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Bioremediation of Industrial Solid Wastes

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Authors' contributions

This work was carried out in collaboration between all authors. Author PNIN participated in all operations of this manuscript. Author CCCO wrote the first draft of the manuscript. Authors PNIN and CCCO designed the study and wrote the protocol performed. Author IOE managed the analyses of the study and managed the literature searches. Author PNIN revised the manuscript and she is responsible for all information presented. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Use of *Pleurotus ostreatus* as a veritable tool in the bioremediation of empty fruit bunches**,** pulp and paper wastes from respective industries.
Place and Duration of Study: Samples of pure culture *Pleurotus ostreatus* was obtained from National Roots Crops Research Institute Laboratory Umudike, Umuahia and transported to the laboratory of Starline Nigeria Group of Companies Aba, both in Abia state, Nigeria where it was used to carry out experimental work from July to September, 2013.
Methodology: The two substrates (empty fruit bunch (EFB) and paper pulp wastes) were dried in the sun for one week and further dried in Gallen Kamp hot box oven (DGH-9053A) at 50ºC for 4hours. Samples were ground, sieved and powder obtained used for proximate composition determination. Approximately 1cm^2 blocks of pure culture of *Pleurotus ostreatus* mycelium previously collected and maintained on Malt extract agar (MEA) was used to inoculate sterilized wheat grain already soaked in water for 36hours, mixed with 2.5% calcium carbonate and 1% gypsum during spawn preparation. The empty fruit bunches and paper wastes were reduced to smaller sizes of 2-5cm. The respective substrates were mixed with lime and soaked in water for 4h to obtain moisture level of 70%. The substrates (2kg) was bagged in heat resistant polythene and sterilized for

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3hours at 80°C. Approximately 40g individual sterilized substrate was inoculated with 2% spawn adopting multilayered technique. After inoculation, holes were made on the bags to ensure aeration, kept inside a disinfected room at 25-28ºC. When *Pleurotus* mycelium had completely colonized the substrates, bags were removed, colonized substrate transferred to sunlight illuminated room at 28°C, moisture content of 75-85% and adequate watering of the mushroom for 21 days. The process was repeated in combinations of other substrates like wheat, straw and sorghum respectively with each of the substrates (EFB and Paper Waste); all prepared at 70:30% ratio respectively.

Results: Among the different treatments, Empty fruit bunch gave maximum yield of 201.5g/kg of substrate on 14th day and total yield of 675.1g/kg of dry substrate. The nutritional content of *Pleurotus ostreatus* grown on different substrate and substrate combination ranges from 83.5-91.4% moisture, 18.3-23.5% protein and 6.04-6.86% ash contents. The combination of EFB + sorghum best supported the bioremediation process of Empty fruit bunch.

Conclusion: *Pleurotus ostreatus* could serve as bioremediation tool for industrial solid wastes like EFB and paper. It can also alleviate poverty by providing greater income for most unemployed youth at no cost.

Keywords: Bioremediation; empty fruit bunch; nutritive content; paper waste; Pleurotus ostreatus.

1. INTRODUCTION

Over the years pollution and management of vast quantities of wastes generated by various industrial activities have been major problems experienced by developing countries. More challenging is the unsafe disposal of these wastes into the ambient environment and biodegradation problems associated with it. These have resulted to alternative means of reclaiming the environment via bioremediation, phyto-remediation and air pollution control. Bioremediation involves the use of microorganisms to breakdown complex materials into simple end products. These micro-organisms have evolved a host of enzymes that aid in biodegradation of natural products within the ecosystem. This microbial clean-up (Bioremediation) method removes a number of other harmful pollutants and perhaps is the most environmentally safe process used today [1-3].

Oil palm (*Elaeis guineensis*) tree is a valuable economic crop widely grown in Nigeria. Its Empty fruit bunch (EFB) is a major solid waste in palm oil industries with 20–25% EFB being generated for every tonne of oil palm fresh fruit bunch (FFB) processed in the mill [4,5]. EFB contains lignocellulosic materials which consist of 45-50% cellulose, 25-35% of hemicellulose and lignin [6,7]. Lignin and its derivatives can form highly toxic and recalcitrant compounds. The industrial application of EFB have not been reported to our knowledge, it is rather found littered everywhere in palm oil industrial areas in Nigeria hence this research. Studies have shown that 80% EFB is used as fuel in palm oil mills thereby generating great environmental hazard to the host communities and this practice has been discouraged [8]. Other ways of disposing EFB like composting incur additional operational cost and technology. However, in the south east of Nigeria EFB is used locally to prepare local delicacies like abacha (sliced boiled cassava), ugba (oil bean salad), ukwa (breadfruit) and production of local black soap because of its high potassium content [9]. The brown colored filtrate from the ash of EFB is used as a sauce for preparing a local delicacy known as 'Otong' which is used in eating meat and spicing soup in south-south region of Nigeria [5]. Many researchers have reported the use of lignocellulosic source of EFB as substrate in cellulase production [5,10,11] while few

