INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0263/2018/20–12–2763–2769
DOI: 10.17957/IJAB/15.0831
http://www.fspublishers.org

Full Length Article

Seaweed Amended Rice Straw Substrate and its Influence on Health Related Nutrients, Trace Elements, Growth and Yield of Edible White Elm Mushroom (*Hypsizygus ulmarius*)

Martha Kasiku Hausiku* **and Hupenyu Allan Mupambwa**
Sam Nujoma Marine and Coastal Resources Research Centre, Sam Nujoma Campus, University of Namibia, P. O. Box 462, Henties Bay, Namibia
*For correspondence: mkhausiku@unam.na; hkasiku@rocketmail.com

Abstract

Edible mushrooms have been widely used for a long time as a source of food and beneficial health related nutrients. With mushrooms being saprophytic feeders, the nutrient content of edible mushrooms is widely dependent on the substrate composition. This study thus looked at the possibility of improving the quality of rice straw substrate by amending it with seaweeds and its influence on substrate biological efficiency (BE), mushroom (*H. ulmarius)* health related nutrients and trace metals contents. The results indicated that incorporation of 5% seaweeds resulted in the highest total yield with 22% higher BE than that of the control; whilst incorporation of 10 and 20% seaweeds reduced total yield and BE by 14 and 22%, respectively, compared to the control. Similarly, incorporation of seaweeds at 5% level resulted in significantly the highest crude protein concentration in the mushrooms. However, this high concentration in crude protein did not result in a direct increase in the 9 essential amino acids (EAA) determined in this study, with the control actually having a higher concentration of most of these EAA. Incorporation of seaweeds resulted in higher concentration in trace metals such as Na and K, which are beneficial to human health. Among the heavy metals determined, Cd and As significantly increased under the seaweed amended treatments, though their concentrations were below the maximum permissible levels set for food materials. The results of this study clearly showed that seaweed incorporation into rice straw at 5% level optimized the substrate conditions resulting in the highest mushroom yield, minerals and protein content. Further studies should evaluate the influence of specific seaweeds incorporated into different substrate with several oyster mushroom species on final health related nutrients and heavy metal accumulation.

Keywords: Biological efficiency; Essential amino acids; Heavy metals; Proximate analysis; Trace metals

Introduction

Edible mushrooms have been widely used for a long time as a source of food due to their pleasant taste and potential health benefits. The cultivation of mushrooms presents an economically viable biotechnology for the utilization of ligno-cellulose rich materials to produce high quality and nutritional food (Kayode *et al.,* 2015). Edible mushrooms such as the *Pleurotus* mushrooms have been grown using different agro-industrial wastes like maize cobs; wheat, rice, soya bean straw; cotton stalks; sugar cane remnants; banana waste; among others (Silva *et al.,* 2012). The nutrient content of edible mushrooms is widely dependent on the quality of the substrate (Hoa *et al.,* 2015). This creates an opportunity to further enhance the food value of culinary mushrooms by enriching the growing substrates with nutritionally beneficial materials. However, unlike vegetables and medicinal mushrooms, research on the nutritional value and chemical composition of culinary mushrooms has been very limited (Deepalakshmi and Mirunalini, 2014). In preliminary studies done in Namibia, *Pleurotus* mushrooms grown on media enriched with seaweeds showed higher levels of iodine relative to those grown in the non-enriched media (Molloy *et al.,* 2003; Kaaya *et al.,* 2012).

Most parts of Namibia are under arid to desert conditions which greatly limits terrestrial biological production making organic waste from agriculture and other activities very limited (Molloy *et al.,* 2003). However, unlike the terrestrial environment, the marine environment of Namibia is extremely productive due to the Benguela upwelling system which promotes some of the highest growth rates of marine organisms such as seaweeds (Molloy *et al.,* 2003). Due to the high productivity of the Namibian marine environment, seaweeds often collect along the coast, creating waste material that consumes lots of time to clean up (Tsanigan, 2009). However, the potential economical and health benefits of these seaweeds remains untapped, particularly in Africa (Kaaya *et al.,* 2012). In Asia,

