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Full Length Research Paper

Tree diversity and carbon stock in urban area of Senegal and their implications to human health and well-being

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Urban green spaces (UGSs) have become essential to meet environmental requirements and improve the quality of life of inhabitants thanks to their role on air pollution, heat islands and climate change. The aim of this study was to characterize the biological diversity of UGSs in the commune of Fann Point E Amitié and their importance for human health. A floristic inventory was carried out to assess tree diversity in these UGSs, and mapping combined with an allometric model was used to estimate carbon stocks. Surveys were also carried out to gather residents' perceptions of the contribution of UGSs to health and well-being. A total of 509 trees, belonging to 55 species and 50 genera, were surveyed. The total quantity of carbon for the whole municipality is estimated at 21.16 tons. Almost all those surveyed recognized the importance of UGSs for a variety of reasons, but the main one was their positive impact on health and relaxation. These results show that beyond the beauty of their landscape, UGSs should be perceived through their role in biodiversity conservation thanks to the number of species they host, in climate change mitigation thanks to the carbon sequestration by trees, and their role in human health.

Key words: Biodiversity, carbon stock, urban trees, health, Dakar, Senegal.

INTRODUCTION

Plants in the city are allies for human health and the well-being of residents. Today, they represent one of the essential elements not only for the quality of the living environment, but also for the attractiveness of territories (Laïlle et al., 2013; Kouassi et al., 2018). They are essentially made up of all natural or artificial urban vegetated spaces better known as urban green spaces (Selmi et al., 2013; Tavin and Leseur, 2016). These green spaces can be private or public (urban parks, private gardens, vegetated roofs and walls, linear trees, wastelands and vacant spaces, riparian forests, etc). UGSs provide cities and their inhabitants with numerous ecosystem services (Tavin and Leseur, 2016; Mehdi et al., 2013, 2017) and benefits that contribute to improve their living environment but also to the physical and mental health of populations (Laïlle et al., 2013).

UGSs enable thermal regulation and the reduction of heat island impacts thanks to evapotranspiration (FAO, 2018). In addition, UGSs contribute to the fight against air pollution by fixing airborne particles (Arnold et al., 2011;

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Mehdi et al., 2013; Clergeau, 2019). According to Bolund and Hunhammar (1999), around 85% of air pollution can be filtered by plants. Similarly, urban trees contribute considerably to climate change mitigation, notably through photosynthesis (Vergriete and Labrecque, 2007). However, despite their importance, urban trees are subject to numerous pressures due to galloping demographics. Dakar, like other African cities, is not immune to this reality (Konaté et al., 2021).

Indeed, due to its geographical position, Dakar is today a major hub of economic activity and one of the main destinations for national and international internal migrants (ANSD, 2020b, a), with a concentration of 23%, or almost a quarter of the Senegalese population (ANSD, 2020a, b). In fact, it is a suffocating city, with concrete replacing trees almost everywhere. The remaining native trees on public roads, sidewalks and in parks are being replaced by exotic species due to their faster growth rates (FAO, 2018; Sy et al., 2022). The result is a strong anthropization of landscapes in these urban and peri-urban spaces, with consequences for the loss of natural ecosystems and the degradation of vegetation (Bolon et al., 2018; Charahabil et al., 2018). In addition, high traffic density and the poor condition of a large number of vehicles lead to high carbon monoxide (CO) emissions (Sivertsen et al., 2005; Demay, 2011), which increase air pollution problems. Industrial facilities, mainly located in the eastern part of the city, are also sources of air pollution. Industrial facilities, mainly located in the eastern part of the city, are also sources of emissions of atmospheric particles, sulfur dioxide (SO_2), volatile organic compounds, (VOCs) and nitrogen oxides (NOx) (Sivertsen et al., 2005). Various studies have reported heavy pollution in the city of Dakar, as in most large conurbations (Zoma, 2022; Ndong, 2019; Salao et al., 2017; Demay, 2011; Kerbachi et al., 2009; Guereirro et al., 2005; Sivertsen et al., 2005).

This atmospheric pollution is thought to be responsible for various pathologies such as allergies and numerous respiratory diseases (Boutaric and Lascoumes, 2008). Neukirch et al. (1998) and Segala et al. (1998) have shown that a 2 to 4% reduction in respiratory volume is linked to air pollution, particularly in children. According to these authors, decreases of 4 to 8% in ventilatory performance are noted in asthmatic subjects as soon as pollution indicators reach 50 µg/m^3. Studies have also shown the effect of ambient temperature variation on the risk of gestational diabetes and adverse glycemic outcomes (Preston et al., 2020). Such phenomena are sparking growing interest in the conservation of urban biodiversity and its services to improve the quality of the living environment and, above all, human health. Thus, this study investigates the state of biodiversity in the city and its implication for human health. Its aim was to characterize the floristic diversity of green spaces and analyze their importance for human health, particularly in the commune of Point E-Fann-Amitié (PFAM), at the crossroads of numerous social and economic interactions.

MATERIALS AND METHODS

Study area

Point E-Fann-Amitié municipality (PFAM) is located in the south-west of the capital and is part of the district of Dakar-Plateau-Gorée. It is located between latitudes 14° 40′ 33″ and 14° 42′ 29″ North and longitudes 17° 28′ 37″ and 17° 27′ 07″ West (Figure 1). It is bounded to the north by the commune of Mermoz-Sacré-Cœur, to the south by the municipality of Gueule Tapée-Fass-Colobane and to the north-east by the municipality of Grand Dakar. The PFAM covers an area of 5 km² and has 18,841 inhabitants (ANSD, 2013). Part of the Dakar-Plateau-Gorée district in the Dakar department, it comprises the following neighbourhoods: Amitié 1, Amitié 2, Zone B Ballon, Zone B Bâtiment, Sicap Rue X, Point E, Fann Résidence, Fann Hock. It belongs to the northern Sudanese climate zone and is influenced by the Atlantic Ocean through its maritime facade, which results in the presence of the maritime trade winds, causing coolness and almost permanent humidity.

Collection of floristic data

To characterize green spaces and their floristic diversity, a prospective visit was carried out in the commune of Fann-Point E-Amitié. This visit made it possible to classify green spaces according to their typology (private garden, public garden, aligned tree). An inventory of the flora was then carried out with a view to understanding the diversity of species in these spaces. Sampling is based on a reasoned choice, considering the size of the commune, its urban context and its residential character. Public green spaces were chosen as being more accessible for the study. In accordance with the work of Niang (2005), the nearest individual method was applied to inventory woody flora. The first individual is chosen at random, and the next is the one closest to the previously selected individual. As soon as two individuals are equidistant, a random selection is made. All individuals with a circumference ≥ 9.5 cm at 1.30 m from the ground are measured with a tape measure, and all those with circumferences below this pre-count threshold are considered juveniles, that is, part of the natural regeneration. Tree's height was estimated based on the distance from the tree's base to the top's high point. For ornamental plants and herbaceous plants, a qualitative inventory is carried out. Pictures are taken and their identification is made on the online database of the Conservatory and Botanical Gardens (CJB) of Geneva. The work of Dieng (2014) was also used as a basis for identifying ornamental species. A total of 2 public gardens and 5 (tree alignments) alleys were inventoried.

Estimating carbon stocks

Carbon stock estimation was done using the remote sensing method and also based on the allometric model proposed by Mbow et al. (2013).

For the remote sensing method, a Sentinel 2A image with a resolution of 10 m on the 6 of February 2022 was used. The data acquired by remote sensing underwent a number of pre-processing and processing operations, including geometric and radiometric corrections.

Firstly, radiometric corrections were used to improve the visual quality of the image. Secondly, as the downloaded image had no spatial reference, we proceeded by geo-referencing using an image-by-image approach. This mainly involves rotating or translating one image relative to the other until the control points (landmarks) coincide. Furthermore, different indices such as Normalized Difference Vegetation Index (NDVI) and Ratio Vegetation Index (RVI) were analysed to determine vegetation indices such as Normalized Difference Vegetation Index (NDVI) and

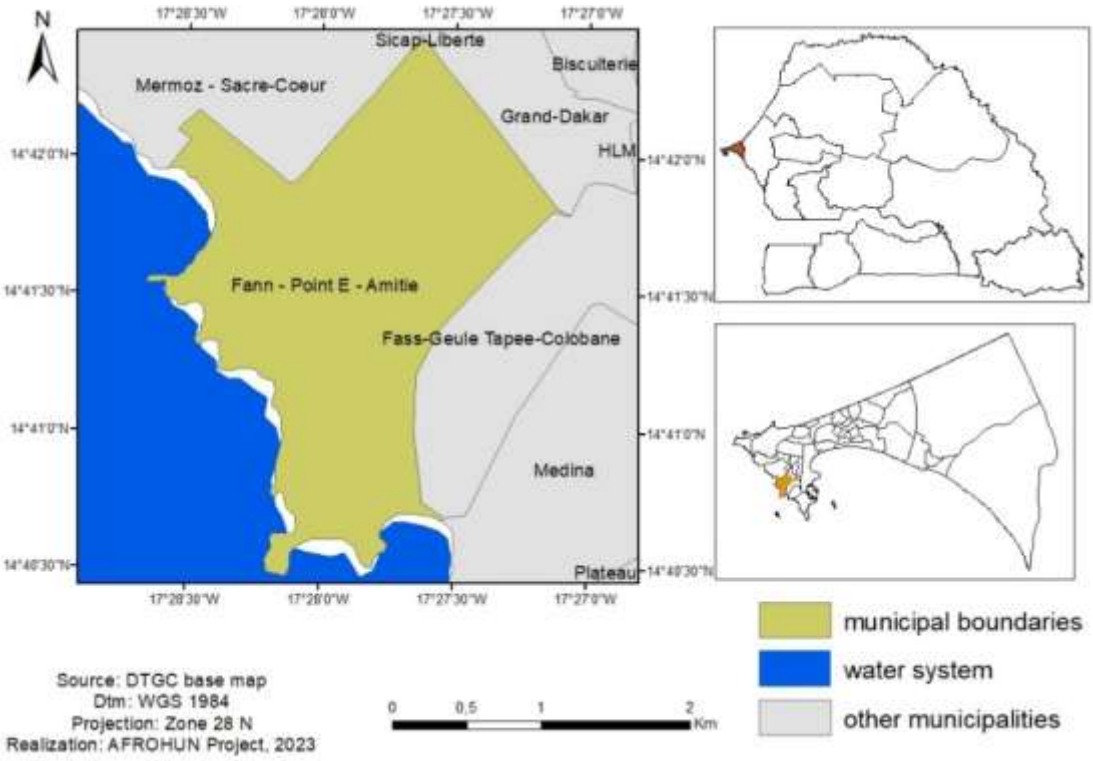

Figure 1. Location of Fann-Point E-Amitié city, Dakar, Segegal.
Source: Authors

Ratio Vegetation Index (RVI) using mathematical combinations of the red band and the PIR (Near Infra-Red) band. NDVI is a vegetation indexing method that describes the density of vegetation as an indicator of the presence and condition of vegetation in satellite images.

Its formula is:

(1)

where PIR: Near Infra-Red; Red: red Band.

The ratio vegetation index (RVI) represents the quotient of Near Infrared to Red and highlights the presence of vegetation in the images (Jordan, 1969). To calculate the above-ground biomass, the formula of Das and Singh (2012) was applied:

where (*t.ha-1*): aerial biomass and RVI: quotient vegetation index.

Information extracted from the images was used to determine the average biomass per pixel on the image. This biomass was then multiplied by the number of pixels in the area to determine the total biomass (Henrick, 2019).

To estimate the amount of total carbon (Goussanou et al., 2018) formula's was used:

$$Ct = Bt \times 0.487 \quad (3)$$

where Ct: total carbon and Bt: total biomass.

As for the allometric model (Mbow et al., 2013), it allows to estimate the above-ground biomass for each tree (i), by the following formula (Mbow et al., 2013):

where BA: above ground biomass; DBH: diameter at breast height. Conventionally, the average carbon content of woody biomass is 50% of the total biomass.

Socio-economic data collection

Surveys carried out among residents of the PFAM made it possible to study the population's perception of the importance of green spaces in relation to human health and well-being. A sampling based on the total number of households allowed to calculate the study population according to the Yamane (1967) formula.

where *N* = 3858 the total number of households; ***e*** = the level of precision (10%) and ***n*** = sample 1 size.

In total, 97 households were surveyed in order to obtain quantitative and qualitative data. In addition, individual semi-structured interviews were conducted with resource persons, including health personnel, students and faculty, given the importance of the university in the commune.

Data analysis

The floristic data were analysed using the package vegan of RStudio software 4.2.1 version, while the survey data were analysed using ibm SPSS statistics 24 software. The qualitative

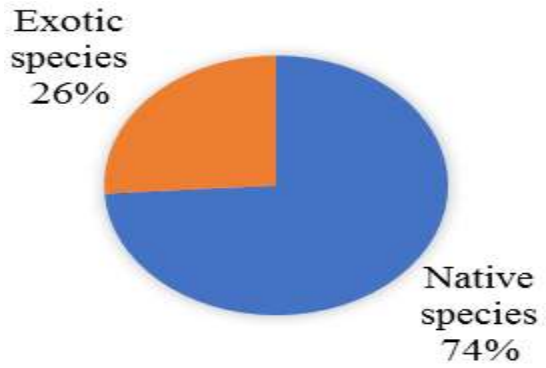

Figure 2. Proportion of native and exotic species.
Source: Authors

data were analysed using content analysis. Multiple comparison between the districts using ANOVA test and a multiple pairwise-comparison using Tukey HSD (Tukey Honest Significant Differences) was performed to determine if the mean difference between districts are statistically significant.

RESULTS

Species richness and tree diversity

A total of 509 trees belonging to 55 species in 50 genera and 32 botanical families were recorded. Native species are more represented (74.71%) than introduced species (25.85%) (Figure 2). The main families are Fabaceae (10 species), Apocynaceae (5 species), Arecaceae (4 species), and Euphorbiaceae (3 species). Moraceae and Nyctaginaceae contain 2 species each, while the rest of the families contain only one species each (Figure 3).

Moreover, the analysis of the distribution of species according to the main categories of green spaces allowed to list 15 species on average in the alleys against 8 species in the public gardens (Figure 4). Considering the presence of species according to the different districts of the commune, it appears that Fann residence is richer in species with an average value of 15 species. In Fann Hock and at Point E, the presence of 8 and 5 species, respectively was noted, while in Amitié and Zone B, the trees listed belong to only one species (Figure 5).

Similar letters in the boxplots provided from the ANOVA test show no significant difference of mean species richness among districts of the FPAM (Figure 5) even if the highest number of species is found in Fann résidence (15 species) and the lowest in Amitié and Zone B with one species.

A similar variation of Shannon diversity index (Figure 6) is noted without any significant difference among the districts of the FPAM.

Figure 7 shows the species with numbers exceedingly at least 5 trees. Of these, *Azadirachta indica* is the most abundant species with more than 250 trees. This is followed by *Samanea saman, Cordia sebestena, Peltophorum pterocarpum* and *Cocos nucifera* with numbers around 3 dozen each. Apart from *Mangifera indica* and *Terminalia mantaly*, whose numbers are close to 20 trees, the rest of the species represented in this Figure 7 do not exceed 5 trees each.

Figure 8 shows that the predominance of *A. indica* is more evident in the neighbourhoods of Fann Résidence, Amitié and Point E, while in the neighbourhood of Zone B, the abundance of *S. saman* is more noticeable. In Fann Hock, *Cordia sebestena* is relatively more present compared to the other species.

In the FPAM, the height of trees varies greatly from one district to another (Figure 9b). Trees taller than 10 m are more common in Zone B and relatively rare in the other districts. However, trees less than 10 m tall are more common in Amitié. Tree diameter also varies according to the districts of the FPAM (Figure 9a). This figure shows the presence of large trees with a diameter of around 100 cm, particularly in Zone B, while trees with a diameter of around 50 cm are more frequent in Amitié. The trees in Fann Résidence and Point E have a relatively similar diameter structure, while in Fann Hock, trees with a smaller diameter are more frequent.

Most of the trees sampled are shade and ornamental trees (Figure 10). Apart from the Amitié district, ornamental species are present everywhere. As for shade species, they were found in all neighbourhoods, although there are more shade species in the Fann Résidence and Fann Hock districts.

Carbon stocks of trees in the districts of FPAM

The NDVI and RVI vegetation indices were calculated from the satellite image of the study area (Figure 11). For the NDVI, the values are between -0.14 and 0.5. Negative values correspond to surfaces covered by water and/or mineralised surfaces. For bare soil and built-up areas, the NDVI shows values close to 0. Values greater than or equal to 0.2 and represented by dots on the map, refer to the presence of vegetation. With the RVI, the results show a quantity of plant biomass equivalent to 43.44 tonnes. This represents about 21.16 tons of carbon.

According to the allometric model, carbon stocks vary by district (Figure 12). In this figure, the width of the violin describes how often this value appears in the data set. Larger regions of the heat chart indicate values that occur more frequently. The red dots represent the average amount of carbon and this Figure 12 indicates that the amount of carbon in most trees varies between 0.02 and 7.4 t in the districts of Fann hock, Fann residence and Point E and the mean amount of carbon per tree is 0.78 t. However, in Amitié district, there is no variation. In the FAPM, considering districts, the values of carbon stocks can drop to 0.007 t or reach 403 t depending on tree abundance.

