızılırmak River, Türkiye	A	144	0.0230	2.860	0.850	Birecikligil et al. (2016)
Beyşehir Lake, Türkiye	A	1868	0.0175	2.959	0.925	Dereli and Dinçtürk (2016)
Madatapa Lake, Georgia	F	67	1.7900	2.990	0.930	Japoshvili et al. (2017)
	M	38	1.7300	2.930	0.950	
	A	141	-1.800	2.980	0.930	
Pantelimon II Lake, Romania	A	50	0.0396	2.758	0.844	Stavrescu-Bedivan et al. (2018)
Onaç Creek, Türkiye	A	127	0.0097	3.187	0.989	İnnal et al. (2019)
Lower Sakarya River, Türkiye	A	179	0.0264	2.870	0.970	Reis et al. (2019)
Doroudzan Dam, Iran	A	16	0.0151	3.090	0.946	Paighambari et al. (2020)
Gölcük Lake, Türkiye	A	20	0.0144	3.104	0.937	Güçlü and Küçük (2021)
Küçükler Reservoir, Türkiye	A	15	0.0015	3.808	0.864	
Buldan Reservoir, Türkiye	A	21	0.0025	3.636	0.913	
Demirköprü Reservoir, Türkiye	A	5	0.0003	4.346	0.963	
Marmara Lake, Türkiye	A	56	0.0167	3.032	0.985	
Afşar Reservoir, Türkiye	A	15	0.0115	3.123	0.963	
Karamenderes River, Türkiye	A	117	0.0079	3.255	0.990	Yalçın Özdilek and Partal (2022)
Aras River, Türkiye	A	233	0.0090	3.259	0.998	Çiçek et al. (2022)
Asi River, Türkiye	A	34	0.0140	3.070	0.863	
Batı Akdeniz, Türkiye	A	95	0.0160	3.034	0.990	
Çoruh River, Türkiye	A	38	0.0230	2.830	0.978	
Doğu Akdeniz, Türkiye	A	19	0.0110	3.156	0.998	
Fırat River, Türkiye	A	159	0.0130	3.060	0.971	
Konya, Türkiye	A	29	0.0170	2.982	0.981	
Seyhan, Türkiye	A	32	0.0090	3.171	0.949	
Asi River, Türkiye	**A**	**88**	**0.0138**	**3.082**	**0.961**	**Present study (2022)**

The wide ecological tolerance of exotic fish species causes them to multiply rapidly in inland waters (Güngör, 2012). In this regard, LWR is a valuable tool for fish stock assessment (Keyombe et al., 2015; Acarlı et al., 2022). The value of "a" in the LWR equation is expected to be between 0.001-0.050 for natural fish populations (Froese, 2006). The estimated value of "a" in this study is consistent with the expectations, as in most studies conducted in Turkish inland waters (Table 2). Çiçek et al. (2022) reported this value as 0.014 for this region. Alagöz Ergüden (2015a) reported higher "a" values for the *C. gibelio* population in the Seyhan River. Similarly, Bulut et al. (2013) reported higher "a" values for the female population of *C.*

gibelio from the Seyitler Dam Reservoir. Japoshvili et al. (2017) reported a value of -1.8 from Madatape Lake, South Georgia. The differences in the "a" value may have resulted from the sampled environment and seasonal variations (Karadurmuş, 2022).

Fulton's condition factor (*K*) is a practical tool while evaluating the feeding and spawning activity of fish (Karadurmuş, 2022). The *K* value differs depending on the predation rate, presence of disease in the environment, food availability, and abiotic environmental factors such as pH, temperature, pollution status of the environment (Wang et al., 2017). These growth-limiting conditions cause deviations from the theoretical value of 1; whereas, favorable growth conditions cause *K* values higher than 1 (Ujjania et al., 2012). In this study, the *K* value varied between 1.25 and 2.33 indicating that *C. gibelio* grows in a harmony with the environmental conditions in the Asi River. Likewise, Çiçek et al. (2022) stated that this exotic species has been established successfully in this region and different habitats in Türkiye.

Conclusion

This study provides the valuable data on the length-weight relationship and relative condition factor of *C. gibelio* from the Asi River. The findings of the present paper revealed that *C. gibelio* showed an isometric growth and this region is relatively suitable for the growth of this species. Therefore, these data will be a valuable background for further biological studies and local fisheries management strategies. Nevertheless, more comprehensive studies are required for a better understanding of the population dynamics of the species, as researches on fish biology are essential to understand the status of fish populations in different habitats.

Acknowledgment

The authors are thankful to Dr. Ece Kılıç (İskenderun Technical University, Türkiye) for her valuable supports and suggestions.

Conflict of Interest

The authors declare that there is no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Estimates of Combining Ability and Association among Morpho-Agronomic Traits of Single Cross Maize (*Zea mays* L.) Hybrids

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ARTICLE INFO

Article History

Received: 22.09.2022

Accepted: 15.11.2022

First Published: 19.12.2022

Keywords

Combining ability

Correlation

Genotypic

Grain yield

Path coefficient

Phenotypic

ABSTRACT

Screening genotypes have a crucial role to increase the efficiency of selections in plant breeding program. Therefore, this study was emphasized to determine combining ability and the association between traits among themselves and yield. The experiment was conducted at Haramaya University Research Station (Raare) for two years (2018 and 2019) using 4x7 alpha-lattice design with three replications. Pooled analysis of variance revealed highly significant ($p \leq 0.01$) variations among crosses for grain yield and related traits. According to the result of combining ability analysis, parental line L3 was identified as a good general combiner for grain yield, ear diameter, 1000-kernel weight, and days to maturity. Similarly; L1, L2, and L8 proved as the best general combiner for number of kernels per row. Crosses L1×L6, L3×L5, L4×L6, L4×L8, and L5×L7 were found good specific combiners for 1000-kernel weight. Furthermore, the cross L5×L6 was the best specific combiner for ear diameter, whereas L4×L7 for both number of kernels per row and 1000-kernel weight. Likewise, the crosses L1×L5, L3×L8, L6×L7, and L7×L8 were identified as the best specific combiner towards earliness. Moreover, thousand kernel weight showed significant positive correlation with grain yield, conversely, days to anthesis, days to silking, ear aspect, and *Puccinia sorghi* exhibited significant negative correlation with grain yield at genotypic and phenotypic levels. Ear length, 1000-kernel weight, number of kernel rows per ear, and *Turcicum* leaf blight had positive direct effect on grain yield at genotypic and phenotypic level. In general, the result presented in the study might be useful for further breeding process to improve the productivity of maize.

Please cite this paper as follows:

Mogesse, W., & Zeleke, H. (2022). Estimates of combining ability and association among morpho-agronomic traits of single cross maize (*Zea mays* L.) hybrids. *Journal of Agricultural Production*, *3*(2), 78-87. https://doi.org/10.56430/japro.1178621

Introduction

Maize (*Zea mays* L.) has a crucial role for human diet as well as animal nutrition, especially in developing countries. It is the most important and widely grown cereal crop in Ethiopia next to teff. Maize belongs to the grass family Poaceae and historically believed that the crop was originated from Mexico and introduced to Ethiopia around the late 17th century (Haffangel, 1961). Even if the introduction of maize to Ethiopia is a recent phenomenon, it dominates the total cereal production of the country and provides livelihood food security. Maize crop has a versatile use, wider genetic variability, high grain yield per unit area and can grow in a wide range of environments. Maize together with wheat and rice comprise a major component of the human diet, accounting for an estimated 42 percent of the World's food calories and 37 percent of protein intake [average 2016-18 (FAO, 2021)]. As a result, maize is considered the strategic crop to secure food self-sufficiency and the growing demand for maize has steadily increased in Ethiopia. Therefore, to address the growing demand of farmers, there is a need to improve the productivity of maize through breeding. Studies on genetic parameters and the correlation between yield and yield-related traits are pre-requisite to plan a meaningful breeding program and thereby enhance the productivity of the crop (Reddy & Jabeen, 2016).

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The foremost requirement in any hybrid breeding program is identification and selection of diverse parents that have good general and specific combining ability with other parents for yield and yield contributing traits (Fasahat et al., 2016). Indeed, diallel mating system is one of the suitable means to detect the performance of offspring, as well as the parents and progeny performance can be statistically separated into components relating to GCA and SCA (Murtadha et al., 2018). Combining ability analysis is the most powerful biometrical tool in identifying the better combiners and measure the relative capacity of the parental line to transmit genetic information to its offspring for the development of outstanding hybrids. The role of combining ability in maize breeding for selection of better parents as it discloses to the mode of inheritance for various plant traits and determines the nature and magnitude of gene action involved in the inheritance of the traits has been well documented (Gissa et al., 2007; Abdel-Moneam et al., 2009; Gouda et al., 2013; Mogesse et al., 2020).

In genetic breeding, plant selection can be conducted directly or indirectly by studying linear relationships among traits, such relationships can be determined by correlation coefficient and by complementary methods, such as path analysis. Therefore, understanding the interrelationship of quantitative characters with grain yield is crucial to determine the efficiency of selection and providing the basis for planning an effective breeding program. Correlation is the linear association between two variables (Gomez & Gomez, 1984) measured by correlation coefficient, which is required in plant breeding to quantify the degree of genetic and non-genetic association between two or more traits. Moreover, the coefficient of correlations aids in determining the level of relationship between two separate traits as well as the levels at which these traits are mutually variable (Johnson et al., 1955). A positive correlation coefficient indicates simultaneous relationship between dependent and independent traits in selection (Eleweanya et al., 2005). The low phenotypic correlation could arise due to the modifying effect of the environment on the association character at genetic level (Alake et al., 2008).

Information like association among traits and the relative contribution of each trait is required to determine selection criteria in plant breeding. Therefore, path coefficient analysis, which was proposed by Wright (1921) used to partition correlation coefficient into direct and indirect effects and provides information on the actual contribution of a trait to the yield. Furthermore, path coefficient analysis produces a clear picture of a set of independent variables on the dependent variable and provides realistic relationship of the characters, which helps in identifying traits that are useful as selection criteria to improve crop yield (Milligan et al., 1990; Chaudhary et al., 2017; Mogesse, 2021). Thus, correlation and path coefficient analysis are important tools to assist breeders in defining priority traits for selection, quantifying the level of relationships between the traits, provide reliable and useful information on the nature, extent, and direction of selection (Mallikarjuna et al., 2011; Zeeshan et al., 2013; Nabila et al., 2017; Wedwessen & Wolde, 2020; Mogesse, 2021). Thus, to improve the production and productivity of maize as well as tackling newly emerging challenges, continuous maize research is required. As a result, widely adapted maize varieties that suit different cropping systems and farming conditions are required to enhance food self-sufficiency, cash generation, and poverty reduction in the country. Therefore, the research aimed to verify combining ability and association of morpho-agronomic traits to practice indirect selection for further breeding process.

Materials and Methods

Descriptions of Study Area

The study was undertaken at the research farm of Haramaya University (Raare), which is located at an altitude of 2020 meter above sea level, latitude 9º26' N and longitude 42º3' E. The area received an average annual rainfall 727 mm with minimum and maximum average temperatures of 8.99 ºC and 25.15 ºC, respectively during 2018/19 cropping season.

Experimental Design and Field Management

Eight maize inbred lines were obtained from Haramaya university maize research program, and crossed in a half-diallel mating design (Griffing's Method IV, Model I) during 2017 cropping season. The resulting twenty-eight single cross F_1 maize hybrids were evaluated in 2018 and 2019 cropping seasons using 4x7 alpha-lattice design in three replications. The experiment comprised two rows per plot with 5.1 m long and 0.75 m inter and 0.30 m intra row spacing. An alley left 1.5 m between blocks. During planting, two seeds per hole were planted to ensure enough stand and later thinned after two weeks of emergence (when seedlings were 3-4 leaf stage) to have a single healthy seedling per hole and to retain 44,444 plants stand ha^{-1}. Urea and NPS fertilizers were applied at the recommended rates. Moreover, all other necessary field management practices were carried out as per the recommendation of the study area and the crop.

Data Collection and Analysis

Field data on phenology, growth, grain yield and yield related traits, and disease reaction were recorded on plot and individual plant basis at the appropriate plant growth stages. Data related to days to anthesis (DA), days to silking (DS), days to maturity (DM), and grain yield (GY) were recorded on the plot basis, whereas, plant height (PH), ear height (EH) were recorded from five randomly selected plants, and number of kernel rows per ear (NKRE), number of kernels per row (NKR), ear length (EL), ear diameter (ED) and 1000-kernel weight from five randomly selected ears leaving border plants of each row. The average was taken as the mean value of the treatment. Grain yield was measured from entire ears of each experimental unit. Moreover, grain yield was adjusted to 12.5% moisture content and converted yield per plot into ton per hectare.

Data obtained from field measurement were subjected to analysis of variance using the PROC MIXED procedure of SAS (2002) to test the level of significance resulting from 8x8 diallel cross. The traits, which showed significant difference were subjected to diallel analysis using AGD-R (Analysis of Genetic Designs in R). Significant genotypic variance of each trait was further partitioned to GCA, SCA, and experimental error. Diallel analysis of variance was conducted to estimate general combining ability (GCA) and specific combining ability (SCA) following Griffing's approach Model I, Method IV [n(n−1)/2], which only includes one set of crosses with neither reciprocals nor parents as suggested by Griffing (1956). The estimates of genotypic and phenotypic correlation were computed by the method described by Singh and Chaundry (1985).

Results and Discussion

Analysis of Variance

Analysis of variance revealed that there were significant differences among crosses for ear diameter, 1000-kernel weight, anthesis-silking interval, grain yield, days to maturity, number of kernels per row, and number of kernel rows per ear suggesting that the presence of variability among crosses. The traits which showed significant difference were subjected to genetic analysis of variance to determine GCA and SCA variance. The combined analysis of GCA exhibited highly significant difference for number of kernels per row implying that additive gene action contributes to the expression of the trait, whereas the mean squares of SCA were also significant for thousand kernel weight indicating non-additive gene action contributes to the expression of the trait (Table 1). Several studies (Gissa et al., 2007; Abdel-Moneam et al., 2009; Gouda et al., 2013; Mogesse et al., 2020) showed that significant GCA and SCA effects. The SCA sums of squares were higher than GCA sums of squares for ear diameter, anthesis-silking interval, and number of kernels per row implying that non additive gene action is important for controlling the inheritance of these traits. The results were in parallel with the previous reports of Elmyhum (2013) and Sugiharto et al. (2018). On the other hand, GCA sums of squares were higher than SCA for number of kernel rows per ear, grain yield, number of days to maturity, and thousand kernel weight that implies additive gene action is important in controlling the inheritance of this trait. The results were comparable with the previous reports of Wende (2013) and Tessema et al. (2014). Significant year effects were observed for the traits of ear diameter, grain yield, anthesis-silking interval, and number of kernels per row. The mean squares of crosses × year interaction effect were also significant for all traits, except anthesis-silking interval. Similar results were reported by Bello and Olawuyi (2015) in their previous studies on gene action, heterosis, correlation, and regression estimates in developing hybrid cultivars in maize. Similarly, significant GCA × year interaction was observed for days to maturity, number of kernels row per ear, and 1000-kernel weight; while significant positive SCA × year interactions were found for all traits studied, except anthesis-silking interval and number of kernel rows per ear.

Table 1. Pooled analysis of variance, mean and CV for grain yield and yield component in single crosses F_1 maize hybrids evaluated at Haramaya, Ethiopia in 2018 and 2019.

Source of Variation	df	ED	ASI	DM	GY	NKR	NKRE	TKW
Year (Y)	1	1.880**	4.124**	34.118	105.695**	98.296**	1.030	303.843
Crosses (C)	27	0.247	0.366	71.116	6.957	20.946	2.107	4945.718
GCA	7	0.216	0.310	96.683	14.686	13.944**	5.742	6854.160
SCA	20	0.264	0.395	65.742	4.803	23.945	0.875	4761.619*
Crosses × Y	27	0.340**	0.196	67.868**	9.071*	20.396**	2.814**	3721.877**
GCA × Y	7	0.644	0.167	138.857*	12.916	4.450	8.313**	9649.455**
SCA × Y	20	0.327**	0.207	46.657**	8.446*	26.536**	0.899	2083.011**
Error	96	0.144	0.192	16.626	4.929	8.605	0.882	728.425
Grand Mean		4.593	3.274	164.786	8.870	38.929	12.651	364.235
Min		3.517	3.000	142.000	5.033	27.800	10.300	304.870
Max		5.250	3.667	170.667	10.883	43.567	15.867	416.170
CV (%)		6.513	10.572	0.696	21.736	5.204	3.107	8.855

GY: Grain yield (t ha^{-1}), NKRE: Number of kernels row per ear, DM: Days to maturity, ASI: Anthesis-silking interval, ED: Ear diameter, NKR: Number of kernel per rows, TKW: Thousand kernel weight, **Significant at p<0.01 level of probability, and *Significant at p<0.05 level of probability.

Estimates of GCA Effects

According to the result of GCA analysis in Table 2, significant differences were observed among lines for various traits. Significant positive GCA effect (0.635) for grain yield was observed in L3. Thus, L3 was proven as a good general combiner for yield and could be used extensively in hybrid breeding programme with view to increase the yield level. However, L1 and L5 proved as poor general combiner for grain yield. On the other hand, significant positive GCA effects for number of kernel rows per ear were observed in L1, L2, and L8 implying that they are good general combiners and might be selected for obtaining high yielding hybrids, while L3, L5, L6, and L7 exhibited significant negative GCA effect for number of kernel rows per ear, which may contribute undesirable trait for grain yield improvement. Significant positive GCA effects for 1000-kernel weight were obtained from L2 and L3; therefore, these lines could be considered as potential parents for genetic improvement of grain yield through 1000-kernel weight. Conversely, parental lines L1 and L8 showed highly significant negative GCA effect for 1000-kernel weight implying that they are poor general combiners to improve grain yield through 1000-kernel weight. Positive and significant GCA effect for ear diameter was detected in parental lines L3 and L8 showing the tendency of the parents to enhance ear diameter and thereby improve grain yield. Significant negative GCA effect for days to maturity was observed in L2 and L7 depicting that these lines have genes for earliness, which also contributes desirable trait in improving maize maturity period. In contrary, L3 showed significant positive GCA effect for days to maturity, implying that the tendency of the parent to contribute delay in maturity to their progeny. Significant negative GCA effect for anthesis-silking interval was observed in L5 towards desirable direction, whereas L6 showed significant positive GCA effect for anthesis-silking interval. The results were in agreement with the earlier reports of Amiruzzaman et al. (2013), Hosana et al. (2015), Genet et al. (2017), and Gemechu et al. (2018).

Table 2. Estimates of general combining ability effects (GCA) for grain yield and yield related trait of maize inbred lines evaluated at Haramaya, Eastern Ethiopia.

Parent	ASI	DM	ED	NKR	NKRE	TKW	GY
L1	-0.097	-0.944	-0.073	0.672	0.255*	-26.341**	-0.918*
L2	0.014	-2.222**	-0.015	0.267	0.566**	10.959**	0.268
L3	-0.014	3.139**	0.099*	0.722	-0.270*	17.748**	0.635*
L4	0.042	0.444	-0.070	-0.681	0.035	0.731	0.199
L5	-0.153*	0.694	-0.042	0.561	-0.259*	4.251	-1.093*
L6	0.125*	-0.500	-0.051	-0.525	-0.273*	4.412	0.310
L7	-0.014	-1.306*	0.022	-0.289	-0.534**	1.070	0.418
L8	0.097	0.694	0.130*	-0.728	0.480**	-12.830**	0.182
SE (gi)	0.048	0.450	0.042	0.323	0.104	2.975	0.245

GY: Grain yield (t ha^{-1}), TKW: Thousand kernel weight, NKRE: Number of kernels row per ear, NKR: Number of kernels per rows, DM: Days to maturity, ASI: Anthesis-silking interval, ED: Ear diameter, **Significant at p<0.01 level of probability, and *Significant at p<0.05 level of probability.

Estimates of SCA Effects

The estimate of specific combining ability (SCA) effects of the crosses for grain yield and yield related traits are illustrated in Table 3. Among all crosses evaluated in this experiment, L1×L6, L3×L5, L4×L6, L4×L8, and L5×L7 possess significant desirable positive SCA effect for 1000-kernel weight, that implies these well combined hybrids might be used for obtaining higher 1000-kernel weight and thereby improve grain yield. Conversely, the crosses L1×L4, L1×L5, L2×L3, L4×L5, L6×L7, and L7×L8 exhibited significant negative SCA effect for thousand kernel weight, suggesting the tendency of the hybrid to decrease the trait. Significant positive SCA effects for number of kernels per row and 1000-kernel weight were observed in L4×L7, indicating the tendency of the hybrids to improve grain yield. Conversely, L3×L8, L4×L5, and L6×L7 exhibited significant negative SCA effect for number of kernels per row, implying that the tendency of poor hybrids combinations to improve the trait. The results were comparable with the finding of Hassan et al. (2019).

Positive and significant SCA effects were observed in L5×L6 for ear diameter, implying that was the best specific combiner for higher ear diameter and might be used for obtaining high yielding hybrids. Conversely, L1×L5 and L6×L7 exhibited significant negative SCA effect for ear diameter. Significant negative SCA effects for days to maturity were observed in L1×L5, L3×L8, L6×L7, and L7×L8 towards earliness. Conversely, L1×L8, L3×L7, and L6×L8 had significant positive SCA effect for days to maturity towards lateness. Significant negative SCA effects for anthesis-silking interval were observed in L2×L4, L4×L6, and L7×L8. Conversely, L2×L6 and L4×L8 showed significant positive SCA effect for anthesis-silking interval.

Table 3. Estimates of specific combining ability (SCA) effects for yield and yield related trait of maize inbred lines evaluated in eastern Ethiopia.

Cross	ASI	DM	ED	NKR	NKRE	TKW	GY
L1×L2	-0.190	-2.286	-0.073	0.133	0.479	17.030	1.463
L1×L3	0.004	-1.813	-0.137	1.044	0.115	0.775	-0.704
L1×L4	-0.052	-0.119	0.066	-1.487	0.209	-38.992**	-1.784*
L1×L5	0.143	-3.702*	-0.278*	-2.095	-0.363	-29.912**	0.591
L1×L6	0.032	2.492	0.263	-0.242	0.184	25.327*	0.388
L1×L7	0.004	2.464	0.208	1.455	0.029	8.069	-0.187
L1×L8	0.060	2.964*	-0.050	1.194	-0.652	17.702	0.233
L2×L3	0.060	2.798	-0.145	-0.717	-0.096	-23.459*	0.477
L2×L4	-0.329*	-1.341	0.125	-1.448	-0.502	11.275	-0.687
L2×L5	0.032	-0.758	-0.187	0.644	-0.208	0.738	0.355
L2×L6	0.587**	-1.563	-0.012	0.663	-0.460	-10.456	-0.448
L2×L7	-0.107	2.075	0.066	0.527	0.201	11.886	-0.523
L2×L8	-0.052	1.075	0.225	0.199	0.587	-7.014	-0.637
L3×L4	0.198	-0.536	0.177	0.696	0.451	8.069	0.696
L3×L5	-0.107	1.381	0.150	0.555	-0.238	23.616*	1.005
L3×L6	-0.218	0.909	-0.042	-0.126	-0.091	-6.445	-0.915
L3×L7	0.087	3.214*	0.152	0.805	0.170	7.013	0.260
L3×L8	-0.024	-5.952**	-0.156	-2.256*	-0.310	-9.570	-0.820
L4×L5	0.004	-1.591	-0.198	-2.576*	-0.227	-53.634**	-1.059
L4×L6	-0.440**	1.437	-0.139	1.410	0.070	32.322**	1.055
L4×L7	0.198	0.909	0.072	2.924**	-0.069	19.680*	0.563
L4×L8	0.421*	1.242	-0.103	0.480	0.067	21.280*	1.216
L5×L6	-0.079	1.520	0.283*	1.769	0.631	16.919	-0.404
L5×L7	-0.107	2.825	0.111	1.433	0.142	36.828**	-0.612
L5×L8	0.115	0.325	0.119	0.271	0.262	5.444	0.124
L6×L7	0.282	-8.313**	-0.464**	-5.365**	-0.427	-56.650**	0.469
L6×L8	-0.163	3.520*	0.111	1.891	0.092	-1.017	-0.145
L7×L8	-0.357*	-3.175*	-0.145	-1.779	-0.046	-26.825**	0.030
SE (ij)	0.151	1.407	0.131	1.012	0.324	9.312	0.766

GY: Grain yield (t ha^{-1}), NKRE: Number of kernels row per ear, DM: Days to maturity, ASI: Anthesis-silking interval, ED: Ear diameter, NKR: Number of kernel per rows, TKW: Thousand kernel weight, **Significant at $p<0.01$ level of probability, and *Significant at $p<0.05$ level of probability.

Genotypic and Phenotypic Correlation

Grain yield is a complex quantitative trait, which is influenced by several component traits (Crosbie & Mock, 1981). The cumulative effects of such traits determine the yield. Therefore, to determine such relationships, correlation analyses were used, which enables to describe the direction and magnitude of relationship of targeted characters with attribute traits. The result obtained from correlation analysis showed that grain yield had significant positive genotypic correlation with 1000-kernel weight and ear diameter. This implies that 1000-kernel weight and ear diameter tend to enhance grain yield. Conversely, days to tasseling, days to silking, *Turciccum* leaf blight, plant aspect, ear aspect and *Puccinia sorghi* showed negative genotypic correlation with grain yield (Table 4).