To cite this paper: Hausiku, M.K. and H.A. Mupambwa, 2018. Seaweed amended rice straw substrate and its influence on health related nutrients, trace elements, growth and yield of edible white elm mushroom (*Hypsizygus ulmarius*). *Int. J. Agric. Biol*., 20: 2763–2769

seaweeds are considered a rich source of nutrients and health related bioactive compounds which are included in the traditional diet as an important marine medicinal food (Besada *et al.,* 2009; Rajapakse and Kim, 2011). Though seaweeds contain lipids in small amounts not exceeding 5% of their dry weight, the essential fatty acids from two biologically important groups *i.e.,* omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) represent the significant part of seaweed lipids (Misurcova *et al.,* 2011). Recently, these seaweeds have been identified to be functional food and nutraceuticals with several human health benefits (Rajapakse and Kim, 2011). Apart from these fatty acids, seaweeds also contain essential amino acids, functional polysaccharides; vitamins and minerals (Rajapakse and Kim, 2011). Due to these health related benefits of seaweeds, they have potential as amendments for the traditional ligno-cellulose based mushroom substrates, an area where research is very limited. Though seaweeds have been evaluated as potential mushroom substrate amendments, much of the work has only focused on improving the mushroom yield and creating a use of the unwanted seaweeds.

There is dearth of information on the improvement on health related nutrients in mushrooms grown on substrates that have been enriched with beneficial materials such as seaweeds. Again, with mushrooms being saprophytic feeders, there are possibilities that following enrichment of the substrate with seaweeds, trace metals may accumulate within their fruiting bodies to levels that may be above acceptable levels (Mleczek *et al.,* 2016). Therefore, this research aimed to evaluate the influence of seaweed-rice straw based substrate on the quantity of healthy related nutrients and the accumulation of trace/heavy metals in edible mushrooms.

Materials and Methods

Source of Materials

The dried rice straw used in this study was collected from the Kalimbeza rice project in the Zambezi Region of Namibia. Seaweeds were collected along the Henties Bay coastal area of Namibia, comprising of a uniform mixture of three seaweeds *i.e., Ulva* spp., *Laminaria* spp. and *Glacilariopsis* spp. The collected seaweeds were initially washed using fresh water before being air dried and grinded using a mechanical grinder into smaller pieces. The seaweeds and the rice straw were stored at room temperature under dry conditions prior to the start of the study. The selected characteristics of the seaweed and rice straw used in this study are shown in Table 1.

Pure cultures of the mushroom species *Hypsizygus ulmarius* popularly known as the White elm mushroom was sourced from Aloha Medicinals Inc., USA. A portion of the mushroom pure culture was then used to develop the spawn used in this study. Initially, the mushroom pure culture was aseptically transferred into petri dishes with potato dextrose agar which had been sterilized at 121°C using an autoclave whose pressure was maintained at 1.5 psi for 15 min. The petri dishes were then incubated at 25°C for up to 10 days, until the mycelium had fully colonized the agar. The mycelia from the fully colonized petri dishes were then used to further develop spawn using soaked and sterilized wheat grains. All this spawn development was done under laminar flow hood with a UV lamp sterilizer in the mushroom research laboratory at the University of Namibia, Sam Nujoma Campus.

Treatments, Experimental Design and Substrate Preparation

The treatments in this study were based on the level of seaweed incorporation into the rice straw substrate. This was done at four levels *i.e.,* 0 (control); 5; 10 and 20% on a dry weight to weight basis, following recommendations of Kaaya *et al.* (2012). All treatments were replicated 3 times, with each experimental unit having a 1.5 kg dry weight of substrate. Before effecting the treatments, the different rice straw and seaweed mixtures were initially soaked in tap water overnight then allowed to fully drain. After this draining, the moistened substrate was then packed into polythene plastics. The packed substrate bags belonging to the different treatments were then pasteurized in a steam pot which was at 100°C for 3 h and these were allowed to cool under aseptic conditions overnight. Finally, the cooled substrate was all inoculated with spawn of *H. ulmarius* at a spawning rate 2% on a weight to weight basis.

Experimental Conditions

The inoculated mushroom bags where incubated in the mushroom research house, at the University of Namibia, Sam Nujoma Campus, under controlled temperature and humidity conditions. During the vegetative stage, the inoculated bags were incubated at 26°C for a period of 4 weeks until full colonization was achieved. Following full colonization, a uniform number of perforations were made on each bag and the temperature in the room was lowered to 16°C, and humidity increased to 95%, to allow for primordia formation. This was done for 7 days, before temperature was readjusted to 18°C, for the fruiting stage. The harvested mushrooms were weighed and then oven dried at 50°C, ground using a blender and stored in zipper plastics. The harvested samples were then analyzed for the various properties as outlined below.

