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The effects of germanium and selenium on growth, metalloid accumulation and ergosterol content in mushrooms: experimental study in *Pleurotus ostreatus* and *Ganoderma lucidum*

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Abstract

The aim of this study was to examine the effects of substrate supplementation with germanium (Ge) and selenium (Se), alone or combined, on the growth, element accumulation and ergosterol content in the fruiting bodies of the mushroom species *Ganoderma lucidum* and *Pleurotus ostreatus*. Both species were grown in four independent experimental systems with different concentrations of germanium powder (1, 5 and 10 mM), selenite [Se(IV)] and selenate [Se(VI)] (0.1, 0.4 and 0.8 mM of each Se species). Se supplementation caused no observable changes to morphology while the addition of Ge induced changes in size and/or colour, especially for *P. ostreatus*. A synergistic effect of Ge supplementation on Se accumulation in fruiting bodies of *G. lucidum* was observed only at the two highest levels of supplementation with both elements. Even though inorganic Se was used to enrich the experimental substrates, organic Se compounds dominated in the fruiting bodies. Increasing levels of Ge in the substrates elevated the content of organic selenium in the fruiting bodies of *G. lucidum*. No such trend was recorded in *P. ostreatus*. The maximum mean Ge levels in *P. ostreatus* and *G. lucidum* reached up to 70 and 80 mg kg^{-1}, respectively. However, the bioaccessibility of this element was low, in the range 5.4–6.6% (*P. ostreatus*) and 0.4–7.6% (*G. lucidum*). Ergosterol content in the fruiting bodies of both species displayed the highest values in all the experimental systems supplemented with Se. The addition of Ge and Se + Ge resulted in losses of ergosterol content in comparison to cultivation with Se supplementation and control. The obtained results show the significant role of Ge–Se interaction in the accumulation of these elements in the studied mushroom species.

Keywords Edible mushrooms · Supplementation of substrate · Germanium · Selenium · Fortification of fruiting bodies

Introduction

The fruiting bodies of approximately 200 wild-growing and cultivated mushroom species are consumed in many countries as a delicacy but also due to increased interest in their biological qualities and potential use as functional foods or a source of novel pharmaceuticals [1–3].

Mushrooms are well known for their abilities to accumulate various metals and metalloids in their fruiting bodies, including those significant for human nutrition as well as toxic elements. This phenomenon can be used in biofortification strategies in mushroom cultivation and result in their increased nutritional value or biological activity. A number of studies have already demonstrated that this approach can be employed to enrich the fruiting bodies of selected species with elements such as copper in *Ganoderma lucidum* [4]; iron (Fe) in *Pleurotus ostreatus*, *P. cornucopiae*, *P. djamor*,

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P. pulmonarius or *P. djamor v. roseus* [5]; lithium (Li) in *G. lucidum*, *P. eryngii* and *P. ostreatus* [6, 7]; potassium (K) in *P. eryngii*, *Flammulina velutipes* or *Hypsizygus marmoreus* [8]; selenium (Se) in *A. bisporus*, *P. eryngii*, *P. ostreatus*, *Pholiota nameko* [9, 10] and zinc (Zn) in *G. lucidum* and *P. ostreatus* [11]. Particular attention has been paid to substrate supplementation with inorganic Se salts as such an approach appears to potentially increase antioxidant, immunomodulatory and anticancer activities of selected mushrooms species [12, 13].

On the other hand, the abilities of mushrooms to uptake and concentrate elements from the ambient environment requires the establishment of high standards of cultivation conditions to minimize any risk of contamination and ensure consumer safety [1, 14]. A number of experimental studies have already shown that selected cultivated species can accumulate toxic metals (e.g. Hg) or metalloids (e.g. As) if there are high levels available in substrate, and that this can further affect the nutritional value of fruiting bodies [15, 16].

For this reason, several recent studies have investigated the content of toxic or potentially toxic elements in various cultivated mushroom species originating from a variety of geographical locations [14, 17]. Most of these studies have found levels of germanium (Ge) below detection limits while some others have detected it at levels ranging from a few µg kg^{-1} in *Phellinus* sp. to as much as approx. 1 mg kg^{-1} in *Ganoderma* sp. [18, 19]. Considering that Ge is a rare metalloid which is extracted in its inorganic form from mostly non-ferrous metallic ores for use in semiconductors and other electronic devices, it is likely that its levels in cultivated substrates are generally very low. However, little is known about how Ge could affect mushroom growth and chemical composition if it was to occur at an increased level in the ambient environment. There are no documented specific physiological roles of Ge in biological systems, including mushrooms and it is considered as a non-essential element. In humans, inorganic forms of Ge (Ge$_{in}$) are associated with toxicity. Experimental models have shown that its organic forms (Ge$_{org}$) may in turn display antioxidant, immunomodulatory and anticancer properties although the use of Ge-containing supplements is currently disregarded [20].

It is important to study how increased Ge content can affect cultivated mushrooms for various reasons: (1) Ge is used in thousands of electronic applications mostly as a transistor metal, it is produced globally with the highest input from China, the United States, Russia, Taiwan, Belgium, Germany and Canada, while the worldwide use of electronics and production of associated waste is on the rise [21], (2) the other potential input of Ge into the environment is the combustion of coal and lignite, which has been estimated to exceed Ge emissions from industrial production by two orders of magnitude; consequently a gradual increase in Ge availability can be expected at certain locations [22], (3)

it has been claimed anecdotally by non-scientific sources that mushrooms such as *P. ostreatus* can be a source of biologically active Ge, (4) screening of some commercially available mushrooms belonging to the *Ganoderma* genus has revealed increased levels of Ge in fruiting bodies [19, 23]. However, information on the kinetics of Ge uptake and accumulation in mushrooms and its potential effects on their growth, morphology, nutritional value and biological activity are literally not known. Apart from studies on Ge uptake, accumulation and associated effects, it is also relevant to investigate whether it can interfere with other elements present in the substrate [24]. In general, a limited number of studies have specifically assessed interactions between elements added to the mushroom substrate [25, 26]. As shown in plants, the presence of Se to a certain threshold level may have beneficial effects in mitigation of stress induced by toxic elements such as cadmium or mercury while at significantly increased Se content additive effects on toxicity may be seen as demonstrated, e.g. in case of lead [27–29]. However, there are essentially no data on how Se could interact with Ge in plants and mushrooms. Considering that there is an increased interest in Se-enrichment of cultivated mushroom species through substrate supplementation, it appears rational to investigate whether Se and Ge can interact with each other, what the potential outcomes of such an effect for cultivated mushrooms might be and whether it could affect the quality of the final product. Additionally, that is also important due to an aspect of the use of mushrooms enriched with ultra-trace elements such as Ge for developing novel dietary supplements and therapies [30].

