An overview of the lithium content and lithiation of the cultivable macrofungal species, *Agaricus bisporus* and *Pleurotus* spp.

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A B S T R A C T

Background: Lithium (Li) therapy has long been used as an effective treatment for bipolar affective disorders and research continues on its reported benefits in treating neurodegenerative brain diseases. However, it also elicits side-effects which may be related to the form and quantity of dosage. The mycelium and fruiting bodies of popular edible macrofungi, *A. bisporus* and *P. ostreatus* have shown promising results in the ability to enrich Li and could potentially serve as an alternative, more calibrated and bio-accessible source.

Scope and approach: This review is focused to feature results from studies that have been carried out, both on the natural occurrence of Li in wild and cultivated common edible *Agaricus* spp. and *Pleurotus* spp. mushrooms as well as on the lithation of their mycelia and fruiting bodies.

Key findings and conclusions: Lithium occurs ubiquitously in wild *Agaricus* and *Pleurotus* fungi at relatively low concentrations, typically ranging from < 0.02 to ~1.0 mg kg^{-1} dry weight (dw). Cultivated, retailed specimens show similar (0.03–~0.5 mg kg^{-1} dw) concentrations. In contrast, the lithiated mycelia of *P. ostreatus* achieved a maximum Li concentration of ~1600 mg kg^{-1} dw, and the edible fruiting bodies of *A. bisporus* and *P. ostreatus* were found to be lithiated to levels of ~40 mg kg^{-1} dw and ~10–~100 mg kg^{-1} dw. The Li concentration of 38 mg kg^{-1} dw achieved for *A. bisporus* using Li_2O compost fortification represents around 200 to 400-fold enrichment relative to the control or retail mushrooms. The process of compost fortification did not introduce contaminant elements such as Cd, Hg, Pb, above the regulation levels within the EU, or above those typically seen in the retail products. Such enriched mushrooms consumed as part of the diet, would allow a more controlled release of Li in the digestive system because of the longer digestion period (as compared to Li salts which are rapidly absorbed) which could potentially reduce or remove some of the side effects that have been reported. More targeted studies are required in order to clarify the absorption and pharmacokinetics of Li contained in these enriched mushrooms.

1. Introduction

The mycelium (vegetative body) of macrofungi (Macromycetes) is a buildup of threadlike and branching filamentous or plektenchymatic structured hyphae, which penetrate the surrounding substrate absorbing water and nutrients including mineral constituents. The hyphae secrete enzymes and chelating agents into the substrate to facilitate the absorption of the nutrients by diffusion and active transport, and translocation mediated by transporter genes. Certain macrofungi can also form structures made of a dense mass of hyphae such as rhizomorphs (mycelial cords, which also act as an absorption and translocation organ

of water and nutrients) or sclerotia (a hard and dense mass of filaments which functions as a depot for absorbed nutrients and biosynthesized products – antioxidants, polysaccharides, glucans, proteins, some provitamins and vitamins). During favorable conditions, macrofungi form fruiting bodies commonly called mushrooms, but are also referred to as carpophores, sporocarps, fruit bodies, toadstools, etc. These spore-producing structures, that emerge seasonally may be epigeous (growing above the ground in soil or on dead wood in trees, including those that are still living) or hypogeous (developing underground) as in the case of truffles. They are commonly considered as the "major" (and edible) organ of fungi and can also accumulate mineral constituents.

Depending on the feeding behavior of particular species, macroelements and trace elements of various nature can be shared between a fungus and its symbiont as in the case of mycorrhizal fungi or retained entirely by the fungus to fulfil its own physiological requirements as in the case of the saprotrophs. Fungi are able to absorb the full range of mineral constituents from the substrate environment and translocate these to the emerging fruiting bodies. Apart from the major nutritional elements, potassium (K), phosphorous (P) and sulphur (S), a number of other elements also usually occur in fruiting bodies and mycelia (or sclerotia) in much lower but varying amounts depending on the species, physiological requirements, natural geochemistry of the soil bedrock and any history of anthropogenic pollution. Species-specific differences in contents of the metallic, metalloid and nonmetal elements, e.g. Ag, Cd, Cu, Fe, Hg, V, Zn, As or Se, has been documented for numerous fungi – as reviewed by Falandysz and Borovi˘cka (2013). An interesting feature of a number of macrofungi is the species- or class (genus)-specific ability to accumulate some minerals to a greater extent than is typical or to hyperacumulate particular minerals. Fungi, raised in a polluted or artificially fortified substrate can - apart from As, Ag, Cd, Hg, Ni or radiocaesium ($^{134/137}$Cs) – also bioconcentrate other minerals such as Se and Li to a substantially greater extent than is typical for the species (Assunçao et al., 2012˜ ; Barcan et al., 1998; Bhatia et al., 2013; Bressa et al., 1988; Falandysz, 2008 and, 2013; Falandysz et al., 1994; Faria et al., 2018; Favero et al., 1990; Klimaszewska et al., 2016; Nunes, 2014; Pankavec et al., 2021c, 2021a; Turło et al., 2010).

