horticulturae

MDPI

Article

Combined Dairy Manure-Food Waste Digestate as a Medium for *Pleurotus djamor*—Mineral Composition in Substrate and Bioaccumulation of Elements in Fruiting Bodies

Agnieszka Jasinska [1,2,*], Raghavendra Prasad [2], Jolanta Lisiecka [2], Michal Roszak [3], Ketil Stoknes [1], Miroslaw Mleczek [4] and Przemyslaw Niedzielski [5]

1 Lindum AS, 3036 Drammen, Norway
2 Department of Vegetable Crops, Poznan University of Life Sciences, 60-594 Poznan, Poland
3 Soppas, 3125 Tønsberg, Norway
4 Department of Chemistry, Poznan University of Life Sciences, ul. Wojska Polskiego 75, 60-625 Poznan, Poland
5 Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, 61-614 Poznan, Poland
* Correspondence: jasinska.a@gmail.com or agnieszka.jasinska@up.poznan.pl; Tel.: +48-60-576-5040

Abstract: The present investigation aimed to study the utilisation of combined dairy manure-food waste digestate as a substrate (experimental mushroom substrate—EMS) for *Pleurotus djamor* (strain 2708, Mycelia) cultivation. Considering the lack of scientific information about the influence of elements concentration in growing substrates on the bioaccumulation of elements in cultivated mushrooms and their residual concentrations in substrates left after cultivation (spent mushroom substrate—SMS), a multi-elemental analysis of 38 elements was carried out. In the study, inductively coupled plasma optical emission spectrometry (ICP OES) was used for elemental analysis. The *P. djamor* cultivated on EMS resulted in a yield of 196.50 g/bag, achieving a biological efficiency (BE) of 39.90%. High variability in the elemental concentrations among substrates both before and after mushroom cultivation was evident. The studied elements accumulation in *P. djamor* was in an increasing trend in three subsequent flushes and was also reflected in the bioconcentration factors (BCFs). The highest BCF (2.35) was determined for Fe. Interestingly, the BCF values for all studied trace elements with detrimental health effects were lower than 1.00. The estimated daily intake (EDI) reflected that the *P. djamor* fruiting bodies grown on EMS can serve as an excellent dietary source of essential major and trace elements: Ca, Mg, Na, Mn, Mo, Ni, Se and Zn. On the other hand, EDI values for K, Cu, Fe, Ag, Ba, Cd, Al, Sb and Sr were greater than the referred guideline values corresponding to higher intake. Overall, the study presented an insight into elemental accumulations and demonstrated the potential utilisation of combined dairy manure-food waste digestate.

Keywords: bioconcentration factor; dietary intake; digestate; elemental composition; growing substrate; inductively coupled plasma optical emission spectrometry; *Pleurotus djamor*

Citation: Jasinska, A.; Prasad, R.; Lisiecka, J.; Roszak, M.; Stoknes, K.; Mleczek, M.; Niedzielski, P. Combined Dairy Manure-Food Waste Digestate as a Medium for *Pleurotus djamor*—Mineral Composition in Substrate and Bioaccumulation of Elements in Fruiting Bodies. *Horticulturae* **2022**, *8*, 934. https://doi.org/10.3390/horticulturae8100934

Academic Editor: Dragan Žnidarčič

Received: 1 September 2022
Accepted: 7 October 2022
Published: 11 October 2022

1. Introduction

Mushroom cultivation is a biotechnological process, during which agro- and industrial waste residues are bioconverted into edible biomass of high nutritional value [1]. Mushrooms have been used as food since ancient times and are appreciated for their delicious flavor and health-promoting properties [2–4]. They are considered both a functional food and source of nutraceuticals [5,6]. In recent years, it is noticeable that the global production of mushrooms has been steadily increasing. It is estimated that the value of mushroom production in the world has reached nearly USD 17 billion in the year 2021, and it is further expected to reach nearly USD 20.4 billion by 2027 [7].

P. ostreatus (white oyster mushroom) is one of the most widespread mushrooms in the world and the *P. djamor* (pink oyster mushroom) is an exotic species popular for its

pink coloration, fibrous texture, and high antioxidant potential [8,9]. The consumption of *Pleurotus* is increasing due to the taste, nutritional and medicinal properties [5,10,11].

In line with the above-mentioned, because of their simple and low-cost production technology, with high biological efficiency, *Pleurotus* spp. are popular and widely cultivated mushrooms throughout the world [12,13]. *Pleurotus* can be cultivated on various conditions and substrates, including plant residues and agro-industrial by-products [11–14]. However, the rational choice of the substrate is one of the most crucial aspects of *Pleurotus* cultivation [15].

In recent years, there is a growing interest in easily available and low-cost alternative materials for *Pleurotus* cultivation [16,17]. Especially, the potential re-utilisation of materials derived from agro-industrial waste streams is of great significance [18–20]. Several studies have demonstrated the possible cultivation of *Pleurotus* spp. on non-traditional substrates, including spent mushroom substrate [21], agave bagasse [22], olive-mill wastes [23], winery and olive will waste [24], rice-bran and food waste compost [25] as well as anaerobically digested plant and agro-residues [26]. The cultivation of *Pleurotus* spp. on straw-based and other agro-waste-derived substrates has been extensively studied [11,18,27,28], while the scientific reports on utilising non-traditional substrate substitutes derived from neglected waste streams, for instance, spent mushroom substrates [21] or combined dairy manure-food waste digestate [20], for mushroom cultivation is still in their infancy.

Anaerobic digestion (AD) is a plausible approach for organic waste management and nutrient recycling, and such technology closely interacts with the food–energy–water (FEW) nexus [29]. AD is an anaerobic biological process that converts organic matter to biogas and digestate [30]. It is being used to degrade various types of organic waste, including lignocellulosic biomass, food waste and animal manure [31]. One such material is dairy manure-food waste digestate. Anaerobic co-digestion of dairy manure and food waste is practiced in The Magic Factory in Sem, Norway, where AD generates an effluent called 'digestate'. This digestate is nitrogen- and organic-matter-rich material which has been investigated as a component of growing substrates for mushroom cultivation [26,32–34].

Among various agro-industrial wastes, the possible utilisation of original digestate (OD) derived from combined dairy manure-food waste can stand out due to the nutritional composition [35], easy availability [32] and relatively low price. On the other hand, depending on the input to the biogas digesters, the digestate can be rich in mineral elements such as nonessential elements, trace elements and heavy metals, various organic pollutants as well as other unwanted compounds which make its usefulness in direct food production (including vegetables and mushrooms) questionable [36–39]. In line with these opportunities and challenges, the lack of scientific information on the valorisation of such digestate is prominent.

As mentioned earlier, even though in mushroom production agro/farm-wastes are converted into valuable food, at the same time, many organic residues are generated after the commercial mushroom production. For every kilogram of fresh mushrooms produced, approximately 5 to 6 kg of residual material (substrate/by-product) is left off [40,41]. This material is known as spent mushroom substrate (SMS). SMS is often considered as a farm/agro-waste [42,43]. To reduce the problems associated with accumulating amounts of SMS, the potential utilisation of this material in subsequent mushroom production is the best and most economically viable method [21,44], which can result in sustainable use of waste resources, generating a circular economy [44–47].

It has been reported that the trace metal concentrations in mushrooms are significantly higher than those in agricultural plants, vegetables and fruits [48]. The most commonly evaluated elements in mushrooms are: Ca, K, Fe, P, Cu, Se, Pb, Cd and Hg; less commonly: Mg, Mn, Al, Cr, Ni and B; very rarely: Na, Ag, Cs, Co, Ba, Sr, Ti, U, Th and V [49–64]. The lack of scientific information on the elemental content in cultivation substrates and their effects on the mushroom production process is evident [65]. Hence, studying the possible utilisation of digestate from combined dairy manure-food waste in the cultivation of popular *P. djamor* and the determination of elemental content in cultivation substrate and mushroom fruiting bodies will be of great significance.

The present investigation aimed to use the original digestate (OD) from anaerobic digestion of combined dairy manure-food waste as a substrate for *P. djamor* (2708, Mycelia) cultivation. The study was carried out to evaluate: (i) the potential use and suitability of a designated agro-industrial waste for *P. djamor* cultivation; (ii) the elemental composition of substrates both before and after mushroom cultivation; (iii) the content of 38 elements accumulated in mushroom fruiting bodies.

In the present investigation, the yield, biological efficiency (BE), dry matter (DM) and bioconversion efficiency (BCE) of *P. djamor* fruiting bodies were also determined. The elemental content in cultivation substrate and mushroom fruiting bodies for major essential elements (MEEs)—Ca (calcium), K (potassium), Mg (magnesium), Na (sodium))—essential trace elements (ETEs)—B (boron), Co (cobalt), Cu (copper), Cr (chromium), Fe (iron), Mn (manganese), Mo (molybdenum), Ni (nickel), Se (selenium), Zn (zinc))—trace elements with detrimental health effect (TEWDHE)—Ag (silver), As (arsenic), Ba (barium), Be (beryllium), Cd (cadmium), Hg (mercury), Tl (thallium))—and nutritionally nonessential elements (NNEs)—Al (aluminum), Bi (bismuth), Ce (cerium), Cs (cesium), Ga (gallium), Ge (germanium), Li (lithium), Nd (neodymium), Pr (praseodymium), Sb (antimony), Sr (strontium), Ta (tantalum), Te (tellurium), Ti (titanium), V (vanadium), W (tungsten), Zr (zirconium)—were determined by inductively coupled plasma optical emission spectrometry (ICP OES). Further, the bioconcentration factors (BCFs) were calculated, and the dietary intake of each element determined as estimated daily intake (EDI) was compared versus its respective needs/limitations. To the best of our knowledge, the presented work is one of its first kind evaluating 38 multi-elemental contents of cultivation substrate (experimental mushroom substrate—EMS), mushroom fruiting bodies and spent mushroom substrate (SMS) to give a deeper insight into using the digestate-based substrate for *P. djamor* cultivation.

2. Materials and Methods

The experiment was performed at the climate-controlled mushroom growing chamber of the R&D Department of Lindum AS, Drammen, Norway. Composting was medium scale (20–40 kg of experimental mushroom substrate per batch) in reactors resembling commercial conditions. Two batches, one in February 2018 and one in March 2019, were made representing combinations of materials and ratios. The amounts were estimated by the dry matter of substrates.

2.1. Preparation of Mushroom Substrate

Experimental mushroom substrate (EMS) consisted of wheat straw—source separated fresh food waste (80%) and dairy manure (20%) original digestate (OD) from anaerobic digestion treatment processes, hot compost—(for triggering the composting process) and gypsum (to maintain the suitable compost structure and stable pH of the outcoming substrate around 7.0–7.3). The composts' compositions were based on dry matter (DM) of substrates before processing and were evened to obtain ±30% of DM of the substrate; the initial C/N ratio was 27:1. The EMS was composted for 10 days and pasteurised. A detailed description of the substrate preparation, composting procedure, physical and chemical properties of base materials and EMS are provided in the Supplementary Materials.

2.2. Mushroom Cultivation

2.2.1. Inoculation

After pasteurisation of the substrate, 2 kg of substrate was filled into the cultivation bags, later cooled to room temperature and inoculated with the granular spawn of *P. djamor* (2708, Mycelia, Deinze, Belgium). Bags were randomised and incubated at 25 °C in a dark room with internal air circulation and passive ventilation with no humidification. After the spawn run in the EMC, cuts in the bags were made using the sterile carpet knife to enable primordia formulation.

2.2.2. Pinning

The temperature inside the growing chamber was maintained at 25 ± 2 °C for 15 days, then reduced by 5 °C for the next 5 days for initiation of pinning. The air humidity was maintained at 85–95% during fruiting body development. The cultivation chambers received LED light (6000 K), and the room was ventilated to maintain the CO_2 concentration below 1000 ppm.

