academicJournals

Vol. 13(22), pp. 2208-2214, 28 May, 2014
DOI: 10.5897/AJB2014.13733
Article Number: E70DF0244897
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Heavy metal and proximate composition associated with the composting of cassava (*Manihot esculenta*) peels used in the cultivation of mushrooms in Ghana

Obodai Mary[1]*, Ofori Hayford[1], Dzomeku Matilda[1], Takli Richard[1], Komlega Gregory[1], Dziedzoave Nanam[1], Mensah Deborah[1], Prempeh Juanita[1] and Sonnenberg Anton[2]

[1]Council for Scientific and Industrial Research (CSIR)-Food Research Institute, PO Box M20, Accra, Ghana.
[2]Plant Research International, Wageningen, Netherlands.

Received 17 February, 2014; Accepted 8 May, 2014

Changes in the heavy metal content and proximate composition during the 28 day composting of cassava peels used in the cultivation of the oyster mushrooms *Pleurotus ostreatus* strain EM-1 was studied. Significant dry weight variations of cellulose, hemicellulose and fat contents were observed from day 0 to 12. Decreases from day 12 to 28 had the values of 15.4, 57.6 and 56.12%, respectively, while lignin, protein and crude fibre values showed a gradual increase from day 0 to 28, with maximum values of 23.73, 49 and 73%, respectively. Cyanide content however showed a reduction from the initial 3.89 to 2.01 mg/L by day 12 and a marginal increase of 16 by day 28. This was however not detected in the mushroom harvested. The levels of heavy metal content in composted cassava peels in decreasing order was iron (Fe), manganese (Mn), zinc (Zn), lead (Pb) and copper (Cu) while that for uncomposted cassava was Fe, Zn, Pb, Mn and Cu. Levels of Cu, Mn, Pd and Zn in mushroom samples analysed were in agreement with reported values in literature. Of all the heavy metals examined, iron accumulated excessively, indicating that *P. ostreatus* strain EM-1 is a good bio-accumulator of Fe.

Key words: Cassava, composting, heavy metals, production.

INTRODUCTION

Production of mushrooms has increased over the years in several countries from a few metric tons to thousands of tons. In China, for example, which is the largest producer of various mushrooms in the world, there has been a 224.3% increase in production over a ten year period (2000-2010) (Li, 2012). These increases in production have grown as a result of the fact that mushrooms are no longer only consumed for its flavor and nutritional benefits but also for functional properties they exhibit. These include among others properties such as being anti-mutagenic, anti-tumoral and anti-viral (Garcia-Lafuente et al., 2011). These functional characteristics are mainly due to their chemical composition (Manzi et al., 2001).

In Ghana, this increase in trend has shown no exception and mushroom production has grown at a steady rate from 120 tons in 2010 to approximately 300 metric tons to date. *Pleurotus ostreatus* mushroom is the main

*Corresponding author. E-mail: obodaime@yahoo.com. Tel: +233 (0) 207 930 703.

species that is produced and is presently cultivated on sawdust. There are however other agricultural by-products available that can be used in mushroom production in both urban and peri-urban areas of Ghana and the subtropics. These include among others, cassava peels, plantain leaves and corn cobs. Among these, cassava peels is the most abundant and has the greatest potential as a substrate for mushroom production. At present, cassava by-product such as peels are in large part unexploited and often discarded along roads and on fields, thus forming a nuisance in the environment.

Cassava (*Manihot esculenta* Crantz) is the sixth most important food crop produced annually globally (FAOSTAT, 2010), and is a staple food for approximately 800 million people (FAO/IFAD, 2000; Lebot, 2009). The annual production of cassava in Ghana is approximately 14.2 million metric tonnes (MT) (FAO, 2013). The peel is a by-product of processing the roots for starch, cassava flour and "gari" (a fermented cassava meal product) constituting 11% of the root, with approximately 3.6 million MT of peels and discharged parts and 400,000 MT (dry matter basis) of peels produced annually (Baah et al., 2011; FAO, 2013).

Cultivation of mushroom in Ghana is carried out on composted sawdust of *Triplochiton scleroxylon* or a combination of *Triplochiton scleroxylon* and *Chlorophora excels*a. Composting is a controlled self-heating, aerobic solid phase biodegradative process of organic materials (Ryckeboer et al., 2003). It is a solid-waste fermentation process, which exploits the phenomenon of microbial degradation and mineralization (Mckinley and Vestal, 1984).