Therefore, the present study aimed to examine the effects of substrate supplementation with Se and Ge, alone or in combinations on their accumulation in the fruiting bodies and the content of ergosterol a mushroom provitamin, in *P. ostreatus* and *G. lucidum*.

Materials and methods

Characteristics of experimental materials

The growing substrate was prepared from a mixture of alder and beech sawdust (1:1 vol) supplemented with 20% of wheat bran, 5% of corn flour, 3% of soybean meal, 1% of chalk and 1% of gypsum in relation to the sawdust dry matter. The mixture was dampened with distilled water to a moisture content of 45%. Next, the substrate was placed in bags (polypropylene foil) and sterilised at a temperature of 121 °C for 1 h. As soon as the temperature fell below 25 °C, Se salts were dissolved, and Ge powder was stirred in sterile water. The Se solutions and Ge suspension were added to the substrate, which was mixed intensely in an automatic stirrer for 15 min (POLYMIX PX-SR 90 D, Kinematica

AG, Littau-Luzern, Switzerland). After the addition of the solutions, the substrate moisture was 60%. Selenium was applied either as sodium hydrogen selenite [$NaHSeO_3$; Se(IV)] or sodium selenate [Na_2SeO_4; Se(VI)], both from Acros Organics (New Jersey, USA), and germanium as Ge powder (> 99.999% trace metal basis) from Sigma-Aldrich. The following concentrations of Se and Ge were applied to the substrate: Se—0.1, 0.4 and 0.8 mM L^{-1} of each Se species, Ge—1, 5 and 10 mM dm^{-1}. The elements were added in three experimental systems: S_1 [0.1 mM Se(IV) + 0.1 mM Se(VI) and 1 mM of Ge], S_2 [0.4 mM Se(IV) + 0.4 mM Se(VI) and 5 mM of Ge] and S_3 [0.8 mM Se(IV) + 0.8 mM Se(VI) and 10 mM of Ge]. The substrates with element(s) addition were mixed using a POLYMIX PX-SR 90 D stirrer (KINEMATICA AG, Littau-Luzern, Switzerland) with *Pleurotus ostreatus* spawn (mycelium on wheat grain) in the amount of 10% of substrate weight and placed in bags (polypropylene foil, bag size 17 × 28 cm). Each bag contained 400 g of the substrate. Incubation was conducted at a temperature of 25 °C and 80–85% air relative humidity until the substrate had become completely covered with mycelium. The bags were placed in a cultivation chamber with the top part of the foil cutoff. The relative humidity of air was maintained at 85–90% and temperature at 16 ± 1 °C. The cultivation was additionally lit with fluorescent light of 500 lx intensity for 10 h a day and aerated in such a way as to maintain CO_2 concentration below 800 ppm. Fruiting bodies were harvested successively as they matured.

Sample preparation and analysis of Ge and Se

Sample collection

Samples were collected, weighed, dried in an electric dryer (SLW 53 STD, Pol-Eko) at 50 ± 2 °C for 48 h, weighed again for dry weight determination and then ground for 0.5 min in a Cutting Boll Mill 200 (Retsch GmbH, Haan, Germany) to obtain powdered samples and finally treated in the extraction procedure.

Extraction procedure

The extraction procedure was based on the work of Gonzálvez et al. [31], modified for the conditions needed for hyphenated system determination and described previously [32]. 1.000 ± 0.005 g of the sample was put into a glass flask containing 10 mL of 1 mol L^{-1} phosphoric acid and extracted in an ultrasonic bath (30 min at ambient temperature). Next, the solution was filtered using paper filter washed with 200 mL of water and 20 mL of phosphoric buffer (pH 5.5) or centrifuged. The pH of the solution was adjusted at 6–6.5 by the addition of 10 mol L^{-1} solution of sodium hydroxide. Finally, the solution was diluted to 20 mL

by phosphate buffer. The Se species in the acid extracts were determined by HPLC-HG-AAS immediately after the extraction procedure.

Instrumentation

Hyphenated high-performance liquid chromatography with hydride generation atomic absorption spectrometry detection (HPLC-HG-AAS) systems has been constructed and described in our previous papers [32, 33]. The hyphenated analytical system consisted of a Shimadzu liquid chromatograph (LC-10A) equipped with a HPLC pump (LC-10AT), vacuum degasser unit (GT-104), Rheodyne PEEK valve (IDEX, USA) and an anion-exchange column Supelco LC-SAX1 (250 mm, 4.6 mm i.d., resin particle size 5 μm) thermostated by a column oven (CTO-10ASvp). The chromatographic run was isocratic at 3 mL min^{-1} with an injection volume of 200 μL. The measurements were performed with a Model SpectraAA 220FS spectrometer (Varian, Australia) with an UltrAA Se hollow cathode lamp.

Analytical procedure

The selenium species Se(VI) does not form a volatile hydride, so to get an analytical signal in the HG-AAS system it was necessary to perform a preliminary reduction of Se(VI) to Se(IV). The reduction was carried out on-line by heating the sample (90–100 °C) with the reducing agent: 0.5 mol L^{-1} thiourea solution in 10 mol L^{-1} hydrochloric acid. The eluate from the chromatographic column was joined with the stream of the reducing agent (flow rate 1 mL min^{-1} from the peristaltic pump) through a T-shape coupling and directed to the Tygon capillary loop (inner diameter 0.82 mm) heated in a water bath. The capillary loop outlet was connected to the hydride generation system. For both analytical systems PEEK transfer tubing of the eluent from the LC column to the hydride generation unit was inserted into a Tygon sleeve. The continuous hydride generation system (VGA-77, Varian) consisted of a manually controlled, peristaltic four-channel pump with Tygon tubing (0.6 mm i.d.), one reaction coil (PTFE tubing 0.8 mm i.d., 75 cm length) and three-way connectors. The gas–liquid separator was made of glass, and the dead interior volume was 3 mL. For the atomization of the Se hydrides (detected at 196.0 nm), a heating controller, electrothermally heating mantle and a quartz tube (ETC-60, Varian) heated to 900 °C were used. The chromatograms were displayed on the screen of a personal computer using the signal graphics option of the AAS software (Varian), and as a separate print out of the screen. Peak areas or height were calculated by the AAS software.