2.2.3. Harvesting

Mushrooms were picked manually at the maturity stage (when the edges of most mushrooms in the cluster started to open), the clusters were harvested as a whole. After the last cluster from the bag was harvested, the holes in the bags were cleaned from residues of mushrooms allowing the second flush of mushrooms to appear. Yields were determined as the weight of the harvested mushrooms from the complete cropping period per fresh weight of substrate at inoculation (i.e., per bag). A representative sample of each crop (approx. 100 g) was also weighed, dried and prepared for qualitative analysis. The detailed information about bag cultivation is provided in the Supplementary Materials.

2.3. Analysis of Raw Materials, Experimental Mushroom Substrate (EMS), Spent Mushroom Substrate (SMS) and Mushroom Tissues

2.3.1. Sampling and Homogenisation

Fresh samples (300 g) of thoroughly mixed EMS were taken as composite samples by combining 10 random sub-samples. From this, fresh samples were taken for immediate pH and EC (electrical conductivity) measurement (repeated three times). The remaining samples were dried and weighed for dry matter determination, later combined and subsequently homogenised in a blender. This was used for ash content analysis. Details on sampling, equipment and methods are provided in Supplementary Materials.

2.3.2. Analytical Methods

Sample Collection and Preparation Procedure

All reagents used in the study were of the analytical grade of purity (99.9%), and deionised ultrapure water (Milli-Q, Millipore, Saint Luis, MI, USA) was used. The 65% nitric acid was used for sample preparation (Merck, Darmstadt, Germany).

Collected samples of substrates and mushroom fruiting bodies (caps and stipes together) were dried preliminary at 45 ± 2 °C for 120 h in an electric oven (SLW 53 STD, Pol-Eko, Wodzisław Śląski, Poland) and ground in a laboratory Cutting Boll Mill PM 200 (Retsch GmbH, Haan, Germany). Accurately weighted 0.500 ± 0.001 g of a dry sample of substrate or mushroom was digested by concentrated nitric acid in close Teflon containers in the microwave sample preparation system Mars 6 Xpress (CEM, Matthews, NC, USA). After, digestion samples were filtered through filter papers and diluted with water to a final volume of 15.0 mL. Each of the samples was analysed in triplicate using the whole sample preparation procedure.

Element Contents in Substrate and Mushroom

The inductively coupled plasma optical emission spectrometry (Agilent 5110 ICP OES, Agilent, Santa Clara, CA, USA) was used for 38 elements determination. The mode of the simultaneously axial and radial view of plasma has been allowed by the synchronous vertical dual view (SVDV). For multi-elemental determination, the common conditions were used: Radio Frequency (RF) power 1.2 kW, nebuliser gas flow 0.7 L/min, auxiliary gas flow 1.0 L/min, plasma gas flow 12.0 L/min, viewing height for radial plasma observation 8 mm, detector CCD (Charge Coupled Device) temperature −40 °C, signal accusation time 5 s for 3 replicates. For sample digestion, the microwave sample preparation system Mars 6 (CEM, Matthews, NC, USA) was followed.

Analytical Method Validation

The detection limits have been determined on the level of 0.0X mg/kg dry weight (DW) or better for all elements determined (follow IUPAC recommendation as 3-sigma criteria). The uncertainty for the total analytical procedure (including sample preparation) was below the level of 20%. Due to the lack of the certified reference material in accordance with the sample's matrix, the traceability was checked using reference materials CRM S-1—loess soil; CRM NCSDC (73349)—bush branches and leaves; CRM 2709—soil; CRM 405—estuarine sediments; CRM 667—estuarine sediments and the recovery (80–120%) was acceptable for most of the elements determined. For not certified elements, the recovery in the standard addition method has been defined.

To consolidate the number of replications while maintaining randomisation, every individual sample was analysed in triplicate. All element contents are given as milligrams per kilogram of dry matter (DM).

2.4. Yield and Biological Efficiency

The yield (g/bag) and dry weight of *P. djamor* fruiting bodies (g·100 g fresh mushroom weight^{-1}) were measured. At the end of the experiment, the recorded data were used to calculate biological efficiency (BE). Biological efficiency was calculated based on the formula suggested by Yang et al. [66]: BE (%) = weight of fresh mushrooms harvested per bag/weight of dry substrate per bag × 100.

2.5. Bioconcentration Factors (BCFs)

The BCFs for major essential elements (MEEs): Ca, K, Mg, Na; essential trace elements (ETEs): B, Co, Cu, Cr, Fe, Mn, Mo, Ni, Se, Zn; trace elements with detrimental health effect (TEWDHE): Ag, As, Ba, Be, Cd, Hg, Tl; nutritionally nonessential elements (NNEs): Al, Bi, Ce, Cs, Ga, Ge, Li, Nd, Pr, Sb, Sr, Ta, Te, Ti, V, W, Zr were calculated as the ratio of their concentration in mushrooms from subsequent flushes to the content in the cultivation substrate based on digestate.

2.6. Dietary Intake and Health Risk Assessment of P. djamor Consumption

To estimate the daily intake of elements through *P. djamor* mushroom consumption, the following equation (E.P.A. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures): EDI = CM × CR/BW, as given by Koutrotsios et al. [65] was used, where EDI stands for the estimated daily intake of elements (mg·kg^{-1}·d^{-1}), CM is the concentration of elements in mushrooms (based on dry weight) and CR corresponds to one serving of mushrooms, which is 250 g of fresh weight equal to 25 g of dry matter [67], and BW represents the average body weight of adults (60 kg)—in agreement with the EU Scientific Committee for the Food Adult Weight parameter [68]. Values of TDI (tolerance daily intake) and RDI (reference daily intake) were adopted from Koutrotsios et al. [65] and based on reports and research findings [69–74].

2.7. Statistical Analysis

The study was carried out in three replicates as a one-factor design, where the influence of EMS was evaluated for *P. djamor* cultivation. The data obtained from the experiment were subjected to a one-way analysis of variance (*ANOVA*). The differences in means were later assessed using Tukey's HSD at a 95% confidence level ($p < 0.05$). Results are presented as mean ± standard deviation (SD). The statistical analyses were performed using SPSS 22 (IBM Corporation, Armonk, NY, USA) software for Windows. The visualisation of data was performed using OriginLab 2022 software for Windows (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Yield, Dry Matter and Biological Efficiency of P. djamor

The total yield (flush one, two and three combined) (g/bag), dry matter (g), per cent dry matter and biological efficiency (BE) are given in Table 1. In the present investigation, the experimental mushroom substrate (EMS) based on digestate resulted in the yield of 196.50 g of *P. djamor* fruiting bodies. The dry matter of *P. djamor* fruiting bodies was 24.20 g, corresponding to 12.52%. The BE of the substrates reflects the ability of a specific mushroom strain to grow on the substrate. In the present investigation, the BE (%) of *P. djamor* (2708, Mycelia) cultivated on EMS was 39.30%.

Table 1. The yield, dry matter and biological efficiency (BE) of *P. djamor* cultivated on EMS.

Yield (g)	196.5 ± 43.6
Dry matter (g)	24.20 ± 4.18
Dry matter (%)	12.52 ± 1.87
Biological efficiency (%)	39.30 ± 8.72

3.2. Elemental Concentration in the EMS (Experimental Mushroom Substrate) Used for the Cultivation of P. djamor

In the present investigation, the concentrations for 38 elements; major essential elements (MEEs): Ca, K, Mg, Na; essential trace elements (ETEs): B, Co, Cu, Cr, Fe, Mn, Mo, Ni, Se, Zn; trace elements with detrimental health effect (TEWDHE): Ag, As, Ba, Be, Cd, Hg, Tl; nutritionally nonessential elements (NNEs): Al, Bi, Ce, Cs, Ga, Ge, Li, Nd, Pr, Sb, Sr, Ta, Te, Ti, V, W, Zr in the EMS at inoculation were determined and are presented in Table 2. Among many, one of the main factors influencing the concentration of elements in mushrooms is their availability in the cultivation substrates. The results indicated that the EMS was a rich source of all MEEs: Ca, K, Mg and Na; some ETEs: Fe, Mn and Zn. In the EMS, studied TEWDHE ranged between <0.01 mg·kg^{-1} DM (Ag and As) and 0.62 mg·kg^{-1} DM (Cd). Among the studied TEWDHE, only the concentration of Ba was higher (40.85 mg·kg^{-1} DM). As for the NNEs, the content in the substrate decreased in the following order: Sr > Al > Te > W > Ti > Sb > Pr > Bi > Ta > Ce > Nd > Ge > V > Cs > Ga > Li.

3.3. Elemental Concentration in the SMS (Spent Mushroom Substrate) after P. djamor Cultivation

At the end of the *P. djamor* cultivation, the residual elemental analysis of SMS was carried out for MEEs, ETSs, TEWDHE and NEE (Table 2). Among studied MEEs, the residual concentrations of Ca, K, Mg and Na in SMS were observed to be greater than the initial amount of these elements detected in the EMS. Similarly, among ETEs, the content of B, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn in SMS was relatively greater than the initial values in EMS. Particularly, the amount of Fe in SMS increased nearly 4.5 times in comparison to the initial value detected in EMS.

Table 2. Element's concentration in the substrate (EMS) used for the cultivation of *P. djamor* and in the substrate after cultivation (SMS).

	Element (mg·kg^{-1} DM)									
	Major Essential Elements (MEEs)									
				Ca	K	Mg	Na			
EMS				1420	11,000	1270	1950			
SMS				2130	17,000	2520	2910			
	Essential trace elements (ETEs)									
	B	Co	Cr	Cu	Fe	Mn	Mo	Ni	Se	Zn
EMS	2.67	0.39	35.2	1.99	28.8	51.5	0.47	0.06	1.10	47.3
SMS	5.43	0.47	47.4	3.78	130	79.1	0.59	3.03	1.99	82.0

Table 2. *Cont.*

Element (mg·kg^{-1} DM)										
Major Essential Elements (MEEs)										
Trace elements with detrimental health effects (TEWDHE)										
		Ag	As	Ba	Be	Cd	Hg	Tl		
EMS		<0.01	<0.01	40.85	0.05	0.62	0.04	0.29		
SMS		0.01	0.2	60.39	0.06	0.66	0.04	1.63		
Nutritionally nonessential elements (NNEs)										
	Al	Bi	Ce	Cs	Ga	Ge	Li	Nd	Pr	Sb
EMS	59.3	0.53	0.45	<0.01	<0.01	0.35	<0.01	0.38	1.35	1.85
SMS	250	1.13	0.21	6.15	0.01	0.77	0.25	0.26	2.07	3.38
			Sr	Ta	Te	Ti	V	W	Zr	
EMS			60.4	0.52	12.05	2.05	0.31	3.61	0.25	
SMS			85.3	0.56	17.42	12.6	0.76	3.30	0.53	

EMS—experimental mushroom substrate, SMS—spent mushroom substrate.

3.4. Elemental Concentration in Fruiting Bodies of P. djamor

Among the studied TEWDHE, the amount of Ag and Hg in SMS remained at the same level as initial values determined in EMS at the beginning of *P. djamor* cultivation. While, a slight increment in concentrations of As, Ba, Be, Cd and Tl in SMS was noticed, reflecting the residual amount of the above-mentioned elements in SMS.

The concentration of Ce, Nd and W among NNEs in SMS was relatively lower when compared to the initial values determined in EMS. While the concentration of all other studied NNEs in SMS slightly increased. Particularly, the residual concentration of Al and Ti in SMS was observed to be approximately four- and six-folds greater than the initial values determined in EMS.