Biological Efficiency (BE)

The BE of the different treatments was determined by weighing the fresh whole cluster of fruiting bodies without removing the stalks using the following equation.

$$BE\ (\%) = 100 \times \frac{\text{Total mushroom fresh weight across all flushes}}{\text{Substrate dry weight}}$$

Table 1: Selected characteristics of materials of the seaweed and rice straw samples used in this study

Characteristics	Parameter	Seaweed	Rice straw
Proximate analysis	Total ash (%)	26.8 ± 6.0*	23.0 ± 3.3
	Fat (%)	1.2 ± 0.3	0.4 ± 0.02
	Crude protein (%)	14.6 ± 0.3	1.95 ± 0.4
	Crude fiber (%)	8.8 ± 0.2	39.2 ± 3.0
	Soluble carbohydrate (%)	18.6 ± 6.3	28.2 ± 3.3
Total elemental concentration	Ca (g kg^{-1})	8.87 ± 0.3	1. 8 ± 0.3
	K (g kg^{-1})	4.7 ± 0.06	10.4 ± 0.5
	Na (g kg^{-1})	26.0 ± 0.2	2.5 ± 0.02
	Mg (g kg^{-1})	4.7 ± 0.06	0.8 ± 0.09
	P (g kg^{-1})	3.5 ± 0.07	0.4 ± 0.004
	Fe (mg kg^{-1})	227.7 ± 20.4	497.1 ± 9.8
	Cd (mg kg^{-1})	2.95 ± 0.2	0.3 ± 0.04
	Cr (mg kg^{-1})	BDL	2.2 ± 0.9
	Ni (mg kg^{-1})	3.3 ± 1.1	4.5 ± 0.9
	Zn (mg kg^{-1})	27.0 ± 5.3	30.8 ± 1.8
	As (mg kg^{-1})	56.9 ± 0.9	1.34 ± 0.09

*****Figures are means ± Standard deviation; BDL = below detectable limits

Proximate Analysis

The proximate analysis was done by an independent agricultural laboratory under the Ministry of Agriculture, Water and Forestry in Namibia. Crude protein content was determined using the Kjeldhal method; crude fat was determined by Soxhlet, using diethyl ether as a solvent; total ash was determined by weighing the samples before and after burning at 500°C for 24 h in a muffle furnace, whilst crude fiber content was determined by the acid-detergent method which involved treating the dried sample (1 g) with acid-detergent solution for 1 h to digest non-fiber components. The percentage of all the fractions (crude protein, crude fat and ash) were added together and subtracted from 100 to obtain the total carbohydrate percentage. These above methods were all based on the Association of Official Analytical Chemists (AOAC, 1996).

Essential Amino Acids Analysis

Amino acid analysis was done by an independent laboratory in South Africa, the Central Analytical Facility at the University of Stellenbosch, employing methods described by Sharma *et al.* (2014). Briefly, a 0.1 g powdered mushroom sample was digested using 6M HCl then diluted 2.5 times using dilute NaOH. A 10 μL of the diluted sample was then used for derivatization with the results being calculated as grams per 100 grams of sample.

Trace Element Analysis

The dried and grinded mushroom samples were digested using a mixture of Nitric acid and Perchloric acid on a block digester for total elemental analysis as described by AgriLASA (2004). The digested samples were then analyzed for the following elements, Na, K, Mg, Ca, Cd, Cr, Fe, As, Ni, P and Zn using an ICP-OES (model iCAP 6000 Series; Thermo Fisher Scientific).

Data Analysis

The data collected were analyzed using a one-way Analysis of Variance (ANOVA) using JMP Version 12.0.1 statistical software (SAS Institute, Inc., Cary, North Carolina, USA, 2010). Where statistical significance was observed, means were then separated using Fischer's Protected Least Significant difference at $P < 0.05$.

Results

Effects of Seaweed Amended Substrate on Mushroom Yield and Biological Efficiency

Two parameters that measure the substrate biological productivity which are bilogical efficiency and total yield were determined in this study. There were significant differences ($P < 0.0013$) observed amongst treatments for both total yield and biological efficiency (BE). Incorporation of seaweed into rice straw at 5% level resulted in the highest total yield and BE, whilst the 20% treatment showed the least (Fig. 1). It was interesting to note that, for both total yield and BE, incorporation of seaweed at 5% resulted in a 22% higher yield relative to the control. However, when seaweed was incorporated at 10 and 20%, a reduction of 14 and 22%, respectively, in total yield and BE was observed relative to the control. On average, as the seaweed incorporation level increased from 5%, the yield and BE decreased by 19%. Incorporation of seaweed into the rice straw at 0 (control); 5; 10 and 20% resulted in a BE of 103; 125; 89 and 81%, respectively.