reports prior to this research is known to have used EFB as a substrate for the cultivation of *Pleurotus* spp [12-14].

Another source of industrial solid wastes is generated from pulp and paper manufacturing plants. Pulp and paper are manufactured from lignocellulosic raw materials from wood, recycled paper and agricultural residues. These wastes are usually disposed through landfill and incineration processes [15]. These methods of disposal could be harmful to ecosystem and cause diseases to man. Many studies have been reported on the treatment of pulp and paper mill effluents using biological method such as conventional aerobic, anaerobic treatment and use of white-rot fungi in the treatment [16-18]. Recently, mushrooms are regarded as the most profitable and environment-friendly method for recycling of the vast lignocellulosic waste substrates [19,20].

Pleurotus ostreatus belong to the class of *Basidiomycetes* known as 'oyster mushroom' and mostly cultivated in the tropics using micro molecules of cellulose and starch for their growth [21]. The life cycle starts from spores and germinates to form a mass of branched hyphae of mycelia on a substrate under favourable conditions [22]. They are edible mushroom also used for both bioremediation and medicinal purpose. The first successful attempt to grow *Pleurotus ostreatus* for human consumption was made by [23] in Germany. *Pleurotus* species are rich source of vitamin C, B-complex (thiamin, riboflavin, folic acid and niacin pantothenic acid and biotin), minerals (Ca, P, Fe, K, Cu and Na) and protein [24- 27]. Also its high content in potassium: Sodium ratio makes it an ideal food for patients suffering from hypertension and heart diseases. *Pleurotus* is a proficient lignin-degrading mushroom and can grow well on different types of lignocellulolosic materials which offers one of the most realistic and economic method for the bioremediation of agro-industrial wastes [28,29]. This technology can equally control air pollution associated with burning of these solid wastes and reduces environmental pollution due to unutilized agricultural wastes.

The introduction of Environmental Air Quality Regulation in Nigeria is yet be fully implemented unlike some developing countries such as Malaysia that make use of alternative disposal or management method for EFB and other solid wastes hence this research on better ways of converting these industrial solid wastes to an economically viable material which would also help to alleviate poverty in the country.

Therefore, the aim of this research to use *Pleurotus ostreatus* as veritable tool in the bioremediation of empty fruit bunches, pulp and paper wastes from industries.

2. MATERIALS AND METHODS

2.1 Sample and Pure Cultures Collection

The Industrial wastes (EFB and paper) used as substrates was generated from Starline palm oil mill industries and Starline corrugated packaging Owerrinta respectively, both in Abia State, Nigeria. The pure culture of *Pleurotus ostreatus* was obtained from National roots Crops Research Institute Laboratory Umudike, Umuahia and was transported to the laboratory of Starline Nigeria Group of Companies Aba, both in Abia state, Nigeria. The cultures were maintained on 2% malt extract agar slants at 4ºC prior to use for microbiological examination.

2.2 Preparation of Substrates for Proximate analysis

The two substrates (empty fruit bunch and paper pulp wastes) used were examined, debris removed, milled and used for proximate analysis as described by [22] with slight modification. The substrates were dried in the sun for one week and further dried in Gallen Kamp hot box oven (DGH-9053A) at 50ºC for 4hours. Samples were ground using National model kitchen electric blender and sieved through a 1mm sieve to obtain a powdered processed sample, which was used for determination of the proximate composition.

