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Determination of the Concentration of Essential Elements in Pleurotus Ostreatus Cultivated on Valisneria Arthiopica as a Supplementary Substrate to Sawdust using Instrumental Neutron Activation Analyses

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Abstract: Mushrooms are excellent nutritional and medicinal sources in the environment. This study has sought to conduct an analysis of the concentration of the essential elements in the mushroom sample that was cultivated on three supplementary substrate compositions (25% *Vallisneria aethiopica* and 75% *Tripochton scleroxylon*, 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* and 75% *Vallisneria aethiopica* and 25% *Tripochton scleroxylon*) using Instrumental Neutron Activation Analysis (INAA) at the Ghana Research Reactor-1 facility(GHARR-1). The concentrations of the elements were detected in *Pleurotus ostreatus* cultivated on three different percentage substrate compositions of a mixture of *Vallisneria aethiopica* and *Triplochiton scleroxylon*. The mixture of 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* was best for most of the cultivation of oyster mushrooms since most of the elements detected in the samples attained the highest concentration in this substrate. The validity of the INAA technique for determination essential elements was checked by analyses of SRM 1566b (Oyster tisue) and Peach leaves 1547, respectively. The mean concentrations of the nutritional elements (Al, Cs, Ca, Cu, Cr, Cl, Zn, Br, Hg, Th, Fe, K, Mg, Mn, Na and V) were determined in mushrooms cultivated on three different substrate mixtures.

Key words: Instrumental neutron activation analyses, major element, mushroom, substrate, trace element

INTRODUCTION

Mushrooms are used in the preparation of many delicacies all over the world. Oyster mushrooms (*Pleurotus ostreatus*) are best known medically for their cardiovascular and cholesterol controlling benefits. They contain mevinolin and related compounds which are potent competitive inhibitors of 3-hydroxy 3 methyl glutaryl coenzyme (A reductase), the major rate limiting enzyme in cholesterol biosynthesis. A mushroom is the fleshy, spore-bearing fruiting body of a fungus, typically produced on soil or on its food source. The standard for the name "mushroom" is the cultivated white bottom mushroom, Agaricus bisponus, hence the word mushroom is most often applied to fungi that have stem, a cap and gills on the underside of the cap. However, "mushroom" can also refer to a wide variety of gilled fungi with or without stems and the term is used more generally to describe both the fleshy fruiting bodies of some Ascomycota and the woody or leathering fruiting bodies of some Basisdiomycota (Sherman *et al*., 1954-1955). Mushrooms are heterotrophic due to the fact that they lack chlorophyll and unlike flowering plants, are unable to produce their own food. As a result of their heterotrophic nature, they are dependent on their substrates for their nutritional requirements such as carbon, nitrogen, water and minerals (Rajaratnam *et al*., 1997). All species of mushrooms take several days to form primordial mushroom fruit bodies. It takes as long as a week for an oyster mushroom to grow from a pinhead to maturity depending on temperature and humidity. When the cap margin stars to turn up, the fruiting body is past its prime. The cultivated mushrooms as well as the common field mushrooms initially form a minute fruiting body referred to as the pin stage because of their small size and shape. The primordial forms at the ground level in the lawn in humid spaces under the thatch and after heavy rainfall or in dewy conditions balloon to full size in a few hours, release spores and their collapse. Previous study shows that the growth of mushroom is dependent on the medium that the mushroom is grown on, the pH value of the medium and the temperature at which the mushroom is growing. (Wuest *et al*., 1985). According to Tagwira *et al*. (1998), the addition of 10% water hyacinth supplement to a sawdust substrate and groundnut shells increased oyster mushroom production 250 and 221%, respectively. It was

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Fig. 1: Preparation of the *Vallisneria aethiopica*