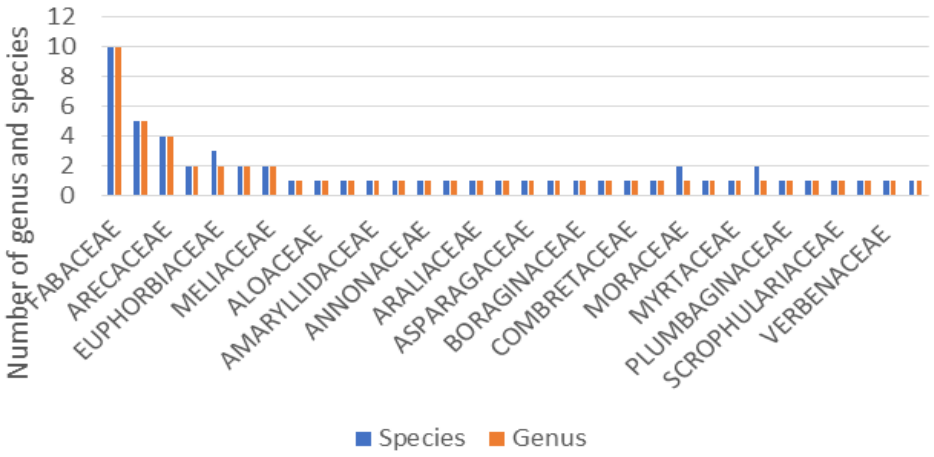

Figure 3. Number of genus and species by botanical families.
Source: Authors

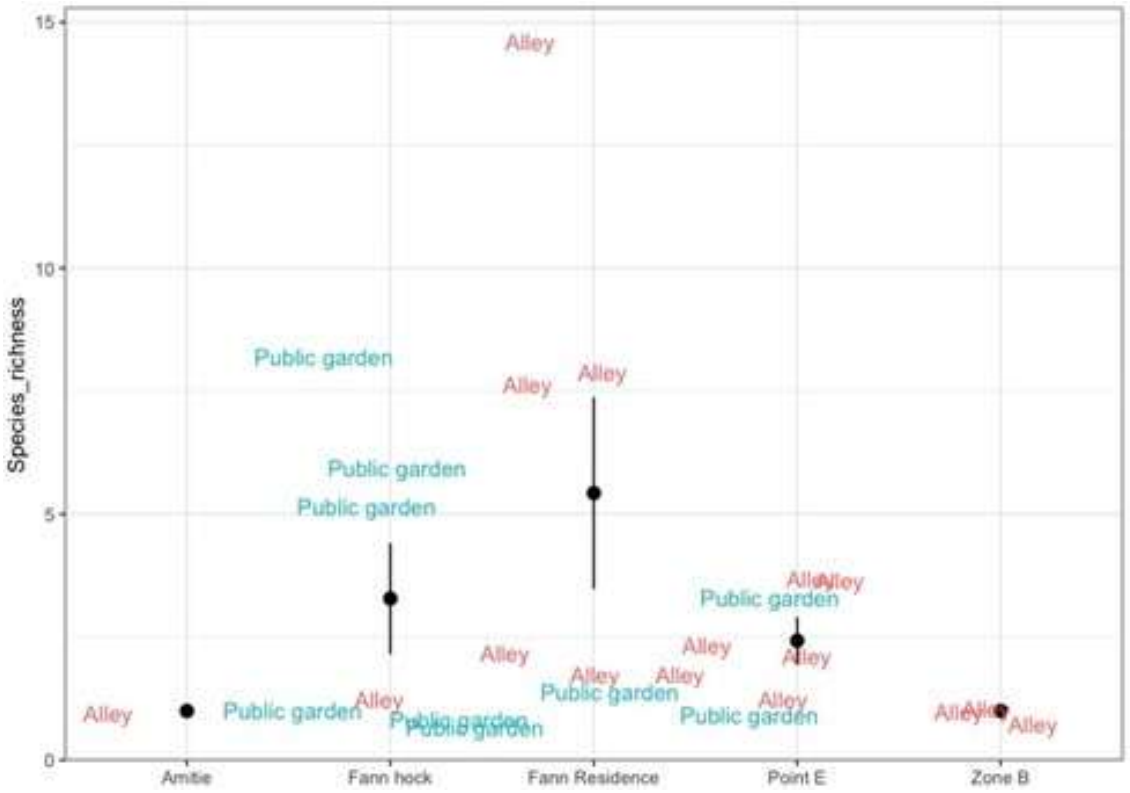

Figure 4. Variation in species richness by type of green space and districts of FPAM.
Source: Authors

Figure 13 shows a variation in carbon stocks by localities but there is no significant difference among the districts of the FPAM. The largest carbon stock is found in Amitié, with around 7.9 t of carbon. The other localities follow with 7.8 t for Fann Résidence, 7.6 and 7.5, respectively for Fann hock and Zone B. Point E comes last, with a carbon content of around 7 tonnes.

Attractions and benefits of UGS in the FPAM

The urban green spaces of the FPAM are places that are

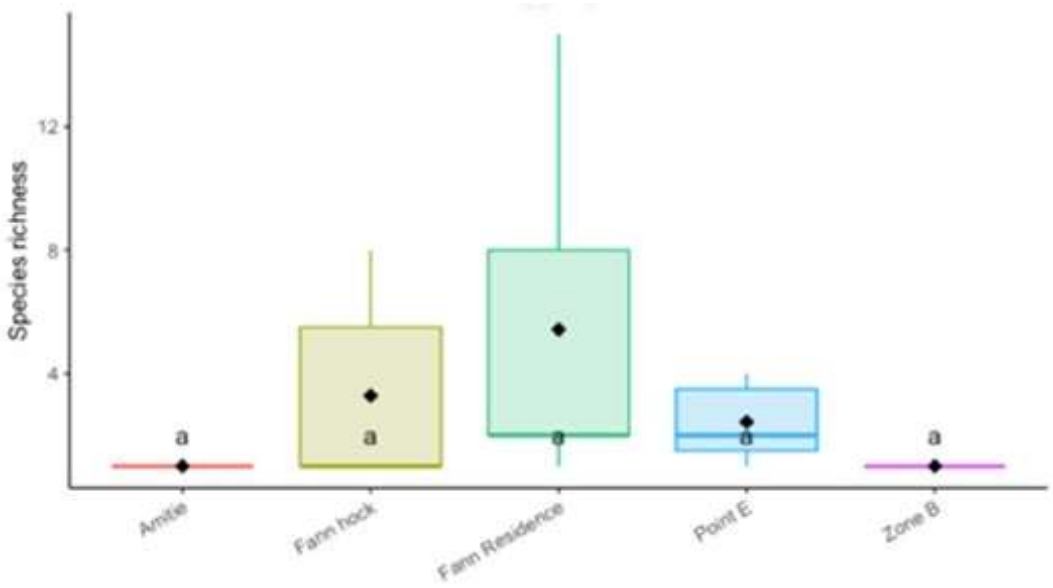

Figure 5. Species richness by district of the FPAM.
Source: Authors

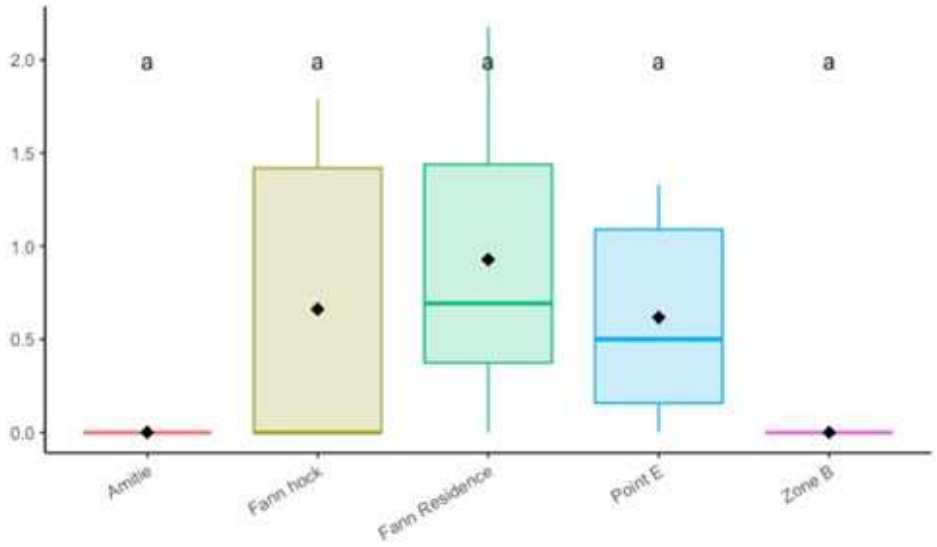

Figure 6. Shannon index diversity by district of the FPAM.
Source: Authors

frequented by men (76%) and women (24%) for various reasons (Figure 15). Among the respondents, 26.4%, associate green spaces with a break from the city/a way to reconnect with nature, relax and unwind. To this can be added, that urban green space is conceived as a very attractive and aesthetic place (by 24.2% of the respondents) where one breathes pure air (10%), but also a place free of noise (Figure 14), calm and without nuisance (18.2%). The search for peace and quiet also points in this direction. Spending time with the family (10.4%), doing sports (3.9%) or even having fun and other activities (6.9% of the respondents) also express the idea of a change of atmosphere.

Moreover, the majority of respondents (69%) believe that UGS play an important role in health and well-being. Users also emphasised other benefits such as physical activity and the creation of social links, but also mentioned ecological impacts (air purification, reduction of urban heat islands, carbon sequestration, etc).

In addition, we were able to observe the importance of

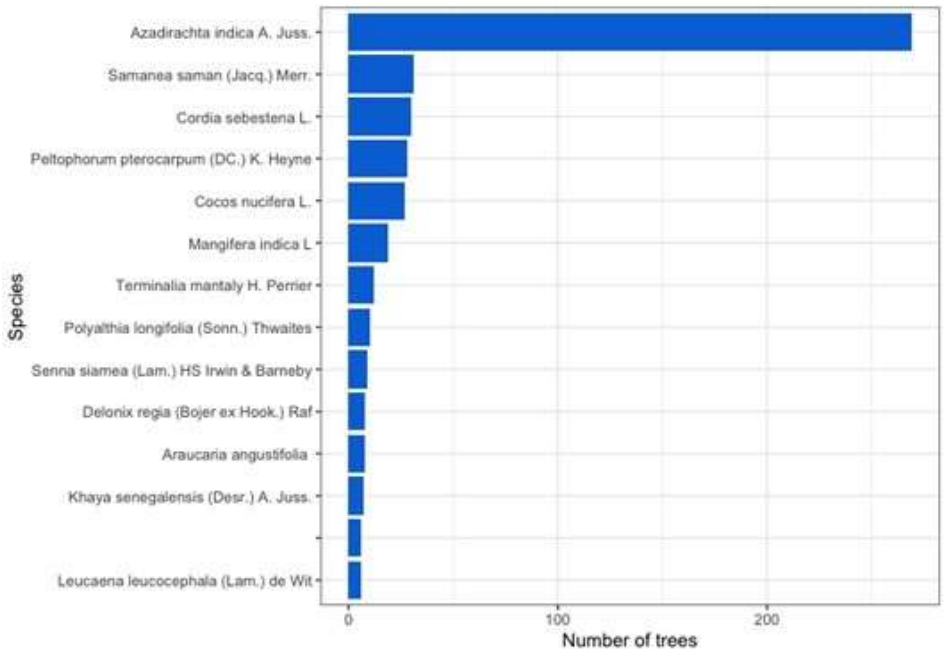

Figure 7. Abundance of main species of FPAM.
Source: Authors

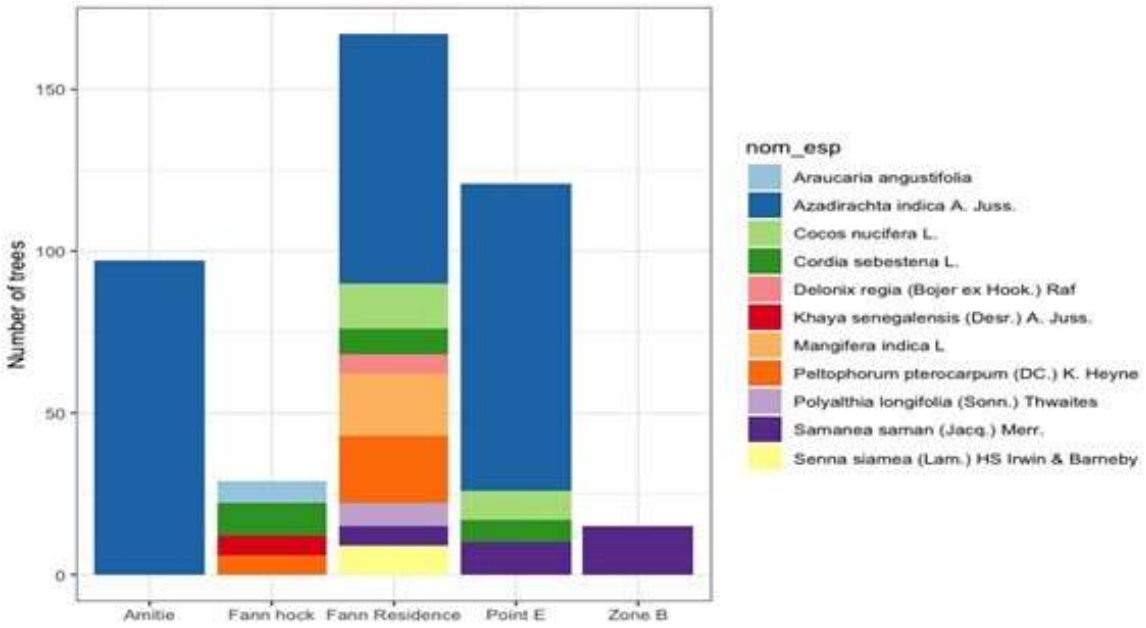

Figure 8. Distribution of main tree species abundance by districts of FPAM.
Source: Authors

individual uses of green spaces, in particular the strengthening of social ties.

Many people see UGS as a meeting place. Thus, during the semi-structured interviews, one researcher maintained that *"urban green spaces are not only indispensable for aesthetics, but also necessary for the balance of biodiversity, including human health".* The majority of respondents (97.9%) felt that UGSs are

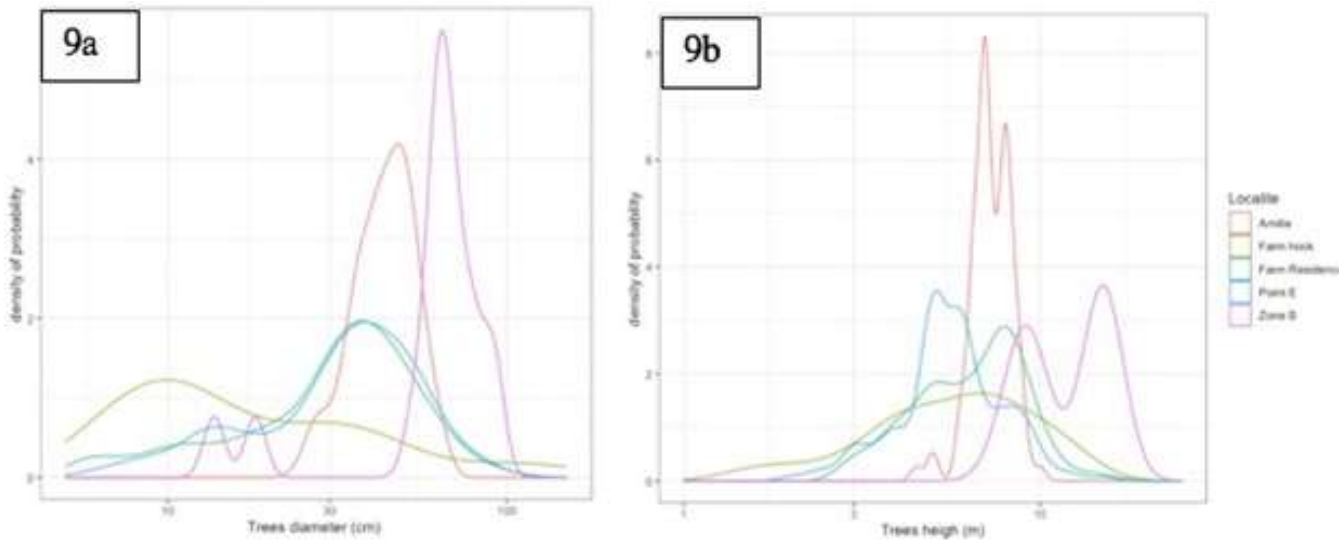

Figure 9. Tree diameter *(9a)* and height structure *(9b)* of tree species by districts of FPAM.
Source: Authors

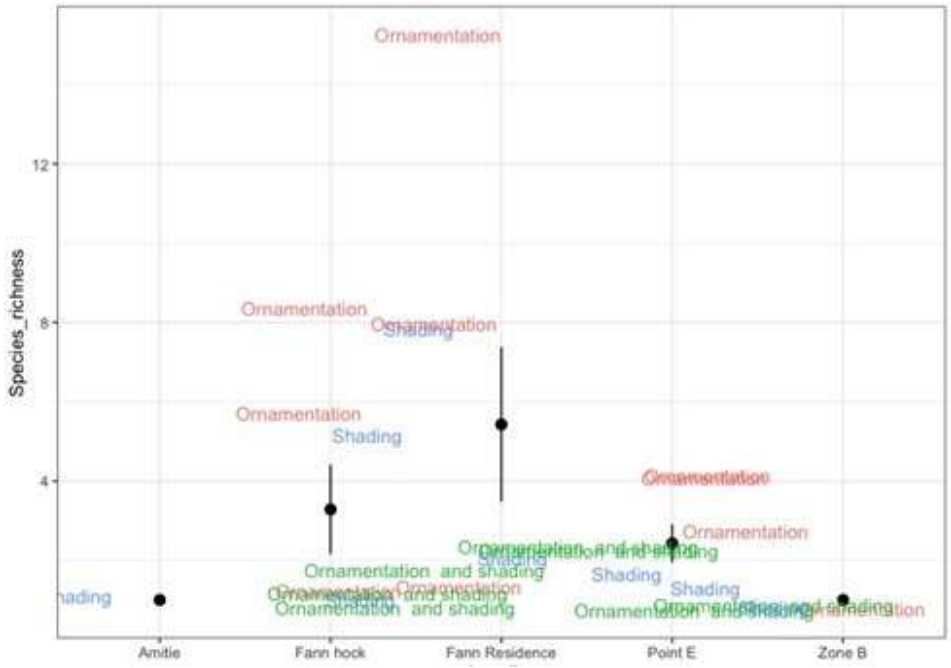

Figure 10. Species richness variation according to their role in the districts of FPAM.
Source: Authors

important in daily life because of the many services they provide. Sixty-nine percent (69%) of the respondents felt that UGSs play an important role in health and well-being.

These trends are confirmed by some of the resource persons we interviewed. According to some of them, UGSs play a role of thermal regulator that positively impacts health. For example, one health professional we met stated that the shading provided by UGSs acts as a protector against sunrays. In addition, there are other benefits related to medicinal uses. In this sense, another interviewee said*: 'From a medicinal point of view, the bark of Khaya senegalensis tree is used to treat certain illnesses, but also from a cultural point of view, because*

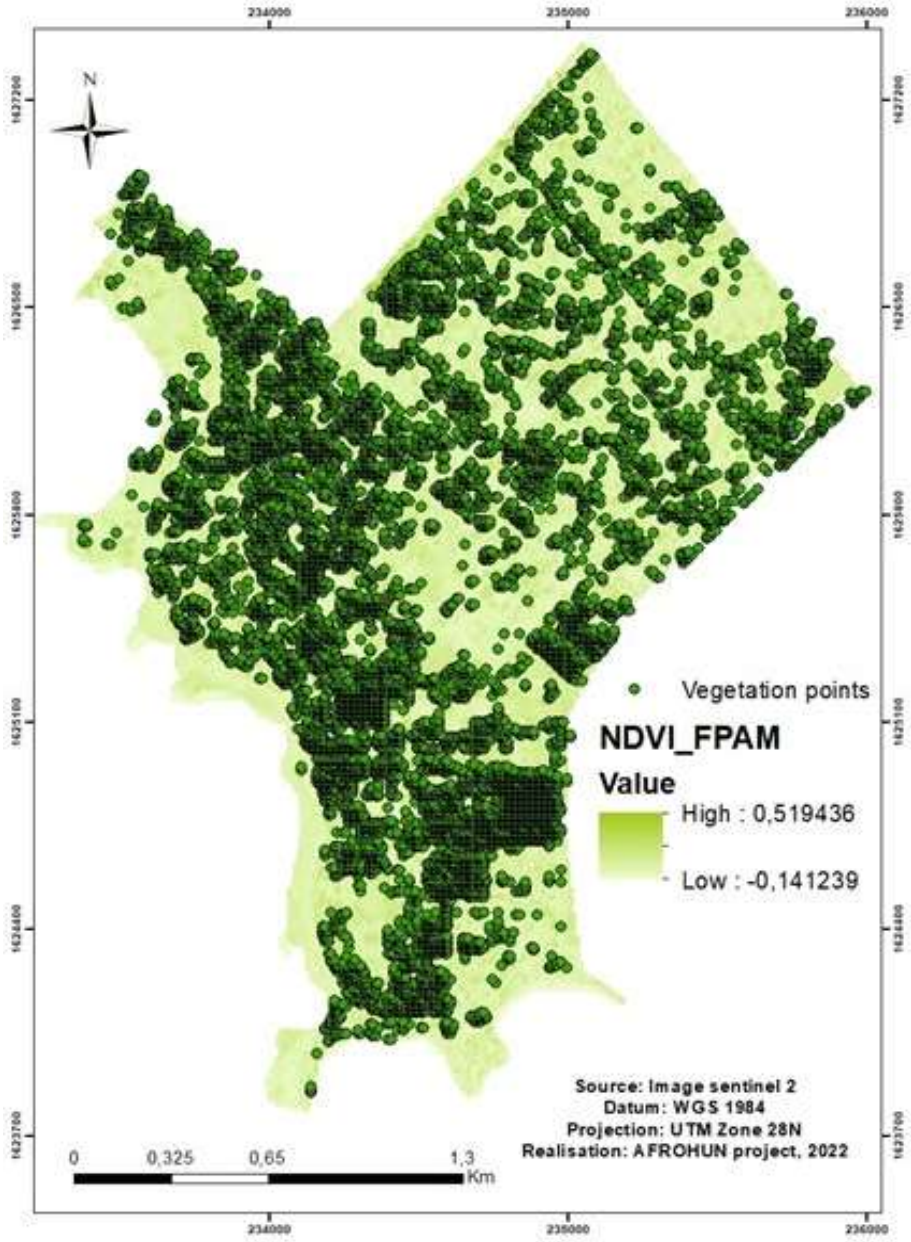

Figure 11. Vegetation map of the FPAM.
Source: Authors

the bark can be used to get rid of the evil eye, and the resin is also taken to be used as glue or for clothing'. According to another person: "*Green spaces help to reduce some forms of stress, which is why many residents report a feeling of internal well-being after spending a few hours in the public gardens".*

DISCUSSION

This study has shown that the FPAM has a fairly rich diversity of flora, concentrated in particular in the municipality's alleys and public gardens. A total of 55 species in 50 genera and 32 families were recorded in the municipality. These values are higher than those found by Sy et al. (2022) at Cheikh Anta Diop University alleys and the 26 woody species, 25 genera and 13 families found in Hann Zoological and Forestry Park (Cissé, 2021). In contrast, the study by Charahabil et al. (2018) conducted in the city of Ziguinchor (southern part of Senegal) found a higher species richness with 132 species in 95 genera and 32 families. Elsewhere in the city of Lomé, values of 93 species belonging to 79 genera and 47 families were found (Polorigni et al., 2015).