2.2.1 Spawn preparation

Clean wheat grains were commercially sourced from a Ariaria local market at Aba, Abia State, Nigeria. Two kilograms of dry wheat grain was soaked in water for 36hours to absorb about 65% moisture. The grain was mixed with 2.5% calcium carbonate and 1% gypsum and packaged in different clean white glass containers of about 500ml volume [19] modified. Each glass container was three-quarter filled with spawn substrate. The packaged spawn substrate content was then sterilized in autoclave at 121ºC for 30mins. The sterilized spawn substrates were allowed to cool and aseptically inoculated with approximately 1cm^2 blocks of mycelium previously maintained on MEA. Thereafter, it was incubated at a temperature of 25±2ºC at 150rev/min for 48hours to quicken mycelia development.

2.2.2 Preparation of substrates for *Pleurotus ostreatus* cultivation

The empty fruit bunches were first reduced to smaller sizes in Hammer mill then mechanically chopped into 2-5cm long bits as described [30] while the carton (paper) wastes were cut to same size manually. The respective substrates were mixed with lime and soaked in water for 4hours; excess water was drained and maintained at optimum moisture level of 70 percent. Approximately 2kg of the substrate was transferred into a heat resistant polythene bag of 100µm thickness (60x35cm size) and stoppered with a cotton plug respectively. The substrates were sterilized for 3hours, at temperature of 80ºC in an ESPEC industrial oven (PHH-302) model. The sterilized substrates were allowed to cool overnight in preparation for inoculation. 2% spawn was used to inoculate approximately 40g sterilized substrate adopting multi-layered technique respectively. After inoculation, holes were made on the bags to ensure aeration and were kept inside a disinfected room house where the temperature and humidity were maintained at room temperature of about 25-28ºC for quick colonization of the substrate (EFB and paper wastes) respectively. After *Pleurotus* mycelium had completely colonized the substrates, the bags were removed and colonized substrate transferred to sunlight illuminated room where temperature and humidity of 28ºC and 75-85% were maintained through adequate watering of the mushroom for 21days. The process was repeated with a combination of wastes from wheat straw and sorghum respectively with each of the substrates (EFB and Paper Wastes); all prepared at 70:30% ratio respectively.

2.3 Determination of pH

The pH of substrates was determined after milling and soaking 10g of each (EFB and paper wastes) in 50ml distilled for 10 minutes using pH meter (Orion 410A+ Thermo Electron Corporation model). Preparation of adjusted pH was carried out later using Citrate-Phosphate buffer solution 5.0, 5.5, 6.0 and 6.5 as prepared by [31].

2.4 Proximate Analysis

2.4.1 Protein content

The Kjeldahl method was used for determination of protein content was as described by [22,32] and multiplying the resultant nitrogen content with factor of 6.25. About 0.2g of the respective samples was mixed with 25ml Conc H_2S0_4 and selenium (catalyst) in a digestion flask. The mixture was heated to clear solution in a fume cupboard. The clear solution (digest) was diluted to 100ml and subsequently used for analysis. Distillation process was carried out on 10mls digest mixed with 45% (w/w) NaOH solution in Kjeldahl apparatus. The distillate was collected into 10ml of 4% boric acid solution with 3 drops of mixture indicator (Methyl red indicator and bromocressol green). A total of 50ml of distillate was collected and titrated against 0.02N H_2S0_4 solution. This process was repeated in the blank.

The N_2 content was calculated using the formula below:

$$\% N_2 = \frac{100 \times (N \times 14 \times V_1 (T-X).}{W \times 100 Va}$$

Where

W = Weight of sample
N = Normality of the titrant
V_1 = Digest volume
Va = Volume of digest distilled
T = Titration vol of sample
X = Titration vol.Blank
% Protein = % Nitrogen x 6.25

2.4.2 Moisture content

The moisture content was determined by the gravimetric method of [22,26] using 1g of each substrate into separate previously weighed porcelain dish. It was dried in the oven at 105ºC for 3hours, cooled in a dessicator and re-weighed. Drying, cooling and weighing were repeated at I hr intervals until constant weight was achieved. The weight of moisture loss was determined and expressed as percentage of the sample measured. Similar process was repeated in the case of moisture content determination of *Pleurotus ostreatus*.

It was calculated using this formula;

$$\text{Moisture content \%} = \frac{\text{Weight of fresh sample} - \text{weight dry sample}}{\text{Weight of fresh sample}} \times 100$$

2.4.3 Determination of total carbohydrate

Total carbohydrate was determine using Anthrone Method as described by [33].