The number of validation parameters characterising the analytical method were determined. The limits of detection

0.01 mg kg^{-1} of dry matter both for Se(IV) and Se(VI) and the uncertainty of results (expressed as relative standard deviation, RSD) at the level of 10% for both selenium forms were obtained. As it was impossible to estimate the measurement traceability because of the lack of any certified reference materials for the determination of Se$_{in}$ species, the recovery of each Se species was measured upon addition of a standard to the sample. A recovery of 96–105% of each species was satisfactory.

Total selenium determination

Total selenium concentration (Se$_{total}$) was measured with the use of the inductively coupled plasma optical emission spectrometer Agilent 5110 ICP-OES (Agilent, USA). The standard conditions were used: Radio Frequency (RF) power 1.2 kW, nebulizer gas flow 0.7 L min^{-1}, auxiliary gas flow 1.0 L min^{-1}, plasma gas flow 12.0 L min^{-1}, Charge Coupled Device (CCD) temperature −40 °C, axial plasma view, accusation time 5 s, 3 replicates. The analytical wavelengths were 196.026 and 209.426 nm for Se and Ge, respectively. The limit of detection 0.01 mg kg^{-1} of dry matter for Se and 0.04 mg kg^{-1} of dry matter for Ge with the uncertainty of results (measured as RSD) at the level of 5.0% were obtained. Traceability was measured with the standard addition procedure. The concentration of the organic selenium (Se$_{org}$) was calculated as the difference between total selenium concentration (Se$_{total}$) and the concentration of inorganic selenium (Se$_{in}$) compounds [a sum of Se(IV) and Se(VI)].

Gases and reagents

Compressed argon gas of N-50 purity (99.999%) obtained from Linde (Poland) was employed as the carrier gas for Se vapour to the quartz cell without further purification. Water was redistilled and further purified with the water purification system Milli-Q (Millipore, USA). Standard selenium solutions (1000 mg Se L^{-1}) were prepared by dissolving appropriate amounts of sodium selenite (Na$_2$SeO$_3$), sodium selenate (Na$_2$SeO$_4$) and selenomethionine, obtained from Sigma-Aldrich (USA). Commercial selenium standard (Romil, England) was used for total selenium and germanium determination. The standard stock solutions were stored in glass bottles at 4 °C in darkness. Low concentration standards obtained by dilution of the stock solutions were prepared daily. Sodium tetrahydroborate(III), used as reducing solution (3% w/w), was prepared daily by dissolving NaBH$_4$ (Merck) in high-purity water, stabilised with 1% (w/w) NaOH (Merck) solution to decrease its rate of decomposition. The solution was used without filtration. The HCl solution was of the highest quality grade (Suprapure, Merck). Thiourea of the analytical quality grade was obtained from

Merck. The buffered mobile phase was prepared by mixing disodium hydrophosphate (Na$_2$HPO$_4$) 100 mmol L^{-1} with potassium dihydrophosphate (KH$_2$PO$_4$·2H$_2$O) 10 mmol L^{-1} obtained from Merck.

Determination of ergosterol

Ergosterol extraction and determination were carried out according to Perkowski et al. [34]. Briefly, about a hundred milligrams of powdered mushroom samples were mixed with pure methanol and NaOH. After irradiation twice in a microwave oven and cooling, HCl was added. The extraction was repeated three times. The mixed extracts were dried under a nitrogen atmosphere, redissolved in 1 mL of pure methanol, and filtered through syringe filters (Fluoropore Membrane Filters). The determination of ergosterol was performed on an ACQUITY UPLC H-Class System with a PDA eλ Detector (Waters Corporation, Milford, MA, USA) and an Acquity UPLC HSS T3 C$_{18}$ column (150 mm × 2.1 mm, particle size 1.8 μm), (Waters, Ireland). Isocratic elution with a mobile phase consisting of methanol (85%), acetonitrile (10%) and water (5%) was carried out with a flow of 0.5 mL min^{-1}.

Statistical analysis

The results are presented as mean values from triplicates and the standard deviation calculated by STATISTICA 12 software (StatSoft, USA). The differences in ergosterol content were analysed with one-way analysis of variance (ANOVA) followed by the post hoc Tukey's test ($P < 0.05$).

The bioaccessibility (%) of Ge and Se in the various experimental models applied in the present study was calculated using the formula: $x = \frac{\text{content in fruiting bodies}}{\text{initial substrate content}} \times 100$.

Results

Characteristics of biomass of fruiting bodies

The biomass of both *G. lucidum* and *P. ostreatus* from the substrates enriched with only Se was not related to any important changes in the morphology of these mushroom species (Figs. 1, 2). Similar observations for mushrooms growing in the substrate enriched with Ge were recorded, except for *G. lucidum* fruiting bodies (lower size) treated with the highest addition of the metalloid. The combined exposure of *G. lucidum* to Ge and Se caused significant changes in the morphology (lower size than in the control and changes in colour) of their fruiting bodies growing under systems S$_2$ and S$_3$. Similar results were also observed for *P. ostreatus* for the two highest concentrations of both Se forms and Ge additions. Fruiting bodies growing under S$_2$ were

