# International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-8, Issue-6, June 2022

# Preface

We would like to present, with great pleasure, the inaugural volume-8, Issue-6, June 2022, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

**Environmental Research:**

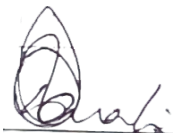
*Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.*

**Agriculture Research:**

*Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.*

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

Mukesh Arora
(Managing Editor)



Dr. Bhagawan Bharali
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# Fields of Interests

| Agricultural Sciences | |
|---|---|
| Soil Science | Plant Science |
| Animal Science | Agricultural Economics |
| Agricultural Chemistry | Basic biology concepts |
| Sustainable Natural Resource Utilisation | Management of the Environment |
| Agricultural Management Practices | Agricultural Technology |
| Natural Resources | Basic Horticulture |
| Food System | Irrigation and water management |
| **Crop Production** | |
| Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth | Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed |
| Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas. | Hay and Silage (Forage crop) Production |
| Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.) | Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries) |
| Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans | Berry crops: strawberries, blueberries, raspberries |
| Sugar crops: sugarcane. sugar beets, sorghum | Potatoes varieties and production. |
| **Livestock Production** | |
| Animal husbandry | Ranch |
| Camel | Yak |
| Pigs | Sheep |
| Goats | Poultry |
| Bees | Dogs |
| Exotic species | Chicken Growth |
| **Aquaculture** | |
| Fish farm | Shrimp farm |
| Freshwater prawn farm | Integrated Multi-Trophic Aquaculture |
| **Milk Production (Dairy)** | |
| Dairy goat | Dairy cow |
| Dairy Sheep | Water Buffalo |
| Moose milk | Dairy product |
| **Forest Products and Forest management** | |
| Forestry/Silviculture | Agroforestry |
| Silvopasture | Christmas tree cultivation |
| Maple syrup | Forestry Growth |
| **Mechanical** | |
| General Farm Machinery | Tillage equipment |
| Harvesting equipment | Processing equipment |
| Hay & Silage/Forage equipment | Milking equipment |
| Hand tools & activities | Stock handling & control equipment |
| Agricultural buildings | Storage |

| Agricultural Input Products | |
|---|---|
| Crop Protection Chemicals | Feed supplements |
| Chemical based (inorganic) fertilizers | Organic fertilizers |
| **Environmental Science** | |
| Environmental science and regulation | Ecotoxicology |
| Environmental health issues | Atmosphere and climate |
| Terrestric ecosystems | Aquatic ecosystems |
| Energy and environment | Marine research |
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| Theoretical production ecology | horticulture |
| Breeding | plant fertilization |

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## Dr.Chiti Agarwal

Dr. Chiti Agarwal works as a postdoctoral associate at the University of Maryland in College Park, Maryland, USA. Her research focuses on fungicide resistance to fungal diseases that affect small fruits such as strawberries. She graduated from North Dakota State University in Fargo, North Dakota, with a B.S. in biotechnology and an M.S. in plant sciences. Dr. Agarwal completed her doctorate in Plant Pathology while working as a research and teaching assistant. During her time as a graduate research assistant, she learned about plant breeding, molecular genetics, quantitative trait locus mapping, genome-wide association analysis, and marker-assisted selection. She wants to engage with researchers from many fields and have a beneficial impact on a larger audience.

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## Mr. Bimal Bahadur Kunwar

He received his Master Degree in Botany from Central Department of Botany, T.U., Kirtipur, Nepal. Currently working as consultant to prepare CCA-DRR Plan for Hariyo Ban Program/CARE in Nepal/GONESA.

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# Soil organic Carbon Stock affected by different cropping system of Prayagraj District, Eastern Uttar Pradesh, India

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Received:- 10 May 2022/ Revised:- 25 May 2022/ Accepted:- 08 June 2022/ Published: 30-06-2022


***Abstract—*** *Cropping system is an effective agricultural practice which play crucial role in soil carbon stabilization, soil health and fertility as well as in sequestering atmospheric $CO_2$ in soil for long period of time. With these considerations in mind, a research was conducted in the Prayagraj district of eastern Uttar Pradesh to evaluate how major agricultural systems affect soil carbon stock. The major cropping system includes Wheat-Wheat, Mustard- Mustard, Rice-Wheat and Rice-Mustard Soil samples were collected from eight tehsil of Prayagraj district randomly from depth 0-15 cm and 15-30 cm depth. The findings show that soil organic carbon store in rice-wheat cropping systems is higher than in other cropping systems.*

***Keywords— Cropping system, Soil carbon stock, Atmospheric $CO_2$, Carbon stabilization.***

## I. INTRODUCTION

The concentration of $Co_2$ in earth's atmosphere is currently at nearly 418.96 ppm in 2022 and rising **(Mauna Loa observatory/NOAA).** This represents a 47 percent increase since the beginning of industrial age when the concentration was near 280 ppm. $CO_2$ increase caused by primarily human activities because carbon produced by burning of fossil fuels **(Eawaran *et al.*, 1993)**. Industry, transportation, and home use currently emit roughly 10 Gt CO2 into the atmosphere each year. Increasing SOC in agricultural systems has been considered as a possible solution to mitigate climate change, e.g., via removing atmospheric carbon dioxide ($CO_2$) into the long-lived C pool as it occupies 40% of the earth land surface **(Smith 2008)**. Soil are important global carbon pools as it constitutes the third largest carbon pool estimated at 2500 Pg to 1 m depth so soil system has the potential to sequester significant amount of carbon by improved management, which could significantly offset fossil fuels GHG emissions **(Lal 2004).** Through changed agricultural techniques, it is proposed, much of this carbon can be restored to domesticated soils and thus serve as a significant tool to mitigate climate change, Soil carbon sequestration implies transferring atmospheric $CO_2$ into long-lived pools and storing it in soil securely for long term to either mitigate or defeat global warming and avoid dangerous climate change so it is not immediately re-emitted **(Lal *et al.*, 1995)**. Simply we can say that soil carbon sequestration is the process of transferring carbon dioxide from the atmosphere into the soil through crop residues and other organic solids, and in a form that is not immediately reemitted **(Jones 2007)**. More recently, increasing crop rotational diversity has been shown to play a major role in increasing SOC storage and ecosystem functions, driven by enhanced root C input, soil microbial diversity, and soil aggregate stability **(McDaniel *et al.*, 2014).** Crop rotations can be further improved by incorporation of perennial forages with extensive root systems to increase root C input and physical protection in soil aggregates, resulting in SOC sequestration **(Varvel 2000, Kelley *et al.*, 2003).**

## II. MATERIALS AND METHODS

### 2.1 Study site

The field experiments were carried out at Prayagraj district located between 24° 47' to 25° 47' N latitudes and 81° 19'E to 82° 21'E longitudes. It covers an area of 5246 $km^2$. This district lies in the southern part of the state in the Gangetic plain and adjoining Vindhyan Plateau of India. The district comprises of eight tahsils, namely Sadar tehsil, Soraon, Phulpur, Handia, Bara, Karchana, Koraon and Meja.Tahsil.

Table showing names of site description along with latitude and longitude of the Tehsil.

**TABLE A**

**TABLE SHOWING NAMES OF SITE DESCRIPTION**

| | | |
|---|---|---|
| **1** | **Meja** | V1- Samahan, V2- Bisahijanpur, V3- Chatva upakhar, V4- Luter |
| **2** | **Saroan** | V 1- Naranyanpur, V2- Abdalpur, V3- Sarsa, V4- Gohri |
| **3** | **Karchana** | V1- Rampur Talika, V2- Majhua, V3- Chuppepur, V4- Moongari |
| **4** | **Handiya** | V1- Siyandih, V2- Bhadwan, V3-Birapur kasaudhan, V4- Shankerpur |
| **5** | **Karoan** | V1- Pacheda, V2- Semri baghrai, V3- Patharlal, V4- Taroan |
| **6** | **Bara** | V1- Barakhas, V2- Nivi, V3- Jharsa, V4- Sehunda |
| **7** | **Phulpur** | V1- Serdeh, V2- Karihar, V3- Kaserua, V4- Andhawa |
| **8** | **Sadar** | V1- Bamroli, V2- Jhalwa, V3- Fulwa, V4-Mandari |

### 2.2 Soil Sampling and Analysis

Analysis of soil was done during grand growth of the crops. The soil samples were randomly collected from eight Tehsil of Prayagraj district and from each Tehsil four village were selected for the sampling. Soil sample were collected from each village with different cropping system i.e., Wheat-Wheat, Mustard- Mustard, Rice-Wheat and Rice- Mustard from 0-15 cm and 15-30 cm depth. The soil was returned to the laboratory after sample and air-dried at room temperature. The dirt was lowered in weight and sieved through a 2 mm mesh before being used for analysis.

Analysis of Bulk density (g/cc) by **Muthuvel, *et al.*, (1992)**, Organic carbon (%) and soil total organic carbon by **Walkley and Black (1947)** and soil organic carbon stock by **Batjes (1996).**

### 2.3 Carbon stock Analysis

#### 2.3.1 Bulk density x soil depth x total organic carbon

Total organic carbon = %OC x 1.3

According to walkey and black method 77% is Organic matter

100÷77=1.29

**Statistical analysis** of variance F4 way classification was used and their after for comparing two objects together the value of critical difference was also analyzed.

## III. RESULTS AND DISCUSSION

Table 1 shows the bulk density (g/cc) value of different cropping system at grand growth of Prayagraj district. The maximum value of bulk density was found 1.25 (g/cc) of village Fulwa in Sadar tehsil at 15-30 cm depth of rice-mustard cropping and minimum value of bulk density was found 1.00 (g/cc) of village Karihar in Phulpur tehsil at 0-15 cm depth of muatard-mustard crop.

Table 2 shows the organic carbon (%) value of major crops at growing stage of Prayagraj district. The maximum value of organic carbon (%) was found 0.92 (%) of village Bisahijanpur in Meja tehsil at 0 to 15 cm depth of rice- wheat cropping system and minimum value of organic carbon (%) was found 0.11(%) of village Bhadwan in Handiya tehsil at 15 to 30 cm depth of wheat-wheat cropping system.

Table 3 shows the total organic carbon (%) value of major crops at grand growth stage of Prayagraj district. The maximum value of total organic carbon was found 1.18 (%) of village Kaserua in Phulpur tehsil at 0 to 15 cm depth of rice-wheat cropping system and minimum value of carbon stock was found 0.14 (%) in Handiya tehsil of village Bhadwan in Handiya tehsil at 15 to 30 cm depth of wheat-wheat cropping system.

Table 4 shows the total organic carbon stock (t/ $ha^{-1}$) value of major crops at grand growth stage of Prayagraj district. The maximum value of total organic carbon stock (t/ $ha^{-1}$) was found 38.13 (t/ $ha^{-1}$) of village Kaserua in Phulpur tehsil at 0 to 15 cm depth of rice-wheat cropping system and minimum value of total organic carbon stock (t/ $ha^{-1}$) was found 5.06 (t/ $ha^{-1}$) of village Bhadwan in Handiya tehsil at15 to 30 cm depth of wheat-wheat cropping system.