Lithium was recognized as a treatment for manic-depressive illness in 1949 and used since the 1960s (Cade, 1949; Moncrieff, 1995). Li drugs, typically in the form of carbonate, citrate, and other salts were used to treat or prevent episodes of abnormal excitation or frenzied behaviour, mood disorders, and in high doses of 150–360 mg, to treat bipolar disorder (Leung, 1970; Marshall, 2015; www, 2021a and 2021b). However, treatment with the salts has too short a residence time (rapid clearance from the body) and has shown side effects in some patients such as excessive urination, renal dysfunction, nausea, diarrhea, thyroid and parathyroid gland dysfunction, neurological conditions (lethargy, ataxia, confusion, agitation), etc. (Rust et al., 2018). An alternative means of administering Li may be through the consumption of Li-rich foods in the diet or through popular and commonly available foods such as mushrooms that have been enriched with Li. Dietary Li is thought to be released far more slowly, during the digestive process and may thus overcome or reduce both drawbacks of rapid clearance as well as side effects from the high and acute nature of the dose. This is an area of active research, as is investigation into the possible essentiality of Li for neurological function in human and animals (McIntyre et al., 2001; Moncrieff, 1995; Soares & Gershon, 1998).

Many edible mushrooms, particularly wild species have been shown to contain Li, although the contents are generally very low (Falandysz et al., 2017c; Giannaccini et al., 2012; Vetter, 2005; Zhang, Barałkiewicz, Han´c, et al., 2020). Among cultivated species, the common button mushroom, *Agaricus bisporus* (J.E. Lange) Imbach*,* is a popular food (Report, 2019), sold worldwide as a fresh,

frozen or preserved product and recent studies have shown that it can bio-accumulate substantial amounts of Li from fortified substrates. Other common, edible mushroom species that have been studied for a similar purpose are *Pleurotus ostreatus* (Jacq.) P. Kumm. (oyster mushroom) and *Pleurotus* spp. The objective of this review is to feature results from studies that have been carried out, both on the natural occurrence of Li in wild and cultivated mushrooms as well as on the lithation of some fungal species. **2. Lithium in wild and cultivated *A. bisporus* and *P. ostreatus***

The lithium content in the fruiting bodies of *A. bisporus* and *P. ostreatus* (Table 1) and some other species of the genus *Agaricus* and *Pleurotus* was first studied by Vetter (1989). This was followed by studies on cultivated *A. bisporus*, *P. ostreatus* and *Lentinula edodes* (Berk.) Pegler as well as thirty eight wild species (altogether 171 samples) from localities in Hungary using inductively coupled plasma – mass spectroscopy (Vetter, 1994, 2005).

It is clear from the data compiled in Table 1, that lithium does not occur in abundance in *A. bisporus* and *P. ostreatus*, in general, showing concentrations that range from below detection limits (usually < 0.02 mg kg^{-1}) to 0.5 mg kg^{-1}. This low level of occurrence is not limited to *Agaricus* and *Pleurotus* as recently reported occurrences in other species appear to concur, remaining below the 0.5 mg kg^{-1} level. A recent study (Zhang, Barałkiewicz, Han´c, et al., 2020), investigating wild fungal species from soils with a polymetallic background from South-Western China showed Li concentrations of 0.25 ± 0.06 mg kg^{-1} dw in *Boletus speciosus* Frost; 0.27 ± 0.09 mg kg^{-1} dw in *Boletus umbriniporus* Hongo and 0.27 ± 0.04 mg kg^{-1} dw in *Hemileccinum impolitum* (Fr.) Sutara ˇ (previous name *Boletus impolitus* Fr.). Earlier, Falandysz et al. (2017), studying the sclerotia of the medicinal fungus *Wolfiporia cocos* (Schwein.) Ryvarden et Gilb., also from South-Western China reported a relatively lower concentration of 0.022 ± 0.027 mg kg^{-1} dw (median 0.010 mg kg^{-1} dw). This low level of Li occurrence was also seen in other wild fungal species from European locations, with concentrations of 0.09 ± 0.02 mg kg^{-1} dw in the Man on Horseback, *Tricholoma equestre* (L.) P. Kumm., mushroom (Rzymski & Klimaszyk, 2018), and 0.012 ± 0.003 to 0.075 ± 0.062 mg kg^{-1} dw in the caps of Fly Agaric, *Amanita muscaria* (L.) Lam., (Falandysz et al., 2020).