3.4.1. Content of Major Essential Elements (MEEs) in Fruiting Bodies of P. djamor

The results of the *P. djamor* elemental analysis indicated that the concentration of all studied MEEs significantly differed among subsequent flushes (Table 3). The most abundant element recorded in *P. djamor* mushrooms in all three flushes was K, followed by Ca, Mg and Na. Between three subsequent flushes, the content of Ca in *P. djamor* fruiting bodies was highest in flush three (1270 mg·kg^{-1} DM). Similarly, the content of Mg (718 mg·kg^{-1} DM) and Na (406 mg·kg^{-1} DM) was also significantly superior in flush three. While the content of K in flush two and three was significantly superior to that of flush one. A very particular flush-specific pattern was recorded concerning the studied major element accumulation in fruiting bodies of *P. djamor*, where an increasing trend of accumulation was distinct among subsequent flushes.

Table 3. Concentrations of MEEs in the fruiting bodies of *P. djamor* cultivated on EMS.

Element (mg·kg^{-1} DM)	**Flush I**	**Flush II**	**Flush III**
Ca	880 ± 168 ^{b} *	1110 ± 41.7 ^{ab}	1270 ± 44.1 ^{a}
K	11,000 ± 130 ^{b}	13,000.20 ± 400 ^{a}	14,000.20 ± = 300 ^{a}
Mg	526 ± 35 ^{c}	622 ± 293 ^{b}	718 ± 38 ^{a}
Na	218 ± 34 ^{b}	274 ± 29 ^{b}	406 ± 28 ^{a}

* Data represent mean ± SD (n = 3); means corresponding to the same element are compared between flushes; identical letters denote no significant difference between mean values; different letters indicate the significant differences among mean values for elements according to Tukey's HSD at $p < 0.05$.

3.4.2. Content of Essential Trace Elements (ETEs) in Fruiting Bodies of P. djamor

Significant differences were recorded for ETEs content in the *P. djamor* fruiting bodies (Table 4). The elemental content in *P. djamor* was recorded to be the highest in flush three

for Fe (191 mg·kg^{-1} DM) and Se (2.44 mg·kg^{-1} DM) when compared to previous flushes. While the content of B and Zn in flush two (2.74 mg·kg^{-1} DM and 78.0 mg·kg^{-1} DM) and three (3.37 mg·kg^{-1} DM and 93.5 mg·kg^{-1} DM), respectively, were observed to be significantly superior to the content of *P. djamor* in flush one. Like the MEEs, distinct increasing accumulation of B, Fe, Se and Zn in *P. djamor* fruiting bodies were noticed for subsequent flushes. While the no-particular flush-specific pattern for increasing accumulation was detected for Co, Cr, Cu, Mn, Mo and Ni among the three flushes and the determined values for these elements were not statistically different.

Table 4. Concentrations of ETEs in the fruiting bodies of *P. djamor* cultivated on EMS.

Element (mg·kg^{-1} DM)	Flush I	Flush II	Flush III
B	0.93 ± 0.92 ^{b} *	2.74 ± 0.48 ^{a}	3.37 ± 0.17 ^{a}
Co	0.56 ± 0.05 ^{a}	0.80 ± 0.07 ^{a}	0.99 ± 0.08 ^{a}
Cr	0.34 ± 2.6 ^{a}	0.39 ± 1.3 ^{a}	0.42 ± 0.5 ^{a}
Cu	11.3 ± 0.7 ^{a}	13.8 ± 0.8 ^{a}	15.0 ± 0.4 ^{a}
Fe	110 ± 22 ^{c}	147 ± 15 ^{b}	191 ± 7 ^{a}
Mn	5.96 ± 0.68 ^{a}	6.74 ± 0.21 ^{a}	7.76 ± 0.45 ^{a}
Mo	0.01 ± 0.00 ^{a}	0.03 ± 0.03 ^{a}	0.20 ± 0.11 ^{a}
Ni	0.01 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}	0.10 ± 0.03 ^{a}
Se	0.38 ± 0.32 ^{c}	1.58 ± 0.44 ^{b}	2.44 ± 0.14 ^{a}
Zn	48.7 ± 8.2 ^{b}	78.0 ± 7.1 ^{a}	93.5 ± 1.3 ^{a}

* Data represent mean ± SD (n = 3); means corresponding to the same element are compared between flushes; identical letters denote no significant difference between mean values; different letters indicate the significant differences among mean values for elements according to Tukey's HSD at $p < 0.05$.

3.4.3. Content of Trace Elements with Detrimental Health Effects (TEWDHE) in the Fruiting Bodies of *P. djamor*

The content of studied TEWDHE in *P. djamor* fruiting bodies are given in Table 5. The element content for As and Ba of *P. djamor* fruiting bodies exhibited significant difference among subsequent flushes, where the content of As and Ba in flush two (0.62 mg·kg^{-1} DM and 2.06 mg·kg^{-1} DM) and flush three (0.88 mg·kg^{-1} DM and 2.32 mg·kg^{-1} DM), respectively, were greater than the content determined from *P. djamor* fruiting bodies in flush one. The flush-specific increasing accumulation of elements in mushroom fruiting bodies was only determined for As and Ba. The content of the rest of the studied TEWDHE across different flushes did not differ significantly.

Table 5. Concentrations of TEWDHE in the fruiting bodies of *P. djamor* cultivated on EMS.

Element (mg·kg^{-1} DM)	Flush I	Flush II	Flush III
Ag	0.06 ± 0.04 ^{a} *	0.12 ± 0.01 ^{a}	0.16 ± 0.02 ^{a}
As	0.14 ± 0.20 ^{b}	0.62 ± 0.06 ^{a}	0.88 ± 0.19 ^{a}
Ba	1.58 ± 0.30 ^{b}	2.06 ± 0.04 ^{a}	2.32 ± 0.13 ^{a}
Be	0.04 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}
Cd	0.24 ± 0.06 ^{a}	0.35 ± 0.01 ^{a}	0.51 ± 0.15 ^{a}
Hg	0.01 ± 0.00 ^{a}	0.02 ± 0.01 ^{a}	0.05 ± 0.01 ^{a}
Tl	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}

* Data represent mean ± SD (n = 3); means corresponding to the same element are compared between flushes; identical letters denote no significant difference between mean values; different letters indicate the significant differences among mean values for elements according to Tukey's HSD at $p < 0.05$.

3.4.4. Content of Nutritionally Nonessential Elements (NNEs) in the Fruiting Bodies of *P. djamor*

In the present study, among the studied NNEs in the fruiting bodies of *P. djamor*, the content of Cs in mushroom fruiting bodies was significantly different and superior in flush three (58.4 mg·kg^{-1} DM) in comparison with flushes one and two (Table 6). Except for the content of Cs among subsequent flushes, none of the studied NNEs was found to be statistically different. No flush-specific increasing pattern of elemental content in *P. djamor* mushrooms among different flushes was noticed for studied NNEs except for Cs.

Table 6. Concentrations of NNEs in the fruiting bodies of *P. djamor* cultivated on EMS.

Element (mg·kg^{-1} DM)	Flush I	Flush II	Flush III
Al	5.41 ± 0.66 ^{a} *	7.37 ± 0.99 ^{a}	8.26 ± 0.23 ^{a}
Bi	0.34 ± 0.14 ^{a}	0.70 ± 0.18 ^{a}	0.97 ± 0.03 ^{a}
Ce	0.02 ± 0.01 ^{a}	0.04 ± 0.00 ^{a}	0.05 ± 0.01 ^{a}
Cs	2.48 ± 4.29 ^{c}	31.8 ± 14.9 ^{b}	58.4 ± 17.4 ^{a}
Ga	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
Ge	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.16 ± 0.12 ^{a}
Li	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
Nd	0.01 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}
Pr	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}
Sb	1.52 ± 0.13 ^{a}	1.96 ± 0.25 ^{a}	2.43 ± 0.23 ^{a}
Sr	2.66 ± 0.49 ^{a}	3.45 ± 0.07 ^{a}	3.91 ± 0.41 ^{a}
Ta	0.01 ± 0.00 ^{a}	0.05 ± 0.03 ^{a}	0.13 ± 0.04 ^{a}
Te	2.56 ± 0.42 ^{a}	3.17 ± 0.27 ^{a}	3.93 ± 0.26 ^{a}
Ti	0.06 ± 0.03 ^{a}	0.11 ± 0.01 ^{a}	0.15 ± 0.01 ^{a}
V	0.01 ± 0.00 ^{a}	0.03 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}
W	2.91 ± 0.53 ^{a}	3.61 ± 0.16 ^{a}	3.94 ± 0.06 ^{a}
Zr	0.11 ± 0.02 ^{a}	0.13 ± 0.01 ^{a}	0.14 ± 0.00 ^{a}

* Data represent mean ± SD (n = 3); means corresponding to the same element are compared between flushes; identical letters denote no significant difference between mean values; different letters indicate the significant differences among mean values for elements according to Tukey's HSD at $p < 0.05$.

3.5. Bioconcentration Factor (BCF) of Elements from EMS into the Fruiting Bodies of P. djamor

The BCFs of major essential elements (MEEs), essential trace elements (ETEs), trace elements with detrimental health effects (TEWDHE) and nutritionally nonessential elements (NNEs) were calculated as the ratio of their concentration in *P. djamor* fruiting bodies from individual flush to the respective content in the cultivation substrate based on digestate (Figure 1).

In the present study, BCF values differed among all three flushes, as seen in Figure 1A–C. The BCFs for MEEs ranged from 0.26–1.18 for flush one, 0.33–1.46 for flush two and 0.49–1.58 for flush three. The range of BCFs for ETE's in flushes one, two and three was 0.01–1.21, 0.01–1.81 and 0.08–2.35. In flushes one, two and three, the range of BCFs for TEWDHE was 0.003–0.33, 0.01–0.48 and 0.02–0.71. The range of BCFs of NNEs in *P. djamor* fruiting bodies was 0.001–0.41, 0.001–1.07 and 0.001–1.33 in flush one, two and three, respectively.

The BCF values higher than 1.00 was noted for Fe, Cu and K for fruiting bodies obtained from flush one. While, the BCFs were higher than 1.00 for Fe, Zn, Cu, K, W and Sb from flush two. The BCF for Fe was the highest (2.35), which was greater than 2.00. The value was followed by BCFs of Zn, Cu, K, Se, Sb, W, B, Ca and Mg which were greater than 1.00 as calculated from the mushroom fruiting bodies from flush three. In contrast, BCFs for other MEEs, ETEs, TEWDHE and NNEs were observed to be lower

than 1.00. Furthermore, the BCF value for all studied trace elements with detrimental health effects (TEWDHE): Ag, As, Ba, Be, Cd, Hg and Tl were lower than 1.00. Our results demonstrated that the bioaccumulation of elements in fruiting bodies of *P. djamor* varies among subsequent flushes, which are reflected in presented BCF values.