Fig. 2: The picture of the *Oyster mushroom* after cultivation and sawdust mixture

established that water hyacinth is a good supplementary substrate for the production of oyster mushrooms. The objective of this study is to conduct an analysis of the concentration of the essential elements in the mushroom sample that was cultivated on three supplementary substrate compositions (25% *Vallisneria aethiopica* and 75% *Tripochton scleroxylon*, 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* and 75% *Vallisneria aethiopica* and 25% *Tripochton scleroxylon*) using Instrumental Neutron Activation Analysis (INAA) at the Ghana Research Reactor-1 facility (GHARR-1). This study will also help to establish *Vallisneria aethiopica* (an aquatic plant found along the Black Volta Lake) as a supplementary substrate for the production of oyster mushrooms. *Pleurotus ostreatus* is amongst the earliest and easiest to cultivate mushroom. It is a delicious edible wavy-leaf-shaped mushroom found throughout the North Temperate Zone, almost always on dead hard wood (angiosperm) trees). It can also be easily cultivated on a variety of substrates. The mushrooms thrive in tropical climates, are 10% protein, and they can grow prolifically when they have plenty of water. In the wild, it is often found in abundance in October but it is found every month from March to November in Wisconsin and every month of the year in more southerly locations. (Stamets, 2000). *Pleurotus ostreatus* grown on sawdust seems to be the perfect solution for protein-starved diets across Africa, where millions of children die every year from protein deficiency.

MATERIALS AND METHODS

Instrumentation: Sample irradiations for neutron activation analysis were carried out in the 30 kW Miniature neutron source reactor (MNSR) at a neutron flux of 5×10^{11} neutrons per cm^2.s. The reactor is situated at the Ghana Atomic Energy Commission, Kwabenya-Accra, Ghana.

Study design: The sampling and cultivation of the mushrooms occurred between April, 2011 and July, 2011 on the Ghana Atomic Energy Commission site at Kwabenya - Accra, Ghana. Coarse saw dust (from *Tripochton scleroxylon*) was selected from a local saw mill so that it would not pack too densely (if it does, the mycelium would not get enough air). The saw dust was mixed with *Vallisneria aethiopica* in three different percentage compositions (25% *Vallisneria aethiopica* and 75% *Tripochton scleroxylon*, 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* and 75% *Vallisneria aethiopica* and 25% *Tripochton scleroxylon*). The various saw dust compositions were then enriched with bran, a nitrogen supplement. This is to help increase the mushroom yield. The saw dust was then sterilized in an autoclave so that all existing microscopic competitors were eliminated. Oyster mushrooms require a bit more humidity and fresh air than the white variety. They grow well on a range of agricultural and wood waste products, including hardwood chips, chopped cereal straws or corn cobs. After the growing medium was pasteurized and cooled, it was inoculated; that is, mixed with spawn and packed into long, tubular shaped plastic bags. It was incubated in semi-dark conditions and exposed to high relative humidity (85-95%) in a wooden box. Holes were punched in the bags to allow the mycelium to breathe and the bags were hung up or set on racks in the growing rooms. After about 14 days, the mushrooms popped out through the holes and were harvested. Fig. 1 and 2 show the process of mixing the substrate and the cultivated oyster mushroom, respectively.

Sample preparation: The mushroom, *Pleurotus ostsreatus* was weighed using the Metiler Toledo. The *Pleurotus ostreatus* was divided into three parts, ie.that cultivated on 25, 50 and 75% *Vallisneria aethiopica* substrate respectively. The mushroom samples were washed with water and cut in small pieces with plastic knife and put in Petri plates or plastic recipients. The samples were then freeze-dried for 10 to15 h in a freeze-dryer. After freeze-drying process, the samples were grounded and homogenized in an industrial blender into

Table 1: Results of standard reference material (peach leaves 1547 & SRM 1566b oyester tissue) showing reported and measured values used for validation