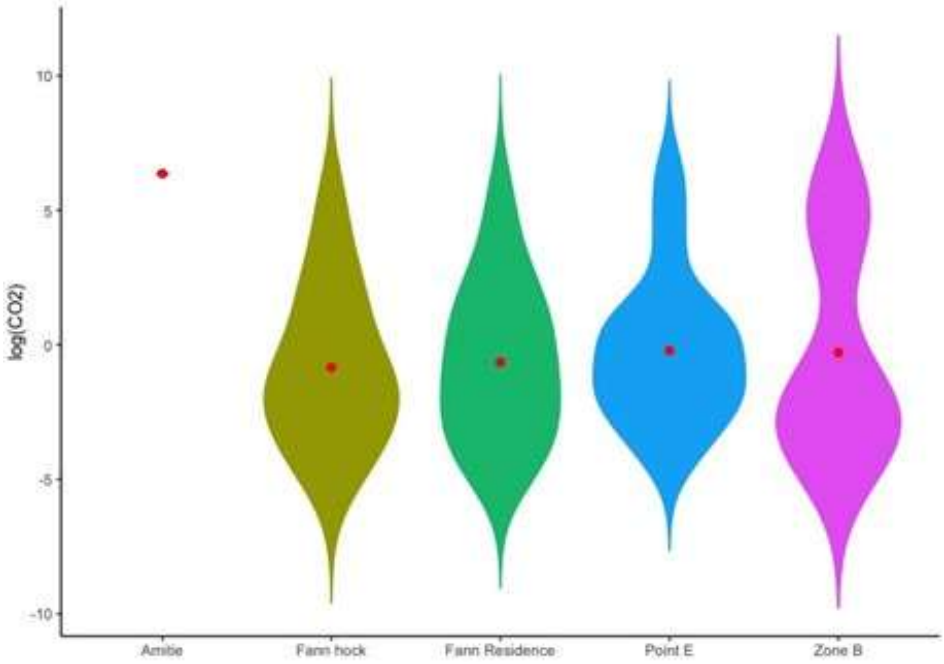

Figure 12. Carbon stocks variation among trees by district of the FPAM.
Source: Authors

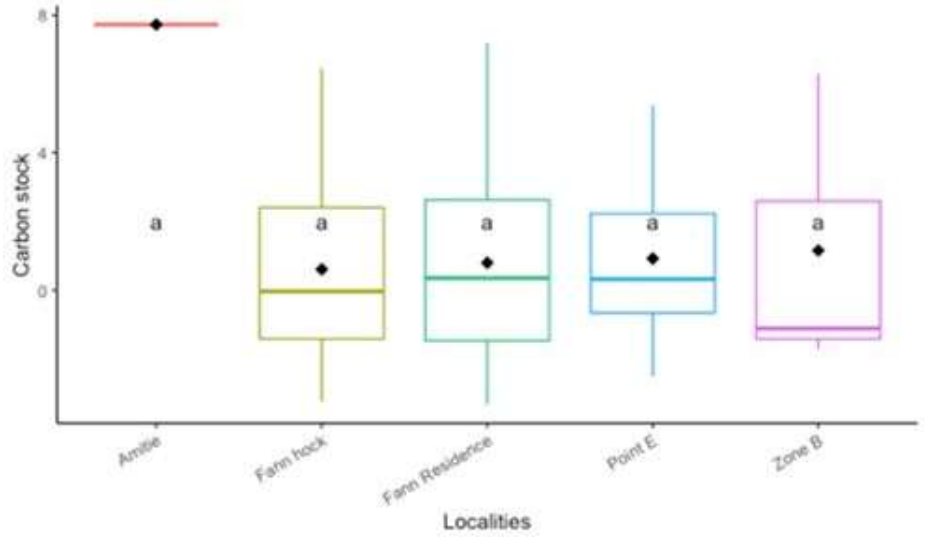

Figure 13. Carbon stocks variation by district of the FPAM.
Source: Authors

These different results show that cities, despite their high anthropisation, concentrate many species and can thus contribute to biodiversity conservation. This result was confirmed by Obrist et al. (2012) who showed that biodiversity was high in well-structured built environments with green spaces. Even if urbanisation remains a threat to biodiversity, the presence of green spaces in the form of trees and public gardens in the municipality is an asset for urban biodiversity. Moreover, according to Dejean et al. (2019), biodiversity in urban areas is an opportunity for climate change.

Carbon stocks of the vegetation in the commune of FPAM estimated by remote sensing were about 21.16 t of carbon. These values are different from those found by

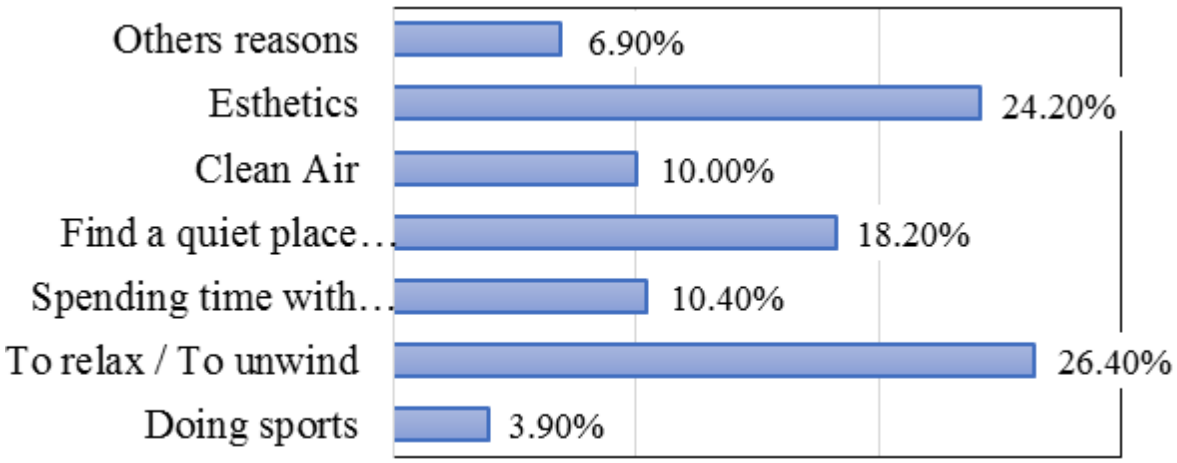

Figure 14. Main reasons for using the urban green spaces.
Source: Authors

Figure 15. People talking in the green spaces.
Source: Authors

Gomgnimbou et al. (2019) in the city of Bobo Dioulasso, ranging from 6.44 to 713.97 tha^{-1} depending on the green spaces. This could be explained by the use of different methodological approaches, notably the variation in spatial scale, given that these authors calculated the carbon stock for each green space, whereas this study is at the municipality scale. However, the results allowed us to deduce that the municipality had a significant carbon sequestration potential. The relatively high carbon stock values obtained with the allometric model were related to the presence of large-diameter trees in certain districts of the municipality. Thus, the perception of trees in the city must also be related to their carbon stock, especially in a context where climate change is a major issue in cities (Bolon et al., 2018). The absorption of CO_2, the main greenhouse gas, by trees makes it possible to partially offset carbon dioxide emissions in urban areas (Asselin, 2022). In cities, rapid urbanisation has also led to urban heat islands, which are a major manifestation of climate change. The effect of vegetation on heat islands is reflected in the atmosphere of part of the solar radiation and the projection of shade on the surrounding surfaces (Vergriete and Labrecque, 2007). Trees contribute to the reduction of greenhouse gases through carbon sequestration and help to cool the air. This lowering of temperatures is linked to shading and the phenomenon of evapotranspiration (Mehdi et al., 2017). Moreover, the leaf surface of plants represents a deposition zone for

dust and other smoke particles that are harmful to health (Mehdi et al., 2017).

The importance of green spaces in the FPAM for health and well-being was underlined by almost all respondents (97.9%). Various reasons for using these green spaces were mentioned, the main one being to relax and unwind. A similar study conducted in Yamoussoukro by Hervé et al. (2019) had revealed that the shading of green spaces was the main reason for their attraction. This is due to the fact that temperatures are high in these municipalities and people find coolness in the shade of trees. Sixty-nine per cent (69%) of respondents believed that green spaces played an important role in health and well-being. This can be explained by the many services provided by green spaces in enabling physical activity, relaxation, carbon sequestration, reducing heat islands and improving air quality. Thus, Pascal et al. (2019) considered that greening was a means of improving the health and quality of life of inhabitants. Observation revealed that urban green spaces, particularly public gardens, participated in the creation of social links. The same observation was also made by Blanc et al. (2012) in urban parks open to the public in the municipalities of Nantes and Angers (France). Furthermore, results revealed that the urban green spaces of the FPAM were privileged places for private meetings.

Most of the trees sampled were shade and ornamental trees that embellish the landscape of FPAM. This result is in agreement with the study of Qureshi et al. (2013) in Karachi (Pakistan) and Shackleton et al. (2015) in the settlements of Bele and Tzaneem (Limpopo Province in South Africa) who had demonstrated the contribution of green spaces accompanying roads in the decoration of cities. In addition to enhancing the urban landscape, one of the main benefits of urban trees is their contribution to health and well-being. They reduce concentrations of air pollutants and make the environment healthier (Codjo et al, 2022) as air pollution is implicated in many respiratory diseases (Robardet, 2021).

Urban green spaces are also associated with risks of insecurity linked mainly to the poor management of these facilities. About 23% of respondents perceived the presence of UGSs as places where banditry is prevalent, especially public spaces without night lighting, which are favourite places for alcoholics. In this sense, a student found in the public garden of Fann Hock said, *"I often come here to learn my lessons because it is the ideal place where I concentrate best, but there is insecurity at times and especially the noise generated by the alcoholics who frequent this place, which disturbs my concentration".* This is also the case in the study of the floristic diversity of urban plant formations by Sehoun et al. (2020) in the south of Benin, who also showed that the lack of night-time lighting in these areas leads to insecurity. Also, other problems related to the lack of maintenance, toilets and the transformation of these spaces into places of trade are as many nuisances in these public urban green spaces. In addition, green spaces are perceived as places where roaming dogs frequent and mosquitoes proliferate. That's why Bolon et al. (2018) have pointed out the health risks and challenges of urban greening, especially at the human-animal interface.

In view of all issues related to urban green spaces, their planning and management must be part of a holistic perspective, particularly through a One Health approach (Smos, 2020). Ecosystems degradation favour the emergence of diseases (zoonoses, infectious diseases) and numerous studies have confirmed the benefits of green spaces at all stages of life, both psychologically and sociologically as well as physiologically (Robardet et al., 2021; Vida, 2011; Kjell et al., 2019; Laurence, 2019).

Conclusion

Tree species richness and tree diversity in urban green spaces and their carbon stocks in Fann-Point E-Amitié municipality present interesting assets for conserving urban biodiversity and increasing the resilience of the Senegalese capital to climate change. In addition, this study has shown the attraction of green spaces and the importance that populations attach to them for various reasons, the main one being health. Given the many benefits and advantages, there is a need for greater awareness of the importance of urban green spaces in our individual and collective health. Furthermore, to meet the challenges of sustainable development in cities, it is necessary to reconsider the role of urban green spaces in strategies of biodiversity conservation, climate change and public health. Further the one health approach remains a promising way forward as trees in cities are favourable to health determinants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

An exploratory study of the impact of commingled biochar on removal of total petroleum hydrocarbon (TPH) from crude oil polluted soil

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An experimental scrutinization of bioremediation of crude oil polluted soil using furnace pyrolyzed comingled-biochar containing poultry litter, pine wood and rice straw char made at different proportions was carried out in the present study. The experiment was performed in five stages which include soil investigation, biochar production, characterization, and total petroleum hydrocarbon (TPH) remediation via green house. The purpose of this investigation is to evaluate the impact of biochar blend on TPH removal. The result showed that the efficiency of bioremediation was affected significantly by the acidic soil. Therefore, the commingled biochar seems promising in remediation of crude oil polluted soil and addition to soil nutrient. The highest TPH removal (46.74) was found in experimental run 12, which also had the highest level of independent variables (15 g of poultry litter-PL, 6 g of rice straw-RS, and 3 g of pine wood-PW char). This suggests that PL is more effective in the biochar mix than RS and PW. However, the efficiency of biochar-blended cleanup of soil varied depending on the biochar source and pyrolysis process as captured in the design of experiment using response surface methodology (RSM) *via* design expert. Biochar blend application to soils allows the development of microbial communities which are particularly important for nutrient cycling which leads to bio-stimulation enhancing the removal of TPH.

Key words: Biochar blend; furnace pyrolysis; bioremediation; crude oil polluted soil; experiment.

INTRODUCTION

Oil spills have occurred as a result of old infrastructure and poor maintenance on the side of industries (Vidal, 2014). According to the Nigerian government, 7,000 spills of crude oil took place between 1970 and 2000 (Vidal, 2014). Additionally, weathering can encourage soil pore blockage, which can lead to long-term consequences such soil death and decreased biota bioactivity and pollutant degradation (Lominchar et al., 2018). As a

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result, an efficient cleanup method is required to satisfy the needs of the impacted areas within the Niger-Delta Region (Amnesty International, 2009). Even though numerous methods for cleaning up soil contaminated by crude oil have been suggested, there is still a need for efficient, environmentally acceptable methods for removing hydrocarbons. Several researchers recommended bioremediation, which is an effective, affordable, and environmentally sound method (Wu et al., 2016). However, studies have shown that adding biochar to soil can be a successful technique for the simultaneous remediation, generation of bioenergy, long-term carbon sequestration, and enhancement of soil quality (Su et al., 2016). Previous research has shown that biochar has a stronger ability to lower total and bioavailable Polycyclic-Aromatic Hydrocarbons (PAHs) concentrations in multicomponent polluted soils than green waste compost (Beesley et al., 2010). For example, the total and bioavailable PAHs are reduced by 31.8 and 34.1%, respectively, in polluted soils amended with biochar (Gomez-Eyles et al., 2011).

However, the percentage of reduction varied based on the pyrolysis settings, feedstock type, particle size and application rate of the biochar, contact time, and soil and organic pollutant characteristics. According to several research, biochar can significantly lower freely dissolved PAH concentrations (Kumari et al., 2014; Oleszczuk et al., 2014b). In order to reduce PAH bioaccumulation in turnip, Khan et al. (2015) discovered that different types of biochar (5%) were most effective in the following order: peanut shell biochar (84%) > soybean straw biochar (70%) > rice straw biochar (55%) > sewage sludge biochar (36%). However, the removal processes are often governed by the interactions of these pollutants with different attributes of biochar (Tan et al., 2015). Kong et al. (2018) used sawdust and wheat straw biochar synthesized at 300 and 500°C for remediation petroleum polluted soil. Despite its potential benefits, biochar has not been utilized on a large scale yet as well as combination of different biomasses. The primary cause is related to variations in the material characteristics from the biomass sources such as surface area (SA), aromaticity, cation exchange capacity (CEC), pH, nutritional contents, and porosity (Spokas, 2010). Furthermore, the properties of biochar made from various pyrolysis processes and feedstock types vary significantly, which has an impact on bioremediation. In view of this, the methods to solve some of the difficulties faced by traditional pyrolysis were taken into consideration. For example, in continuous-mode, furnace pyrolyzer is a device that consists of a cylindrical oven that is maintained at a consistent temperature (Ndukwu and Horsfall, 2020). However, the advantage of furnace pyrolysis is the temperature uniformity with high efficiency and energy saving potential (Batista et al., 2018).

To the best of our knowledge, the use of comingled biochar for amendment of crude oil-polluted soil is a new application. Therefore, this article uses an experimental technique to look into the impact of a biochar blend from furnace pyrolysis on TPH removal from crude oil-polluted soil.

MATERIALS AND METHODS

Description of study area

A sample of crude oil polluted soil from the Niger Delta Region was collected at Kpuite in Tai Local Government Area (LGA) of Rivers State, Nigeria. Kpuite is a town located within latitude 4°43'37" N and longitude 7°17'4" E (Available from: https://dailypost.ng/2013/10/10/oil-spill-hits-ogoni-again/, accessed on June 21, 2022). Tai LGA has estimated population of 194,732 people, with the majority of residents belonging to the Ogoni ethnic group (Okon and Ogba, 2018). It has a total area of 159 km^2, an average temperature of 25°C with significant crude oil and natural gas deposits and is home to a number of oil mining companies. It is recognized for growing a variety of crops such as vegetables, bananas, plantains, and cassava, thus farming is thriving there as well. Figure 1 depicts a map of the research area.

Soil sampling and characterization

The crude oil polluted soil samples were collected using soil auger at a depth of 30 cm in Kpuite, homogenized and then poured into a sack bag. The physicochemical properties analyzed include pH, nitrogen, potassium, moisture content, temperature, electric conductivity, cation-exchange capacity and phosphorus content. In addition, the total petroleum hydrocarbon (TPH) and microbes present in the polluted soil were also determined.