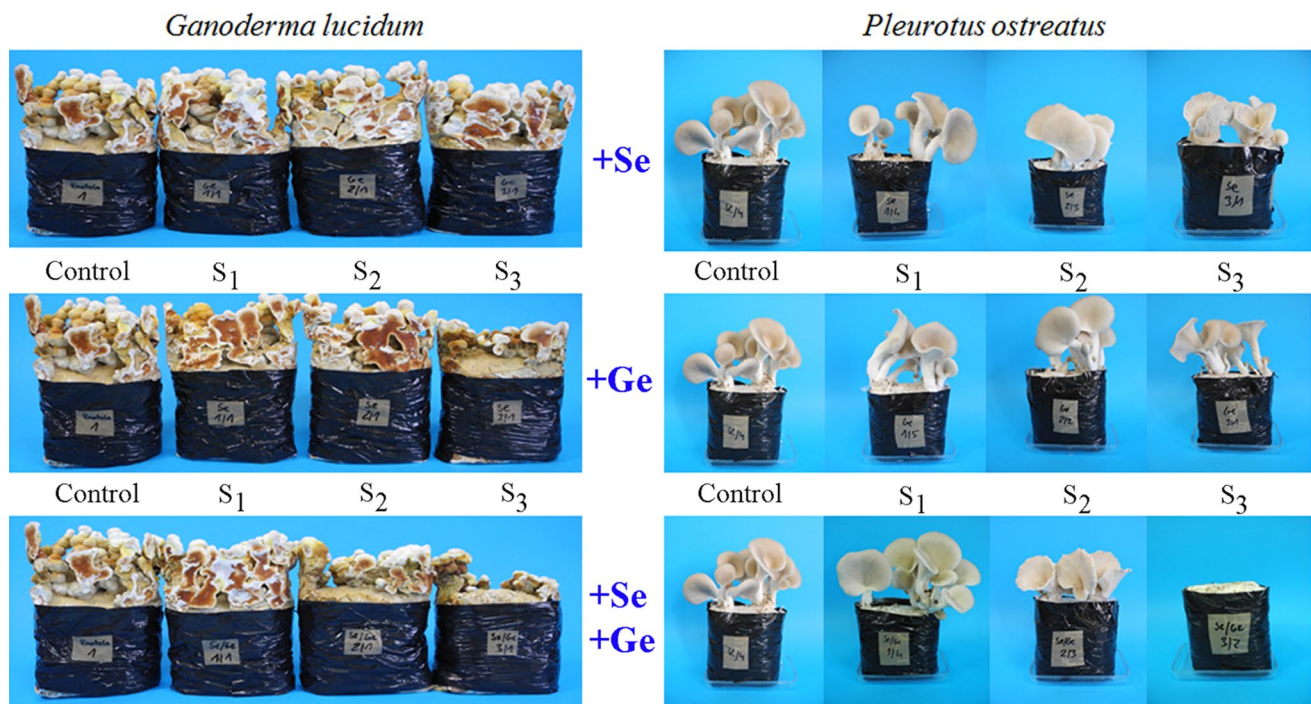*Ganoderma lucidum*　　　　　　　　　　　　　*Pleurotus ostreatus*

Fig. 1 Morphology of *Ganoderma lucidum* and *Pleurotus ostreatus* fruiting bodies growing under particular experimental systems

whiter (not visible in Fig. 1) and characterised by a lower size than the control, while *G. lucidum* growing under S_3 was not able to yield.

The biomass of three independent controls of *G. lucidum* was in the range 72.7–74.1 g per 400 g of substrate, while for *P. ostreatus* it was 81.3–81.9 g, which indicated stable conditions of growth (Fig. 2). Addition of Se, Ge or Ge + Se to the substrate of *G. lucidum*, did not cause any significant decrease in the biomass of this mushroom (89.2; 86.2 and 70.3% of control for systems enriched with Se, Ge and Ge + Se), respectively. A similar result was observed for *P. ostreatus* growing in substrates supplemented with Se only (85.1% of control).

The exposure of *P. ostreatus* to Ge in its highest concentration (S_3) caused a significant decrease of biomass (59% of control). The biomass of *P. ostreatus* exposed to Se + Ge in system S_2 was significantly lower than for the control and S_1 (72.6% of control), which suggested that *P. ostreatus* is more sensitive to Ge + Se addition than *G. lucidum*.

Content of Ge and Se in fruiting bodies

The content of Ge in *G. lucidum* increased with the increase of the metalloid concentration in the substrate (Fig. 3a). Addition of Se stimulated the accumulation of Ge in systems S_2 and S_3, where the content of Ge in mushrooms supplemented with two elements was significantly higher than under the supplementation with only Ge

(64.5 and 113%, respectively). A different tendency was observed for *P. ostreatus* fruiting bodies (Fig. 3c). Cultivation of this species in the substrate with Se or Ge + Se did not cause any significantly higher/lower accumulation of Ge.

Selenium content in *G. lucidum* fruiting bodies increased with the increase of the element in the substrate with the same significant differences as observed for Ge (Fig. 3b). Significantly higher contents of Se were found in fruiting bodies growing under S_2 and S_3 systems enriched with Ge + Se than in those supplemented with Ge only (by 132 and 37.6%, respectively). The content of Se in *P. ostreatus* increased with the increase of the metalloid in the substrate but as for Ge in *G. lucidum*; there were no significant differences in Se content in fruiting bodies cultivated in both kinds of substrates (enriched with one or two metalloids).