Many prior studies have indicated that more diversified crops improve soil organic carbon sequestration compared to mono cropping systems **Gan YT *et al.*, (2015); Campbell CA *et al.*, 2005)**. Stabilization of soil organic carbon under various cropping systems; rice-wheat farming stabilises more soil organic carbon even without fertilizer **(Stevenson, 1965; Paustian *et al.*, 1992) and Brar and Benbi (2009).**

**TABLE 1**

**BULK DENSITY OF SOILS OF DIFFERENT CROPPING SYSTEM AT 0-15 CM AND 15-30 CM DEPTHS AT GROWING STAGE OF MAJOR CROPS OF 2017-18.**

| Tehsils | Cropping system | (0-15 cm) depth | | | | Mean | (15-30 cm) depth | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | | V1 | V2 | V3 | V4 | |
| Meja | Wheat/Wheat | 1.11 | 1.01 | 1.02 | 1.11 | 1.06 | 1.20 | 1.11 | 1.18 | 1.14 | 1.16 |
| | Mustard/Mustard | 1.10 | 1.08 | 1.10 | 1.11 | 1.10 | 1.22 | 1.18 | 1.25 | 1.18 | 1.21 |
| | Rice/ wheat | 1.08 | 1.01 | 1.11 | 1.01 | 1.05 | 1.14 | 1.11 | 1.18 | 1.11 | 1.14 |
| | Rice/Mustard | 1.25 | 1.01 | 1.11 | 1.11 | 1.12 | 1.18 | 1.11 | 1.18 | 1.20 | 1.17 |
| Saroan | Wheat/Wheat | 1.14 | 1.11 | 1.18 | 1.11 | 1.14 | 1.20 | 1.22 | 1.25 | 1.18 | 1.21 |
| | Mustard/Mustard | 1.10 | 1.20 | 1.11 | 1.12 | 1.13 | 1.18 | 1.24 | 1.17 | 1.25 | 1.21 |
| | Rice/ wheat | 1.08 | 1.11 | 1.11 | 1.10 | 1.10 | 1.18 | 1.20 | 1.23 | 1.18 | 1.20 |
| | Rice/Mustard | 1.00 | 1.08 | 1.01 | 1.11 | 1.05 | 1.18 | 1.18 | 1.11 | 1.16 | 1.16 |
| Karchana | Wheat/Wheat | 1.18 | 1.14 | 1.10 | 1.18 | 1.15 | 1.20 | 1.20 | 1.24 | 1.24 | 1.22 |
| | Mustard/Mustard | 1.10 | 1.11 | 1.11 | 1.11 | 1.11 | 1.17 | 1.18 | 1.20 | 1.18 | 1.18 |
| | Rice/ wheat | 1.11 | 1.11 | 1.01 | 1.11 | 1.08 | 1.18 | 1.15 | 1.17 | 1.25 | 1.19 |
| | Rice/Mustard | 1.18 | 1.14 | 1.11 | 1.11 | 1.14 | 1.22 | 1.25 | 1.18 | 1.18 | 1.21 |
| Handiya | Wheat/Wheat | 1.11 | 1.08 | 1.01 | 1.01 | 1.05 | 1.11 | 1.18 | 1.14 | 1.20 | 1.16 |
| | Mustard/Mustard | 1.18 | 1.11 | 1.14 | 1.18 | 1.15 | 1.20 | 1.18 | 1.25 | 1.33 | 1.24 |
| | Rice/ wheat | 1.12 | 1.01 | 1.06 | 1.02 | 1.05 | 1.20 | 1.18 | 1.17 | 1.20 | 1.19 |
| | Rice/Mustard | 1.18 | 1.10 | 1.11 | 1.10 | 1.12 | 1.20 | 1.17 | 1.25 | 1.20 | 1.21 |
| karoan | Wheat/Wheat | 1.18 | 1.11 | 1.11 | 1.04 | 1.11 | 1.20 | 1.20 | 1.18 | 1.17 | 1.19 |
| | Mustard/Mustard | 1.11 | 1.08 | 1.10 | 1.11 | 1.10 | 1.22 | 1.18 | 1.18 | 1.18 | 1.19 |
| | Rice/ wheat | 1.16 | 1.10 | 1.11 | 1.06 | 1.11 | 1.25 | 1.18 | 1.20 | 1.11 | 1.19 |
| | Rice/Mustard | 1.08 | 1.11 | 1.01 | 1.07 | 1.07 | 1.11 | 1.18 | 1.11 | 1.18 | 1.15 |
| Bara | Wheat/Wheat | 1.18 | 1.14 | 1.01 | 1.10 | 1.11 | 1.25 | 1.24 | 1.16 | 1.20 | 1.21 |
| | Mustard/Mustard | 1.14 | 1.18 | 1.11 | 1.16 | 1.15 | 1.20 | 1.25 | 1.18 | 1.25 | 1.22 |
| | Rice/ wheat | 1.18 | 1.11 | 1.05 | 1.08 | 1.11 | 1.25 | 1.20 | 1.19 | 1.20 | 1.21 |
| | Rice/Mustard | 1.11 | 1.20 | 1.16 | 1.11 | 1.15 | 1.20 | 1.25 | 1.20 | 1.33 | 1.25 |
| Phulpur | Wheat/Wheat | 1.01 | 1.01 | 1.00 | 1.08 | 1.02 | 1.11 | 1.11 | 1.12 | 1.18 | 1.13 |
| | Mustard/Mustard | 1.08 | 1.00 | 1.11 | 1.11 | 1.08 | 1.18 | 1.20 | 1.21 | 1.25 | 1.21 |
| | Rice/ wheat | 1.01 | 1.10 | 1.01 | 1.10 | 1.05 | 1.11 | 1.14 | 1.11 | 1.17 | 1.13 |
| | Rice/Mustard | 1.11 | 1.12 | 1.08 | 1.11 | 1.11 | 1.18 | 1.19 | 1.20 | 1.20 | 1.19 |
| Sadar | Wheat/Wheat | 1.08 | 1.11 | 1.16 | 1.15 | 1.13 | 1.18 | 1.18 | 1.20 | 1.20 | 1.19 |
| | Mustard/Mustard | 1.14 | 1.08 | 1.11 | 1.11 | 1.11 | 1.20 | 1.18 | 1.20 | 1.24 | 1.21 |
| | Rice/ wheat | 1.11 | 1.18 | 1.17 | 1.18 | 1.16 | 1.20 | 1.20 | 1.25 | 1.20 | 1.21 |
| | Rice/Mustard | 1.18 | 1.11 | 1.08 | 1.18 | 1.14 | 1.25 | 1.18 | 1.11 | 1.25 | 1.20 |

**TABLE 2**
**SOILS ORGANIC CARBON (%) OF DIFFERENT CROPPING SYSTEM AT 0-15 CM AND 15-30 CM DEPTHS AT GROWING STAGE OF MAJOR CROPS OF 2017-18.**

| Tehsils | Cropping system | (0-15 cm) depth | | | | Mean | (15-30 cm) depth | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | | V1 | V2 | V3 | V4 | |
| Meja | Wheat/Wheat | 0.4 | 0.85 | 0.56 | 0.51 | 0.58 | 0.3 | 0.46 | 0.4 | 0.49 | 0.41 |
| | Mustard/Mustard | 0.38 | 0.67 | 0.49 | 0.32 | 0.47 | 0.22 | 0.45 | 0.39 | 0.4 | 0.37 |
| | Rice/ wheat | 0.45 | 0.92 | 0.73 | 0.7 | 0.70 | 0.36 | 0.62 | 0.51 | 0.54 | 0.51 |
| | Rice/Mustard | 0.40 | 0.78 | 0.69 | 0.38 | 0.56 | 0.29 | 0.51 | 0.48 | 0.51 | 0.45 |
| Saroan | Wheat/Wheat | 0.47 | 0.75 | 0.48 | 0.3 | 0.50 | 0.28 | 0.66 | 0.39 | 0.2 | 0.38 |
| | Mustard/Mustard | 0.32 | 0.6 | 0.23 | 0.6 | 0.44 | 0.22 | 0.32 | 0.36 | 0.12 | 0.26 |
| | Rice/ wheat | 0.69 | 0.41 | 0.73 | 0.72 | 0.64 | 0.40 | 0.12 | 0.56 | 0.50 | 0.40 |
| | Rice/Mustard | 0.61 | 0.62 | 0.60 | 0.71 | 0.64 | 0.43 | 0.40 | 0.39 | 0.51 | 0.43 |
| Karchana | Wheat/Wheat | 0.35 | 0.49 | 0.72 | 0.45 | 0.50 | 0.23 | 0.31 | 0.54 | 0.31 | 0.35 |
| | Mustard/Mustard | 0.42 | 0.47 | 0.7 | 0.31 | 0.48 | 0.22 | 0.38 | 0.48 | 0.2 | 0.32 |
| | Rice/ wheat | 0.37 | 0.52 | 0.75 | 0.48 | 0.53 | 0.29 | 0.40 | 0.58 | 0.35 | 0.41 |
| | Rice/Mustard | 0.50 | 0.51 | 0.76 | 0.36 | 0.53 | 0.32 | 0.40 | 0.56 | 0.31 | 0.40 |
| Handiya | Wheat/Wheat | 0.37 | 0.25 | 0.6 | 0.43 | 0.41 | 0.3 | 0.11 | 0.29 | 0.22 | 0.23 |
| | Mustard/Mustard | 0.31 | 0.27 | 0.32 | 0.3 | 0.30 | 0.21 | 0.21 | 0.23 | 0.25 | 0.23 |
| | Rice/ wheat | 0.40 | 0.31 | 0.61 | 0.47 | 0.45 | 0.36 | 0.20 | 0.59 | 0.31 | 0.37 |
| | Rice/Mustard | 0.36 | 0.30 | 0.34 | 0.31 | 0.33 | 0.27 | 0.28 | 0.28 | 0.30 | 0.28 |
| karoan | Wheat/Wheat | 0.71 | 0.42 | 0.42 | 0.37 | 0.48 | 0.48 | 0.25 | 0.31 | 0.30 | 0.34 |
| | Mustard/Mustard | 0.33 | 0.27 | 0.45 | 0.32 | 0.34 | 0.23 | 0.20 | 0.33 | 0.23 | 0.25 |
| | Rice/ wheat | 0.71 | 0.47 | 0.42 | 0.40 | 0.50 | 0.48 | 0.28 | 0.34 | 0.30 | 0.35 |
| | Rice/Mustard | 0.36 | 0.28 | 0.50 | 0.29 | 0.36 | 0.27 | 0.20 | 0.34 | 0.28 | 0.27 |
| Bara | Wheat/Wheat | 0.42 | 0.40 | 0.46 | 0.61 | 0.47 | 0.28 | 0.21 | 0.35 | 0.42 | 0.32 |
| | Mustard/Mustard | 0.48 | 0.41 | 0.52 | 0.62 | 0.51 | 0.37 | 0.32 | 0.34 | 0.30 | 0.33 |
| | Rice/ wheat | 0.46 | 0.40 | 0.45 | 0.61 | 0.48 | 0.34 | 0.27 | 0.37 | 0.48 | 0.37 |
| | Rice/Mustard | 0.50 | 0.43 | 0.57 | 0.68 | 0.55 | 0.38 | 0.35 | 0.47 | 0.60 | 0.45 |
| Phulpur | Wheat/Wheat | 0.57 | 0.53 | 0.87 | 0.72 | 0.67 | 0.46 | 0.38 | 0.70 | 0.52 | 0.52 |
| | Mustard/Mustard | 0.76 | 0.80 | 0.88 | 0.71 | 0.79 | 0.40 | 0.69 | 0.61 | 0.50 | 0.55 |
| | Rice/ wheat | 0.58 | 0.57 | 0.91 | 0.78 | 0.71 | 0.56 | 0.40 | 0.81 | 0.61 | 0.60 |
| | Rice/Mustard | 0.76 | 0.83 | 0.88 | 0.71 | 0.80 | 0.65 | 0.67 | 0.70 | 0.60 | 0.66 |
| Sadar | Wheat/Wheat | 0.52 | 0.58 | 0.52 | 0.70 | 0.58 | 0.42 | 0.50 | 0.38 | 0.56 | 0.47 |
| | Mustard/Mustard | 0.52 | 0.58 | 0.42 | 0.61 | 0.53 | 0.41 | 0.48 | 0.32 | 0.45 | 0.42 |
| | Rice/ wheat | 0.57 | 0.60 | 0.51 | 0.72 | 0.60 | 0.50 | 0.47 | 0.42 | 0.56 | 0.49 |
| | Rice/Mustard | 0.56 | 0.57 | 0.48 | 0.67 | 0.57 | 0.48 | 0.50 | 0.41 | 0.51 | 0.48 |