Grain yield showed significant positive phenotypic correlation with 1000-kernel weight and anthesis-silking interval, whereas days to anthesis, days to silking, *Puccinia sorghi*, and ear aspect were exhibited significant negative phenotypic correlation with grain yield. The results were in parallel with the report of Reddy and Jabeen (2016), who highlighted that significant positive correlation of grain yield with 1000-kernel weight and ear diameter. The traits, days to silking and days to anthesis were exhibited highly significant negative correlation with grain yield at genotypic and phenotypic levels, which indicated that breeding for earliness has the potential of increasing yield unlike selection for flowering. The results were comparable with the report of Mhoswa et al. (2016).

Thousand kernel weight exhibited significant positive correlation with ear length, ear diameter, plant height, ear height, days to maturity, and number of kernels per row and hence, selection for these traits will help in further improvement of the given trait. On the contrary, the trait like days to anthesis, days to silking, *Turciccum* leaf blight, plant aspect, ear aspect, and *Puccinia sorghi* were negatively correlated with 1000-kernel weight at phenotypic and genotypic level. Likewise, number of kernels per row showed significant positive association with plant height, ear length, ear diameter, ear height, days to maturity, and 1000-kernel weight at genotypic and phenotypic level, which implies that the trait needs to be considered for indirect selection to improve the trait simultaneously. Conversely, days to anthesis, days to silking, *Puccinia sorghi*, plant aspect, and ear aspect were negatively correlated with number of kernels per row at genotypic and phenotypic level.

Ear diameter manifest significant positive association with plant height, days to maturity, ear height, ear length, number of kernels per row, number of kernel rows per ear, and 1000-kernel weight at genotypic and phenotypic level, indicating the possibility of simultaneous improvement of the trait, whereas days to anthesis, days to silking, plant aspect, and ear aspect were negatively correlated with ear diameter at genotypic and phenotypic level. The results were comparable with the report of Abenezer et al. (2020), who highlighted significant positive correlation of ear diameter with plant height, ear height, number of kernels per row, and 1000-kernel weight. Likewise, ear length exhibited significant positive correlation with ear height, plant height, days to maturity, ear diameter, number of kernels per row, and 1000-kernel weight at genotypic and phenotypic levels. Conversely, days to anthesis, days to silking, plant aspect, ear aspect, *Turciccum* leaf blight, and *Puccinia sorghi* were negatively correlated with ear length at phenotypic and genotypic level.

Days to maturity exhibited significant positive correlation with plant height, ear height, ear diameter, ear length, number of kernels per row, number of kernel rows per ear, and 1000-kernel weight at genotypic and phenotypic levels, implying that simultaneous selection of this trait might bring an improvement to grain yield at the expense of maturity period, while days to anthesis, days to silking, plant aspect, ear aspect, *Turciccum* leaf blight, and *Puccinia sorghi* were negatively correlated with days to maturity at genotypic and phenotypic level. Therefore, the positive and significant genotypic and phenotypic association of days to maturity with plant height and ear height tends to improve their height and maturity period simultaneously. The results were supported by the report of Mogesse (2021).

Number of kernel rows per ear, ear length, ear diameter, 1000-kernel weight, plant height, ear height, days to maturity showed positive and significantly inter correlated with each other. Accordingly, increasing ear diameters tends to increase the number of kernel rows per ear and consequently increases the grain yield. Likewise, increasing ear length tends to increase number of kernels per row, and thereby increase grain yield significantly. Therefore, selection for these traits will help in grain yield improvement.

Genotypic Path Coefficient Analysis

Genotypic path coefficient analysis was conducted to determine direct and indirect effects of secondary traits on grain yield (Table 5). According to the result of path coefficient analysis; days to anthesis, plant aspect, ear height, number of kernel rows per ear, 1000-kernel weight, ear length, *Turciccum* leaf blight, and days to maturity exhibited positive direct effects on grain yield, whereas days to silking, number of kernels per row, ear diameter, plant height, ear aspect, and *Puccinia sorghi* exhibited negative direct effects on grain yield at genotypic level. The results were comparable with earlier reports of Pandey et al. (2017) and Nabila et al. (2017).

The highest positive direct effect on grain yield was exhibited by *Turciccum* leaf blight followed by days to maturity, ear height, and days to anthesis at genotypic level. On the other hand, *Puccinia sorghi* had highest indirect effect on grain yield through days to anthesis followed by ear diameter through *Puccinia sorghi* and ear aspect, and days to silking through number of kernels per row.

Days to anthesis showed positive direct effect and significant negative association with grain yield at genotypic level. As a result, the direct effects are positive and the correlation coefficients are significantly negative, the indirect causal factors which are having positive effects *viz.*, ear diameter, plant aspect, plant height, *Turciccum* leaf blight, and number of kernels per row along with days to anthesis are to be considered simultaneously during selection.

The direct negative effect of ear diameter was more than compensated by its indirect effects hence resulting in positive correlation with grain yield, implying that grain yield can be improved through ear diameter indirectly. Positive indirect effects through days to silking, ear height, ear length, days to maturity, number of kernel rows per ear, ear aspect, and 1000-kernel weight are the possible causes of positive correlation between ear diameter and grain yield. Therefore, these characteristics should be taken into account if selection is made through ear diameter. Similar conclusion was also drawn by Mogesse (2021).

Number of kernel rows per ear, ear height, ear length, 1000-kernel weight, and days to maturity exhibited positive direct effects and also had positive association with grain yield. Therefore, number of kernel rows per ear, ear height, ear length, 1000-kernel weight, and days to maturity are important traits contributing to total grain yield and could be used as reliable indicators in indirect selection for grain yield as they have positive relationship to yield.

Table 4. Estimate of phenotypic (below diagonal) and genotypic (above diagonal) correlations for yield and yield related traits in maize.

Traits	DA	DS	ASI	PS	ET	PA	PH	EH	DM	EA	EL	ED	NKR	NKRE	TKW	GY
DA	**1.000**	0.993**	-0.277	0.871**	0.494**	0.775**	-0.800**	-0.787**	-0.667**	0.615**	-0.811**	-0.682**	-0.737**	-0.128	-0.663**	-0.580**
DS	0.982**	**1.000**	-0.151	0.771**	0.479**	0.712**	-0.784**	-0.780**	-0.592**	0.605**	-0.848**	-0.596**	-0.764**	-0.206	-0.624**	-0.501**
ASI	-0.178	-0.065	**1.000**	-0.691**	-0.147	-0.162	-0.031	-0.046	-0.136	-0.103	-0.128	0.052	-0.180	-0.034	-0.005	0.778**
PS	0.598**	0.572**	-0.359	**1.000**	0.695**	0.593**	-0.768**	-0.788**	-0.784**	0.371	-0.461*	-0.429*	-0.458*	-0.308	-0.409*	-0.670**
ET	0.309	0.299	-0.130	0.665**	**1.000**	0.435*	-0.643**	-0.761**	-0.571**	0.442*	-0.494**	-0.340	-0.288	-0.459*	-0.445*	-0.449*
PA	0.598**	0.617**	-0.020	0.527**	0.415*	**1.000**	-0.781**	-0.999**	-0.820**	0.744**	-0.789**	-0.864**	-0.884**	-0.383*	-0.726**	-0.395*
PH	-0.661**	-0.680**	0.019	-0.628**	-0.543**	-0.715**	**1.000**	0.922**	0.695**	-0.424*	0.804**	0.464*	0.744**	0.276	0.635**	0.126
EH	-0.641**	-0.657**	0.023	-0.578**	-0.556**	-0.798**	0.861**	**1.000**	0.677**	-0.680**	0.874**	0.664**	0.805**	0.216	0.809**	0.250
DM	-0.582**	-0.605**	-0.115	-0.664**	-0.491**	-0.676**	0.697**	0.651**	**1.000**	-0.510**	0.586**	0.728**	0.606**	0.296	0.556**	0.253
EA	0.515**	0.518**	-0.001	0.353	0.354	0.690**	-0.395*	-0.604**	-0.552**	**1.000**	-0.770**	-0.833**	-0.717**	-0.545**	-0.575**	-0.522*
EL	-0.731**	-0.751**	-0.092	-0.417*	-0.414*	-0.724**	0.755**	0.794**	0.667**	-0.698**	**1.000**	0.619**	0.887**	0.216	0.824**	0.323
ED	-0.540**	-0.541**	0.044	-0.372	-0.292	-0.721**	0.456*	0.592**	0.656**	-0.757**	0.605**	**1.000**	0.676**	0.577**	0.617**	0.432*
NKR	-0.665**	-0.693**	-0.131	-0.381*	-0.212	-0.799**	0.671**	0.693**	0.619**	-0.639**	0.871**	0.645**	**1.000**	0.216	0.723**	0.115
NKRE	-0.109	-0.115	0.022	-0.294	-0.316	-0.401*	0.270	0.215	0.392*	-0.492**	0.141	0.597**	0.162	**1.000**	-0.030	0.223
TKW	-0.590**	-0.599**	-0.038	-0.340	-0.363	-0.592**	0.598**	0.731**	0.556**	-0.556**	0.836**	0.554**	0.680**	-0.024	**1.000**	0.457*
GY	-0.504**	-0.455*	0.455*	-0.429*	-0.263	-0.330	0.108	0.242	0.217	-0.463*	0.270	0.370	0.100	0.182	0.436*	**1.000**

GY: Grain yield, DA: Days to anthesis, ASI: Anthesis-silking interval, ED: Ear diameter, EH: Ear height, EL: Ear length, NKR: Number of kernels per row, PH: Plant height, NKRE: Number of kernel rows per ear, DS: Days to silking, TKW: Thousand kernels weight, DM: Days to maturity, PA: Plant aspect, EA: Ear aspect, PS: *Puccinia sorghi*, ET: *Turcicum* leaf blight caused by *Exserohilum turcicum* (Pass.), **Significant at $p<0.01$ level of probability, and *Significant at $p<0.05$ level of probability.

Table 5. Partitioning genotypic in to direct (diagonal bold) and indirect (off diagonal) effects of different traitson grain yield of maize.

Traits	DA	DS	PS	ET	PA	PH	EH	DM	EA	EL	ED	NKR	NKRE	TKW	rg
DA	**0.441**	-0.362	-0.016	0.327	0.097	0.476	-0.563	-0.517	-0.719	-0.196	0.205	0.873	-0.170	-0.458	-0.580
DS	0.417	**-0.386**	-0.330	0.284	0.089	0.447	-0.602	-0.259	-0.307	-0.205	0.054	0.941	-0.274	-0.370	-0.501
PS	0.998	-0.609	**-0.314**	0.865	0.074	0.417	-0.566	-0.607	-0.433	-0.111	0.759	0.165	-0.409	-0.899	-0.670
ET	0.701	-0.421	-0.508	**0.683**	0.054	0.186	-0.311	-0.242	-0.316	-0.119	0.601	0.731	-0.510	-0.977	-0.449
PA	0.666	-0.212	-0.172	0.167	**0.125**	0.441	-0.621	-0.435	-0.368	-0.191	0.529	0.248	-0.309	-0.261	-0.395
PH	-0.754	0.656	0.777	-0.724	-0.097	**-0.845**	0.832	0.538	0.495	0.195	-0.820	-0.890	0.367	0.396	0.126
EH	-0.707	0.239	0.324	-0.041	-0.125	-0.702	**0.466**	0.124	0.238	0.115	-0.174	-0.046	0.161	0.377	0.250
DM	-0.296	0.006	0.191	-0.532	-0.102	-0.283	0.345	**0.638**	0.354	0.142	-0.287	-0.539	0.394	0.222	0.253
EA	0.118	-0.050	-0.858	0.185	0.093	0.782	-0.350	-0.395	**-0.168**	-0.186	0.472	0.822	-0.724	-0.263	-0.522
EL	-0.789	0.472	0.066	-0.324	-0.099	-0.484	0.136	0.254	0.499	**0.242**	-0.095	-0.254	0.287	0.411	0.323
ED	-0.345	0.018	0.993	-0.911	-0.108	-0.856	0.318	0.564	0.972	0.150	**-0.769**	-0.717	0.767	0.355	0.432
NKR	-0.537	0.364	0.061	-0.772	-0.110	-0.373	0.399	0.269	0.611	0.215	-0.195	**-0.541**	0.287	0.437	0.115
NKRE	-0.439	0.468	0.452	-0.232	-0.048	-0.509	0.33	0.129	0.426	0.052	-0.021	-0.549	**0.229**	-0.065	0.223
TKW	-0.283	0.112	0.646	-0.193	-0.091	-0.172	0.406	0.231	0.371	0.199	-0.091	-0.837	-0.040	**0.197**	0.457

DA: Days to anthesis, ASI: Anthesis-silking interval, ED: Ear diameter, EH: Ear height, EL: Ear length, NKR: Number of kernels per row, PH: Plant height, NKRE: Number of kernel rows per ear, DS: Days to silking, TKW: Thousand kernels weight, DM: Days to maturity, PA: Plant aspect, EA: Ear aspect, PS: *Puccinia sorghi*, and ET: *Turcicum* leaf blight caused by *Exserohilum turcicum* (Pass.).

Phenotypic Path Coefficient Analysis

The phenotypic path coefficient analysis was done to determine direct and indirect effects of secondary traits on grain yield. The results of phenotypic direct and indirect effects of secondary traits on grain yield are illustrated in Table 6. According to phenotypic path coefficient analysis number of kernel rows per ear, ear length, days to silking, *Turciccum* leaf blight, and 1000-kernel weight exhibited positive direct effects on grain yield; whereas days to anthesis, days to maturity, number of kernel per row, ear diameter, ear aspect, plant aspect plant height, ear height, and *Puccinia sorghi* showed negative direct effect on grain yield at phenotypic level, implying that selection for high yield can be carried out indirectly through yield components. Among all the highest positive direct effect on grain yield was exhibited by 1000-kernel weight followed by days to silking and ear length at phenotypic level. The results were in line with the earlier report of Rafiq et al. (2010). On the other hand, plant height had highest indirect effect on grain yield through days to anthesis (0.911) followed by ear height (0.883) through days to anthesis and plant aspect (0.836) through number of kernels per row. Therefore, these traits could be considered as the main components for selection in further breeding process to improve grain yield. A negative direct effect was observed in plant height, plant aspect, ear height, ear aspect, days to maturity, days to anthesis, ear diameter, number of kernels per row, and *Puccinia sorghi* on grain yield. The results were in accordance with the previous report of Mogesse (2021). Moreover, plant height, ear height, days to maturity, ear diameter, and number of kernels per row exhibited positive phenotypic correlations with grain yield. The trait, days to 50% silking exhibited positive direct effects and significant negative association with grain yield at phenotypic level. Since the direct effects are positive and the correlation coefficients are significantly negative, the indirect causal factors which are having positive effects *viz*., plant height, ear height, days to maturity, and number of kernels per row along with days to 50% silking are to be considered simultaneously during selection.

Table 6. Partitioning phenotypic in to direct (diagonal bold) and indirect (off diagonal) effects of different traits on grain yield of maize.

Traits	DA	DS	PS	ET	PA	PH	EH	DM	EA	EL	ED	NKR	NKRE	TKW	rg
DA	**-0.473**	0.511	-0.359	0.050	-0.472	0.166	0.248	0.177	-0.185	-0.084	0.060	0.352	-0.014	-0.481	-0.504
DS	-0.368	**0.596**	-0.247	0.048	-0.487	0.171	0.182	0.144	-0.166	-0.086	0.060	0.203	-0.015	-0.488	-0.455
PS	-0.825	0.473	**-0.432**	0.107	-0.416	0.158	0.341	0.202	-0.114	-0.048	0.041	0.398	-0.038	-0.277	-0.429
ET	-0.426	0.247	-0.287	**0.161**	-0.328	0.136	0.329	0.149	-0.114	-0.048	0.032	0.222	-0.041	-0.296	-0.263
PA	-0.823	0.510	-0.228	0.067	**-0.790**	0.179	0.471	0.205	-0.222	-0.083	0.080	0.836	-0.051	-0.482	-0.330
PH	0.911	-0.562	0.271	-0.088	0.564	**-0.251**	-0.509	-0.212	0.127	0.087	-0.051	-0.702	0.035	0.487	0.108
EH	0.883	-0.543	0.250	-0.090	0.630	-0.216	**-0.591**	-0.198	0.194	0.091	-0.066	-0.726	0.028	0.596	0.242
DM	0.803	-0.500	0.287	-0.079	0.534	-0.175	-0.385	**-0.304**	0.177	0.077	-0.073	-0.648	0.050	0.453	0.217
EA	-0.710	0.428	-0.153	0.057	-0.545	0.099	0.357	0.168	**-0.321**	-0.080	0.084	0.669	-0.063	-0.454	-0.463
EL	0.807	-0.621	0.280	-0.067	0.571	-0.190	-0.469	-0.203	0.224	**0.215**	-0.067	-0.911	0.018	0.682	0.270
ED	0.744	-0.447	0.161	-0.047	0.569	-0.114	-0.350	-0.199	0.243	0.070	**-0.111**	-0.675	0.077	0.451	0.370
NKR	0.461	-0.573	0.164	-0.034	0.286	-0.168	-0.410	-0.188	0.205	0.100	-0.072	**-0.047**	0.021	0.354	0.100
NKRE	0.151	-0.095	0.127	-0.051	0.317	-0.068	-0.127	-0.119	0.158	0.016	-0.066	-0.170	**0.128**	-0.019	0.182
TKW	0.813	-0.495	0.147	-0.059	0.467	-0.150	-0.432	-0.169	0.179	0.096	-0.062	-0.712	-0.003	**0.815**	0.436

DA: Days to anthesis, ASI: Anthesis-silking interval, ED: Ear diameter, EH: Ear height, EL: Ear length, NKR: Number of kernels per row, PH: Plant height, NKRE: Number of kernel rows per ear, DS: Days to silking, TKW: Thousand kernels weight, DM: Days to maturity, PA: Plant aspect, EA: Ear aspect, PS: *Puccinia sorghi*, and ET: *Turcicum* leaf blight caused by *Exserohilum turcicum* (Pass.).

Conclusion

Information on combining ability of parent and relationship among traits are required to increase the efficiency of selection in plant breeding programs. Therefore, the research aimed to quantify combining ability and the relationships between grain yield and yield contributing traits. According to combining ability analysis of variance L2 and L7 were found the best general combiner towards earliness. Likewise, parental line L3 identified as good general combiner to improve grain yield, 1000-kernel weight, ear diameter, and lateness in maturity. Therefore, L3 might be used in hybrid breeding program to obtain high yielding hybrids. Similarly, L2 was proved as the best general combiner for number of kernel row per ear and 1000-kernel weight. L1 and L8 were found good general combiner for number of kernel row per ear. Therefore, based on SCA effects, it might be concluded that L1, L2, L3, and L8 could be used for obtaining high yielding hybrids. On the other hand, crosses L1×L6, L4×L6, L4×L8, and L5×L7 exhibited significant positive SCA effects for 1000-kernel weight. Similarly, the cross L5×L6 was good specific combiner for ear diameter, while L4×L7 for both number of kernels per row and 1000-kernel weight. Moreover, the crosses L1×L5, L3×L8, L6×L7, and L7×L8 showed significant negative SCA effect for days to maturity that depicted the contribution of genes for earliness. According to correlation and path coefficient analysis, grain yield exhibit significant positive correlation with anthesis-silking interval and 1000-kernel weight at genotypic and phenotypic level. However, ear aspect, days to tasseling, days to silking, and *Puccinia sorghi* showed negative and significant correlations with grain yield. On the other hand, number of kernel rows per ear, 1000-kernel weight, and ear length exhibited positive direct effect on grain yield at genotypic and phenotypic levels. Thus, these traits can be considered as the main components for selection in hybrid breeding program with a view to obtaining high yielding hybrids.

Conflict of Interest

The authors declare that they have no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Pb (II) Recovery by Trout Bones: Adsorption, Desorption and Kinetic Study

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ARTICLE INFO

Article History
Received: 24.10.2022
Accepted: 21.11.2022
First Published: 22.12.2022

Keywords
Adsorption kinetics
Desorption
Lead removal
Rainbow trout

ABSTRACT

Heavy metal removal from the water was studied by using fish bones produced in the trout farm of Atatürk University Faculty of Fisheries. Fish bones used as adsorbent were obtained from rainbow trout (*Oncorhynchus mykiss*). Trout bone was used in its natural form. According to the experimental results that maximum Pb (II) adsorption capacity of rainbow trout bones was 188.16 mg/g. The Langmuir, Freundlich, and Temkin isotherm models were applied to describe the adsorption of Pb (II) on trout bones. Langmuir and Freundlich isotherm models were found more favourable than Temkin with the correlation coefficients of 0.999, 0.999, and 0.857, respectively. Controllable factors used in this study were solution pH, temperature, adsorbent dosage, mixing speed, and initial Pb (II) concentration. The optimum working parameter values for Pb (II) adsorption using trout bones were found to be 5.5, 30 ºC, 3 g/L, 200 rpm, and 10 mg/L for pH, temperature, adsorbent concentration, stirring speed, and initial Pb (II) concentration, respectively. The adsorption kinetics of Pb adsorption by trout bones was modelled using the pseudo-first order and the pseudo-second order kinetics equations. The results indicate that, pseudo-second-order kinetic model gives more favourable results (R^2_{mean} = 0.997) than pseudo-first-order (R^2_{mean} = 0.971). Fish bones were characterized by some instrumental analyses such as SEM, EDS, FTIR, and zeta potential measurements. In the regeneration phase of the study, maximum desorption efficiency was 95.86% at pH 1.5.

Please cite this paper as follows:

Kocadagistan, B. (2022). Pb (II) recovery by trout bones: Adsorption, desorption and kinetic study. *Journal of Agricultural Production*, *3*(2), 88-99. https://doi.org/10.56430/japro.1193955

Introduction

Heavy metals are more commonly known as metals and their compounds having an atomic density greater than 4±1 g/cm^3. Cu, Zn, Hg, Cd, Pb, Sn, Mn, As, Cr, Co, Ni, and Ag can be counted as heavy metals, which are considered the most common toxic mineral pollutants in water and soil systems (Nadeem et al., 2006). Many of these substances have been blacklisted by various international organizations as they cause soil and water pollution (Edelstein & Ben-Hur, 2018; L. Liu et al., 2018; Chen et al., 2021). Heavy metals cause significant damage to human health because of their accumulation properties and difficult decomposition. Methods such as chemical precipitation, electrochemical and redox removal, ion exchange, adsorption and membrane separation are frequently used to remove heavy metals from wastewater (Demirbas, 2008). Lead is one of the most detected pollutants in aqueous environments and soils. It is used as a raw material for a wide variety of industries, especially battery manufacturing (Abdelhafez & Li, 2016; L. Liu et al., 2018; Chen et al., 2021). Lead concentrations in most bodies of water on the planet are above the EPA's action level of 15 ppb. Removal of this toxic metal from aquatic environments such as drinking water, stream water, groundwater, and wastewater is very important for human life (Abdelhafez & Li, 2016). The type, form, and concentration of lead or any other heavy metal in water and wastewater play a decisive role in the selection of treatment processes to be designed to remove them.

In the literature, there are many traditional and new wastewater treatment methods such as chemical precipitation (Kumar et al., 2021), micro, ultra, and nanofiltration (Abdullah

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et al., 2019), electrochemical oxidation (Martínez-Huitle & Panizza, 2018), ion exchange (Dąbrowski et al., 2004), and adsorption (Senthil Kumar & Gayathri, 2009; Basu et al., 2017; R. Liu et al., 2018; Lu et al., 2019; Zhu et al., 2019) for lead removal from water and wastewater. Among these methods, adsorption is one of the most used techniques in heavy metal removal due to its high efficiency and low cost (Hayati et al., 2017).

One of the factors limiting the widespread use in adsorption processes is the determination of adsorbent with high adsorption capacity but low cost. Therefore, adsorbents obtained from natural sources have attracted great interest (Silva-Yumi et al., 2018). There are many heavy metal adsorption studies in the literature, especially with adsorbents obtained from natural sources such as agricultural wastes and their modified forms (Dubey & Gopal, 2007; Bansal et al., 2009; Qiao et al., 2019; Yu et al., 2022), wheat straw (Khan et al., 2021), biochar (Q. Wang et al., 2018; Guan et al., 2022), clay (Xie et al., 2018), tea plant waste (Ibrehem, 2019).

In heavy metal removal processes by adsorption, another important point is that heavy metal ions retained by adsorption are not released back into the nature after the process. Because the metal binding mechanism is mostly by chemical adsorption, the adsorbed metal can be desorbed under suitable conditions (Tongtavee et al., 2021). Performing desorption in a controlled manner can turn this situation into an advantage and the materials left behind after adsorption can be recovered in an efficient, economical, and environmentally friendly way. Also, desorption is an environmentally friendly technique with low energy consumption and due to the reversible nature of most adsorption processes, adsorbents can be used repeatedly with simple desorption methods (Charoenchai & Tangbunsuk, 2022).

Aquaculture is an alternative food that meets the protein needs of the increasing world population. Fish bones are an important part of the wastes generated in fish production facilities, which are becoming increasingly widespread throughout the world. Global production of aquatic animals was around 178 million tons in 2020. About 51% (90 million tons) of this amount was fisheries. In addition, 63 percent (70 percent from caught fishing and 30 percent from aquaculture) of the total production was harvested in marine waters and 37 percent (83 percent from aquaculture and 17 percent from hunting) from inland waters (FAO, 2022). In 2021, 335,644 tons of aquaculture production in Türkiye took place in the seas and 136,042 tons in inland waters. The most important fish species grown were trout with 135,732 tons in inland waters, sea bass with 155,151 tons and sea bream with 133,476 tons in seas (TÜİK, 2022). In addition, aquaculture contributes more than 53 percent to world aquaculture production, which is worth 232 billion USD (FAO, 2022).

In this study, rainbow trout bones (RTB) was selected and tested as an adsorbent for waste recycling. It is thought that it is very interesting to use the bones of rainbow trout, which is a fish species that can live in clean waters, to clean the water.