Determination of soil pH

From the evenly mixed soil sample in the sack bag, 10 g sieved (5 mm) and air-dried soil was weighed and placed into a 50 ml beaker, followed by addition of 25 ml distilled water. The liquid was manually stirred with a glass rod for 30 min before being allowed to settle for 1 h. A pH meter with electrode (Kent EIL 7055) was placed in the polluted soil sample to determine the pH.

Determination of nitrogen

A 0.0001g of soil sample was placed into a Kjeldahl digestion flask. The weight of sample contained 0.1 *N* acid and between 14 – 56 mg of nitrogen. The sample weight taken was less than 2.2 g. To the flask 0.7 g of HgO was added with 25 mL of H_2SO_4 and 15 g of K_2SO_4. The flask was placed in an inclined position and gradually heated until frothing stopped, then quickly boiled, causing the condensate to develop around halfway up the flask's neck. The solution was boiled for 2 h until it became transparent, and then 50 mL of standard acid was added. The receiving flask was then filled with 6 drops of indicator solution. The absorbing mixture was cooled to room temperature before adding about 200mL of water and cooling the flask contents. The total nitrogen in soil was calculated from Equation 1:

$$\text{Total nitrogen (\%)} = \left(\left(\frac{(A - B) \times N \times 0.01401)}{C} \right) \times 100 \right) \quad (1)$$

Where A is the standard NaOH solution needed for blank titration (mL), B is the standard NaOH solution necessary for sample final

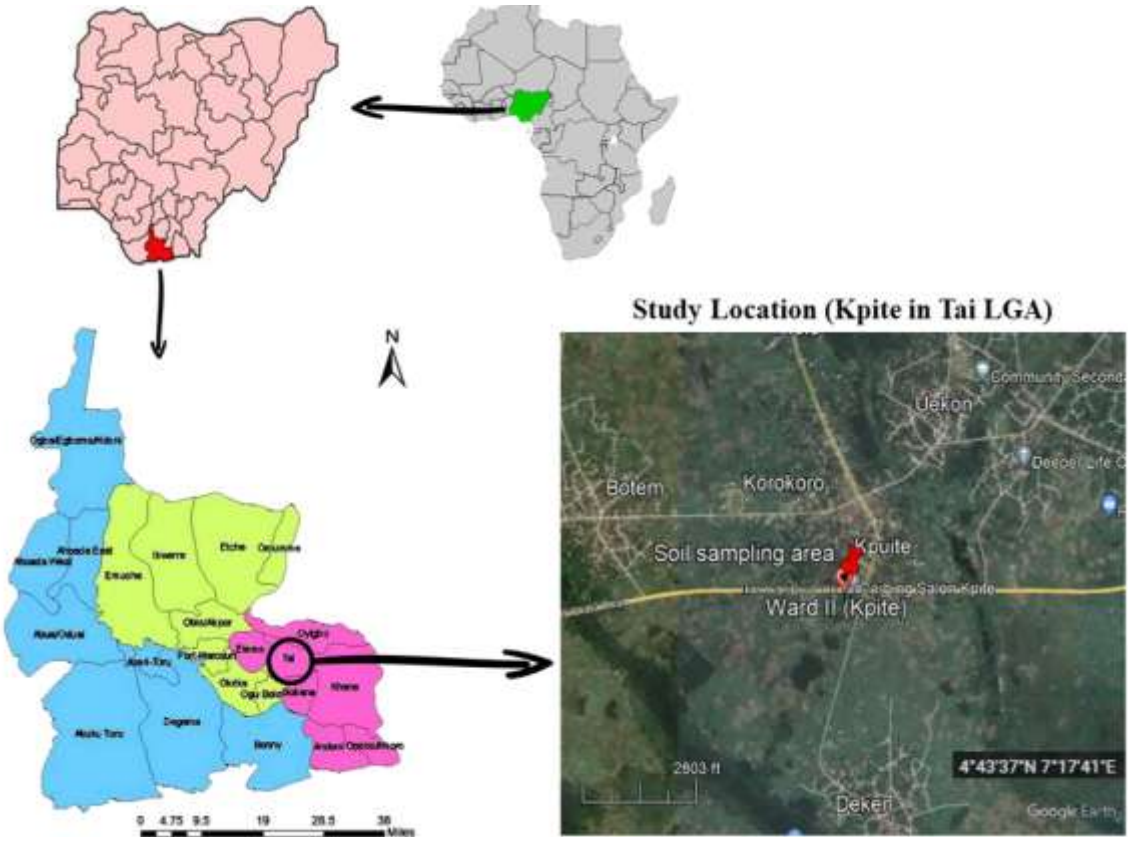

Figure 1. Map of study area showing the soil sampling point.
Source: Google Map, 2023

volume of around 10 mL. The material was chilled and diluted without being allowed to dry. Then the total phosphorus content was determined with acid wash water in wash tubes.

Determination potassium

A 125 mL Erlenmeyer flask was filled with a 2.5 g sample of air-dried polluted soil and then mixed with 50 mL of the 0.10 regular hydrochloric acid extraction solution before shaken automatically for 15 min. A 50 mL Erlenmeyer flask was used to fill the soil suspension after it has been filtered through Whatman No. 12 folded filter paper. Potassium concentration was measured in the soil solution extract without further dilution using a Beckman Model B Flame Spectrophotometer.

Then percentage transmittance of each potassium standard reference solution and the soil solution extract was measured. Referring to a calibration curve created by mapping the readings for percent transmittance against the potassium concentrations of the five reference solutions, the amount of potassium in the soil solution extract was calculated.

Determination of moisture content

The gravimetric method with oven drying was the technique used to determine the water content of the crude oil polluted soil. The soil sample was collected in a moisture-can and the wet weight recorded. It was then dried in an oven at 105°C for 24 to 48 h before being reweighed. The amount of water lost was then computed as a percentage of the dry soil's bulk.

Determination of Cation-Exchange-Capacity (CEC)

3 g of 1 mm air-dried soil sample was placed in a 250 ml Erlenmeyer flask, and 100 ml of 1 *N* NH_4OAC (pH = 7.0) solution was added. The flask was shaken thoroughly by hand and allowed to stand overnight (cover the flask mouth with parafilm). The mixture was filtered with light suction using a Bchner funnel and no. 2 filter paper and then poured into a clean flask. A small (25 ml) portion was added at a time. The presence of Ca^{2+} was checked, and the vacuum closed. Then the funnel was lifted carefully out of flask, 3 drops of filtrate was transferred from the funnel end into a test tube, with additional 3 drops 1 *N* NH_4Cl, 3 drops 1:1 NH_4OH, and 3 drops 10% ammonium oxalate. No precipitate indicates the completion of filtering.

The soil was filtered with light suction using 200 mL 1 *N* NH_4Cl followed by 100 mL 0.25 *N* NH_4Cl. In addition, it was washed with 200 ml of isopropyl alcohol, following a small (25 mL) portion at a time. Then 10 drops of the filtrate and 10 drops of 0.1 *N* $AgNO_3$ were added to a clean test tube. At a time when the chloride is no longer present, the collection flask was emptied and cleaned while the filtrate was discarded. In addition, the soil was filtered with 300 mL of 10% NaCl (in 6 portions). The filtrate was kept in a clean bottle for CEC determination. Then 20 mL of the filtrate was transferred into a microkjeldahl flask, a spoon of MgO powder was added. In addition, 40 mL of the solution was distilled and added into 5 ml of 2% H_3BO_3. Finally, the boric acid solution was titrated

with standard H_2SO_4 (0.01 *N*). The CEC of the soil was calculated from Equation 2:

$$\mathrm{CEC}\left(\text{in meq.}\frac{1}{100\text{g}}.\text{soil}\right) = \mathrm{V} \times 0.001\mathrm{N} \times \left(\frac{300\text{mL}}{20\text{mL}}\right) \times 100\text{g.}\left(\frac{1}{\mathrm{Ms}}\right) \quad (2)$$

where, V is the volume of 0.001 N H_2SO_4 spent for titration, in ml, 300 mL is the total volume of 10% NaCl, used to substitute the NH^{4+}, 20 mL is the volume of filtrate used for distillation and Ms is the weight of the soil sample used.

Determination of Total Petroleum Hydrocarbon (TPH)

TPH analyses are defined by the following methods of soil sample collection, extraction, cleanup, separation, and quantification:

Soil extraction

An amber glass bottle was filled with 10 g of soil sample. The soil sample in the glass bottle was also mixed with anhydrous sodium sulphate (Na2SO4) according to USEPA procedure 8015C. The sample was then swirled. Na2SO4 was added to the soil sample to draw out the moisture. The soil sample was given 300 g/ml of a surrogate (1-chlorooctadecane) standard. After adding 30 ml of dichloromethane (DCM) to the sample as an isolating solvent, the bottle carrying the soil sample was tightly corked and moved to a manual shaker. Details of this method can be found in the report of Alinnor and Nwachukwu (2013).

Soil cleanup

A glass column was used to clean the sample. As part of the column preparation process, glass cotton was placed into the column. DCM was used to dissolve silica gel, which was then added to the column as slurry. "After the addition of anhydrous Na2SO4, pentane was added to the column. In a beaker, a concentrated sample extract was mixed with cyclohexane and put onto the column. The sample extract was eluted with pentane, and the eluted material was collected in a beaker under the column. Following elution, the column was washed with DCM. The eluted sample was placed in a fume closet at room temperature overnight to allow for evaporation."

TPH separation and detection

TPH was extracted and identified in soil samples using an Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) equipment (USEPA method 8015C). The concentrated sample eluted from the column was then injected into the GC vial in the amount of 3 µl. The GC's micro-syringe was cleaned three times with blank DCM before taking the sample for analysis. After that, the sample was utilized to rinse the micro-syringe once more. The sample was then injected into the column to separate the chemicals in the sample. Following separation, the compounds were passed through a flame ionization detector. FID is used to identify the compounds in the sample. TPH concentration was determined in milligrams per kilogram using a particular chromatogram.

Determination of microbes present in soil

Total heterotrophic bacteria (THB): Microbial analysis of heterotrophic bacteria was accomplished by weighing ten grams (10 g) of crude oil polluted soil sample with an analytical balance (Metter weighing balance PB3002 Switzerland) and combining with 90 ml of sterile distilled water to form the stock suspension. A 10-fold dilution in stages of the soil sample was performed. 1 ml of the diluted sample was then plated on nutritional agar for bacteria and potato dextrose agar for fungi count using the pour plate method. Nutrient agar plates were incubated at 37°C for 24 h and PDA plates at 282^{o}C for 72 h. After incubation, discrete colonies of culture were counted on potato dextrose and nutrient agar plates and the unit was expressed in cfu/g.

Hydrocarbon utilizing fungi (HUF) and hydrocarbon utilizing bacteria (HUB)

Ten grams of soil samples (contaminated) was serially diluted in 90 ml of sterile distilled water. An aliquot portion (0.1 ml) from dilution 105 was inoculated on pre-sterilized surface dried nutrient agar medium and uniformly spread to obtain discrete and countable colonies (Cheesbrough, 2000). In the Bushnelli Haas agar (BHA) medium supplemented with crude oil, a comparable quantity of the 103 was inoculated (vapour phase method). The plates inoculated with the suspension from the dilutions were incubated at room temperature for 24 - 48 h hydrocarbon degrading bacteria and fungi.

The vapour-phase inoculation method was used for Bushnelli Haas Agar (BHA). The procedure involved covering the lid of the petri dish with sterile filter paper that had been moistened with crude oil. The colonies were counted after incubation using standard methods (Cheesbrough, 2000).

Determination of soil temperature

A thermocouple temperature probe was used to determine the temperature of the soil sample.

Determination of Electric Conductivity (EC)

This experiment was carried out using a two-electrode conductivity meter. A 100 g of the soil sample was measured into a beaker and the conductivity meter was turned on. The electrode of the conductivity meter was immersed into the sample. The reading of the conductivity was displayed on the screen of the conductivity meter and the result was recorded. The electrode was then removed from the beaker containing the sample and rinsed with distilled water. This same procedure was repeated for other samples collected and the results recorded.

Soil textural analysis

A 50 g of crude oil polluted soil sample was measured, dried at room temperature, grounded with wooden roller and sieved through 2 mm mesh. The particle size distribution was ascertained by using Bouyoucos hydrometer method according to Gee and Bauder (1986) and Okon and Ogba (2018).

Procedure for biochar production

Collection of biomasses

Pine wood (PW) were obtained from the Timber Saw-Mill at Mile 2 Diobu, Port Harcourt, poultry litter (PL) was collected from a poultry farm at Igwuruta, Rivers State, while rice straw (RS) was collected from a rice farm at Abakaliki, Ebony State all in Nigeria. The feedstocks were collected at different locations due to availability

Figure 2. The biomass samples (a) pine wood (b) poultry litter and (c) rice straw.
Source: Authors

and accessibility. The feedstocks (PW), (RS) and (PL) were washed and dried separately at 60°C for 24 h to fully remove the water. RS and PL were pulverized to pass through a 10-mesh (2 mm) sieve in order to reduce their initial particle size while PW feedstock was hammer-milled and pelletized to approximately 6 mm at a wood processing plant. PW treatments were converted into cylindrical pellets by removing the pure components and total moisture content with deionized H_2O and pelletizing with a pellet mill fitted with a 6-mm die and roller set. The processed feedstocks were then separated and stored in separate bins and the biomass sample is presented in Figure 2.

Furnace assisted pyrolysis of biomass

The biochar samples were created from various feedstocks at pyrolysis temperatures ranging from 400 to 500℃ and at different residence time using an Electric Vulcan furnace A130 model with a gas expeller (rice straw (RS) = 400℃ for 1 h., poultry litter (PL) = 500℃ for 1 h 30 min, pine wood pellets (PW) = 500℃ for 2 h). Furnace assisted pyrolysis was selected due to some advantages such as temperature uniformity and high efficiency and energy saving (Allyson, 2011).

The function of the air expeller is to remove flue gases during pyrolysis. The feedstock was stored in a stainless-steel tube reactor that was tightly closed using the closing caps on both ends. The closing cap features a gas intake on one end and a vent for pyrolytic fumes on the other. The electric furnace was set to the specified temperature during pyrolysis. After completion of the residence time, the pyrolyzed samples were cooled down to room temperature, stored and labeled as RS-BC-400, PL-BC-500, and PW-BC-500 according to pyrolysis temperature and residence time. The pyrolysis experiment was done in batches since the furnace is very small. After pyrolysis, the PL and RS biochars were passed through a 2-mm screen, and the material that remained on the sieve is known as "pellets." A portion of the pellets were ground such that they could pass through a 0.42-mm sieve; this particle size is known as "dust." Pyrolysis residence time was between 1 to 2 h for the selected feedstock. In order to characterize, biochars were ground to <0.30 mm. The electric furnace used for the pyrolysis is shown in Figure 3. The sample of biochar produced from the Vulcan furnace is presented in Figure 4.

Biochar characterization

After shaking for 2 h at 200 rpm, the pH of each ground biochar sample was determined using a 1:2 (v/v) biochar/deionized H2O mixture. The ash, C, and N contents of biochars on an oven dry weight basis was assessed at Giolee Global Resources Laboratory at Stadium Road, Port Harcourt, Rivers State, Nigeria utilizing the American Society for Testing and Materials (ASTM) D1372 and 3176 standard combustion procedures (ASTM, 2006). The P and K contents were measured on an oven dry weight basis using Environmental Protection Agency (EPA) method 3052, an oven-assisted acid digestion procedure (US EPA, 1996), and quantified using an inductively coupled plasma mass spectrometer, as described by Novak et al. (2009).

Bioremediation experiment design

The bioremediation experiment was designed using Box-Behnken Design (BBD) applying response surface methodology (RSM) in Design-Expert 13. RSM is a popular method for studying a process in which the response of interest is affected by different variables, with the goal of optimizing the response (Olatunji et al., 2021). It is a helpful tool because it allows for the evaluation of the effects of several factors and their interactions on one or more response variables (Olatunji et al., 2022). The parameters that affect the process (Poultry litter biochar, Rice straw biochar and Pine wood biochar all measured in grams) are called the independent variables, while the response (TPH) is called dependent variable. The BBD technique in RSM was used in the design due to the number of independent variables. This technique accepts a minimum of three independent variables, which in this study are pine wood biochar, poultry dropping biochar and rice straw biochar. The BBD technique is known for very high level of accuracy when used for predictions. In adopting RSM, selection of contributing parameters, their levels and proper experimental design are essential. RSM is a collection of methods for building empirical studies of the connections involving a response and a number of input parameters.

Since PL char, RS biochar, and PW biochar (g) are all quantifiable independent variables. It is presumptive that the independent variables are continuous and subject to minor experimental error. Usually a second-order model is utilized to find a suitable approximation for the functional relationship between independent variables and the response surface (Olatunji et al., 2021b). This is expressed in Equation 3:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^{\,2} + \sum_{i} \sum_{j} \beta_{ij} X_i X_j + \varepsilon \quad (3)$$

Where: ε is the random error.

The experimental range and design values are presented in Tables 1 and 2.

Bioremediation of crude oil polluted soil

400 g of crude oil polluted sample was placed in different plastic bottles, labelled 1 to 17 and control. Biochars (PW, PL and RS),

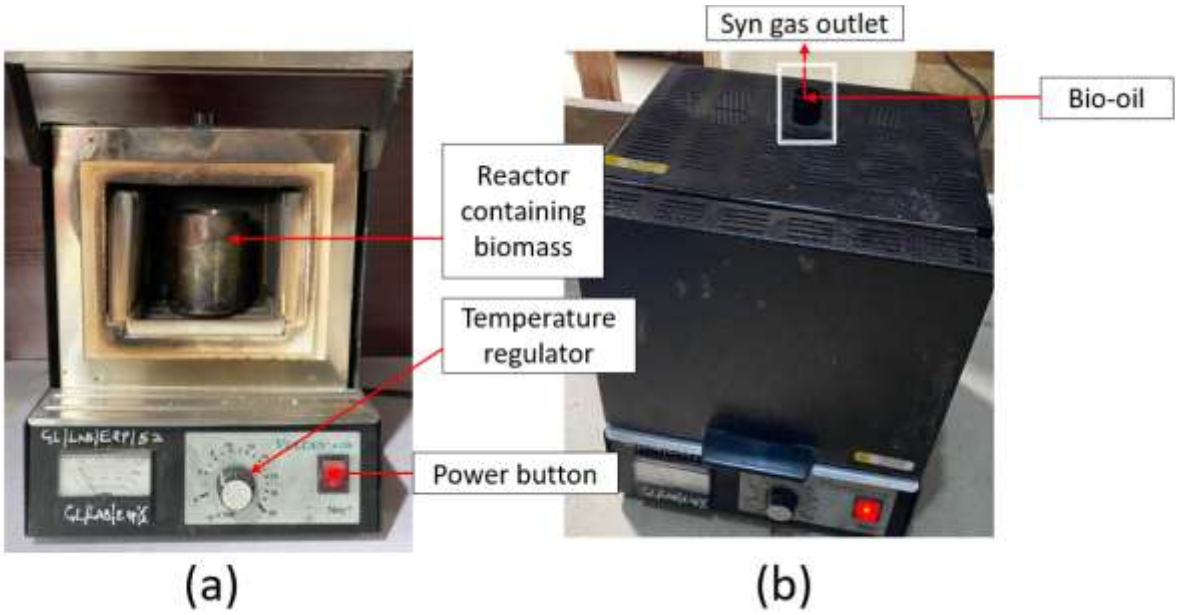

Figure 3. The "Vulcan Furnace A-130" used for biomass pyrolysis (a) open (b) closed.
Source: Authors

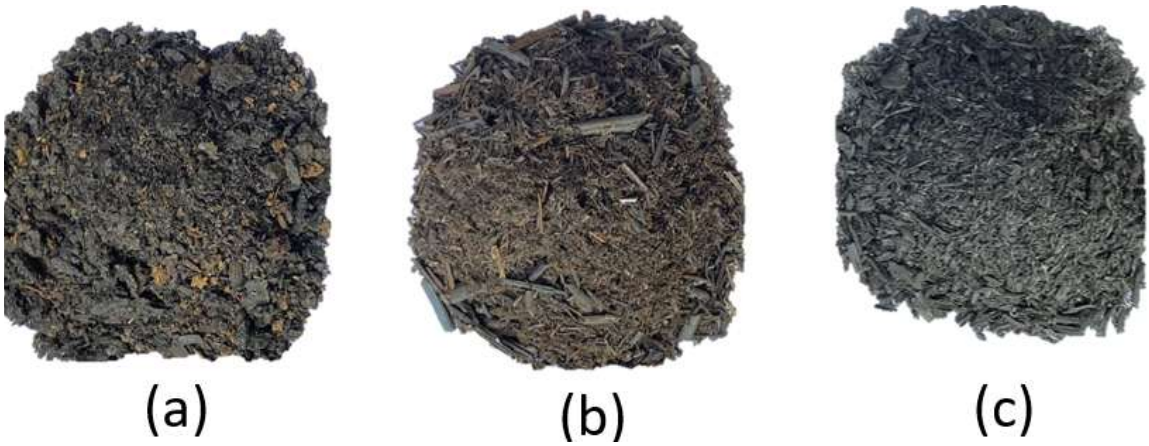

Figure 4. Biochar produced (a) poultry litter (b) rice straw and (c) pine wood.
Source: Authors

were added to each of the crude oil polluted soil in appropriate proportions as indicated on RSM experimental design. The soil was mixed twice weekly to provide sufficient aeration and moistened by the addition of water twice every week to adjust the water holding capacity throughout the experimental period. In the control sample water was also added twice a week except biochar according to Agarry and Ogunleye (2012a).