**TABLE 3**
**SOILS TOTAL ORGANIC CARBON OF DIFFERENT CROPPING SYSTEM AT 0-15 CM AND 15-30 CM DEPTHS AT GROWING STAGE OF MAJOR CROPS OF 2017-18.**

| Tehsils | Cropping system | (0-15 cm) depth | | | | Mean | (15-30 cm) depth | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | | V1 | V2 | V3 | V4 | |
| Meja | Wheat/Wheat | 0.52 | 1.11 | 0.73 | 0.66 | 0.39 | 0.39 | 0.60 | 0.52 | 0.64 | 0.54 |
| | Mustard/Mustard | 0.49 | 0.87 | 0.64 | 0.42 | 0.29 | 0.29 | 0.59 | 0.51 | 0.52 | 0.47 |
| | Rice/ wheat | 0.59 | 1.20 | 0.95 | 0.91 | 0.47 | 0.47 | 0.81 | 0.66 | 0.70 | 0.66 |
| | Rice/Mustard | 0.52 | 1.01 | 0.90 | 0.49 | 0.38 | 0.38 | 0.66 | 0.62 | 0.66 | 0.58 |
| Saroan | Wheat/Wheat | 0.61 | 0.98 | 0.62 | 0.39 | 0.36 | 0.36 | 0.86 | 0.51 | 0.26 | 0.50 |
| | Mustard/Mustard | 0.42 | 0.78 | 0.30 | 0.78 | 0.29 | 0.29 | 0.42 | 0.47 | 0.16 | 0.33 |
| | Rice/ wheat | 0.90 | 0.53 | 0.95 | 0.94 | 0.52 | 0.52 | 0.16 | 0.73 | 0.65 | 0.51 |
| | Rice/Mustard | 0.79 | 0.81 | 0.78 | 0.92 | 0.56 | 0.56 | 0.52 | 0.51 | 0.66 | 0.56 |
| Karchana | Wheat/Wheat | 0.46 | 0.64 | 0.94 | 0.59 | 0.30 | 0.30 | 0.40 | 0.70 | 0.40 | 0.45 |
| | Mustard/Mustard | 0.55 | 0.61 | 0.91 | 0.40 | 0.29 | 0.29 | 0.49 | 0.62 | 0.26 | 0.42 |
| | Rice/ wheat | 0.48 | 0.68 | 0.98 | 0.62 | 0.38 | 0.38 | 0.52 | 0.75 | 0.46 | 0.53 |
| | Rice/Mustard | 0.65 | 0.66 | 0.99 | 0.47 | 0.42 | 0.42 | 0.52 | 0.73 | 0.40 | 0.52 |
| Handiya | Wheat/Wheat | 0.48 | 0.33 | 0.78 | 0.56 | 0.39 | 0.39 | 0.14 | 0.38 | 0.29 | 0.30 |
| | Mustard/Mustard | 0.40 | 0.35 | 0.42 | 0.39 | 0.27 | 0.27 | 0.27 | 0.30 | 0.33 | 0.29 |
| | Rice/ wheat | 0.52 | 0.40 | 0.79 | 0.61 | 0.47 | 0.47 | 0.26 | 0.77 | 0.40 | 0.47 |
| | Rice/Mustard | 0.47 | 0.39 | 0.44 | 0.40 | 0.35 | 0.35 | 0.36 | 0.36 | 0.39 | 0.37 |
| karoan | Wheat/Wheat | 0.92 | 0.55 | 0.55 | 0.48 | 0.62 | 0.62 | 0.33 | 0.40 | 0.39 | 0.44 |
| | Mustard/Mustard | 0.43 | 0.35 | 0.59 | 0.42 | 0.30 | 0.30 | 0.26 | 0.43 | 0.30 | 0.32 |
| | Rice/ wheat | 0.92 | 0.61 | 0.55 | 0.52 | 0.62 | 0.62 | 0.36 | 0.44 | 0.39 | 0.46 |
| | Rice/Mustard | 0.47 | 0.36 | 0.65 | 0.38 | 0.35 | 0.35 | 0.26 | 0.44 | 0.36 | 0.35 |
| Bara | Wheat/Wheat | 0.55 | 0.52 | 0.60 | 0.79 | 0.36 | 0.36 | 0.27 | 0.46 | 0.55 | 0.41 |
| | Mustard/Mustard | 0.62 | 0.53 | 0.68 | 0.81 | 0.48 | 0.48 | 0.42 | 0.44 | 0.39 | 0.43 |
| | Rice/ wheat | 0.60 | 0.52 | 0.59 | 0.79 | 0.44 | 0.44 | 0.35 | 0.48 | 0.62 | 0.47 |
| | Rice/Mustard | 0.65 | 0.56 | 0.74 | 0.88 | 0.49 | 0.49 | 0.46 | 0.61 | 0.78 | 0.59 |
| Phulpur | Wheat/Wheat | 0.74 | 0.69 | 1.13 | 0.94 | 0.60 | 0.60 | 0.49 | 0.91 | 0.68 | 0.67 |
| | Mustard/Mustard | 0.99 | 1.04 | 1.14 | 0.92 | 0.52 | 0.52 | 0.90 | 0.79 | 0.65 | 0.72 |
| | Rice/ wheat | 0.75 | 0.74 | 1.18 | 1.01 | 0.73 | 0.73 | 0.52 | 1.05 | 0.79 | 0.77 |
| | Rice/Mustard | 0.99 | 1.08 | 1.14 | 0.92 | 0.85 | 0.85 | 0.87 | 0.91 | 0.78 | 0.85 |
| Sadar | Wheat/Wheat | 0.68 | 0.75 | 0.68 | 0.91 | 0.55 | 0.55 | 0.65 | 0.49 | 0.73 | 0.60 |
| | Mustard/Mustard | 0.68 | 0.75 | 0.55 | 0.79 | 0.53 | 0.53 | 0.62 | 0.42 | 0.59 | 0.54 |
| | Rice/ wheat | 0.74 | 0.78 | 0.66 | 0.94 | 0.65 | 0.65 | 0.61 | 0.55 | 0.73 | 0.63 |
| | Rice/Mustard | 0.73 | 0.74 | 0.62 | 0.87 | 0.62 | 0.62 | 0.65 | 0.53 | 0.66 | 0.62 |

**TABLE 4**

**SOILS ORGANIC CARBON STOCK (T/ HA) OF DIFFERENT CROPPING SYSTEM AT 0-15 CM AND 15-30 CM DEPTHS AT GROWING STAGE OF MAJOR CROPS OF 2017-18.**

| Tehsils | Cropping system | (0-15 cm) depth | | | | Mean | (15-30 cm) depth | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | | V1 | V2 | V3 | V4 | |
| Meja | Wheat/Wheat | 17.32 | 33.15 | 22.28 | 22.08 | 23.71 | 14.04 | 19.91 | 18.41 | 21.79 | 18.54 |
| | Mustard/Mustard | 16.30 | 28.22 | 21.02 | 13.85 | 19.85 | 10.47 | 20.71 | 19.01 | 18.41 | 17.15 |
| | Rice/ wheat | 18.95 | 35.88 | 31.60 | 27.30 | 28.43 | 16.01 | 26.84 | 23.47 | 23.38 | 22.42 |
| | Rice/Mustard | 19.50 | 30.42 | 29.87 | 16.45 | 24.06 | 13.35 | 22.08 | 22.09 | 23.87 | 20.35 |
| Saroan | Wheat/Wheat | 20.90 | 32.47 | 22.09 | 12.99 | 22.11 | 13.10 | 31.40 | 19.01 | 9.20 | 18.18 |
| | Mustard/Mustard | 13.73 | 28.08 | 9.96 | 26.21 | 19.49 | 10.12 | 15.48 | 16.43 | 5.85 | 11.97 |
| | Rice/ wheat | 29.06 | 17.75 | 31.60 | 30.89 | 27.33 | 18.41 | 5.62 | 26.86 | 23.01 | 18.47 |
| | Rice/Mustard | 23.79 | 26.11 | 23.40 | 30.74 | 26.01 | 19.79 | 18.41 | 16.88 | 23.07 | 19.54 |
| Karchana | Wheat/Wheat | 16.11 | 21.79 | 30.89 | 20.71 | 22.37 | 10.76 | 14.51 | 26.11 | 14.99 | 16.59 |
| | Mustard/Mustard | 18.02 | 20.35 | 30.30 | 13.42 | 20.52 | 10.04 | 17.49 | 22.46 | 9.20 | 14.80 |
| | Rice/ wheat | 16.02 | 22.51 | 29.25 | 20.78 | 22.14 | 13.35 | 17.94 | 26.47 | 17.06 | 18.70 |
| | Rice/Mustard | 23.01 | 22.67 | 32.90 | 15.58 | 23.54 | 15.23 | 19.50 | 25.77 | 14.27 | 18.69 |
| Handiya | Wheat/Wheat | 16.02 | 10.53 | 23.40 | 16.77 | 16.68 | 12.99 | 5.06 | 12.89 | 10.30 | 10.31 |
| | Mustard/Mustard | 14.27 | 11.69 | 14.23 | 13.81 | 13.50 | 9.83 | 9.66 | 11.21 | 12.97 | 10.92 |
| | Rice/ wheat | 17.47 | 12.21 | 25.22 | 18.70 | 18.40 | 16.85 | 9.20 | 26.92 | 14.51 | 16.87 |
| | Rice/Mustard | 16.57 | 12.87 | 14.72 | 13.30 | 14.36 | 12.64 | 12.78 | 13.65 | 14.04 | 13.28 |
| karoan | Wheat/Wheat | 32.67 | 18.18 | 18.18 | 15.01 | 21.01 | 22.46 | 11.70 | 14.27 | 13.69 | 15.53 |
| | Mustard/Mustard | 14.29 | 11.37 | 19.31 | 13.85 | 14.70 | 10.94 | 9.20 | 15.19 | 10.58 | 11.48 |
| | Rice/ wheat | 32.12 | 20.16 | 18.18 | 16.54 | 21.75 | 23.40 | 12.89 | 15.91 | 12.99 | 16.30 |
| | Rice/Mustard | 15.16 | 12.12 | 19.50 | 12.10 | 14.72 | 11.69 | 9.20 | 14.72 | 12.89 | 12.12 |
| Bara | Wheat/Wheat | 19.33 | 17.78 | 17.94 | 26.17 | 20.31 | 13.65 | 10.16 | 15.83 | 19.66 | 14.82 |
| | Mustard/Mustard | 21.34 | 18.87 | 22.51 | 28.05 | 22.69 | 17.32 | 15.60 | 15.65 | 14.63 | 15.80 |
| | Rice/ wheat | 21.17 | 17.32 | 18.43 | 25.69 | 20.65 | 16.58 | 12.64 | 17.17 | 22.46 | 17.21 |
| | Rice/Mustard | 21.65 | 20.12 | 25.79 | 29.44 | 24.25 | 17.78 | 17.06 | 22.00 | 31.12 | 21.99 |
| Phulpur | Wheat/Wheat | 22.23 | 20.67 | 33.93 | 30.33 | 26.79 | 19.93 | 16.47 | 30.58 | 23.93 | 22.73 |
| | Mustard/Mustard | 32.01 | 31.20 | 38.13 | 30.74 | 33.02 | 18.41 | 32.29 | 28.79 | 24.38 | 25.97 |
| | Rice/ wheat | 22.62 | 24.45 | 35.49 | 33.46 | 29.01 | 24.24 | 17.78 | 35.06 | 27.83 | 26.23 |
| | Rice/Mustard | 32.93 | 36.25 | 37.07 | 30.74 | 34.25 | 29.91 | 31.09 | 32.76 | 28.08 | 30.46 |
| Sadar | Wheat/Wheat | 21.90 | 25.11 | 23.52 | 31.40 | 25.48 | 19.33 | 23.01 | 17.78 | 26.21 | 21.58 |
| | Mustard/Mustard | 23.12 | 24.43 | 18.18 | 26.41 | 23.03 | 19.19 | 22.09 | 14.98 | 21.76 | 19.50 |
| | Rice/ wheat | 24.68 | 27.61 | 23.27 | 33.13 | 27.17 | 23.40 | 22.00 | 20.48 | 26.21 | 23.02 |
| | Rice/Mustard | 25.77 | 24.68 | 20.22 | 30.83 | 25.37 | 23.40 | 23.01 | 17.75 | 24.86 | 22.26 |

| | Due to depth | Due to cropping system | Due to tehsil | Due to village |
|---|---|---|---|---|
| Result | S | S | S | S |
| S. ed. | 0.61 | 0.87 | 1.23 | 0.87 |
| CD at 5% | 1.22 | 1.73 | 2.44 | 1.73 |

## IV. CONCLUSIONS

The current study indicates that different cropping systems have significant impact on soil organic carbon stock. Soils under different cropping systems showed best results than monoculture crops. The study indicates that rice-wheat cropping system shows higher organic carbon stock than all other cropping systems and lowest in mustard-mustard cropping system.

