Original Research Article

Effect of continuous iron pollution on morphology and DNA profile of *Pleurotus tuber-regium*

ABSTRACT

Aims: Iron, the fourth most abundant micronutrient in the soil has been classified as a metal of environmental concern, the study was to assess the effect of continuous iron pollution on growth and the molecular profile of *Pleurotus tuber-regium*.
Study design: Experimental research design.
Place and Duration of Study: The Bells University of Technology, between December 2019 and October 2020.
Methodology: *P. tuber-regium* sclerotia procured locally were seeded into 1 kg soil polluted with 20, 40, 60, and 80 mg kg^{-1} $FeSO_4$ respectively. Watering was done with the respective Fe solutions for 59 days at the rate of 100ml/48hrs. The Stipe length, pileus diameter, fruitbody weight, and days for fruitbody emergence were recorded for each treatment. The concentration of iron in soil and mushroom was determined by Atomic Absorption Spectrophotometer (AAS). DNA extracted from the fruitbodies was subjected to Random Amplified Polymorphic DNA (RAPD) analysis using five RAPD primers and a cluster analysis was performed.
Results: Fruiting was achieved in all soils (0, 20, 40, 60, 80 mg kg^{-1}) after 23, 17, 50, 58, 59 days respectively. Iron had no significant effect on stipe length while pileus diameter and fruitbody weight increased only at 20mg kg^{-1}. Iron concentration in the soil and mushroom increased as Fe pollution was increased. The bioaccumulation factor was higher in contaminated soil and was between 0.78 and 1.08. The RAPD primers amplified 53 band sizes ranging from 100 - 700 bp and the DNA was clustered in a major group. The percentage polymorphism was 16.9% indicating a low level of genetic variation.
Conclusion: The result indicated that continuous Fe pollution at the stated concentrations did not have any significant effect on the DNA profile of *Pleurotus tuber-regium* but the morphological growth of the mushroom was enhanced at low iron contamination levels.

Keywords: DNA profile, Iron, Morphology, Pleurotus tuber-regium, contamination

1. INTRODUCTION

Soil is a reservoir for many organic, inorganic, synthetic, and toxic substances. The quality of soil has received attention due to the increased industrialization and mechanization which have given rise to the accumulation of pollutants in the environment. The most frequent contaminant of soil is heavy metals. Heavy metals are persistent in soil and a threat to

environmental and public health due to their potential reactivity, toxicity, and mobility [1,2]. Though, some heavy metals, for instance, copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn) are biologically essential and are needed for some physiological functions.

Iron is the fourth most abundant micronutrient in the soil and has been classified alongside Cu, Cd, and Pb as a metal of most immediate concern [3]. Iron is an integral part of several enzymes, participates in redox reactions, and is an essential part of hemoglobin, a protein in human blood which transports oxygen from the lungs to other tissues. Iron is needed in definite proportion and could be toxic at a high threshold. Due to its use in engineering, construction, and automobiles, iron accumulates in the soil and could be of concern. Most soils contained appreciable quantities of iron higher above the WHO standard and the highest concentration was found in the soils obtained from different auto-mechanic locations in Nigeria [4,5]. Food chain contamination with heavy metals has been a source of worry due to potential accumulation in biosystems and could lead to a number of disorders.

Mushrooms are saprophytic fungi and their use as food and alternative source of protein is well recognized in African culture as they contain appreciable protein, fiber, minerals, and vitamins; hence are referred to as nutraceuticals and health food [6]. Mushrooms grow on soil and substrates and produce a wide range of enzymes for the breakdown of complex organic matter which they absorb into their cells [7]. They have a very effective mechanism for bioaccumulation of heavy metals from the ecosystem higher than agricultural crops [8,9]. *Pleurotus tuber-regium,* a tuberous edible mushroom that produces fruitbody from a sclerotium has served as food and has also been employed in the bioremediation of toxic hydrocarbon, crude oil, diesel, and heavy metals [10,11,12,13]. *P tuber-regium* bio-accumulated higher amounts of Fe than Cu in its sporophore in a study conducted by [14]. The lack of proper environmental pollution management coupled with the economic importance of oyster mushrooms as food and medicine in Africa has raised concerns about the impact of heavy metal pollution on edible mushrooms. Therefore, the effect of heavy metals on food safety and marketability, crop growth, and the phytotoxic and environmental

health of organisms need to be investigated. The study was to assess the effect of continuous iron pollution on the growth and genetic profile of *Pleurotus tuber-regium*.