As shown in Table 1, the bioaccessibility of Ge was generally low, although higher in *P. ostreatus*. In *G. lucidum* it increased with increasing initial Ge content. The simultaneous addition of Se to the substrate resulted in increased accumulation of Ge in *G. lucidum* fruiting bodies, particularly at higher initial content. A similar observation was made for Se in both species—the simultaneous presence of Ge increased the content of Se in fruiting bodies. Higher bioaccessibility of Se was noted for *P. ostreatus*.
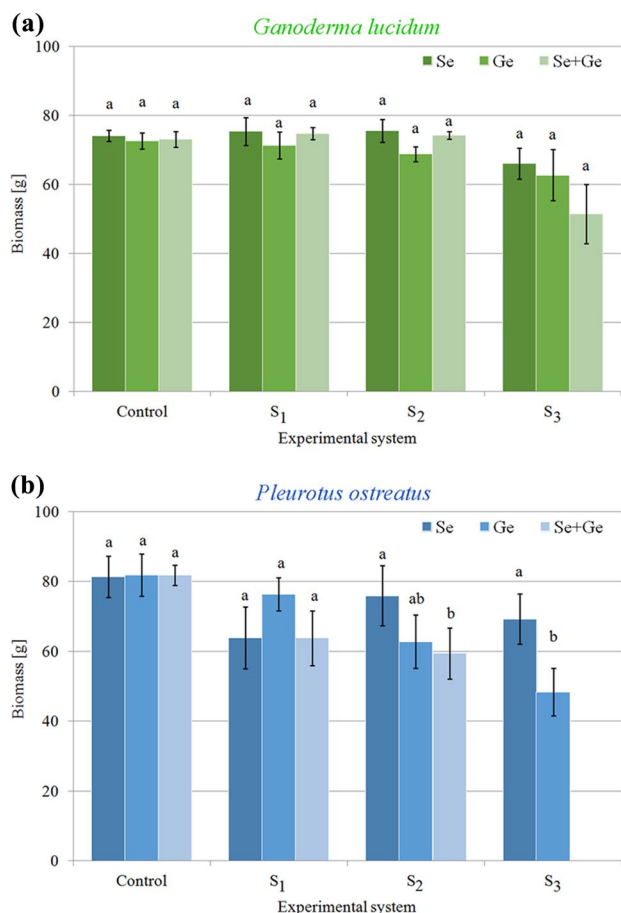
Fig. 2 Biomass (g) of *Ganoderma lucidum* (**a**) and *Pleurotus ostreatus* (**b**) fruiting bodies

Content of Se species in fruiting bodies

Accumulation of Se added to the substrate as Se(IV) and Se(VI) in the same concentrations resulted in different concentrations of these forms in fruiting bodies (Fig. 4). Despite the addition of inorganic Se forms to the substrate, the dominant form in both mushroom species and all four experimental systems was Se_{org}. The proportion of Se_{org} in *G. lucidum* growing in the substrate enriched with Se only (Fig. 4a) increased from 62.7% (S_1) to 87.1% (S_3) of total Se content. The addition of Ge and Se to the substrate caused a higher increase of Se_{org} participation [from 56.0% (S_1) to 91.6% (S_3)] in the total content of the metalloid. It is worth underlining that cultivation of *G. lucidum* in substrates with different additions (S_1–S_3) of the same concentration of both Se forms was related to the similar ratio of their contents (1.7; 0.7 and 2.0 for S_1, S_2 and S_3, respectively) in fruiting bodies. The addition of Ge to the substrate did not change this ratio for mushrooms growing under S_1 and S_2 systems (1.6 and 1.4, respectively), while for S_3 the ratio was 5. This could suggest that the presence of Ge in higher concentration

could improve Se_{org} accumulation by *G. lucidum* fruiting bodies.

The accumulation of Se forms in *P. ostreatus* fruiting bodies was diverse with the highest proportion in fruiting bodies growing in the substrate with Se addition only [from 70.7% (S_3) to 82.7% (S_2) of total Se content (Fig. 4c) but also with Ge and Se addition jointly [from 75.7% (S_1) to 77.6% (S_2) of total Se content], (Fig. 4d). The ratio of Se(IV)/Se(VI) in mushrooms growing in substrates enriched with only Se was similar for systems S_1 and S_3 (3.3 and 3.1, respectively) and lower for S_2 (1.4). Addition of Ge to the substrate did not cause the ratio of both Se forms to be 4.1 and 3.9, respectively, for S_1 and S_2 systems.

Content of ergosterol

Ergosterol contents were 325–337 and 180–186 mg kg^{-1} dm in *G. lucidum* and *P. ostreatus*, respectively, within the triplicate control variant (Fig. 5). Only the addition of Se for *G. lucidum* in the experimental system S_1 resulted in an elevation of ergosterol content (367 mg kg^{-1} dm) in comparison to the control. In all the experimental systems, high content of ergosterol was observed only if Se was added (S_1, S_2, and S_3).

Supplementation with Ge resulted in a significant drop of ergosterol to 77.2 mg kg^{-1} dm in S_3. Supplementation of *G. lucidum* with combined Se and Ge lead to a further reduction of the content, although in comparison to Ge supplementation the decrease was significant only for S_1. Fruiting bodies of *P. ostreatus* in all experimental systems contained lower ergosterol content in comparison to the control. In experimental systems S_1 and S_2 the highest ergosterol content was observed for Se supplementation, whereas the addition of Ge caused a decrease of ergosterol level. The Se and Ge addition resulted in a decrease in ergosterol content. However, significant changes in ergosterol content between Ge and Se + Ge supplementation were confirmed only in S_1. In the experimental system S_3, a similar content of ergosterol was determined for both Se and Ge additions.