Therefore, present study shows the different cropping system have a potential to enhance soil organic carbon and helpful in soil carbon stabilization.

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# Assessment of Severity of Termites Attack in Adekunle Ajasin University Akungba Akoko Campus, Ondo State, Nigeria

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Received:- 13 May 2022/ Revised:- 05 June 2022/ Accepted:- 11 June 2022/ Published: 30-06-2022


***Abstract—*** *Termites' attack has been a major problem for wooden structures and buildings. The severity of termites' attack on the AAUA campus area using Triplochiton scleroxylon wood as bait was examined. Identification of prevalent termite species as well as the soil's physical properties were studied in twenty sampled locations. Defect-free wood samples of Triplochiton scleroxylon dimensioned 35 × 35 × 450mm according to ASTM D3345-17 (2017) were buried halfway in the soil and exposed to termite attacks for 12 weeks (3 months) in an established timber graveyard at the locations. A weekly visual assessment of the stakes was done in accordance with the ASTM D 3345 rating scale and a gravimetric weight loss assessment was carried out after the period of exposure. Data obtained were used to produce termites severity probability map of the campus area was prepared using ArcGIS software and following the USDA standard color codes. Six termites species identified were: Ancistrotermes cavithorax (Isoptera: Macrotermitidae; termite), Odontotermes pauperan (Isoptera: Macrotermitidae; fungus-growing termite), Microtermes species (Isoptera: Termitidae; termite), Trinervitermes species (Isoptera: Termitidae; Trinervitermes), Macrotermes subhyalinus (Isoptera: Termitidae; Rambur), and Amitermes evuncifer (Isoptera: Termitidae; amitermes). The result showed that soil properties ranged from 7.19±0.02 to 19.78±0.03% for the moisture content, 28.82±0.02 to 51.72±0.02% for water holding capacity, 1.08±0.01 to 1.76±0.01 for the bulk density, while the soil organic matter values across the locations ranged from 6.08±0.02 to 21.29±0.04, however, only the water holding capacity has a moderate positive correlation with the severity of termite activities. The termite infestation probability map revealed that almost every part of the AAUA campus showed termite activities ongoing with a varying degree.*

***Keywords— Subterranean termites, Termites severity probability map, GIS technology, Wood protection.***

## I. INTRODUCTION

Wood is a traditional building material used for a variety of applications, such as fencing, decking, cladding, and construction of domestic dwellings, it has found applications in the construction of heavy load-bearing structures like jetties, bridges and industrial buildings, etc. (Ritter 1990). However, whenever wood is exposed directly or indirectly to environmental factors, it requires protective measures against weathering and bio deteriorating agents, like fungi, insects (termites), and bacteria.

Termites are an important factor in the forest and its associated ecosystems including micro-human-modified environments; contributing immensely to soil formation, and fertility through cellulosic biomass degradation processes (Ssemagand*a et al*, 2011). They are social insects of the order of *Isopteran* with about 3,000 known species of which 75% are classified as soil-feeding termites (Grimalkin and Engle, 2005). They live in colonies consisting of workers, soldiers, a queen, and a king which collectively form well-organized social formations.

Termites play important contrasting ecological roles in reworking the soil profile and the destruction of material meant for building construction, agriculture, and forestry (Lee and Wood, 1971; Milked and Mike, 1982; Joni; Gummier and Nyanganji, 2005). As polymorphic social insects, they live in self-constructed mounds called termitaria, whose destructive activities are usually higher during the dry season or drought compared to the rainy season, lowland rather than highlands, and in plants cultivated under stress and such are referred to as predictable 'ecosystem engineers' (Rajeev and Sajeev., 1998).

It has been discovered that builders, developers, site buildings without the initial assessment of the prevalence of termites which is a major wood pest. This has led to frequent construction failures of the roof, ceiling, and other wood structures. This study, therefore, was carried out to determine the prevalence of termites using AAUA campus as a case study to advise developers on what needs to be done before putting up a structure.

## II. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in Adekunle Ajasin University Akungba Akoko (AAUA which is between latitudes $7^0$ 28' 9.15' to $7^0$ 29'' 15.18' North of the equator and longitude $5^0$ 44'' 15.96' to $5^0$ 46'' 14.78' East of the Greenwich Meridian (Fig.1). It is situated in Akoko South West Local Government Area of Ondo State. Akungba Akoko town. (Allen, 2012)

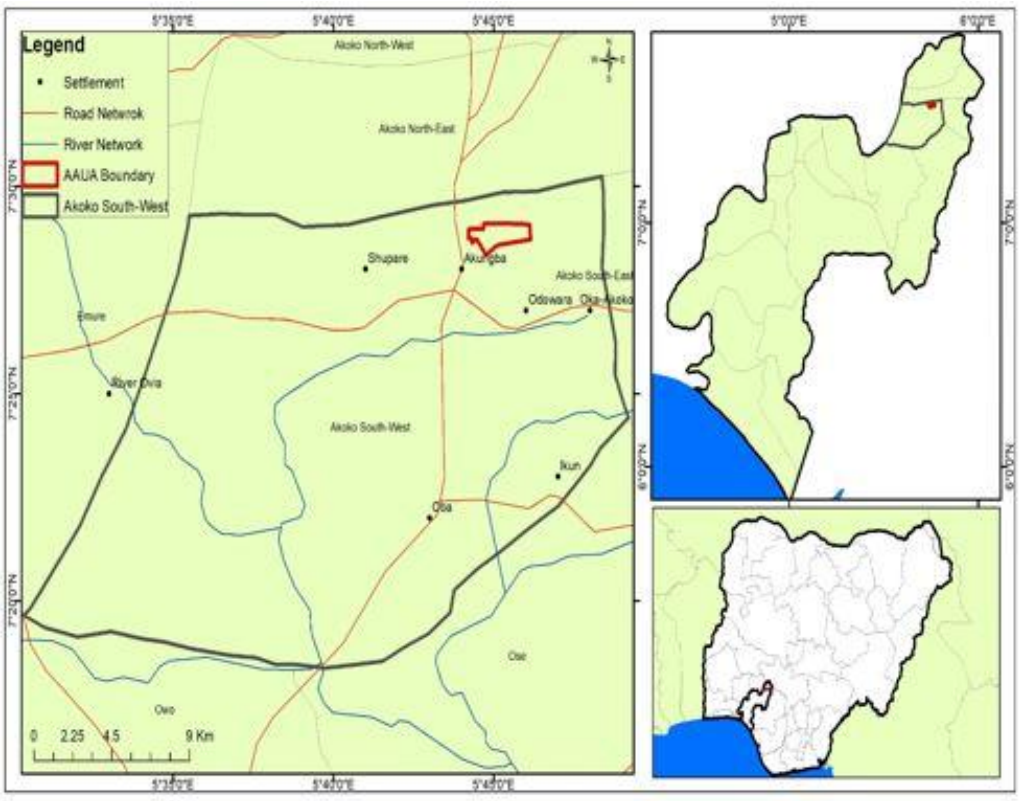


**FIGURE 1: Map of the study area**

**FIGURE 2: Sample Field Plots (Timber graveyard)**

The ecological zone which used to be a rainforest is gradually becoming derived savannah due to erratic rainfall patterns resulting from climate change. The zone has a mean annual rainfall of 1250mm and the average temperature is between $18^0$C and $35^0$C. The area is characterized by Precambrian Basement rocks such as grey gneiss, quartzo-feldspathic gneiss, charnockite; granite gneiss; and porphyritic gneiss (Okpoli, 2015).

### 2.2 Materials

The site sampling and plot selection were carried out with the use of a georeferenced AAUA Map obtained from the Center for Space Research and Application (CESRA) FUTA using ArcGIS software. The sample stakes used as bait for this study were obtained from *Triplochiton scleroxylon* (Obeche) wood known for its moderate susceptibility to termite attack. The wood species were purchased from a local timber market in Akungba-Akoko, Ondo State, and taken to the wood workshop of the Department of Forestry and Wood Technology Federal University of Technology Akure, where it was converted into standard sample sizes. The other materials that were used include moisture meter, microscope, oven-dryer, core sampler, digger among others.

### 2.3 Methods

#### 2.3.1 Sites Selection

A hard copy of the AAUA campus map was obtained from the Physical Planning Department of the university. The map was scanned, digitized, and geo-referenced at the Center for Space Research and Application (CESRA) FUTA using ArcGIS software. The map was divided into grids of regular points which were numbered serially after which, the coordinates (Table 1) of twenty randomly selected points were obtained and exported to the geo-referenced map to ascertain their exact locations on the school campus.

**TABLE 1**
**COORDINATES OF THE SELECTED LOCATIONS WITH THEIR SITE NAMES**

| Location | Latitude(°) | Longitude(°) |
|---|---|---|
| **1** | 7.4797 | 5.7375 |
| **2** | 7.4808 | 5.7377 |
| **3** | 7.4791 | 5.7394 |
| **4** | 7.4813 | 5.7411 |
| **5** | 7.4822 | 5.7425 |
| **6** | 7.4783 | 5.7444 |
| **7** | 7.4811 | 5.7389 |
| **8** | 7.4813 | 5.7502 |
| **9** | 7.4761 | 5.745 |
| **10** | 7.4786 | 5.7486 |
| **11** | 7.4775 | 5.7542 |
| **12** | 7.4733 | 5.7436 |
| **13** | 7.4794 | 5.7597 |
| **14** | 7.483 | 5.7533 |
| **15** | 7.4839 | 5.7575 |
| **16** | 7.4844 | 5.7522 |
| **17** | 7.4813 | 5.7613 |
| **18** | 7.483 | 5.7633 |
| **19** | 7.4811 | 5.7477 |
| **20** | 7.4822 | 5.7675 |

The georeferenced map of AAUA also superimposes the boundary layer for plot sampling. After the plot sampling, a reconnaissance survey ground-truthing) was carried out to assess the ground conditions of each location before the establishment of the timber graveyard in each of these selected locations.

#### 2.3.2 Wood sample selection and Preparation

*Triplochiton scleroxylon* (Obeche) wood was processed to 35×35× 450 mm according to ASTM D3345-17 (2017) and one hundred samples were obtained. All the samples were labelled for easy identification and their initial weight was obtained

using a weighing balance, after which they were oven-dried at a temperature of $103\pm2^0$C for twenty-four (24) hours until a constant weight was obtained. The oven-dried weight of each sample was also obtained and this served as the initial weight of each of the samples with respect to weight loss assessment.

### 2.3.3 Field Test

The field (Timber graveyard) test was carried out at the20 locations within the AAUA campus. Each of the selected sites was cleared with wood shavings spread to stimulate termites' activities. Five wood stakes were buried to a depth of 225mm below the ground surface and at the spacing of 1000 × 1000 mm from each other (Fig. 11). The weekly visual assessment was carried out for twelve (12) weeks to assess the severity of termite attack on the wood samples in each of the selected twenty (20) locations and ratings were done according to ASTM D 3345-17 (2017). At the end of the twelve (12) weeks testing period, the entire sample was withdrawn and re-weighed using a gravimetric method to assess the level of degradation by termites.

### 2.3.4 Termites Collection and Identification

Termites' specimens which include workers 'and soldiers' were collected at each location using a plastic insect specimen bottle filled with 10ml of ethanol. The collected specimens were taken to the Center for Termites` Research, Identification, and Management, Department of Biology, the Federal University of Technology Akure for proper identification.