2. MATERIALS AND METHODS

2.1 Materials

Pleurotus tuber-regium sclerotia were purchased from a local market in Ota Ogun State Nigeria and identified by the Waste Utilization Department (Mushroom unit) of Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. Loamy soil was collected from the premises of the Bells University of Technology Ota, Ogun State, Nigeria.

2.2 Preparation of soil and inoculation of sclerotia

The loamy soil was dried at 80^{o}C for 72 h and sieved with 0.1 mm mesh size. The dried soil (1kg) was measured into perforated plastic bowls measuring 20 x 10 cm. Different concentrations of $FeS0_4$ $7H_2O$ (20, 40, 60, 80 mg kg^{-1}) were prepared and uncontaminated soil (0 mg kg^{-1}) served as the control. The soil was moistened with the respective iron sulfate solution. *Pleurotus tuber-regium* sclerotium (30g) soaked in sterile water for 18 h was seeded in each of the bowls. Watering was done with 100ml of the iron sulfate solution every 48 h. The stipe length, cap diameter, fresh weight, and period of sporophore emergence were observed and recorded in mm and days respectively.

2.3 Determination of Fe concentration in the soil and mushroom

The percent iron concentration in soil and mushroom was determined by Atomic Absorption Spectrophotometer according to [15].

2.4 DNA extraction

DNA was extracted with CTAB according to the method [16] and subjected to Random Amplified Polymorphic DNA (RAPD) analysis using five decamer RAPD primers, OPT01, OPT04, OPB10, OPB12, and OPB13 [17]. Amplified PCR products were electrophoresed in

1% agarose gel and visualized by staining with ethidium bromide solution (0.5µg/ml) while banding patterns were photographed using a Bio-Rad UV Trans-illuminator. The gels were scored for the presence or absence of reproducible bands and cluster analysis was performed by the unweighted pair group method of arithmetic (UPGMA).

3. RESULTS AND DISCUSSION

3.1 Effect of continuous iron pollution on the morphology of *Pleurotus tuber-regium*

The effect of continuous iron pollution on the morphology of *P. tuber-regium* is presented in (Fig. 1). Matured fruit bodies of *Pleurotus tuber-regium* were produced in all the soils, including the control and Fe contaminated soils. However, the emergence of the fruit body was fastest in 20 mg kg^{-1} Fe contaminated soil and delayed in soils with higher contamination. Fruiting occurred after 17 days of planting in 20 mg kg^{-1} contaminated soil unlike in other soils which took place after 50, 58, and 59 days respectively.