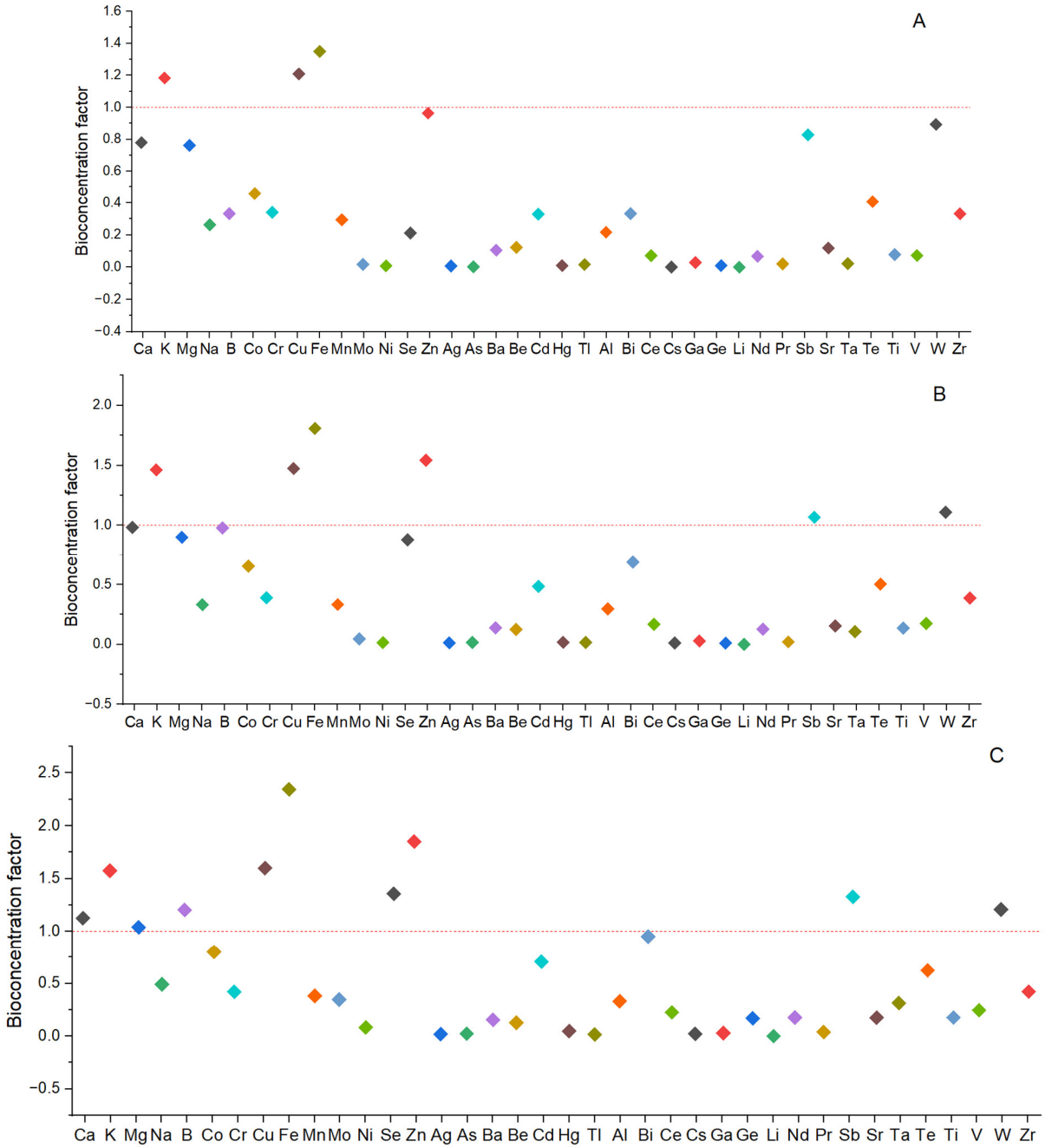

Figure 1. Scatterplot illustrating bioconcentration factor (BCF) from flush I (**A**), flush II (**B**) and flush III (**C**) of major essential elements (MEEs): Ca, K, Mg, Na; essential trace elements (ETEs): B, Co, Cu, Cr, Fe, Mn, Mo, Ni, Se, Zn; trace elements with detrimental health effect (TEWDHE): Ag, As, Ba, Be, Cd, Hg, Tl; nutritionally nonessential elements (NNEs): Al, Bi, Ce, Cs, Ga, Ge, Li, Nd, Pr, Sb, Sr, Ta, Te, Ti, V, W, Zr in *P. djamor* mushroom fruiting bodies cultivated on EMS. The red-dotted lines represent a reference line for BCF (marked at 1.00), the presented BCF values for elements in respective graphs below the line are less than 1.00 and above the reference line are greater than 1.00. The scatterplots for BCF were generated using the OriginLab 2021 software for Windows (OriginLab Corp., Northampton, MA, USA).

4. Discussion

4.1. Effect of the EMS (Experimental Mushroom Substrate) on the Cultivation of P. djamor

The most important aspect of *Pleurotus* cultivation is the rational choice of the substrate [15]. In line with this, the mineral content, C/N ratio, dry matter and pH of the substrate are the most important factors for mycelium colonisation and development of fruiting bodies. Therefore, the yield and biological efficiency of mushrooms are largely dependent on the substrate composition [75]. The yield of *P. djamor* cultivated on EMS (combined dairy manure-food waste digestate) obtained in the present study was relatively higher than *P. djamor* yields reported by Atila [76] on oak sawdust (87.5 g) and sunflower head residue (136.4 g). In line with this, relatively lower yields were reported by Hasan et al. [77], where *P. djamor* was cultivated on substrates mixes based on sugarcane bagasse supplemented with wheat bran at 0% (156.2 g) and 30% (154.7 g). However, the achieved mushroom yield in our study was lower than that of substrate mixes based on other organic materials such as safflower hay (258.1 g), bean straw (258.1 g) [76], sawdust (301 g) and coir pith (256.80 g) [78] and sugarcane bagasse supplemented by wheat bran at 10% (379.5 g), 20% (299.2 g), 40% (255.4 g) and (276.7 g) [77].

The BE value reported in the present investigation (39.30%) was relatively higher than that of BE values reported by Hasan et al. [77], where sugarcane bagasse supplemented with 30%, 50%, 40%, 0% and 20% resulted in BE of 20.62%, 20.822%, 34.053%, 36.893% and 39.89%. The BE values reported in our study were nearly comparable with the findings of Salmones et al. [79], where *P. djamor* IE-121 strain resulted in BE of 40.4% on the traditional wheat straw substrate and *P. djamor* IE-218 strain resulted in BE of 40.2% on coffee pulp.

It should be underlined that, by far, this is the first attempt on the cultivation of *P. djamor* on digestate-based substrate resulting in a yield of 196.5 g and BE of 39.30% (Table 2). The research focused on using digestate-based materials for other *Pleurotus* species cultivation is also still limited. A study by Chanakya et al. [26] on two species *P. florida* and *P. flabellatus* cultivated on paddy straw and coir pith both supplemented with 30% of anaerobically digestate waste resulted in BE for *P. florida* of 231% and 148% and 209% and 188%, respectively. *Pleurotus ostreatus* cultivation on sawdust or sawdust and soy hull with 30% and 50% digestate-based substrates was investigated by O'Brien et al. [32]. For sawdust and digestate substrate, the BE was from 25% to 63%, but for sawdust, soy hull and digestate, the BE was higher, ranging from 48% up to 115%. On the contrary, Hultberg et al. [80], in the cultivation of *P. ostreatus* on peat- (70%) and digestate- (30%) based substrate, BE of 50.9% was obtained. While, in our study *P. djamor* cultivated on digestate-based substrate (EMS) reached 39.30%. According to Stamets [81], the BE of mentioned *Pleurotus* species is generally higher than this of *P. djamor* and is likely to exceed 100%. Therefore, BE obtained on EMS in our study can be considered as preliminary findings, supporting the possibility of *P. djamor* cultivation on such non-traditional substrate achieving nearly BE of 40%.

Corresponding to Kurtzman and Zadrazil [82], K, Fe and Mg are the most important minerals for the cultivation of *Pleurotus*. Similarly, Zn, Mn, Cu, Cr and Mo are mentioned as the essential trace elements for the growth of several mushroom species [83–85]. Therefore, the elements: Ca, K, Mg and Na (MEEs) and B, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn (ETEs) are naturally present in raw materials most used for the preparation of the mushroom cultivation substrate, such as cereals (rice, wheat, corn) bran, hardwood sawdust or sugar cane bagasse [86]. In our study, the MEEs and ETEs were found in abundance in the EMS (Table 2), indicating the suitability of EMS for the cultivation of *Pleurotus* mycelium and fruiting bodies development. The elemental composition of EMS obtained in our study are comparable with the findings of Hoa et al. [75] and Sales-Campos et al. [86]. In their studies, the cultivation substrates were based mostly on traditional raw materials. Our results are also in line with the findings of Hultberg et al. [80], where peat-digestate-based substrate was studied. The EMS in our study was especially rich in Na and Mo (1955.08 and 0.47 $mg \cdot kg^{-1}$ DM, respectively), but considerably lower in Fe in comparison withHultberg et al. [80], who reported that the Fe in their substrate was mainly from ferric chloride, an additive in biogas production [87].

To our best knowledge, there is no information on the upper limit values for concentrations of elements in mushroom cultivation substrate. Therefore, the values from the

experiment were compared with the limit values for concentrations of heavy metals in soil ($mg \cdot kg^{-1}$ of dry matter in a representative sample, soil with a pH of 6 to 7): Cd (1–3), Cu (50–140), Hg (1–1.5), Ni (30–75), Pb (50–300) and Zn (150–300). None of the above-mentioned elements of the initial cultivation substrate (EMS) exceeded the limits of these concentrations. Therefore, it can be inferred that it is safe to use the original digestate-based compost as a substrate component for mushroom cultivation.

4.2. Value of Spent Mushroom Substrate (SMS)

The spent mushroom substrate (SMS) obtained in this study showed higher values of MEEs and ETSs than initial EMS because of the residues of mycelium and further degradation and decomposition of the substrate components (Table 2). During mushroom cultivation, the composting process continues, enhanced by the microbiological activity of the fungus metabolism [88]. The level of SMS degradation is influenced not only by physical, chemical, environmental and biological factors, but also by the genetics of *Pleurotus* species, according to Oliviera [89], cited by Sales-Campos et al. [86]. Remarkably, our study showed the amount of Fe in SMS was 4.5-fold greater than in EMS. These findings are in line with Sales-Campos et al. [86], where the level of Fe was over three times higher in SMS than in the initial substrate. Based on this, it can be inferred that the higher concentration of Fe in mushroom fruiting bodies, higher BCF and higher EDI (estimated daily intake) values are associated with the higher Fe concentration during mushroom cultivation and the higher residual value reflected at the end of cultivation in SMS. An unusual pattern in SMS was observed in a study by Hultberg et al. [80], where typically elevated levels of Ca, K, Mg, P, Zn and Fe decreased after the cultivation of *P. ostreatus*. The substrates used in this experiment were also non-traditional for mushroom cultivation, containing peat in 70% and digestate sludge in 30%, initially containing lower amounts of elements than in traditional substrates; therefore, it is assumed the elements were built up in the mushroom body and/or lost by leaching.

The elevated content of Al, As, Ba, Be, Cd, Ti, and Tl can be explained by decreasing dry matter of substrates as a result of composting processes [90–92]. Generally, dry matter loss of the substrate corresponds with biological efficiency and yield of mushrooms, which is directly proportional to the carbon loss into the atmosphere due to mushroom respiration as well as assimilation into the mushroom fruit body [26]. Such a relative increase in the mineral content in SMS after *Pleurotus* ssp. cultivation was described also by Zadrazil [93] and Silva et al. [94].

Like EMS, for SMS, the obtained values were compared with the limit values for concentrations of heavy metals in soil (mg/kg of dry matter in a representative sample, soil with a pH of 6 to 7): Cd (1–3), Cu (50–140), Hg (1–1.5), Ni (30–75), Pb (50–300) and Zn (150–300). The limits were not exceeded for any element in any of the examined SMS, which allows us to infer that it is safe to re-use SMS as the agricultural amendment [95,96]. However, the EU directive 86/278/EEC shows instead limit values for amounts of heavy metals which may be added annually to agricultural land, based on a 10-year average (kg/ha/yr): Cd (0.15), Cu (12), Hg (0.1), Ni (3), Pb (15), Zn (30); therefore, one should be aware of those limitations while using digestate-derived SMS for soil amendment or other agricultural purposes.