The main purpose of composting to a mushroom grower is to prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of other microorganisms. In several successive steps, microbial communities consume the more easy degradable organic components generating a substrate that is stable and increased in the fibrous components, humified forms and inorganic products, generating heat as a metabolic waste product.

Heavy metal concentration in mushroom is considerably higher than those in agricultural crop plants, vegetables and fruit. This suggests that mushrooms possess a very effective mechanism that enables them to readily take up some heavy metals from the ecosystem (Zhu et al., 2010), due to their dense mycelial system, which infiltrates the substrate (Garcia et al., 2005). The accumulation of heavy metals in mushrooms has been found to be affected by environmental and fungal factors. Environmental factors are organic matter content, pH and metal concentration in soil and fungal factors such as species of mushroom, morphological part of fruit body, developmental stages, age of mycelium, intervals between fructifications and biochemical composition (Radulescu et al., 2010).

This study was conducted to determine for the first time the changes in the heavy metal content and proximate composition during the composting of cassava peels, thus making it suitable for mushroom cultivation in Ghana and the heavy metal contents of mushrooms produced.

MATERIALS AND METHODS

Mushroom culture and spawn preparation

Cultures of *P. ostreatus* (Jacq.ex.Fr.) Kummer strain EM -1 originally from Mauritius and maintained on Malt Extract Agar slants were used to prepare sorghum grain spawn (Oei, 1991).

Cassava substrate preparation

Compost was prepared by the outdoor single-phase solid waste fermentation. Freshly milled dried cassava (*M. esculenta*) peels from a mixture of *Afisiafi* and *Bankye Hemaa* (local names) weighing 193 kg, obtained from the Volta Region of Ghana were mixed with rice bran (10% w/w) and lime (0.5% w/w) and composted as described by Obodai et al. (2007). The mixture was then stacked into a heap of about 0.8 m high and 1.0 m wide at the base and left to compost for 28 days with regular turning every 4 days. Before turning, temperatures were read and samples were taken from the core region of the compost with a pair of sterile forceps and its chemical and cyanide compositions evaluated. All samples were taken in duplicates.

At 28 days of composting, samples of the compost were adjusted to approximately 68 - 70% (Buswell, 1984) and then supplemented with rice bran (12%) and lime (0.5%). The mixtures were bagged, sterilized, incubated and mushrooms harvested as described by Obodai et al. (2007).

Chemical analysis

Samples of composting cassava peels taken at four days intervals from the central portion of the heap were put into sterile bags and quantitative estimation of crude fibre, cellulose, hemicellulose, lignin and fat were carried out using the standard methods as described by AOAC (2005). Lignin and cellulose were determined by acid detergent fibre (ADF) method (AOAC, 2005). Hemicellulose content was estimated by neutral detergent solution using 0.5 g of dried sample (AOAC, 2005). The difference between the acid detergent fibre and neutral detergent fibre gave the value for hemicellulose content. Crude fibre values were determined by AOAC (2005) method and calculated as:

$$\text{Crude fibre} = \frac{\text{Loss in weight on ignition (A - B)}}{\text{Initial sample weight}} \times 100$$

Where, A = Initial weight of sample before ignition, and B = final weight of sample after ignition.

To calculate total nitrogen in the samples, the specimens were dried at 60°C and analysed by the Micro kjeldahl Method (AOAC, 2005). To obtain crude protein value, nitrogen content values were multiplied by a factor of 6.25. For cyanide determinations, the method was in accordance with Obiri et al. (2007).