2.4.4 Ash content

The ash content method was carried out as described by [26,34] using furnace incineration. 3g sample (substrate/ mushroom) was ashed in a Gallenkamp furnace in previously ignited and cooled porcelain dishes of known weight at 550ºC for 6hours. After cooling in desiccators, it was re-weighed. The weight of the ash obtained was expressed as a percentage of the weight of sample analyzed.

Calculation of percentage ash was based on the formula below:-

$$\% \text{ Ash} = \frac{100\ (X_2 - X_1)}{X}$$

Where

X_1 = Weight of empty porcelain dish
X_2 = Weight of porcelain dish + ashes
X = Weight of sample

2.4.5 Crude fibre determination

Crude fibres of both substrate and mushroom samples were respectively determined according to [22] method and calculated using the formulae below:-

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W} \times 100$$

Where

W_2 = Weight of empty porcelain dish + sample after boiling, washing and drying.
W_3 = Weight of porcelain dish + sample after ashing
W = Weight of sample

2.4.6 Yield and biological efficiency

Total weight of the mushroom harvested from all the three flushes were weighed as total yield of mushroom. The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula Chang [35].

$$\text{B.E.\%} = \frac{\text{Fresh weight of mushroom}}{\text{dry weight of substrate}} \times 100$$

where B.E. is Biological Efficiency.

3. RESULTS AND DISCUSSION

3.1 Growth Support of *Pleurotus ostreatus* on Nutrutional Content of Industrial Solid Wastes

The proximate composition of unsterilized and harvested (EFB and paper wastes) substrates is presented in Tables 1 and 2 respectively. The experiment was carried out to show possible use of both nutritional and chemical contents of 100% industrial solid used during the period

of this research. EFB showed the higher level of MC (23.60±1.2), dry mass, (56.85),CHO (67.50), protein content(2.78±1.08),nitrogen(2.78±1.08) hemicelluloses (28.50±0.7) and cellulose contents (52.43±1.06) than paper wastes. However, least level of ash content (4.87±0.09) and pH6.08±0.07 was observed during the proximate composition determination of unsterilized substrate Table 1.

The proximate composition of substrates after harvest of *Pleurotus ostreatus* in Table 2 showed that paper wastes had higher moisture content reduction of 17.64% against 7.63% in EFB also there was pH reduction in pH on both substrates. Similarly, there was no significant difference in percentage reduction of protein content (15.1%; 15%) and carbohydrate content (1.78%; 1.71%) in both EFB and paper wastes nutritional utilization respectively. The results of Tables 1 and 2 show that the cultivation of *Pleurotus ostreatus* on these substrates had significant effect on the ash content. This is in accordance with a report [22] on improved ash content of substrates like sawdust, cotton waste, palm oil chaff and dry plantain leaf.

3.2 Total Yield of Three Flushes of *Pleurotus ostreatus*

The yield of *P. ostreatus* cultivated on different substrates is represented in Table 3. Maximum yield of *P. ostreatus* was revealed on use of EFB + Sorghum straw (732g/kg substrate) while paper wastes supported the yield of *P. ostreatus* minimally (446.1g/kg substrate). *Pleurotus ostreatus* was grown on various combination of substrates (EFB, paper, EFB + wheat mill, paper+ wheat mill, EFB+ sorghum mill and paper+ sorghum mill) so as to improve the yield on the two major substrates under study. In all substrates under study, the highest yield of *Pleurotus ostreatus* was observed on the 7^{th} day.

Result concurs with previous studies which have reported the use of saw dust and sorghum in the growth of *P. ostreatus* [12,19,30,36]. Saw dust was reported to have shown maximum average yield of 646.9g similar to findings [37]. The range of substrates support for the cultivation of *P. ostreatus* should be attributed to its ability to synthesize relevant hydrolytic and oxidative extracellular enzymes [38]. They are known for their remarkable enzymatic complex capable of degrading lignin variety of wood [17].

3.3 Effect of Substrates on Nutritional Content of *Pleurotus ostreatus*

The average nutritive contents and biological efficiency of the *Pleurotus ostreatus* harvested in three flushes on different substrates is shown in Table 4. *Pleurotus ostreatus* grown on EFB + Sorghum straw showed the highest nutritive content of 91.4% moisture, 23.5% protein, carbohydrates 55.8%, crude fiber 8.04% and biological efficiency of 73.2%.