Effects of Seaweed Amended Substrate on Mushroom Nutrient Composition

Proximate composition: The proximate analysis for the mushroom samples determined five parameters which were crude protein (CP), soluble carbohydrates, crude fiber, fat and total ash, with significant differences being observed amongst treatments in all parameters as shown in Table 2. The crude protein was significantly higher in the treatment in which seaweed was incorporated at 5%; whilst, the control had the lowest. Relative to the control, the CP for the 5% treatment was 22% more. However, though CP was highest within the 5% treatment, this was not significantly different from the 10 and 20% treatments which also resulted in a 21 and 19% more CP, relative to the control. Unlike for yield parameters, incorporation of seaweed from 10 to 20%, only resulted in a 1.7% decrease in CP content, relative to the 5% treatment. As the seaweed level increased from 5 to 20%, the concentration of soluble carbohydrates decreased (Table 2) Compared to the control treatment, mushrooms from the 5% seaweed, 10% seaweed and 20% seaweed treatments had 12, 15 and 52% lower soluble carbohydrate contents, respectively.

Table 2: Proximate analysis in mushroom samples grown on rice straw substrate substituted with different levels of seaweed

Treatment	Crude protein (%)	Soluble carbohydrates (%)	Crude fiber (%)	Fat (%)	Total ash (%)
Control	17.37b	11.91a	19.42a	1.48c	7.69c
5% seaweed	21.22a	10.53a	15.75b	1.98a	8.35a
10% seaweed	21.02a	10.13a	18.74a	1.79b	8.03b
20% seaweed	20.68a	5.75b	19.40a	1.63bc	8.46a
P value	0.0064	0.0031	0.0064	0.0017	0.0026
CV %	4.58	12.24	4.83	4.83	1.81

* Values in the same column followed by a different lowercase letter are significantly different at $P < 0.05$

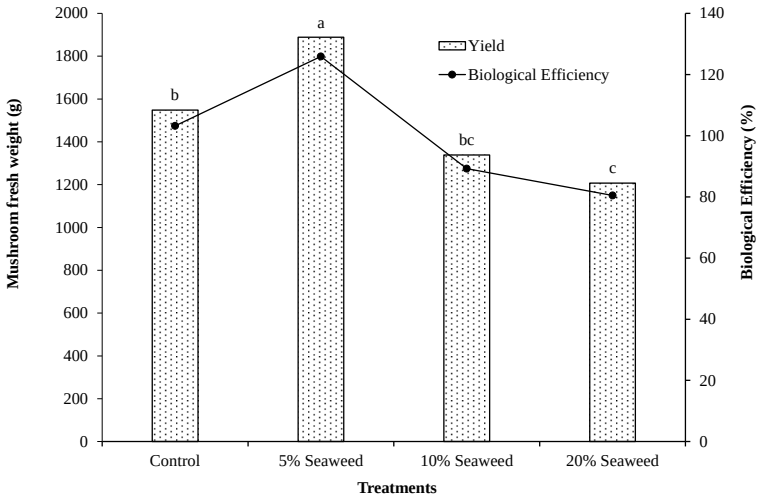

Fig. 1: Variations in total fresh yield and biological efficiency in mushrooms (*H. ulmarius*) grown on rice straw substrate amended with different levels of seaweeds. Columns showing different lowercase letters are significantly different at $P < 0.05$

For the crude fiber, the 5% seaweed treatment showed a significantly lower content, whilst there were no significant differences between the control, the 10% seaweed and 20% seaweed treatments. Compared to the control treatment, incorporation of seaweed at 5% resulted in a 19% significantly lower crude fiber. Similar to crude protein, incorporation of seaweed resulted in a significantly higher crude fat (CF) content in the mushroom samples, with the 5% seaweed having the highest CF, followed by 10% seaweed and 20% seaweed treatments (Table 2). Incorporation of seaweed into rice straw at 5% resulted in 33% more CF compared to the control. On the other hand, when seaweed was incorporated at 10 and 20%, on an average 9% decrease in CF was observed relative to the 5% seaweed treatment. Though there were significant differences amongst treatments on total ash (TA), there was no consistent trend observed with the highest TA being recorded in the 20% seaweed treatment (Table 2).