The identification procedure involved both internal and external morphology assessment of the obtained specimens. Internal morphology was carried out after enteric valve armature of the termites were removed, dissected, and fixed in alcoholic Boun's fluid, after which they were dehydrated in dioxane and observed under a scanning electron microscope while external morphology for the termite's identification was carried out after the termites were fixed in dehydrated ethanol series (70 to 100%), the termites were observed under scanning electron microscope using the Mandible, Antenna, Pronotum, Labrum, Hyaline tip Fontanelle and postnotumfor clearer identification.

### 2.3.5 Soil Properties Determination

Soil physical properties from the study sites (Bulk density, Moisture content, and Organic matter) were determined from soil samples obtained from the twenty (20) selected locations following standard laboratory procedures.

### 2.3.6 Preparation of Termite Severity Probability Map

At the end of the weekly assessments period, weight loss values and data obtained from the ASTM D3345-17 visual rating assessment for each location were used to prepare a termite infestation/severity probability map using the IDW function in ArcGIS software's Spatial Analyst for data interpolation with a moderate weighting value and following USDA standard color codes to assign the different severity levels (Peterson *et al.,* 2006):

### 2.3.7 Experimental Design and Data Analyses

The experimental design used for the research is Complete Randomized Design (CRD) with 20 selected locations constituting the treatment. The data obtained from the fieldwork was analyzed using the Statistical Package for Social Sciences (SPSS) version 21. Descriptive statistics of the investigated variables were obtained, while analysis of variance (ANOVA) ($\alpha = 0.05$) was carried out to determine if there were significant differences in the investigated variables as observed in the twenty (20) selected locations and the mean separation was carried out using Duncan New Multiple Range Test (DMRT) where a significant difference is observed.

## III. RESULTS

### 3.1 Termite Identification

The results revealed that six termite species are prevalent within the AAUA campus area with the *Ancistrotermes cavithorax* and *Microtermes spp.* having the most abundant termite species prevalent within the campus area. This result supports the works of Harris (1971) and Akande (1992) who reported that termite species are wide across vegetation zones in Nigeria.

Termite identification carried out across the twenty selected locations within the AAUA campus area in Table 2 and Figure 3 showed that *Ancistrotermes cavithorax* species is the most abundant termite species found within the AAUA campus, with its presence identified eight (8) out of the eighteen locations where termite species were obtained, followed by the *Microtermes species* which was identified in seven out of the eighteen locations within the campus area, and *Trinervitermes species* which

was identified in four (4) out of the eighteen locations, while *Odontotermes pauperan, Macrotermes subhyalinus,* and *Amitermes evuncifer* had a minor presence in the campus area; as was identified in just one location respectively within the campus area.

**TABLE 2**
**TERMITE SPECIES IDENTIFIED IN THE 20 SELECTED LOCATIONS ACROSS THE AAUA CAMPUS AREA**

| location | Termite species | | | | | |
|---|---|---|---|---|---|---|
| | *Odontotermes pauperan* | *Trinervitermes species* | *Ancistrotermes cavithorax* | *Microtermes species* | *Macrotermes subhyalinus* | *Amitermes evuncifer* |
| Location 1 | - | + | - | - | - | - |
| Location 2 | - | + | - | - | - | - |
| Location 3 | - | - | - | - | - | - |
| Location 4 | + | - | - | - | + | - |
| Location 5 | - | - | - | - | - | - |
| Location 6 | - | - | + | - | - | - |
| Location 7 | - | + | - | - | - | - |
| Location 8 | - | + | - | - | - | - |
| Location 9 | - | - | + | - | - | - |
| Location 10 | - | - | + | + | - | - |
| Location 11 | - | - | + | + | - | - |
| Location 12 | - | - | + | - | - | - |
| Location 13 | - | - | + | - | - | - |
| Location 14 | - | - | - | - | - | + |
| Location 15 | - | - | + | - | - | - |
| Location 16 | - | - | - | + | - | - |
| Location 17 | - | - | - | + | - | - |
| Location 18 | - | - | - | + | - | - |
| Location 19 | - | - | - | + | - | - |
| Location 20 | - | - | + | + | - | - |

***Where ''+'' means presence and ''–'' means the absence***

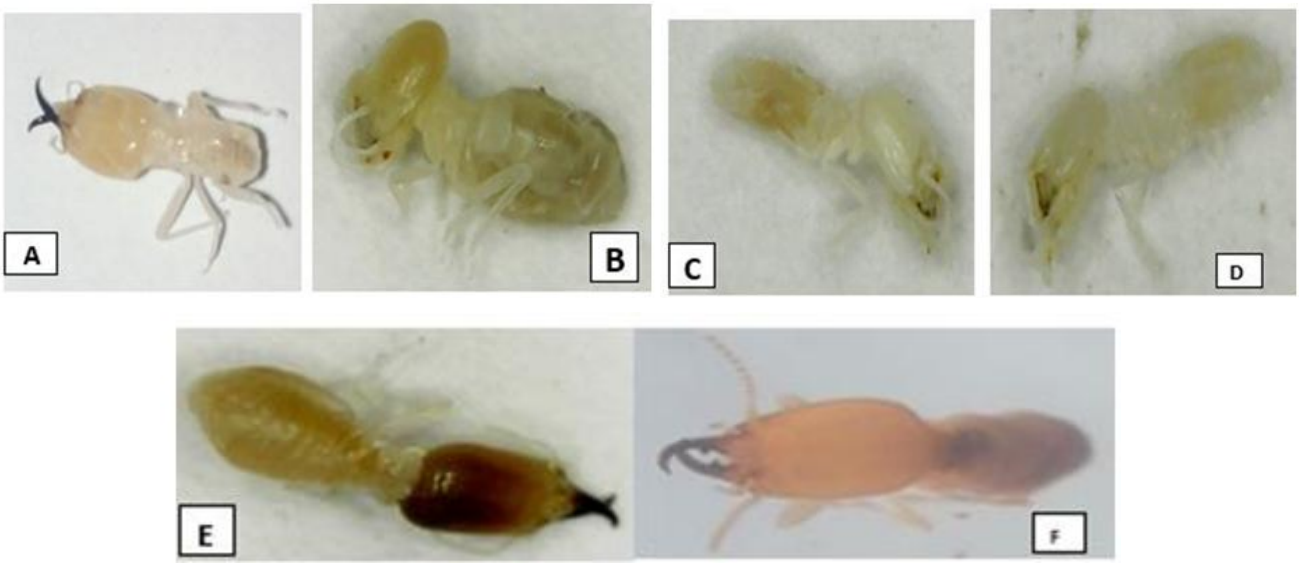


**FIGURE 3: Termite species identified at the locations used for this study. A: *Ondototermes pauperan,* B: *Trinervitermes spp.,* C: *Ancistrotermes cavithorax,* D: Microtermes *spp.,* E: Macrotermes *subhyalinus* F: Amitermes *evuncifer***


**FIGURE 4: Evidence of Termite activities at some of the selected study sites.**

### 3.2 Relationship between Termite Severity and Soil Properties

The result of the investigated soil properties carried out in this study showed that soil properties viz bulk density, moisture content, soil organic matter, and soil water holding capacity varied from one location to another, although the values were not significantly different for some locations, and is believed to have played a role in the distribution of the termite species identified within the campus area. Concerning bulk density (**fig 5**), it was observed that location 18 had the highest bulk density of 1.76±0.01g/cm$^3$while location 2 had the least bulk density of (1.08±0.01g/cm$^3$).

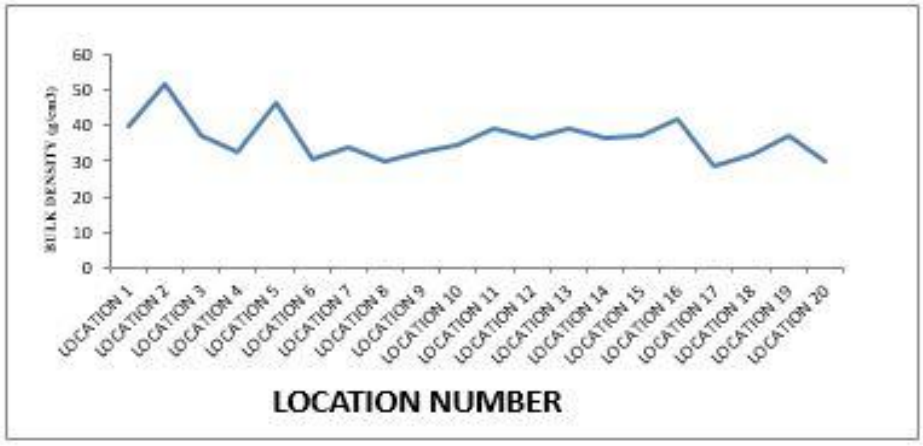


**FIGURE 5: Bulk density of soil samples obtained from the 20 selected locations across the AAUA campus area**

The moisture content (**fig 6**) of the soil samples from the 20 selected locations in the AAUA campus area, shows that location 3 has the highest moisture content value of (19.78±0.03) while location 8 (7.19±0.02) had the lowest moisture content value.


**FIGURE 6: Moisture content (%) of soil samples was obtained from the 20 selected locations across the AAUA campus area.**

The soil organic matter (**fig 7**) content in the 20 selected locations within the AAUA campus, ranged from 21.29±0.04%location 2 to (6.08±0.02) location 1. A similar result is observed with the soil water holding capacity (**fig 8**) of the soil in the 20 locations, which ranged from51.72±0.02% location 2 to 28.82±0.02% for location 17.


**FIGURE 7: Organic matter content (%) of soil samples obtained from the 20 selected locations across the AAUA campus area.**

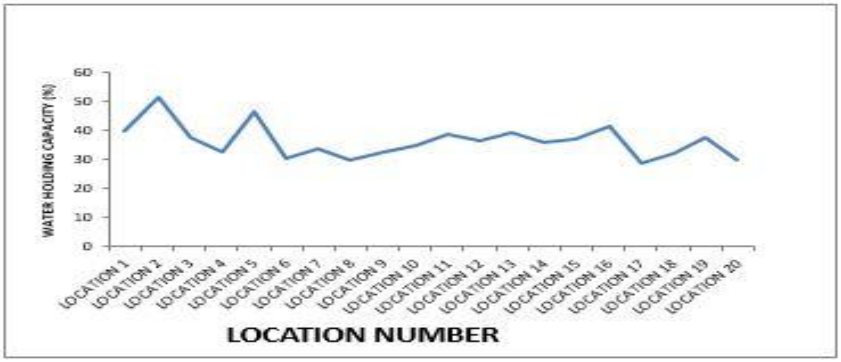


**FIGURE 8: Water holding capacity (%) of soil samples obtained from the 20 selected locations across the AAUA campus area.**

Although the values were not significantly different for some locations and are believed to have contributed to the distribution of the termite species identified within the campus area, as well as the severity of their activities as revealed by the spearman's rank correlation (Table 3) between the investigated soil properties in the 20 selected locations across AAUA campus and termite severity of attack; measured as a function of the visual ratings of the wood samples in these locations with *Ancistrotermes cavithorax* and the *Microtermes spp* adapting to a greater range of variations.

From the results, as shown in **Table 3**, it can be observed that there is a moderate positive correlation between the visual rating of wood samples observed in the twenty selected locations within the AAUA campus and their water holding capacity, which was statistically significant at 0.05 probability level ($rs = 0.510$, $P = 0.05$).