The plastic bottles were then incubated at room temperature (varied from 27 to 33^{o}C) and kept in a wooden greenhouse made with dimensions; Length =140 cm, Width =55 cm, and Depth =110 cm as shown in Figure 3. Each plastic bottle (diameter = 15 cm and height = 8 cm) was labelled (SS+BCB) and the mixture was rigorously stirred to ensure nutrients and bacteria homogeneity. In order to keep the soil salinity at tolerable levels, plastic saucers were used to prevent loss of water from beneath the bottle. Each bottle's crude oil-contaminated soil was added with varying quantities of PL (5 - 15g), RS (2 - 6g), and PW (2 - 4g) as specified on the RSM experimental design (Table 1). The range was selected according to the reports of Agarry and Ogunleye (2012b). In addition, 400g of the crude oil polluted soil was collected from the homogenized portion and used as control sample. In the control sample biochar was not added. The green house used for the experiment is presented in Figure 5.

In total, 17 microcosms and a control sample was set-up and left to bioremediate for 30 days. All microcosms were mixed manually twice per week to enhance oxygenation and kept moist during the 30-day experimental period.

Efficiency of crude oil removal was assessed after 30-days by measuring the total petroleum hydrocarbon content (TPH) of remediated soil.

Table 1. Variable levels and the experimental limit.

Factors	High level	Medium level	Low level
Poultry litter, grams [A]	15	10	5
Rice straw, grams [B]	6	4	2
Pinewood, grams [C]	4	3	2

Source: Authors

Table 2. Full-factorial BBD for the three independent variables.

Experimental Runs	Poultry litter, grams [A]	Rice straw, grams [B]	Pinewood, grams [C]
1	10	2	2
2	15	2	3
3	10	6	2
4	10	4	3
5	10	4	3
6	15	4	4
7	5	6	3
8	10	4	3
9	10	6	4
10	5	2	3
11	10	4	3
12	15	6	3
13	10	4	3
14	5	4	2
15	10	2	4
16	15	4	2
17	5	4	4
Control	-	-	-

Source: Authors

Total Petroleum Hydrocarbon (TPH) analysis

After the bioremediation period of 30 days, 20 grams of each soil sample was obtained from the bulk mixture and dried at room temperature for 72 h for TPH analysis using FLUORAT-02 analyzer via fluorometric method. The extraction solvent for the TPH was hexane and 460 nm-wavelength absorbance measurements were made with a UV-visible spectrophotometer. 5 g of the soil was placed in 200 ml beaker and 150 ml of toluene was added. The mixture was stirred continuously for 30 min, left to stand in a fume cupboard for 2 h and then filtered using Whatman No 42-filter paper. The residue, (soil), was allowed to dry in an oven at 50°C. TPH concentrations were then measured from a calibration curve prepared by plotting measured absorbance at different initial concentrations of TPH against each other. The percentage of total petroleum hydrocarbons degraded was measured after 30 days using Equation 5:

$$\%\text{TPH removal} = \frac{C_0 - C_f}{C_0} \times 100$$

Where C_0 = initial TPH concentration in soil (g/Kg) and C_f = final concentration of TPH in bioremediated soil (g/Kg).

RESULTS AND DISCUSSION

Physicochemical properties of soil

The results of the physicochemical properties are presented in Tables 3 and 4. From the results, it was observed that the electrical conductivity (EC) value is 73.4 µS/cm. This indicates that the polluted soil is non-saline, as the electrical conductivity (EC) is below 4000 µS/cm (Miller and Donahue, 1995) and does not exceed the critical value of 2000 µS/cm (Miller and Donahue, 1995; Okon and Ogba, 2018). This indicates that the soil does not have salinity problem prior to remediation. Qin et al. (2012) suggested that salinity had great impact on bioremediation of petroleum hydrocarbon. High salinity suppresses the growth of microbes which is capable of limiting the rate of biodegradation (Ebadi et al., 2018).

Figure 5. Greenhouse used for bioremediation experiment.
Source: Authors

Table 3. Physical and chemical features of soil contaminated by crude oil.

Parameter	Value
pH	4.720
Temperature (℃)	28.50
Moisture Content (%)	21.49
Electrical Conductivity ($\mu S/cm$)	73.40
Cation Exchange Capacity (meq/100 g)	6.200
Total Nitrogen (%)	0.690
Potassium (%)	0.279
Total phosphorus (%)	21.02

Source: Authors

Table 4. Soil textural classification prior to remediation.

Parameter	Value (%)
Sand	62.81
Silt	20.59
Clay	16.60

Source: Authors

Despite this, the most readily available type of nitrogen in soil is ammonium. However, as shown in Table 3, the total nitrogen concentration of the polluted soil sample is 0.690%. When compared to the medium range of 0.10 to 0.45% (Brady and Weil, 1996) for soils in the research area, this figure is considered high. The crude oil polluted soil had an acidic pH (4.72), a temperature of 28.5 (°C), a moisture content of 21.49 (%), a cation exchange capacity of 6.2 (meq/100g), and potassium, total nitrogen, and available phosphorus concentrations of 0.279, 0.69, and 21%, indicating that soil nutrition was deficient and imbalanced. The texture of the sand, silt, and clay fractions varied in the study area.

However, the texture of the soil determines how much water can be held in it, how readily it can be tilled, how much aeration it receives, and how fertile it is (FPDD, 1990). Sand generated on loosely consolidated coastal plain sand and sandstones contributes to the soil's high sand content. But the crude oil spill's influence which increased substantially the percentage of sand had a negative impact on the soil quality in the impacted areas. This is due to possible high oil drainage into the lower horizon of the soil, which results in an aeration issue as oil accumulates in the air pores and obstructs the easy movement of nutrients to the soil.

Bacteria and fungi in soil degrading hydrocarbons prior to remediation

Table 5 displays the findings of the total petroleum hydrocarbon-degrading bacteria and fungi in the crude oil-polluted sample. It was discovered that complete heterotrophic and hydrocarbon-using bacteria available are 5.8 (10^3 cfu/g) and 5.7 (10^2 cfu/g) respectively. This

Table 5. Soil microbial analysis.

Microbe	Value
Total Heterotrophic Bacteria (10^3cfu/g)	5.8
Total Heterotrophic Fungi Count (10^2cfu/g)	1.3
Hydrocarbon Utilizing Bacteria (10^2cfu/g)	5.7
Hydrocarbon Utilizing Fungi (10^2cfu/g)	0.3

Source: Authors

indicates a promising bioremediation process if bacteria are stimulated by biochar. However, hydrocarbon utilizing fungi are between 0.3 - 1.3 (10^2 cfu/g). The variation in cfu/g from 0.3 to 5.8 cfu/g of the different species indicates a higher content of hydrocarbon utilizing bacteria (HUF) and can be attributed to the high level of moisture in the polluted soil.

Total heterotrophic and hydrocarbon utilizing bacteria are 5.8 (10^3 cfu/g) and 5.7 (10^2 cfu/g) respectively (Table 5). This indicates a promising bioremediation process if bacteria are stimulated by biochar. However, hydrocarbon utilizing fungi are between 0.3 and 1.3 (10^2 cfu/g).

Soil total petroleum hydrocarbon (TPH) content

The initial TPH concentration of the crude oil polluted soil is given as 1405 mg/kg. This value indicates a significant level of pollution in the study area.

Physicochemical characteristics of biomass

The results of the physicochemical analysis of raw biomass before pyrolysis are presented in Table 6. It was observed that the initial pH and moisture content of pinewood (PW) char was 3.53 and 12.33 (%) respectively. This shows that PW biomass is acidic which may not be favorable for bioremediation of acidic soil with pH. Also, PL had acidic pH of 4.92 while rice straw (RS) has a higher pH of 7.22. However, the pH of PW and PL are expected to increase after pyrolysis and also considering the blend biochar from poultry litter and rice straw biomasses (Figure 6).

It was observed that the pH of pine wood increased from 3.53 to 3.6, still indicating an acidic condition, while the pH of poultry litter increased significantly from 4.92 to 7.16, and the pH of rice straw char increased from 7.22 to 8.23. Consequently, the pH of the biochar is now favorable for bioremediation. However, their nitrogen is still low ranging from 0.12 to 0.15% for all three biochars. The ash content of pine wood, rice straw and poultry litter char were 18.96, 5.48 and 25.6% respectively. This shows that the physicochemical properties of biochar are affected by pyrolysis temperature, and residence time. According to Chatterjee et al. (2020), increasing the pyrolysis temperature led to greater C and ash contents, lower N contents, and higher pore volume and micro surface area.

Bioremediation experimental result

The result of a 30-day bioremediation experiment carried out in a wood green house with different biochar blend making 17 experimental runs and 1 control are presented in Table 6. The experimental analysis was based on the reduction of TPH content of the polluted soil and pH. A significant reduction of TPH was observed for all the different proportions of blended biochar after the 30-days period. However, pH of all the different proportions was within the range of 6.26 to 6.91.

The interaction effect of the commingled biochar on TPH removal

In Figure 7a, the 2D contour and 3D surface plot shows the effect of interaction between PL char and RS char (g). This plot demonstrates that both PL char and RS char have positive mutual impact on the biodegradation of TPH. Similarly, in Figure 7b, the contour plot indicates that mutual but negative effect of TPH removal. However, at a fixed weight (g) of RS char, it was observed that increase in weight (g) of PL biochar resulted in higher TPH degradation. This is an indication that PL biochar had a significant and positive impact on TPH removal more than RS char, whereas PW biochar resulted in negative effect due it its physicochemical properties such as low pH (3.60). Although, PL and RS biochar had higher pH values 7.16 and 8.23 which caused the significant and positive effect in the remediation process.
In Figure 7c, the 2D contour plot illustrates the interaction effect between PW char and RS char (g) on the degradation process. The plot indicates a negative effect without mutual influence between PW and RS char on the degradation process. Moreover, as PW char increases, it is observed that the decrease in the weight (g) of RS char leads to lower TPH reductions. It was revealed that the highest TPH (46.74) removal occurred in experimental run 12 which shows the highest level of independent variables such as 15 g of PL, 6 g of RS and 3 g of PW char respectively. This indicates that PL in the biochar mix is more effective than RS and PW.

Ducey et al. (2015) utilized feedstocks in four ratios (100% pine chip; 80:20 mixture of pine chip to poultry litter; 50:50 mixture of pine chip to poultry litter; 100% poultry litter) prior to pyrolysis and soil amendment as a biochar product. Their results demonstrated significant shifts in microbial community composition in response to biochar amendment, the effects of which were greatest with 100% poultry litter biochar. This agrees with the

Table 6. The independent variables and residual TPH after 30-days bioremediation period.

Experimental runs	PL biochar (g)	RS biochar (g)	PW biochar (g)	TPH (%)
1	10	2	2	39.90
2	15	2	3	43.93
3	10	6	2	38.07
4	10	4	3	35.55
5	10	4	3	35.30
6	15	4	4	42.77
7	5	6	3	35.44
8	10	4	3	34.56
9	10	6	4	36.04
10	5	2	3	33.25
11	10	4	3	39.33
12	15	6	3	46.74
13	10	4	3	41.01
14	5	4	2	26.38
15	10	2	4	32.17
16	15	4	2	38.59
17	5	4	4	27.91
Control	-	-	-	*11.30*

Source: Authors

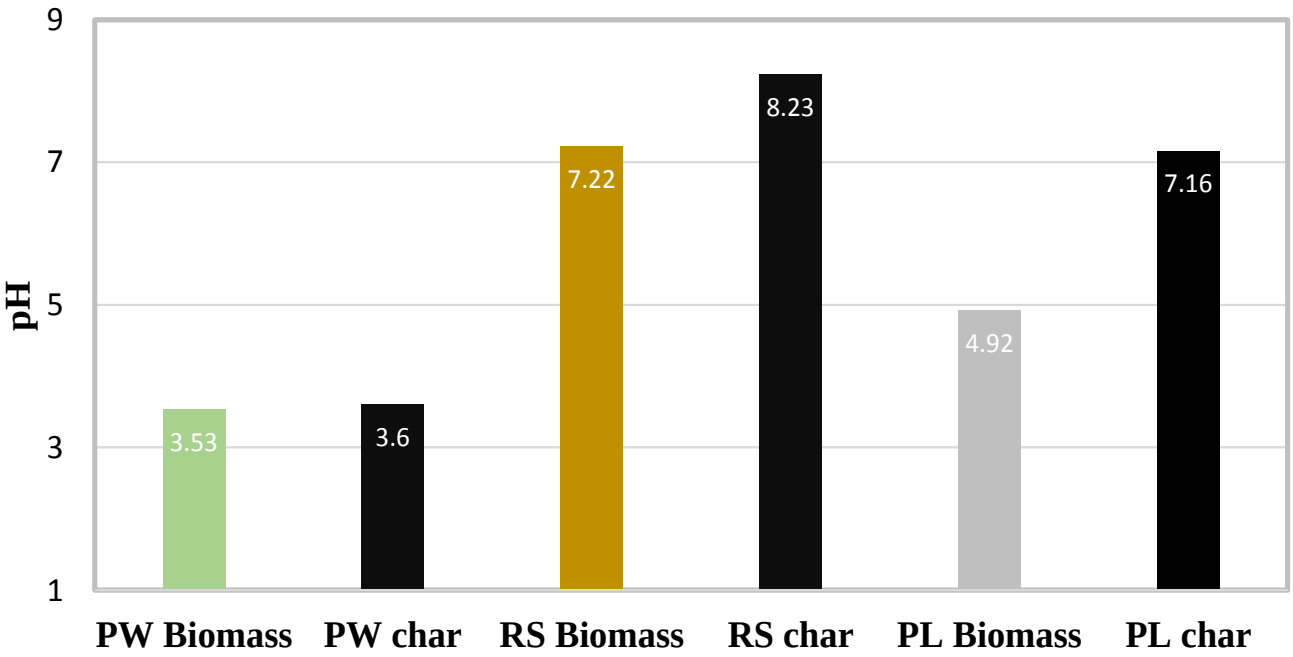

Figure 6. Variation pH of biomasses and biochar produced via furnace assisted pyrolysis.
Source: Authors

findings of this research work were soil sample remediated with high amounts of PL char showed high % TPH removal (Table 6). Saeed et al. (2021) found that the soil analysis showed a crude oil degradation efficiency of 34% for biochar derived from a single biomass.

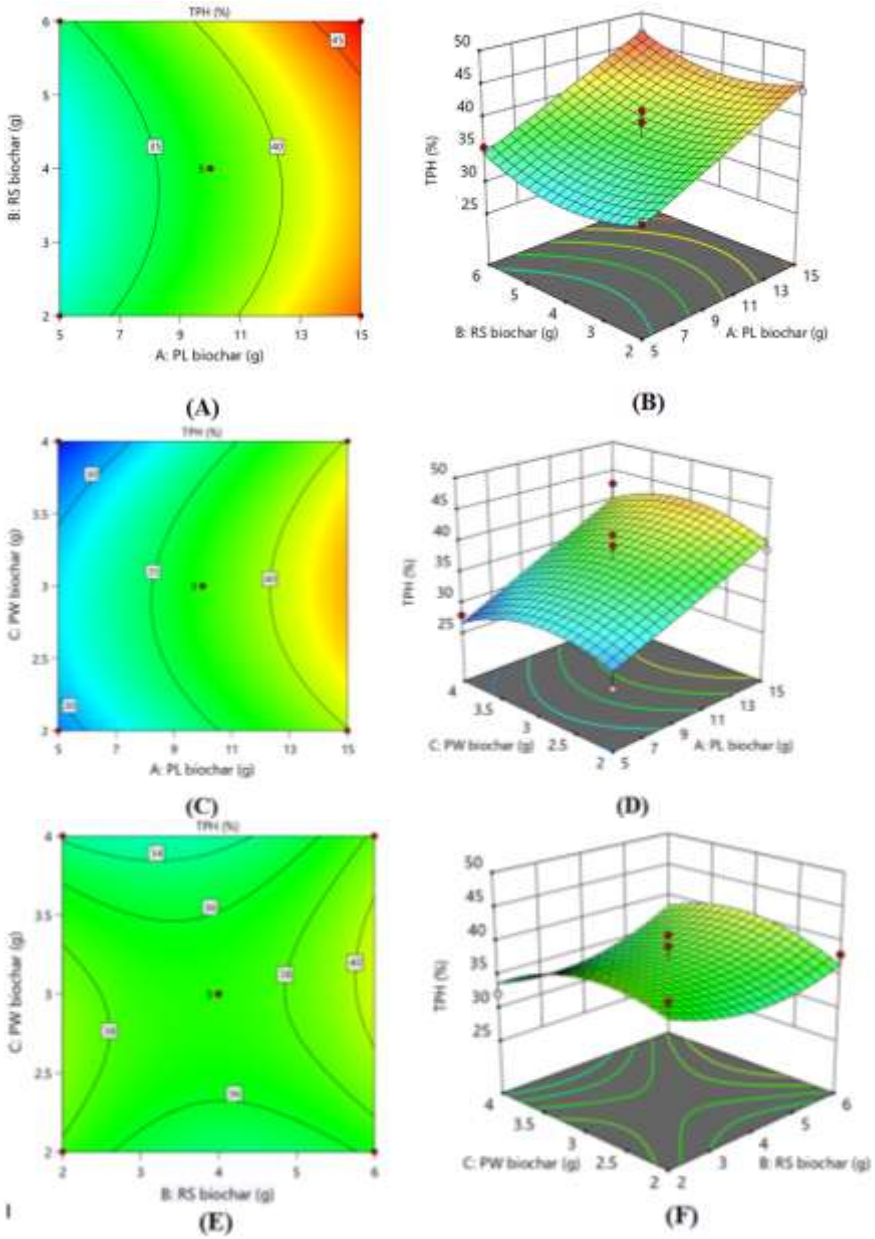

Figure 7. 2D contour and 3D surface plot (A – B) effect of PL biochar and RS biochar (C – D) effect of PL biochar and PW biochar (E – F) effect of RS biochar and PW biochar on TPH degradation.
Source: Authors

Therefore, the biochar blend seems promising for the remediation of crude oil-polluted soil and as an addition to soil nutrients. The effectiveness of biochar-facilitated soil remediation was case specific, changing with the biochar source, amendment rate as captured in the design of experiment using response surface methodology (RSM) *via* design expert. Biochar blend application to soils allows the development of microbial communities (that is, mycorrhizal fungi) which are particularly important for nutrient cycling (Lambers et al., 2008) which leads to bio-stimulation enhancing the removal of TPH.