Heavy metal determination using atomic absorption spectrophotometer (AAS) analysis

Samples of dried uncomposted and composted cassava peel at 28

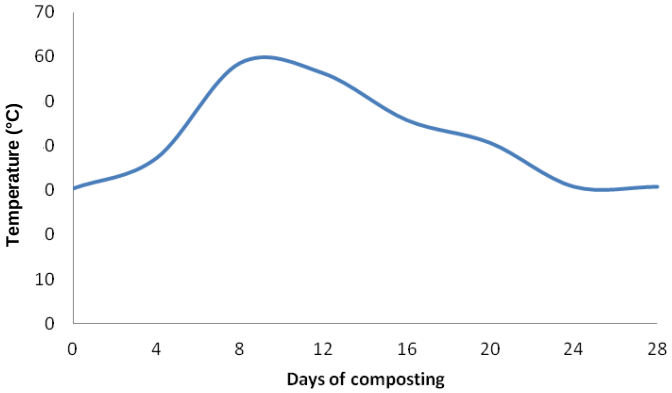

Figure 1. Tempearture changes during composting of cassava peels over a 28 day period.

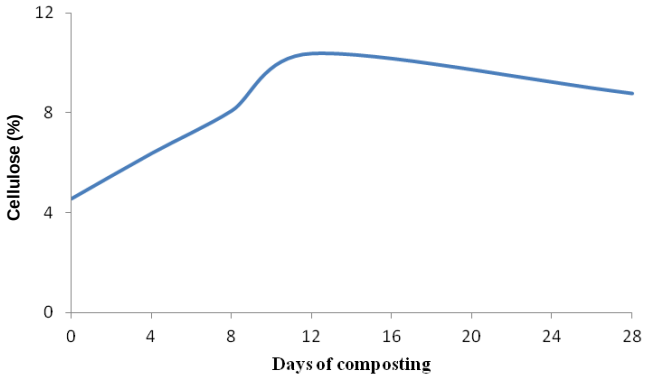

Figure 2. Changes in cellulose content during composting of cassava peels over a 28 day period.

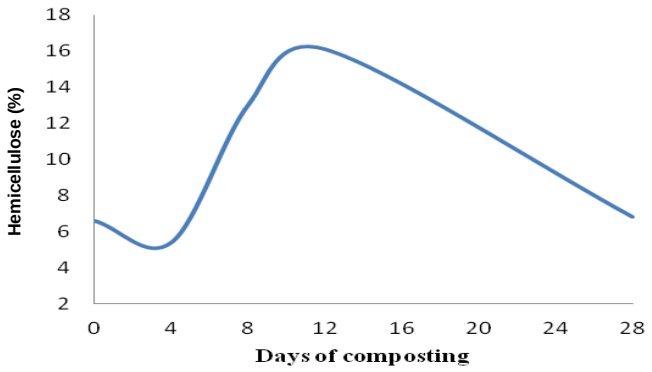

Figure 3. Changes in hemicellulose content during composting of cassava peels over a 28 day period.

days of composting, mycelia from *P. ostreatus* on composted cassava (PEM) after full spawn run, mycelia from *P. ostreatus* on uncomposted cassava (PEM1) after full spawn run, fresh mushroom from composted cassava, and mushroom from uncomposted cassava were milled using stainless steel laboratory blender. The dry ashing method was used for atomic absorption spectrophotometer (AAS) analysis (AOAC, 2005). All glasswares were washed with 1% nitric acid followed by demineralised water. Three grams (3 g) each of the above named samples were weighed into platinum crucibles. The crucible and the test portion were placed in the Muffle furnace at a temperature of 550°C for 8 h. The crucible with ash was put in a desiccator to cool. Five milliliters (5 ml) of nitric acid of mass fraction not less than 65%, having a density of approximately ρ (HNO_3) = 1 400 mg.mL^{-1} was added, ensuring that all the ash came in contact with the acid and the resultant solution heated on hot plate until the ash dissolved. Ten milliliters (10 ml) of 0.1 mol.L^{-1} nitric acid was added and filtered into 50 ml volumetric flask. The resultant solution was topped up to the mark with 0.1 mol.L^{-1} nitric acid. Blank solution was treated the same way as the sample. Buck Scientific 210VGP Flame AAS (Buck Scientific, Inc. East Norwalk, USA) was used to read the absorbance values at appropriate wavelength of the interested metal in the sample solution. Cathode lamps used were copper (Cu) (wavelength 324.8 nm, lamp current 1.5 mA), iron (Fe) (wavelength 248.3 nm, lamp current 7.0 mA), manganese (Mn) (wavelength 279.5 nm, lamp current 3.0 mA), lead (Pb) (wavelength 217.0 nm, lamp current 3.0 mA) and zinc (Zn) (wavelength 213.9 nm, lamp current 2.0 mA). Air/acetylene gas was used for all the analysis. The metal content of the samples were derived from calibration curves made up of minimum of three standards.