The aim of this study is to investigate the usability of RTB for Pb (II) removal via adsorption. In this way, it is thought that an effective and reusable natural novel metal ion adsorbent will be presented to the field of application. Regeneration processes have not been studied much in order to reuse the adsorbents used in adsorption studies, which are frequently encountered in the literature. It is thought that the study will contribute to the literature in terms of waste minimization and evaluation. In order to achieve this aim, the effects of operating parameters such as pH, temperature, stirring speed, time, adsorbate, and adsorbent concentrations on adsorption were investigated in detail. In addition, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and Brunauer-Emmett-Teller (BET) analyses for rainbow trout bones were made and presented to the literature. With the help of kinetic models, the adsorption behaviour of these bones was also tried to be revealed.

Materials and Methods

Chemicals

In experimental studies, aqueous $Pb(NO_3)_2$ was used as a stock solution at a concentration of 1000 mg/L Pb (II). HCl and NaOH solutions were used for pH adjustment and HNO_3 solutions were used for regeneration experiments. All chemicals were analytical reagent grade from E. Merck, Darmstadt, Germany.

Adsorbent

In the study, artificial pond rainbow trout bones and vertebrae were used as adsorbent and this material was obtained from Atatürk University Fisheries Faculty Inland Water Fish Application and Research Centre.

RTB were first separated from their meat as much as possible, washed with hot water many times to remove their fat, and then dried in an oven at 105 ºC by washing with distilled water. Then, to remove organic residues (oil and fatty acids), ethanol was added and mixed in a shaker for two hours at room temperature. This process was repeated three times and the bones were washed with distilled water and dried. After this process, all RTB that became brittle were powdered and kept in a desiccator from a humid environment until the experiments were carried out. Mineral and chemical composition of RTB were given in Table 1.

Table 1. Mineral composition of RTB lipid free dry matter (Toppe et al., 2007).

Mineral		**Quantity**
Calcium	g/kg	147
Phosphorus	g/kg	87
Magnesium	g/kg	2.4
Iron	mg/kg	32
Zinc	mg/kg	126
Copper	mg/kg	0.9
Chromium	mg/kg	6.7
Sodium	g/kg	5.8
Potassium	mg/kg	7.7
Selenium	mg/kg	-
Iodine	mg/kg	2.5
Chlorine	g/kg	4.2
Fluorine	g/kg	0.09
Arsenic	mg/kg	1.2
Cadmium	mg/kg	0.02
Mercury	mg/kg	0.01
Lead	mg/kg	-

Analyses

CrisonpH25+ brand pH meter device was used. Samples were centrifuged before analyses with Nüve NF 1200R centrifuge device. Pb (II) concentrations were measured at 283.3 nm wavelength, 10 mA current and 0.5 nm slit width by using a Shimadzu AA6800 atomic adsorption spectrophotometer. Scanning Electron Microscopy (ZEISS SIGMA 300), Fourier-Transform Infrared Spectroscopy (Bruker VERTEX 70v), Brunauer-Emmett-Teller (Micromeritics 3 Flex), and Zeta potential (Malvern Zetasizer Nano ZSP) analyses were performed to reveal adsorbent characterization. These analyses were carried out in the laboratories of Atatürk University Eastern Anatolia High Technology Application and Research Centre (DAYTAM).

Analyses throughout the study were carried out in triplicate and carried out according to the standard methods (Rice et al., 2012). The results presented in the article are the average of repetitions. In addition, error function analyses were used in isotherm and kinetic model calculations for the reliability of the results.

Adsorption Experiments

Adsorption experiments were carried out with 100 mL Erlenmeyer flasks. During the experiments, initial Pb (II) concentrations were between 10 and 200 mg/L. The pH values were used between 2 and 12 and the adsorbent concentrations were between 0.25 and 3 g/L. Solution temperatures were maintained between 20 ºC and 40 ºC in a thermoregulated shaker (Edmund Bühler Incubator HoodTH15) and mixing speeds and times were applied in the range of 100 to 300 rpm and 1 to 300 minutes, respectively.

Adsorbed Pb (II) amount onto per unit weight of adsorbent (mg/g) is calculated with the following equation (Bardestani et al., 2019):

$$q_e = \frac{(C_o - C_e).V}{m} \quad (1)$$

where, C_o and C_t are the Pb (II) concentrations (mg/L) at time 0 and t, respectively; V is the volume of the Pb (II) solution (L); and m is the weight of material (g).

Desorption Experiments

The suitability of an adsorbent for regeneration is an important factor in addition to its properties such as adsorption capacity or cost. For this reason, the RTB used in this study were subjected to desorption experiments to measure whether they could be reused or not, in addition to examining their adsorption capacity.

Desorption experiments continued with the process of taking the adsorbent into a 0.1 M HNO_3 solution/distilled water mixture after the adsorption process reached equilibrium and regenerating it under mechanical stirring at 200 rpm for 8 hours at room temperature. The amount of lead transferred to the solutions in this way was measured at different time intervals.

In order to reveal the pH effect, the pH range of the regeneration solution was changed as in the adsorption process. In the study, adsorption and desorption processes were considered as a whole and this procedure was carried out for all samples immediately after the completion of the adsorption process.

Results and Discussion

The Effect of Operational Parameters

pH affects the electronic equilibrium on non-covalent bonds and destabilizes the electronic configuration in the medium. Therefore, it is one of the most important physicochemical parameters in adsorption processes. Low pH solutions contain high concentrations of hydronium cations and compete for a significant number of functional groups interacting with light metals or other metal cations (Cid et al., 2020). As the pH increases, the more negatively charged ligands are depleted and then metallic cations are attracted to the cell surface, resulting in binding (Yun et al., 2001). Finding the optimum adsorption pH is very important as pH affects the removal of metal ions from aqueous solutions by influencing the chemistry of metals and changing the surface charge of particles (Asadi et al., 2020). At the same time, pH is an important parameter in desorption, although not as much as in adsorption, since the amount of heavy metal recovered as a result of the desorption process also depends on the amount of heavy metal retained by the adsorption process. General desorption is controlled by both metal desorption and re-adsorption reactions.

In metal ion adsorption processes, as the pH rises to the neutral zone and above, metal compounds begin to precipitate in the solution; on the contrary, the adsorbent surface is positively charged and does not attract metal ions. In both cases, the adsorption efficiency falls outside the optimum value. Therefore, Zeta Potential values of adsorbent against pH are very helpful to find the optimum pH value. In a solution containing Pb (II) ions, below pH 3.30, the dominant type is Pb (II) ions in the solution, while lead will begin to precipitate as $Pb(OH)_2$, as the pH starts to rise (Awual & Hasan, 2019).

Zeta Potential values of RTB for different pH values are given in Figure 1. As seen in the figure, positive and negative peak values were measured as +18 mV and -236 mV at pH 2 and 14, respectively.

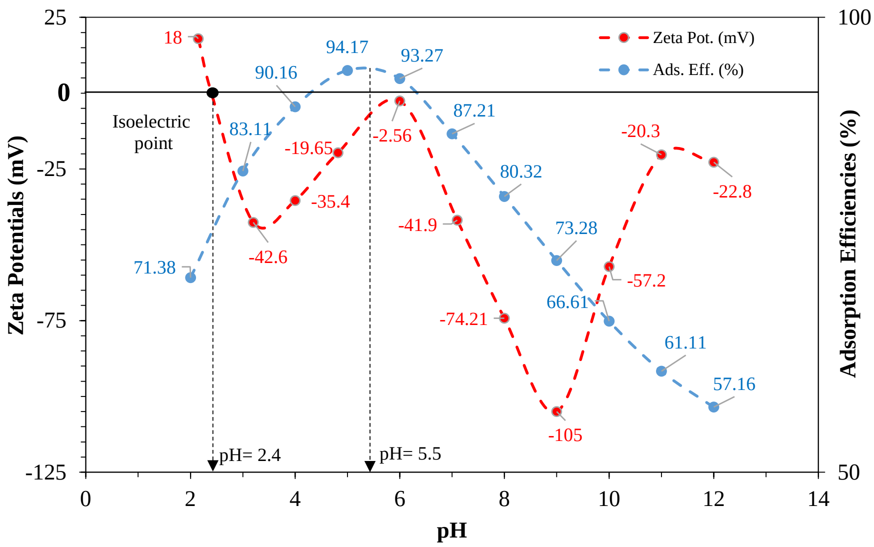

Figure 1. pH versus zeta potential and adsorption efficiencies graph of RTB.

As can be seen from the graph, the zeta potential values of RTB at different pH values have a wide range from +18 mV to -105 mV. However, when evaluated together with the adsorption efficiencies [for 3 g/L adsorbent, 10 mg/L initial Pb (II) concentration, and 30 ºC conditions] obtained at these pH values, it is seen that the highest efficiency is obtained at pH 5.5. The zeta potential value was measured as -7 mV at the pH value where this yield was obtained. Theoretically, at higher negative potential values, positively charged lead ions should bind more to the adsorbent. However, as mentioned above, this is not always the case in metal chemistry. Because at high pH values (pH 5 and above) where higher potential values are obtained, precipitation occurs in the solution and the adsorption efficiency decreases (Y. Wang et al., 2013). Therefore, pH 5.5 was chosen as the optimum value in the study.

Lead concentrations of 10, 25, 50, 100, and 200 mg/L were applied to evaluate the effect of the initial Pb (II) concentration on adsorption. Adsorption efficiencies versus time at different initial Pb (II) concentrations are given in Figure 2.

As seen in Figure 2, the amount of Pb (II) remaining in the solution increased with increasing initial concentration, and the adsorption efficiency decreased at the same rate. Maximum adsorption efficiencies were found as 87.4, 84.56, 85.21, 80.79, and 70.5% for 10, 25, 50, 100, and 200 mg/L initial Pb (II) concentrations, respectively. According to these results, the optimum initial Pb (II) concentration was determined as 10 mg/L. The case of higher adsorption efficiency at low initial concentrations can be explained by the fact that when the initial concentration of metal ions is low, there are many adsorption sites on the adsorbent surface and metal ions can fully react with the adsorption sites. Therefore, the removal rate is higher (Chen et al., 2021).

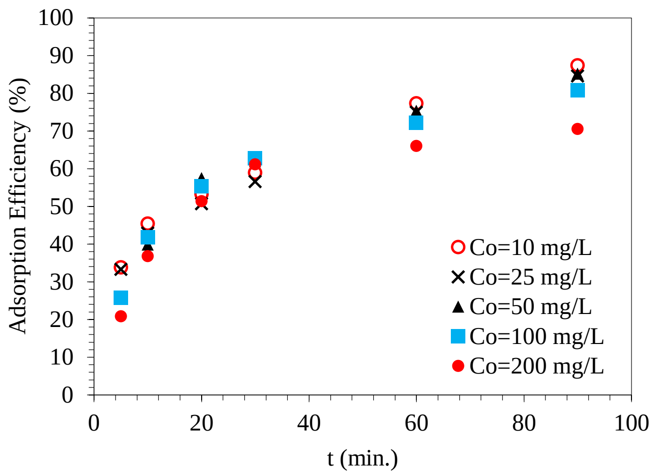

Figure 2. Pb (II) removal efficiencies versus time for different initial Pb (II) concentration (m = 3 g/L, pH = 5.5, T = 30 ºC, agitation speed = 200 rpm).

Figure 3 shows the change in the Pb (II) removal rates when the adsorbent dosage is changed from 1 mg/L to 3 mg/L. As can be seen in Figure 3, the concentration of Pb (II) remaining in the solution decreases (removal rates increases) as the adsorbent dosage increases from 1 to 3 g/L due to the presence of more adsorbent surface area in the solution. With the increasing adsorbent concentration, there was not a big difference in the removal efficiencies at equilibrium, but the highest yield was obtained with 3 g/L RTB concentration. As it can be seen when Figure 3 is examined, while the removal efficiency is about 78% at 0.25 g/L adsorbent concentration, this value reached a maximum of 87.4% at 3 g/L.

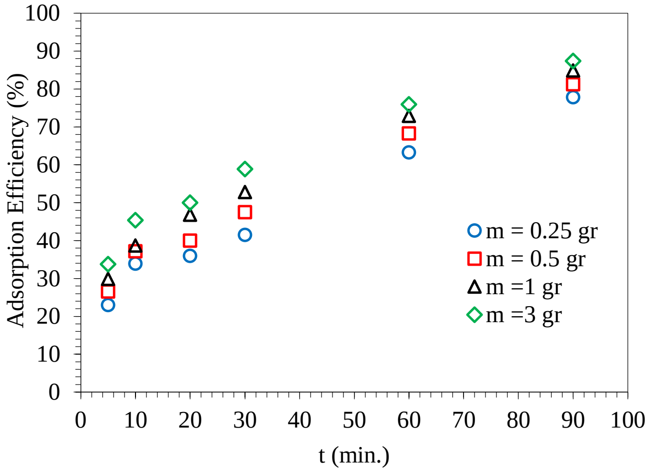

Figure 3. Pb (II) removal efficiencies versus time for different adsorbent concentration (C_o = 10 mg/L, pH = 5.5, T = 30 ºC, agitation speed = 200 rpm).

Shaker speeds were varied between 100 rpm and 300 rpm to reveal how the mixing speed affected the adsorption. As can be seen from Figure 4, the amounts of Pb (II) concentrations remaining in the solution are lower at 100 and 200 rpm than at 250 and 300 rpm. The highest removal efficiency was obtained at 200 rpm and decreased with increasing speed, since high mixing speeds caused the boundary layer to decrease and the bed resistance to decrease, resulting in a decrease in the amount of adsorbed material (Taha et al., 2016).

Temperature has a significant effect on the adsorption efficiency. In the study, the experiments were carried out at three different temperatures ranging from 20 to 40 ºC. As can be seen in Figure 5, the temperature increases from 20 ºC to 30 ºC positively affected the adsorption efficiency. however, the efficiency decreases as the temperature rises from 30 ºC to 40 ºC. This is due to the exothermic nature of the process or the reduced effect of physical forces at high temperature (Kennedy et al., 2007).

As a result of the data obtained in studies examining the effects of operational parameters on adsorption, the most suitable operating parameter values for Pb (II) adsorption using RTB, temperature, adsorbent concentration, mixing speed, and initial Pb (II) concentration were 30 ºC, 3 g/L, 200 rpm, and 10 mg/L, respectively.

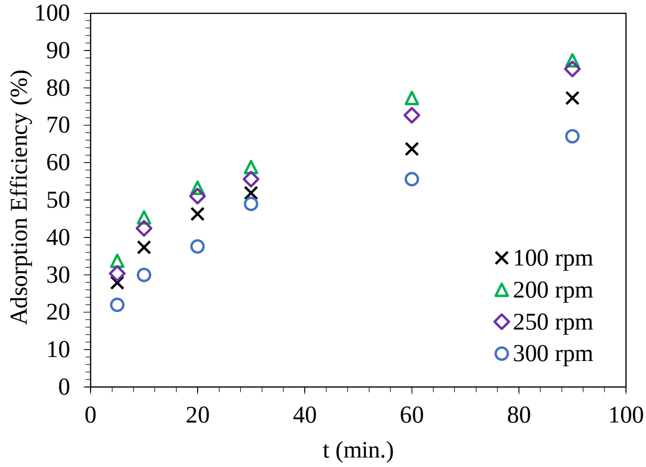

Figure 4. Pb (II) removal efficiencies versus time for different agitation speed (C_o = 10 mg/L, m = 3 g/L, pH = 5.5, T = 30 ºC).

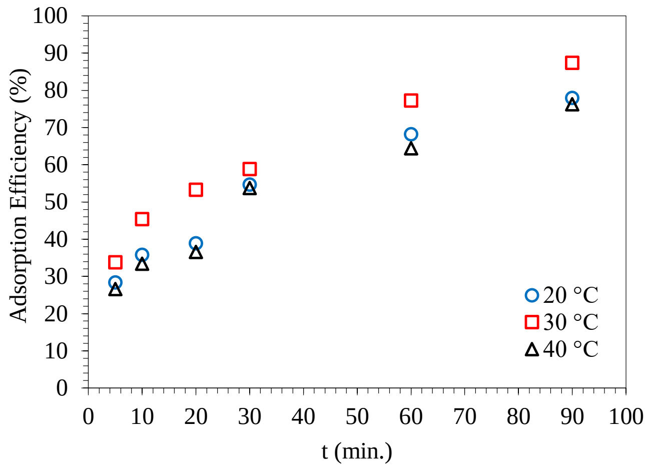

Figure 5. Pb (II) removal efficiencies versus time for different temperature (C_o = 10 mg/L, m = 3 g/L, pH = 5.5, agitation speed = 200 rpm).

Adsorbent Characterization

Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FTIR), and Brunauer-Emmett-Teller (BET) analyses were performed to examine the characteristic structure of RTB. BET and Langmuir surface area of RTB are 1.86 and 2.08 m^2/g, respectively. The other values obtained by BET analyse are given in Table 2.

Table 2. BET analyse values of RTB.

Parameter	Size
BET surface area (m^2/g)	1.8660±1.4511
Langmuir surface area (m^2/g)	2.0847
g-Plot micropore area (m^2/g)	0.9102
g-Plot out surface area (m^2/g)	0.9558
Mean particle size (ɳm)	3.215, 3.546
Mean particle diameter (ɳm)	1.4410
Maximum pore volume (cm^3/g)	0.176931750

SEM analysis is a frequently used method for surface morphology analysis of an adsorbent and was used to examine the structure of RTB and to reveal the changes that may occur after adsorption in this study. SEM images of RTB before and after adsorption are given in Figure 6.

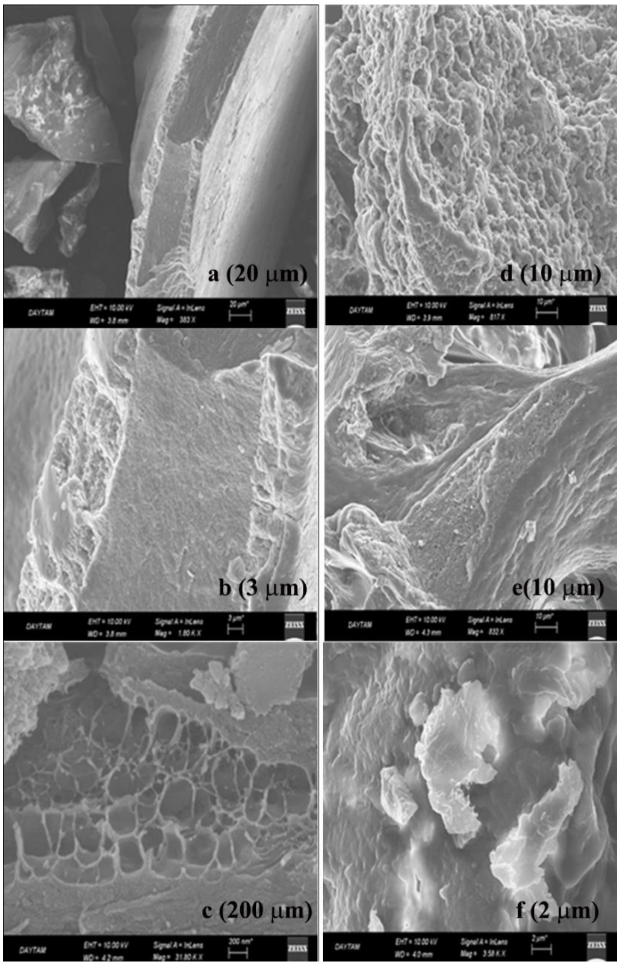

Figure 6. SEM images of RTB before (a, b, and c) and after (d, e, and f) adsorption.

The porous structure of the RTB surface is shown in Figure 6. The pores are quite heterogeneous and have sharp edges. When the pre- and post-adsorption images are compared, it is seen that the structure morphology changes slightly after adsorption but still does not deteriorate much.

The FTIR spectroscopy graph is given in Figure 7. As can be seen from Figure 7, FT-IR spectrum peaks of raw RTB have upper or lower transmittance values than those of after adsorption.

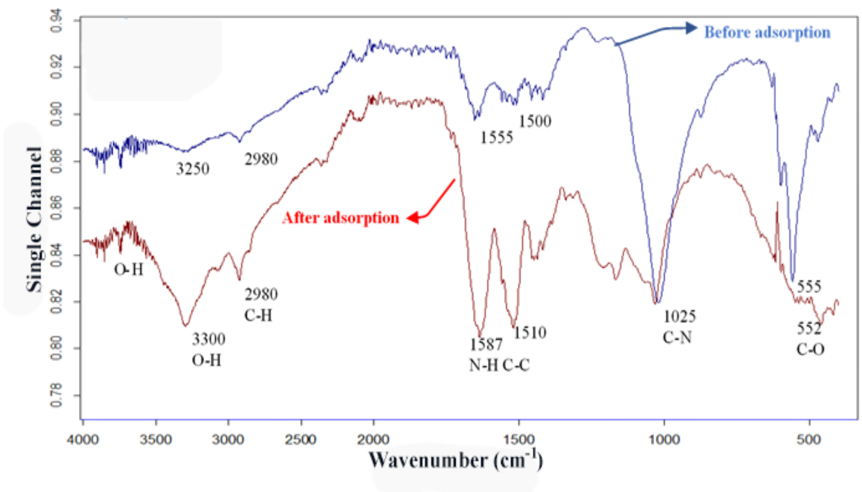

Figure 7. FTIR spectrum of RTB before and after Pb (II) adsorption.

It was observed that the values of the peaks, which were 3250, 2982, 1155, 1500, 1020.552 before Pb (II) adsorption, shifted to 3300, 2980, 1587, 1510, 1025.555 cm^{-1} values after adsorption corresponded to the O-H and C-H groups. These groups can generally be alcohol, phenol, carboxyl acids. It has been observed that C-H and C-C groups may be present in the band range of 500-1000 cm^{-1} at 552-1020 cm^{-1} peaks before adsorption and at 552-1025 cm^{-1} peaks after adsorption. C=O and C=N functional groups were observed at 1510 and 1587 cm^{-1} values.

Adsorption Isotherms

Adsorption isotherms represent the amount of substance adsorbed per unit of adsorbent as a function of equilibrium concentration in solution at constant temperature. In this study, three isotherm equations; namely Langmuir, Freundlich, and Temkin were tested. The equations for these isotherms (Senthil Kumar & Gayathri, 2009) are given below.

Langmuir model equation:

$$q_e = \frac{q_m K_L C_e}{1+K_L C_e} \quad (2)$$

where, q_m is the maximum adsorbate uptake capacity (mg/g) and K_L is the Langmuir constant related to the energy of adsorption (L/mg).

Freundlich model equation:

$$q_e = K_F C_e^{\frac{1}{n}} \quad (3)$$

where, K_F is Freundlich constant related to biosorption capacity (L/g) and $1/n$ is the heterogeneity factor.

Temkin model equation:

$$q_e = B ln A C_e \quad (4)$$

where, $B = RT/b$, b is the Temkin constant related to heat of sorption (J/mol), A is the Temkin isotherm constant (L/g), R is the gas constant (8.314 J/mol.K), and T is the absolute temperature (K) of solution.

Besides, the essential property of the Langmuir isotherm can be expressed in terms of the dimensionless constant separation factor R_L and values between $0<R_L<$ represent a favourable adsorption (Al-Ghamdi et al., 2019).

$$R_L = \frac{1}{1+K_L C_o} \quad (5)$$

In the batch equilibrium experiments, the temperature was 20 to 40 ºC, the adsorbent dosage was 0.25 to 3 g/L, and the initial Pb (II) concentration was 10 to 200 mg/L. Figure 8 shows the linear model plots of the isotherms for 30 ºC, pH 5.5, and 200 rpm operating conditions. Isotherm model parameters are given in Table 3.

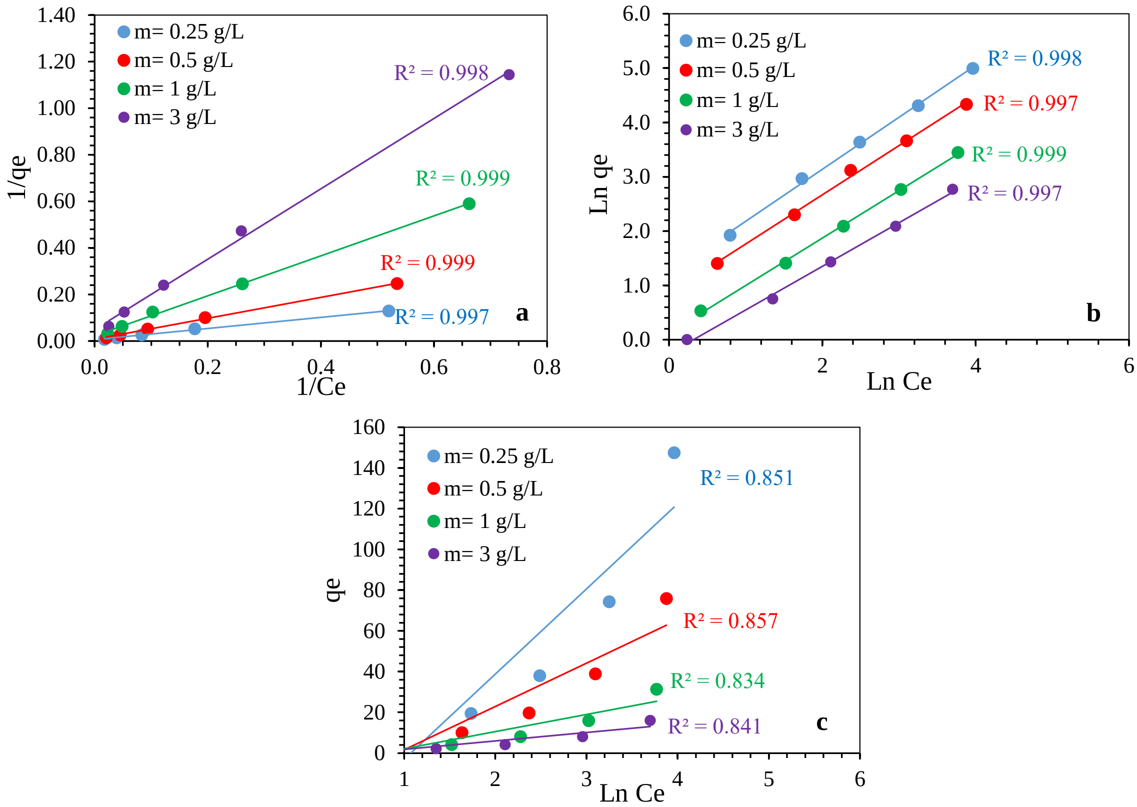

Figure 8. Langmuir (a), Freundlich (b), and Temkin (c) isotherm model plots 30 ºC, pH 5.5, and 200 rpm agitation speed operating conditions.