Biochar's toxicity to the environment and human health

While biochar is made from bio-based materials, it is essential to note that hazardous chemicals from feedstock or created products during pyrolysis still exist

and pose environmental concerns (Kusmierz and Oleszczuk, 2014a). Organic fractions such as polyaromatic hydrocarbons and volatile organic chemicals, as well as inorganic heavy metal fractions and persistent free radicals, are among the harmful substances (Zheng et al., 2018). As a result, this section highlights the significance of assessing biochar risk evaluation from the perspectives of the pyrolysis process, feedstock, and potential dangers in biochar handling, as well as the methods utilized in risk evaluation. The chemical, physical, and structural properties of biochar are influenced by feedstock parameters and pyrolysis time and temperature, which are critical in understanding biochar functionality. However, biochar use has been associated to several soil application concerns, such as biochar being poisonous, enabling greenhouse gas emissions, reducing pesticide efficiency, and affecting soil bacteria (Ndirangu et al., 2019). Potential hazards result from pyrolysis conditions promoting the formation of certain traits and functional groups as well as contaminated feedstock. Through the food chain, these created hazardous chemicals are a threat to human health. Ndirangu et al. (2019) asserts that determining the toxicity levels is the first stage in the risk management of hazardous biochar; however, this step was not completed in the current study due to financial and time restraints. However, it can be taken into account in future research for evaluating the biochar blend's toxicity.

Proposed approach to promote the recovery of soils attacked by hydrocarbons

Due to its wide availability of the requisite biomass, sustainability, cost-effectiveness, high efficiency, significant internal surface area, and ideal physicochemical qualities such as pH, nutrient, etc., biochar mix has shown a good potential to treat crude oil-polluted soil. However, utilizing biochar blends may increase soil fertility, while reducing TPH level and recycle agricultural waste (Zahed et al., 2021). The usage of biochar blended from various feedstocks is still relatively new, nevertheless. Since the biochar blend would have a balance in the mixture of the properties (for example, the pH of RS char is 8.23, PW char is 3.6, and PL char 7.16) it has been proved to be successful in the remediation of TPH from acidic soil (pH = 4.72). The goal is to increase the remediation efficiency. As a result, the biochar blend evaluated in this study is strongly recommended to aid in the recovery of soils impacted by hydrocarbons.

Conclusion

The remediation of soil with biochar-blend has been demonstrated to have an effect not only on the soil physicochemical properties such as pH, but also in the removal TPH at a fast rate. The blend (PW, PL and RS biochar) is suitable for acidic soil. This comes in the form of increased soil aggregates with concomitantly increased water retention capabilities, improved soil pH levels, as well as increased available nutrients. It was observed that PW char did not perform very well due its low pH (3.6). In further studies it can be removed in the biochar blend, while considering more of PL and RS char.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of acid pre-treatment and two-stage oxygen-assisted fermentation on the production of vinegar from lignocellulose biomass peel of pineapple

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The valorization of lignocellulosic waste stands as a promising avenue to bolster sustainable food production and consumption within a circular economy framework. This study centered on the production of vinegar from pineapple peels through a two-stage fermentation process aided by oxygen. The pineapple peels underwent sorting, washing, drying, and subsequent grinding into a powder. This powder was subjected to hydrolysis using dilute sulphuric acid, followed by primary alcoholic fermentation utilizing Saccharomyces cerevisiae. The resulting fermented must was then subjected to oxidation in a second stage, facilitated by Acetobacter aceti, with varying concentrations of oxygen. A central composite design involving three factors, fermentation time, bacteria inoculum, and oxygen was employed to investigate the impact of these process parameters on the physicochemical attributes (pH, specific gravity, total soluble solids, titratable acidity) and the ferric reducing antioxidant power (FRAP) of the produced vinegar. The acid hydrolysis phase led to a notable rise in total soluble sugars (6 to 11.5 oBrix) and glucose concentration (300 to 580 mg/dL). Primary fermentation resulted in significant reductions in pH (7.02 to 5.38), total soluble solids (11.5 to 6 oBrix), and glucose concentration (580 to 62 mg/dL), accompanied by marked increases in titratable acidity (g/100 ml) and alcohol content (0.6 to 7%). The volume of oxygen demonstrated significant effects on acetic acid content, pH, and specific gravity, with the highest values (4.68 g/100 ml, 4.02, and 1.004, respectively) achieved at the maximum oxygen volume of 100 ml. The FRAP values ranged from 16.7 to 24.97 mg Fe2+ / mg, with the sample lacking oxygen displaying the highest FRAP. Furthermore, fermentation time and bacteria inoculum exerted significant effects on acetic acid content, with an optimal value of 4.43 g/100 ml. Interaction between bacteria inoculum, oxygen volume, and fermentation time also had significant effects on specific gravity.

Key words: Lignocellulose waste-biomass, Pineapple peel, Acid Pre-treatment, Two-stage Fermentation, Vinegar.

INTRODUCTION

The growing concern over sustainable food waste management has gained momentum, particularly in the context of the UN Sustainable Development Goals (UN-SDG). The integration of effective waste management practices, responsible production, and consumption aligns with achieving the objectives of the UN-SDG

framework (Rodić and Wilson, 2017; Lemaire and Limbourg, 2019; Pujara et al., 2019). This convergence highlights the potential for linking waste management concepts with the promotion of good food waste practices. Recent efforts have been dedicated to converting agro-food waste into valuable products, driven by the principles of sustainability (Cheok et al., 2016; Ong et al., 2018; Sindhu et al., 2019; Rico et al., 2020). Lignocellulosic waste-biomass, such as the one discussed in this study, holds promise as an economically viable and readily accessible renewable bioresource, capable of yielding useful bio-products (Bhatia et al., 2020). Among the bio-products derived from lignocellulose waste, vinegar has been successfully produced through fermentation, utilizing various lignocellulosic sources including fruit peels (Roda et al., 2014), which are abundantly consumed worldwide. Consequently, a substantial volume of fruit waste, particularly fruit peels, accumulates (Su et al., 2016). Pineapple (*Ananas comosus*) and other fruit peels, recognized as common fruit waste in Cameroon, illustrate this pattern. Approximately 80% of pineapple components, including peels, crowns, leaves, core, and stems, are discarded during processing, transportation, and storage, ultimately becoming waste (Roda and Lambri, 2019; Zainal Alam et al., 2020). Studies reveal that pineapple peels contain sugars that, although not readily accessible (Roda et al., 2016), can be converted into fermentable forms for producing ethanol and other bio-products (Lucarini et al., 2021) with appropriate pre-treatment. This underscores the potential for transforming waste materials into valuable resources, aligning with sustainability goals and addressing the challenges of food waste.

The main compounds found in lignocellulose biomass are cellulose (35-50%), hemicelluloses (25-30%), and lignin (25-30%) (Anwar et al., 2014). The cellulose and hemicellulose are densely packed in layers of lignin, which hinders the biological digestibility of the cellulose present in lignocellulose biomass (Mosier et al., 2005). This has made the hydrolysis process rate-limiting thereby requiring prior biomass pre-treatment in order to disrupt the lignin layers, thereby exposing the cellulose and hemicellulose, and therefore increases the yield of sugars (Mosier et al., 2005; Kumar et al., 2009).

Acids have been used in biomass pre-treatment with some improvements in the digestion performance. The most common acid in this process has been sulphuric acid, which have been documented to be very potent in the total removal of the hemicellulose component of corn stalk, whole corn stalk, Sorghum stalk, grasses and other lignocelluloses after pre-treatment (Di Cai et al., 2016; Yangyang et al., 2017; Xiaoling et al., 2018). Many raw materials have been use in the production of vinegar, but pineapple peels are promising as a renewable fermentation substrate due to their high sugar contents, up to 8.92% (Ali et al., 2020). Production of vinegar is mostly carried at home scale or cottage industries using natural fermentation (Ezenekwe et al., 2021). In the modern commercial production of vinegar, the generator method and the submerged fermentation method are used. These methods are based on the goal of infusing as much oxygen as possible into the alcohol product to speed up the acetic acid fermentation process. The acetification of alcohol to vinegar from using direct oxygen may have different physicochemical properties. Yet no studies have been available for use of pure oxygen for vinegar fermentation of pineapple peels biomass.

With an increasing demand for vinegar, necessitating the development of an eco-friendly process (Gunjan and Haresh, 2020), it is necessary to optimize the technologies used (Piotrowski and Kubica, 2021). Oxygen assisted fermentation is an invention in the fermentation process wherein substantially pure oxygen is directed into the fermentation medium. Oxygen makes up about 20% of normal atmospheric air (Zheng et al., 2018). When correctly applied, oxygen interacts with both the microorganism in such a way that its health is improved and fermentations encounter less problems (Shea, 2018). The use of oxygen assisted fermentation has been shown, not only to reduce energy consumption but also reduces cycle of time used for the process and reduces contamination of product. The main objective of this study is to evaluate the use of oxygen assisted two-stage solid fermentation and effects of process parameters on the quality of vinegar produced.

MATERIALS AND METHODOLOGY

Collection of raw material

Pineapples peels were gotten from fruit vendors in the Bamenda food market. They were sorted to remove damaged peels, washed with tap water and cut into smaller pieces in order to facilitate drying. The peels were dried in an oven at 60°C for 48 h (Pereira et al., 2022). The dried peels were then ground and sieved using 250 um pore size sieve. The pineapple peels powder served as sample (Casabar et al., 2019).

Acid hydrolysis of sample

Acid penetrates lignin without any preliminary pretreatment of biomass, breaking down the cellulose and hemicellulose polymers to form simple sugar molecules (Lenihan et al., 2010; Satyanagalakshmi et al., 2011). The biomass was mixed with dilute sulphuric acid (H_2SO_4) in the ratio 1:5. The mixture was placed in an autoclave at 121^{o}C and a pressure of 1 bar for 15 min after release of first pressure. The pH of the sample was adjusted using

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a NaOH base. The total soluble solids, glucose level and specific gravity were recorded.

Fermentation

Activation of yeast (Saccharomyces cerevisiae) and inoculation for fermentation

In order to ensure the viability of the yeast and to reduce the duration of the lag phase during fermentation, 10g of the yeast was added into a litre of fresh must and allowed at 30°C. The sample was observed after every 15 min until development of bubbles signifying the start of fermentation and release of CO_2. At this state, it was then used as inoculum during fermentation for wine production. Prior to inoculation, must samples were pasteurised in order to eliminate all vegetative forms of microorganisms. The inoculum was then applied. This phase of fermentation was carried out anaerobically for 96 h at 30°C.

Preparation of bacteria culture for acetic acid fermentation

Acetic acid bacteria were isolated from palm wine. To do this, palm wine put in a sterilized container at room temperature until fruit flies were seen. Culture media was prepared which include: yeast extract, standard medium and enrichment medium, using the different culture techniques. The cultured samples were inoculated on GYC medium and incubated at 37°C.

Preparation of glucose yeast calcium carbonate agar GYC medium (standard medium)

GYC medium is made up of different quantities of reagents (5% glucose, 1% yeast extract, 1% $CaCO_3$ 1.5% agar). Using an electronic balance, 85 g of the medium was measured and dissolved in 1 L of distilled water. It was well mixed then heated until boiling for 1 min to ensure complete dissolution. The solution was cooled to 45-50 ºC and aseptically 70 ml of ethanol was added (Mizzi et al*.,* 2022).

Preparation of enrichment medium

1% mannitol, 0.5% ethanol, 0.3% acetic acid, 1.5% peptone, 0.8% yeast extract were weighed using an electronic balance and diluted in 100 ml of distilled water. The solution was transferred into a sterile bottle and autoclaved at 115°C for 15 min.

Inoculation and incubation of samples

Isolation of *Acetobacter aceti* was accomplished by inoculating the sample, using the pouring method, on standard GYC medium. In an inverted position, the petri dishes were incubated at 37°C, for 48 h. The yellow colonies that developed were sub cultured by streaking on a new media for 48 h. Each distinct bacteria colony was then picked for identification.

The colony characteristics including color, size, shape and elevation were studied after incubation. In order to further identify the bacteria, the cells were tested for their gram reaction, catalase activity, oxidase activity, cell shape and scent. Also, the optical density of the bacteria was measured in order to estimate the concentration of acetic acid bacteria in the culture medium.

Purification of culture isolates

All the colonies, which appeared after 48 h of incubation on the surface of standard medium GYC plates, were examined for morphological characteristics. The required *Acetobactor aceti* colonies were inoculated on the surface of standard GYC medium for specific culture isolates until pure growth was obtained. Then one loop of cells from the pure culture was inoculated into the enrichment medium and *Acetobactor aceti* growth medium was prepared and ready for inoculation into fermentation broth samples.

Optimization of the acetification fermentation process

Prior to acetification, the alcoholic fermented samples were heated to 70°C to kill yeast. *Acetobactor aceti* inoculum was added after cooling to 33°C, temperature at which acetic acid fermentation was carried out.

Effect of two stage oxygen assisted fermentation on the physicochemical and antioxidant properties of vinegar produced

Under conditions of pineapple peel vinegar fermentation temperature of 33°C, 15% inoculation of acetic acid bacteria, 7% alcohol and oxygen saturation of 20, 40, 60, 80 and 100% (Table 1), were investigated the effect of the amount of oxygen on the physicochemical and antioxidant properties of vinegar evaluated. This was compared with samples with no inoculum and no oxygen and that with inoculum but no oxygen.

Experimental design

The experimental design used by Debapriya et al. (2019) and Guo et al. (2018) was modified and applied. Three factors were considered for optimization (oxygen (60-120%), fermentation time (3-7 days) and acetic acid bacteria inoculum (10 -20%) using a two level Central Composite Design (CCD) and Response Surface Methodology (RSM) with coded values A, B, and C, respectively, as shown in Table 2. The acetic acid concentration in the fermentation broth (g/100ml) was analyzed as the main response. Other responses included, ferric reducing antioxidant power (FRAP), pH, Brix, specific gravity and temperature. A total of 16 sets of runs were generated using STATGRAPHICS. Analysis of variance (ANOVA) was used to test the effect of the different factors on response variables and the interaction between the responses and the interaction variables examined using model equations. Error sum of squares (SSE), regression sum of squares (SSR) and corrected sum of squares (SST) were determined using ANOVA analysis. The coefficient of determination, R^2 expressed the polynomial model's fit quality. A confidence level of 95% was presumed to be significant in this study. Based on the effect of three factors, the respective Pareto plots were obtained for both levels.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + E \quad (1)$$

Analysis of vinegar

Physicochemical analysis of samples

Specific gravity: A hydrometer (IP67) was used to measure the specific gravity by dipping directly into the sample. The meter was read and results recorded.

Measuring TSS: Three drops of each sample was placed on the prism of a brix refractometer (RETK-78), such that the sample

Table 1. Variation of oxygen volume/percentage.

Sample	Oxygen saturation (%)	Bacteria inoculum (ml)
1	20	15
2	40	15
3	60	15
4	80	15
5	100	15
6	0	15
7	0	0

covers the entire prism surface. The cover plate was closed and allowed for 30 seconds then TSS was read from the eye piece on the refractometer using the eye. TSS was expressed as brix degree indicating the mass in gram of dry matter for 100g.

Determination of acetic acid content (San, 2005): The acidity was determined by titration as described by Chalchisa and Dereje (2021) with 0.1N NaOH and phenolphthalein as indicator. 0.1ml of phenolphthalein (0.05%) was added to 5 ml sample of vinegar plus 20 ml distilled water in a 250 ml dry conical flask. The titrated volume was noted as soon as the endpoint, a steady pink coloration, was reached.

Determination of pH: The pH of each sample was measured directly using a digital pH meter calibrated with buffer solutions of pH 6.86, 4.01 and 9.18 respectively at 25°C.

Determination of alcohol content: At the end of the alcoholic fermentation, an alcometer was used to measure the percentage alcohol.

Density (**Ademiluyi and Mepha, 2013**)**:** An empty 25 ml pycnometer was weighed and recorded using an electronic balance. The sample was then poured into the pycnometer and covered with the glass cover of the pycnometer. Overflow of the liquid was cleaned and the weight of the pycnometer plus sample was weighed. The mass of the sample was gotten by subtracting the weight of the empty flask from that of pycnometer plus sample. The density (g/ml) was calculated as a ratio of the mass of sample (g) to the volume (25 ml) of the pycnometer.

Determination of antioxidant activity of vinegar: Ferric Reducing Antioxidant Power (FRAP) Assay. 1 ml of sample was placed in a sterilized glass tube and 2.5 ml buffer solution (0.2M Phosphate buffer, pH 6.6) added. 2.5 ml of 1% potassium fericyanide solution was added and vortex. It was then incubated at 50°C for 20 min after which 2.5 ml of 10% TCA acid (Trichloro acetic acid) was added. The resulting mixture was centrifuged at 3000 rpm for 10 min and 2.5 ml of the supernatant was diluted with 2.5 ml of distilled water, then 0.5 ml of 0.1% $FeCl_3$ was added to obtain a blue colour, and the absorbance read at 593 nm against a blank. The absorbance read was compared with the standard calibration curve (Vijayalakshmi and Ruckmani, 2016; Wilawan et al., 2019).

Statistical analysis

The experiments were conducted in triplicates and the mean values calculated. The statistical analysis was performed using STATGRAPHICS centurion version XVII. Analysis of Variance (ANOVA) and probability value (p-value) ≤ 0.05 was considered as a significant difference. R^2-value >70 and/or standard error <10 was used for model validation.

RESULTS AND DISCUSSION

Physicochemical properties of pineapple peels

The pH, TSS, specific gravity and sugar concentration of pineapple peels before and after pre-treatment are presented on Table 3. The sugar concentration was higher than the meter could record after pre-treatment. It was then diluted with distilled water to 580 mg/dl. The pH of neutralization was 7.02. The alcohol content after pre-treatment was 7%.

Acetic acid bacteria culture and identification

Figure 1 shows the acetic acid bacteria growth on a GYC medium (A) and microscopic view after gram staining (B). The morphological characteristics are presented on Table 4. Acetic acid bacteria were able to grow on GYC medium, had a rod shape, flat elevation, smooth surface, gram negative, catalase positive, oxidase negative, looked whitish. The results were similar to that obtained by Mathew et al. (2019), who isolated acetic acid bacteria from previous batch of apple cider vinegar. The different characteristics of the bacteria culture demonstrated that it belonged to *Acetobacter* genus. Results obtained were also similar to that of Zahoor et al. (2006).