In our study, based on the residual elemental concentrations in *P. djamor* SMS based on combined dairy manure-food waste, it is noticeable that even after the cultivation of *P. djamor*, the SMS still holds a significant amount of residual nutrients which can be potentially re-used as a supplement for subsequent mushroom production [97,98], in plant cultivation, animal feed and many more [25,43,99–101].

4.3. Chemistry and Safety of Mushrooms

4.3.1. Effect of EMS (Experimental Mushroom Substrate) on the Content of Elements in *P. djamor* Fruiting Bodies

The present study is so far the broadest investigation of the multi-elemental content of the fruiting bodies of *P. djamor* cultivated on dairy manure-food waste digestate-based substrate. The results of the elemental analysis indicated that the concentrations of studied MEEs, ETEs, TEWDHE and NNEs significantly differed among flushes. As demonstrated, the investigated species show considerable abilities for increased accumulation of certain elements such as: As, B, Ba, Ca, Cs, Fe, K, Mg, Na, Se and for Zn in subsequent flushes.

The elemental content of mushrooms is similar to that of superior plants, where K, P and Mg are the most abundant minerals [25,75,84,85,102–106]. This is also confirmed by our study, where values in flush three increase accordingly Na > Mg > Ca > K (Table 3). Levels of potassium were, however, half of those obtained for wild *P. djamor* by Mallikarjuna et al. [107], but the content of Ca, Na and Mg was four-, two- and one-half-fold greater. Siwulski et al. [108] also report the highest levels of K for cultivated *P. djamor* among other investigated Pleurotus species. In our study, the bioaccumulation of Cr in the fruiting bodies ranged between 0.34–0.42 mg/kg DM in subsequent flushes. The values obtained in our studies were lower than the values reported by Zsigmond et al. [109] (40 mg/kg DM) and Yamac et al. [110] (0.54 mg/kg DM). In addition, mushroom requires trace amounts of some elements such as Fe, Mn, Co, Cu, Cr and Zn since they play an important role in biological systems [111]. This was also confirmed by our study with values increasing from flushes one to three with highest values for (mg/kg DM): Fe 110–191; Zn 48.7–93.5; Cr 0.34–0.42; Cu 11.3–15.0; Mn 5.96–7.76; Co 0.56–0.99 (Table 4). Those findings are in line with Mallikarjuna et al. [107], where the content of ETEs was highest for Fe (148 mg/kg) and decreased accordingly (Zn–92.1; Cu 14.5; Mn 11.2 mg/kg). Whereas, the content of Zn was much higher (311 mg/kg) for cultivated *P. djamor,* as investigated by Siwulski et al. [108]. However, the results of elemental concentrations from our investigation are greatly comparable to the findings of O'Brien et al. [32], who studied the cultivation of *P. ostreatus* using a combined dairy manure-food waste digestate-based substrate. In comparison with our study, the levels of K, Mg and Mn were two-folds greater, while the values of Cu and Zn were in line with our findings. It is noteworthy that the level of B was much higher (5.5 to 40.4 mg/kg, substrate-dependent) compared to our results (0.93–3.37 mg/kg flush-dependent); Cr was scarce, less than 2 mg/kg, like in our study it ranged from 0.34 up to 0.42 mg/kg (flush-dependent). Moreover, our study indicated the accumulation of elements is increasing with a subsequent flush. This might be due to the loss of the substrates DM during the mushroom growing period as well as due to its metabolism and removal of substances from the substrate for the construction of the fruit body [88].

On the other hand, the content of Fe, Cu, Mn, Zn, Pb, Cd and Ni in the environment indicates the level of its pollution [112]. Therefore, the substrate composition plays a significant role in mineral uptake and accumulation in consumable mushroom parts [108]. Generally, the mineral composition of mushrooms reflects their growth conditions. Therefore, the assessment of elemental concentrations in mushrooms can be described with the BCF. BCFs are also called enrichment factors [113]. Living organisms demonstrating BCF values higher than 1.00 are defined as bioaccumulators and are considered efficient absorbers of elements. In line with this, in our study, the *P. djamor* fruiting bodies cultivated on digestate-based substrate bioaccumulated following major essential element: K, essential trace elements: Cu, Fe, Zn and nutritionally nonessential elements: Sb and W, reflecting the enrichment factor (>1.00) in fruiting bodies of *P. djamor* for mentioned elements. Most importantly, none of the studied trace elements with detrimental health effect BCFs were greater than 0.55. The BCF values for TEWDHE, a group of elements especially important in terms of food security, demonstrated that *P. djamor* are not bioaccumulators of such elements, at the same time reflecting the safety consumption. The findings of O'Brian et al. [32] also confirm the safety of mushroom tissues for human consumption if cultivated on the digestate-based substrate.

4.3.2. Consumption of *P. djamor*—Dietary Intake

Mushrooms are known as organisms which accumulate various elements from the cultivation substrate including trace metals [55,114,115]. This characteristic feature of mushrooms has both positive and negative sides. The metal elements are classified as essential—which participate in the physiological processes)—and their long-term deficiency may lead to human health issues and associated diseases. On the other hand, there are metals which are less necessary for life and there are those which exhibit adverse and/or toxic effects on long-term exposure or consumption [116]. However, it should be also taken into consideration that ways for mushroom tissues prepared for human consumption, such as cooking, frying, microwaving, etc., could reduce the bioaccessibility of toxic elements in the human gastrointestinal tract [56].

Depending on the input to the biogas digesters, it can be also rich in mineral elements such as nonessential and trace elements, heavy metals, various organic pollutants and other unwanted compounds, which puts its usefulness in direct food production in vegetables of mushrooms questionable [36–38]. Keeping the questionability and safety consumption of *P. djamor* mushrooms cultivated on non-traditional substrates which are rich in some metal elements such as digestate, the determined levels of all studied elements were compared to the guideline values referring to Adequate Intake (AI) per day for MEEs and ETEs established by the EFSA [117,118]. While, the Al, Cd and Ni were related to their Tolerable Weekly Intake (TWI) mg·kg^{-1} body weight (bw). The guideline value for As corresponds to benchmark-dose level (BDL01) mg·kg^{-1} bw day^{-1}. Guidance level values for MEEs and ETEs were adopted from Mleczek et al. [119], corresponding to a single serving of 250 g fresh weight (=25 g dry weight) of fruit bodies, consumed by an adult. Similarly, the guideline values for TEWDHE were adopted from Siwulski et al. [67], based on EC [120]. While, the TDI and RDI values were derived from WHO [69,71], IRIS [121,122], Bruce and Odin [70], WHO [123] and Martone [72].

In the present study, the wide variability was found in the mushroom's elemental content in subsequent flushes (i.e., flushes one, two and three), which are well-reflected in the wide range of respective EDI values (Table 7). The calculated values for EDI indicated that among subsequent flushes the EDI values for all studied elements increased, maintaining an increasing trend of elemental accumulation.

Table 7. Estimated daily intake (EDI, mg·kg^{-1}d^{-1}) of major essential elements (MEEs), essential trace elements (ETEs), trace elements with detrimental health effect (TEWDHE) and nutritionally nonessential elements (NNEs) through the consumption of one serving (calculated as 250 g F.W., corresponding to 25 g D.W.) of *P. djamor* mushrooms by an adult person (60 kg). TDI—tolerance daily intake (mg·day^{-1}); RDI—Recommended dietary intake (mg·day^{-1}).

Elements	EDI			Guideline Level	TDI	RDI
	Flush I	Flush II	Flush III			
Major essential elements—MEEs						
Ca	367	462	530	950 [118]	Nr *	1000 [71]
K	4540	5610	6040	3500 [118]	nr	
Mg	219	259	299	350 [118]	nr	240 [71]
Na	90.74	114	169	2000 [118]	nr	
Essential trace elements—ETEs						
B	0.39	1.14	1.41	nr	nr	nr
Co	0.23	0.33	0.41	nr	nr	nr
Cu	4.71	5.74	6.23	1.6 [118]	10 [69]	2.2 [69]
Fe	45.9	61.5	79.7	11 [118]	48 [69]	10–50 [69]
Mn	2.48	2.81	3.23	3.0 [118]	11 [69]	3 [69]
Mo	0.01	0.01	0.08	0.065 [118]	nr	0.1–0.3 [69]
Ni	0.01	0.01	0.04	0.0195 [117]	0.720 [69]	
Se	0.16	0.66	1.02	0.070 [118]	320–480 [69]	0.026–0.035 [69]
Zn	20.3	32.5	39.0	11.7 [118]	60 [69]	15-20 [69]

Table 7. *Cont.*

Elements	EDI			Guideline Level	TDI	RDI
	Flush I	Flush II	Flush III			
Trace elements with detrimental health effects—TEWDHE						
Ag	0.03	0.05	0.07	nr	0.005 [121]	nr
As	0.06	0.26	0.37	0.5 [120]	nr	nr
Ba	0.66	0.86	0.97	nr	0.2 [122]	nr
Be	0.02	0.02	0.02		27.6 [70]	nr
Cd	0.10	0.15	0.22	1.0 [120]	nr	nr
Hg	0.01	0.01	0.03	0.1 [120]	nr	nr
Tl	0.01	0.01	0.01	nr	nr	
Nutritionally nonessential elements—NNEs						
Al	2.25	3.08	3.44	1.0 [123]	0.14 [118]	nr
Bi	0.14	0.29	0.41	nr	nr	nr
Ce	0.01	0.02	0.03	nr	nr	nr
Cs	1.03	13.3	24.3	nr	nr	nr
Ga	0.01	0.01	0.01	nr	nr	nr
Ge	0.01	0.01	0.07	nr	nr	nr
Li	0.01	0.01	0.01	nr	nr	1 [72]
Nd	0.01	0.01	0.02	nr	nr	nr
Pr	0.01	0.01	0.01	nr	nr	nr
Sb	0.63	0.82	1.02	nr	0.36 [69]	nr
Sr	1.11	1.43	1.63	nr	0.13 [123]	nr
Ta	0.01	0.02	0.06	nr	nr	nr
Te	1.07	1.33	1.63	nr	nr	nr
Ti	0.03	0.05	0.06	nr	nr	nr
V	0.01	0.02	0.02	nr	nr	nr
W	1.21	1.51	1.64	nr	nr	nr
Zr	0.05	0.06	0.06	nr	nr	nr

* nr—Values not referred. Guidance level values for MEEs and ETEs were adopted from Mleczek et al. [119], corresponding to a single serving of 250 g FW (=25 g DW) of fruit bodies; the guideline values for TEWDHE were adopted from Siwulski et al. [67], based on EC [120]; the TDI and RDI values were derived from WHO [69,71], Bruce and Odin [70], Martone [72], IRIS [121,122] and WHO [123].

Among the studied MEEs, the EDI values in all subsequent flushes for Ca, Ma and Na were below the guideline level values corresponding to AI [118]. Whereas, for K, the EDI of *P. djamor* mushrooms was more than the referred AI value [118]. In our study, the estimated EDI values indicated that the *P. djamor* cultivated on digestate-based substrate can serve as a good source of MEEs—Ca and Mg fulfilling RDI [71].

Concerning evaluated ETEs, the EDI values for Cu in all three subsequent *P. djamor* flushes exceeded the AI [118], TDI and RDI [69]. Similarly, for Fe, the estimated EDI values from flushes two and three were higher than the AI [118], TDI and RDI [69]. Moreover, the EDI for Fe from flush one *P. djamor* fruiting bodies was below the TDI [69], serving as an excellent source of Fe fulfilling the RDI [69]. Similarly, the *P. djamor* fruiting bodies were an excellent source of essential trace elements including Mn, Mo, Ni, Se and Zn represented by EDI values, which can satisfy the respective dietary requirements [69,118].