Table 3. Isotherm model parameters of Pb (II) adsorption on RTB for various adsorbent dosages at 30 ºC.

Parameters	Values			
m (g/L)	0.25	0.5	1.0	3.0
Langmuir				
q_m (mg/g)	188.16	140.19	44.94	20.38
K_L (L/mg)	0.022	0.016	0.026	0.033
R^2	0.997	0.999	0.999	0.998
ARE	6.213	5.675	8.147	5.121
SSE	0.027	0.016	0.031	0.024
X^2	0.321	0.267	0.334	0.286
Freundlich				
K_F (L/g)	3.411	2.355	1.136	0.778
1/n	0.956	0.907	0.872	0.802
R^2	0.998	0.997	0.999	0.997
ARE	7.150	6.666	7.175	6.045
SSE	0.033	0.027	0.029	0.031
X^2	0.463	0.341	0.339	0.317
Temkin				
A (L/g)	0.342	0.397	0.476	0.575
b (J/mol)	62.28	122.35	310.36	631.66
R^2	0.851	0.857	0.834	0.841
ARE	8.662	6.791	9.124	7.821
SSE	0.161	0.078	0.082	0.101
X^2	0.527	0.395	0.429	0.463

It can be seen from Table 3 that the R^2 correlation coefficients of the Langmuir and Freundlich are higher than the Temkin. On the other hand, R^2 values obtained for Langmuir model (0.998 on average) were slightly higher than Freundlich (0.997 on average) for the trials performed under the same conditions. As can be seen from Table 3, the correlation coefficients of the Temkin isotherm model have the smallest values obtained in the study and it stands out as the least suitable model for the adsorption of lead on RTB among other models examined in this study (between 0.834 and 0.857). Therefore, it can be said that Langmuir and Freundlich models are suitable for the adsorption of Pb (II) on RTB. On the other hand, it can be said that the Pb (II) adsorption on RTB is favourable since K_L values of Langmuir and *1/n* values of Freundlich were smaller than 1 for all operating conditions (Zhang et al., 2019). For all conditions in this study, the calculated dimensionless constant R_L was between 0 and 1. This reveal that adsorption is the favourable. Error analyses also confirm this situation due to smaller error values and shown in Table 3.

Adsorption Kinetics

Pseudo-first-order and pseudo-second-order kinetic models were used to reveal the reaction rate. And to make the results more useful, these models were analysed in both linear and nonlinear forms. Equations of these models are given in Eq. (6) and (7), respectively (Senthil Kumar & Gayathri, 2009).

$$q_t = q_e[1 - \exp(k_1 t)] \quad (6)$$

$$q_t = \frac{q_e^2 k_2 t}{1+(k_2 q_e t)} \quad (7)$$

where, k_1 (1/min) and k_2 (g/mg.min) are the adsorption rate constants of first-order and second-order kinetic models, respectively; q_e is the equilibrium adsorption uptake (mg/g); and q_t is the adsorption uptake (mg/g) at time *t* (min). Results of the kinetic study are given in Table 4 for 30 ºC. Figure 9 shows the linearised pseudo-first-order (a) and pseudo-second-order (b) model plots at 30 ºC, pH 5.5, and 200 rpm agitation speed operating conditions. Error analyses results for kinetic adsorption are given in Table 5.

Table 4. Kinetic model parameters for 30 ºC.

M (g)	C_o (mg/L)	$q_{e,exp}$ (mg/g)	Pseudo-First-Order			Pseudo-Second-Order		
			k_1	R^2	$q_{e,cal}$	k_2	R^2	$q_{e,cal}$
	10	31.160	0.0228	0.960	24.554	0.0019	0.997	34.756
	25	77.360	0.0245	0.951	61.944	0.0008	0.996	83.084
0.25	50	151.960	0.0314	0.952	120.344	0.0003	0.996	184.097
	100	297.000	0.0387	0.973	233.426	0.0002	0.999	349.598
	200	589.800	0.0461	0.997	532.839	0.0001	0.998	716.875
	10	16.260	0.0252	0.972	12.554	0.0039	0.997	18.458
	25	39.760	0.0272	0.969	30.985	0.0015	0.991	44.983
0.5	50	78.640	0.0292	0.924	57.713	0.0006	0.994	92.504
	100	155.680	0.0346	0.969	115.227	0.0004	0.999	180.359
	200	303.460	0.0406	0.989	254.319	0.0002	0.999	363.920
	10	8.490	0.0271	0.985	6.438	0.0062	0.992	9.734
	25	20.430	0.0293	0.986	15.370	0.0031	0.993	22.973
1	50	40.270	0.0353	0.970	30.102	0.0014	0.999	46.590
	100	79.430	0.0325	0.960	55.483	0.0008	0.998	90.323
	200	156.710	0.0346	0.974	120.760	0.0003	0.999	184.523
	10	2.913	0.0293	0.987	2.041	0.0322	0.993	3.154
	25	7.047	0.0297	0.986	5.024	0.0116	0.996	7.743
3	50	13.927	0.0346	0.975	10.006	0.0045	0.999	15.882
	100	26.930	0.0324	0.967	18.237	0.0026	0.998	30.255
	200	53.210	0.0345	0.977	40.709	0.0010	0.999	62.388

According to the pseudo-first-order kinetic model, the adsorption rate is controlled by the liquid membrane diffusion. On the other hand, the pseudo-second-order kinetic model assumes that the rate is controlled by chemical adsorption. From Table 4, it is seen that the pseudo-second-order kinetic model correlation coefficients are higher than the pseudo-first-order correlation coefficients. Therefore, it can be said that the pseudo-second order kinetic model is more suitable for the adsorption of Pb (II) on RTB. The calculated q_e values of model ($q_{e,cal}$) were closer to the experimental measured q_e values ($q_{e,exp}$) in pseudo-second-order model, which indicates that the adsorption is controlled by chemical adsorption. Moreover, the smaller the adsorption rate constants (k), the stronger the affinity of the adsorbent region, so the adsorption process is faster and more convenient (Chu et al., 2019). The pseudo-second-order kinetic model rate constants obtained in this study were the smallest, which is further proof that this kinetic model is more suitable.

Table 5. Error analysis results of kinetic adsorption for 30 ºC.

C_o (mg/L)	10	25	50	200
Pseudo-First-Order				
ARE	26.6	33.1	36.8	41.9
SSE	0.531	0.618	0.771	0.734
X^2	3.27	3.21	2.98	2.69
Pseudo-Second-Order				
ARE	8.21	7.75	6.32	8.13
SSE	0.223	0.193	0.136	0.097
X^2	0.421	0.367	0.441	0.384

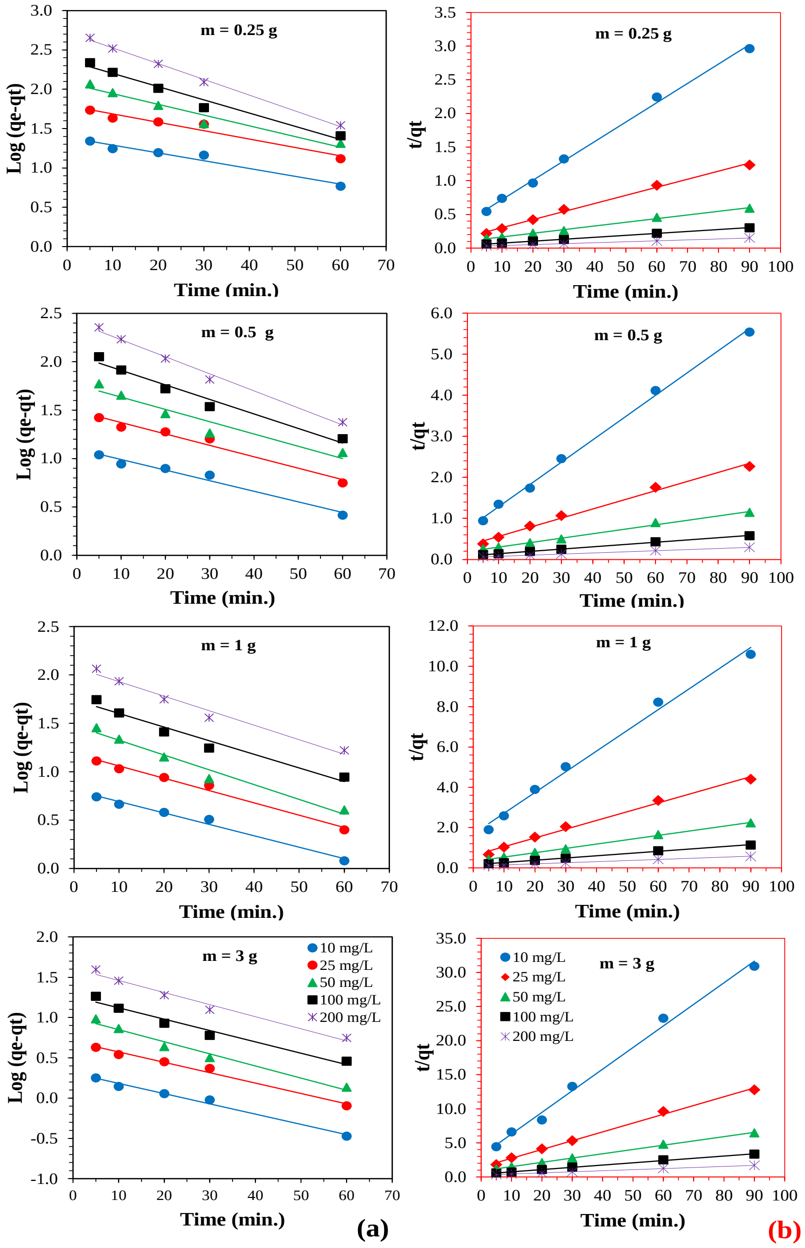

Figure 9. Pseudo-first-order (a) and pseudo-second-order (b) kinetic model plots at 30 ºC, pH 5.5, and 200 rpm agitation speed operating conditions.

Regeneration

For the measurement of desorption capacity of RTB samples used in adsorption, samples obtained from batch adsorption experiments were filtered through filter paper and rinsed with distilled water 3 times to remove free Pb (II) and placed in solutions brought to different acidic pH values using 0.1 M HNO_3. Samples were taken from the solutions and Pb (II) analyses were performed. As a result of the analysis, the desorption ratio RTB was calculated with the following equation (Eq. 8):

$$Des.ratio = \frac{VC}{mq_e} x\ 100\% \qquad (8)$$

where, V is the volume of the desorption solution (L); C is the Pb (II) concentration in the desorption solution (mg/L); m is the amount of the adsorbent in the desorption experiment (g); and q_e is the amount of Pb (II) adsorbed onto the adsorbent in the adsorption (mg/g) [Lu et al., 2019]. Desorption rates versus pH graphs were given in Figure 10.

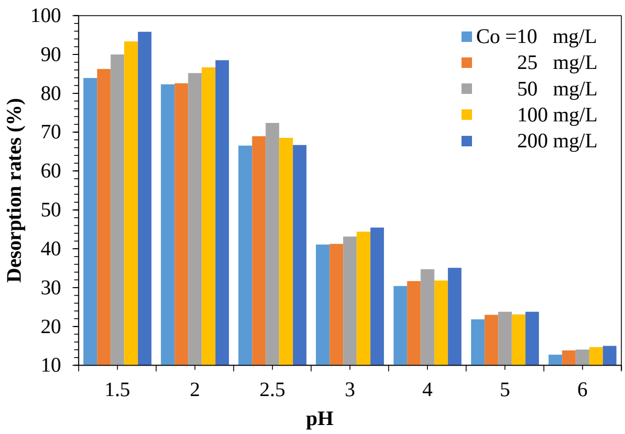

Figure 10. Desorption rates of RTB versus pH graph for 3 g/L adsorbent dosage.

As can be clearly seen from Figure 8, the desorption of lead from RTB is highly related to the pH of the solution. In this study, the maximum desorption rate was 95.85% at pH 1.5 for 3 g/L sorbent concentration. The desorption rates decreased rapidly (14.99% at pH 6) with increasing pH. In addition, desorption efficiencies increased with increasing adsorbent concentrations and initial Pb (II) concentrations. According to these results, it can be said that RTB is a reusable sorbent for removal of Pb (II).

Conclusion

The RTB exhibited a good adsorption performance for Pb (II) with q_{max} of 188.16 mg/g. Maximum adsorption occurred at 30 °C for 3 g/L adsorbent and 10 mg/L initial Pb (II) concentration. It was observed that the adsorption process was pH dependent and the optimum pH for Pb (II) removal was 5.5. Since the Langmuir isotherm model correlates better than the Freundlich model for the adsorption of Pb (II) on the RTB, the adsorption can be described as monolayer and reversible. According to pseudo-second-order kinetic data, the predominant adsorption process of Pb (II) was attributed to chemical reactions. Since Pb (II) can be desorbed with an efficiency of over 95%, RTB has been found to be a stable and reusable adsorbent. Although the relatively low surface area of the adsorbent used did not affect the adsorption efficiency much in this study, attempts to increase this area may result in higher heavy metal adsorption efficiencies.

Further studies in combination with different heavy metal species will be beneficial in terms of the widespread use of RTB in heavy metal adsorption.

Acknowledgment

This research was carried out in Atatürk University Engineering Faculty Environmental Engineering Department research laboratories. The authors would like to thank the staff of Atatürk University East Anatolia High Technology Application and Research Centre (DAYTAM).

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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RESEARCH ARTICLE

Trend Analysis and Macroeconomic Variable Determinants of Egg Production in Nigeria

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ARTICLE INFO

Article History
Received: 13.09.2022
Accepted: 05.12.2022
First Published: 21.12.2022

Keywords
Egg
Macroeconomics
Nigeria
Production
Trend

ABSTRACT

The daily animal protein intake of an average Nigerian is far less than the World Health Organization recommended minimum standard. The egg is the cheapest and most affordable animal protein source in Nigeria, but its production has not been able to match the increasing demand capacity. Premised on the above facts and to identify ways to intensify egg production, the study was specifically designed to examine the trend in egg production and establish the relationship between egg production and selected macroeconomic fundamentals in addition to other variables in Nigeria. The study used time series data from the period 1961 to 2020. The data were sourced from the Food and Agricultural Organization (FAO), the World Bank, and the Central Bank of Nigeria. The Augmented Dickey-Fuller unit root test and ADF-GLS unit root test were used to confirm the stability of the series. The Engle-Granger two-step technique was used to test for the cointegration of the series. The empirical finding showed that the amount of credit disbursed to the agricultural sector, per capita income, and the quantity of maize and chicken meat produced are positive determinants of egg production in both long and short-run periods. In contrast, the consumer price index (inflation rate) relates negatively in both periods to egg production. To upsurge egg production in the country, it is recommended that more credit facilities be injected into the agricultural sector. At the same time, the inflation rate is maintained at a minimal rate in the country.

Please cite this paper as follows:

Akpan, S. B., & Nkanta, V. S. (2022). Trend analysis and macroeconomic variable determinants of egg production in Nigeria. *Journal of Agricultural Production*, *3*(2), 100-109. https://doi.org/10.56430/japro.1174674

Introduction

Egg production in Nigeria has a long history and is now an important component of the poultry sub-sector, constituting about 30% of the total poultry production (Esiobu et al., 2014). The supply and demand for eggs in the country have witnessed a tremendous improvement over the years (FAO, 2022; see Table 1). Several reasons are accounted for by the country's expanding production and utilization of poultry eggs. The case for the mounting population with the corresponding increase in demand capacity for egg products is obvious. Besides, the difficulties and security challenges encountered during the production and transportation of other animal sources of protein, such as goats, cows, sheep, and pigs across regions in the country, have additionally stirred interest in egg production as an efficient alternative and more reliable source of animal protein. Furthermore, egg production is an integral part of many youth empowerment programmes across the country, perhaps because of its sustainable nature (Ajani et al., 2015; Price, 2019; UNDP, 2020; Ajala et al., 2021). Moreover, the egg being the cheapest source of animal protein, is found almost everywhere in the country, which further exaggerates intensified production. Subsequently, several home-based poultry farms have metamorphosed into small-to-medium-sized commercially viable poultry farms across the country (Netherlands Enterprise Agency, 2020). According to FAO

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(2021), the bulk of the poultry (80%) is produced by rural households in developing societies, hence constituting a sustainable source of livelihood activity. In Nigeria, egg production has supported the livelihood activities of more than 10 million rural and urban households. Additionally, about 16.80% of the country's population (representing over 33.6 million people) is in the age range of 15 and 35 years and is regarded as youth. According to analysts, a youthful population incentivises production (Adeniran et al., 2018; Nmeregini et al., 2020; Ajala et al., 2021).

Table 1. The contribution of the livestock sub-unit to the agricultural sector and economy and the per capita egg consumption in Nigeria.

Policy Year	Average Livestock Contribution to Agricultural GDP (%)	Average Livestock Contribution to Economy GDP (%)	Average Per Capita Consumption of Egg (Number of Egg/Person)
1981-1985	19.808	3.019	70.68
1986-1990	15.205	3.085	74.08
1991-1995	12.406	2.897	94.49
1996-2000	11.779	3.204	72.88
2001-2005	7.847	2.323	78.30
2006-2010	7.483	1.916	85.44
2011-2015	8.363	1.778	85.99
2016-2021	7.036	1.557	82.72
1981-2020	**11.138**	**2.450**	72.43

Source: Computed by authors. Gross domestic product at the current basic prices (₦' Billion).

The commercial egg industry in Nigeria consists mainly of eggs from hens. The local production only meets 19.84% of the domestic demand (following the American Heart Association recommended one egg per day for a healthy individual), thus creating a huge gap for the industry to expand (FAO, 2022). The country produced about 650 metric tons of egg and 300 metric tons of poultry meat in 2019 (FAO, 2022). Available records revealed that the average egg consumption in Nigeria was approximated at 78.48 eggs per person/annum in 2020 compared to 285.50 eggs/person/annum in the United States of America and 128.2 eggs/person/annum in South Africa (FAO, 2022; USDA, 2022). The deficiency is also prominent in poultry meat uptake among Nigerians. For instance, Nigeria consumes, on average, about 1.9 kg per capita of poultry meat compared to 49.3 kg for the USA, 7.67 for Ghana, and 32.98 kg for South Africa (Ayojimi et al., 2020). Despite the low per capita egg consumption rate in Nigeria, the country is presently witnessing a supply deficit (FAO, 2022). With the anticipated increase in the economic capacities of the population in urban and rural areas, the demand for poultry products is expected to rise due to improved effective purchasing capacities and a high youth population (Adesehinwa et al., 2019; Netherlands Enterprise Agency, 2020). The supply deficit constitutes a serious source of economic loss to the government of Nigeria. For instance, assuming full capacity was achieved in egg production, the sub-unit will generate about ₦800 billion or $1.88 billion US dollars per annum (FAO, 2022).

Given the market potential available in the egg industry, the sub-unit has continued to attract sustainable investments (both local and international investments) following its increasing importance in diverse economic fields. Nutritionally eggs are rich in high-quality proteins, easily digestible fats, carbohydrates, and minerals, including essential vitamins (Huopalahti et al., 2007; Madubuike, 2012; Ogunwole et al., 2015). Besides, poultry egg contains a trace of carbohydrates and all essential amino acids in a satisfactory proportion as required by the body for growth and body repair (Dolberg, 2003; Matt et al., 2009). Furthermore, eggs are raw materials for the pharmaceutical and cosmetic industries (Matt et al., 2009) and are one of the major additives in some food industries, among others.

However, the Nigerian poultry industry has myriad opportunities to explore the unutilized capacity and expand the frontier of value chains in the sub-sector. Currently, the poultry sub-sector contributes about 30% to the agricultural GDP and supports more than 10 million households' income and is a major player in Africa's poultry market. With an estimated 180 million birds (30% layers and 70% broiler), the country's poultry industry is ranked the second largest in Africa. It is worth more than 4.0 billion US dollars, out of which egg production contributes about 1.88 billion dollars (FAO, 2019). With the enormous investment prospects in the country's poultry sub-sector, the country's economy must be positioned adequately to nurture and sustain the thriving of the industry now and in the future. Often, several factors are reported to cause the depressed growth of the country's agricultural sector. The principal factor is the high volatility in the macroeconomic environment (Akpan & Umoren, 2021). The macroeconomic environment comprises monetary, fiscal, exchange rate regimes, and trade policies, including other complementary policies, developed to control production performances in the real and service sectors, among other sectors. The uncertainty of the macroeconomic environment is known to impair growth in agricultural production and have a deteriorating effect on export promotion (Udah et al., 2015; Akpan, 2019; Udoh & Akpan, 2019; Akpan & Udo, 2021; Akpan & Umoren, 2021). Sound macroeconomic policies would birth sustainable and stable macroeconomic fundamentals, which are essential elements needed to achieve the country's short and long terms economic and developmental goals through agricultural innovations and development (Fan et al., 2008). In this dimension, many agricultural economists have documented empirical studies relating agricultural production to some selected macroeconomic fundamentals in Nigeria.

For instance, a positive impact of per capita income and credit to the agricultural sector on agricultural production have been submitted by several authors (Akpan, 2019; Udoh & Akpan, 2019; Akpan et al., 2021). Also, few authors have established an indirect relationship between the annual inflation rate, nominal exchange rate and growth in agricultural production in Nigeria (Oyakhilomen & Grace, 2014; Wasiu & Ndukwe, 2018; Gatawa & Mahmud, 2019; Obiageli, 2020). However, none of these researches has specifically focused on egg production as an independent agricultural output. Based on the time-dependent nature of the economic system and the increasing importance of egg production, there is an overwhelming need to update these batches of information and extend this research dimension to egg production in Nigeria. Based on this assertion, the study was intentionally designed to address the following objectives:

(i) to examine the trend in egg production and

(ii) identify the macroeconomic determinants of egg production in Nigeria.

Materials and Methods

Brief Description of Nigeria

The country is situated in the Gulf of Guinea in sub-Saharan Africa. The country lies 4º and 14º north of the equator and between longitude 3º and 15º east of Greenwich. The country's land mass is about 923,769 km^2 (or about 98.3 million hectares), with 853 km of coastline stretching along the northern edge of the Gulf of Guinea. The population is over two hundred (200) million (National Population Commission, 2021), and the country is gifted with abundant agricultural, mineral, marine, and forest resources. Its several vegetation zones, plentiful rain, surface water, and underground water resources and moderate climatic extremes allow for the production of diverse food, tree, and cash crops all year round. More than sixty percent of the population is involved in agricultural enterprises such as cassava, groundnuts, oil palm, cotton, rubber, cocoa, rice, maize, aquaculture, yams, various beans and legumes, sorghum, carrots, ginger, fruits, onions, tomatoes, melons, and vegetables among others. In addition, artisanal fishery and livestock production such as poultry, goat, sheep, pigs, and cattle thrived in all regions of the country (Federal Ministry of Environment, 2021).

Data Source

The study used **s**econdary or time series data sourced from the World Bank and Food and Agricultural Organization (FAO) as well as the Central Bank of Nigeria. The time series used spanned from 1961 to the year 2020.

Theoretical Framework

The study adopted a simple framework based on the production theory but assumed that production is also affected by non-physical factors. Generally, the theory of production depicts the unilateral relationship between the physical outputs of firm and physical inputs or factors of production. Explicitly a two-input production function can be expressed thus:

$$Q = f(K, L) \tag{1}$$

Note that Q represents the output, and K and L represent capital and labour inputs, respectively. The volume of Q of a firm at any time depends on the quantities of K and L utilized by that firm. Also, the availability of K and L inputs is determined by several factors, including macroeconomic factors and climatic as well as economy's stability, among others. The relationship implies that firm output depends on the physical inputs, while the physical inputs are determined by several non-physical factors within the economy. Explicitly the assumed relationship can be simplified as thus:

$$K, L = f(Economic\ factors, climatic\ factors\ etc.) \tag{2}$$

$$Q = f(K, L, Economic\ factors, climatic\ factors\ etc\ ) \tag{3}$$

By implication, the Q of a firm is also determined by non-physical factors, among others. Therefore, Eq. (3) was expanded to form the structural model used in this study.

Model Specification

Trend analysis of egg production in Nigeria

The exponential trend equation was specified and used to analyse the trend in annual egg production in Nigeria. The specified exponential trend equation is presented in Eq. (4).

$$log_e EGG_t = b_0 + b_1 T + U_t \tag{4}$$

where, T is the time variable expressed in years. To estimate the exponential growth rate in egg production, Eq. (5) was specified according to Akpan et al. (2022) as thus:

$$(r) = (e^{b1} - 1)\ x\ 100 \tag{5}$$

To further extend the trend analysis of egg production in Nigeria, a quadratic trend equation was specified and estimated for egg production. The quadratic trend analysis allows for the determination of the coefficient of acceleration, deceleration and stagnation of egg production in the long run or increase period. Hence, the quadratic trend equation (Akpan et al., 2022) is shown in Eq. (6).

$$log_e EGG_t = b_o + b_1 T_1 + b_2 T_2^2 + u_t \quad (6)$$

If $b_2>0$; the egg production is increasing at decreasing or increasing rate over a double increase of time: when $b_2<0$; the growth rate over double time is not significant.