Li concentrations in macrofungi are low in comparison to other monovalent alkali elements such as caesium (^{133}Cs) (0.004–40 mg kg^{-1} dw) (Randa ˇ & Ku ˇcera, 2004), sodium (Na) (44 ± 14 to 1400 ± 460 mg kg^{-1} dw), rubidium (Rb) (18–230 to 730–1300 mg kg^{-1} dw) or potassium (K) (23,000 ± 4000 to 40,000 ± 3000 mg kg^{-1} dw) (Falandysz & Borovi ˇcka, 2013), as well as radioactive francium (Fr). Caesium and rubidium are not considered to have any physiological function in fungi and are therefore considered as non-essential, as is sodium, which is considered as a nutrient - in small doses - in humans. K on the other hand is a key nutrient for fungi and is homeostatically regulated. In humans, this involves maintenance of blood plasma potassium levels (and therefore the potassium content throughout the body) within narrow concentration limits, despite varying levels of intake through the diet.

3. Lithiated *A. bisporus* and *P. ostreatus*

3.1. Mycelial lithiation in laboratory experiments

Growing fungi in hydroponic systems fortified with mineral constituents such as selenium (Se) or Li, can produce selenized or lithiated mycelia, respectively (Faria et al., 2018; Klimaszewska et al., 2016; Nunes et al., 2015, 2014 and; Slusarczyk et al., 2013´ ; Turło et al., 2010). *In vitro* experiments (conducted in Petri dish plates), showed that *Pleurotus pulmonarius* (Fr.) Quel. was tolerant to Li (20´ –100 mg kg^{-1} in malt extract agar) (Hartikainen et al. (2013), but *P. ostreatus* was sensitive to Li fortification and did not grow, at the higher concentration of 0.5 g L^{-1}

(in the form of LiCl in agar medium, over two weeks of incubation) (Richter et al., 2008). Growth of the mycelial biomass in *Pleurotus djamor* (Rumph. ex Fr.) Boedijn), *Pleurotus eryngii* and *P. ostreatus* exposed to Li (50–270 mg L^{-1} as CH_3COOLi, LiCl, Li_2CO_3, LiOH or Li_2SO_4 in potato dextrose agar) was affected to different extents depending on the species and the Li salt used, with lower impact when LiCl and Li_2SO_4 were used (Nunes et al., 2014, 2015).

The mycelium of *P. ostreatus* cultured in a liquid malt extract (20 g L^{-1}) moderately fortified with Li at a concentration of 40 mg L^{-1}, showed relatively high levels

of accumulation, i.e. at a concentration of up to 1575 mg kg^{-1} dw, when fortified with Li_2CO_3, and up to 550 mg kg^{-1} dw when LiCl was used. Enrichment was less efficient at other - both lower and higher - fortification levels (Table 2). However, depending on the fortification level and the salt (Li_2CO_3 or LiCl) used, the addition of Li to the growth medium appears to have an inhibitory effect on mycelial biomass growth in *P. ostreatus* ($p < 0.01$), with LiCl showing a weaker inhibition (Faria et al., 2018).

3.2. Lithiation of fruiting bodies – efficiency and bioconcentration potential

3.2.1. Agaricus bisporus

The lithiation of *A. bisporus* was investigated in four separate studies that were carried out using a variety of different inorganic lithium salts – Li_2CO_3, $LiNO_3$, LiOH and LiO_2, to fortify the substrate (compost) in which the mushrooms were raised. The experiments were designed to be representative of commercial compost and commonly used growing conditions rather than laboratory based controlled conditions, and therefore, commercially available mushroom growing sets were used. These consisted of a straw and chicken manure based compost, ready- inoculated with phase III mycelia and covered with a peat casing. The Li background in this compost/peat set-up showed a narrow range of concentrations, i.e. 0.10–0.20 mg kg^{-1} dw over the four studies. This unfortified compost was used as the control for the studies and the Li concentrations in the mushrooms grown in this substrate showed a similarly narrow Li concentration range of 0.056 mg kg^{-1} dw for $LiCO_3$ to 0.087 mg kg^{-1} dw for LiO_2, in the whole mushrooms.