**TABLE 3**

**SPEARMAN'S RANK-ORDER CORRELATION MATRIX FOR VISUAL RATINGS AND INVESTIGATED SOIL PROPERTIES OBSERVED IN THE 20 SELECTED LOCATIONS WITHIN THE AAUA CAMPUS**

| | | Moisture Content | Organic Matter | Water Holding Capacity | Bulk Density |
|---|---|---|---|---|---|
| VISUAL RATING | Correlation Coefficient | 0.347 | 0.175 | 0.510* | -0.239 |
| | Sig. (2-tailed) | 0.133 | 0.461 | 0.022 | 0.310 |

**** = Correlation is significant at the 0.05 level (2-tailed)***

## IV. DISCUSSIONS

There was no termite presence in locations 3 and 5 throughout the period of this study hence no species were recorded under them as shown in Table 2. Termites play important ecological roles in reworking the soil profile and the destruction of material meant for building, construction, agriculture, and forestry (Lee and Wood, 1971; Milked and Mike, 1982; Joni, 1990; Black and Ekwakol, 1997; Gummier and Nyanganji, 2005). However, termites are very sensitive to the environmental condition while establishing colony or during foraging activities, therefore has to deal with different soil types together with their properties (Ali et al., 2013: Haverty and Nutting, 1976)

Generally, an adequate moisture content level is necessary for burrowing activities, as well as to ensure longer distances and larger coverage areas. An increase in soil moisture in rates that will not interfere or limit the free movement of the termites, bring an increase in termite activities. **.** The moisture content of all the 20 selected locations across the AAUA campus area in

this study ranged from 7.19% to19.78%, which is well within the range favorable for termite activities. Once inside the building, termites will continue to maintain contact with the ground (for moisture) and the nest center (the center of the communication). (Ali *et al*., 2013; Ghaly and Edwards, 2011; Wong and Lee, 2010; Arab and Costa-Leeonardo, 2005; Su and Puche, 2003; Ahmed, 2000).

Termites feed on a very variety of organic detritus like dry grass, decaying leaves, animal dung, hummus, and living or dead wood (Brossard et al, 2007) due to the ability to decompose lignocellulosic biomass and dead organic matter in tropical and sub-tropical regions. (Jounguet et al, 2002; Mahaney et al. 1999)

Since termites are known to increase the organic matter content of the soil and modify the clay composition of this soil; all in a bid to construct nests that are erosion-resistant (Jouquet et al. 2002). This can then be concluded that both *Ancistrotemes cavithorax*and *Microtermes spp.* are well adapted to soil of both high and low organic matter content, hence, their wide distribution across these locations.

In this study, wood exposed to soil with high water-holding capacity showed low resistance to termites attacks, the result of this work corroborates with the report from Jurgenrius *et al* (1999) and Owoyemi *et al* (2017) revealed that soil properties are one of the factors contributing to the level of attacks of termites when considering the effect of soil bulk density on the rate of termite attacks.

### 4.1 The severity of Termite Activities on the Campus Area

The results of the weight loss assessment of *Triplochiton scleroxylon* wood samples in the 20 selected locations across the AAUA campus area were summarized in **Figure 9**. It was observed that locations with higher weight loss values, have similar low visual ratings, with higher weight loss values recorded for locations where *Ancistrotermes cavithorax*, *Microtermes spp,* and *Macrotermes subhyalinus* species were identified, and the attack increases getting with the presence of *Macrotermes subhyalinus*, and this correlated with the observations of Owoyemi *et al*. (2017) which reported the aggressive nature of this termite species.


**FIGURE 9: Severity of termite activities across the 20 locations within the AAUA campus as measured by the gravimetric weight loss and the ASTM D3345-17 visual rating.**

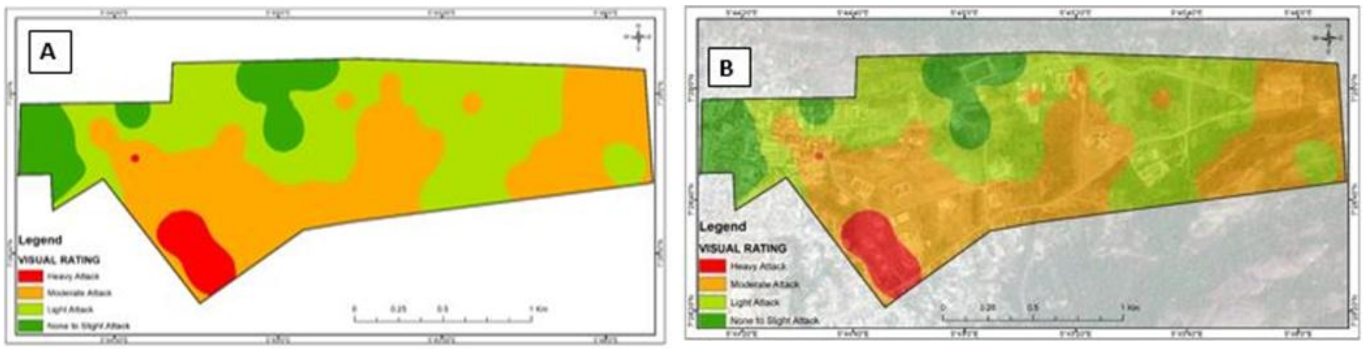


**FIGURE 10: Termite Infestation Probability Map of AAUA campus; A: Map showing the termite severity on the campus-based on the visual ratings of the selected locations, B: Termite severity of the AAUA campus superimposed on the extracted Google earth imagery showing other known features that fall within the campus boundary.**

The result showed that locations 3 and 5 had no evidence of termite presence throughout the 12 weeks of testing, indicating that the weight loss values observed in these locations and invariably in all the 20 selected locations across the campus area had contributions from other wood biodeteriorating agents other than termites. The weight loss recorded in these locations can be due to micro bacterial activities and /or fungi activities which can be favoured by the high organic matter and moisture content. This report corresponds to previous knowledge that apart from the biting action, most termite species maintain a symbiotic interaction with a greater community of microorganisms which helps them in breaking down their food materials (Ali *et al*., 2013; O'Brien and Slaytor, 1982).

### 4.2 Termite Severity Probability Map of AAUA Campus Area

The termite infestation probability map of the AAUA campus prepared using the ArcGIS software is presented in Figure 10, after twelve (12) weeks of exposure across the twenty selected locations within the AAUA campus area. It was observed that termite activities were ongoing in almost all areas within the AAUA campus area; except locations 3 and 5 within which no termite species or foraging activity were observed. These locations could be found around Mass Communication Department (location 3) which is waterlogged during the rainy season, and could be responsible for the absence of termite activities in these areas. Location 5 besides the Faculty of Social and Management Sciences is a very rocky soil area with little or no moisture making it difficult for termites to burrow in the earth making it difficult for termites to thrive.

Areas under light termite activities or probability of light to moderate attacks involve a host of important university installations such as University Eastern Gate, University Senate Building, Entrepreneurship building, beside the V.C Lodge e.t.c. Other areas under moderate attack and severity of termite activities include the incomplete student hostel opposite the University Health Centre, open field opposite Handball court, school Library, New Faculty of Art, the Farm Gate, Teak plantation in front of Advancement Office, the University farm, while the areas that recorded high termite activities with heavy severity of attack include the university sports complex and chemistry laboratory, zenith female hostel.

A sharp transition could be easily be noticed between the regions belonging to the different severity (color-coded) levels (Fig. 10a &b).this implies that almost every part of the AAUA campus area is at the risk of termite attack, Therefore, from the foregoing, adequate caution and design considerations must be taken when constructing buildings within the campus area, putting into consideration construction measures against termite ingress and infestation of the buildings; especially in the areas under moderate to heavy severity of termite attack.

## V. CONCLUSION

The study has established the presence of termites activities within the AAUA campus with *Ancistrotermes cavithorax* most associated with aggressive foraging activities within the campus area co-existing with the *Microtermes species*. Activities of termites have imparted negatively on wooden structures in buildings. The knowledge of its prevalence is important when sitting building in a residential environment. The termite's probability map developed for AAUA will serve as a guide for what pre-constructional methods to adopt in a new environment; while it will also guide on what remedial approaches to take in areas where buildings have been erected already. Studies on termites' severity should be conducted periodically as termites move from one location to another as evidenced in the periodic swarming activities of winged termites.

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# Occurrence of Avian Pox Outbreaks in Wild and Canary Commercial Breedings. Diagnosis through Electron Transmission Microscopy and Histopathology Techniques

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Received:- 18 May 2022/ Revised:- 07 June 2022/ Accepted:- 14 June 2022/ Published: 30-06-2022


***Abstract—*** *Avian pox is one of the major viral diseases that affects canaries and due to its rapid spread, can decimate the entire breeding, causing great economic damages to breeders. Canaripoxvirus species belongs to the Poxviridae family and Avipoxvirus genus. Three main forms characterize the disease, the cutaneous, diphtheric and septicemic, but coryza and tumor forms can also occur. In the period from 2006 to 2021, outbreaks of avian pox occurred in canaries from commercial breeding and Ecological Park, in canaries victims of illegal trade in São Paulo, SP, Brazil. All birds had skin lesions in beaks and feets and sometimes in the eyes and tongue, in addition to anorexia, diarrhea, weight loss and death. Samples of skin lesion fragments, crusts and organs from all birds were processed by negative staining, immunoelectron microscopy and immunocytochemistry techniques. Skin lesions samples were also processed by the histopathology technique. Through the negative staining technique, a larger number of avipoxvirus particles was visualized in all samples of nodular lesions examined and in the sample of canary lung fragments from outbreak 7. Paramyxovirus particles were visualized in samples of liver, lung, heart, gizzard, intestine and proventriculus fragments of canaries 1 and 2 from outbreak 3. In the samples of lungs fragments, pleomorphic formations similar to mycoplasmas, were also visualized. In the samples of lung, heart, gizzard, liver, intestine and tongue fragments, from outbreak 5, the presence of mycoplasma particles was also observed. The presence of aggregates formed by the antigen-antibody interaction characterized the positive result for avipoxvirus obtained in the immunoelectron microscopy technique in all skin lesions samples. In the immunocytochemistry technique the antigen-antibody reaction was strongly enhanced by the dense particles of colloidal gold on avipoxviruses. In the histopathological examination many avipoxvirus inclusion bodies (Bollinger bodies), strongly eosinophilic ring-shaped, marked hyperkeratosis and epithelial hyperplasia were observed in feet skin of canaries. Areas with globular degeneration and monolymphocytic inflammatory infiltrate were also visualized.*

***Keywords— Avipoxvirus, Canary, Transmission electron microscopy, Histopathology.***

## I. INTRODUCTION

Canary breeding is a practice that has become increasingly profitable and widespread in Brazil and plays a fundamental role in preservation of species. The yellow color of the Belgian lineage is the most popular, but the search for new and different tonalities, size, genetic improvement, besides presentation in exhibitions and preservation, are the main objectives of the creators [1].

Avian pox is one of the major viral diseases that affects canaries and due to its rapid spread, can decimate the entire breeding, causing great economic damages to breeders [2]. *Canaripoxvirus* species belongs to the *Poxviridae* family and *Avipoxvirus* genus [3]. Transmission involves insects as vectors, direct contact with aerosols between infected and susceptible birds, ingestion of water, contaminated food and semen transmission [2, 4, 5]. Three main forms characterize the disease, the cutaneous, diphtheric and septicemic, but coryza and tumor forms can also occur [6]. Cutaneous or dry is the most common in passerines, being characterized by the formation of nodules or vesicles in regions devoid of feathers, such as around the eyes, paws, feet and beak and in some cases, papules in the peri and infra-orbital regions, nose, sinus and tongue causing

dyspnea and dysphagia [6]. In this form, the lesions can lead to blindness when they occur around the eyes and obstruct the passage of food or air when they occur around the mouth and esophagus. On the paws and feet, they make perching difficult, often leading to the development of secondary bacterial infections [7]. In the diphtheric or wet form, most commonly seen in parrots, fibronecrotic lesions (white plaques) occur on the membranes of the oral cavity, tongue, pharynx and larynx. Birds may have dyspnea and asphyxia due to laryngeal obstruction. Parrots and macaws can be affected by diphtheric enteritis with myocardial necrosis [6]. Canaries are the species most affected by the septicemic form, evidenced by the absence of skin lesions and the presence of pulmonary lesions [5]. There is also loss of appetite, ruffled feathers, cyanosis and solitude. The disease progresses to desquamative pneumonia with capillary occlusion resulting in dyspnea and death within 3 days, with mortality ranging from 70 to 90% [8]. A fourth form, coryza, affects parrots, which begins with clear nasal discharge that progresses to fibrinous and mucous membranes, followed by conjunctivitis. The form of tumors, represented by skin nodules that evolve into tumors and adenomas, is more commonly observed in canaries [6]. An unusual form of poxvirus was described in a herd of canaries, affecting mainly young animals, whose signs were respiratory distress, loss of feathers and crusts on the head, neck and back, anorexia, weight loss, ruffled feathers and high mortality [9]. Avipoxvirus was also isolated from tongue for canaries, that showed severe localized proliferative glossitis [10]and an outbreak of systemic avian pox associated with B1 subgroup was related among canaries [11]. Other outbreak of canaripox was reported in breeder farms associated with co-infection by *Mycoplasma gallisepticum* [12]. In some birds, avipoxviruses may exist in a latent form [13,14] and may survive for months or even years in dry skin crusts [5]. More than one form can occur simultaneously in the same bird or in different birds from the same farm affected by the disease outbreak [6].