Macroeconomics factors influencing egg production in Nigeria

A time-dependent multiple regression model was specified at the level of variables to determine the long and short-run determinants of egg production. The egg production equation adopted in the study assumed the following implicit Cobb-Douglas form, as shown in Eq. (7).

$$EGG_t = \beta_0 + \beta_1 CRE_t + \beta_2 EXC_t + \beta_3 PCI_t + \beta_4 CPI_t + \beta_5 MEC_t + \beta_6 MAI_t + \mu_t \quad (7)$$

where, EGG_t = Annual aggregate egg (hens) production measured in metric tons; CRE_t = annual credit injected in the agricultural sector (% of GDP); EXC_t = annual exchange rate of naira/dollar (proxy effect of external World influence); PCI_t = GDP per capita (naira/person) (proxy of demand capacity); CPI_t = Consumer price index (proxy of input prices); MEC_t = Meat (chicken) in tons (proxy of egg substitute); MAI_t = Quantity of maize produced in tons (proxy of poultry feed); U_t = the random error term and $U_t \sim$ IID $(0, \delta^2_U)$.

To validate the stability in the long-run function of egg production in Nigeria, the study used the Engle and Granger (1987) two-step technique test. The pre-condition for applying the Engle-Granger two-step standard procedures of the cointegration tests is that the variables specified must be integrated in the same order. The additional condition is that the unit root of the error term from the long-run model should be stationary at its level. If these conditions are fulfilled, then the error correction model (ECM) for the series can be estimated. The ECM represents the short-run production function for eggs in Nigeria. For the short-run production function of egg to hold, it is assumed that all other determinants of egg production are held constant or fixed. The specification of the ECM of egg production is implicitly expressed as in Eq. (8).

$$\Delta \text{LnEGG}_t = \varphi_0 + \varphi_1 \sum_{i=1}^{n} \Delta LnEGG_{t-1} + \varphi_2 \sum_{i=1}^{n} \Delta \text{LnX}_{t-i} + \varphi_3 ECM_{t-1} + U_t \quad (8)$$

Note that specified variables are presented in Eq. (7) and the coefficient (φ_3) of the ECM_{-1} ($-1<\varphi_3< 0$) measures the magnitude of deviation from the long-run equilibrium in period ($_{t-1}$).

Results and Discussion

Descriptive Statistics

The descriptive statistics of the specified variables are presented in Table 2. The findings revealed that the coefficient of variability and index of skewness in the annual per capita income and nominal exchange rate is greater than unity. This implies that the specified variables experienced high annual volatility and persistent increment across the specified period. The coefficient of variability was lowest in the amount of credit disbursed to the agricultural sector. This means that the amount of credit allotted to the agricultural sector over the years skewed positively but did not change significantly. The volatility indices in the quantity of egg, chicken meat and maize produced were less than unity and portrayed minimal variations in annual outputs across the study period. The skewness index for the chicken meat showed a negative sign, meaning that annual production increased at a decreasing rate.

Table 2. The descriptive statistics of variables.

Variable	Min.	Max.	Average	Std. deviation	CV	Skewness
EGG	75000	6.6000e+005	3.4076e+005	2.0149e+005	0.59129	0.27559
PCI	69.272	7.2470e+005	1.2975e+005	2.1162e+005	1.6310	1.5542
EXC	0.54678	306.90	66.541	92.206	1.3857	1.3159
MEC	30000	2.7300e+005	1.4863e+005	71441	0.48067	-0.13854
MAI	4.8800e+005	1.1548e+007	4.5113e+006	3.4666e+006	0.76842	0.42199
CRE	3.7043	19.626	8.5270	3.2587	0.38216	1.3525

Data are derived from FAO and World Bank, 2022.

Trend in Egg Production in Nigeria

The estimated exponential trend equation of egg production is shown in Table 3. The findings showed that egg production in Nigeria relates positively to time. This means that egg production increases with an increase in the time factor. The result revealed an average positive exponential growth rate of 4.02% per year in Nigeria. This growth rate in egg production showed remarkable improvement compared to the 3.90% growth rate per year obtained for Africa in 2017. This implies that several policies and programmes implemented by the government to boost poultry production yielded significant and positive impacts. In addition, the quadratic trend equation reveals that egg production over time increases at a decreasing rate. The pictorial representation of the estimated trend line is fitted to the linear graph of egg production and is shown in Figure 1. The trend in egg production assumed an upward progressive growth from 1961 to 1987. This period corresponds to the pre-structural adjustment programme (SAP). The agricultural policies and programmes then were majorly targeted at developing the agricultural sector at the regional levels.

Table 3. The trend analyses of egg production in Nigeria.

Variable	Coefficient	Standard Error	t-value
Exponential trend equation			
Constant	11.3229	0.0354	319.7***
Time factor	0.0394	0.0010	39.05***
Focal. (1, 58)	1524.664***		
R-squared	0.9634		
Exponential growth rate (%)	4.0186		
Quadratic trend equation			
Constant	11.0736	0.0331	334.3***
Time factor	0.0636	0.0025	25.37***
Time factor squared	−0.0004	3.98e-05	−9.934***
Focal. (2, 57)	2095.67***		
R-squared	0.9866		

***Represent a 1% significance level.

Following the influx of foreign exchange resulting from the crude oil export in the 1970s, import substitution was introduced to establish heavy-duty agro-industries such as flour mills. The period marks the birthing of pronouncing private investment in the poultry industry. The trend in egg production witnessed a sharp depression in 1988 and 1989 and later an upsurge from 1990 to 1995. The trend in egg production in this period was mainly modelled by the policies and programmes of the structural adjustment programme. The remarkable characteristic of this era was the privatisation and commercialization of government-owned agro-enterprises. During this time, private investment in poultry production witnessed a remarkable increase, but improvement in the sub-sector was hampered by increasing volatility in the macroeconomic fundamentals. Also, the industry depended so much on imported inputs, and this caused a sharp decline in egg production in 1997. The post-SAP era, which spanned from 1993, saw the introduction of new agricultural policies, including a ban on importing many poultry essential inputs, the devaluation of the national currency and independence of the poultry sub-sector. Egg production struggles to thrive in the harsh economic weather prevalent in the country. However, from 2010 to 2020, the agricultural transformation agenda was introduced to strengthen private investment in the poultry sub-sector. Enhanced finances, research and several collaborative initiatives were established to boost poultry outputs, including egg production.

This period corresponds to or is noted for a conspicuous increase in egg production until early 2020. The COVID-19 pandemic and persistent increase in feed prices stalled the production capacity of eggs and resulted in a reduction in annual output. In summary, the trend in egg production has shown undulating behaviours from 1961 to 2020, which corresponded to the various impacts of policies and programmes enunciated to improve capacity utilization in the sub-sector. However, the overall growth has been impressive considering the uncertainty that wraps up the entire economic system in the country.

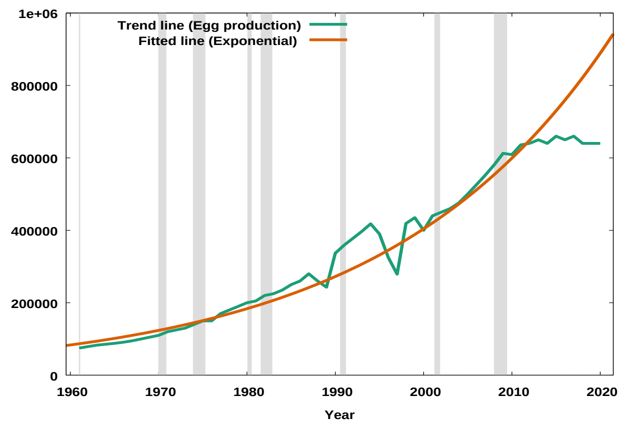

Figure 1. Trend in egg production in Nigeria.

Determinants of Egg Production in Nigeria

Unit root test

The study employed the Augmented Dickey-Fuller (Dickey & Fuller, 1979) test and the ADF-GLS unit root test developed by Elliott et al. (1996) to confirm the unit root of the variables. The results of the unit root test of variables are presented in Table 4. The results revealed that all variables were not stationary at their levels but were stationary at the first difference.

Table 4. Unit root test using ADF and ADF-GLS methods.

	ADF (with constant)				ADF-GLS (with constant and trend)			
	Lag	**Level**	**1st Diff.**	**Decision**	**Lag**	**Level**	**1st Diff.**	**Decision**
EGG	0	-1.6036	-7.8774***	1(1)	0	-1.9968	-7.8774***	1(1)
PCI	0	-0.3684	-6.2718***	1(1)	0	-1.6976	-6.3379***	1(1)
EXC	0	0.2398	-5.9813***	1(1)	0	-1.8439	-6.0820***	1(1)
MEC	0	-3.0267	-7.6005***	1(1)	0	-1.6417	-8.5692***	1(1)
MAI	0	-0.6701	-8.0437***	1(1)	0	-2.1053	-8.1172***	1(1)
CRE	0	-2.8873	-7.8814***	1(1)	0	-0.3302	-7.9262***	1(1)
CPI	0	0.3315	-3.5437***	1(1)	0	-1.6621	-3.6447***	1(1)
Residual	**0**	-4.8357***				-4.8788***		

***Indicate 1% significance level. Variables are expressed in a natural logarithm.

The result for ADF is similar to ADF-GLS unit root tests. The result implies that if variables are used at their levels, the possibility of producing spurious regression estimates is high if variables are used at their levels. Hence, following the recommendations given by Johansen and Juselius (1990) such specifications should be tested for the existence of cointegration. Therefore, the cointegration test was conducted for specified variables in Eq. (7).

The Engle-Granger two-step cointegration test

The unit root test results presented in Table 4 for the error term showed that the null hypothesis for no cointegration is rejected at a 1% probability level of significance, the null hypothesis of no cointegration is rejected. The inference is that there is a long-run equilibrium relationship between egg production and the specified macroeconomic fundamentals as well as other relevant variables in Nigeria.

The long-run determinants of egg in Nigeria

The long-run determinants of egg production function in Nigeria are presented in Table 5. The result revealed that; the per capita income of Nigerians (which proxies demand capacity) has a significant positive influence on egg production. It implies that egg production increases as the demand capacity increases. The probable reason for this finding is traceable to the fact that eggs are affordable and are easily found as well as being preferred by all classes of people in society when their incomes increase, hence one of the normal goods. The result is confirmed by Akpan (2019); Udoh and Akpan (2019), Akpan et al. (2021).

The slope coefficient of chicken meat has a positive significant relationship with egg production in Nigeria. The relationship connotes that as broiler production increase, egg production also increases. This could be related to the fact that the two poultry products often are jointly produced by farmers as a way to ensure all round farm income and avert risks and uncertainties associated with agricultural production. This means that an increase in the production of one enterprise would increase the production of the other enterprise. The result also revealed that the quantity of maize produced and the amount of credit disbursed to the agricultural sector are significant positive determinants of egg production in Nigeria. Maize is one of the major constituents of poultry feed, and its production is critical to the volume of poultry feed. Hence, an increase in the production of maize will increase the possibility of producing more poultry feeds at affordable prices. Affordable poultry feed would enhance egg production by lowering the cost of production. Credit disbursed to the agricultural sector has been established as one of the major stimulants of agricultural growth. It enhances the acquisition of farm inputs and encourages economies of scale in agro-production.

The result corroborates Oyakhilomen and Grace (2014), Wasiu and Ndukwe (2018), Gatawa and Mahmud (2019), Obiageli (2020). On the other hand, the consumer price index, which proxies input prices, relates negatively to egg production in the country. This means that as the consumer price indices increase, the possibility of increasing egg production declines, probably due to the high prices of inputs. The finding agrees with Akpan and Umoren (2021).

Table 5. The long-run estimates of egg production equation.

Variable	Coefficient	Standard Error	t-value	Probability
Constant	4.3856	0.7152	6.132	<0.0001
PCI_t	0.1703	0.0516	3.300***	0.0017
EXC_t	0.0299	0.0304	0.9832	0.3300
MEC_t	0.4813	0.0608	7.920***	<0.0001
MAI_t	0.0564	0.0328	1.718*	0.0917
CRE_t	0.0888	0.0469	1.890*	0.0642
CPI_t	−0.0976	0.0329	−2.967***	0.0061
R-squared	0.986378	Adjusted R-squared		0.984836
Focal. (4, 55)	639.6311***			

Variables are expressed in the natural logarithm. * and *** represent 10% and 1% significance levels, respectively.

The short-run egg production in Nigeria

The error correction model was estimated to capture the dynamics in the egg production equation and identify the speed of adjustment as a response to departure from the stable long-run equilibrium. The study used Hendry (1995)'s technique to obtain an acceptable ECM model for egg production in Nigeria. The ECM estimates are presented in Table 6. The result revealed that the slope coefficient of the error term is negative and statistically significant, thus validating the existence of stable long-run equilibrium for the estimated egg equation. The result also implies that egg production is sensitive to the departure from its equilibrium value in the previous periods. This connotes that the adjustment speed in the egg production equation is the same no matter the shock in the specified explanatory variables.

Table 6. The estimated short-run egg equation in Nigeria.

Variable	Coefficient	Standard Error	t- value	Probability
Constant	0.0165	0.0185	0.8891	0.3783
ΔEGG_{t-1}	0.1776	0.1783	0.9960	0.3241
$\Delta LnPCI_t$	0.1144	0.0637	1.797*	0.0785
$\Delta LnEXC_t$	0.0063	0.0251	0.2493	0.8041
ΔMEC_t	0.1025	0.0744	1.377	0.1748
$\Delta LnMAI_t$	0.0529	0.0314	1.686*	0.0982
$\Delta LnCRE_t$	0.1043	0.0539	1.936*	0.0586
$\Delta LnCPI_t$	−0.0869	0.0346	−2.512**	0.0117
ECM_{t-1}	−0.5946	0.2446	−2.431**	0.0188
Diagnostic tests				
R-squared	0.7320032	Adjusted R-squared		0.60902
F (6, 52)	16.6891***			

* and ** represent 10% and 1% significance level, respectively.

However, the slope coefficient of the ECM (−0.5946) representing the speed of adjustment is consistent with the hypothesis of convergence towards the long-run equilibrium. The diagnostic test for the ECM model revealed an R^2 value of 0.732 and a significant F-calculated, hence assuring the fitness of the estimated equation.

The empirical result shows that the per capita income, the quantity of maize and credit disbursed to the agricultural sector are positive significant short-run determinants of egg production in Nigeria. Alternatively, the consumer price index exerted an adverse influence on egg production in the short-run period.

Stability test

Figure 2 represents the CUSUM with a 95% confidence band generated from the ECM. Note that all residuals are located between the two standard deviation limits at a 5% confidence interval. The result indicates that the short-run model (ECM) is stable, as it is maintained within the 5 per cent significance level within the observation period. Further stability tests are presented in Table 7. The result showed that the estimated short-run model is adequately specified, and the error terms are normally distributed.

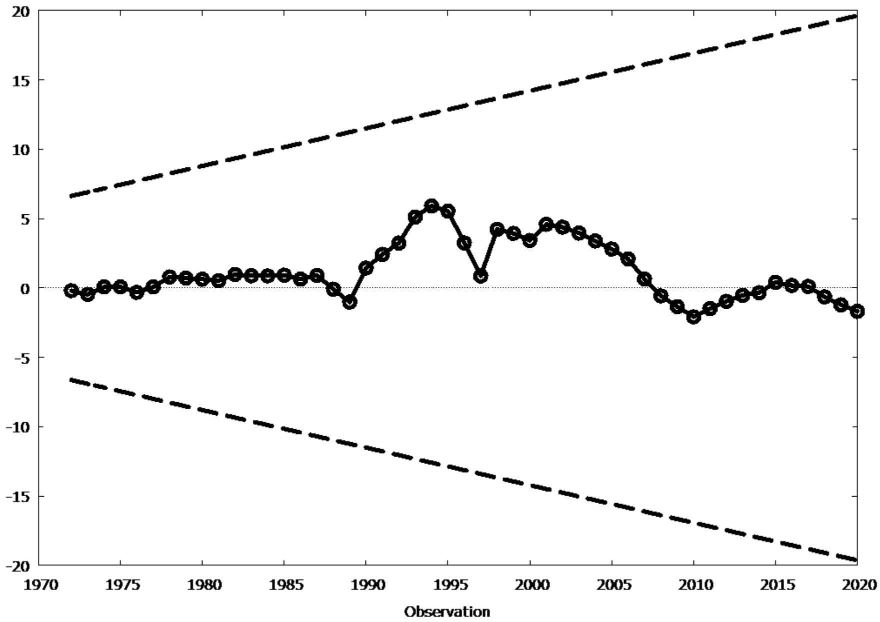

Figure 2. CUSUM plot with 95% confidence band.

Table 7. Test of validity of parameters in ECM.

Test	Value	Inference
RESET test	1.1207	Specification is adequate
Normality test	1.6703	Error normally distributed
White test	17.2485	Heteroscedasticity not significant
CUSUM test	-0.2404	No change in parameters

The result of the Reset test revealed that the estimated equation is adequate with efficient structural rigidity, while heteroskedasticity did not pose a serious problem.

Conclusion

The study has established that egg production has a significant relationship with some key macroeconomic fundamentals, egg substitute (chicken meat) and maize production (a major constituent of animal feeds) in both short and long-run periods in the country. The study also confirmed that the inflation rate (proxy by the consumer price index) relates adversely to egg production in the immediate and long-run periods. The need to increase egg production from the present growth rate of 4.02% is obvious given the devastating levels of the per capita consumption of eggs and the corresponding economic loss due to deficit supply as well as the deficiency in protein consumption across categories of Nigerians. The alarming level of malnutrition and high level of unemployment, as well as poverty, can be curtailed drastically using the weapon of egg production enterprises which many experts attest to as being profitable and sustainable. Within the ambit of this study, an upsurge in egg production can be achieved by improving the per capita income of Nigerians. Providing adequate credit facilities is proven effective in surging egg production. Adopting necessary agronomical techniques to increase maize production is recommended as a pathway to increase egg production in Nigeria. Enhancing value addition in the poultry sub-sector through an increase in chicken meat production would additionally increase egg production. Besides, a reduced and stable inflation rate in the country is necessary and strongly recommended for the survival of egg enterprises in the country.

Conflict of Interest

The authors declare that they have no conflict of interest.

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Morpho-Genetic Characterization of *Abelmoschus* Moench. Accessions

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ARTICLE INFO

Article History
Received: 25.08.2022
Accepted: 23.10.2022
First Published: 21.12.2022

Keywords
Genetic variability
Morphological variability
Okra
Plant characterization
RBCL genotyping

ABSTRACT

This study was carried out to assess the morphological and genetic variability of seven accessions of *Abelmoschus* L. species using morphometric analysis and ribulose bisphosphate carboxylase large chain (RBCL) molecular markers. Using a completely randomized block design, seeds of the okra accessions were planted with three replicates each. During the developmental stage, the morphological features of the accessions were observed and recorded according to the standard descriptor for the crop. Morphologically, all the accessions exhibited a degree of similarities, albeit, at maturity, the leaf, plant height, leaf colour, and leaf shape became distinct. A cluster of the phenotypic characterization was observed at a 3.74 level of coefficient of similarity with two distinct clusters, which were predicted to be *A. esculentus* and *A. caillei*. The percentage variance of the two principal components was 55.12% and 22.69% with corresponding Eigenvalues of 4.11 and 1.69, respectively. Results of the RBCL analysis revealed genetic variability at a 0.80 level of coefficient of similarity. Two distinguishable clusters were observed. Both morphometric and genotyping results suggest that variations exist among and within the seven accessions. In conclusion, there is a need to frequently evaluate plant genetic resources held in gene banks as they may not reflect the whole range of diversity inherent in the species.

Please cite this paper as follows:

Obongodot, N. U., Osawaru, M. E., & Ogwu, M. C. (2022). Morpho-genetic characterization of *Abelmoschus* Moench. accessions. *Journal of Agricultural Production*, *3*(2), 110-123. https://doi.org/10.56430/japro.1166934

Introduction

Abelmoschus Moench. species is an important traditional vegetable crop in the tropical, subtropical, and Mediterranean regions of the world (Staub et al., 1997; Bisht & Bhat, 2006; Ogwu & Osawaru, 2022). It is commonly known as okra and the Angiosperm Phylogeny Group (APG, 2009; Osawaru & Ogwu, 2013) recognized it as a monophyletic group. It is cultivated for its edible fruits, leaves, seeds, floral parts, and woody stem (Bioversity International, 2007; Osawaru et al., 2012). It is one of the oldest cultivated crops, presently grown in many countries of the world (Ariyo, 1993; Oyelade et al., 2003; Ogwu et al., 2016a, 2016b, 2017). There are several species, both cultivated and wild especially within their native range. In West Africa, the species of okra cultivars are of two distinct types, the common okra [*Abelmoschus esculentus* (L.) Moench.] and the West African okra [*Abelmoschus caillei* (A. Chev.) Stevels].

Okra may be classified morphologically using the quantitative and qualitative parameters or descriptors recommended by the International Board of Plant Genetic Resources (IBPGR, 1991) and germination protocol as outlined by Osawaru et al. (2012). These descriptors include plant height, leaf size, leaf colour, stem colour, general aspects of the stem, leaf shape, fruit pubescence, *etc*. The morphological characterization points out the high degree of variations that exist among and between accessions of okra, which further requires evidence using molecular makers to clarify

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(Omonhinmin & Osawaru, 2005; Osawaru et al., 2011, 2014a, 2014b). Several biochemical analyses have proven the presence of various phytochemicals embedded in the species. Diversity classified by phenotypic and morphological characters usually varies with environments and evaluation of traits requires growing the plants to full maturity before identification. Presently, the rapid development of biotechnology has allowed easy analysis of the massive number of loci distributed throughout the genome of the plants to allow for variability distinction and characterization. Molecular markers have also proven to be powerful tools for the assessment of genetic variation and in the elucidation of genetic relationships within and among species (Chakravarthi & Narayaneni, 2006). Molecular markers unlike morphological traits are not disturbed by the environment (Staub et al., 1997). Germplasm characterization, a process that involves recording and compilation of data on important characteristics that distinguish one species from the other and accessions or varieties within species, has been used to enable easy and quick discrimination among phenotypes (Bioversity International, 2007; Das et al., 2012). This process determines the expression of highly heritable characters ranging from morphological or agronomical features to seed proteins or molecular markers. Characterization allows for simple grouping of accessions, developments of core collections, identification of gaps, and retrieval of valuable germplasm for breeding programmes resulting in better insight into the composition of the collection and its genetic diversity (Das et al., 2012).

More so, the characterization of genetic resources is an essential first step in any crop improvement and conservation programme (Adeoluwa & Kehinde, 2011; Ogwu et al., 2014). Characterization and quantification of the germplasm and knowledge of the genetic viability between and among closely related crop varieties are necessary for a cogent use of plant genetic resources (Adeoluwa & Kehinde, 2011). A precise knowledge of genetic diversity among okra germplasm plays a major role in breeding programmes.

Molecular characterization has presently become a more efficient method of distinguishing plant genetic resources. It provides reliable information for assessing, among other factors, the amount of genetic diversity, the structure of diversity in samples and populations (Perera et al., 2000; Shim & Jørgensen, 2000), rates of genetic divergence among populations (Maestri et al., 2002), and the distribution of diversity in populations found in different locations (Perera et al., 2000; Maestri et al., 2002; Figliuolo & Perrino, 2004).This study is set up to investigate variations in the morphological and genetic characteristics of the species and characterize them based on their variation.

Materials and Methods

Study Area

This study was conducted at the experimental garden of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria [6.40°N 5.61°E]. It is located within the humid tropical rainforest zone of Nigeria and belongs to the Af category of Koppen's climatic classification. The climate includes high rainfall up to 200-300 mm of pattern with peaks in July and September respectively, high temperatures ranging between 20-40 °C, and high atmospheric humidity (Omuta, 1980). The RBCL genotyping was carried out at the Bioscience Laboratory of the International Institute of Tropical Agricultural, Ibadan [7.49°N 3.91°E].

Source of Plant Material

Seven accessions of *Abelmoschus* were obtained from the active collection of the national gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan Nigeria (Table 1).

Table 1. Passport data of okra accessions collected from NACGRAB.

Accession	Status	Genus	Source Location	Germplasm Collected
NGB 00297	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00309	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00308	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00302	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00467	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00371	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds
NGB 00387	Landraces	*Abelmoschus*	NACGRAB, Ibadan [7.22°N 3.50°E]	Seeds

Morphological Characterization and Data Collection

After five weeks in the field, qualitative and quantitative morphological data were collected based on the International Board for Plant Genetic Resources (IBPGR, 1991) descriptor list for okra. Data included plant height, leaf length, leaf width, and general growth appearance (*i.e.*, habit) [Table 2]. Leaf shape was characterized according to Charrier (1984) [Figure 1].

Table 2. Qualitative morphological characteristics evaluated in the study and their character state and codes.