Discussion

This is the first study to demonstrate the potential effects of Ge, alone or in combination with Se, on the morphology, growth and chemical constituents of mushroom fruiting bodies. Moreover, information on Ge uptake and accumulation in mushrooms has been limited, thus this study adds to a general understanding of the effects of this metalloid in biological systems. In fact, its content in various foodstuffs has also been rarely assessed and has mostly concerned vegetables and fruits [35]. The highest levels of Ge in commercially available mushrooms were noted
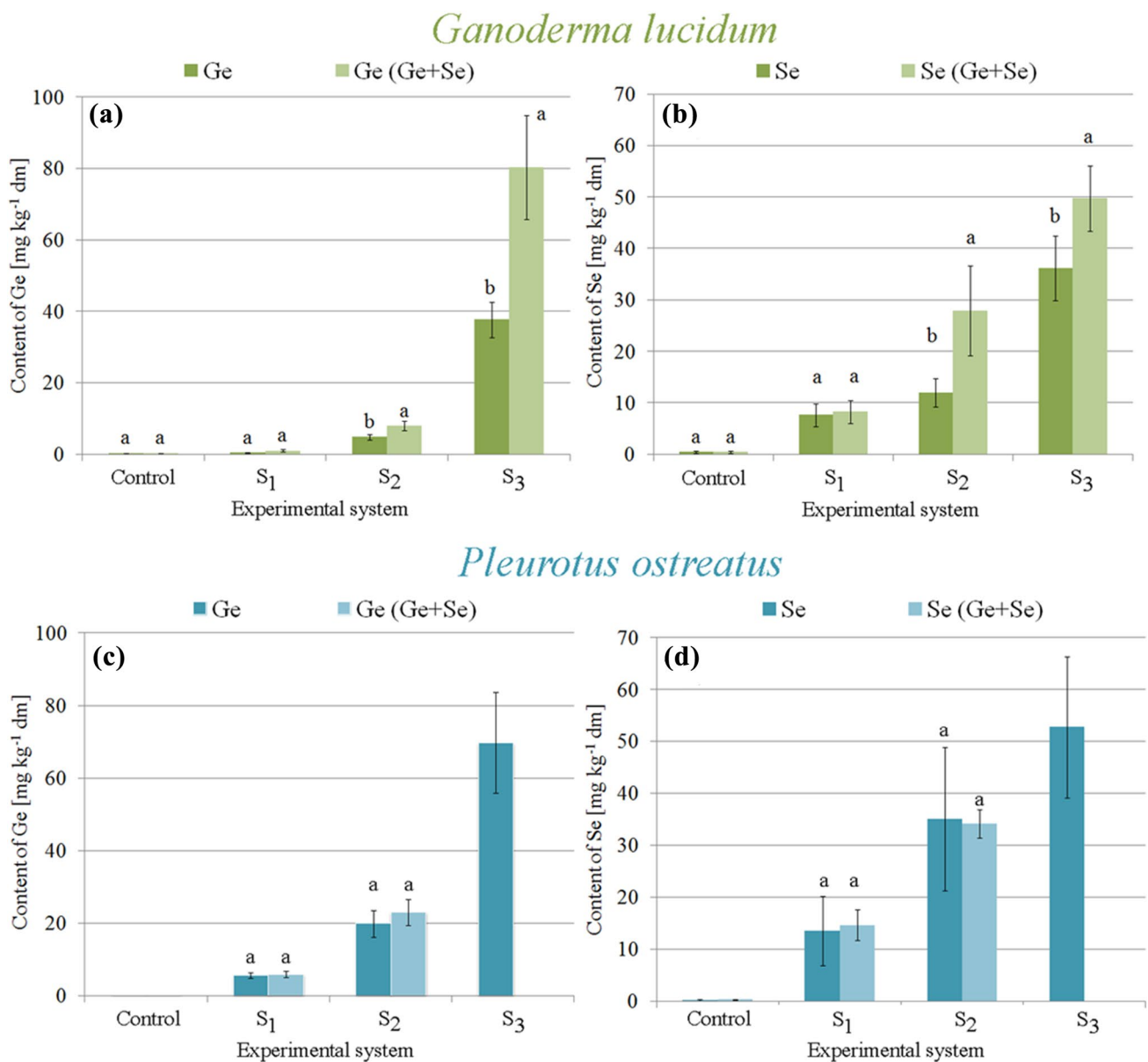
Fig. 3 Content (mg kg^{-1} dm) of Ge and Se in *Ganoderma lucidum* (**a**, **b**) and *Pleurotus ostreatus* (**c**, **d**) fruiting bodies

so far for selected *Ganoderma* species in which it could exceed 1 mg kg^{-1}, while specifically in *G. lucidum*, which was also tested in the present study, it reached approx 0.5 mg kg^{-1} [19, 23]. The present study experimentally suggests that this may result from substrate contamination with Ge, particularly if one considers that Ge bioaccessibility for *G. lucidum* was rather low. However, at low doses, Ge has been experimentally reported to exert potentially positive effects through antioxidant, antimutagenic, and antitumor activities [36], although it should be highlighted that there is no clear evidence for any health-beneficial effects of dietary Ge, and in certain forms, it may be toxic for humans [37]. Overall, the increased Ge

content in commercially cultivated *Ganoderma* sp. and the reasons behind it require further studies to ensure consumer safety.

Although contamination with Ge does not currently represent a major ecological problem, its use in numerous electronic applications, as well as its emission from some combustion processes, suggest that its levels in the surrounding environment will slowly increase [22]. Considering that mushrooms can accumulate various elements from the ambient environment it is important to ascertain to what extent this element can be present in fruiting bodies, whether this can affect mushroom growth and chemistry, and last but not least, whether it can interfere with other

Table 1 The bioaccessibility of Ge and Se for fruiting bodies *P. ostreatus* and *G. lucidum* in various experimental models

Element	Experimental model	Bioavailability (%)	
		Pleurotus ostreatus	*Ganoderma lucidum*
Ge	S1: Ge	5.4	0.4
	S1: Se + Ge	5.6	0.9
	S2: Ge	3.9	0.9
	S2: Se + Ge	4.3	1.5
	S3: Ge	6.6	3.5
	S3: Se + Ge	–	7.6
Se	S1: Se	41.8	23.6
	S1: Se + Ge	45.3	25.3
	S2: Se	27.0	9.2
	S2: Se + Ge	26.4	21.5
	S3: Se	20.3	14.1
	S3: Se + Ge	–	19.2

elements in the substrate such as Se whose use in mushroom biofortification has been discussed in some previous research [9–11, 25, 26].

While Se is a vital element, Ge is a non-essential one that may display certain biological activities but with no known physiological function. As previously shown in *G. lucidum*, the presence of Se in a growing medium can induce toxic effects in *G. lucidum* mycelia while Ge, which is easily taken up and accumulated in fruiting bodies, did not induce any detectable effects in the 10–1200 mg kg^{-1} range [38]. In line with these findings, the present study also highlights that Ge did not significantly alter the biomass and growth of this species except for a slight decrease in yield when it was present in the substrate along with Se at the highest level. It also induced some morphological changes under the highest levels. More profound effects were observed in *P. ostreatus* in which lower biomass was produced after Ge presence in the substrate, but this effect appeared to be further magnified by the presence of Se. Notably, however, no effects were noted up to 5 mM of Ge. The difference in the effect of Ge alone or combined with Se on the growth of *P. ostreatus* and *G. lucidum* is most likely due to the higher bioaccessibility of Ge to the former. Altogether, these observations indicate that although Ge is taken up by mycelia and accumulated in the fruiting bodies of both species, it does not induce any acute toxic effects at levels that could potentially be expected in commercial unaltered substrates. The present study also indicates that mushrooms are likely to be more resistant to Ge presence than plant crops such as wheat and barley, in which toxic effects such as organ necrosis, are already observed in the μM range [39, 40]. A full understanding of the potential mechanisms behind this tolerance, despite an

accumulation of Ge in mushrooms and its translocation to fruiting bodies, would require further studies.