Statistical analysis

The standard deviations on mean values of duplicate samples were analysed using Statistical Package for Social Scientist (SPSS, 2005), version 16.0.

RESULTS AND DISCUSSION

During the composting of the cassava peels, various changes in the chemical components of the peels were observed. Temperatures within the heap became stable from day 24 to 28 (Figure 1). Significant increases of cellulose, hemicellulose and fat contents were observed up to day 12 (10.37, 16.1 and 9.39%, respectively) after which there were gradual declines of 15.4, 57.6 and 56.12%, respectively (Figures 2 to 4). The increase in cellulose, hemicellulose and lignin during the first 12 days will likely due to the consumption of starch by microorganisms. The decrease of these compounds (cellulose and hemicelluloses) in subsequent days indicate that when starch is removed mostly, microorganisms start to degrade also the (hemi) cellulose. Lignin, protein and crude fibre values showed a gradual increase from day 0 to 28, with a maximum value of 23.73, 49 and 73%, respectively (Figures 5, 6 and 7). These changes could be due to the type of microorganisms present in the substrate. Presumably, antibiosis was at play in the composting cassava peel by-product. The composting process involves microbial activity, chemical reactions, aeration, temperature and nutritional factors. Previous work carried out by Obodai et al. (2011) on decomposing sawdust of *Triplochiton scleroxylon* used in the cultivation of *P. ostreatus* showed

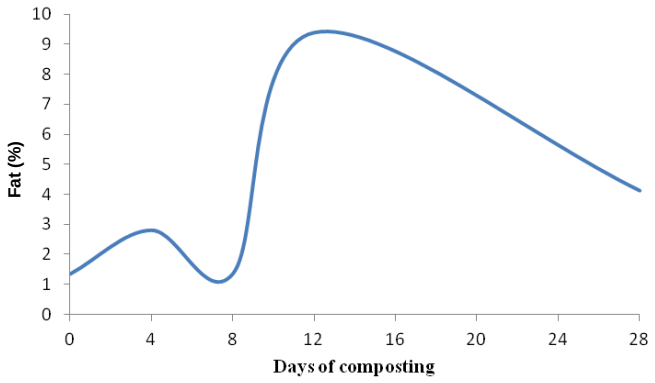

Figure 4. Changes in fat content during composting of cassava peels over a 28 day period.

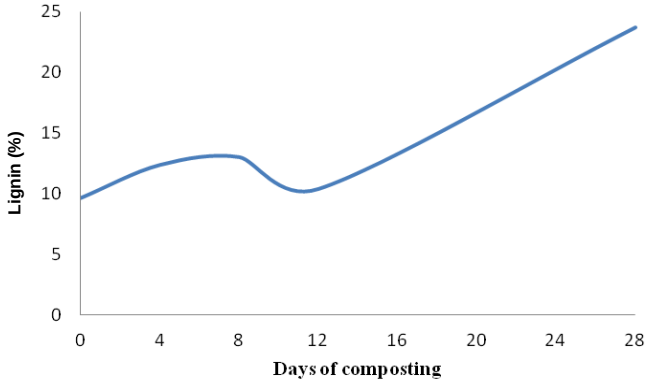

Figure 5. Changes in lignin content during composting of cassava peels over a 28 day period.

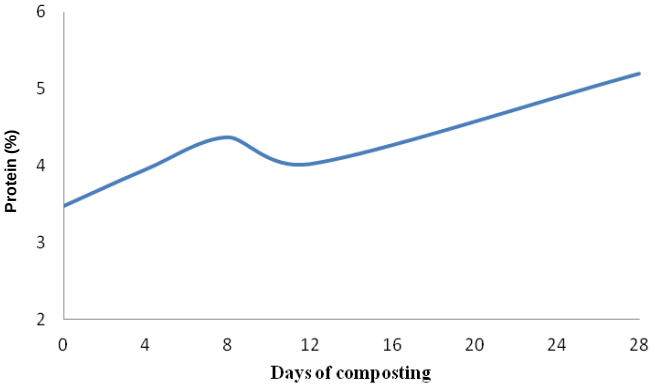

Figure 6. Changes in protein content during composting of cassava peels over a 28 day period.