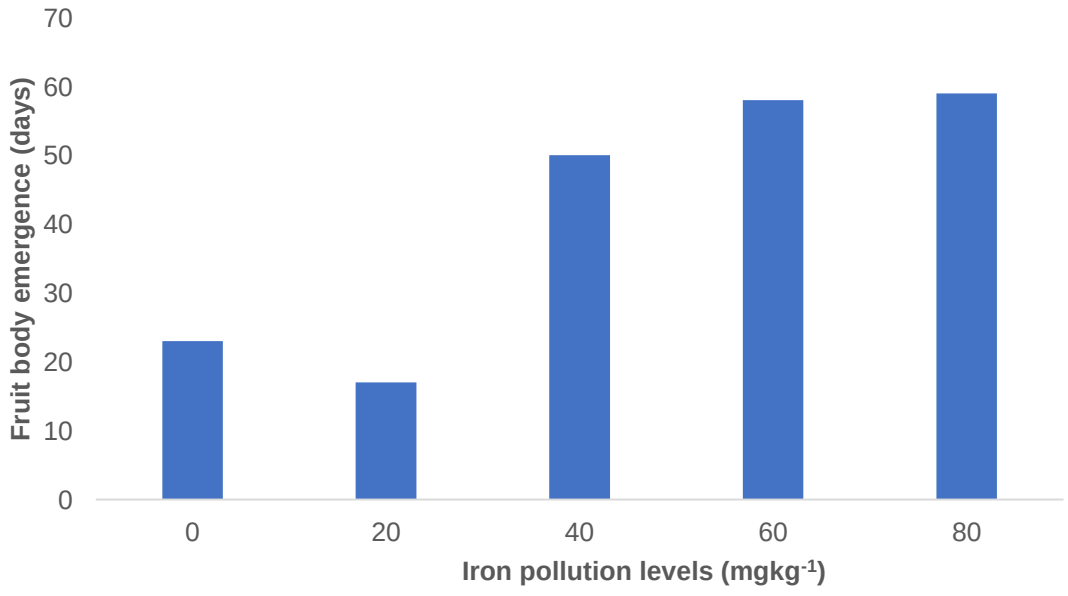

Fig. 1: Effect of continuous iron pollution on *P. tuber- regium* fruit body emergence

The effect of Fe contamination on the fresh weight of *P. tuber-regium,* pileus diameter, and length of the stipe is shown in (Fig. 2). The fresh fruit body weight and pileus diameter increased at 20 mg kg^{-1} and reduced thereafter with increased Fe contamination. Fusun et al [18] observed that fresh and dry weights of shoots of soybean seedlings were highest at 15 mg kg^{-1} Nano Fe application, and further increase caused a drastic reduction in weights. In another study, the iron concentration at 175 mg L^{-1} and higher levels strongly inhibited the mycelial growth of *Pleurotus ostreatus* [19]. This could be attributed to the fact that Fe is an essential micronutrient required by organisms in relatively definite quantities which explained why a low level of iron supported better growth than higher levels. The WHO permissible level of Fe in plants and foods/vegetables is 20 mg/kg and 48 mg/kg respectively [20,21]. Excess iron has been reported to produce stunted growth of roots and tops on some plants and its accumulation to high levels can be toxic [22].