The ability of the studied mushrooms to grow and accumulate Ge in their fruiting bodies may have some potentially interesting industrial applications in its recycling [21, 22]. Due to the commercial value of Ge, its re-processing is economically justified. It is known that tailings, e.g. those originating from zinc mining, can be characterized by increased levels of Ge [41]. Some mushroom species have already been shown to maintain their growth on highly contaminated soils polluted with flotation tailings [42]. Thus, the observation of the present study represents a promising possibility for the potential use of mushrooms to recycle Ge. This would require further more detailed experimental and in-field studies coupled with the development of methods to extract Ge from fruiting bodies, although some approaches in this respect have already been suggested for plant biomass [43].

As recently demonstrated by Goy et al. [44] using scanning electron microscopy, high concentrations of Se in the ambient environment can also lead to various ultrastructural alterations in mushrooms, such as a decrease in hyphal diameter and changes in spore number and shape. As previously established, when Se is added to a substrate at 0.4 μM, it is incorporated into the fruiting bodies without any significant reduction in growth and yielded biomass [25]. As shown in the present study, Se supplemented in inorganic forms was transformed and determined in fruiting bodies in organic selenocompounds. Similar transformations were previously observed by Rzymski et al. [26] in *Agaricus bisporus* and Rathore et al. [45] in *Calocybe indica*. This is an important finding if one considers that the biological role of Se is mainly mediated by its organic form, particularly selenoproteins with selenocysteine at their active centre [46]. This also concerns the human organism in which Se is an essential element of important metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems and regulation of immune function [47–49]. The addition of Se in the overgrown substrate may potentially increase the biological activity of polysaccharides and proteins isolated from *G. lucidum* [50, 51]. In turn, biofortification of *P. ostreatus* with Se was found to increase the protein content and antioxidant properties of the mushrooms [13, 52].

As shown in the present study, Se and Ge were taken up and accumulated in fruiting bodies in a concentration-dependent manner. Supplementation with both elements resulted in decreased ergosterol content in the fruiting bodies of *G. lucidum* and *P. ostreatus*. This effect was, however, much more evident for Ge. Ergosterol is a vitamin D precursor as it can be transformed under UV radiation in a biologically active form of D$_2$—ergocalciferol [53–55]. Although it appears that ergocalciferol has a lower biological effect in humans than cholecalciferol, it may be an important
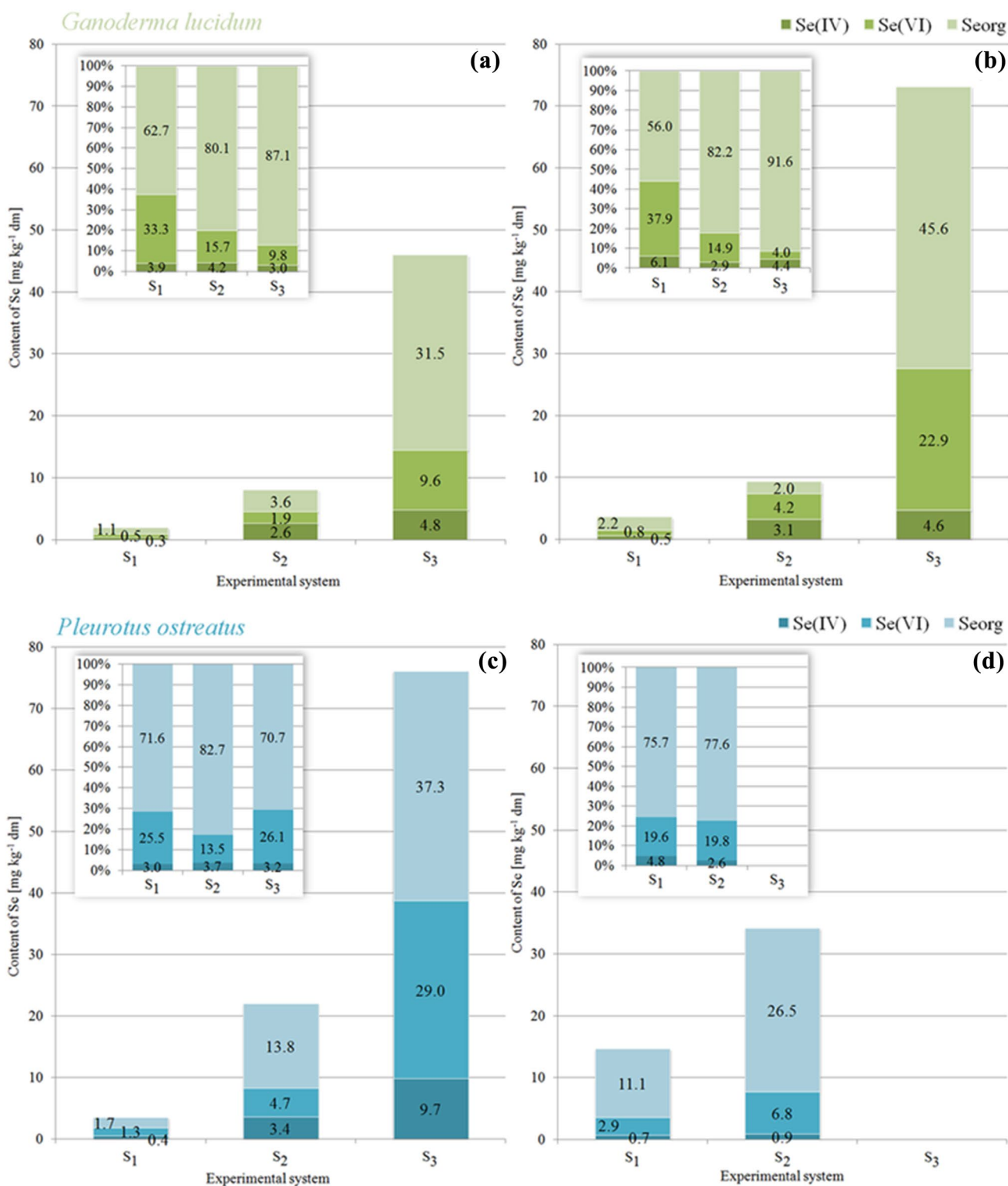
Fig. 4 Content (mg kg^{-1} dm) of Se forms in *Ganoderma lucidum* (**a**, **b**) and *Pleurotus ostreatus* (**c**, **d**) fruiting bodies growing in substrates enriched with Se (**a**, **c**) and Se + Ge (**b**, **d**) with percentage participation of particular Se forms in total content of Se