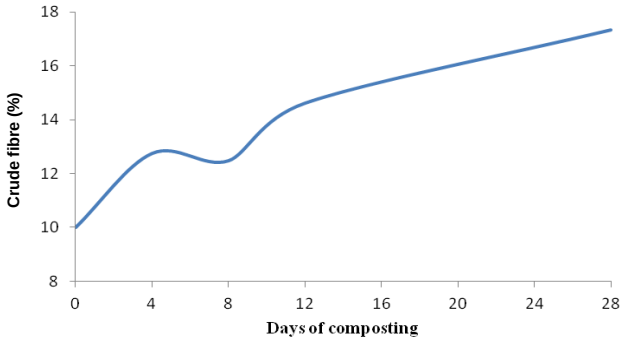

Figure 7. Changes in crude fibre content during composting of cassava peels over a 28 day period.

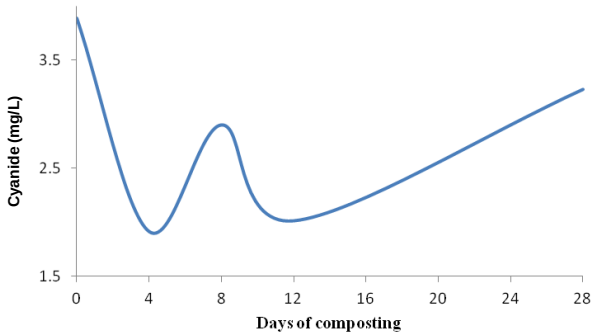

Figure 8. Changes in cyanide content during composting of cassava peels over a 28 day period.

decreasing amounts of cellulose, hemicelluloses and crude fibre as compared to lignin with increasing days of composting and attributed it to these components being easily degradable by fungi and bacteria present as compared to lignin a polymer of aromatic compounds which is very resistant and relatively difficult for cellulolytic organisms to decompose (Insam and de Bertoldi, 2003).

Cyanide however showed a reduction from the initial 3.89 to 2.01 mg/L by day 12 and a marginal increase of 16% by day 28 (Figure 8). This marginal increase in cyanide can probably be attributed to the microorganism at play during that period of composting.

Heavy metals

Metals such as iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are essential metals since they play an important role in biological systems, whereas aluminium (Al) and lead (Pb) are non-essential metals as they are toxic even in traces (Unak et al., 2007). The essential metals can also produce toxic effects when the metal intake is excessively elevated (Al-Khlaifat and Al-Khashman, 2007; Gopalani et al., 2007). Five metals namely, Cu, Fe, Mn, Pb and Zn were analysed in *P. ostreatus* mushroom and in the substrates on which they

Table 1. Heavy metal contents ($mgkg^{-1}$) of cassava peel substrates and mushrooms.

Sample	Cu	Fe	Pb	Mn	Zn
Uncomposted cassava	3.7 ± 0.08[a]	380.22 ± 8.84[b]	21.3 ± 2.63[b]	20.24 ± 1.09[a]	24.06 ± 1.09[b]
Composted cassava	6.96±1.33[a]	592.16±37.97[d]	30.97±1.56[c]	42.46±0.47[b]	38.58±0.92[c]
PEM + composted cassava only	8.14±0.52[a]	388.39±11.23[b]	11.54±3.81[a]	25.48±0.47[a]	34.07±0.66[c]
PEM on uncomposted cassava	5.44±1.07[a]	496.82±19.40[c]	32.58±4.65[c]	36.16±3.29[b]	23.57±0.53[b]
Mushroom from composted cassava	28.72±4.67[c]	70.50±20.89[a]	8.34±2.17[a]	63.23±7.58[c]	13.68±0.57[a]
Mushroom from uncomposted cassava	17.44±0.20[b]	86.67±2.89[a]	3.75±0.24[a]	45.68±2.23[b]	12.03±0.62[a]

Results presented as mean concentrations ± standard deviations. Superscript figures in the same column represent significant or insignificant differences at $p \leq 0.05$ (ANOVA, Duncan test, $p \leq 0.05$). Results are on dry weight basis. Cu = copper; Fe = iron; Pb = lead; Mn = manganese; Zn = zinc.