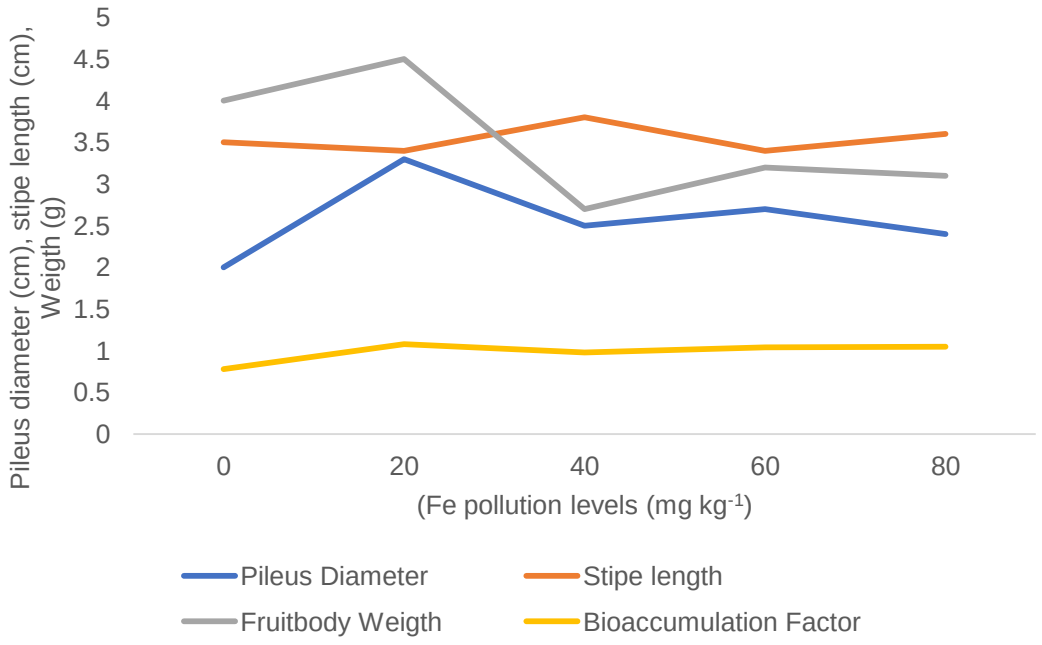

Figure 2: Effect of continuous Fe pollution on the morphology of *P. tuber-regium*

A direct linear correlation between pileus diameter and fruit body weight of *P. tuber-regium* was observed in a study by [23]. Also, a good linear correlation between cap diameter and biological efficiency was observed for mushrooms cultivated on gamma-irradiated and steam sterilized sawdust [24]. This suggested a relationship between the pileus diameter and fresh fruit bodyweight of mushrooms.

The concentration of iron in mushrooms and soil presented in Fig. 3 showed that iron concentration in both soil and mushroom increased as the iron pollution increased. The highest iron concentration was observed at 80 mg kg^{-1}. Values ranged between 1.6 and 20.2 mg kg^{-1} and 2.0 to 19.3 mg kg^{-1} in mushroom sporophore and soil respectively. The iron content recorded in this study was within FAOWHO permissible limits.