Element	Reported value (Peach Leaves 1547)	Measured value (Peach Leaves 1547)	Reported value (SRM 1566b Oyster Tissue)	Measured value (SRM 1566b Oyster Tissue)
Aluminum(Al)	249.00±8.00	250.00±7.90	197.2±6.0	201.6±8.1
Calcium(Ca)	1.56±0.02	1.76±0.03	838±20	836.1±32
Magnesium(Mg)	4300±0.01	4292±0.01	1085±23	1090±47
Sodium(Na)	24±2	23.8±0.19	3297±53	3302±79
Potassium(K)	24300±0.03	23900±0.02	6520±90	6527±62
Bromine(Br)	11.00±1.63	10.90±0.60	-	-
Chromium(Cr)	1.00±0.21	1.14±0.50	-	-
Cesium(Cs)	-	-	-	-
Mercury(Hg)	-	-	0.0371±0.0013	0.0472±0.0024
Iron(Fe)	218.00±14.00	220.00±13.6	205.8±6.8	204.7±5.2
Manganese(Mn)	98.00±3.00	97.00±3.00	18.5±0.2	19.2±0.31
Thorium(Th)	-	-	0.0367±0.0043	0.0415±0.0068
Vanadium(V)	0.37±0.03	0.41±0.40	0.577±0.023	0.604±0.051
Zinc(Zn)	17.90±0.40	19.60±2.15	1424±46	1437.2±69
Chlorine(Cl)	360.00±19.00	358.00±17.00	5140±100	5148±82
Copper(Cu)	3.70±0.40	3.80±0.40	71.6±1.6	72.3±2.0

fine powder. These mushroom samples were stored in pre-cleaned polyethylene vials until analysis. The sample preparation was done in a dry, dust-free environment. The mushroom sample was weighed in six fold with each weighing 200 mg on the metiler balance. The sample was wrapped in a clean polythene film using a pair of forceps. The samples were then packed into polyethylene irradiation capsules. The capsule was heat-sealed. Standard reference materials Peach leaves 1547 (200 mg) and SRM 1566b Oyster tissue (200 mg) were then prepared in the same manner as the mushroom samples. The two standard reference materials with certified values were used to validate the INAA method. The large capsules containing the samples and standards have a diameter of 1.6 cm and a height of 5.5 cm. The values are represented in Table 1.

Sample irradiation and counting: Samples and standards were transferred into the reactor via the pneumatic transfer system at a pressure of 0.6 MPa. The mushroom samples were irradiated for 2 min, 1 h and 4 h for short, medium and long irradiations respectively. At the end of the irradiation, the samples and standards were removed from the reactor and allowed to decay for 24 h and 1 week for medium and long irradiations, respectively. The large irradiation vial containing the radioactive mushroom sample was placed on the Coaxial High Purity Germanium (HPGe) semi-conductor γ-ray detector (Canberra) and the γ-activity of the induced radioisotopes. Measurement time depended on the activities of the induced radioisotopes. This was followed by the measurement of the γ-activity of the induced radioisotopes in the standard reference materials on the same coaxial HPGe γ -ray detector and at the same source-detector distance. A plexiglass source support was mounted on the detector during the measurement in order to ensure easy and reproducible source positioning (De Corte *et al*., 1987). The ORTEC MAESTRO-32 γ-spectroscopy software was used for γ-spectrum acquisition.

RESULTS AND DISCUSSION

Table 1 shows the analytical results obtained for Aluminium, Cesium, Calcium, Chromium, Copper, Chlorine, Zinc, Bromine, Mercury, Thorium, Iron, Potassium, Magnesium, Manganese, Sodium and Vanadium at GHARR-1 laboratory for the reference materials compared with the experimental samples. The values obtained compared favourably well with the recommended values. The experimental samples were within ±5% of the recommended values. The measurement precision specified by the relative standard deviation was within ±4%. The error margins are standard deviations.