All growing experiments were carried out in triplicate, (i.e. 4 studies x 6 fortification levels x 3 replicates) and usually, mushrooms from the first flush of growth were taken for analysis. Additionally, in the case of the Li_2CO_3 study, the second flush was also collected in order to examine the effects of culinary processing and bioavailability *in vitro* (Pankavec et al., 2021b). As shown in Table 3, the composts used in the studies were fortified to achieve concentrations ranging from 1 to 100 mg kg^{-1} dw in the growing substrate. Results for all four studies showed that the mushrooms grew successfully (as per the commercially advertised rate of growth) in all the different fortified and unfortified (control) growing sets. However, when a higher fortification level of 500 mg kg^{-1} dw was used, the Li concentration in the compost appeared to have inhibited fruiting. This resulted in no observable mushroom growth at this fortification level in any of the four studies. However, all of the mushrooms grown in the other Li-salt fortified sets were enriched in comparison to the control. The whole body Li concentrations ranged from 0.49 mg kg^{-1} dw for $LiCO_3$ to 2.0 mg kg^{-1} dw for Li_2O, at the lowest compost fortification level of 1 mg kg^{-1} dw, and reached a maximum concentration range of 16 mg kg^{-1} dw for LiOH to 38 mg kg^{-1} dw for Li_2O.

At the highest level of viable (for growth) compost fortification (100 mg kg^{-1} dw), the levels of Li enrichment achieved in the mushrooms in these four studies were considerably higher than the study controls (0.056–0.087 mg kg^{-1} dw) and cultivated (commercially retailed) mushrooms (Table 1). In terms of the concentrations achieved in the four studies, the uptake was found to be highest when Li_2O fortified compost was used. In these mushrooms, the Li level in the whole fruiting bodies was 38 mg kg^{-1} dw, which corresponds to an enrichment of > 440-fold, relative to the control. The uptake of Li as seen in the fruiting bodies did not however follow a linear trend with increasing compost fortification. This is best represented by the bioconcentration factor (BCF) as visualised by the plot in Fig. 1-B, which shows the highest rate of uptake at the lower compost fortification levels of 5–10 mg kg^{-1} dw, before declining back to levels that are close to unfortified compost. The similarity of the bio-accumulation curves for all of the four studies suggests that the uptake process is similar for all of the Li salts that were used. However, some questions remain unanswered, e.g. as there is no physiological requirement identified (as yet) for Li in the fungi, when does the mycelium begin to uptake this element; is Li absorbed in competition with other elements; is the uptake linear during the whole growing process or does it accelerate with the emergence of the fruiting bodies. The answer to the latter question may have implications for the practical raising of commercial (enriched) *A. bisporus*, in that lower, but repeated fortification may be a more efficient means of enriching the fruiting bodies, rather than higher fortification levels. Another practical question is - as the mushrooms from these experimental sets were found to grow relatively abundantly in the second flush, are they similarly enriched with Li? The bio-

accumulation curves suggest that at the higher fortification levels at least, some of the added Li will be retained by the growing compost.

The distribution of Li between the caps and stipes of non-lithiated (control) *A. bisporus* was close to 1.0 (range of medians; the cap to stipe concentration quotient, called also $Q_{C/S}$ index, ranged from 0.97 to 1.1). The median values of $Q_{C/S}$ in lithiated *A. bisporus* showed greater variations, arising from the fortification level and the type of salt used (overall $Q_{C/S}$ range from 0.62 to 1.3. In the study using $LiNO_3$, all $Q_{C/S}$ values were in favour of the stipes (< 1.0), i.e. from 0.74 to 0.90.

3.2.2. Pleurotus spp

The lithiation of fruiting bodies has also been studied in other edible and cultivable species such as *P. ostreatus* and *P. eryngii.* In a study reported by Assunçao ˜ et al. (2012), *P. ostreatus* was grown on a substrate-based on coffee husk, that was fortified with LiCl at various concentrations (94 mg kg^{-1} dw plus from 62.5 to 500 mg kg^{-1} dw). Li concentrations in the cultivated mushrooms were found to be significantly ($p < 0.05$) influenced by the concentration of the Li salt in the substrate with reported concentrations of up to ~ 138 mg kg^{-1} dw. Curiously, Vieira et al. (2013) have reported a Li concentration of 37.5 mg kg^{-1} dw in lithiated *P. ostreatus* grown in a coffee husk medium that was fortified with LiCl at 500 mg/kg (Table 3). However, the coffee husk substrate used in this experiment was also reported to show a rather high background Li concentration of 94 mg kg^{-1} dw (Table 3). No other data could be found in available literature on background level of Li in coffee husk.