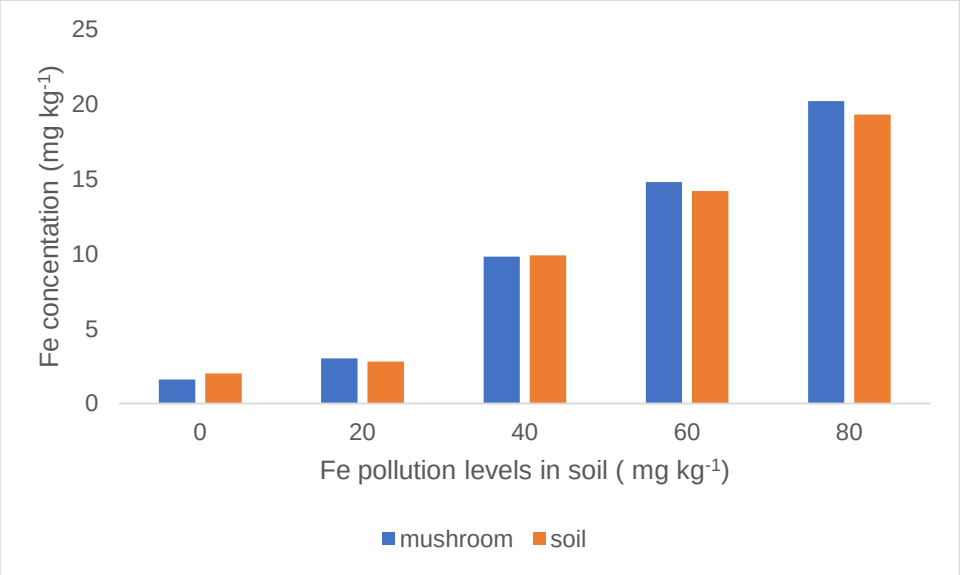

Figure 3: Iron concentration in *Pleurotus tuber-regium* and soil

[25] recorded Fe levels of 0.56 mg kg^{-1} and 4.50 mg kg^{-1} in food crops planted in non-mining areas of Nasarawa State. Iron levels in mushroom samples from Tokat in Turkey ranged from 568 and 3562 mg/kg dry weight [9]; while 8.25 and 58.25 mg kg^{-1} Fe concentrations were found in mushrooms collected from Iselu market in Benin city Nigeria [26]. In another study, the highest iron bioaccumulated in the mycelium of *Pleurotus ostreatus* was 3500 mg kg^{-1} and was produced with an iron addition of 300 mg L^{-1} [19]. The content of metallic elements in many mushroom species was considerably higher than in fruits and vegetables [27]. Variations in iron levels in mushrooms can be attributed to differences in species absorption potential/uptake levels, levels in the substrate, and physicochemical properties of the soil. Iron availability in the soil and uptake is largely determined by the interaction of soil acidity and aeration [28]. At very low soil pH levels and in waterlogged conditions, iron is reduced from its oxidized Fe^{3+} form to its highly soluble and readily available Fe^{2+} form [29]. However, the iron values in the mushroom recorded in this work were within the limits

recommended by international organizations like FAO/WHO expert committee on Food Additives.

The bioaccumulation factor (BF) is an indication of the ability of the mushroom to tolerate and accumulate heavy metals and is measured as a ratio of the metal concentration in the fruitbody to the metal in the soil. The bioaccumulation Factor recorded in this study was higher in polluted soils than in control and was considered significant. The range was between 0.78 and 1.08; the highest was at 20 mg kg^{-1} iron pollution. in a similar study, the highest bioaccumulation of iron was obtained in a culture medium with 150 mg L^{-1} of iron [19]. This suggested that continuous iron pollution at the stated concentrations was tolerated and bioaccumulated by *Pleurotus tuber-regium*. Iron is a microelement needed for metabolic activity in living organisms and the bioaccumulated quantity was still within the permissible limit. Iron concentrations in plants from dumpsites were below the tolerable limits recommended by WHO as reported by [21].

The DNA profile of the mushrooms using the five RAPD primers revealed a total of 53 bands ranging from 7 (OPB-13) to 16 (OPB-10) with sizes in the range of 100 bp to 700 bp. Out of the 53 scorable bands, 44 were monomorphic (83.0%) while 9 were polymorphic (16.9%; Table 1). Figure 4 showed the RAPD amplified pattern with primers OPT-01, OPT-04, and OPB-12. The percentage polymorphism was 16.9% indicating there was not much variation in the DNA profile. This indicated that continuous Fe contamination at the stated concentrations had no significant effect on the DNA profile of *Pleurotus tuber-regium* rather the pileus diameter, fresh weight, and fruit body emergence were enhanced at a lower level of contamination.

Table 1: The five RAPD primers employed for the amplification of DNA for verifying the genetic profile of *Pleurotus tuber-regium*

RAPD primers	Nucleotide Sequence	No of Bands scored	No of polymorphic bands	% poly-morphism
OPT01	5-GGG GCA CTC A–3	13	1	7.6
OPT04	5-CAC AGA GGG A-3	7	2	40.0
OPB10	5- CTG CTG GGA A -3	16	2	14.2
OPB12	5- CCT TGA CGC C -3	10	1	11.1
OPB13	5- TTC CCC CGC T -3	7	3	75.0
TOTAL		53	9	16.9