were grown. Of all the heavy metals examined, Fe accumulated more excessively than Cu, Pb, Mn and Zn. The mean concentration of Fe was 380±8.84, 592.16±37.97, 388.39±11.23, 496.82±19.40, 70.50±20.89, 86.67±2.89 mg/kg (dry weight basis) (Table 1) for uncomposted cassava peels, composted cassava peels, *P. ostreatus* mycelium (PEM) on composted cassava peels, PEM1 on uncomposted cassava peels, mushroom harvested from composted cassava peels and mushroom harvested from uncomposted cassava peels, respectively.

The maximum and minimum Fe values determined in the mushroom samples were 86.67±2.89 and 70.50±20.89 mg/kg, respectively which far exceed the limit of 15 mg/kg set by WHO (1982). Iron (Fe) deficiency anemia for instance affect one third of the worlds population. On the other hand, excessive intake of iron is associated with an increase risk of colorectal cancer (Senesse et al., 2004).

The high values of Fe recorded in the mushrooms can be attributed to the Fe content in the cassava peels before and after composting (Table 1). Also it has been reported that the part of the mushroom analysed, stipe or cap and the age of the mushroom plays a major part in the amount of heavy metal content detected (Radulescu et al., 2010).

Statistically, there was no significant difference in the level of Fe determined in mushroom from composted and uncomposted cassava peels at $p \leq 0.05$. Iron levels reported in this study were however far lower than those reported by other authors. Sesli and Tùzen (1999) reported values such as 31.3-1,190 mg/kg, Isiloğlu et al. (2001) had values such as 180-407 mg/kg and 146-835 mg/kg was reported by Tùzen (2003).

Copper is an essential constituent of some metalloenzymes and is required in haemoglobin synthesis and in the catalysis of metabolic growth (Silvestre et al., 2000). Copper concentrations determined ranged from 3.7±0.08 to 28.72±4.67 mg/kg which are below the safe limit set by World Health Organization (WHO) (40 mg/kg) as copper in foods (WHO, 1982). At $p \leq 0.05$, there was significant difference between the concentrations of Cu in mushroom from composted cassava and mushroom from uncomposted cassava. Cu levels in mushrooms reported in literature are 4.71-51.0 mg/kg (Tùzen et al., 1998), 13.4-50.6 mg/kg (Soylak et al., 2005) and 12-181 mg/kg (Tùzen, 2003).

Lead (Pb) is toxic even at trace levels (Dobaradaren et al., 2010) and the impairment related to Pb toxicity in humans include abnormal size and haemoglobin content of the erythrocytes, hyper stimulation of erythropoisis and inhibition of haem synthesis according to Vonugopal and Lucky (1975). The maximum and minimum values of Pb concentrations determined in the mushrooms were 8.34±2.17 and 3.75±0.24 mg/kg on composted and uncomposted cassava peels, respectively, which were below the 10.0 mg/kg limit that has been set by WHO as Pb content in raw plant materials (WHO, 1982). However, the difference in Pb levels between the mushroom from composted cassava and mushroom from uncomposted cassava was statistically not significant at $p \leq 0.05$. Pb levels reported in literature are 0.75-7.77 mg/kg (Tùzen et al., 1998), 0.40-2.80 mg/kg (Svoboda and Kolac, 2003) and 1.43-4.17 mg/kg (Tùzen, 2003).

According to Unak et al. (2007), Mn is an essential metal and it plays an important role in biological systems such as its presence in metalloproteins. The highest and lowest Mn concentrations determined in both mushrooms from composted cassava and mushroom from uncomposted cassava were 63.23±7.58 and 45.68±2.23 mg/kg, respectively which are far below the toxicity limit between 400-1000 mg/kg of Mn in plant. However, at $p \leq 0.05$, the difference in the levels of Mn between mushroom from composted cassava peels and mushroom from uncomposted cassava peels is significant. Varying ranges of Mn values have been reported such as 14.5-63.6 mg/kg (Isiloğlu et al., 2001), 12.9-93.3 mg/kg (Tùzen, 2003), 14.2-69.7 mg/kg (Soylak et al., 2005).