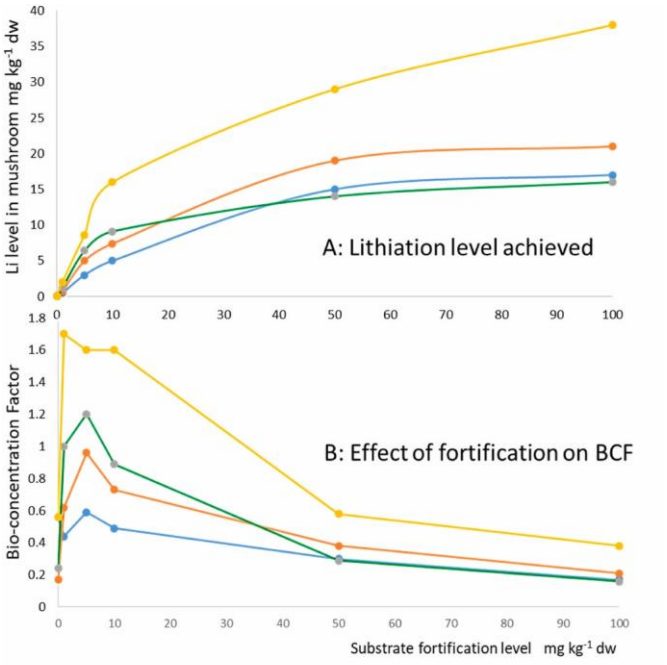
In another study (Koutrotsios et al., 2020), examining the effects of the cultivation substrate on the uptake of different elements in fungi, seven types of substrate were investigated - almond and walnut shells, corn cobs, grape marc plus cotton gin trash, olive mill by-products (leaves and two phase olive mill waste), extracted olive-press cake, date palm tree leaves and pine needles. *P. ostreatus* raised on these substrates contained Li in fruiting bodies at varying concentrations, i.e. in the range from 0.29 ± 0.04 mg kg^{-1} dw when raised in almond and walnut shells and up to 0.97 ± 0.55 mg kg^{-1} dw when grown using pine needles.

The BCF values for Li in the *P. ostreatus* grown in different substrates in the study by Koutrotsios et al. (2020) was assessed as 0.41 (grape marc plus cotton gin trash), 0.47 (almond and walnut shells 1:1 w/w), 0.54 (pine needles), 0.70 (date palm tree leaves), 0.98 (corn cobs), 1.0 (olive mill by-products (leaves and two phase olive mill waste 1:1 w/w) and 3.2 (extracted olive-press cake). These values were in the range reached by lithiated *A. bisporus* in some cases - depending on the salt and level of fortification (Table 3). Koutrotsios et al., 2020 also investigated another fungal species, *Cyclocybe cylindracea* (the Poplar Fieldcap, a species that is not very well known in Northern Europe, but is often found in large quantities, is an excellent edible mushroom and in high demand in other parts of the world). Li concentrations for *C. cylindracea* raised on these substrates were generally higher (apart from two substrates) than *P. ostreatus* and ranged from 0.37 ± 0.08 mg kg^{-1} dw in mushrooms raised on pine needles to 3.87 ± 2.35 mg kg^{-1} dw for those grown using extracted olive-press cake.

Mleczek at al. (2017) studied the effect of lithiation in *P. eryngii* and *P. ostreatus* using an experimentally prepared substrate (in 17 × 25 cm polypropylene foil bags) fortified with Li salts such as Li_2CO_3 and CH_3COOLi (Fig. 2; Table 3). The use of Li_2CO_3 showed a better effect on the lithiation efficiency when compared to CH_3COOLi, although it was more toxic to the mycelia at higher concentrations. The maximum accumulation in the first flush of *P. ostreatus* raised using Li_2CO_3 fortification was 12 mg kg^{-1} dw, with a relatively lower concentration, 8.3 mg kg^{-1} dw, recorded for CH_3COOLi (second flush concentrations were 16 mg kg^{-1} and 5.3 mg kg^{-1} dw, respectively). The lithiation levels achieved using the two salts were reversed in *P. eryngii*, with Li concentration in the fruiting bodies reaching a maximum of 11 mg kg^{-1} dw with Li_2CO_3 fortification and 15 mg kg^{-1} dw with CH_3COOLi (rounded values) (Table 3).