Bacteria viability

The optical density obtained at 600 nm was measured in order to check the viability of bacteria cells in the medium. The optical density increased steadily within 72 h, then decreased (Figure 2). Increase in optical density

Table 2. The experimental design matrix with real variables.

Sample	A	B	C
1	120	3	10
2	90	5	15
3	120	7	20
4	39.5462	5	15
5	60	7	10
6	120	3	20
7	60	3	10
8	90	8.36359	15
9	90	5	23.409
10	90	5	6.59104
11	60	7	20
12	90	1.63641	15
13	90	5	15
14	140.454	5	15
15	60	3	20
16	120	7	10

The general quadratic model with three factors and interactions is depicted in Equation 1.

signifies cell viability. The decrease after 72 h could be due to nutrient depletion in the medium.

Effect of oxygen assisted two stage fermentation on the physicochemical and anti-oxidant properties of vinegar

Effect of oxygen assisted fermentation on pH

As shown in Figure 3, there was a drop in pH as fermentation time increases, with values ranging from 5.38 on day one to 4.06 on day six, with the lowest value of 4.06 observed in sample 5. Samples 6 and 7, which developed in the absence of oxygen, showed no difference in pH throughout the experiment. Even though sample 6 was inoculated with bacteria, there was no effect on the pH as compared to the other samples. Samples 1 to 5 showed a reduction in pH, with sample 5 recording the lowest pH. This indicates that increasing the amount of oxygen leads to a decrease in pH, which could be attributed to the fact that alcohol in the samples was being converted to the weak acid, acetic acid, by the bacteria present. Decrease in pH was in line with the findings of Ezenekwe et al. (2021) on production and physicochemical evaluation of vinegar produced from pineapple and pawpaw fruits with their peels. Raji et al. (2012) also observed a decrease in pH as the fermentation time increased. However, the pH values obtained at the end of this experiment were higher than that of Ezenekwe et al. (2021) and Raji et al. (2012).

Effect of oxygen assisted fermentation on specific gravity

The specific gravity decreased as the fermentation time increased for the first six samples as shown in Figure 4. Values ranged from 1.008 to 1.004. There was no effect on specific gravity for sample seven. These results differ from the results of Ezenekwe et al. (2021), who recorded an increase in specific gravity as the fermentation period increased.

Effect of oxygen assisted fermentation on acetic acid content

Figure 5 presents variation in acetic acid production as a function of oxygen concentration during fermentation. Samples 6 and 7 had very low acetic acid content compared to the other samples. Samples 1-5 showed an increase in acetic acid value and the sample with the highest oxygen concentration had the highest acid content. This could be because there was enough oxygen available for the conversion of alcohol to acetic acid by acetic acid bacteria. Acetification is an aerobic process, and oxygen is critical to the growth of the bacteria (Mas et al., 2014). This therefore suggests that the higher the oxygen saturation, the more acetic acid is being produced during fermentation. This was in line with the results of Sossou et al. (2009), Raji et al. (2012), Ezemba et al. (2021) and Song et al. (2022). Deficiency of oxygen strictly reduces acetic acid production and can lead to cell damage, meanwhile sufficient oxygen supply maintains a reasonable energy metabolism and cell tolerance to improve acetic acid fermentation (Zheng et al., 2018). The results confirm that in order to catalyze the reaction that provides energy, acetic acid bacteria require an adequate supply of oxygen (Spinosa et al., 2015).

Effect of oxygen assisted fermentation on Antioxidant activity

All samples had higher antioxidant activity as compared to the commercial vinegar (Figure 6). Samples without oxygen had the highest antioxidant activity compared to the treated samples. These results indicate that processing alcohol to vinegar reduces the antioxidant activity of the product. These results suggest that the antioxidant activity of pineapple peel vinegar reduces when using pure oxygen. It also suggests that if the oxygen is sufficient then the antioxidant activity will be more compared to insufficient oxygen supply. The difference was not significant among the samples, but it was higher than the commercial vinegar and observed to decrease from the positive and negative controls. However, Bakir et al. (2016) concluded that

Table 3. Physicochemical properties of pineapple peel.

Variable	Before pre-treatment	After pre-treatment and neutralization	After fermentation
pH	/	7.02	5.38
TSS (^{o}brix)	6	11.5	6
SG	1.000	1.012	1.009
Glucose concentration (mg/dl)	300	580	62
TA(g/100ml)	/	/	0.6
Alcohol content (%)	/	/	7

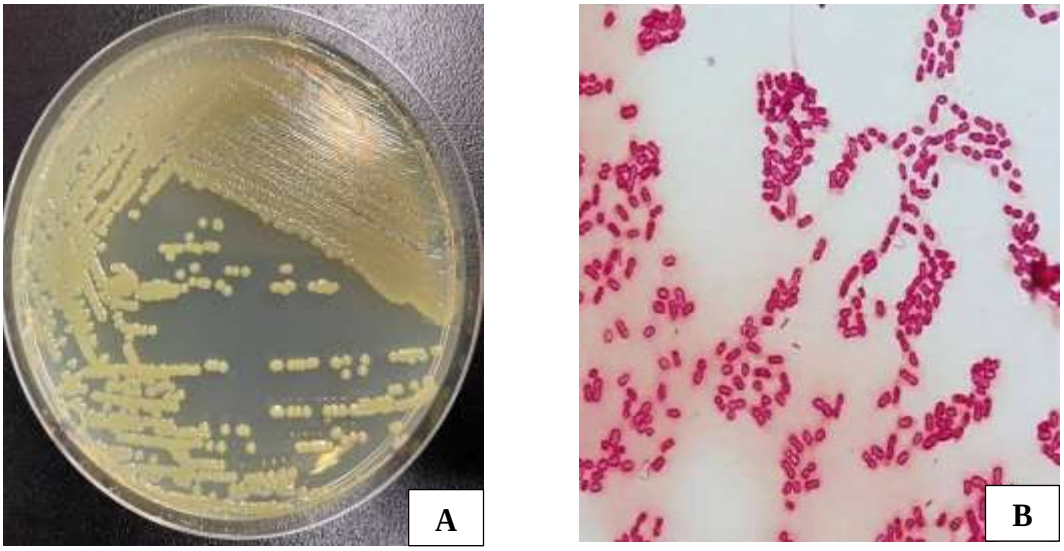

Figure 1. Acetic acid bacteria culture on a GYC medium (A) and Gram Staining (B).

Table 4. Results of bacteria identification.

Character evaluated	Results
Inoculation on GYC medium	Growth observed
Shape	Rod
Color	Whitish
Elevation	Flat
Surface	Smooth and shiny
Gram stain	Gram negative
Catalase	Positive
Oxidase	Negative
Odor	Like that of vinegar
Identity	*Acetobacter*

spectrophotometric methods indicate a strong loss of antioxidant phenolic compounds during the transition from fruit wine to fruit vinegar. The higher antioxidant activity confirms the findings of Kulkarni (2015), who observed that pineapple peel vinegar had a higher antioxidant activity compared to that of the fruit and concluded that pineapple peel vinegar can be produced in large scale and marketed for its therapeutic effects. Also, higher antioxidant capacity found in the pineapple peel vinegar can be explained by the higher

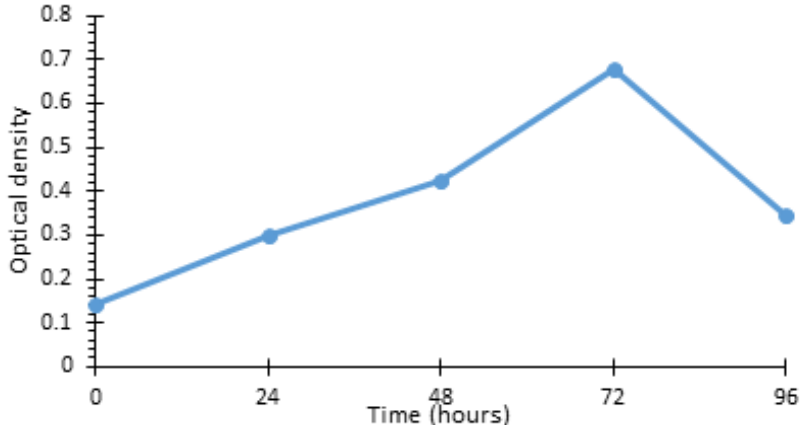

Figure 2. Evolution of optical density of isolated acetic acid bacteria.

concentration of phenolic compounds and organic acids commonly found in the fruits, which have antioxidant activity. Higher values were reported by Fonseca et al. (2018) in blueberry wine and honey for all of the antioxidant methods evaluated. However, the results of Vahos et al. (2020) revealed that alcoholic beverages had the highest antioxidant activity; after acetic fermentation, a decrease in antioxidant potential was observed in all three extractive processes evaluated, including FRAP.

Effect of process parameters on the physicochemical and antioxidant properties of vinegar

A central composite design was used to study the effect of process parameters on the production of vinegar from pineapple peels. The process variables evaluated were the oxygen concentration (A), fermentation time (B) and bacteria inoculum (C). The main responses considered were acetic acid concentration, specific gravity and antioxidant potential (FRAP). Other responses were pH, total soluble solids (TSS), temperature and density. Table 5 presents the experimental outcome of the 16 runs generated by the experimental design.

Effect of process parameters on acetic acid content

The model equation depicting the influence of process parameters on acetic acid concentration is represented by equation 2.

$$Y_{\text{Acetic acid content}} = -10.1444 + 0.0484253A + 2.04056B + 0.603834C - 0.000239605A^2 + 0.000604167AB + 0.000325AC - 0.169258B^2 - 0.009375BC - 0.0155554C^2\ldots \quad (2)$$

With R^2 of 86.22% (greater than 70%), the equation is said to be valid.

Bacteria inoculum (C) and time (B) had significant effects on the acetic acid content of the vinegar, with p-value less than 0.05 (Appendice). Doubling time had a negative effect on acetic acid content, while bacteria inoculum had a positive significant effect (Figure 7). All other factors and interactions showed no significant effect on the acetic acid content. The interaction of time and bacteria inoculum, doubling inoculum and doubling oxygen concentration had negative effects, though not significant, while interaction of time and oxygen, oxygen and bacteria inoculum, oxygen concentration had positive, non-significant effects on the acetic acid concentration.

Chalchisa and Dereje (2021) stated that bacterial inoculum and fermentation time were the most important factors affecting vinegar production.

Increasing the bacteria inoculum increases the acetic acid concentration. This was in line with the results of Saha and Banerjee (2013), who found out that increasing the bacteria quantity during banana vinegar fermentation, increases the acetic acid concentration. In their work, the highest acidity of 4.67 was observed in the sample with highest amount of bacteria inoculum. Production of acetic acid from ethanol depends on the presence of acetic acid bacteria (Song et al., 2022).

Increasing the fermentation time increases the amount of acetic acid in the sample. But doubling the time rather reduces the acetic acid content. This show that time has both a positive and negative effect on acetic acid content of vinegars. Aye (2016) found out that the acidity of the vinegar produced decreased when the fermentation period was longer, and the negative effect observed could be caused by the further oxidation of acetic acid. Also, acetic acid has toxic effects on acetic acid bacteria when the concentration becomes greater than the bacteria can tolerate (Song et al., 2022). The best results are obtained when acetic acid was being maximized. For maximum acetic acid content, the Optimum value = 4.34504, under the optimum conditions of 121.123 oxygen volume, 137 h and 18.9 ml of acetic acid bacteria.

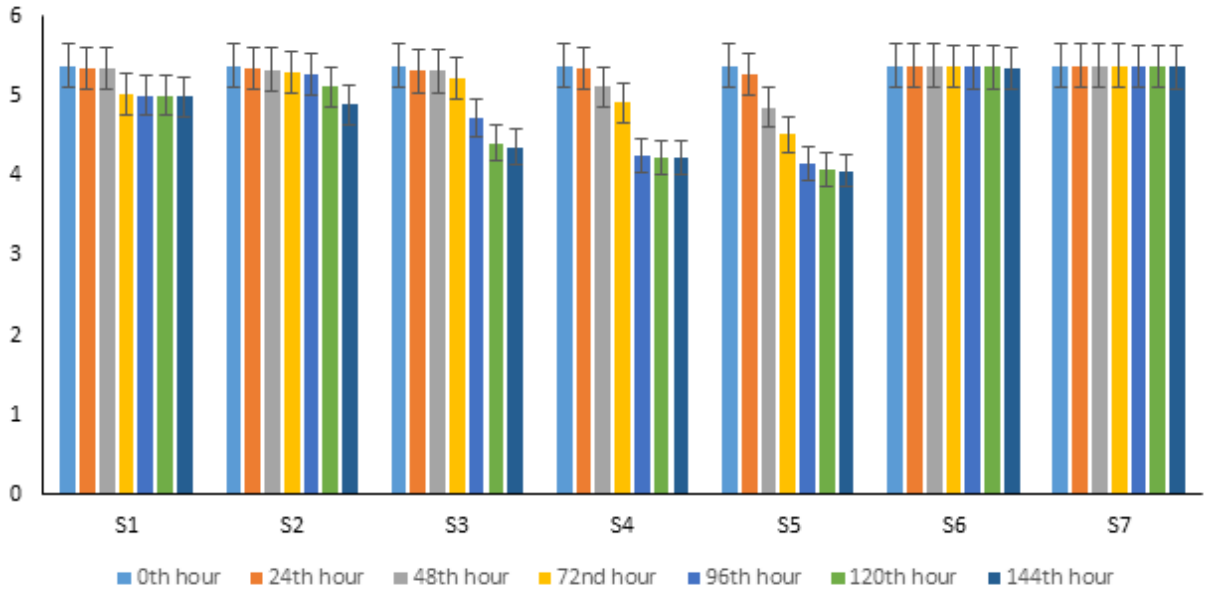

Figure 3. Effect of oxygen assisted fermentation on pH.

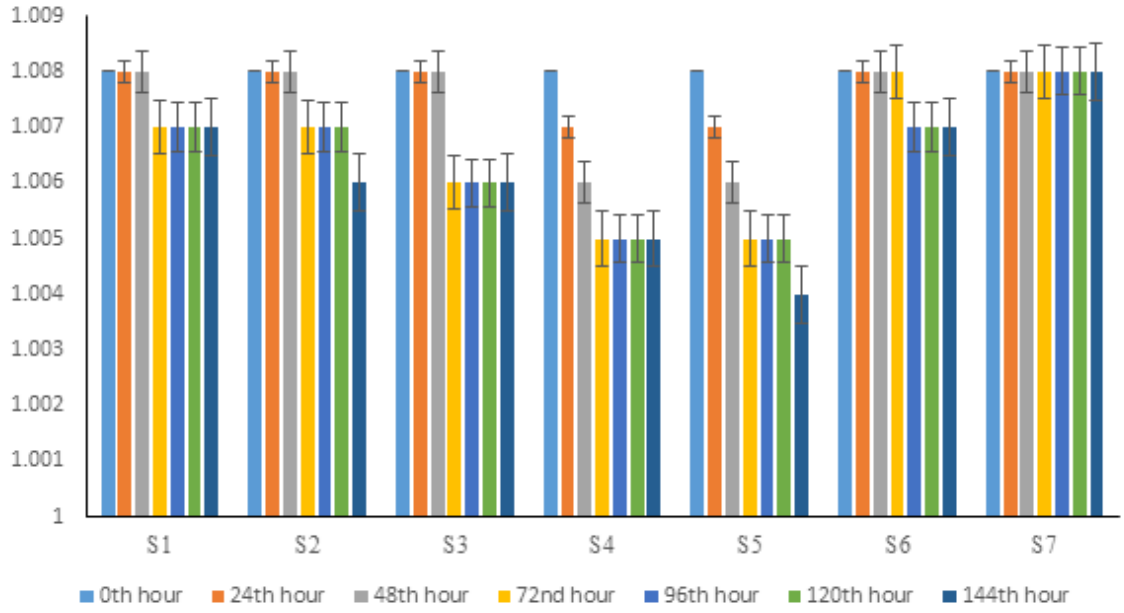

Figure 4. Effect of oxygen assisted fermentation on specific gravity.

The optimum acetic acid content is in line with the recommended value for vinegar production.

Effects of process parameters on pH of vinegar

The model equation predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on pH of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as shown in equation 3:

$$Y_{pH} = 5.96163 - 0.0378167A - 0.0907543B + 0.0592418C + 0.000186719A^2 + 0.0013125AB + 0.000175AC + 0.00179514B^2 - 0.004375BC - 0.0019048C^2 \quad (3)$$

Where pH is the response. The equation is not valid since R^2 is 58.6073, which is less than 70%.

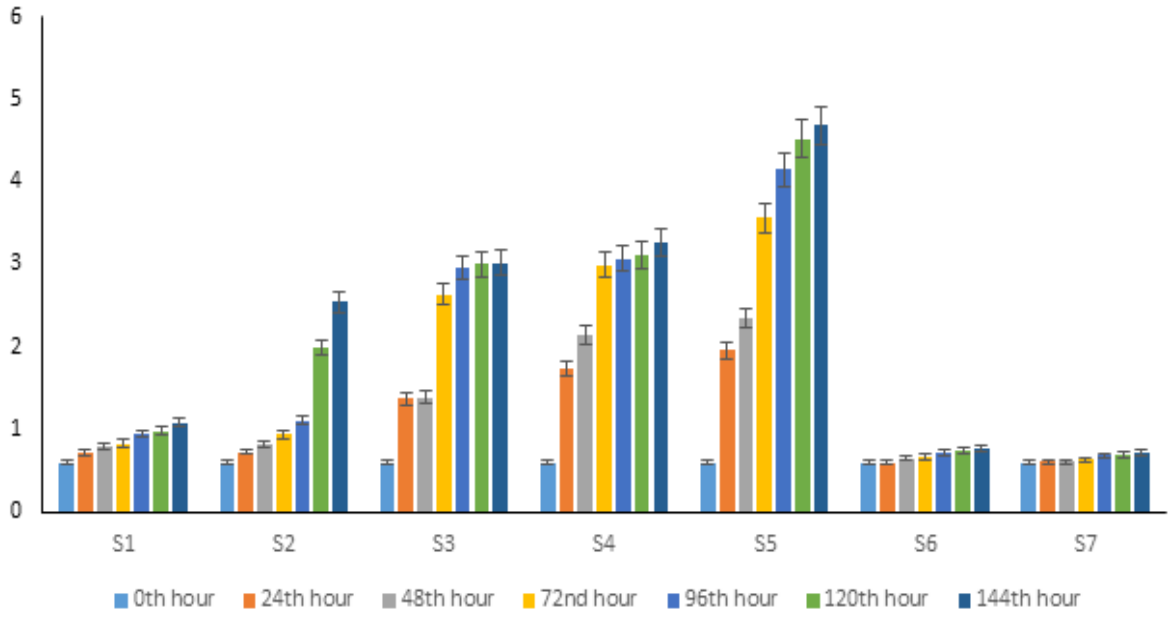

Figure 5. Effect of oxygen assisted fermentation on acetic acid content.