Zinc (Zn) is an essential metal and a component of a wide variety of different enzymes in which it is involved in catalytic, structural and regulatory roles. It constitutes about 33 ppm of adult body weight and is essential as a constituent of many enzymes involved in a number of physiological functions, such as protein synthesis and

energy metabolism (Ma and Betts, 2000). WHO has recommended permissible limit of Zn in foods such as 60 mg/kg (WHO 1982). The maximum and minimum concentration of Zn determined in mushroom samples were 13.68±0.57 and 12.03±0.62 mg/kg, which are below the safe limit of Zn in food set by WHO. There was no significant difference in the concentration of Zn in mushroom from composted cassava peels and mushroom from uncomposted cassava peels at $p \leq 0.05$. The order of heavy metal accumulation in mushrooms harvested from both composted and uncomposted cassava peels was Fe>Mn>Cu>Zn>Pb while it was Fe>Zn>Pb>Mn>Cu, Fe>Mn>Zn>Pb>Cu, Fe>Zn>Mn>Pb>Cu, Fe>Mn>Pb>Zn>Cu for uncomposted cassava peels, composted cassava peels, PEM on composted cassava peels, PEM1 on uncomposted cassava peels, respectively. Of all the heavy metals examined, Fe was accumulated excessively than Cu, Pb, Mn and Zn indicating that *P. ostreatus* strain EM-1 is a good bio-accumulator of iron.

Conflict of Interests

The author(s) have not declared any conflict of interests

ACKNOWLEDGEMENT

This work was funded by the Gains from losses of Root and Tuber Crops (GRATITUDE), an EU funded project

REFERENCES

AOAC (2005). Official methods of analysis of AOAC International, 18th Edition. AOAC International, Gaithersburg, Maryland, USA.

Al-Khlaifat AL, Al-Khashman OL (2007). Atmospheric heavy metal pollution in Aqaba city Jordan using *Phoenix dactylifera* L. leaves. Atmos. Environ. 41:8891-8897.

Baah J, Tait RM, Tuah AK (2011). Selecting browse plants to supplement cassava peel-based diet for peri-urban small ruminants. Small Ruminant Res. 96:36-40.

Buswell JA (1984). Potentials of spent mushroom substrates for bioremediation purposes. Compost 2:31-35.

Dobaradaren S, Kaddafi K, Nazmara S, Ghaedi H (2010). Heavy metals (Cd, Cu, Ni, and Pb) content in fish species of Persian Gulf in Bushehr Port, Iran. A. J. Biotech. 32:6191-6193.

FAO (2013). MAFAP- SPAAA: Monitoring African foods and Agricultural policies: Analysis of incentives and disincentives for cassava in Ghana http://www.fao.org/fileadmin/templates/mafap/documents/technical_notes/GHANA/GHANA_Technical_note_CASSAVA_EN_Feb 2013.pdf

FAO/IFAD (2000). The World Cassava Economy:Facts, Trends and Outlooks, Food and Agriculture Organization of the United Nations and International Fund for agricultural Development. Rome.

FAOSTAT (2010). Food and agricultural Commodities production. Food and Agricultural Organization of the United Nations Statistics Database.

García MA, Alonso J, Melgar MJ (2005) *Agaricus macrospourus* as Potencial Bioremediation Agent in Compost Material Contaminated with Heavy Metals. J. Chem. Tech. Biotech. 80:(3)-325-330.

Garcia-Lafuente A, Moro C, Villares A, Guillamón E, Rostagno MA, D'Arrigo M, Martínez JA (2011). Mushrooms as a source of anti-inflammatory agents. Am. J. Commun. Psychol. 48(1-2):125-141.

Gopalani M, Shahare M, Ramteke DS, Wate SR (2007). Heavy metal content of potato chips and biscuits from Nagpur City, India. B. Environ. Contam. Tox. 79:384-387.

Insam H, de Bertoldi M (2003). Microbiology of the composting process. In: Golueke, C., Bidlingmaier, W., de Bertoldi, M. and Diaz, L. (Eds). Comp. Sci. Tech. 25-47. Elsevier Science Ltd.

Isıloğlu M, Yilmaz F, Merdivan M (2001). Concentrations of trace elements in wild edible mushrooms. Food Chem. 73: 169-175.