Table 2 shows the concentration of the various elements found in the mushroom samples. The concentrations of mercury, thorium and cesium were low. The amount of cesium in drinking water is about 1 μ/L. On the average, a person swallows about 10 μg of stable cesium daily in food and water and inhales about 0.025 μg/day. Plants and animals contain cesium concentrations of about 1-300 mg/g/day (Agency for Toxic Substance and Disease Registry, 2004). The concentrations of inorganic and organic mercury in humans are 0.0003 and 0.0001 mg/kg, respectively (US EPA, 1997). Thorium is required only in small amounts in the body. Increased exposure to thorium may lead to cancer or death. The concentrations of these elements are good for human consumption.

The mixture of 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* was the substrate that recorded the highest concentrations for Vanadium, Manganese, Magnesium, Sodium, calcium, bromine and chromium. However, the 25% *Vallisneria aethiopica* and 75% *Tripochton scleroxylon* mixture substrate and the 75%

Table 2: Mean values (6 values each) of elemental concentration in mg/kg recorded in the mushroom samples cultivated on the various substraste compositions

Elements	25% Vallisineria (Conc. IN mg/kg)	50% Vallisineria (Conc. IN mg/kg)	75% Vallisineria (Conc. IN mg/kg)
Al	600.08±3.20	327.38±2.36	455.13±3.02
Ca	530±90	1440±150	740±120
Mg	1300±180	1830±220	1530±260
Na	420.17±0.06	950±10	315.5±0.05
K	43200±600	31900±400	14200±200
Br	0.74±0.11	1.91±0.88	1.20±0.18
Cr	0.038±0.002	0.04±0.001	0.019±0.01
Cs	*<0.001	*<0.001	*<0.001
Hg	*<0.01	*<0.01	*<0.01
Fe	83.72±4.20	58.1±2.6	102.4±5.3
Mn	28.83±1.76	80.15±2.42	76.47±2.33
Th	*<0.001	*<0.001	*<0.001
V	0.658±0.14	1.140±0.11	0.42±0.15
Zn	11.44±0.6	16.03±0.8	20.01±1.10
Cl	1773±47	337±58	3616±59
Cu	57.229±5.291	29.894±4.028	15.051±4.048

*- Detection limit

Vallisneria aethiopica and 25% *Tripochton scleroxylon* substrate recorded the highest elemental concentrations for aluminium, potassium and copper as well as iron, zinc and chlorine respectively.

A meta-analysis of 32 clinical trials testing the effect of reducing sodium intake on blood pressure, by Cutler *et al.* (1991) concludes that there is no evidence that moderate sodium presents any food hazard. The 4th edition of the Dietary Guidelines for Americans, 1995 urges consumers to choose a diet moderate in salt and sodium and refer to a daily value of 2400 mg of sodium found in the Nutrition facts label. The concentrations for sodium in the mushrooms obtained from the various substrate compositions could be termed as moderate. However the value recorded from the 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* mixture may be good enough for the human consumption.

An intake of between 0.5-1.0 mg/day of vanadium is enough to meet or exceed nutritional requirements without risking toxicity. No more than 1.8 mg/day should be used in people. Since the safety and effectiveness of vanadium have not been thoroughly studied, caution should be exercised when using vanadium as a nutritional supplement (Richard *et al*., 1995). High doses of vanadium (doses greater than 15 mg/day) may cause liver and kidney damage. The concentrations of 0.658±0.14, 1.140±0.11 and 0.420±0.15 mg/kg are rich enough for human consumption.

The estimated exposures were considered by the Committee on Toxicity of Chemicals in food, consumer products and the environment (COT). The highest average bromine concentrations were found in Nuts group (25.8 mg/kg, the fish group (6.7 mg/kg) and the Offal group (0.55 mg/kg). However, in the 1990 Japanese Total Dietary Survey, the bromine concentration for the fish and the shell fish group was 12 mg/kg (Samar *et al*., 2001). The physiological functions of bromine are unclear and there are no recommendations for optimum dietary intake in any species. The Committee on Medical Aspect of Food Policy (COMA) has concluded that bromine is ubiquitous and it is unlikely that any deficiency will occur in humans. However, values of 1.91±0.88, 0.74±0.11 and 1.20±0.18 mg/kg that have been recorded in this work are within range and comparable to values obtained by COT.