Essential Amino Acids Composition

For the essential amino acids (EAA), it was interesting to note that the control treatment resulted in the highest concentrations of histidine, threonine, lysine, tryptophan, methionine and valine (Table 3). Relative to the control treatment, the 5% seaweed treatment showed an average 26% lower concentration in the above six EAA. Similarly, the 10% seaweed treatment also showed an average 28 lower concentration for the above EAA, except for histidine, where there were no significant differences between the control and the 10% seaweed treatment. However, unlike other seaweed treatments, the 20% seaweed treatment actually resulted in mushrooms whose concentration was not significantly different from the control, except for threonine (Table 3). For isoleucine, there were no significant differences observed across all treatments, though the 20% seaweed treatment had the highest concentration. However, for leucine and phenylalanine, there was no consistent trend observed among treatments, with the 20% seaweed treatment having the highest concentration of leucine whilst the 5% seaweed treatment had the highest concentration of phenylalanine.

Effects of Seaweed Amended Substrate on Mushroom Trace Element Composition

For the total elemental concentration, the 5% seaweed treatment showed significantly the highest concentration of Ca, K, Mg and Fe. For these elements, except for Fe, a consistent trend was observed in the

Table 3: Essential amino acids concentrations in mushroom samples grown on rice straw substrate substituted with different levels of seaweed

Essential amino acid (g 100 g^{-1})	Control	5% Seaweed	10% Seaweed	20% Seaweed	*P* value	CV %
Histidine	4.90a*	2.14b	4.83a	3.75a	0.0091	17.9
Threonine	7.99a	5.98bc	5.28c	6.44b	0.0026	7.6
Lysine	8.98a	6.45b	6.08b	8.28a	0.0088	10.2
Tryptophan	6.77a	5.63ab	4.79b	6.72a	0.0312	11.3
Methionine	2.18a	1.86bc	1.64c	2.02ab	0.0051	5.8
Valine	7.82a	6.90b	6.29c	7.42ab	0.0023	3.9
Isoleucine	5.12	5.44	5.11	6.10	ns	8.2
Leucine	7.11c	8.30b	8.20b	9.82a	0.0006	4.3
Phenylalanine	7.10a	7.67a	6.18b	7.25a	0.0346	6.5

*Values in the same row followed by a different lowercase letter are significantly different at $P < 0.05$; ns = not significant at $P < 0.05$

Table 4: Total elemental concentrations of digested mushroom samples grown on rice straw substrate substituted with different levels of seaweed

Element	Control	5% Seaweed	10% Seaweed	20% Seaweed	*P* Value	CV %
Ca (g kg^{-1})	0.24b*	0.35a	0.26b	0.33a	0.0052	8.3
K (g kg^{-1})	30.24b	37.50a	32.16b	36.0a	0.0011	3.6
Mg (g kg^{-1})	1.05b	1.24a	1.05b	1.13b	0.005	3.89
Na (g kg^{-1})	2.57	2.99	2.85	3.1	ns	6.8
P (g kg^{-1})	6.8c	8.6b	8.7b	10.3a	0.0003	4.7
Fe (mg kg^{-1})	47.3b	96.89a	43.64b	49.05	0.0012	15.7
Cd (mg kg^{-1})	0.50b	0.83ab	1.01a	1.08a	0.02	19.5
Cr (mg kg^{-1})	1.71a	1.32a	0.26b	nd	0.0014	37.7
Ni (mg kg^{-1})	2.85	2.53	3.68	2.44	ns	24.9
Zn (mg kg^{-1})	79.50	81.11	67.51	71.07	ns	11.8
As (mg kg^{-1})	1.54c	1.94bc	2.56a	2.23ab	0.019	13.2

*Values in the same row followed by a different lowercase letter are significantly different at $P < 0.05$; ns = not significant at $P < 0.05$; nd = not detected

following order 5% seaweed > 20% seaweed > 10% seaweed > control.

For mushrooms grown on 5% seaweed treatment, the Ca concentration was 46% more than the control, whilst for the 10 and 20% seaweed treatments it was 8 and 9% more than the control (Table 4). In a similar trend, relative to the control, the 5% seaweed; 10% seaweed and 20% seaweed treatments had 24; 6 and 19% more K, respectively. For Mg concentration, the highest concentration was 1.24 g kg^{-1} for the 5% seaweed treatment, which was 18% more than the control, whilst the 20% seaweed had 7% more Mg compared to the control.