4. Effect of lithiation on the co-accumulation of trace elements in *A. bisporus* and *P. ostreatus*

In addition to nutritious and beneficial elements such as Cu, Zn, K, Se etc., the mycelial network of fungi also absorb a range of environmental and geogenic contaminants and elements such as lead (Pb), mercury



Fortification level	Li_2CO_3	$LiNO_3$	LiOH	Li_2O
SLOPE 0-10 mg kg^{-1} dw	0.50	0.76	0.92	1.58
SLOPE 10-100 mg kg^{-1} dw	0.13	0.15	0.11	0.24

Fig. 1. The BCF of Li in *A. bisporus* fruiting bodies grown in commercial compost fortified with different Li salts. Although maximum levels of Li were achieved at 100 mg kg^{-1} dw fortification (A), the most efficient uptake is seen between 5 and 10 mg kg^{-1} dw, as reflected by the slope of regression in figure A and the magnitude of the bio-concentration factors (B).

(Hg) cadmium (Cd) arsenic (As) etc. The process of fortifying a substrate with a particular element or compound can lead not only to enhanced uptake of the target compound(s) but may inadvertently perturb or affect the co-accumulation of other elements present in the substrate which may enhance the nutritional benefits, but equally, also increase the potential toxicity for consumers. It is therefore important to verify, from a safety point of view that the process of lithiation of cultivated edible fungi does not introduce contaminant elements and preferably, does not deplete nutrient elements. Within the EU, the concentration of some of these elements are regulated in edible fungi and although Hg, Cd, Pb, As and Sn are included in the regulations, only Pb and Cd have specified maximum limits. These are set at 0.3 mg kg^{-1} for Pb and at 0.2 mg kg^{-1} for Cd (European commission, 2006). The data in Table 4 are given on a dw basis and would reduce, approximately by a factor of 10 on a ww basis, so it is evident that the concentrations of Pb and Cd in the lithiated *A. bisporus* would be below the regulated limits.

The mass co-accumulation of Ag, Al, As, Ba, Co, Cd, Cs, Cu, Cr, Hg, Li, Mn, Ni, Pb, Rb, Sr, V, Tl, U and Zn in lithiated *A. bisporus* grown in substrate fortified with LiOH, Li_2O, Li_2CO_3 and $LiNO_3$ (Li added in concentration from 1 to 100 mg kg^{-1} dw; Tables 3 and 4) was studied by Pankavec et al. (2021a, 2021b, 2021c, 2021d). The absolute concentration levels of these elements were found to be low in the lithiated mushrooms (Table 4), i.e. occurrences were at the lower end of the ranges reported for non-lithiated or commercial raw mushrooms (Berna´s et al., 2006; Falandysz et al., 1993, 1994; Jaworska, 2015; Vetter, 1989, 1994; Vetter et al., 2005). In some cases, a possible effect of the compost fortification was observed, arising from a statistically significant difference in concentration of certain trace elements in caps or stipes depending on the experiment, i.e. on the level and type of salt used for fortification.

The low levels of occurrence of the trace elements in lithiated and control *A. bisporus* (Table 3) could be associated to the low occurrence levels of these elements found in the compost used for the cultivation experiments. In general, Zn, Cu and Mn are micronutrients and they usually occur at greater concentrations than many other elements in *A. bisporus* (Table 4) and other mushrooms. Al, Ba and Sr that occurred in the Li fortified substrate at a somewhat greater concentration than other elements are weakly bioconcentrated or indeed bio-excluded (BCF < 1) by fungi (Table 4) (Falandysz et al., 2021). On the other hand, toxic elements such as Ag, As, Cd and Hg, (but not Pb), are strongly bioconcentrated (BCF > 1) and can occur in *A. bisporus*, as well as in many other species of macromycetes, at elevated concentrations, if the substrate is contaminated with these heavy metals (Falandysz et al., 1994; Frank et al., 1974; Mędyk et al., 2017; Saba et al., 2016; Zhang, Barałkiewicz, Wang, et al., 2020).