Secondary bacterial and fungal infections contribute to the worsening of the disease [15]. Nonspecific stressors may be associated with viral reactivation [6]. Birds with mild injuries can recover depending on their immunity, becoming carriers and spreading the virus [16].

In Brazil, there are few reports on the occurrence of the disease in wild birds, in captivity or commercial breeding.

An outbreak of the disease occurred in 2003, was reported in an Ecological Park that affected several species, such as cowled-cardinal (*Paroaria dominicana*), white-throated seedeater (*Sporophila caerulescens*) and double-collared seedeater (*Sporophila albogularis*), with high mortality [17], and another outbreak that occurred in 2012 in the same Ecological Park, infected about 45 species of bay-winged-cowbird (*Gnorimopsar chopi*) [18]. Other occurrences signaled the presence of the virus in psittacines [19, 20], in a common barn owl (*Tyto alba*) [21], in penguins (*Spheniscus magellanicus)* [22] and in teal (*Dendrocygna autumnalis*) [23].

In commercial poultry, smallpox is a notifiable disease, being included in list B, among the communicable diseases considered important from a socio-economic and/or sanitary point of view at the national level and whose repercussions on the international trade of animals and products of animal origin are considerable [24].

This research aimed to detect the presence of avian poxvirus during outbreaks occurred in commercially farmed canaries and in an Ecological Park in the State of São Paulo, SP, Brazil, using transmission electron microscopy and histopathology techniques.

## II. MATERIAL AND METHODS

### 2.1 Descriptions of the outbreaks

Outbreak 1 – In the period from the 2006 to 2009, during illegal commercialization of the Brazilian birds, 96 Saffron finches were apprehended and being forwarded to the Tietê Ecological Park, São Paulo, SP, Brazil. Twenty-nine birds presented cutaneous lesions around the beak and legs (fig. 1, arrow). After presenting weight loss, prostration and sudden death the birds were sent to Biological Institute of São Paulo, SP, Brazil, to investigate viral agents.

Outbreak 2 – In March and July 2013, 3 canaries from properties located in Itu, SP and São Paulo, SP, Brazil were sent. The birds had lesions on their feet. No other information was obtained.

Outbreak 3 – In April 2014, 2 canaries from São Paulo, SP, Brazil, were sent for examination. The birds presented diarrhea, weight loss, prostration, lesions on the legs and beaks. During the necropsy, we observed that the organs were hemorrhagic and one of the canaries had a dilated proventriculus.

Outbreak 4 – In March 2017, an outbreak occurred in an intensive sport-type canary farm in the State of São Paulo, SP, Brazil, with 200 animals. Initially 50 to 100 birds died and 50 became ill. The birds had symptoms and clinical signs of progressive emaciation and death within a few days of evolution. A total of 12 canaries, 8 months old, were sent to Biological Institute of São Paulo, SP, Brazil, for research on viral agents. Necropsy showed that the proventriculus, intestines and liver were enlarged, in addition to the presence of the nodular lesions with blood on the legs, feet, beaks and around the eyes (fig. 2, arrow).

**FIGURE 1: Canaries with feet lesions (arrows).**

**FIGURE 2: Canary with bloody lesion on the feet (arrow).**

Outbreak 5 – During the month of June 2018, an outbreak occurred in an intensive farm with 50 Belgian canaries, in Taubaté, SP, Brazil. Suddenly 7 animals became ill and one of them died. Posteriorly, all the animals died. During the necropsy of an animal, we could observe that the liver was friable, enlarged and yellowish, the intestines and lungs were hemorrhagic and the ventriculus had a dark content. It was also observed the presence of severe lesions in the beak, eyes and paws of the bird.

Outbreak 6 – In the period of December 2018, 2 canaries from intensive exploration and sport breeding in São Paulo, SP, Brazil were sent. The number of animals on the farm and the number of sick animals were not reported. At necropsy, it was observed that the intestines and lungs were hemorrhagic and the presence of lesions on the paws.

Outbreak 7 – In November 2021, an outbreak occurred in an intensive sport-type canary breeding, located in Santo André, SP, Brazil, with 70 animals. At the time, 7 birds became ill and 3 died. Two puppies were sent for necropsy, but they arrived very altered and it was not possible to perform the necropsy. The birds had lesions on their legs and beaks. A pool of organs from each bird was made. In February 2022, new animals were affected and a canary was sent for necropsy. The bird had lesions on the tongue, paws and beak. During the necropsy it was observed that the organs were hemorrhagic and the spleen and intestines were dilated.

## III. METHODS

Samples of fragments of nodular lesions and dry crusts and organ fragments from all birds were processed by the negative staining (rapid preparation) technique.

### 3.1 Negative staining technique (rapid preparation)

In the negative staining process, the fragments of nodular lesions, dry crusts and organ fragments, were suspended in phosphate buffer 0.1 M and pH 7.0 and placed in contact with metallic grids. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate [25; 26; 27].

Poxvirus positive samples were processed by immunoelecton microscopy and immunocytochemistry techniques.

### 3.2 Immunoelectron Microscopy Technique (IEM)

The copper grids were prepared as described above, sensitized with a primary virus-specific antibody primary and were washed with phosphate buffer 0.1 M and pH 7.0. Upon incubation with the viral suspension, grids were washed successively with distilled water and negatively contrasted with 2% ammonium molybdate under the same conditions [26;28].

### 3.3 Immunocytochemistry technique.

At the immunolabeling technique with colloidal gold particles for negative staining, the copper grids were placed in contact with viral suspension of the samples and, after removing the excess with filter paper, the same were put on specific primary antibody drops. After further washing in PBS drops, the grids were incubated in protein A drops, in association with 10 nm colloidal gold particles (secondary antibody). Grids were then contrasted with 2% ammonium molybdate [29].

## IV. RESULTS

### 4.1 Negative Staining Technique (Rapid Preparation)

Through the negative staining technique (rapid preparation) a larger number of avipoxvirus particles was visualized in all samples of nodular lesions examined and in the sample of canary lung fragments from outbreak 7. The particles showed irregular distribution of the tubules on the outer membrane (fig. 3, arrow), some enveloped (fig. 4, arrow), measuring on average 350 nm in lenght x 250 nm in diameter. Enveloped, pleomorphic, characteristic paramyxovirus particles (fig. 8, arrow), measuring between 100 and 300 nm in diameter, were visualized in samples of liver, lung, heart, gizzard, intestine and proventriculus fragments of canaries 1 and 2 from outbreak 3. In the samples of lungs fragments, pleomorphic formations similar to mycoplasmas, measuring between 100 and 800 nm, were also visualized. In the samples of lung, heart, gizzard, liver, intestine and tongue fragments, from outbreak 5, the presence of mycoplasma particles was also observed.

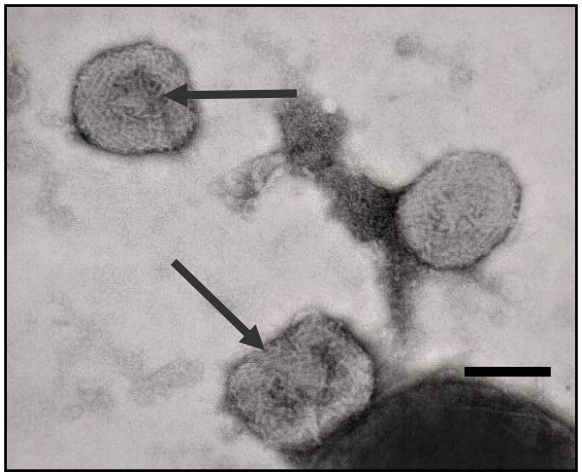


**FIGURE 3: Negatively stained of avipoxvirus particles, ovoide, showing irregular distribution of the tubules on the outer membrane (arrows). Bar: 190 nm.**

**FIGURE 4: Negatively stained of avipoxvirus particle showing outer envelope (arrow). Bar: 270 nm.**

### 4.2 Immunoelectron microscopy

The presence of aggregates formed by the antigen-antibody interaction characterized the positive result for avipoxvirus obtained in the immunoelectron microscopy technique in all skin lesions samples (fig. 5, arrow).

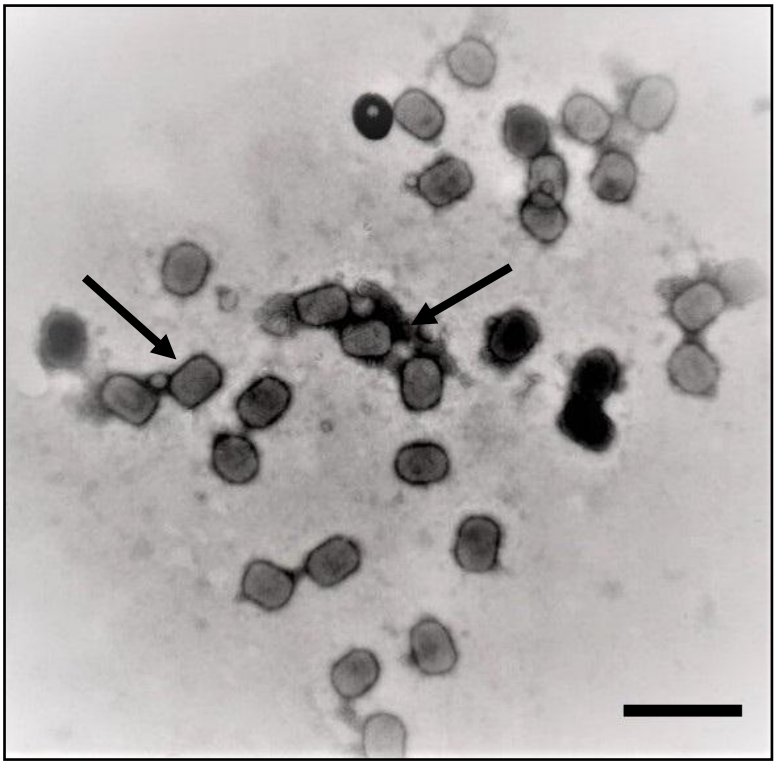
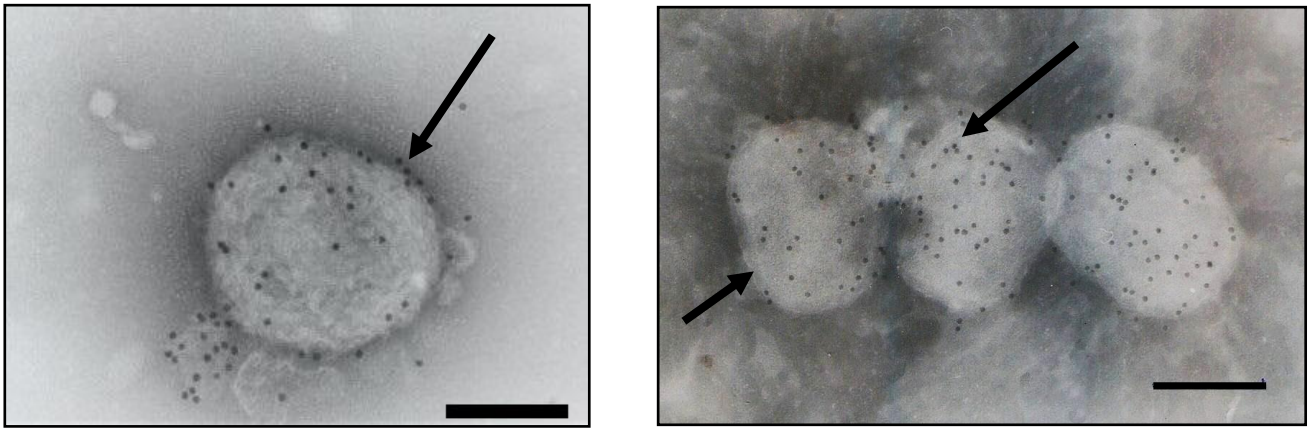


**FIGURE 5: In the immunoelectron microscopy technique the avipoxvirus particles were aggregated by antigen-antibody interaction (arrows). Bar: 690 nm.**

### 4.3 Immunocytochemistry technique

In the immunocytochemistry technique the antigen-antibody reaction was strongly enhanced by the dense particles of colloidal gold on avipoxviruses (figs. 6, 7, arrow).