Among the studied TEWDHE, except for Ag, Ba and Be, the EDI values for As, Cd and Hg were below the established maximum level of intake in food such as mushrooms 0.5, 1.0 and 0.1 $mg \cdot kg^{-1}$ DW, respectively [120]. While, for Ag, the EDI values from all three subsequent flushes of *P. djamor* fruiting bodies were higher than TDI [121], and an established intake of 0.015 $mg \cdot kg^{-1}$ body weight per week was recommended by WHO [67].

For studied NNEs, the EDI for referred elements including Al, Sb and Sr were higher than the TDI [69,123] and the guidelines value [123], respectively. For NNEs, it should be noted that our risk assessment by EDI values was limited to the elements with available reference values including AI, TDIs and RDIs. Almost 90 per cent of studied NNEs lack TDIs or RDIs. In this context, the underlying fact cannot be ruled out that a few, or several, of these NNEs can also appear in dietary intake to a degree that can be unhealthy.

5. Conclusions

The present investigation demonstrates a plausible approach of utilising combined dairy manure-food waste digestate as a substrate for *P. djamor* (2708, Mycelia) cultivation, achieving satisfactory yield and BE. The concentrations of the studied 38 elements exhibited high variability in the initial substrate (EMS), mushroom fruiting bodies and residual substrate (SMS). The amounts of MEEs, ETEs, TEWDHE and NNEs showed an increasing trend of accumulation in mushroom fruiting bodies in the subsequent flushes, demonstrating flush specific trend. In line with this, the BCFs values increased with subsequent flushes, where the highest (>2.00) BCF was recorded for Fe in flush three. Overall, BCF for Fe, Cu, K, Zn, W, Sb, K, Se, B, Ca and Mg were greater than 1.00. Moreover, the BCF values for all studied TEWDHE were lower than 1.00. Considering the questionability of using such non-traditional substrates (digestate) in mushroom production, our study presented that *P. djamor* fruiting bodies grown on EMS can serve as an excellent source of Ca, Mg and Na (MEE's), Mn, Mo, Ni and most importantly Se and Zn (ETE's), which are noteworthy. On the other hand, EDI values for K, Cu, Fe, Ag, Ba, Cd, Al, Sb and Sr were greater than the referred guideline values corresponding to higher intake and associated risks. As a future line of work, evaluating the suitability of digestate-based substrate for other mushroom species is highly recommended.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8100934/s1, Table S1. Raw materials used. Table S2. Experimental mushroom composts (EMC)—at make-up and during Phase I. Table S3. Composition of the experimental mushroom compost after Phase II (at inoculation).

Author Contributions: Conceptualisation: A.J.; methodology: A.J. and K.S.; software: R.P.; validation: A.J., K.S. and R.P.; formal analysis: R.P.; investigation: A.J.; M.R., M.M. and P.N.; resources: A.J., M.R. and R.P.; data curation: A.J. and R.P.; writing—original draft preparation: A.J. and R.P.; writing—review and editing: A.J., R.P. and J.L.; visualisation: A.J., R.P.; supervision: K.S. and J.L.; project administration: A.J.; funding acquisition: A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 751052—VegWaMus CirCrop-H2020-MSCA-IF-2016.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zervakis, G.I.; Koutrotsios, G. Solid-State Fermentation of Plant Residues and Agro-industrial Wastes for the Production of Medicinal Mushrooms. In *Medicinal Plants and Fungi: Recent Advances in Research and Development*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 365–396. [CrossRef]
2. Roncero-Ramos, I.; Delgado-Andrade, C. The beneficial role of edible mushrooms in human health. *Curr. Opin. Food Sci.* **2017**, *14*, 122–128. [CrossRef]
3. Chaturvedi, V.K.; Agarwal, S.; Gupta, K.K.; Ramteke, P.W.; Singh, M.P. Medicinal mushroom: Boon for therapeutic applications. *3 Biotech* **2018**, *8*, 334. [CrossRef] [PubMed]
4. Venturella, G.; Ferraro, V.; Cirlincione, F.; Gargano, M.L. Medicinal Mushrooms: Bioactive Compounds, Use, and Clinical Trials. *Int. J. Mol. Sci.* **2021**, *22*, 634. [CrossRef] [PubMed]
5. Carrasco-González, J.A.; Serna-Saldívar, S.O.; Gutiérrez-Uribe, J.A. Nutritional composition and nutraceutical properties of the *Pleurotus* fruiting bodies: Potential use as food ingredient. *J. Food Compos. Anal.* **2017**, *58*, 69–81. [CrossRef]
6. Ma, G.; Yang, W.; Zhao, L.; Pei, F.; Fang, D.; Hu, Q. A critical review on the health promoting effects of mushrooms nutraceuticals. *Food Sci. Hum. Wellness* **2018**, *7*, 125–133. [CrossRef]
7. Mushroom Cultivation Market by Type (Button Mushroom, Oyster Mushroom, Shiitake Mushroom, Other Types), by Phase, by Region (North America, Europe, Asia Pacific, South America, Rest of the World)—Global Forecast to 2025.

Available online: https://www.researchandmarkets.com/reports/5018601/mushroom-cultivation-market-by-type-button (accessed on 20 July 2022).
8. Acharya, S.; Saha, A.K. Antimicrobial activity of *Pleurotus djamor*. *Indian J. Mycopathol. Res.* **2011**, *2*, 329–332.
9. Maity, G.N.; Maity, P.; Khatua, S.; Acharya, K.; Dalai, S.; Mondal, S. Structural features and antioxidant activity of a new galactoglucan from edible mushroom Pleurotus djamor. *Int. J. Biol. Macromol.* **2021**, *168*, 743–749. [CrossRef]
10. Waktola, G.; Temesgen, T. Pharmacological activities of Oyster mushroom (*Pleurotus ostreatus*). *Novel Res. Microbiol. J.* **2020**, *4*, 688–695. [CrossRef]
11. Raman, J.; Jang, K.Y.; Oh, Y.L.; Oh, M.; Im, J.H.; Lakshmanan, H.; Sabaratnam, V. Cultivation and Nutritional Value of Prominent *Pleurotus* spp.: An Overview. *Mycobiology* **2021**, *49*, 1–14. [CrossRef]
12. Sánchez, C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol. Adv.* **2009**, *27*, 185–194. [CrossRef]
13. Ritota, M.; Manzi, P. *Pleurotus* spp. Cultivation on Different Agri-Food By-Products: Example of Biotechnological Application. *Sustainability* **2019**, *11*, 5049. [CrossRef]
14. Melanouri, E.-M.; Dedousi, M.; Diamantopoulou, P. Cultivating Pleurotus ostreatus and Pleurotus eryngii mushroom strains on agro-industrial residues in solid-state fermentation. Part I: Screening for growth, endoglucanase, laccase and biomass production in the colonization phase. *Carbon Resour. Convers.* **2022**, *5*, 61–70. [CrossRef]
15. Yohannes, B.; Abraham, M.; Bikila, G.; Robel, D.; Getahun, T.; Jale, M.; Malesu, A.; Tsehaynesh, F.; Lalise, D. Selection of appropriate substrate for production of oyster mushroom (*Pleurotus ostreatus*). *J. Yeast Fungal Res.* **2020**, *11*, 15–25. [CrossRef]
16. Daba, A.S.; El nakieb, F.; Hafez, E.E. The effect of Recycling Different Wastes (as a substrate) on Mushroom (*Pleurotus ostreatus*) Fruit Bodies, Morphologically, Genetically and its Metabolites. *Biosci. Res.* **2018**, *15*, 2286–2294.
17. Tesfay, T.; Godifey, T.; Mesfin, R.; Kalayu, G. Evaluation of waste paper for cultivation of oyster mushroom (*Pleurotus ostreatus*) with some added supplementary materials. *AMB Express* **2020**, *10*, 15. [CrossRef]
18. Girmay, Z.; Gorems, W.; Birhanu, G.; Zewdie, S. Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express* **2016**, *6*, 87. [CrossRef]
19. Koutrotsios, G.; Danezis, G.P.; Georgiou, C.A.; Zervakis, G.I. Rare earth elements concentration in mushroom cultivation substrates affects the production process and fruit-bodies content of *Pleurotus ostreatus* and *Cyclocybe cylindracea*. *J. Sci. Food Agric.* **2018**, *98*, 5418–5427. [CrossRef]
20. Jasinska, A.; Wojciechowska, E.; Stoknes, K.; Roszak, M. Bioconversion of Agricultural Wastes into a Value-Added Product: Straw of Norwegian Grains Composted with Dairy Manure Food Waste Digestate in Mushroom Cultivation. *Horticulturae* **2022**, *8*, 331. [CrossRef]
21. Lisiecka, J.; Prasad, R.; Jasinska, A. The Utilisation of *Pholiota nameko*, *Hypsizygus marmoreus*, and *Hericium erinaceus* Spent Mushroom Substrates in Pleurotus ostreatus Cultivation. *Horticulturae* **2021**, *7*, 396. [CrossRef]
22. Koutrotsios, G.; Patsou, M.; Mitsou, E.K.; Bekiaris, G.; Kotsou, M.; Tarantilis, P.A.; Pletsa, V.; Kyriacou, A.; Zervakis, G.I. Valorization of Olive By-Products as Substrates for the Cultivation of *Ganoderma lucidum* and *Pleurotus ostreatus* Mushrooms with Enhanced Functional and Prebiotic Properties. *Catalysts* **2019**, *9*, 537. [CrossRef]
23. Koutrotsios, G.; Tagkouli, D.; Bekiaris, G.; Kaliora, A.; Tsiaka, T.; Tsiantas, K.; Chatzipavlidis, I.; Zoumpoulakis, P.; Kalogeropoulos, N.; Zervakis, G.I. Enhancing the nutritional and functional properties of Pleurotus citrinopileatus mushrooms through the exploitation of winery and olive mill wastes. *Food Chem.* **2022**, *370*, 131022. [CrossRef]
24. Chae, H.J.; Ahn, J.H. Optimization of rice bran and food waste compost contents in mushroom culture medium to maximize mycelial growth rate and fruit body yield of *Pleurotus ostreatus*. *Int. Biodeter. Biodegr.* **2013**, *80*, 66–70. [CrossRef]
25. Chang, S.T.; Lau, O.W.; Cho, K.Y. The cultivation and nutritional value of *Pleurotus sajor*-caju. *Eur. J. Appl. Microbiol. Biotechnol.* **1981**, *12*, 58–62. [CrossRef]
26. Chanakya, H.N.; Malayil, S.; Vijayalakshmi, C. Cultivation of Pleurotus spp. on a combination of anaerobically digested plant material and various agro-residues. *Energy Sustain. Dev.* **2015**, *27*, 84–92. [CrossRef]
27. Garuba, T.; Abdukkareem, K.A.; Ibrahim, I.A.; Oyebamiji, O.I.; Shoyooye, O.A.; Ajibade, T.D. Influence of substrates on the nutritional quality of *Pleurotus pulmonarius* and *Pleurotus ostreatus*. *Ceylon J. Sci.* **2017**, *46*, 67. [CrossRef]
28. Jin, Z.; Li, Y.; Ren, J.; Qin, N. Yield, Nutritional Content, and Antioxidant Activity of *Pleurotus ostreatus* on Corncobs Supplemented with Herb Residues. *Mycobiology* **2018**, *46*, 24–32. [CrossRef]
29. Lin, L.; Xu, F.; Ge, X.; Li, Y. Improving the sustainability of organic waste management practices in the food-energy-water nexus: A comparative review of anaerobic digestion and composting. *Renew. Sust. Energy Rev.* **2018**, *89*, 151–167. [CrossRef]
30. Li, Y.; Park, S.Y.; Zhu, J. Solid-state anaerobic digestion for methane production from organic waste. *Renew. Sust. Energ. Rev.* **2011**, *15*, 821–826. [CrossRef]
31. Mata-Alvarez, J.; Dosta, J.; Romero-Güiza, M.S.; Fonoll, X.; Peces, M.; Astals, S. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew. Sust. Energ. Rev.* **2014**, *36*, 412–427. [CrossRef]
32. O'Brien, B.J.; Milligan, E.; Carver, J.; Roy, E.D. Integrating anaerobic co-digestion of dairy manure and food waste with cultivation of edible mushrooms for nutrient recovery. *Bioresour. Technol.* **2019**, *285*, 121312. [CrossRef] [PubMed]
33. Stoknes, K.; Scholwin, F.; Krzesiński, W.; Wojciechowska, E.; Jasińska, A. Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. *Waste Manag.* **2016**, *56*, 466–476. [CrossRef]