S/N	Parameters Measured	Parameter Key	Character State
1	General aspect of the stem	GAS	1-Erect 2-Medium 3-Procumbent
2	Stem colour	STC	1-Green 2-Green with red patches 3-Purple
3	Stem pubescence	STP	1-Glabrous 2-Slight 3-Conspicuous
4	Nature of branching	BRA	1-Orthotropic stem only 2-Medium 3-Strong
5	Leaf shape	LSH	From 1-11 (see Figure 1)
6	Leaf colour	LC	1-Green 2-Green with red veins 3-Red

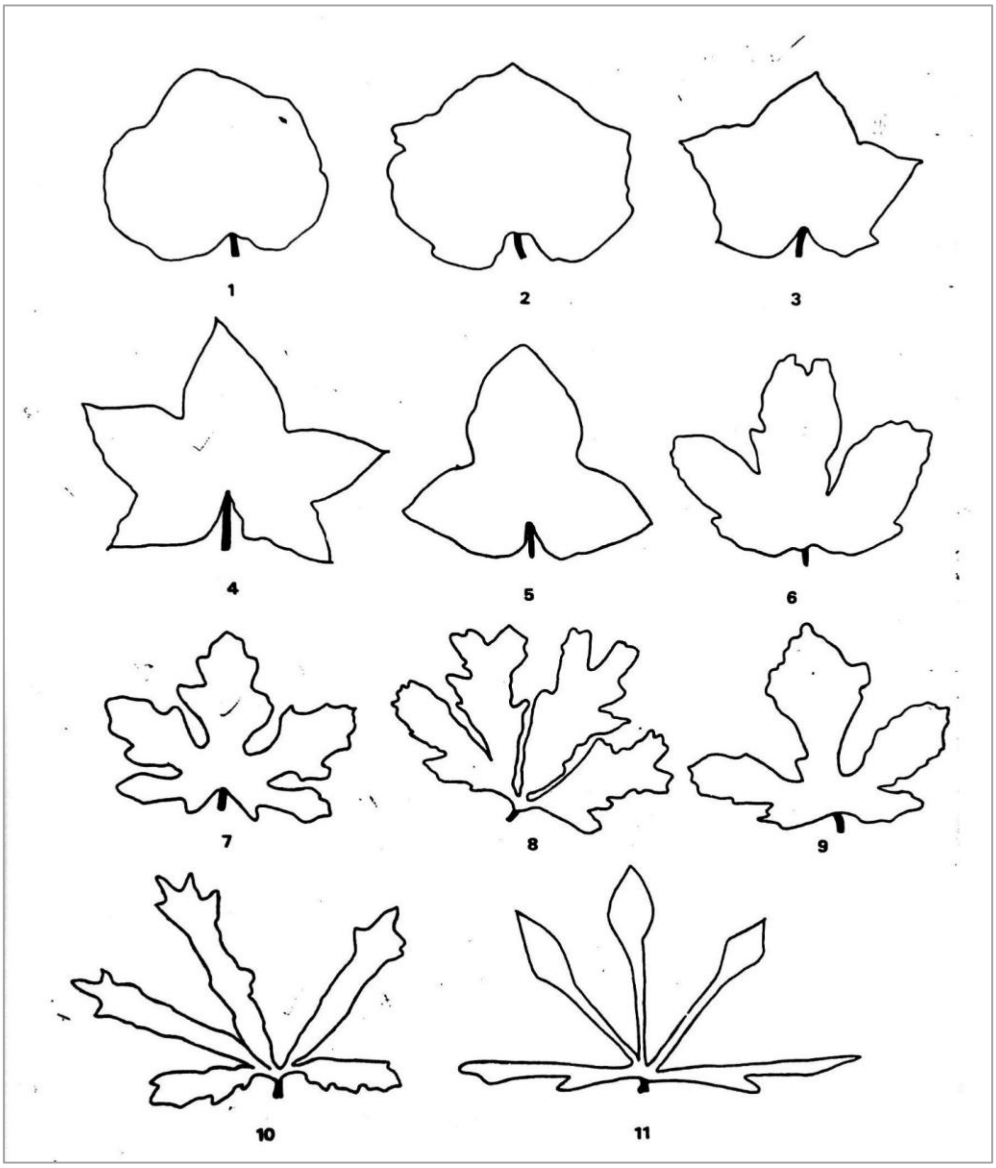

Figure 1. Leaf shapes descriptor key for *Abelmoschus*. Source: Adapted from Charrier (1984).

Ribulose Bisphosphate Carboxylase Large Chain Genotyping

To take into account possible genetic variability within each accession, young leaves of 5 weeks old were genetically analysed using Ribulose bisphosphate carboxylase large chain (RBCL) marker at the Bioscience Laboratory of the International Institute of Tropical Agricultural.

Total genomic DNA was extracted from young leaves (100 mg per accession) of 5 weeks old plants following these procedures. Samples were prepared by putting approximately 100 mg of freeze-dried tissues into an extraction tube. Two steel balls were added each into the tube to enable grinding. The freeze-dried tissue was then ground into a fine powder using Genogrinder-2000. About 450 µl of pre-heated plant extraction buffer was added. The mixture was incubated at 65 °C for 20 min and mixed occasionally by inverting the tubes to homogenize the sample, then the mixture was removed and allowed to cool for 2 mins. 200 µl of ice-cold 5 M Potassium acetate was added and incubated on ice for 20 minutes to precipitate protein. The mixture was Centrifuge at 10000 rpm for 10 mins. The supernatant was transferred into freshly labelled tubes. 450 µl of chloroform isoamylalcohol (24:1) was then added and mixed gently to further precipitate protein and lipids. Again, mixture was centrifuged at 10000 rpm for 10 min and then the supernatant was transferred into freshly labelled tubes. 2/3 volume of ice-cold isopropanol was added to the mixture gently and incubated at -80 °C for 15 mins to precipitate the DNA. The mixture was centrifuged at 10000 rpm for 10 min and the supernatant was decanted until the last drop. Then about 400 µl of 70 % ethanol was added to wash the DNA pellet and centrifuge. The supernatant was decanted until the last drop to remove dry air pellets. 60 µl of ultra-pure water or low salt TE was added to re-suspend the DNA, followed by 2 µl of RNase and then the mixture was incubated at 37 °C for 30-40 minutes. 0.8% agarose gel was prepared to use for checking the DNA quality and removal of RNA. DNA-50 option of the Nanodrop spectrophotometer was used to quantify the DNA concentration. Two universal primers were used for the RBCL-PCR analysis. The PCR mixtures were prepared using 2.5 µl of 10x PCR buffer, 1 µl of 25 mM $MgCl_2$, 1 µl each of forward and reverse primer, 1 µl of DMSO, 2 µl of 2.5 mM dNTPs, 0.1 µl of 5 µl Taq DNA polymerase, and 3 µl of 10 ng/µl DNA. The total reaction volume was made up to 25 µl using 13.4 µl of nuclease-free water. PCR cycling parameters included 9 cycles with an initial denaturing temperature of 94 °C for 5 mins and 15 sec denaturing and afterward an annealing temperature of 65 °C for 20 seconds and finally an extension with 72 °C for 30 seconds. Then 35 cycles with 94 °C for 15 seconds, the annealing temperature of 55 °C for 20 seconds, extension with 72 °C for 30 seconds, final extension at 72 °C for 7 minutes, and then held at 10 °C. After PCR, the amplicons generated were sequenced on ABI3500 Genetic Analyser.

Statistical Analysis

The morphometric analyses, based on measurements of both qualitative and quantitative characters were subjected to multivariate analysis. A matrix was developed based on individual character distribution. Several multivariate approaches were used to compare all the evaluated characters including principal components analysis (PCA), Gower-based non-metric dimensional scaling (NMDS), Gower-based unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, and Pearson (linear) correlation in PAST. Before the analysis, all the characters were normalized using the software's correlation matrix as previously adopted and described by Wahlsteen and Tyler (2019). Pearson's correlation was used to show the relationship between the traits studied. The PCA was used to analyse matrices of several characters and species to get a general overview of the variation in the two groups.

The RBCL matrix generated after sequencing was further analysed using PAST (Palaeontological Statistic) package version 3.24 (Hammer et al., 2019) to compare the similarities and dissimilarities present in the accessions. The principal component analysis was used to analyse matrices of several characters and species to get a general overview of the variation between and among groups. Non-metric dimensional scaling was used to produce a dissimilarity-based index to highlight the taxonomic significance of characters included in a study. Neighbour-joining cluster analysis was used to produce a dendrogram for the accessions.

Results

Reproducible results were obtained from this study for both the RBCL genotyping and morphological characterization. The results from the genetic variability test were complimentary with the morphological characterization. The morphological characteristics were observed in the field weeks after the seeds were propagated. Growth was in stages. Plant height was observed to have a progressive growth pattern in all accessions although accession NGB 00387 was found to have a more rapid growth than the others (Figure 2). Slight similarities were observed between accessions NGB 00297 and NGB 00309; NGB 00302 and NGB 00467; accessions NGB 00371 and NGB 00308 were quite distinct with accession NGB 00308 having a slow growth rate (Figure 2). Leaf length showed a linear growth pattern in all the accessions except NGB 00302, NGB 00371, and NGB 00387, which showed slight similarities in their growth pattern within the first week after germination (Figure 3). The leaf width of the seven accessions showed a distinct growth pattern, as some were much wider than others (Figure 4). Variability among and within the accessions at various stages of their growth was observed. The variations in the qualitative characters of the accessions are presented in Table

3, which further distinguishes them. Leaf shape varied among accessions and this was used to classify the accessions.

The results presented in Figures 5-7 suggest two distinct clusters with each group showing a high level of dissimilarity following the multivariate analysis carried out using data from the morphological traits. Figure 5 shows the principal component analysis for the phenotypic characters of the okra accessions. The two principal components presented represent over 50% of the observed variations within the accessions (Suppl. Table 1). The loadings and scores of the nine principal components are presented in Suppl. Tables 2 and 3, respectively. Figure 6 presents the non-metric dimensional scaling analysis of the accessions. The results support the existence of two clusters based on observed phenotypic dissimilarities within and among the accessions at a stress of 0.103. The loading of each of the NMDS axis is presented in Suppl. Table 4. The R^2 value of axis 1 is 0.851 whereas axis 2 is 0.090. Figure 7 presents the Gower-based UPGMA clustering dendrogram based on the phenotypic traits of the okra accessions. Table 4 presents the correlation matrix of sequence data from the RBCL genotyping of the accessions. Figure 8 shows the principal component analysis of the okra accession prepared from the matrix generated from the RBCL analysis. These results suggested a more in-between and less out of group association from the seven okra accessions with clusters delimited based on okra species into *A. callei* and *A. esculentus*.

Table 3. Qualitative morphological characteristics evaluated in the study and their character state and codes.

		Accessions						
S/N	**Parameters**	**NGB 00297**	**NGB 00309**	**NGB 00308**	**NGB 00302**	**NGB 00467**	**NGB 00371**	**NGB 00387**
1	GAS	Erect	Erect	Erect	Erect	Erect	Erect	Erect
2	STC	Green	Green	Green with red patches	Purple	Green with red patches	Green with red patches	Green with red patches
3	STP	Slight	Slight	Slight	Slight	Slight	Slight	Slight
4	BRA	Orthotropic stem only	Orthotropic stem only	Orthotropic stem only	Strong	Orthotropic stem only	Orthotropic stem only	Orthotropic stem only
5	LSH	Lobed	Cordate	Cordate	Pinnately lobed	Lobed	Cordate	Pinnately lobed
6	LC	Green	Green with red veins	Green with red veins	Green	Green	Green with red veins	Green with red veins

GAS: General aspect of the stem, STC: Stem colour, STP: Stem pubescence, BRA: Nature branching, LSH: Leaf shape, LC: Leaf colour.

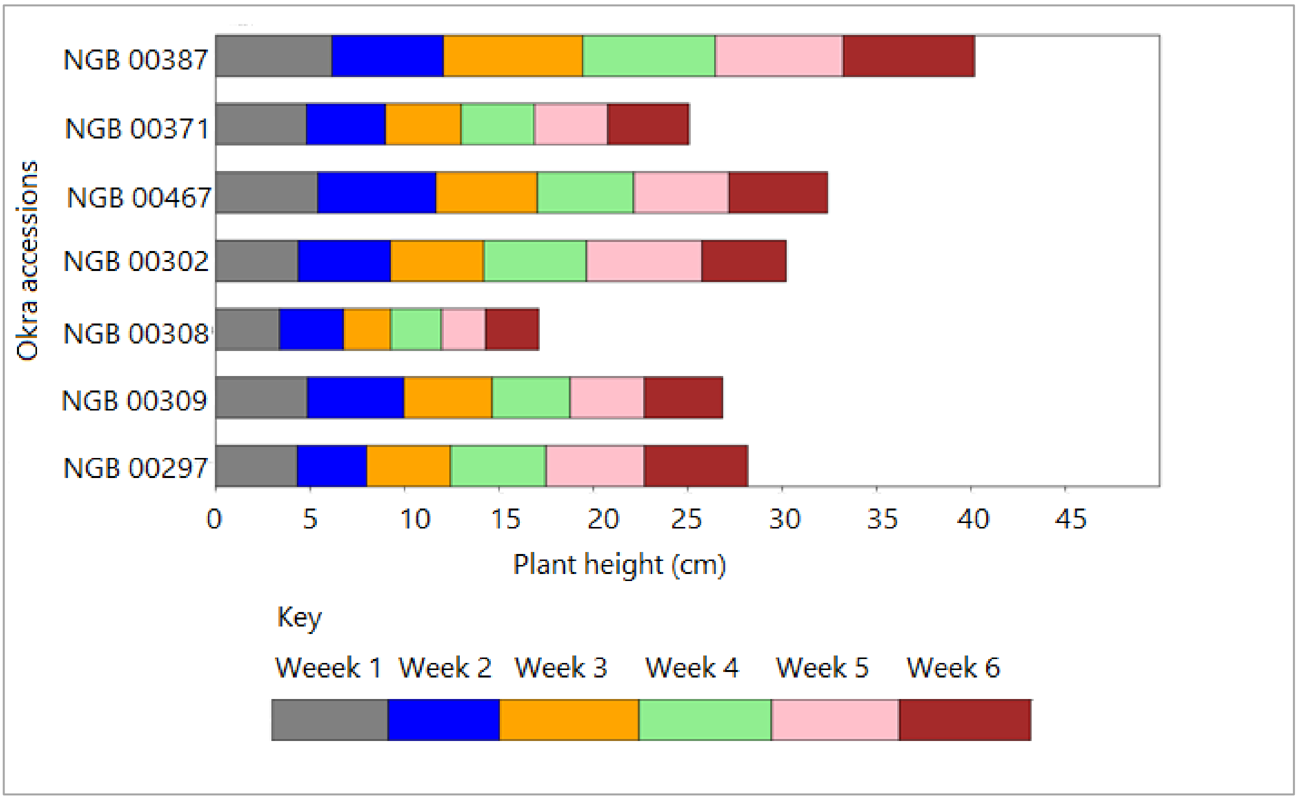

Figure 2. Plant height of okra accessions during the study.

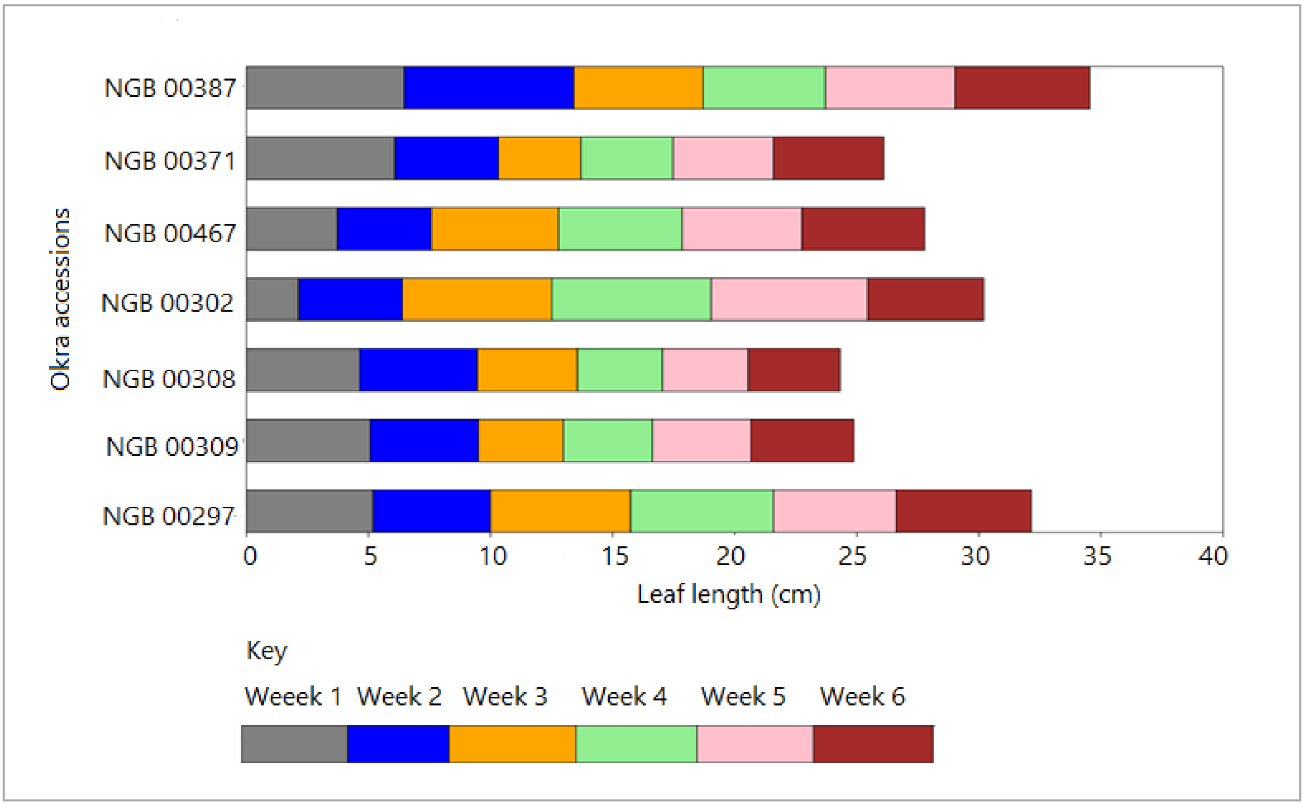

Figure 3. Length of the leaf of *Abelmoschus* accessions during the study.

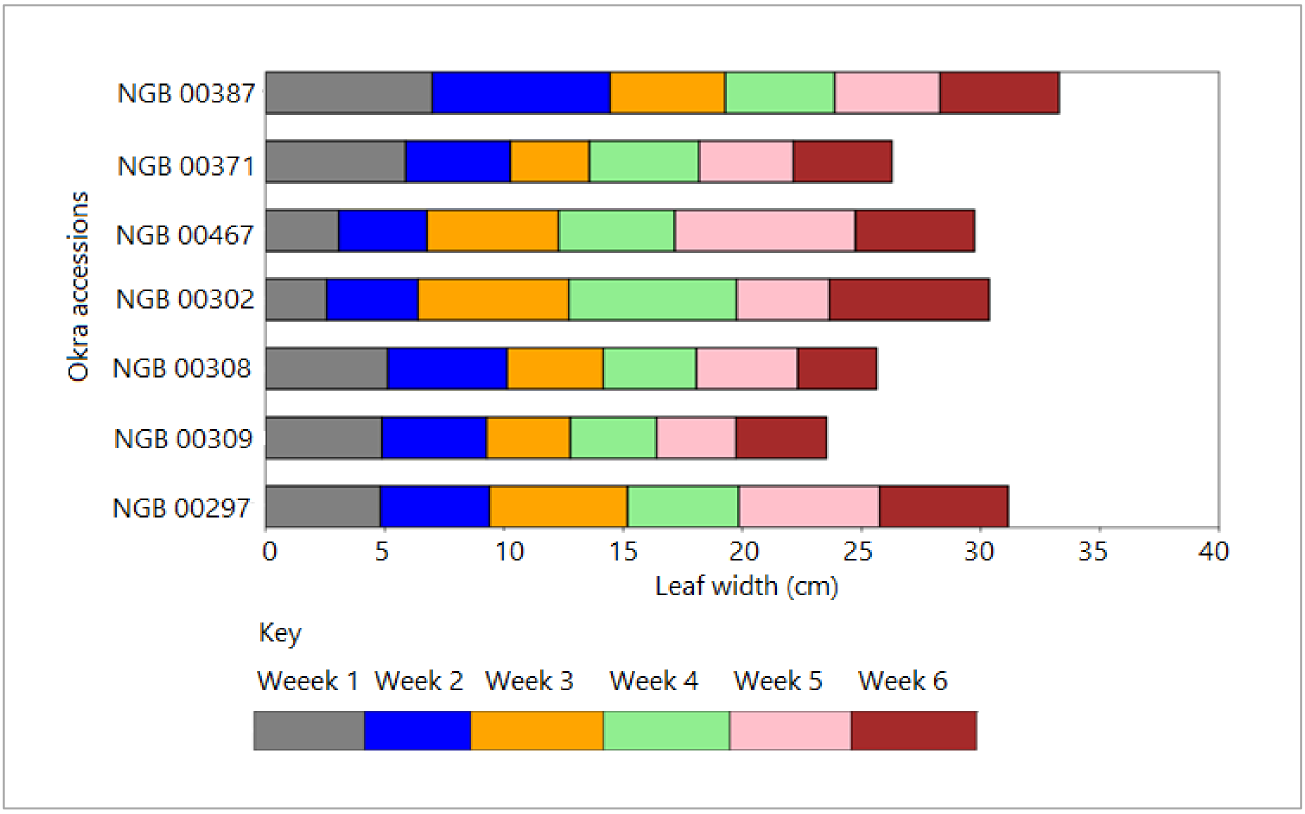

Figure 4. Leaf width of *Abelmoschus* accessions during the study.

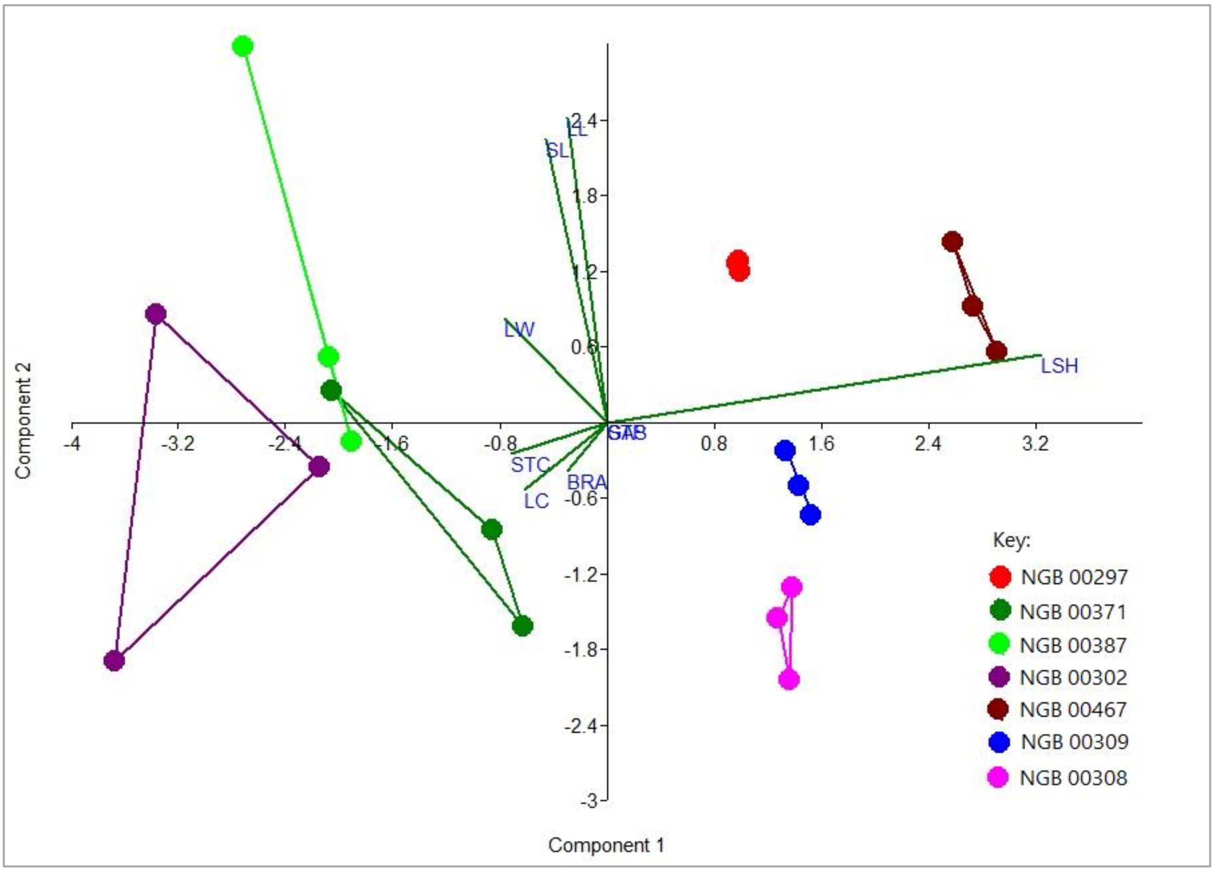

Figure 5. PCA of the phenotypic traits of the okra accessions with Jolliffe cut-off of 0.59963. GAS: General aspect of the stem, STC: Stem colour, STP: Stem pubescence, BRA: Nature branching, LSH: Leaf shape, LC: Leaf colour, LL: Leaf length, LW: Leaf width, SL: Stem length.