There were no significant differences amongst treatments on Na concentration, though the 20% seaweed treatment had the highest concentration of 3.1 g kg^{-1}, whilst the control had the lowest of 2.57 g kg^{-1} (Table 4). Another trend was also observed among the treatments for P and Cd, where the higher the seaweed incorporation level, the higher the concentration (Table 4). For the 5, 10 and 20% seaweed, the concentration of P in the mushrooms grown in these treatments was 26; 28 and 51% significantly more ($P < 0.0003$), respectively, relative to the control. For Cd concentration, the 20% seaweed; 5% seaweed and 10% seaweed levels resulted 166%; 66% and 102% more Cd, compared to the control. Though not similar to the previous trend, the concentration of Fe was also highest in the 5% seaweed treatment, which was 302% higher than the control. However, for the other treatments *i.e.*, 10% and 20%, the Fe concentrations were not significantly different from that of the control. For Cr concentration, the control treatment had the highest concentration, with a consistent decreasing trend as the concentration of seaweeds increased. Relative to the 5% seaweed treatment, the mushrooms from the control treatment had 23% more Cr (Table 4). Though As did not show a consistent trend, it was interesting to observe that, the lowest concentration was observed in the control treatment. A similar inconsistent trend which was not significant was also observed for Ni and Zn, where the highest concentration was observed in the 10% seaweed treatment for Ni and 5% seaweed treatment for Zn.

Discussion

In our study, dried seaweeds were incorporated into rice straw from 0 to 20% (w/w) and the highest yield and biological efficiency (BE) was observed in the treatment in which the seaweed had been incorporated at 5%. Generally, lignocellulosic materials such as rice straw are low in protein and mineral nutrition, and this could explain the higher yield when the rice straw was amended with protein and mineral rich seaweed. Though supplementation of mushroom substrates with nutrient and mineral rich materials like wheat bran has been widely used to increase yield, the ratio should not be too high due to the possibility of yield reduction (Rizki and Tamai, 2011; Alananbeh *et al.*, 2014). This could explain the decrease in yield and BE that

was observed when seaweed incorporation ratio was increased to 10 and 20% levels in our study, as the seaweeds could have contributed too much crude protein (Table 1), which can be detrimental to mycelial growth (Bellettini *et al.,* 2016). Also, at higher levels of seaweed incorporation, mushroom growth and development can be affected by the high concentration of salts such as Na, Ca and K (Kaaya *et al.,* 2012), which were quite high in the seaweed samples used in this study. However, the results of our study were not consistent with observations made by Kaaya *et al.* (2012), where they reported a seaweed incorporation rate of 10% as the highest yielding. This inconsistency clearly indicates that mushroom yield and BE are directly related to mushroom species (Alananbeh *et al.,* 2014), as in our study we used *H. ulmarius* whilst Kaaya *et al.* (2012) used *Pleurotus sajor-caju*.

In this investigation, we evaluated the potential nutritional benefits of seaweeds to improve the proximate composition of edible mushrooms. It was interesting to note that seaweed incorporation significantly enhanced the mushroom protein concentration, with an optimum protein concentration being observed at 5% seaweed incorporation. This clearly showed the positive contribution of the seaweeds to final mushroom protein concentration, as the seaweeds used in this study had 7 times more protein compared to the rice straw (Table 1). Mushrooms have been reported to release enzymes that convert large insoluble components of lignocellulosic materials into soluble-low molecular weight compounds, which can be absorbed by the mushrooms for nutrition (Kurt and Buyukalaca, 2010). This could explain the direct relationship between substrate nutrient content and final mushroom concentration observed in this study. Similar results have been reported by Gupta *et al.* (2013) in which rice straw was amended with varying levels of saponin free detoxified mahua seed cake, with the 20% incorporation ratio giving the highest protein content over the 40% incorporated treatment. Though seaweed incorporation resulted in significantly higher protein concentrations in mushrooms, this was not the case with the essential amino acids (EAA) determined in the current study, as also shown by the lack of correlation between protein content with any of the EAA (data not shown). Similar results were also observed by Mendez *et al.* (2005) in which oyster mushroom amino acid profiles showed no significant differences amongst different substrates. Studies on the protein quality in edible mushrooms such as *H. ulmarius* grown on seaweed amended substrate are not available, and further research required (Li *et al.,* 2017). Apart from proteins, seaweed incorporation also influenced changes in soluble carbohydrates and total ash. A higher seaweed incorporation ratio actually decreased the soluble carbohydrates indicating that the main source of these was the lignocellulosic rice straw. However, the ash content, which is indicative of mineral content, increased as seaweed level increase, supporting why the mushrooms from the seaweed amended substrate had higher mineral levels.