34. Jasinska, A.J.; Wojciechowska, E.; Krzesiński, W.; Spiżewski, T.; Stoknes, K. and Krajewska, K. Mushroom cultivation on substrates with addition of anaerobically digested food waste. *Acta Hortic.* **2016**, *1123*, 199–206. [CrossRef]
35. De Groot, L.M.; Bogdanski, A.K. *Bioslurry = Brown Gold? A Review of Scientific Literature on the Co-Product of Biogas Production*; FAO: Rome, Italy, 2013.
36. Spielmeyer, A.; Ahlborn, J.; Hamscher, G. Simultaneous determination of 14 sulfonamides and tetracyclines in biogas plants by liquid-liquid-extraction and liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 2513–2524. [CrossRef]
37. Zirkler, D.; Peters, A.; Kaupenjohann, M. Elemental composition of biogas residues: Variability and alteration during anaerobic digestion. *Biomass Bioenerg.* **2014**, *67*, 89–98. [CrossRef]
38. Ali, S.S.; Nessem, A.A.; Sun, J.; Li, X. The effects of water hyacinth pretreated digestate on Lupinus termis L. seedlings under salinity stress: A complementary study. *J. Environ. Chem. Eng.* **2019**, *7*, 103159. [CrossRef]
39. Lehmann, L.; Bloem, E. Antibiotic residues in substrates and output materials from biogas plants–Implications for agriculture. *Chemosphere* **2021**, *278*, 130425. [CrossRef]
40. Ma, Y.; Wang, Q.; Sun, X.; Wang, X.; Su, W.; Song, N. A Study on Recycling of Spent Mushroom Substrate to Prepare Chars and Activated Carbon. *BioResources* **2014**, *9*, 3939–3954. [CrossRef]
41. Hanafi, F.H.M.; Rezania, S.; Mat Taib, S.; Md Din, M.F.; Yamauchi, M.; Sakamoto, M.; Hara, H.; Park, J.; Ebrahimi, S.S. Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): An overview. *J. Mater. Cycles Waste Manag.* **2018**, *20*, 1383–1396. [CrossRef]
42. Phan, C.W.; Sabaratnam, V. Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 863–873. [CrossRef]
43. Rinker, D.L. Spent mushroom substrate uses: Technology and applications. In *Edible and Medicinal Mushrooms: Technology and Applications*; Zied, D.C., Pardo-Giménez, A., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2017; pp. 427–454. [CrossRef]
44. Zied, D.C.; Sánchez, J.E.; Noble, R.; Pardo-Giménez, A. Use of Spent Mushroom Substrate in New Mushroom Crops to Promote the Transition towards A Circular Economy. *Agronomy* **2020**, *10*, 1239. [CrossRef]
45. Gern, R.M.M.; Libardi Junior, N.; Patrício, G.N.; Wisbeck, E.; Chaves, M.B.; Furlan, S.A. Cultivation of *Agaricus blazei* on *Pleurotus* spp. spent substrate. *Braz. Arch. Biol. Technol.* **2010**, *53*, 939–944. [CrossRef]
46. Ashrafi, R.; Mian, M.H.; Rahman, M.; Jahiruddin, M. Recycling of Spent Mushroom Substrate for the Production of Oyster Mushroom. *Res. Biotechnol.* **2014**, *5*, 13–21.
47. Noonsong, V.; Puttakun, N.; Tinsirisuk, M.; Seephueak, P. Recycling of spent *Pleurotus* compost for production of the *Agrocybe cylindracea*. *Mycosphere* **2016**, *7*, 36–43. [CrossRef]
48. Mleczek, M.; Siwulski, M.; Stuper-Szablewska, K.; Rissmann, I.; Sobieralski, K.; Goliński, P. Accumulation of elements by edible mushroom species: Part, I. Problem of trace element toxicity in mushrooms. *J. Environ. Sci. Health Part B* **2013**, *48*, 69–81. [CrossRef]
49. Cocchi, L.; Vescovi, L.; Petrini, L.E.; Petrini, O. Heavy metals in edible mushrooms in Italy. *Food Chem.* **2006**, *98*, 277–284. [CrossRef]
50. Györfi, J.; Geösel, A.; Vetter, J. Mineral composition of different strains of edible medicinal mushroom *Agaricus subrufescens* Peck. *J. Med. Food* **2010**, *13*, 1510–1514. [CrossRef]
51. Liu, B.; Huang, Q.; Cai, H.; Guo, X.; Wang, T.; Gui, M. Study of heavy metal concentrations in wild edible mushrooms in Yunnan Province, China. *Food Chem.* **2015**, *188*, 294–300. [CrossRef]
52. Largeteau, M.L.; Llarena-Hernández, R.C.; Regnault-Roger, C.; Savoie, J.M. The medicinal Agaricus mushroom cultivated in Brazil: Biology, cultivation and non-medicinal valorisation. *Appl. Microbiol. Biot.* **2011**, *92*, 897–907. [CrossRef]
53. Mleczek, M.; Magdziak, Z.; Gąsecka, M.; Niedzielski, P.; Kalač, P.; Siwulski, M.; Sobieralski, K. Content of selected elements and low-molecular-weight organic acids in fruiting bodies of edible mushroom Boletus badius (Fr.) Fr. from unpolluted and polluted areas. *Environ. Sci. Pollut. R.* **2016**, *23*, 20609–20618. [CrossRef]
54. Muñoz, A.H.S.; Corona, F.G.; Wrobel, K.; Soto, G.M.; Wrobel, K. Subcellular distribution of aluminum, bismuth, cadmium, chromium, copper, iron, manganese, nickel, and lead in cultivated mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*). *Biol. Trace Elem. Res.* **2005**, *106*, 265–277. [CrossRef]
55. Rzymski, P.; Mleczek, M.; Niedzielski, P.; Siwulski, M.; Gąsecka, M. Cultivation of *Agaricus bisporus* enriched with selenium, zinc and copper. *J. Sci. Food Agr.* **2017**, *97*, 923–928. [CrossRef]
56. Sun, L.; Liu, G.; Yang, M.; Zhuang, Y. Bioaccessibility of cadmium in fresh and cooked *Agaricus blazei* Murill assessed by in vitro biomimetic digestion system. *Food Chem. Toxicol.* **2012**, *50*, 1729–1733. [CrossRef]
57. Schlecht, M.T.; Säumel, I. Wild growing mushrooms for the Edible City? Cadmium and lead content in edible mushrooms harvested within the urban agglomeration of Berlin, Germany. *Environ. Pollut.* **2015**, *204*, 298–305. [CrossRef]
58. Vetter, J. Chemical composition of fresh and conserved Agaricus bisporus mushroom. *Eur. Food Res. Technol.* **2003**, *217*, 10–12. [CrossRef]
59. Vetter, J. Data on sodium content of common edible mushrooms. *Food Chem.* **2003**, *81*, 589–593. [CrossRef]
60. Vetter, J.; Lelley, J. Selenium level of the cultivated mushroom *Agaricus bisporus*. *Acta Aliment. Hung.* **2004**, *33*, 297–301. [CrossRef]
61. Vetter, J. Arsenic content of some edible mushroom species. *Eur. Food Res. Technol.* **2004**, *219*, 71–74. [CrossRef]