The current recommendation for the adequate daily intakes of calcium are 500 mg for children aged 1-3 years, 800mg for children between 4 and 8 years, 1300 mg for adolescents aged 9-18 years, 1000 mg for adults between 19 and 50 years and 1200 mg for 51 years and older (Recommended Dietary Allowances, 1989) . The results recorded lie within the recommended range and thus the mushrooms can be recommended for humans.

The levels of Mg in the mushroom sample were highest (1830±220 mg/kg) for the samples that used 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* mixture as their substrate. However, the values obtained for all the samples were higher than the Recommended Dietary Allowance (RDA) of 420 mg (Recommended Dietary Allowances, 1989). The consumption of mushrooms can be recommended by doctor for their patients in order to get the recommended dosage of magnesium.

Manganese is a very essential nutrient found in many foods such as grains, cereals and even tea. The amount of manganese in a typical diet appears to be enough to meet the daily needs (about 1-10 mg/day).). Manganese is an essential nutrient for the secretion of several enzymes. The concentrations in the mushroom samples are high enough to meet the daily requirements (28.83±1.76, 80.15±2.42 and 76.47±2.33 mg/kg).

Most unprocessed foods typically contain less than 5 mg/kg aluminum. Higher concentrations (mean levels 5 to10 mg/kg) were often found in breads, cakes and pastries (with biscuits having the highest levels), some

vegetables (with mushrooms, lettuce and corn salad having the highest levels), dairy products, sausages, shellfish, sugar-rich foods baking mixes, (EFSA Journal, 2008). Evaluation on the combined evidence from several studies in mice, rats and dogs that used dietary administration of aluminum compounds. In these studies the lowest-observed-adverse-effect levels (LOAELs) for effects on neurotoxicity, testes, embrytoxicity and the developing nervous system were 52, 75, 100 and 50 mg aluminum/kg per day, respectively. The lowest aluminum concentration among the results obtained is 327.38±2.36 mg/kg by the substrate. 50% *Vallisneria aethiopica* with 50% *Triplochiton scleroxylon*. This is considerably good as to the other two results.

The amount of copper found in the human body (50 to 120 mg) would probably fit on the head of a pin, but such a tiny quantity doesn't prevent this mighty mineral from performing impressive feats to promote optimal health (Whitney, 1990). According to the U.S. National Academy of Sciences, doctors who order 30 mg of iron or more everyday should also suggest taking 15 mg of zinc and 2 mg of copper along with the iron. The results obtained had 57.229±5.291 mg/kg which of course is within the stipulated level above it is also the highest among the results.

The suggested level for fortification of zinc staples is 30-70 mg/kg of flour. The zinc levels in the breast milk show a progressive decline during the course of lactation from 4 mg/L in the first week to 0.5 mg/L at 9 months, complementary foods with high content of absorbable zinc are required to satisfy the growing needs. (Krebs *et al*., 1995) The result of 20.1±1.10 mg/kg is slightly below the suggested level above the highest among the results obtained. This implies that the 20.0±1.10 mg/kg is considerably good for human and such as rich in zinc.

To ensure that enough of the mineral is available, says Lenore Hodges, Ph.D., R.D., director of nutritional services and patient care at Florida Hospital in Orlando, your daily intake should at least meet the recommended dietary allowance (Hochwald, 1999). For premenopausal women that means 15 mg daily. Men and post menopausal women need only 10 mg but pregnant women should consume 30 mg because iron is important for foetal development. The results of iron recorded were higher than the recommended level above.

Chlorine has been shown to decrease body weight notably with high doses (Human health fact sheet, August 2005). The concentrations of Cl in the mushroom samples were high and could be said to be desirable by humans.