**FIGURES 6, 7: Antigen-antibody interaction strongly enhanced by the dense gold particles over the avipoxvirus (big arrow). Observe enveloped particles in fig. 7 (minor arrow). Bar. fig. 6: 160 nm; Bar. fig. 7: 200 nm.**

### 4.4 Histopathology technique

In the histopathological examination many avipoxvirus inclusion bodies (Bollinger bodies), strongly eosinophilic ring-shaped (fig. 9), marked hyperkeratosis and epithelial hyperplasia (fig. 10) were observed in feet skin of canaries. Areas with globular degeneration and monolymphocytic inflammatory infiltrate were also visualized (fig. 11).

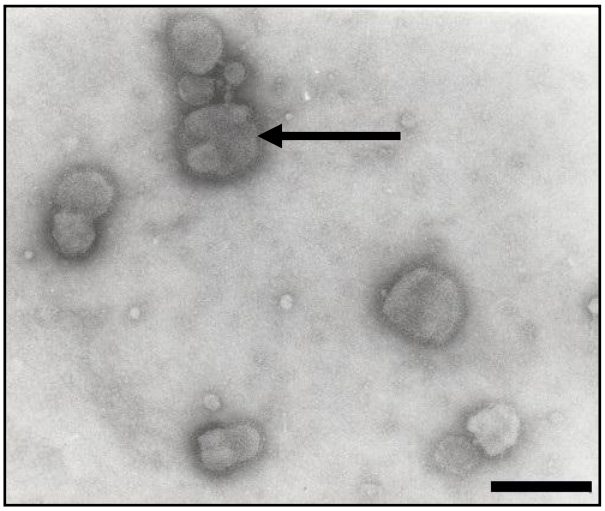


**FIGURE 8: Negatively stained of enveloped, pleomorphic, characteristic paramyxovirus particles, measuring between 100 and 300 nm in diameter (arrow). Bar: 430 nm.**

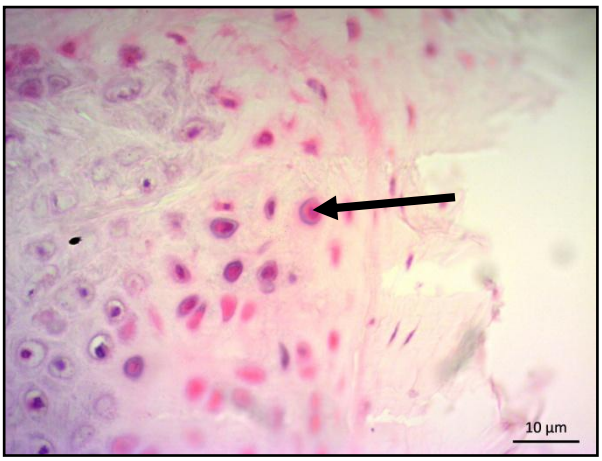



**FIGURE 9: Photomicrograph of canary feet skin, showing many inclusion bodies (Bollinger bodies), ring-shaped and strongly eosinophilic (arrow). X630.**

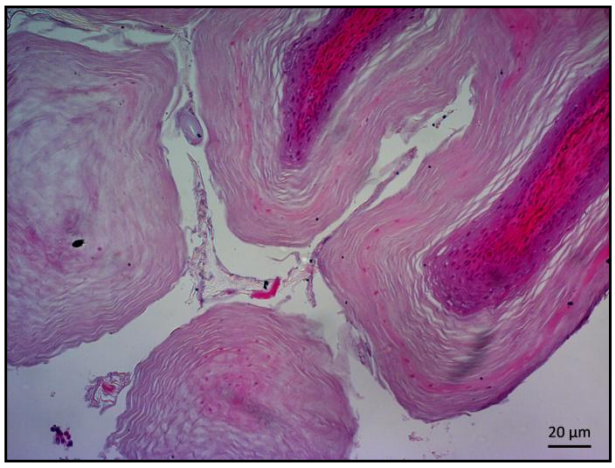



**FIGURE 10: Photomicrograph of canary feet skin, showing marked hyperkeratosis and epithelial hyperplasia. X200**

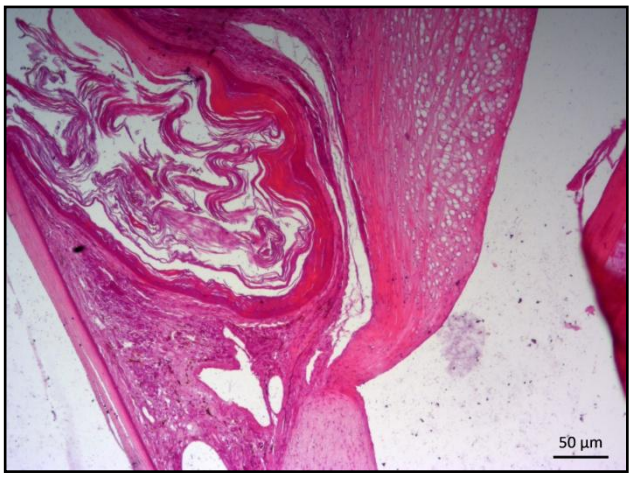



**FIGURE 11: Photomicrograph of canary feet skin, where epithelial hyperplasia with areas of globular degeneration and monolymphocytic inflammatory infiltrate is observed. X100**

## V. DISCUSSION

In this work we report the occurrence of outbreaks of avian poxvirus in commercial canary farms and in an Ecological Park in the State of São Paulo, SP, Brazil. Similar outbreaks have been reported worldwide, causing unfortunate animal losses with consequent damage to breeders and nature.

Several forms of the disease affect canaries, the septicemic form with the absence of skin lesions and the presence of lung lesions [5], tumors [6; 7; 30] and an uncommon form, characterized by respiratory problems, associated with loss of feathers and crusts on the head, neck and back, with high mortality [9]. The cutaneous or nodular form, however, is the most prevalent among the creations in Brazil, leading to the death of most flock canaries.

All the canaries in our study had cutaneous lesions on the feet, beak and sometimes in the eyes, in agreement with the report of other authors, in canaries [9; 12; 30; 31; 32; 33] and in other avian species, such as, *Gnorimopsar chopi* [18], *Paroaria dominicana*, *Sporophila caerulescens* and *Sporophila albogularis* [17]; *Tersina viridis* [34]; *Tyto alba* [21]; hummingbirds [35]; *Parus major* [36]; *Gymnorhina tibicen* [37]; house sparrow [38]; pigeons [39]; *Bubo virginianus* [40]; *Dendrocygna autumnalis* [23]; *Corvus macrorhynchos* [41] and in Psittacines [19].

Two canaries in our study, from outbreaks 5 and 7, had pink to reddish lesions on the tongue. Catania et al. [10] also verified the presence of similar nodular formations on the tongue of the canaries in their study, however, they did not find any other type of lesion or sign of pneumonia. The canaries from outbreak 4 had lesions with blood in the feet. Sheron-Bochler et al. [42] described hemorrhagic lesions in the head of a Southern giant petrel infected by avipoxvirus, while lesions in the lungs and liver were described in a great horned owl by Echenique et al. [40]. Flamingos with nodular lesions on their beaks not showed other clinical signs [43].

Anorexia, diarrhea, weight loss and death were the clinical signs most commonly observed in the canaries in our study, also observed in canaries by other authors [9; 11; 12; 33]. The presence of diarrhea has been described in bay-winged cowbird, cowled -cardinal, white-throated seedeater, double-collared seedeater and swallow tanager [17; 18; 34]. Most of the canaries in our research had bleeding organs and dilated intestines; one animal showed an enlarged spleen. Reza et al. [30] reported the presence of an enlarged spleen and liver in the canaries of their study.

Mycoplasma particles were seen in the lungs of 2 canaries from outbreak 3 and in organ fragments from a canary from outbreak 5. Concurrent infection of Avipoxvirus with *Mycoplasma gallisepticum* was also reported in the air sacs of two canaries by Shaib & Barbour [12]. Co-infections or secondary infections in diseases initiated by viral agents may provide a more serious condition, leading to increases in the mortality rate [44]. In mixed infections, mycoplasma can affect the physical barriers of the upper and lower airways, causing changes in tracheal cilia and mucosal destruction, aggravating respiratory disease [12].

Paramyxovirus particles were also observed in the canaries organ fragments from outbreak 3. Avipox and PMV-1 infections were responsible for high mortality of houbara bustards from a rehabilitation center in Pakistan. The association of PMV-1 and diphtheric and septicemic avipox was observed mainly in houbara bustards victims of illegal trade [45]. Serotype 2, however, is the most common in passerines, causing weight loss, severe pneumonia and diarrhea [5,6]. Stress conditions in birds due to transport, temperature changes, and poor nutrition can favor higher mortality [11] making birds more susceptible to PMV-1 infection, in addition to activating latent avipoxvirus infections [5; 46].

The viral presence was confirmed by the application of transmission electron microscopy techniques. A large number of poxvirus particles showing irregular distribution of the tubules on the outer membrane measuring 350 x 250 nm and enveloped particles, were visualized in all lesions samples examined by negative staining technique. These viral morphological aspects were also observed by this technique in skin lesions of canaries [10; 32], Australian magpie [37], penguin [42], partridge [47], bay-winged cowbird, cowled-cardinal, white-throated seedeater, double-collared seedeater and swallow tanager [17; 18; 34].

In the immunoelectron microscopy technique, the poxvirus particles were aggregated forming an immune complex, result also confirmed by other authors who used this technique to agglutinate poxvirus particles in swinepox cases [48]; herpesvirus in non-human primates [49]; swine coronavirus [50]; paramyxovirus, retrovirus and coronavirus in owls [51].

The detection of the viral antibody was also performed by the application of the immunocytochemistry technique, which enhancedly marked the avipoxvirus particles by protein-A-gold. Other researchers have reported similar results in *Gnorimopsar chopi, Paroaria dominicana, Sporophila caerulescens, Sporophila albogularis* and *Tersina viridis* [17;18;34].

In the histopathological examination of the skin of canaries, the most relevant results, such as inclusion bodies (Bollinger bodies), hyperkeratosis, epithelial hyperplasia and monolymphocytic infiltrate, are in agreement with the findings of other authors in canaries [9; 11; 12; 30; 32; 33], in barn owl [21]; great horned owl [40]; duck [23]; penguin [22; 42], [47], jungle crow [41] and in turkeys [52].

Considering that there is no specific treatment available for the disease, in infected captive birds, biosecurity standards should be established for flocks, such as the practice of quarantine before the introduction of new birds into the aviary, isolation of birds when infected, elimination of vector mosquitoes by installing screens on windows and application of low toxicity insecticides [6]. Adequate vaccination of all birds helps to contain the spread of the disease [10]. The use of caustic pencil to cauterize the lesions, associated with an optimized diet with vitamin and mineral supplements and probiotics can be useful in the recovery process [53].

In the presence of outbreaks of viral diseases, transmission electron microscopy has been extremely useful, acting in the rapid detection of multiple agents [54; 55]. The application of the techniques was paramount for the diagnosis of avian pox and the immediate adoption of prophylactic measures, prevention and control of the disease in the canary breedings.

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