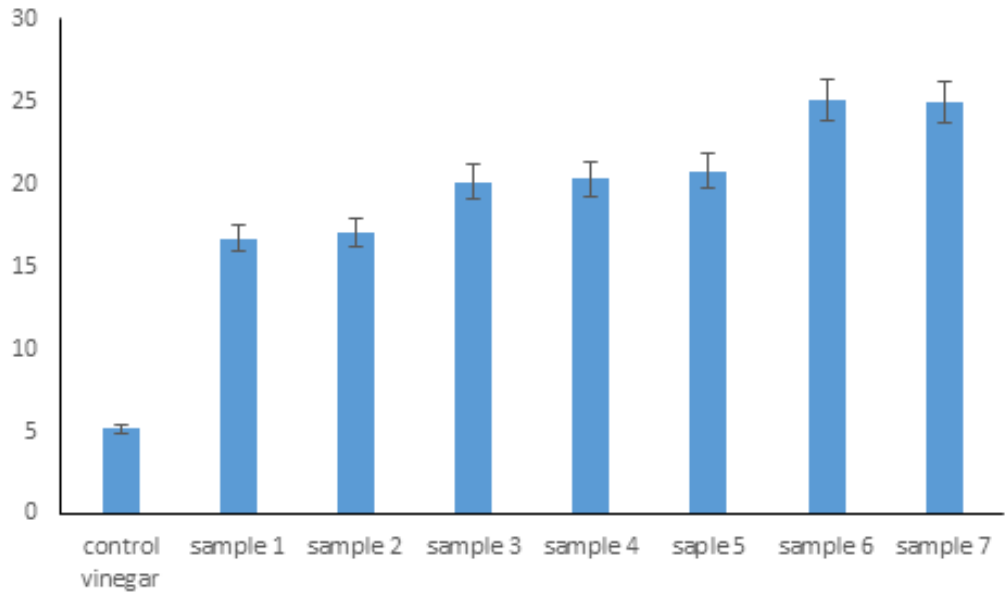

Figure 6. Effect of oxygen assisted fermentation on antioxidant activity.

No factor had a significant effect on the pH. Time, doubling bacteria inoculum, interaction of oxygen concentration and bacteria inoculum and doubling oxygen concentration had negative non-significant effects on the pH of the vinegar produced. Doubling Time had a positive non-significant effect on the pH of the sample, together with oxygen concentration, bacteria inoculum, interaction of time and oxygen concentration and interaction of time and inoculum (Figure 8). This reveals that there is a decrease in pH with increasing length of time which is in line with the report of Ezenekwe et al. *(*2021). The decrease in pH could be attributed to the fact that the alcohol present is being oxidized to acetic acid, which then lowers the pH of the sample. The effect of process parameters on pH is in line with the findings of Mizzi et al. (2022), with no significant difference between the initial and final pH values. Therefore, pH does not provide a good indication of the fermentation process.

Table 5. Effect of process parameters on the physicochemical and antioxidant activities of vinegar produced by two stage oxygen assisted fermentation of pineapple peel biomass.

		Responses					
Runs	**acetic acid concentration** (g/100ml)	**specific gravity**	**FRAP**	**pH**	**TSS (%)**	**Temperature (°C)**	**Density**
1	1.5	1.007	24.7	4.66	5.5	31.2	1.2056
2	3.34	1.006	17.03	4.55	5.7	30.5	1.2059
3	3.4	1.007	23.9	4.98	5.8	31.2	1.2073
4	1.99	1.008	14.43	4.48	6	30.1	1.2045
5	2.2	1.007	19.7	4.83	6	30.4	1.2085
6	2.99	1.006	20	4.54	5	31	1.2037
7	1.56	1.008	28.9	4.47	5.8	30.3	1.2066
8	3.24	1.007	14.9	4.83	6	31.3	1.209
9	3.97	1.007	14.9	4.48	5.2	30	1.2031
10	1.56	1.008	15.6	4.55	6	29.7	1.2072
11	3.12	1.006	18.5	4.76	6	31	1.2053
12	0.66	1.008	14.33	5.89	5	31.5	1.0057
13	4.26	1.006	14.9	4.48	5.4	31.9	1.204
14	4.52	1.007	16.6	4.69	5.8	31.7	1.2038
15	2.5	1.007	21.6	4.72	5.4	29.9	1.2049
16	2.64	1.008	18.1	4.76	6	31	1.2104

The results for each response was generated separately and fitted into the model equation CCD, then analyzed separately.

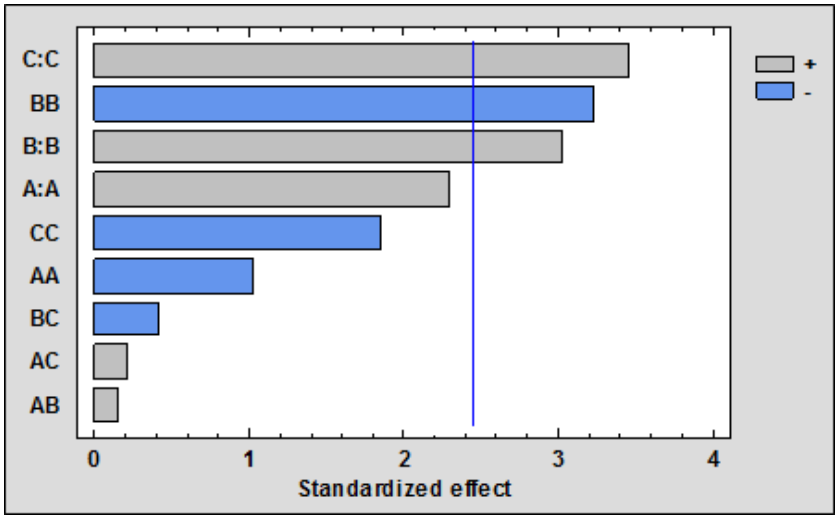

Figure 7. Pareto diagram showing the effects of process parameters on the acetic acid content of the vinegar produced.

Optimize Response for pH was 5.4 with processing conditions of 122.5 oxygen volume, 1.6 day and 14.7 ml inoculum

Effects of process parameters on TSS/Brix

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on TSS/Brix of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as presented in equation 4:

$$Y_{TSS} = 7.69267 - 0.0298656A - 0.0503593B - 0.082121C + 0.000141053A^2 + 0.00104167AB - 0.00025AC - 0.00361831B2^2 + 0.00875BC + 0.000835283C^2 \quad (4)$$

Four effects have *p*-values less than 0.05, indicating that

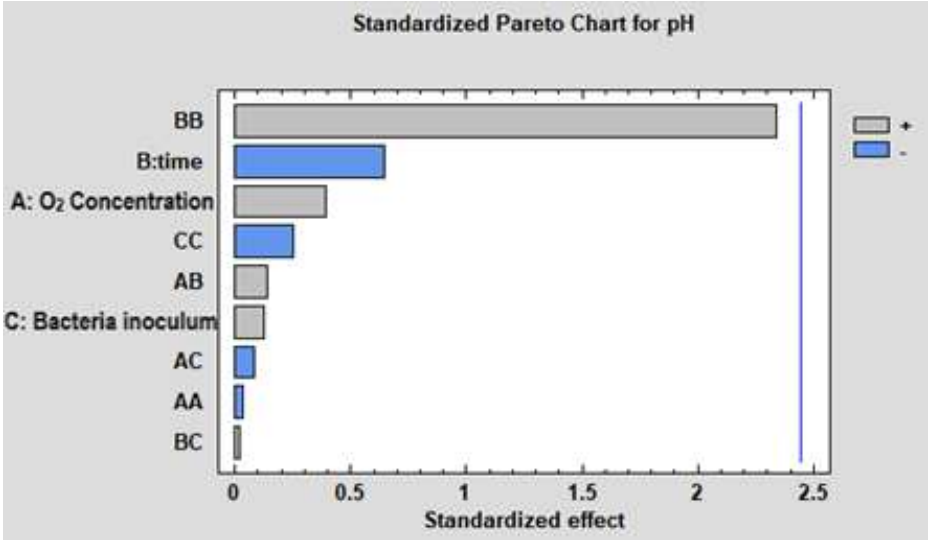

Figure 8. Pareto diagram showing the effects of process parameters on pH of vinegar.

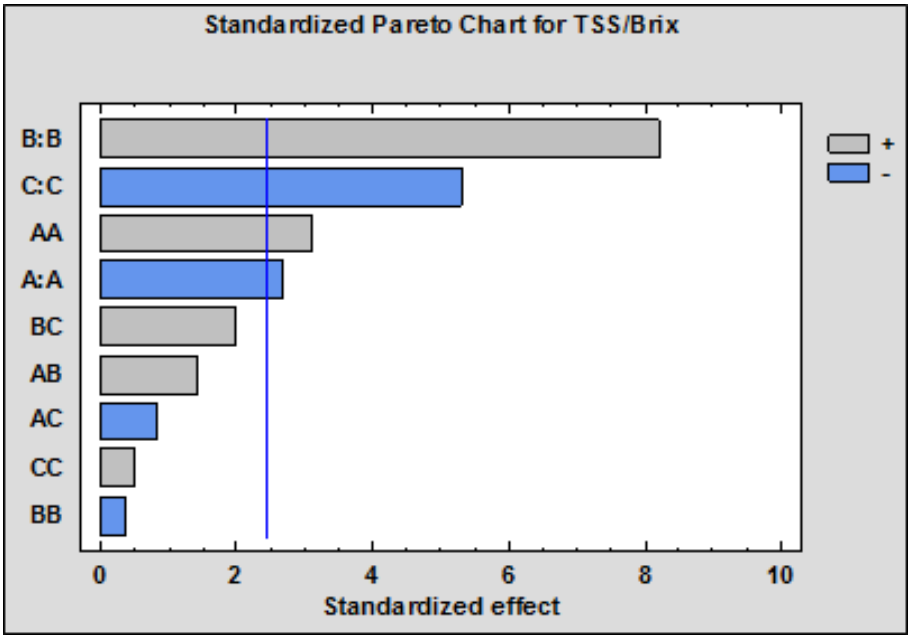

Figure 9. Pareto diagram showing effects of process parameters on TSS/Brix of vinegar.

they are significantly different at the 95.0% confidence level. Bacteria inoculum had a negative significant effect on the TSS, together with oxygen concentration, while time and doubling oxygen concentration had significant positive effects (Figure 9). The TSS values however were higher than that obtained by Akarca et al. (2020), who had mean values of 3.63±0.07 °Brix.

Bacteria inoculum had a significant negative effect on the total soluble solid of the product with a p-value of 0.0018. This indicates that increasing the amount of bacteria reduces the TSS in the vinegar. The minimum brix value obtained was 5.2% and the maximum was 6%.

Oxygen volume had a significant positive effect on the total soluble solid of the product with a *p*-value of 0.0366. This indicates that increasing the amount of oxygen will increase the TSS in the vinegar. However, doubling the oxygen volume had a significant negative effect on the TSS of the product, with a *p*-value of 0.0213. Time had a

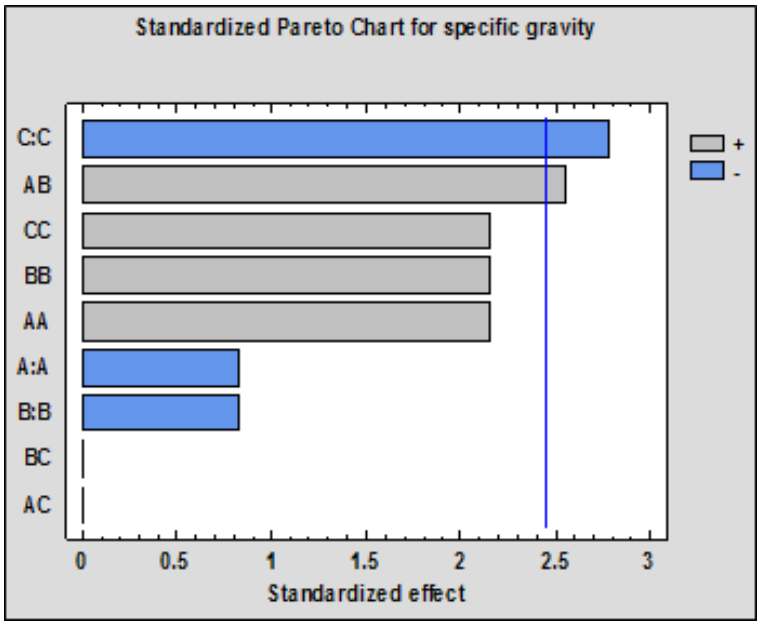

Figure 10. Effects of process parameters on specific gravity of vinegar.

significant positive effect on the TSS of the product with a *p*-value of 0.002. This indicates that increasing the time will increase the TSS in the vinegar.

Effects of process parameters on specific gravity

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on specific gravity of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as shown in equation 5.

$$Y_{Specific\ gravity} = 1.02125 - 0.000124304A - 0.00179323B - 0.000554406C + 4.36292E\text{-}7A^2 + 0.00000833333AB + 0.0AC + 0.000098166B^2 + 0.0BC + 0.0000157066C^2 \quad (5)$$

The equation is valid since the R-squared = 79.4904%, greater than 70%. The significant effects of process parameters on vinegar specific gravity are illustrated by a Pareto diagram shown in Figure 10. Bacteria inoculum had a significant negative effect on the specific gravity of the vinegar produced while interaction of time and oxygen concentration showed a positive significant effect on the specific gravity of vinegar produced. Other factors and interactions had no significant effect. These results show that increasing bacteria inoculum will reduce the specific gravity of the vinegar.

Effect of Bacteria inoculum on the specific gravity of vinegar: As the amount of bacteria increases, the specific gravity reduces. Bacteria inoculum has a significant negative effect on the specific gravity of the vinegar produced with p-value of 0.0319.

Effect of time and oxygen concentration on specific gravity: As the amount of oxygen increases, together with time, the specific gravity also increases as shown in Figure 11. They have a significant positive effect with a p-value of 0.0430.

Effects of process parameters on ferric reducing antioxidant activity of vinegar

The model predicting the effects of fermentation time, bacteria inoculum and oxygen concentration on FRAP of prepared vinegar is a second order polynomial equation with linear, interaction and quadratic terms as depicted by equation 6:

$$Y_{FRAP} = 86.7586 - 0.503245A - 8.22198B - 3.49431C + 0.00159595A^2 + 0.02AB + 0.008AC + 0.27954B^2 + 0.2075BC + 0.0537067C^2 \quad (6)$$

Figure 12 is a standardized pareto chart showing the influence of factors on FRAP. No significant effect was observed on the antioxidant activities of the vinegar, although the values were higher than that of commercial vinegar. However, time and bacteria inoculum had negative effects on the ferric reducing antioxidant activity of the vinegar. On the other hand, every other factor and interaction had positive effects. Results of multiple response optimization analysis revealed optimum

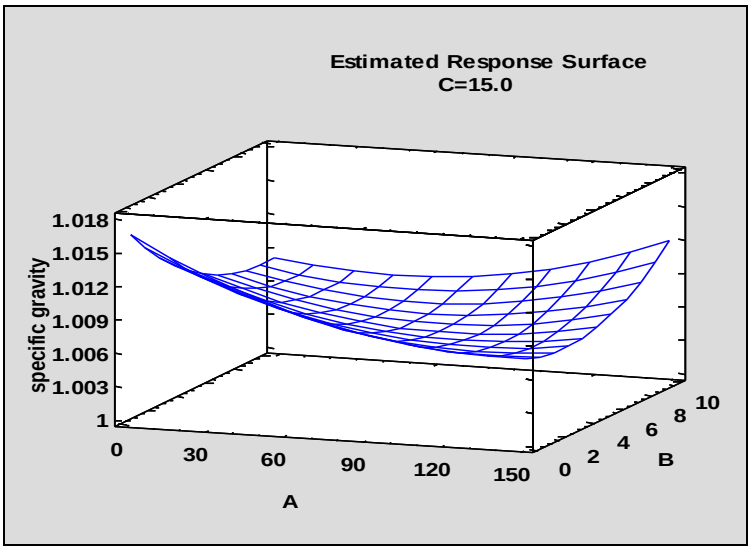

Figure 11. Effect of time and oxygen concentration on specific gravity of vinegar.

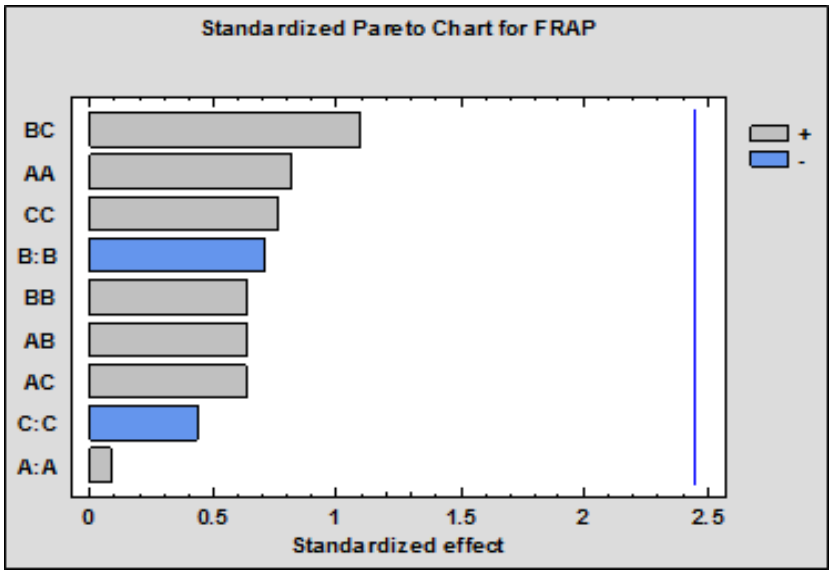

Figure 12. Pareto diagram showing the effects of process parameters on ferric reducing antioxidant activity of vinegar.

conditions of 8 days, 18.81 bacteria inoculum volume and 140 ml oxygen saturation and predicted optimum pH, TSS, temperature, acetic acid content, specific gravity and density to be 5.21, 6.0, 31.6, 3.1, 1.007 and 1.19882 respectively. The predicted optimum ferric reducing antioxidant activity was 28.97. The predicted acetic acid concentration which was the main response fell below the standard. This could be because of other factors. However, the predicted acetic acid content was closer to that of Jang et al. (2009), with a predicted acetic acid content of 3.77%.

Conclusion

The main objective of this research was to contribute to the utilization of food waste by creating vinegar through a two-stage fermentation process that involves the use of pineapple peels, a type of lignocellulose biomass. This innovative approach incorporates the assistance of oxygen to enhance the fermentation process. The findings of the study demonstrated that this two-stage oxygen assisted fermentation technique had significant impacts on key attributes of the produced vinegar,

including pH, specific gravity, and acetic acid content. Interestingly, the research indicated that the ferric reducing antioxidant activity of the produced vinegar remained unaffected by the fermentation process. While this activity did not exhibit significant changes, it's noteworthy that the values surpassed those observed in commercial vinegar products. The study also investigated the influence of various process parameters—namely, time, bacteria inoculum, and oxygen volume—on several physicochemical properties of the vinegar, such as acetic acid content, specific gravity, total soluble solids (TSS), and temperature. Notably, these parameters had varying effects on the studied properties. Importantly, the pH of the vinegar, as well as its density and antioxidant activity, were found to be unaffected by the tested parameters. Despite the potential additional cost associated with using pure oxygen, the two-stage oxygen assisted fermentation technique presents itself as a promising method for industrially producing vinegar from pineapple peels and other agro food waste. This approach offers several advantages, including its environmentally friendly nature, shorter production time and the product is free from contamination.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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