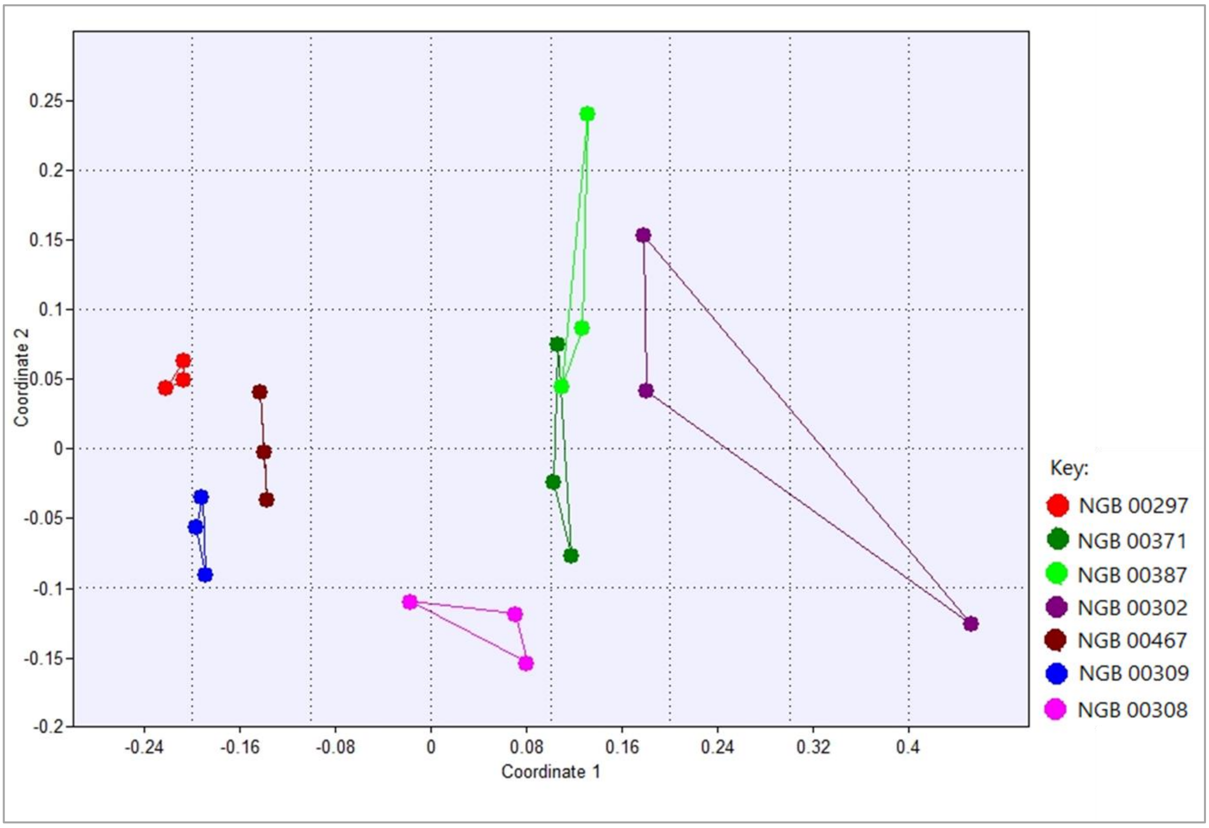

Figure 6. Gower-based NMDS of the phenotypic characters of the okra accessions.

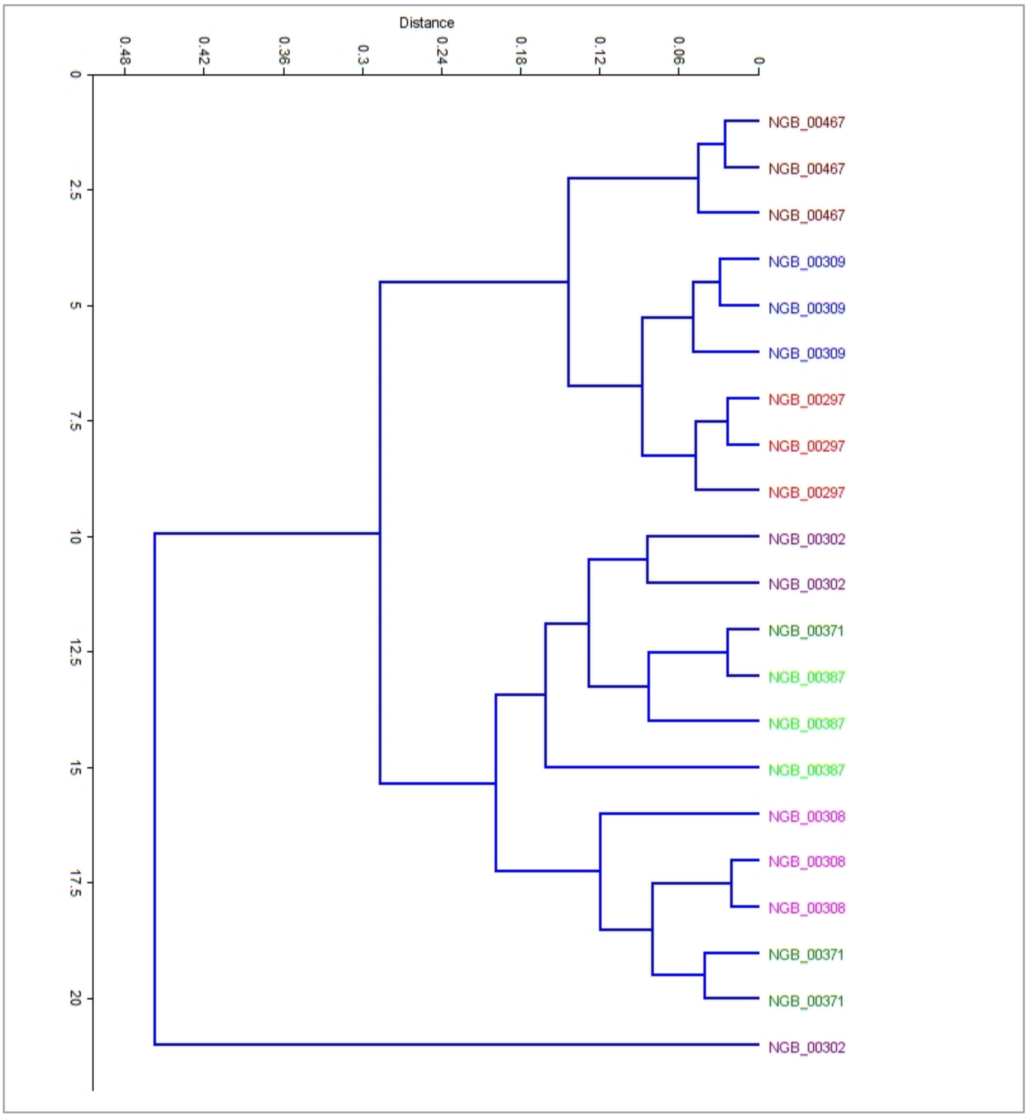

Figure 7. Gower-based UPGMA clustering analysis of the phenotypic characters of the okra accessions with a Cophenetic correlation of 0.87754.

Table 4. Pairwise correlation analysis of RBCL genotyping sequence data.

Accessions*	00302ᵃ	00371ᵃ	00387ᵃ	00387ᵇ	00308ᵃ	00297ᵃ	00308ᵇ	00309ᵇ	00297ᵇ	00467ᵃ	00302ᵇ
00302ᵃ	0	1.12	0.54	0.625	0.594	0.597	0.6	0.605	0.599	0.608	0.684
00371ᵃ	1.12	0	1.66	1.745	1.714	1.717	1.72	1.725	1.72	1.728	1.804
00387ᵃ	0.54	1.66	0	0.085	0.054	0.057	0.06	0.065	0.06	0.068	0.144
00387ᵇ	0.625	1.745	0.085	0	0.072	0.075	0.078	0.083	0.078	0.087	0.162
00308ᵃ	0.594	1.714	0.054	0.072	0	0.003	0.006	0.011	0.006	0.014	0.09
00297ᵃ	0.597	1.717	0.057	0.075	0.003	0	0.004	0.009	0.004	0.012	0.088
00308ᵇ	0.6	1.72	0.06	0.078	0.006	0.004	0	0.005	0.005	0.014	0.09
00309ᵇ	0.605	1.725	0.065	0.083	0.011	0.009	0.005	0	0.01	0.019	0.094
00297ᵇ	0.599	1.72	0.06	0.078	0.006	0.004	0.005	0.01	0	0.009	0.084
00467ᵃ	0.608	1.728	0.068	0.087	0.014	0.012	0.014	0.019	0.009	0	0.076
00302ᵇ	0.684	1.804	0.144	0.162	0.09	0.088	0.09	0.094	0.084	0.076	0

*All accession names start with NGB. Superscripts a and b represent sample replicates 1 and 2, respectively.

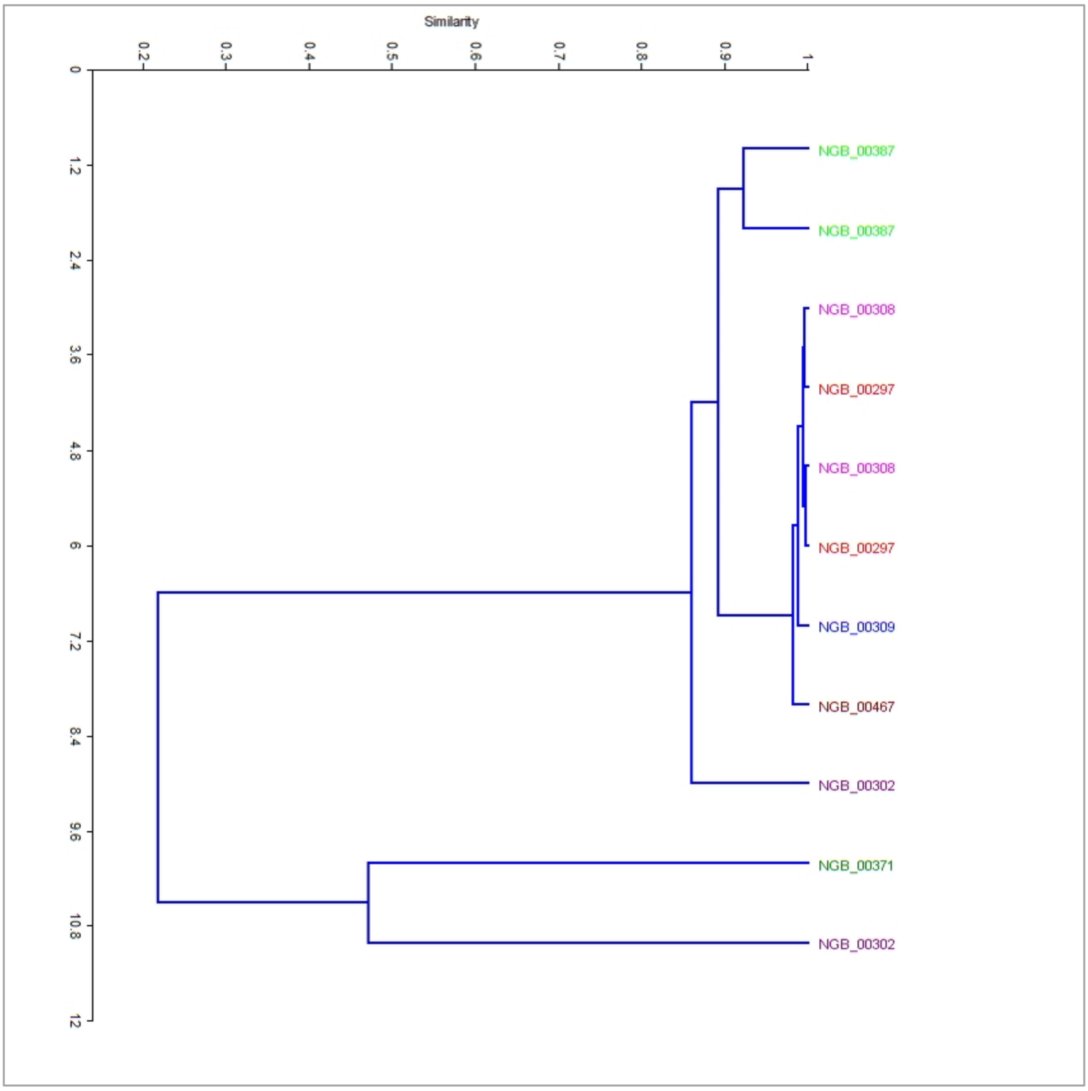

Figure 8. Bray Curtis-based UPGMA clustering dendrogram of the genetic relationship among the okra accessions with a Cophenetic correlation value of 0.981.

Discussion

The results of this study are indicative of the fact that all accessions of the Okra exhibited significant variation in morphological traits (quantitative traits), but minimal variation in qualitative traits, among the accessions investigated. This correlates with the results of (Omalsaad et al., 2014) stating that the latter traits are not useful for studying the genetic diversity of okra germplasm. Observation of significant differences among the quantitative traits is, however, an indication that genetic diversity exists among the accessions. More so, morphological characteristics are an important tool for the evaluation of plants for systematic classification and breeding. These characteristics can in some cases complement the molecular and biochemical basis of characterizing the plant germplasm although it is not enough to set them apart. From the studies, all seven accessions had an erect, and strong stem. The stem colour was green in accessions NGB 00297 and NGB 00309. Accessions NGB 00308, NGB 00467, NGB 00371 and NGB 00387 identified as okra showed red pigments on green stem; while stem pigmentation was wholly purple in accession NGB 00302. Hairiness (pubescence) was slightly less pronounced on stems of all seven accessions so there was variation in the stem pubescence of the okra accessions.

Moderate orthotropic branching was observed in accessions NGB 309, NGB 308, NGB 00297, NGB 00467, NGB 00371, NGB 00387 identified as okra, and strongly branched in NGB 302. Leaf shape was cordate in accessions NGB 00309, NGB 00308, and NGB 00371. In accessions NGB 00297 and NGB 00467 it was found to be lobed. Accessions NGB 00302 and NGB 387 identified as Okra had pinnately lobed leaf shapes. The leaf shape changed as the plant progressively germinated in all accessions. Leaf colouration was observed to be green in all accessions although red pigmentations were present on the leaf base, veins, and midribs of accessions NGB 00387 which was identified as okra, NGB 00308, NGB 00371, and NGB 00309. These findings are consistent with the reports of (Omonhinmin & Osawaru, 2005; Aladele et al., 2008; Oppong-Sekyere et al., 2011). Although a great similarity existed, there were marked morphological variations between members of the two groups.

The quantitative attributes assessed in this study were recorded before flowering. These attributes were leaf width, length of leaf, and stem length. Hazra and Basu (2000) reported that days to bud emergence and plant height at maturity, among other morphological traits, are some of the most variable traits of okra that are necessary for selection programmes aimed at improving desirable traits. The leaves showed linear growth in length in all accessions except for accession NGB 00302. The length of leaf for accessions NGB 00371 and NGB 00387 was slightly similar. The leaf width was generally distinct for all accessions. The plant height of the accessions evaluated was also significantly different. The height of the plant can potentially affect yield as those that are taller are usually subject to wind damage in the event of heavy seasonal rains.

Results from the multivariate analysis are in concordance. A cluster phenotype of the morphological traits was observed at a 3.75 level of similarity forming two distinct clusters. The following accessions were grouped into one: NGB 00309, NGB 00308, NGB 297, and NGB 00467 with the other group having NGB 00302, NGB 00371, and the accession identified as Okra being characterized into this group (such as NGB 00387).

RBCL markers were used to determine a genetic variation within available accessions of *Abelmoschus*. The results from this work proved that RBCL markers are useful tools for genetic variation study in *Abelmoschus*. This study presented a detailed genetic distinctiveness and relationship among the accessions. A moderate level of variability was observed although accession NGB 00371 shows a much wider disparity. Variation generally, is a very important element in breeding programmes (Omonhinmin & Osawaru, 2005; Akinyele & Temikotan, 2007). The use of genetic assays in the identification of plant germplasm has become more efficient and useful for the better characterization of plant genetic resources. Hence it is clear from the result that some okra germplasm used in this study belongs to a restricted germplasm pool. However, the accessions that were found to share common similarities further show a narrow genetic base as reported by Kumar et al. (2017). High similarities among the accessions as reported by Hamon and Koechlin (1991) were expected due to their high self-pollinating properties (Hamon & Koechlin, 1991; Kumar et al., 2017). Gulsen et al. (2007) and Sharma et al. (2015) also reported a similar result establishing a 100% similarity among grapefruit cultivars. This arrayed the fact that though the accessions are independent species, there are bound genetically one to another and may share similar proximate and anti-nutrient properties which further characterizes them. The collection of this data will give the scientists, breeders, and geneticists with adequate information on the allelic similarities present in gene bank materials and to further develop more trials of these accessions. According to documented publications, this is the first report on genetic analysis using the RBCL marker in the characterization of *Abelmoschus* species in Nigeria.

Conclusion

Okra is a plant of immense importance because of the food and nutrition value as well as a source of potential raw material for diverse industrial processes. The okra accessions assessed in the current study showed variations and can be distinguished into *A. esculentus* and *A. caillei*. The diversity and distinction enumerated in the study further outline their potential and give credence to the role they play in plant breeding programmes, food security, and nutritional security among other economic utilization. To further investigate the genetic variation among plant genetic resources, there is a need to frequently evaluate and utilize okra germplasm and other crop germplasm present in gene banks by breeders, researchers, scientists, biologists, and geneticists to unravel the diverse values embedded in these genetic resources as many germplasms with these traits remain unutilized. Genetic diversity has in recent times been acknowledged as a specific area that can contribute to food and nutritional security. Thus, a better understanding will help to determine what to conserve, when, and how to conserve. The diversity indicated can further be utilized in heterosis breeding, transgressive breeding, and introgression of alien genes for specific traits. Nevertheless, the research seeks to contribute to the conservation and breeding of okra germplasm within Nigeria. It is therefore recommended that further studies be carried out to incorporate diverse characterization techniques, secondary phytochemicals, proximate nutrients, and anti-nutrient composition of the germplasm to obtain a more robust result.

Acknowledgment

The authors wish to acknowledge the assistance of the Germplasm unit of the International Institute of Tropical Agriculture, Ibadan for providing laboratory space and resources as well as the gene bank unit of the National Centre for Genetic Resources and Biotechnology, Ibadan for providing the okra accessions used in the study.

Conflict of Interest

The authors declare that there are no competing interests.

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Supplementary Information

Supplementary Table 1. Principal component summary of the morphological characteristics of the okra accessions.

PC	Eigenvalue	% variance
1	4.11067	55.124
2	1.69206	22.69
3	0.97432	13.066
4	0.343987	4.6129
5	0.277947	3.7273
6	0.055448	0.74356
7	0.002711	0.03636
8	1.47E-32	1.97E-31
9	0	0

Supplementary Table 2. Principal component summary of the morphological characteristics of the okra accessions.

Accessions	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
NGB 00297	-1.2409	1.3748	0.53249	-0.76737	-0.55359	-0.10833	0.039873	4.10E-17	0
NGB 00297	-1.2063	1.3424	0.19817	0.56943	-0.44858	-0.00573	-0.04747	9.11E-17	0
NGB 00297	-1.2339	1.2835	0.34432	0.044642	-0.49674	-0.05163	-0.02713	6.67E-17	0
NGB 00309	-1.6025	-0.13603	-0.03121	0.32758	-0.55292	-0.21601	-0.06222	-8.62E-18	0
NGB 00309	-1.6974	-0.41344	-0.4172	0.77635	-0.51136	-0.24383	-0.00133	-5.04E-18	0
NGB 00309	-1.8078	-0.63985	-0.20232	-0.51153	-0.62179	-0.3831	0.10207	-6.82E-17	0
NGB 00308	-1.6799	-1.2024	0.018234	-0.76391	-0.47763	0.53448	-0.03406	-2.97E-16	0
NGB 00308	-1.5125	-1.5005	-0.42136	-0.1636	0.51227	0.24687	0.086307	-4.34E-16	0
NGB 00308	-1.6058	-1.9852	-0.44142	0.13567	0.49389	0.22675	-0.00697	-4.54E-16	0
NGB 00302	3.1883	0.81797	-0.50523	-0.12513	0.43318	-0.23098	-0.02538	4.18E-16	0
NGB 00302	1.9439	-0.36527	-0.1411	-0.8616	0.56661	-0.27898	-0.06867	9.98E-17	0
NGB 00302	3.3615	-1.8583	3.5698	0.51781	-0.22031	0.011755	0.024518	-7.15E-17	0
NGB 00467	1.8839	-0.45369	-0.71434	-0.10557	0.65811	-0.27652	0.04039	1.29E-16	0
NGB 00467	-2.7567	1.5166	1.1147	-0.12199	0.97437	0.006615	-0.01792	-5.05E-16	0
NGB 00467	-2.9027	1.002	0.66087	0.53052	1.0158	-0.03199	0.012588	-5.08E-16	0
NGB 00371	0.61083	-0.81829	-0.29272	-0.31982	-0.05836	0.093855	-0.09542	2.59E-17	0
NGB 00371	1.8175	0.26783	-0.46484	-0.22946	-0.25414	0.084282	-0.03487	3.13E-16	0
NGB 00371	0.37672	-1.591	-0.77428	0.002029	-0.0525	-0.01184	-0.02356	-5.28E-18	0
NGB 00387	1.8747	0.50583	-1.2721	1.6332	-0.05283	0.21402	0.020473	4.05E-16	0
NGB 00387	2.5215	2.9919	0.088454	-0.4375	-0.11125	0.41579	0.047518	4.71E-16	0
NGB 00387	1.6677	-0.13887	-0.84895	-0.12978	-0.24222	0.00453	0.071248	2.96E-16	0

Supplementary Table 3. Principal component summary of the morphological characteristics of the okra accessions.

Character	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
GAS	0	0	0	0	0	0	0	0	1
STC	0.25356	-0.10248	0.09066	-0.03629	0.91748	-0.27104	0.020708	-1.55E-16	0
STP	7.72E-19	-2.19E-17	2.19E-17	-5.53E-18	2.79E-17	1.61E-16	1.79E-16	1	0
BRA	0.081776	-0.10983	0.36639	0.15053	-0.07926	0.0212	0.90422	-1.50E-16	0
LSH	-0.90765	0.16531	0.17886	0.039828	0.29821	0.15579	0.045547	-2.03E-16	0
LC	0.17574	-0.1575	-0.11289	-0.12321	0.21494	0.93241	0.028211	-1.94E-16	0
SL	0.15415	0.62817	-0.25198	0.7001	0.11188	0.1116	0.055102	6.84E-17	0
LL	0.09623	0.68596	-0.07446	-0.68219	0.021562	-0.01307	0.22055	2.05E-17	0
LW	0.2032	0.2453	0.86242	0.061131	-0.06193	0.14071	-0.35694	5.95E-18	0

GAS: General aspect of stem, STC: Stem colour, STP: Stem pubescence, BRA: Nature branching, LSH: Leaf shape, LC: Leaf colour, LL: Leaf length, LW: Leaf width, SL: Stem length.

Supplementary Table 4. Loadings of the NMDS axis.

Accession	Axis 1	Axis 2
NGB_00297	-0.23029	-0.00682
NGB 00297	-0.21822	0.013605
NGB 00297	-0.21551	0.011861
NGB 00309	-0.1892	-0.05276
NGB 00309	-0.19256	-0.07175
NGB 00309	-0.18955	-0.09693
NGB 00308	-0.01221	-0.17571
NGB 00308	0.063642	-0.1196
NGB 00308	0.073395	-0.14289
NGB 00302	0.21052	0.13782
NGB 00302	0.18856	0.028531
NGB 00302	0.51797	-0.10229
NGB 00467	-0.16017	0.079782
NGB 00467	-0.174	0.11852
NGB 00467	-0.16493	0.095566
NGB 00371	0.091822	-0.03085
NGB 00371	0.093821	0.057657
NGB 00371	0.10699	-0.07791
NGB 00387	0.13624	0.074822
NGB 00387	0.16732	0.22637
NGB 00387	0.096355	0.032964

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JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

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RESEARCH ARTICLE

Determining the Yield and Yield Components of Some Local Potato Genotypes Grown in the North Eastern Anatolia Region

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ARTICLE INFO

Article History
Received: 25.11.2022
Accepted: 25.12.2022
First Published: 28.12.2022

Keywords
Adaptation
Local variety
Solanum tuberosum L
Yield components

ABSTRACT

This study was carried out in 2018 to examine the yield and yield components of 8 potato genotypes (Kanursu, Aşkar, Çamlı, Akbulut, Yukarıkızı, Incili, Erikdibi, and Nisantası) and 2 potato varieties (Agria and Lady Olympia) procured in the ecological conditions of Bayburt, Turkey. The experimental work was conducted in trial ground at the Experimental Station of the University of Bayburt (Turkey). The research was designed in random blocks with three replications. As a result of the study, the highest number of stems per plant (6.7), tuber yield per hectare (15.19 tons), number of tubers per plant (8.47), and tuber yield per plant (357.01 g) were found to be in the genotype Akbulut; the highest ratio of large tuber (28.27%) in the genotype Konursu; and the highest ratio of medium tuber (85.22%) in the genotype Çamlıkoz. This study; Although the best potato yield was obtained from the Akbulut genotype on a regional basis, these data are a pioneer for other future studies to determine the cultivar candidates needed in the development of domestic potato cultivars.

Please cite this paper as follows:

Gül, V., & Sefaoğlu, F. (2022). Determining the yield and yield components of some local potato genotypes grown in the North Eastern Anatolia Region. *Journal of Agricultural Production*, *3*(2), 124-130. https://doi.org/10.56430/japro.1210278

Introduction

Potato, one of the most common and important plants in the world, is the main staple food for the majority of the world's population and has various health benefits. It is the fourth most important human food product in the world after rice, corn, and wheat (Karam et al., 2009; Kandil et al., 2011). The high nutritional content, ability to adapt to marginal environments, relative ease of cultivation, low cost and high productivity are the features that make potato one of the most important foods and sources of income for developing countries.