The concentration of minerals that is found in mushrooms is directly influenced by substrate quality, time of fruiting body, and genetic factors among others (Alananbeh *et al.,* 2014; Bellettini *et al.,* 2016). However, lignocellulosic materials like the rice straw used in this study are low in mineral content as evidenced by results of Table 1, thus such substrates may require additives to provide them with different minerals (Bellettini *et al.,* 2016). In this study, incorporation of seaweeds into rice straw generally resulted in higher levels of almost all trace elements, except for Cr. However, similar to the yield and BE results, an optimum level for trace metal absorption was also observed at 5% seaweed incorporation ratio. Contrary to other elements evaluated, for the heavy metals Cd and As, an increase in seaweed incorporation ratio resulted in a direct increase in the concentration of these metals in the harvested mushrooms. What was interesting was that, of all heavy metals, it was the same Cd and As, whose concentration was significantly high in the seaweed compared to the rice straw (Table 1). Amongst the trace metals determined in our study, Cd and As have been reported to be the most toxic with potential to disrupt several metabolic processes in the human body, though these were well below the maximum permissible levels set for food materials (Muchuweti *et al.,* 2006; Zhu *et al.,* 2011; Bvenura and Afolayan, 2012). It is plausible that the presence of Cd and As in high concentrations in the 10 and 20% treatments could have played a critical role in decreasing the yield and BE observed in this study, as they affect the biodegradation process and growth of fungi through inhibition of cellulolytic and hemi-cellulolytic enzymes (Alananbeh *et al.,* 2014). However, the high concentration of K and Na found in mushrooms where seaweeds were incorporated has been reported to be beneficial as it makes such mushrooms ideal for heart and hypertension patients (Alananbeh *et al.,* 2014).

Conclusion

The results of this study clearly showed that seaweed incorporation into lignocellulosic material like straw can significantly increase the mushroom yield, biological efficiency, mineral and crude protein concentrations. However, incorporation of seaweeds into rice straw substrate did not influence the mushroom essential amino acids concentrations. It was clear in this study that a 5% seaweed incorporation ratio resulted in the most appropriate substrate conditions to improve mushroom nutrition. Though seaweed incorporation resulted in significantly higher trace metal and heavy metal concentrations, the levels of the trace metals should actually be beneficial to human health, whilst the heavy metals were below the maximum permissible limits. Further studies should evaluate the influence of specific seaweeds incorporated into different substrate with several edible mushroom species on final health related nutrients.

Acknowledgments

This study was funded by the University of Namibia and United Nations University's Institute for Natural Resources in Africa (UNU-INRA). The authors would like to thank Aloha Medicinals Inc. for donating the mushroom pure culture used in this research; Mr Lineekela Kandjengo for assisting with seaweed identification and Messrs Berthold Handura; Johan De Klerk; Sending Toelath Kharuxab for assisting with experimental establishment.

References

AOAC, 1996. *Official Methods of Analysis of the Association of Official Analytical Chemist*, 16th edition. Washington DC, USA

Agri Laboratory Association of Southern Africa (AgriLASA), 2004. *Soil Handbook*. Pretoria (South Africa): Agri Laboratory Association of Southern Africa

Alananbeh, K.M., N.A. Bouqellah and N.S. Al Kaff, 2014. Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi J. Biol. Sci.*, 21: 616–625

Bellettini, M.B., F.A. Fiorda, H.A. Maieves, G.L. Teixeira, S. Avila, P.S. Hornung, M.A. Junior and R.F. Ribani, 2016. Factors affecting mushroom *Pleurotus spp*. *Saud. J. Biol. Sci.*, https://www.sciencedirect.com/science/article/pii/S1319562X16301814

Besada, V., J.M. Andrade, F. Schultze and J.J. Gonzalez, 2009. Heavy metals in edible seaweeds commercialized for human consumption. *J. Mar. Syst.*, 75: 305–313

Bvenura, C. and A.J. Afolayan, 2012. Heavy metal contamination of vegetables cultivated in home gardens in the Eastern Cape. *S. Afr. J. Sci.*, 108: 1–6