P. ostreatus cultivated using coffee husk fortified with 500 mg kg^{-1} of LiCl (Assunçao et al., 2021, ˜ Table 3), showed similar concentrations of K, P, S, Mg, Ca, Fe, and Zn as non-lithiated, control mushrooms. In detail, K occurred at 19 ± 3 and 14 ± 3 mg kg^{-1} dw (rounded values), Phosphorus at 4.7 ± 1.0 and 4.6 ± 1.0 mg kg^{-1} dw, Sulphur at 3.3 ± 1.6 and 3.2 ± 1.3 mg kg^{-1} dw, Magnesium at 1.0 ± 0.1 and 1.1 ± 0.1 mg kg^{-1} dw, Calcium at 0.47 ± 0.24 and 0.35 ± 0.10 mg kg^{-1} dw, Iron at 0.17 ± 0.04 and 0.16 ± 0.04 mg kg^{-1} dw and Zinc at 0.10 ± 0.03 and 0.09 ± 0.01 mg kg^{-1} dw, while Li was at ~ 138 and ~25 mg kg^{-1} dw, respectively (Assunçao ˜ et al., 2021, Table 3).

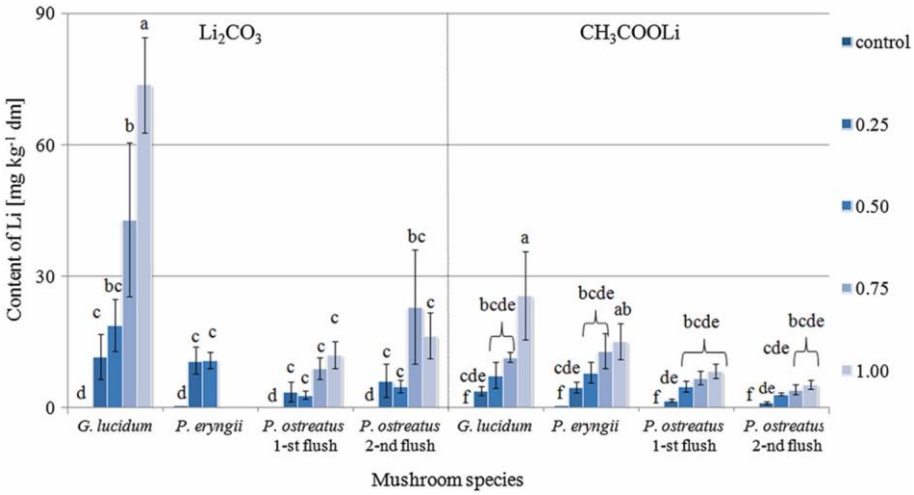


Fig. 2. Lithium concentration (mg kg^{-1} dw) in fruiting bodies of mushrooms (*Ganoderma lucidum*, *P. eryngii* and *P. ostreatus*) cultivated in Li fortified substrate (fortification level of 0.25–1.0 mM of Li_2CO_3 and CH_3COOLi - from light-blue to dark-blue, respectively; no data was reported on the background concentration of Li in the control substrate) (adapted from Mleczek et al., 2017).

5. Potential advantages of using lithiated mushrooms as a treatment

The physiological function of Li and its essentiality in humans remains to be established. A study on rodents has suggested a dietary requirement that influences litter sizes and behavioural effects (Pickett & O'Dell, 1992). However, few studies have been carried out in other animal models (Anke et al., 2005) and deficiencies remain, in the understanding of the biochemical processes that support any nutritional and medicinal effects in humans, even though a provisional RDA of 1000 µg Li day^{-1} has been suggested (Schrauzer, 2002). The use of Li in the treatment of many neurological conditions is well known, such as the beneficial effects of microdose Li on neuronal function, plasticity and repair, and on other functions (e.g. as a nutrient required for B12 and folate transport, the ability to stimulate the proliferation of stem cells, etc.). However, side-effects of Li treatment (the most common ones are nausea, parageusia, dry mouth, diarrhea, mild tremor, fatigue and drowsiness) are also seen in some patients and additionally, others may be intolerant to the treatment for different reasons such as allergy, heart disease, kidney problems, an underactive thyroid gland, low levels of sodium, Addison's disease and Brugada syndrome, etc.). These side-effects are only apparent in some cases and to some extent at least, this may relate to the magnitude of the administered dose and subsequent acute effects on renal function (Rust et al., 2018; www, 2021a and 2021b).

The combined evidence of the beneficial effects of treatment with Li and the side-effects suffered by some patients has prompted a search for more benign ways of administering lithium. Li is absorbed from the gastrointestinal tract after oral administration of Li-salt based drugs, and although initially circulated by blood to various tissues, the vast majority (in excess of 95%) is excreted through the urinary system. The lithiation of a food that allows a slower release of Li through a longer digestion period in the human gut is likely to result in a more calibrated absorption and may reduce or remove some of the side effects that have been reported in some patients. The *in vitro* simulation of gastrointestinal digestion of the Li contained in lithiated *P. ostreatus* was used to predict the accessibility of this form of Li in the digestive tract (Assunçao ˜ et al., 2012). The accessibility was found to be much higher than that for the salt, Li_2CO_3 and may be associated with the chelation of Li to organic compounds (Assunçao et al., 2012˜ ; Elless et al., 2000) in the fruit bodies as compared to the inorganic form of the drug. *In vitro* methods are designed to simulate processes that occur in the human body, but the digestive system is relatively far more complex, and more targeted studies are required on the absorption and pharmacokinetics of Li enriched foods in the digestive system.