About 500 thousand tons of seed potatoes (Basic-1 and Basic-2) are needed for potato production in Turkey (Öztürk & Polat, 2017). These seed potatoes are procured from the Netherlands, Germany, and the United States, and reproduced, certified, and delivered to the producers in Turkey. While the rate of using certified seed is around 25-30% in Turkey, this rate is 95-100% in other potato producing countries (Yılmaz, 2014). The lack of native potato varieties is one of the reasons that cause Turkey's foreign dependency in terms of potato seed. Although Turkey has the conditions suitable for potato cultivation at all times of the year (in terms of agricultural lands and climate), it lacks the native varieties that can be used commercially. Therefore, many previous studies emphasized the problems of not having a native variety (Öztürk et al., 2008; Çalışkan et al., 2010; Şanlı & Kardoğan, 2012). Selecting the right variety for the right region is a very important parameter, as well as taking many measures to grow a high yielding and

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quality potato. Farming seasons are short in the interior and high parts of Turkey (May-September). This makes it important to identify the early varieties with high adaptation to these regions. In order to grow potatoes with high quality and high tuber yield, it should be determined which native potato variety has good characteristics for which region (Arioglu et al., 2005). In the literature, there are some studies carried out to determine the effects of climate and environmental factors and identify new varieties with high quality and yield characteristics (Akkale et al., 2010; Merga & Dechassa, 2019). In order to produce high yielding potato tubers with high marketing and processing quality, it is very important to identify the best performing varieties on a regional basis (Bilate & Mulualem, 2016; Habtamu et al., 2016; Bekele & Haile, 2019). In their agricultural performance study conducted in Bornova, İzmir on nine local potato genotypes obtained from Eastern Anatolia, Yıldırım and Öztürk (2016) reported that the local genotype Posof yielded the best results (Plant height: 60.5 cm, number of stems: 4.9, number of tubers: 11.2, plant yield: 762.7 g, and plot yield: 3.8 kg). Today, many variety generation studies are being carried out for potato, and these varieties are transferred to different regions to identify the varieties with good adaptability, high quality and yield.

The purpose of the present study was to identify the local potato varieties available in the region, to compare them in terms of yield and quality components, and to make a suggestion about the best variety candidate.

Materials and Methods

Trial Establishment

This study was carried out in the trial area of Department of Organic Agricultural Management, Bayburt University in 2018. Figure 1 shows the average precipitation, temperature, and relative humidity for 2018 and other years.

The soil of the trail area was found to be loamy and slightly alkaline and have a pH value of 7.75, a total salt content of 0.047% (salt-free), an organic matter content of 0.99% (very low), a lime content of 9.3%, a phosphorus content of 11.44 kg da$^{-1,}$ and a potassium content of 80.3 kg da^{-1} (high).

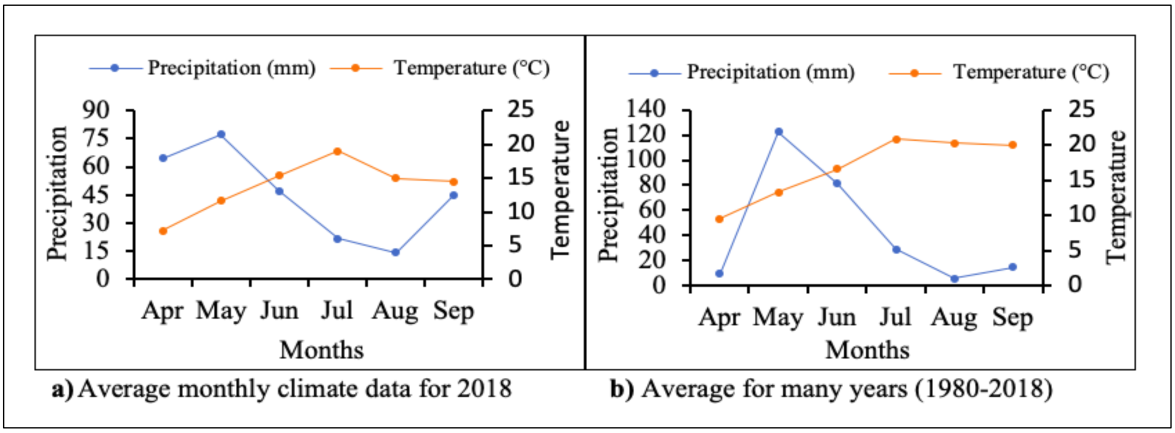

Figure 1. Average monthly temperature, precipitation values at the experimental site in 2018, 2019 and long-term period (1982-2017).

Experimental Layout and Treatments

This study was designed in random blocks with three replications and carried out in 2018 to examine the yield and yield components of 8 potato genotypes (Kanursu, Askar, Çamlı, Akbulut, Yukarıkızı, Incili, Erikdibi, and Nisantaşı) and 2 potato varieties (Agria and Lady Olympia) procured from Bayburt, Turkey (Figure 2). The Agria variety, which is intensively grown in the region, is used as medium late, edible and industrial, while the Lady Olympia variety is used as medium early and industrial.

Seeding and Product Application

Stable manure (30 tons per hectare, 0.9% kg N, 0.5% P_2O_5, and 0.3 kg K_2O) was used as fertilizer. The fertilizer was uniformly applied to the seed bed before planting. The potato plants were planted manually on April 15, 2018, leaving 35 cm between plants and 70 cm between rows.

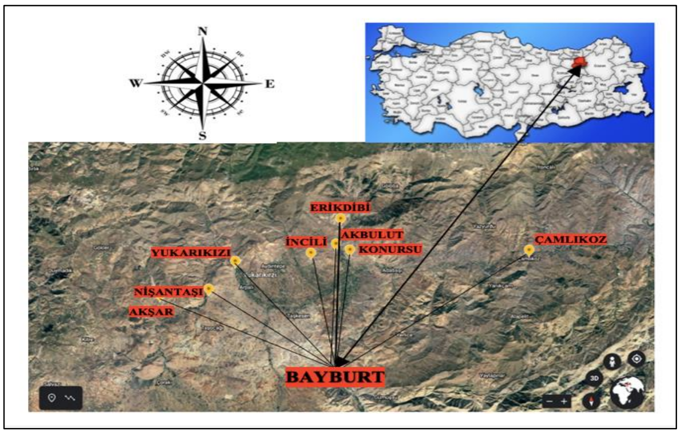

Figure 2. Showing of local potato seed samples obtained from Bayburt region on the map.

Trial Maintenance and Monitoring

Weed control was carried out mechanically during the cultivation period. The experimental area was irrigated regularly and uniformly three times in every 20 days until the end of August as of the end of June against drought stress.

Harvesting

The potato genotypes and varieties were harvested on September 3rd, 2018 when the following conditions were realized: the green parts turned yellow and dry, the stolons separated from the main plant, and the tubers had a certain size and skin with a normal thickness and could not be peeled. The following parameters were examined within the scope of the study: plant height (cm), number of stems per plant, number of lateral branches, number of tubers per plant, tuber yield per plant (g), single tuber weight (g), tuber yield per hectare (tons ha^{-1}), tuber width (cm) and tuber length (cm), and large tuber ratio (%) (>50 mm), medium tuber ratio (%) (>30 mm, <50 mm), and small tuber ratio (%) (<30 mm).

Statistical Analysis

The data obtained as a result of the research were analysed using SPSS software package. The differences between the means were determined using Duncan Multiple comparison test.

Results and Discussion

Statistically significant differences (p≤0.01) were found to exist between the eight native genotypes and two varieties of potatoes grown in Bayburt in terms of morphological and agronomic characteristics. Table 1 shows the sources of variance and the levels of statistical significance for these characteristics.

Table 1. The results of analysis of variance for some heavy metal concentrations in potato genotypes in Bayburt, Turkey.

Mean Square/Source of Variation	Genotype	Error	LSD	CV (%)
df	9	18		
Plant Height	8.44**	20.04	7.69	19.64
Stem Number	44.37**	0.11	0.58	30.06
Side Stem Number	32.97**	6.66	4.44	22.56
Hectare Tuber Yield	44.53**	0.71	1.45	36.99
Tuber Number Per Plant	22.42**	0.27	0.89	29.68
Large Tuber Ration	96.87**	1.27	1.94	30.61
Middle Tuber Ration	248.58**	1.58	2.16	16.51
Small Tuber Ration	175.28**	0.86	1.59	54.81
Tuber Width	7.01**	0.09	0.52	12.24
Tuber Length	16.66**	0.18	0.73	16.11
Tuber Yield Per Plant	50.68**	359.68	32.60	36.49
Single Tuber Weight	34.89**	7.86	4.82	23.18

*,** significant at p<0.01; CV: Coefficient of variations.

Morphological and Agronomic Characteristics

The mean values of the morphological and agronomic characteristics of the local potato genotypes and the commercial potato varieties are given in the Figures 3, 4, 5, and 6. According to the results of the study, the genotype İncili was found to have the highest plant height (51.3 cm) and tuber width (4.91 cm); the genotype Akbulut was found to have the highest number of stems per plant (6.7), tuber yield per hectare (15.19 tons), number of tubers per plant (8.47), tuber yield per plant (357.01 g); the genotype Konursu was found to have the highest ratio of large tuber (28.27%) and the highest ratio of small tuber (25.6%); the genotype Çamlıkoz was found to have the highest number of lateral branches (52.4) and the highest ratio of medium tuber (85.22%); and the genotype Erikdibi was found to have the highest tuber length (7.73 cm) and the highest single tuber weight (58.85 g). On the other hand, the variety Agria was found to have the lowest number of lateral branches (29.8) and the lowest number of tubers per plant (3.39); the variety Lady Olympia was found to have the lowest plant height (28.6 cm) and the lowest tuber length (4.94 cm); the genotype Konursu was found to have the lowest tuber yield per hectare (3.42 tons), the lowest ratio of medium tuber (46.14%), the lowest tuber yield per plant (83.86 g); the genotype Akşar was found to have the lowest tuber width (3.39 cm) and the lowest single tuber weight (25.98 g); the genotype Nişantaşı was found to have the lowest number of stems per plant (2.8); and the genotype Yukarıkızı was found to have the lowest ratio of large tuber (6.81%) and the lowest ratio of small tuber (4.78%). It was observed that the potato genotypes of Bayburt region had superior characteristics compared to the varieties, and especially the potato genotype Akbulut had better agricultural characteristics than the other genotypes and varieties.

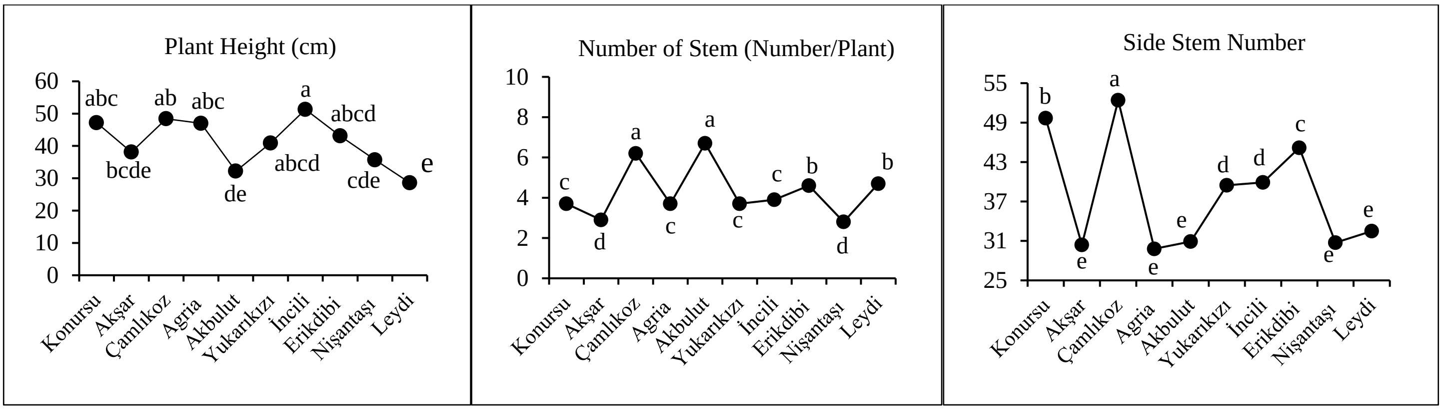

Figure 3. Plant height (left), stem number (middle), and side stem number (right) of potato genotypes.

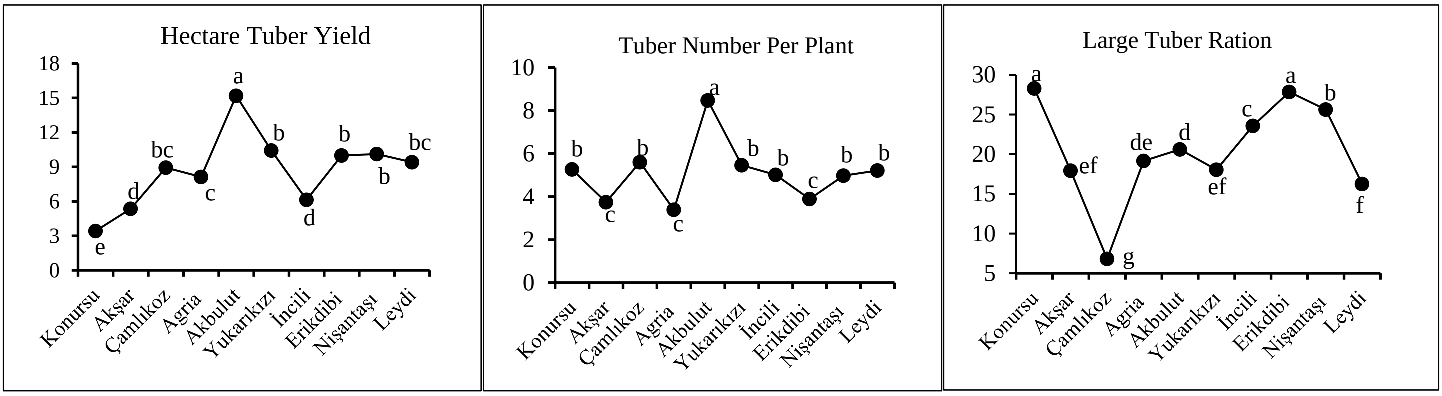

Figure 4. Hectare tuber yield (left), tuber number per plant (middle), and large tuber ration (right) of potato genotypes.

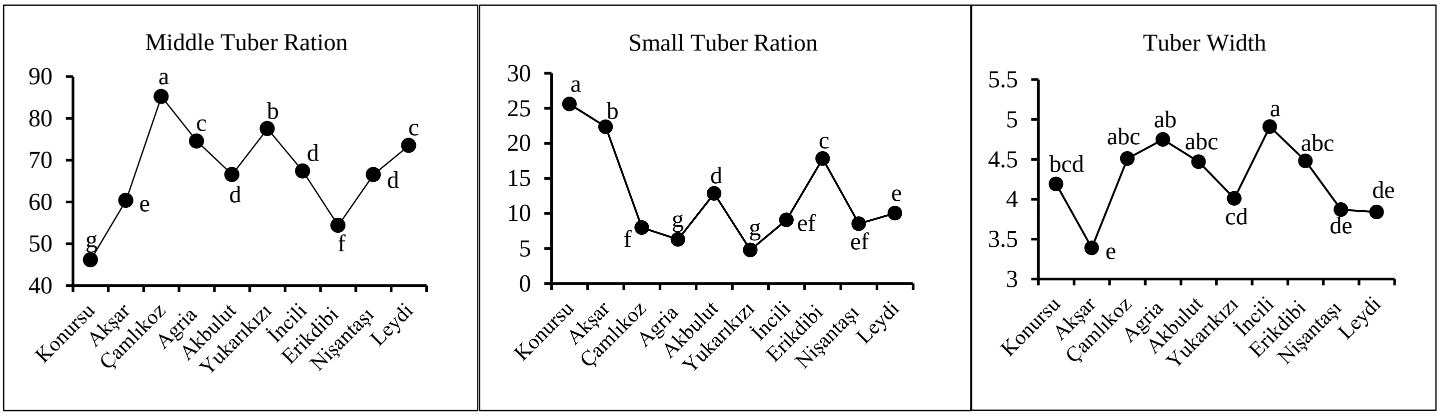

Figure 5. Middle tuber ration (left), small tuber ration (middle), and tuber width (right) of potato genotypes.

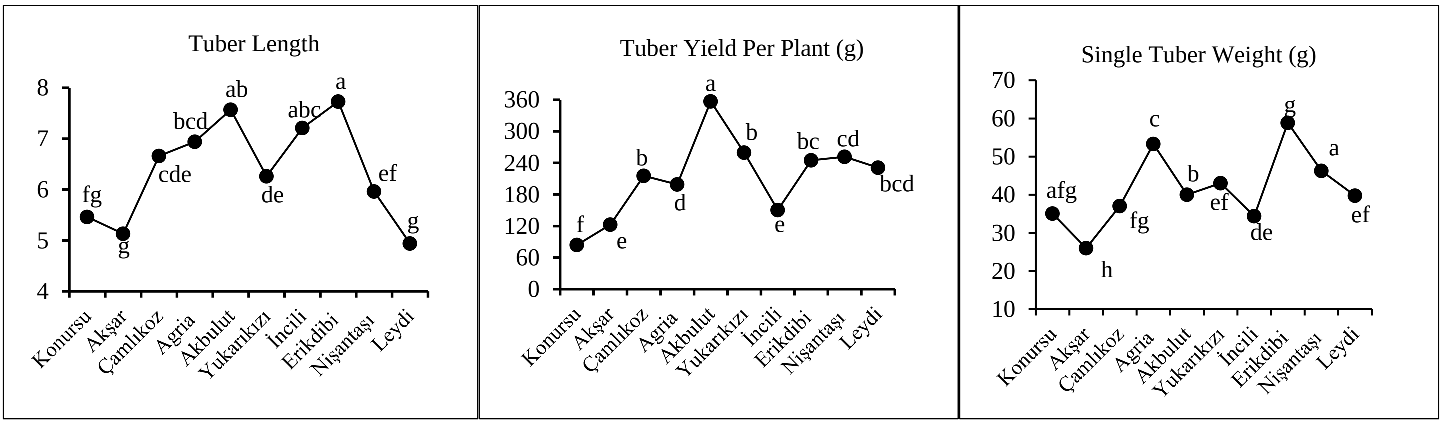

Figure 6. Tuber length (left), tuber yield per plant (middle), and single tuber weight (right) of potato genotypes.

The morphological and agronomic characteristics we found were similar to those reported by some researchers (Kimoone et al., 2005; Ekin, 2009; Cerit & Kaynak, 2010; Kara, 2016; Boydak & Kayantaş, 2017), but lower than those reported by some others (Ekin, 2009; Şanlı & Karadoğan, 2012; Yalçın & Tunçtürk, 2018; Çöl & Akınerdem, 2017; Merga & Dechassa, 2019). This difference might be due to the differences between ours and the previous studies in terms of soil properties, environmental and climatic factors. As a matter of fact, many studies asserted that the low yield was due to the reasons such as soil poor in organic matter, heavy textured soil, genetic characteristics of the varieties, environmental and climatic factors (Karaca Öner & Aytaç, 2016; Boydak & Kayantaş, 2017; Yalçın & Tunçtürk, 2018; Arslan, 2019). It is very important for farmers to use the potatoes they have grown as seeds in determining the potato varieties adapted to a region. Potato is very sensitive to biotic (such as diseases and pests) and abiotic (such as climate and environmental factors) conditions of the region where it is grown (Simakov et al., 2008; Hirsch et al., 2013). Moreover, not only the tuber yield, which is a key parameter in potato, but also the parameters such as number of tubers, tuber size, and plant height vary depending on the climate and environmental factors of the region where the plant is grown (Graveland, 2014). Yılmaz and Tugay (1999) asserted that better quality seeds should be used rather than the physiologically damaged tubers being used in the region for a long time, and that potato tubers grown in high altitudes had higher yields.

Correlation Coefficients

When the Pearson correlation results for the parameters were examined, it was found that there was a high positive correlation between the plant height and the number of stems (r=0.71**), the number of stems and the number of lateral branches (r=0.73**), the tuber yield per hectare and the tuber yield per plant (r=0.99**); a moderate positive correlation between the plant height and the number of lateral branches (r=0.50**) and the tuber width (r=0.51**), the tuber yield per hectare and the number of tubers per plant (r=0.58**), the number of tubers per plant and the tuber yield per plant (r=0.56**), the tuber width and the tuber length (r=0.66**), the tuber length and the single tuber weight (r=0.50**); a high negative correlation between the ratio of medium tuber and the ratio of large tuber (r=-0.83**), the ratio of medium tuber and the ratio of small tuber (r=-0.86**); a moderate negative correlation between the ratio of small tuber and the tuber yield per plant (r=-0.507**); a weak negative correlation between the plant height and the tuber yield per hectare (r=-0.48**) and the tuber yield per plant (r=-0.47**), the tuber yield per hectare and the ratio of small tuber (r=-0.48**) with a significance of $p \leq 0.01$ (Table 2). Moreover, there was a weak positive correlation between the number of stems and the tuber width (r=0.43*); the tuber yield per hectare and the ratio of medium tuber (r=0.39*), the tuber length (r=0.45*), and the single tuber weight (r=0.41*); the ratio of small tuber and the ratio of large tuber (r=0.44*); the ratio of medium tuber and the tuber yield per plant (r=0.40*); the tuber width and the single tuber weight (r=0.36*); the tuber length and the tuber yield per plant (r=0.44*); the tuber yield per plant and single tuber weight (r=0.45*) with a significance of $p \leq 0.05$ (Table 2).

Table 2. Correlation coefficients between observed characteristics.

	PH	SN	SSN	HTY	TNPP	LTR	MTR	STR	TW	TL	TYPP	STW
PH	1											
SN	0.71**	1										
SSN	0.50**	0.73**	1									
HTY	-0.48**	-0.30^{ns}	-0.32^{ns}	1								
TNPP	-0.33^{ns}	0.05^{ns}	0.01^{ns}	0.58**	1							
LTR	0.04^{ns}	0.21^{ns}	-0.08^{ns}	-0.17^{ns}	-0.12^{ns}	1						
MTR	-0.04^{ns}	-0.25^{ns}	-0.10^{ns}	0.39*	0.13^{ns}	-0.83**	1					
STR	0.03^{ns}	0.20^{ns}	0.21^{ns}	-0.48**	-0.11^{ns}	0.44*	-0.86**	1				
TW	0.51**	0.43*	0.25^{ns}	0.12^{ns}	0.07^{ns}	0.09^{ns}	0.12^{ns}	-0.29^{ns}	1			
TL	0.32^{ns}	0.27^{ns}	0.13^{ns}	0.45*	0.24^{ns}	0.14^{ns}	0.07^{ns}	-0.26^{ns}	0.66**	1		
TYPP	-0.47**	-0.31^{ns}	-0.32^{ns}	0.99**	0.56**	-0.16^{ns}	0.40*	-0.507**	0.12	0.44*	1	
STW	0.08^{ns}	0.02^{ns}	-0.02^{ns}	0.41*	-0.22^{ns}	0.28^{ns}	0.03^{ns}	-0.31^{ns}	0.36*	0.50**	0.45*	1

* and ** significant at 0.05 and 0.01 probability levels by t test, respectively. ns: Not significant, PH: Plant height, SN: Stem number, SSN: Side stem number, HTY: Hectare tuber yield, TNPP: Tuber number per plant, LTR: Large tuber ration, MTR: Middle tuber ration, STR: Small tuber ration, TW: Tuber width, TL: Tuber length, TYPP: Tuber yield per plant, STW: Single tuber weight.

In their study, examining the response of potato to an environment in subtropical agricultural ecology, Molahlehi et al. (2013) reported that there was a high positive correlation between the tuber yield and agricultural applications. Sattar et al. (2007) carried out a correlation analysis to examine the effect of genetic variation on yield and yield parameters in 28 potato genotypes and reported that there was a significant positive correlation between the tuber yield per plant and the number of tubers per plant and the mean tuber yield. This result is similar to our results. Correlation analysis helps to identify the superior characteristics of plants and improve their genetic characteristics in yield, especially when potato genotypes are evaluated genetically (Leilah & Al-Khateeb, 2005; Bello et al., 2006; Haydar et al., 2009; Gedamu et al., 2010). Similarly, based on the Pearson correlation results, it can be asserted that there were significant correlations between the tubers of all the potato genotypes depending on genetic characteristics.

It was observed that there were significant differences between the local potato genotypes grown under the conditions of Bayburt region and two commercial potato varieties in terms of yield and yield parameters. When the Pearson correlation results for the parameters that show the quality of genotypes and varieties were examined, it was found that the tuber yield per hectare, one of the important parameters, was highly positively correlated with the tuber yield per plant ($r=0.99^{**}$) and moderately positively correlated with the number of tubers per plant ($r=0.58^{**}$) with a significance of $p \leq 0.01$. Based on the yield and quality parameters, it was observed that the local potato genotypes were adapted to the conditions of the region better than the commercial potato varieties.

Conclusion

The highest number of stems per plant (6.7), tuber yield per hectare (15.19 tons), number of tubers per plant (8.47), and tuber yield per plant (357.01 g) were found to be in the genotype Akbulut; the highest ratio of large tuber (28.27%) in the genotype Konursu; and the highest ratio of medium tuber (85.22%) in the genotype Çamlıkoz. In conclusion, we are of the opinion that the high-altitude regions of Northeastern Anatolia, where the study was carried out, are suitable for potato cultivation. This study will contribute to the future studies designed to identify the local genotypes and varieties suitable for the region and help the farmers who grow potato in the region in their efforts for achieving better yield and quality.

Acknowledgment

We are very thankful to the locals for allowing us to carry out the research in regions of Northern Türkiye.

Conflict of Interest

The authors have no conflicts of interest.

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Use a 12-point Times New Roman font, including the references, table headings and figure captions, double-spaced and with 25 mm margins on all sides of A4 size paper throughout the manuscript. The text should be in single-column format.

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In-text citation (Narrative):

…The results of Bliss (2022) support…

…Sönmez and Taştan (2020) indicated

that…
…According to the method of Öztürk et al. (2021)…

In-text citation (In parenthesis):
…It was found to be isometric (Öztürk, 2018)…
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Two or more works in the same parenthesis:
…extremely toxic for the environment (Sönmez, 2018, 2019; Öztürk et al., 2020a; Kadak & Taştan, 2021)…

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Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

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