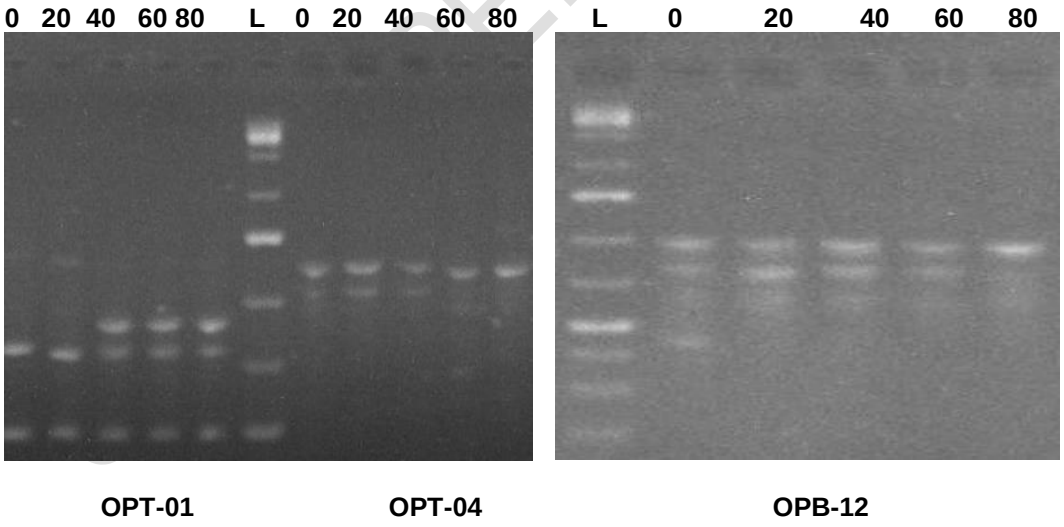

Fig. 4: Gel profiles of RAPD amplification with primers OPT-01, OPT-04, and OPB-12. L- 100 bp DNA ladder; Lanes 0 - 80 (Fe pollution levels in mg kg^{-1})

4. CONCLUSION

The study showed that continuous iron pollution at 20 mg kg^{-1} enhanced the pileus diameter, fresh fruit body weight, and fruit body emergence of *Pleurotus tuber-regium* while higher concentrations caused a subsequent decrease. Iron pollution at the studied concentrations showed no variation in the DNA profile.

REFERENCES

1. Zafar S, Aqil F, Ahmad I. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour. Technol.2006;**98:**2557–2561.

2. Moldoveanu AM. Assessment of Soil Pollution with Heavy Metals in Romania. Environmental Risk Assessment of soil contamination, ch.9, InTech, Rijeka/Croatia.; 2014

3. FAO/WHO. Evaluation of Certain Food Additives: Seventy-first Report of the Joint FAO/WHO Expert Committee on Food Additives. 2010:956

4. Idugboe SO, Tawari-Fufeyin P, Midonu AA. Soil pollution in two auto-mechanic villages in Benin City, Nigeria. IOSR J Environ Sci Toxicol Food Technol 2014;8:9-14.

5. Abidemi OO. Levels of Pb Fe Cd and Co in soils of automobile workshop in Osun State, Nigeria. J Appl Sci Environ Management 2011;15(2):10-15
Doi: 10.4314/jasem.v15i2.68510

6. Isikhuemhen SO, LeBauer DS. Growing *Pleurotus tuber-regium.* Mush World Publication 2004:264–274.

7. Stamets P. Mycelium Running. How mushrooms can help save the world,1st Edn. Tenspeed Press, Berkeley/Toronto. 2005:339.

8. Turkdogan MK, Fevzi K, Kazim K, Ilyas T, Ismail U. Heavy metal in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environ. Toxicol. Pharmacol **2003;**13:175–179.

9. Turkekul I, Elmastas M, Tuzen M. Determination of iron, copper, manganese, zinc, lead, and cadmium in mushroom samples from Tokat. Food Chem 2004;84:389-392.

10. Isikhuemhen OS, Anoliefo GO, Oghale OI. Bioremediation of crude oil polluted soil by the white-rot fungus, *Pleurotus tuber-regium* (Fr.) Sing. Environ Sci Poll Res 2003;10:108-112.

11. Adenipekun CO. Bioremediation of engine-oil polluted soil by *Pleurotus tuber-regium* Sing., a Nigerian white-rot fungus. Afr J Biochem 2008;7:55-58.