Lebot V (2009). Tropical Root and Tuber crops: Cassava, Sweet Potato, Yams and Aroids, CABI, Wallingford, UK.

Li Y (2012). Present development situation and tendency of edible mushroom industry in China. Mush. Sci. 18:3-9.

Ma J, Betts NM (2000). Zinc and Copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individual 9CSF-II). J. Nutr. 130:2838-2843.

Manzi P, Aguzzi A, Pizzoferrato L (2001). Nutritional value of mushrooms widely consumed in Italy. Food Chem. 73: 321-325.

McKinley VL, Vestal JR (1984). Biokinetic analysis and succession of microbial activity in decomposition of municipal sewage sludge. App. Environ. Microb. 47:933-941.

Obiri S, Dodoo D K, Okai-Sam F, Essumang D K (2007). Determination of free cyanide and total cyanide concentrations in surface and underground waters in Bogoso and its surrounding areas in Ghana. Bull. Chem. Soc. Ethiop. 21(2):213-220.

Obodai M, Amoa-Awua W, Odamtten GT (2011). Physical, chemical and fungal phenology associated with the composting of 'wawa' sawdust (*Triplochiton scleroxylon*) used in the cultivation of oyster mushrooms in Ghana. Int. Food Res. J. 17:229-237.

Obodai M, Dzomeku M, Awotwe B, Takli RK, Narh D (2007). Manual on mushroom cultivation technology in Ghana. CSIR-FRI Technical Report

Oei P (1991). Manual on mushroom cultivation: techniques, species and opportunities for commercial application in developing countries. CTA, Wageningen, The Netherlands

Radulescu C, Stihi C, Popescu I V, Busuioc G, Gheboianu AI, Cimpoca VG, Dulama ID, Diaconescu M (2010). Determination of heavy metals content in wild mushrooms and soil by EDXRF and FAAS techniques. Ovidus Uni. Ann. Chem. 21(1):9-14.

Ryckeboer J, Mergaert J, Vaes K, Klammer S, Clercq DE, Coosemans D, Insam JH, Swings J (2003). A survey of bacteria and fungi occurring during composting and self-heating processes. Ann. Microb. 53(4):349-410.

Senesse P, Meance S, Cottet V, Faivre J, Boutron-Ruault MC (2004). High dietary iron and copper and risk of colorectal cancer: a case – control study in Burgundy, France. Nutr cancer. 49:66-71.

Sesli E, Tuzen M (1999). Levels of trace elements in the fruiting bodies of macrofungi growing in the east Black sea region of Turkey. Food Chem. 65(4):453-460.

Silvestre MD, Lagarda MJ, Farra R, Martineze-Costa C, Brines J (2000). Copper, iron and zinc determination in human milk using FAAS with microwave digestion. Food Chem. 68:95-99.

Soylak M, Saracoglu S, Tüzen M, Mendli D (2005). Determination of trace metals in mushroom samples from Kayseri, Turkey. Food Chem. 92:649-652.

SPSS 16 for Windows (2005). SPSS 16 for Windows. Chicago. Illinois, USA

Svoboda L, Kalac P (2003). Contamination of two edible *Agaricus* spp. mushrooms growing in a town with cadium, lead and mercury. Bull. Environ. Contam. Toxicol. 71:123-130.

Tüzen M (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Micro Chem. J. 74:289-297.

Tüzen M, Özdemir M, Demirbas A (1998). Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. Food Chem. 63 (2):247-251.

Unak P, Lambrecht FY, Biber FZ, Darcan S (2007). Iodine measurements by isotope dilution analysis in drinking water in Western Turkey. J. Radioanalytical Nuclear Chem. 273:649-651.

Vonugopal B, Lucky T (1975). Toxicity of non-radioactive heavy metals and their salts in heavy metals toxicity, safety and hormology. (Ed.) F Coulston. Academic Press, Georg Thieme, Stuttgart, New York.

World Health Organaiztion (WHO) (1982). Evaluation of Certain Foods Additives and Contaminants (Tweenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report series, No. 683 Geneva.

Zhu F, Qu L, Fan W, Qiao M, Hao H, Wang X (2010). Environmental Monitoring Assessment. DOI 10.1007/s10661.01-1728-5