CONCLUSION

The various compositons of substrates are good for the cultivation of edible mushrooms as the concentrations of the essential element found in the mushroom samples were within range of the recommended dietary allowance. The mixture of 50% *Vallisneria aethiopica* and 50% *Tripochton scleroxylon* was best for most of the cultivation of mushrooms since most of the elements detected in the samples attained the highest concentration in this substrate. It is highly recommended that *Vallisneria aethiopica* substrate be used for mushroom production since the mushroom yield from this substrate contains most of the essential nutrients that are essential for human growth. Other mushroom species could be cultivated on the substrate compositions to compare their nutritional contents in order to make recommendations. Mushroom cultivation should be done on a large scale in Ghana as a better substitute for meat and fish because it is less expensive, medicinal and healthier. Mushroom cultivation could offer a solution for poverty alleviation. Unlike the agronomic crops, the set up cost for mushroom production are low. The used compost that remains after harvesting the mushrooms may still be recycled for use as animal feed and soil conditioner. It can also serve as a boost to the economy when cultivated commercially because there is ready market for its use.

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REFERENCES

Cutler, J., D. Follman, P. Eliot and I. Suh, 1991. An overview of randomised trials of sodium reduction and blood pressure. Hypertension, 17: 127-133.

De Corte, F., A. Simonits, A. De Wispelaere and J. Hoste, 1987. Accuracy and Applicability of the k_0 Standardization Method. J. Radio Analytical Chem., 113: 145-165.

European food safety authority Journal (EFSA Journal), 2008. Safety of Aluminum from dietary Intake, Scientific Opinion of the panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) 754: 1-34.

Hochwald, L., 1999. All about Iron-Importance of Iron in Human Nutrition. Vegetarian Times.

Human Health Fact Sheet, 2005.

Krebs, N.F., C.J. Reidinger, S. Hartley, A.D. Robertson and K.M. Hambidge, 1995. Zinc supplementation during lactation: Effects on maternal status and milk zinc concentrations. American J. Clin. Nutr., 61: 1030-1036.

Rajaratnam, N., C. Katopodis, S. Wu and M.A. Sabur, 1997. Hydraulics of resting pools for Denil fishways. J. Hydraul. Eng. ASCE, 123(7): 632-638.

Recommended Dietary Allowances, 1989. 10th Edn., National Academy Press, Washington, D.C., pp: 1.

Richard, I., O. Broux, V. Allamand, F. Fougerousse, N. Chiannilkulchai, N. Bourg, L. Brenguier, C. Devaud, P. Pasturaud and C. Roudaut, 1995. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular type 2A. Cell, 81: 27-40.

Samar, M.M., M.S., Neirat, S.L. Resnik and A. Pacin, 2001. Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology. Food Additives Contaminants, 18: 1004-1010.

Sherman, J.F., D.J. Taylor and H.W. Bond, 1954-1955, Puromycin. III. Toxicology and Pharmacology. *Antiobiotics Annual*, pp: 757-765.

Stamets P., 2000. Growing Gourmet and Medicinal Mushrooms. 3rd Edn., Ten Speed Press, Berkeley, CA. U.S.A., pp: 552.

Tagwira, A., A. Mphuru, S.T., Chang, A. Mswaka, G. Pauli and K. Mshigeni, 1998. Effect of Supplementing Substrates with Water Hyacinth for Mushroom Production, Retrieved from: http://www.zeri.org/news/1998/november/nov98.htm, Zeri News letter, November.

United States Environmental Protection Agency (US EPA) , 1997. Mercury Study Report to Congress. EPA Washington, DC.

Whitney, H.R., 1990. Understanding Nutrition. 5th Edn., ISBN: 0314578315.

Wuest, P.J., M.D. Duffy and D.J. Royse, 1985. Six Steps to Mushroom Growing. The Pennsylvania State University Extension Bulletin, Special Circular, pp: 268.