source for certain diet groups such as vegans and vegetarians [56]. Therefore, it appears that Ge can directly or indirectly alter sterol synthesis in mushrooms, an effect previously

established for various antifungal agents. Considering the pivotal role of ergosterol for membrane fluidity and structure [57], the contamination of mushrooms with Ge can result
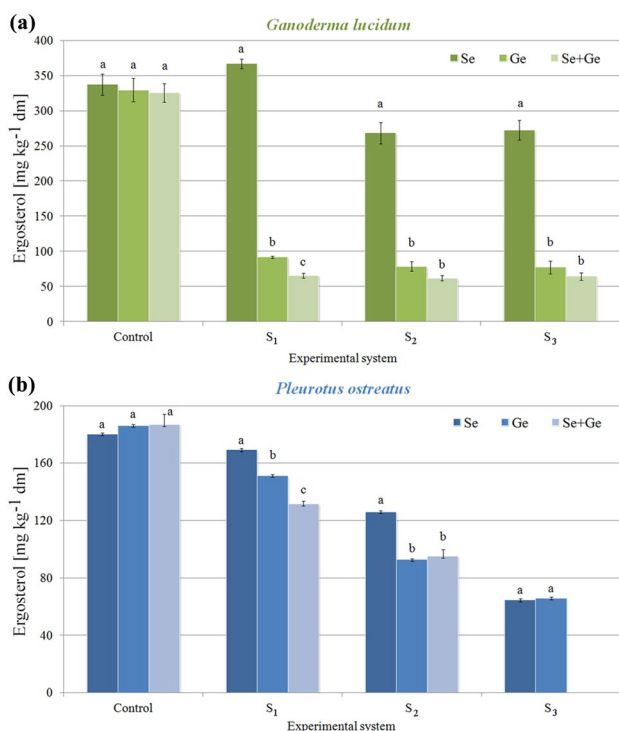
Fig. 5 Content (mg kg^{-1} dm) of ergosterol *Ganoderma lucidum* and *Pleurotus ostreatus* growing in substrates enriched with Se, Ge and Se+Ge

in unappreciated consequences for their growth and physiology. To the best of our knowledge, this is the first time that such effects of Ge have been observed. Further studies would be required to fully elucidate the mechanism(s) of Ge toxicity in mushrooms and whether its impact on ergosterol content represents a cause or effect.

Even though some studies using in vivo experiments have indicated that certain forms of Ge (e.g. b[beta]-bis(carboxyethyl) germanium sesquioxide, known as Ge-132) can have potentially beneficial immunomodulatory effects [58] one should bear in mind that cases of human poisonings, some of which resulted in renal failure and a fatal outcome, associated with Ge intake (including Ge-132 supplements) have also been documented in the literature [37, 59]. Prolonged in vivo exposures to Ge resulted in impaired function of liver and kidney, and myopathy in rodents [60, 61]. The present study shows that Ge can be effectively accumulated if present in the substrate at high levels. Its content, reaching 80 mg kg^{-1} and exceeding 60 mg kg^{-1}, was observed in *G. lucidum* and *P. ostreatus*, respectively. These levels are much higher than usually noted for foodstuffs [35]. Nevertheless, one should note that our study only assessed the total element content. The forms in which Ge can be present in mushrooms remain to be explored. It is plausible that it may form compounds with Se such as Ge selenide [62], although it is unknown whether such a phenomenon

occurs in mushrooms if high levels of Se and Ge are simultaneously available. The bioavailability of Ge in mushrooms, similar to other food products, is also unexplored. It has been shown in vivo in rabbits that Ge oxides are characterized by low oral bioavailability ($< 10\%$), and even lower values were noted for organic compounds [63].

It should be remembered that the present study has an experimental nature and does not suggest that commercially produced mushrooms may be contaminated with Ge. In fact, recent analyses indicate that Ge is undetectable in various cultivated mushrooms such as *Agaricus bisporus*, *Amauroderma rude*, *Auricularia auricula-judae*, *Auricularia nigricans*, *Ganoderma lucidum*, *Lentinula edodes*, *Lignosus rhinocerus*, *Ophiocordyceps sinensis*, *Pleurotus ostreatus*, *Sparassis crispa*, *Tremella fuciformis*, *Wolfiporia cocos*, *Volvariella volvacea* and *Pleurotus* sp. [14, 19]. Finally, despite anecdotal claims that consumption of mushrooms containing a high content of Ge may alleviate health concerns, there is no experimental or clinical evidence of such properties, and the present study cannot be used to support them.

Conclusions

Little attention has so far been given to studies of the interaction between Ge and Se. Therefore, the obtained results indicate the need for such studies in the future. The proper intake of Se in a balanced human diet plays a significant role. Thus, the possible presence of increased Ge concentration in the substrate can be related to a significantly higher content of Se in mushroom fruit. Visible symptoms such as changes in size or colour of mushroom bodies will be present, but a clear synergistic effect during exposure to higher concentrations of Ge in the substrate may be related with a negative influence on humans through the toxicity of both elements. The studied mushroom species were grown in substrate enriched with inorganic Se salts only and the dominant forms in *G. lucidum* were organic Se compounds. This tendency strengthened with increased concentration of Ge in the substrate, thereby illustrating the significant role of Ge compounds in the accumulation of Se in *G. lucidum*, which was not the case for *P. ostreatus*. Additionally, co-supplementation of the substrate with Ge or cultivation of mushrooms with Ge only resulted in a reduction of ergosterol content, which highlights the significance of the interaction between these elements. Due to the important role of ergosterol in fungi the presence in the substrate of some metalloids, especially Ge, may have a negative impact on the growth and physiology of the fungus.

Compliance with ethical standards

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