12. Ogunbayo AO, Bello RA, Nwagbara I. Bioremediation of engine oil-contaminated site J EmergTrends Eng Appl Sci 2012;3(3):483-489

13. Ndimele CC, Ndimele PE, Chukwuka KS. Accumulation of heavy metals by wild mushrooms in Ibadan Nigeria. J Health Pollut 2017;7(16):26-30. Doi: 10.5696/2156-9614-7.16.26

14. Anyakorah CI, Jinadu T. The effect of continuous contamination of soil with heavy metals on growth of *Pleurotus tuber-regium*. Curr Res Environ Appl Mycol 2015;5(4):362-366.

15. AOAC. Official Methods of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC. 2005.

16. Chen WW, Yang JL, Qin C, Jin CW, Mo JH, Ye T. Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis*. Plant Physio 2010;**154**:810– 819.

17. Ekun VS, Adenipekun CO, Ogunkanmi LA, Ojuederie OB, Igwe DO. Molecular characterization of *Auricularia* spp from south-western Nigeria using Random Amplified Polymorphic DNA (RAPD) markers. Niger J Biotech 2018;35:1-5. DOI: 10.4314/njb.v35i1.5

18. Fusun G, Halil IY, Tugba HG, Murat S. Effects of iron sources and doses on plant growth criteria in soybean seedlings. EURasia J Soil Sci. 2019;8(4):298-303. DOI 10.18393/ejss.582231.

19. Almeida SM, Umeo SH, Marcante RC, Yokota ME, Valle JS, Dragunski DC, et al. Braz J Microbiol 2015;46:195-200.

20. Afzal Shah, Abdul Niaz, Nazeef Ullah, Ali Rehman, Muhammad Akhlaq, Muhammad Zakir, et al. Comparative Study of Heavy Metals in Soil and Selected Medicinal Plants. J Chem 2013;2013:5-11

21. Opaluwa OD, Aremu MO, Ogbo LO, Magaji JI, Odiba IE, Ekpo ER. Assessment of heavy metals in water, fish and sediments from UKE stream, Nasarawa state, Nigeria, Curr World Environ 2012;7(2):213-220

22. Connoly EL, Guerinot ML. Iron Stress in Plants. Genome Biol 2002;3(8):1024.1-1024.4 Doi: 10.1186/gb-2002-3-8-revews1024.1-reviews1024.4

23. Anyakorah CI**,** Nwude D, Jinadu T. Lead accumulation in oyster mushroom, *Pleurotus tuber-regium* (Sing) from a continuously lead contaminated soil. Mycosphere 2015;6(2):145–149.

24. Kortei NK, Odamtten GT, Obodai. Correlations of cap diameter (pileus width), stipe length and biological efficiency of *Pleurotus ostreatus* (Ex.Fr.) Kummer cultivated on gamma-irradiated and steam sterilized composted sawdust as an index of quality for pricing. Agric Food Secur 2018;7:35. https://doi.org/10.1186/s40066-018-0185-1

25. Aremu MO, Atolaiye BO, Labaran L. Environmental implication of metal concentration in soil, plant foods and pond in area around the derelict Udege mines of Nasarawa State, Nigeria. Bull Chem Soc Ethiop 2010;24(3):351–360.

26. Udochukwu U, Nekpen BO, Udinyiwe OC, Omeje FI. Bioaccumulation of Heavy metals and pollutants by edible mushroom collected from Iselu market Benin-city. Int J Curr Microbiol App Sci 2014;3(10):52-57.

27. Kalac P. Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000–2009. Food Chem 2010;122:2–15.

28. Thomine S, Lanquar, V. Iron Transport and Signaling in Plants. In: Geisler, M., Venema, K. (eds) Transporters and Pumps in Plant Signaling. Signaling and Communication in Plants, vol 7. Springer, Berlin, Heidelberg. 2011:99-131. https://doi.org/10.1007/978-3-642-14369-4_4

29. Fageria NK, Baligar VC, Wright RJ. Iron nutrition in plants: An overview on the chemistry and physiology of its deficiency and toxicity. Pesq Agropec Bras Brasflia 1990;25:553-570.