Results of the average nutritive contents and biological efficiency of the *Pleurotus ostreatus* harvested in three flushes on different substrates as presented in Table 4 is in line with report of previous study on use of sorghum as good substrate for cultivation of *P. ostreatus* [30]. The MC was more in EFB constituted substrate than paper substrates. Similarly BE % was more in all EFB constituted substrates with highest in EFB+ sorghum and least at EFB (100%) only. The ash content varied between 6.04% on paper wastes to 7.05% on EFB +wheat straw while the protein contein varied between 18.3% on paper wastes to 23.5% on EFB+Sorghum. Moisture contents of P. *ostreatus* grown on different substrates compared favorably with the result of [24] but varied in the Ash and protein contents of 0.63% and 0.92% respectively in *P. ostreatus*. The variation may be attributed to the environmental factors, methodology and possible substrates used for the growth of this mushroom [24,26].

However, the minimal nutritive content of 83.5% moisture,18.3% protein, 48.4% carbohydrate, 6.25% crude fibre and B.E 44.6% was observed on mushroom (*Pleurotus ostreatus*) harvested from paper wastes. It was observed that nutritional contents of the mushroom grown on the co-substrate sorghum was enhanced confirming previous findings [37,38] of 90% and 90.4% report on moisture content of mushroom grown on Empty Fruit Bunch.

Table 1. Proximate composition of the unsterilized substrates used in the cultivation of *Pleurotus ostreatus*

Properties	EFB	Paper wastes
Lignin	16.86±2.04	18.54±0.04
CHO	67.50±1.09	52.60±2.03
MC	23.60±1.2	1.70±0.08
pH	6.08 ±0.07	7.60±1.07
N	1.09 ±0.15	0.40±0.05
Dry mass	56.85±0.7	21.47±2.16
Protein Content	2.78±1.08	0.20±0.09
Ash content	4.87±0.09	5.60 ±1.3
Hemicellulose	28.50±0.7	23.60±1.06
cellulose	52.43±1.06	35.00±1.08

MC: Moisture content; CHO: Carbohydrate content, N: Nitrogen (Mean ± Std. Dev) of triplicate samples, All properties in percentage except pH

Table 2. Proximate composition of substrates after harvest of *Pleurotus ostreatus*

Properties	EFB	Paper wastes
Ligin	16.56±1.04	18.2±1.04
CHO	66.3±1.45	51.7±3.06
% MC	21.8±1.23	1.4 ±0.4
pH	4.65±0.07	5.09±1.07
N	0.53±0.15	0.32±0.05
Dry mass	58.25±0.72	23.17±1.16
Protein Content	2.36±1.25	0.17±0.88
Ash content	5.07 ±0.09	4.8±1.3
hemicellulose	27.77±0.70	23.32±1.25
cellulose	52.03±1.06	34.6±0.18

MC: Moisture content; CHO: Carbohydrate content, N: Nitrogen, (Mean ± Std. Dev) of triplicate samples, All properties in percentage except pH

Table 3. Effect of substrates on average yield of *Pleurotus ostreatus*

Substrate	Yield (g/kg) dry substrate			Total yield
Type	7^{th} day	14^{th} day	21^{st} day	(g/Kg)
EFB	330.3±1.57	201.5±0.96	143.3±0.61	675.1±0.87
Paper wastes	216.7±0.69	117.9±1.08	111.5±0.75	446.1±1.48
EFB+ wheat mill	375±1.18	221.2±1.32	99.2±1.06	695.4±2.04
Paper + wheat mill	206±0.58	184.5±1.86	92.0±2.05	482.5±1.51
EFB +Sorghum mill	375±1.18	197.2±1.32	159.8 ±1.61	732±0.83
Paper+ sorghum mill	268.0±1.58	158.0±1.86	70.0± 2.15	496±2.58

(Mean ± Std. Dev) of triplicate samples

Table 4. Effect of substrates in nutritional content of *Pleurotus ostreatus*

Substrates	% age Moisture Content	% age protein	% age carbohyd rates	% crude fiber	% age ash content	% BE
EFB	89.6	20.4	53.1	7.86	6.82	67.5±1.02
Paper wastes	83.5	18.3	48.4	6.25	6.04	44.6±1.05
EFB + wheat straw	90.3	22.2	53.9	6.75	7.05	69.5±2.05
Paper+ wheat straw	86.7	19.6	50.7	6.55	6.85	48.2±1.85
EFB+ Sorghum straw	91.4	23.5	55.8	8.04	6.77	73.2±1.07
Paper+sorghum straw	86.3	20.1	50.2	7.05	6.43	49.6±0.07