Deepalakshmi, K. and S. Mirunalini, 2014. *Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties. *J. Biochem. Technol.*, 5: 718–726

Gupta, A., S. Sharma, S. Saha and S. Walia, 2013. Yield and nutritional content of *Pleurotus sajor caju* on wheat straw supplemented with raw and detoxified mahua cake. *Food Chem.*, 141: 4231–4239

Hoa, T.H., C. Wang and C. Wang, 2015. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, 43: 423–434

Kaaya, G.P., P.N. Kadhila-Muandingi, H.R. Lotfy and K.E. Mshigeni, 2012. Determination of optimum seaweed concentration for mushroom cultivation and the ability of mushrooms to absorb iodine. *Afr. J. Agric. Res.*, 7: 3673–3676

Kayode, R.M.O., T.F. Olakulehin, B.S. Adedeji, O. Ahmed, T.H. Aliyu and A.H.A. Badmos, 2015. Evaluation of amino acid and fatty acid profiles of commercially cultivated oyster mushroom (*Pleurotus sajor-caju*) grown on gmelina wood waste. *Nig. Food J.*, 33: 18–21

Kurt, S. and S. Buyukalaca, 2010. Yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. ostreatus* and *P. sajor-caju*) cultivated on different agricultural wastes. *Bioresour. Technol.*, 101: 3164–3169

Li, H., Z. Zhang, M. Li, X. Li and Z. Sun, 2017. Yield, size, nutritional value, and antioxidant activity of oyster mushrooms grown on perilla stalks. *Saud. J. Biol. Sci.*, 24: 347–354

Mendez, L.A., C.A.S. Castro, R.B. Casso and C.M.C. Leal, 2005. Effect of substrate and harvest on the amino acid profile of Oyster mushroom (*Pleurotus ostreatus*). *J. Food Compos. Anal.*, 18: 447–450

Misurcova, L., J. Ambrozova and D. Samek, 2011. Seaweed lipids as Nutraceuticals. *In: Advances in Food and Nutrition Research*, Vol. 64, pp: 339–355. Se-Kwon, K (ed.). Academic Press, Burlington, Massachusetts, USA

Mleczek, M., P. Niedzielski, M. Siwulski, P. Rzymski, M. Gasecka, P. Golinski, L. Kozak and T. Kozubik, 2016. Importance of low substrate arsenic content in mushroom cultivation and safety of final food product. *Eur. Food Res. Technol.*, 242: 355–362

Molloy, F.J., A.T. Critchley, L. Kandjengo and K.E. Mshigeni, 2003. The use of the valuable oyster mushroom, *Pleurotus sajor-caju*, for conversion of waste materials produced from seaweed and brewing industries: preliminary investigations. *Ambio*, 32: 76–78

Muchuweti, M., J.W. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J.N. Lester, 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agric. Ecosyst. Environ.*, 112: 41–48

Rajapakse, N. and S.K. Se Kim, 2011. Nutritional and digestive health benefits of seaweed. *In: Advances in Food and Nutrition Research*, Vol. 64, pp: 17–28. Se-Kwon, K. (ed.). Academic Press, Burlington, Massachusetts, USA

Rizki, M. and Y. Tamai, 2011. Effects of different nitrogen rich substrates and their combination to the yield performance of oyster mushroom (*Pleurotus ostreatus*). *World J. Microbiol. Biotechnol.*, 27: 1695–1702

Sharma, G., S.V. Attri, B. Behra, S. Bhisikar, P. Kumar, M. Tageja, S. Sharda, P. Singhi and S. Singhi, 2014. Analysis of 26 amino acids in human plasma by HPLC using AQC as derivatizing agent and its application in metabolic laboratory. *Amino Acids*, 46: 1253–1263

Silva, M.C.S.D., J. Naozuka, J.M.R.D. Luz, L.S.D. Assuncao, P.V. Oliveira, M.C.D. Vanetti, D.M.S. Bazzolli and M.C.M. Kasuya, 2012. Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chem.*, 131: 558–563

Tsanigan, S.M., 2009. Evaluating Namibian macrophytic *algea* as dietary sources for South African abalone (*Haliotis midea*). *Ph.D.* Dissertation. University of Stellenbosch, South Africa

Zhu, F., L. Qu, W. Fan, M. Qiao, H. Hao and X. Wang, 2011. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environ. Monit. Assess.*, 179: 191–199

(Received 17 February 2018; Accepted 04 June 2018)