However, mushrooms are likely to take relatively longer to digest because of the high carbohydrate content. These carbohydrates are reported as being resistant to human digestive enzymes (Cheung, 2013), which allows a longer digestion interval and could delay the solubilisation of Li. Delayed release may overcome the issue of rapid excretion observed with administered Li salts (Leung, 1970) and prolong the therapeutic action while simultaneously avoiding any hazardous peaks in Li concentration during treatment.

6. Conclusion

Lithium (Li) therapy has been seen as a mood stabiliser in the treatment of bipolar affective disorders for over half a century, but in recent years there has been a growing understanding of the beneficial effects of Li on a number of neurological (neuronal function, plasticity and repair) and other functions (e.g. as a nutrient required for B12 and folate transport, the ability to stimulate the proliferation of stem cells, etc.). However, Li treatment also elicits side-effects in some cases although to some extent at least, this may relate to the magnitude of the administered dose and subsequent acute effects on renal function. However the reduction of side effects is particularly important for neurological disorders because of the prolonged period of therapy.

As a mass drug administration (MDA) consideration, a longer running debate continues - on the possible benefits of low intakes of Li added to the water supply, on mood stabilisation, temperament and reduced suicide rates. This addition would provide a weak source of Li to the daily diet as commonly consumed foods such as various meats, dairy products, seafood, eggs and vegetables have low Li contents. Similarly, wild edible mushrooms also show low concentrations of Li. In order to boost the dietary intake of Li, studies have developed strategies to enrich some foods during growth, such as the lithiation of mycelia and fruiting bodies (mushrooms) of popular edible and cultivated fungi such as *Agaricus bisporus, Pleurotus eryngii, Pleurotus ostreatus* and *Pleurotus* spp. by fortifying the growing medium. These studies have generally encountered success with enrichment of the fruiting bodies being achieved at concentrations that are considerably greater than those seen in retail foods (or in wild mushrooms).

The enrichment of the fruiting bodies in response to increasing levels of fortification does not appear to be a linear function of the compost concentration, and a threshold level for Li tolerance in the substrate in *A. bisporus* is seen at concentrations over 100 mg Li kg^{-1} compost dw, above which fruiting is inhibited. Data on the bioconcentration factor (BCF) calculated from some of these studies shows that the highest rate of Li uptake occurs at lower compost fortification levels, e.g. 5–10 mg kg^{-1} dw, and reduces at higher levels of fortification. Enrichment of the fruiting bodies however, is sustained at higher levels of compost fortification, and at a level of 100 mg kg^{-1} dw, a Li concentration of 38 mg kg^{-1} dw was achieved for *A bisporus* grown on commercial compost that had been fortified with Li_2O. This compares favourably to the level of around 0.5 mg kg^{-1} dw seen in many species of wild mushrooms and in retail *A. bisporus*, and represents a ~400-fold enrichment relative to the control mushrooms grown in unfortified compost.

From a food/medicinal safety point of view, it was also verified that

the process of compost fortification did not introduce contaminant elements such as Hg, Cd, Pb, As and Sn that are present in commercial compost and are regulated in food within the EU. Where this was studied, e.g. in *A bisporus*, it was confirmed that the concentrations of Pb and Cd were below the regulated levels for mushrooms.

The mushrooms enriched in this way could therefore potentially be seen as a safe, low dose, bio-accessible therapy for the treatment of some neurological disorders. In addition to their nutritional

content, as well as being considered as a healthy food, they could provide a source of lithium that may be better retained by the body due to slower release during the digestion process. An *in vitro* simulation of gastrointestinal digestion of the Li contained in such mushrooms found that the Li associated with the enriched mushrooms was much higher than that for the Li salts traditionally used for treatment. More targeted studies are required in order to clarify the absorption and pharmacokinetics of Li contained in these enriched mushrooms.

Contribution

Jerzy Falandysz, Alwyn R. Fernandes and Daniela Meloni: the authors contributed equally to this work.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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