3.4 pH Variation on Yield of *Pleurotus ostreatus*

Effect of pH on the yield of *Pleurotus ostreatus* on EFB and paper for the three flushes is demonstrated in Figs. 1 and 2. *P. ostreatus yield* was highest for both substrates at pH 5.0. While EFB showed highest yield of 418g/kg of substrate on the 14th day at pH 5 with total yield of 793.4g/kg, paper wastes showed its best yield on 7th (229g/kg) at pH5 with total yield of 514.5g/kg. However, pH 6.0 shows lowest support on the yield on both substrates. pH ranges as seen in Figs. 1 and 2 are contrary to a report by [39] on optimal growth condition of *P. ostreatus* mycelium at pH=6.0 using whey permeate. It can therefore be inferred from that many factors can affect the yield of *P. ostreatus as* reported by [40].

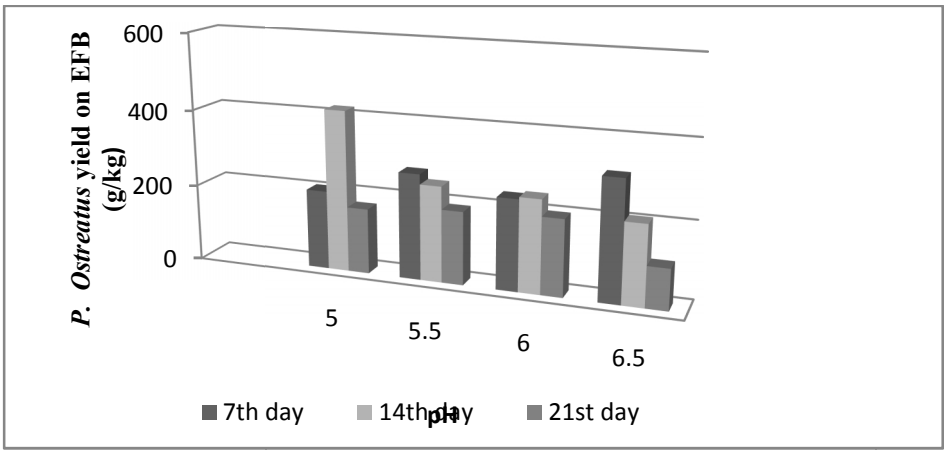

Fig. 1. Effect of pH on yield of *Pleurotus ostreatus* on EFB

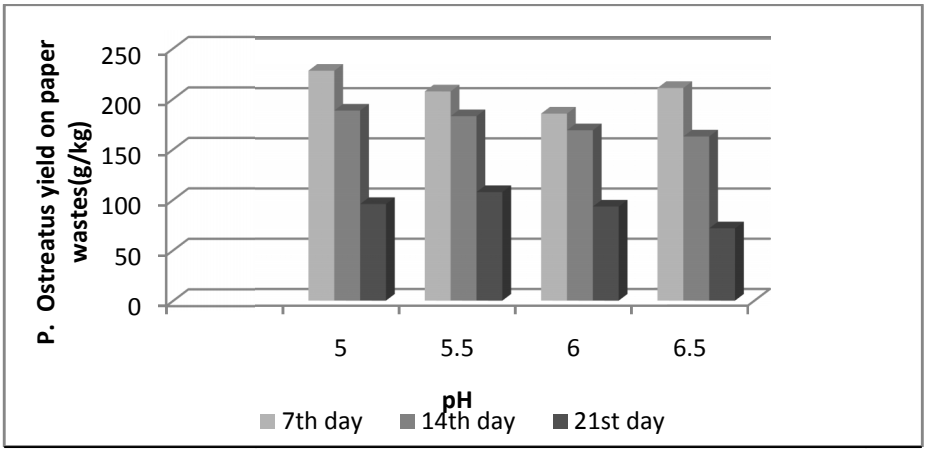

Fig. 2. Effect of pH on yield of *Pleurotus ostreatus* on paper wastes

4. CONCLUSION

Air pollution associated with incineration of Empty Fruit Bunch and paper waste makes it imperative to find an alternative for the control of pollution through bioremediation process. The result from this research work reveals that these industrial wastes that pose great danger to the larger society can turn to wealth through cultivation of *Pleurotus ostreatus* on it. Similarly this will provide an employment opportunity to the unemployed youths in the society thereby reducing crime the benefits of commercializing *Pleurotus ostreatus* cultivation in Nigeria using EFB and paper wastes will not only reduce the toxic effect that might be associated with the accumulation of these wastes in the environment but will improve the diet of an average Nigeria thereby increasing her food security and revenue also bridges the gap of rural-urban mushroom cultivation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adriaens P, Hickey WJ. In: Stone DL (Ed), Biotechnology for the Treatment of Hazardous Waste. – Lewis Publishers. Ann Arbor. 1993;97-120.
2. Beg MU, Saeed T, Al-Muzaini S, Beg KR, Al-Bahloul M. Distribution of Petroleum hydrocarbon in Sediment from Coastal Area Receiving Industrial Effluents in Kuwait. Ecotox and Env. Safety. 2003;54:47-55.
3. Van Hamme JD, Singh A, Ward OP. Recent Advances in Petroleum Microbiology – Micro. & Mol. Bio. Rev. 2003;67(4):503-549.
4. Bakar RA, Darus SZ, Kulaseharan S, Jamaluddin N. Effects of ten year application of empty fruit bunches in an oil palm plantation on soil chemical properties. Evaluation of locally available substrates for cultivation of oyster mushroom (*Pleurotus ostreatus*) in Jimma, Ethiopia. Nut. Cycl. Agro. Eco. Springer Science Business Media B.V.; 2010.
5. Udoetok IA. Characterization of ash made from oil palm empty fruit bunches (OEFB) Int. J. Env. Sci. 2012;3(1):518-524.

6. Deraman M. Carbon Pellets Prepared From Fibers Of Oil Palm Empty Fruit Bunches: 1. A Quantitative X-Ray Diffraction Analysis, PORIM Bulletin, Palm Oil Res. Inst. Malaysia.1993;26.
7. Ewulonu C, Igwe IO. Properties of Oil Palm Empty Fruit Bunch Fibre Filled High Density Polyethylene. Intl. J. Eng. & Tech. 2012;3(6):458-471.