62. Vetter, J.; Hajdú, C.S.; Gyorfi, J.; Maszlavér, P. Mineral composition of the cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. *Acta Aliment. Hung.* **2005**, *34*, 441–451. [CrossRef]
63. Wisitrassameewong, K.; Karunarathna, S.C.; Thongklang, N.; Zhao, R.; Callac, P.; Moukha, S.; Ferandon, C.; Chukeatirote, E.; Hyde, K.D. *Agaricus subrufescens*: A review. *Saudi J. Biol. Sci.* **2012**, *19*, 131–146. [CrossRef]
64. Zhu, F.; Qu, L.; Fan, W.; Qiao, M.; Hao, H.; Wang, X. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environ. Monit. Assess.* **2011**, *179*, 191–199. [CrossRef]
65. Koutrotsios, G.; Danezis, G.; Georgiou, C.; Zervakis, G.I. Elemental Content in *Pleurotus ostreatus* and Cyclocybe cylindracea Mushrooms: Correlations with Concentrations in Cultivation Substrates and Effects on the Production Process. *Molecules* **2020**, *25*, 2179. [CrossRef]
66. Yang, W.; Guo, F.; Wan, Z. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi J. Biol. Sci.* **2013**, *20*, 333–338. [CrossRef]
67. Siwulski, M.; Niedzielski, P.; Budka, A.; Budzyńska, S.; Kuczyńska-Kippen, N.; Kalač, P.; Sobieralski, K.; Mleczek, M. Patterns of changes in the mineral composition of Agaricus bisporus cultivated in Poland between 1977–2020. *J. Food Compos. Anal.* **2022**, *112*, 104660. [CrossRef]
68. Aloupi, M.; Koutrotsios, G.; Koulousaris, M.; Kalogeropoulos, N. Trace metal contents in wild edible mushrooms growing on serpentine and volcanic soils on the island of Lesvos, Greece. *Ecotoxicol. Environ. Saf.* **2012**, *78*, 184–194. [CrossRef]
69. World Health Organization. *Guidelines for Drinking—Water Quality*, 4th ed.; World Health Organization (WHO): Geneva, Switzerland, 2011; pp. 104–108.
70. Bruce, R.M.; Odin, M.; World Health Organization. *Beryllium and Beryllium Compounds*; World Health Organization: Geneva, Switzerland, 2001.
71. World Health Organization; Food and Agricultural Organization of the United Nations. *Vitamin and Mineral Requirements in Human Nutrition*; World Health Organization: Geneva, Switzerland, 2004.
72. Martone, G. Nutritional lithium. *J. Clin. Psychiatry Neurosci.* **2018**, *1*, 3–4.
73. National Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances. *National Research Council Recommended Dietary Allowances*; The National Academies Press: Washington, DC, USA, 1989.
74. World Health Organization. Strontium and Strontium Compound. Concise International Chemical Assessment Document 77. 2010. Available online: https://apps.who.int/iris/bitstream/handle/10665/44280/9789241530774_?sequence=1 (accessed on 17 August 2022).
75. Hoa, H.T.; Wang, C.L.; Wang, C.H. The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **2015**, *43*, 423–434. [CrossRef]
76. Atila, F. Evaluation of suitability of various agro-wastes for productivity of *Pleurotus djamor*, *Pleurotus citrinopileatus* and *Pleurotus eryngii* mushrooms. *J. Exp. Agric. Int.* **2017**, *17*, 1–11. [CrossRef]
77. Hasan, M.T.; Khatun, M.H.A.; Sajib, M.A.M.; Rahman, M.M.; Rahman, M.S.; Roy, M.; Miah, M.N.; Ahmed, K.U. Effect of Wheat Bran Supplement with Sugarcane Bagasse on Growth, Yield and Proximate Composition of Pink Oyster Mushroom (*Pleurotus djamor*). *Am. J. Food Sci. Technol.* **2015**, *3*, 150–157. [CrossRef]
78. Selvakumar, P.; Rajasekar, S.; Babu, A.G.; Periasamy, K.; Raaman, N.; Reddy, M.S. Improving biological efficiency of *Pleurotus* strain through protoplast fusion between *P. ostreatus* var. florida and *P. djamor* var. roseus. *Food Sci. Biotechnol.* **2015**, *24*, 1741–1748. [CrossRef]
79. Salmones, D.; Mata, G.; Waliszewski, K.N. Comparative culturing of *Pleurotus* spp. on coffee pulp and wheat straw: Biomass production and substrate biodegradation. *Bioresour. Technol.* **2005**, *96*, 537–544. [CrossRef]
80. Hultberg, M.; Oskarsson, C.; Bergstrand, K.J.; Asp, H. Benefits and drawbacks of combined plant and mushroom production in substrate based on biogas digestate and peat. *Environ. Technol. Innov.* **2022**, *28*, 102740. [CrossRef]
81. Stamets, P. *Growing Gourmet and Medicinal Mushrooms*, 3rd ed.; Ten Speed Press: Berkley, CA, USA, 2000.
82. Kurtzman, R.H.; Zadrazil, F. Physiological and taxonomic considerations for cultivation of *Pleurotus mushrooms*. In *Tropical Mushrooms: Biological Nature and Cultivation Methods*; Chang, S.T., Quimio, T.H., Eds.; Chinese University Press: Hong Kong, China, 1982; pp. 299–348. ISBN1 9-62-201264-7. ISBN2 978-9-62201-264-6.
83. Molena, O. O Moderno Cultivo de Cogumelos. Nobel: São Paulo, Brazil, 1986; p. 170. ISBN 852130377.
84. Miles, P.G.; Chang, S.T. *Mushrooms Biology: Concise Basics and Current Developments*; Word Scientific: Singapore, 1997; p. 194. ISBN 9-81-022877-5.
85. Chang, S.T.; Miles, P.G. *Edible Mushrooms and Their Cultivation*; CRC Press: Boca Raton, FL, USA, 1989.
86. Sales-Campos, C.; Fereira da Eira, A.; Teixeira de Almeida Minhoni, M.; Nogueira de Andrade, M.C. Mineral composition of raw material, substrate and fruiting bodies of *Pleurotus ostreatus* in culture. *Interciencia* **2009**, *34*, 432–436. Available online: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-18442009000600013 (accessed on 6 July 2022).
87. Kutter, P.; Weissböck, A.D.; Leitner, V.; Jäger, A. Examination of commercial additives for biogas production. *Agron. Res.* **2015**, *13*, 337–347. Available online: https://agronomy.emu.ee/vol132/13_2_8_B5.pdf (accessed on 10 July 2022).
88. Rajarathnam, S.; Bano, Z. Biopotentialities of basidiomacromycetes. *Eur. J. Appl. Microbiol. Biotechnol.* **1992**, *37*, 233–361. [CrossRef]
89. Oliveira, H.C.B. Avaliação de Três Substratos com Diferentes Granulométricas, para o Cultivo de Duas Linhagens de *Pleurotus ostreatus* (Jacq.:Fr.) Kummer. Master's Thesis, Universidade Federal do Ceará, Fortaleza, Brazil, 2000; 89p.
90. Richard, T.L. Municipal solid waste composting: Physical and biological processing. *Biomass Bioenerg.* **1992**, *3*, 163–180. [CrossRef]
91. Hanc, A.; Szakova, J.; Ochecova, P. Differences in the mobility of Cd, Cu, Pb and Zn during composting of two types of household bio-waste collected in four seasons. *Bioresour. Technol.* **2014**, *168*, 204–213. [CrossRef]

92. Hanc, A.; Szakova, J.; Svehla, P. Effect of composting on the mobility of arsenic, chromium and nickel contained in kitchen and garden waste. *Bioresour. Technol.* **2012**, *126*, 444–452. [CrossRef]
93. Zadrazil, F. Cultivation of *Pleurotus*. In *The Biology and Cultivation of Edible Mushrooms*; Ghang, S.T., Hayes, W.A., Eds.; Academic Press: New York, NY, USA, 1978; pp. 521–557. [CrossRef]
94. Silva, S.O.; Costa, S.M.G.; Clemente, E. Chemical composition of *Pleurotus pulmonarius* (Fr.) Quél., substrates and residue after cultivation. *Braz. Arch. Biol. Technol.* **2002**, *45*, 531–535. [CrossRef]
95. Stewart, D.P.C.; Cameron, K.C.; Cornforth, I.S. Effects of spent mushroom substrate on soil chemical conditions and plant growth in an intensive horticultural system: A comparison with inorganic fertiliser. *Soil Res.* **1998**, *36*, 185–198. [CrossRef]
96. Medina, E.; Paredes, C.; Bustamante, M.A.; Moral, R.; Moreno-Caselles, J. Relationships between soil physico-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. *Geoderma* **2012**, *173*, 152–161. [CrossRef]
97. Pardo-Giménez, A.; Pardo-Gonzalez, J.E.; Cunha Zied, D. Evaluation of harvested mushrooms and viability of *Agaricus bisporus* growth using casing materials made from spent mushroom substrate. *Int. J. Food Sci. Technol.* **2011**, *46*, 787–792. [CrossRef]
98. Pardo-Giménez, A.; Picornell Buendia, M.R.; de Juan Valero, J.A.; Pardo-Gonzalez, J.E.; Cunha Zied, D. Cultivation of *Pleurotus ostreatus* using supplemented spent oyster mushroom substrate. *Acta Hortic.* **2012**, *933*, 267–272. [CrossRef]
99. Jasińska, A. Spent mushroom compost (SMC)–Retrieved added value product closing loop in agricultural production. *Acta Agrar. Debr.* **2018**, *150*, 185–202. [CrossRef]
100. Aldoori, Z.T.; Al-Obaidi, A.S.; Abdulkareem, A.H.; Abdullah, M.K. Effect of dietary replacement of barley with mushroom cultivation on carcass characteristics of Awassi lambs. *J. Anim. Health Prod.* **2015**, *3*, 94–98. [CrossRef]
101. Baek, Y.C.; Kim, M.S.; Reddy, K.E.; Oh, Y.K.; Jung, Y.H.; Yeo, J.M.; Choi, H. Rumen fermentation and digestibility of spent mush (*Pleurotus ostreatus*) substrate inoculated with *Lactobacil brevis* for Hanwoo steers. *Revista Colombiana de Ciencias Pecuarias* **2017**, *30*, 267–277. [CrossRef]
102. Strmisková, G.; Strmiska, F.; Dubravicky, J. Mineral composition of oyster mushrooms. *Nahrung* **1992**, *36*, 210–212. [CrossRef]
103. Vetter, J. Mineral element content of edible and poisonous macrofungi. *Acta Alim.* **1990**, *19*, 27–40. Available online: https://www.researchgate.net/profile/JanosVetter/publication/284105599_Mineral_element_content_of_edible_and_poisonous_macrofungi/links/5abdebe145851584fa6fdda0/Mineral-element-content-of-edible-and-poisonous-macrofungi.pdf (accessed on 15 August 2022).
104. Vetter, J. Mineral elements in the important cultivated mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*. *Food Chem.* **1994**, *50*, 277–279. [CrossRef]
105. Wang, D.; Sakoda, A.; Suzuki, M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Bioresour. Technol.* **2001**, *78*, 293–333. [CrossRef]
106. Bernás, B.; Jaworska, G.; Lisiewka, Z. Edible mushrooms as a source of valuable nutritive constituents. *Acta Sci. Polon. Technol. Alim.* **2006**, *5*, 5–20. Available online: https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-963ba13a-95a8-4bf1-ad64-74dcb80a7689 (accessed on 5 August 2022).
107. Mallikarjuna, S.E.; Ranjini, A.; Haware, D.J.; Vijayalakshmi, M.R.; Shashirekha, M.N.; Rajarathnam, S. Mineral composition of four edible mushrooms. *J. Chem.* **2013**, *2013*, 805284. [CrossRef]
108. Siwulski, M.; Mleczek, M.; Rzymski, P.; Budka, A.; Jasińska, A.; Niedzielski, P.; Kalac, P.; Gąsecka, M.; Budzynska, S.; Mikołajczak, P. Screening the multi-element content of *Pleurotus* mushroom species using inductively coupled plasma optical emission spectrometer (ICP-OES). *Food Anal. Method* **2017**, *10*, 487–496. [CrossRef]
109. Zsigmond, A.R.; Kántor, I.; May, Z.; Urák, I.; Héberger, K. Elemental composition of Russula cyanoxantha along an urbanization gradient in Cluj-Napoca (Romania). *Chemosphere* **2020**, *238*, 124566. [CrossRef]
110. Yamaç, M.; Yildiz, D.; Sarikürkcü, C.; Çelikkollu, M.; Solak, M.H. Heavy metals in some edible mushrooms from the Central Anatolia, Turkey. *Food Chem.* **2007**, *103*, 263–267. [CrossRef]
111. Ouzouni, P.K.; Petridis, D.; Koller, W.D.; Riganakos, K.A. Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chem.* **2009**, *115*, 1575–1580. [CrossRef]
112. Mustafa, S.; Sibal, S.; Mustafa, T.; Durali, M. Determination of trace metals in mushroom samples from Kayseri, Turkey. *Food Chem.* **2005**, *92*, 649–652. [CrossRef]
113. Pei, D.; Xie, H.; Song, H.; Xu, H.; Wu, Y. Bioconcentration factors and potential human health risks of heavy metals in cultivated *Lentinus edodes* in Chengdu, People's Republic of China. *J. Food Prot.* **2015**, *78*, 390–395. [CrossRef]
114. Kalač, P. Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000–2009. *Food Chem.* **2010**, *122*, 2–15. [CrossRef]
115. Falandysz, J.; Borovička, J. Macro and trace mineral constituents and radionuclides in mushrooms: Health benefits and risks. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 477–501. [CrossRef]
116. Zoroddu, M.A.; Aaseth, J.; Crisponi, G.; Medici, S.; Peana, M.; Nurchi, V.M. The essential metals for humans: A brief overview. v*J. Inorg. Biochem.* **2019**, *195*, 120–129. [CrossRef] [PubMed]
117. EFSA. Scientific opinion on the risks to public health related to the presence of nickel in food and drinking water. Panel of contaminants in the food chain (CONTAM). *EFSA J.* **2015**, *13*, 4002. [CrossRef]
118. EFSA. *Dietary Reference Values for Nutrients. Summary Report*; EFSA Supporting Publication; EFSA: Parma, Italy, 2017; p. e15121.
119. Mleczek, M.; Siwulski, M.; Budka, A.; Mleczek, P.; Budzyńska, S.; Szostek, M.; Rzymski, P. Toxicological risks and nutritional value of wild edible mushroom species -a half-century monitoring study. *Chemosphere* **2021**, *263*, 128095. [CrossRef] [PubMed]

120. EC. *Commission Regulation (EC) No 629/2008 of 2 July 2008 Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs*; European Commission: Brussels, Belgium, 2008.
121. IRIS (Integrated Risk Information System). Silver. Reference Dose for Oral Exposure. 1991. Available online: https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=99 (accessed on 15 July 2022).
122. IRIS (Integrated Risk Information System). Barium and Compounds. Reference Dose for Oral Exposure. 2005. Available online: https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=10 (accessed on 15 July 2022).
123. EFSA Panel on Contaminants in the Food Chain (CONTAM). Safety of aluminium from dietary intake. Scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC). *EFSA J.* **2008**, *754*, 1–34.