8. Pensamut V, Pongrit , Intarangsi C. The Oil Palm, Department of Alternative Energy Development and Efficiency, Ministry of Energy, Thailand; 2003.
9. Lim B. The New Straits Times of Thursday. December 28; 2000.
10. Akamatsu I, Kobayashi Y, Kamishima H, Hassan K, Mohd Yusoff MN, Hushin M, Hassan AH. Industrial Utilization of Oil Palm By-Products II: Thermo-Mechanical Pulping of Empty Fruit Bunches. Cell. Chem. & Tech. 1987;21:191-197.
11. Rajoka MI, Malik KA. Cellulase production by *Cellulomonas biazotea* cultured in media containing different cellulosic substrates. Biores. Tech.1997;59(1):21-27.
12. Rizki M, Tamai Y. Effects of different nitrogen rich substrates and their combination to the yield performance of oyster mushroom (*Pleurotus ostreatus*). World Journ. Of Microbiol. Biotech. 2011;27:1695-1702.
13. Schuchardt F, Darnoko D, Guritno P. Composting of empty oil palm fruit bunch (EFB) with simultaneous evaporation of oil mill waste water (POME). In: Proceedings of 2002 International Oil Palm Conference. 2002;1-9.
14. Sudirman LI, Sutrisna A, Listiyowati S, Fadli L, Tarigan B. The potency of oil palm plantation wastes for mushroom production. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7) Section: Waste conversion, substrates and casing. 2011;378-384.
15. Suriyanarayanan S, Mailappa AS, Jayakumar D, Nanthakumar K, Karthikeyan K, Balasubramanian S. Studies on the Characterization and Possibilities of Reutilization of Solid Wastes from a Waste Paper Based Paper Industry. Glob. J. Env. Res. 2010;4(1):18-22.
16. Teng TT, Ahmad AL, Wong SS, Zuhairi A,Najafpour G. Treatment of pulp and paper mill wastewater by polyacrylamide (PAM) in polymer induced flocculation; 2005.
17. Wu V, Xiao YZ, Yu HQ. Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm, Biores. Tech. 2005;96:1357–1368.
18. Dalentoft E, Thulin P. The use of aerobic selectors in activated sludge systems for treatment of wastewater from the pulp and paper industry, systems for treatment of wastewater from the pulp and paper industry, Water Sci. Technol. 1997;35:181–188.
19. Gume B, Muleta D, Abate D. Evaluation of locally available substrates for cultivation of oyster mushroom (*Pleurotus ostreatus*) in Jimma, Ethiopia. Afric. J. Microbiol. Res. 2013;7(20):2228-2237.
20. Rai RD, Ahlawat OP. Edible fungi: Biotechnological approaches. Appl. Mycol. Biotechnol. 2002;2:87–111.
21. Banjo NO. Cultivation of Edible Mushroom. The newsletter of Industrial Microbiologist of Nigeria. 1998;4:3.
22. Dike KS, Amuneke EH, Ogbulie JN. Cultivation of *Pleurotus ostreatus*: An edible mushroom from agro base waste products, J. Microbiol. Biotech. Res., 2011;1(3):1-14
23. Falck R. About the walkulter of the oyster mushroom (*Agaricus ostreatus*) on deciduous trees stumps for forestry. Jogdwes. 1917;49:159-165.
24. Caglarirmak N. The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus* sp.) and an estimated approach to the volatile compounds. Food Chem. 2007;105(3):188-1194.
25. Justo MB, Guzman GA, Mejia EG, Diaz CLG, Martinez G, Corona EB. Chemical composition of three Mexican strains of mushroom (*Pleurotus ostreatus*). Archivos Latinoamericanos de Nutricon. 1998;48(4):359-363.

26. Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L. Nutrients in edible mushrooms: An interspecies comparative study. Food Chem. 1999;65:477–482.
27. Sturion GL, Oetterer M. Chemical composition of edible mushrooms (*Pleurotus sp.)* originated from crops in different substrates. Ciênc Tecnologies. Alim. 1995;15(2):189-193.
28. Bano Z, Shasirekha MN, Rajarathan S. Improvement of the bioconversion and biotransformation efficiencies of the oyster mushroom (*Pleurotus sajor-caju*) by supplementation of its rice straw with oil seed cakes. Enz. & Microbiol. Tech. 1993;15:985-989.
29. Cohen R, Persky L and Hadar Y. Biotechnological applications and potential of wood degrading mushrooms of the genus *Pleurotus*. Applied Micro. & Biotech. 2002;58:582-594
30. Patil SS. Cultivation of *Pleurotus Sajor-Caju* on Different Agro Wastes. Science Research Reporter. 2012;2(3):225-228.
31. Gbolagade JS. Bacteria associated with cultures of *Psathyrella atroumbonata* (Pegler) and *Schizophyllum* commune (Fr. Ex. Fr.). Afric. Jour. of Biomed. Res. 2004;7:139-143.
32. James CS. Analytical Chemistry of Foods, Chapman and Hall, New York. 1st Edition; 1995.
33. Fasidi IO, Kadiri M. Effect of sporophores maturity on chemical compoisiton of *Volvariella esculenta* (Mass) Singer, a Nigerian edible mushroom. Die Nahrung. 1993;37:269-273.
34. Gbolagade J, Ajayi A, Oku I, Wankasi D. Nutritive Value of Common Wild Edible Mushrooms from Southern Nigeria. Glob. Journ of Biotech. & Biochem. 2006;1(1):16-21.
35. Chang ST, Lau OW, Cho KY. The cultivation and nutritive value of *P. sajor-caju*. European J. Appl. Micro. Biotech. 1981;12:58-62.
36. Hami H. Cultivation of oyster mushroom. (*Pleurotus* spp.) On saw dust of different woods. M.S.c. Thesis, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan; 1990.
37. Kavitha B, Rajannan G , Jothimani P. Utilization Of Empty Fruit Bunch Of Oil Palm As Alternate Substrate For The Cultivation Of Mushroom. Int. J. Sci., Env and Tech. ISSN 2278-3687. 2013;2(5):839–846.
38. Pathmashini L, Arulnandhy V, Wilson Wijeratnam RS. Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) On Sawdust. Cey. J. Sci. (Bio. Sci.). 2008;37(2):177-182.
39. Ghibom B, Minkyung S, Seungyong L, Seokhwan H. Response surface analysis of solid state growth of *Pleurotus ostreatus* mycelia utilizing whey permeate, Biotech. Letters. 2005;27:1537–1541.
40. Andrej G, Mirjan V, Jure P. Cultivation Techniques and Medicinal Properties of *Pleurotus* spp. Food Technol. Biotechnol. 2007;45(3):238–249.

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