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ORIGINAL ARTICLE

Evaluation of the yield and heavy metal bioaccumulation in the fruit body of *Pleurotus ostreatus* grown on sugar mill wastewaters

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Abstract

Sugar mill wastewater (SMWW) is one of the agro-industrial wastewaters that is generated in huge quantities every year. In this study, the effect of substrate moistening with different SMWW concentrations on the cultivation cycle and yield of *Pleurotus ostreatus* and health risks potential were evaluated. Purposely, five different concentrations of SMWW including 0 (control using fresh water supply) 25, 50, 75, and 100% were tested as wetting agents in *P. ostreatus* cultivation. The yield and biological efficiency (BE%) increased with increasing concentration of SMWW. The yield of strain Homegreen (PO-Holl) increased from 118.01 ± 4.2 g kg^{-1} (control) to 235.0 ± 8.5 g kg^{-1} in 100SMW treatment, whereas that of strain HK-35 (PO-HK35) was increased from 196.5 ± 4.8 g kg^{-1} (control) to 239.2 ± 4.5 g kg^{-1} and 236.3 ± 4.1 g kg^{-1} in 25TP:75SMW and 100SMW treatments, respectively. However, the heavy metal accumulation by *P. ostreatus* strains was also strongly correlated to the increase in SMWW concentration. On the other hand, these values were below the recommended daily intake (RDI) set by the FAO/WHO. Moreover, the values of the Healthy Risk Index (HRI) of all heavy metals were less than 1. The results showed that the use of SMWW as a wetting agent can reduce the use of fresh water and inputs of additive materials in *P. ostreatus* cultivation, while keeping mushroom production safe and sustaining the management of such wastewaters.

Keywords Agro-industrial wastewaters · Heavy metals · Mushroom cultivation · Oyster mushroom · Wastewater management

1 Introduction

The world is facing a serious water scarcity problem [1]. The agriculture sector alone consumes about 70% of accessible fresh water to meet the world's growing food demand [2]. With a projected global population of 10 billion by 2050, this situation is expected to worsen as food demand will increase by 60% [3]. This problem forces humanity to search for alternative water sources that can be used efficiently for both drinking and irrigation purposes [4, 5]. Since the creation of new freshwater resources was not considered possible, the idea of reusing the wastewaters came to the fore [6, 7]. On the other hand, wastewater elimination by biological treatments is one of the remarkable issues at present in wastewater management [8–10].

The global mushroom industry is one of the fastest growing crop industries [11]. *Agaricus bisporus* is the most common cultivated mushroom in the world, followed by *Lentinula edodes*. The third most cultivated species is *Pleurotus ostreatus*, accounting for about 25% of worldwide mushroom production [12]. Large amounts of freshwater are used for substrate preparation in commercial mushroom cultivation. For example, the amount of freshwater used to produce 1 kg of *A. bisporus* is about 200 L [13], the same applies to *L. edodes* [14]. The use of freshwater for substrate moistening puts a huge load on our clean water resources.

A large volume of sugar mill wastewater (SMWW) is generated during the manufacture of sugar [15]. It is one of the most important risk factors which potentially causes environmental health damage in sugar-producing countries, especially due to its high total organic matter and heavy metal content. Therefore, proper management of SMWW is necessary to prevent the impact it may have on the environment [16].

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The nutritional content of the substrate used in mushroom production significantly affects the yield and quality of the mushroom; therefore, additive materials with high nitrogen content must be added to substrates in the preparation of the mushroom-growing medium [17]. SMWW is a rich source of organic matter and macro–micro-elements. The use of SMWW in mushroom cultivation can provide substantial benefits by saving the production cost to farmers as well as protecting environmental health and increasing yields. However, SMWW contains a considerable amount of heavy metals [16]. Heavy metals have major toxic and harmful effects on human health at low levels [18].

Sustainable use of nutrient-rich wastewaters such as dairy wastewater and olive mill wastewater for the cultivation of some mushroom species including *Pleurotus* spp. was reported in the literature previously [17–25]. Although some references concerning the utilization of sugar mill wastewaters for *A. bisporus* cultivation are available [26–28], the use of sugar mill wastewaters in *P. ostreatus* cultivation in the literature is limited. Moreover, there is little information on the contents of macro- micro-element and toxic elements of the fruitbody grown on substrates wetted by SMWW, and the Health Risk Index (HRI) values of these elements. Therefore, there is a need to investigate the potential of SMWW in increasing the mushroom yield and investigate the potential danger of the consumption of mushrooms grown on substrates rich in heavy metal content to human health.

The objectives of this work were (1) to determine the influence of substrate moistening with different SMWW concentrations on the spawn running time (SRP), days to primordia initiation (DPI), days to first harvest (DFH), yield, and biological efficiency (BE) of *P. ostreatus* strains; (2) to compare the effect of the different treatments on the macro- and micro-element contents of *P. ostreatus* strains; (3) to investigate the uptake amount of heavy metals by two *P. ostreatus* strains grown on substrates moistened with different SMWW concentrations; and (4) to estimate the potential health risk associated with the consumption of these mushrooms.

2 Material and methods

2.1 Materials

Tap water (TW) was supplied from the water supply of Mushroom Cultivation Laboratuvary (Ahi Evran University), and sugar mill wastewater (SMWW) was obtained from a sugar production plant (Tutgu Food Ltd., Kırşehir Sugar Plant) located in Mucur, Kırşehir, Turkey.

Fresh wheat straw was provided from a farm in Mucur, Kırşehir, Turkey. Two commercial strains belonging to *P. ostreatus* (PO-HK35 and PO-Homegreen) were used in the experiments. The strains were pre-cultured on malt extract agar medium (MEA) and incubated at 25 °C in the dark. After colonization, the mycelium cultures were maintained at 4 °C. The spawn production method outlined by Atila [29] was utilized for spawn preparation.

2.2 Substrate preparation

Wheat straw was used as a substrate material in the cultivation of *P. ostreatus*. Soaking waters were prepared by supplementing the tap water with various levels of SMWW, viz, 0% SMWW, 25% SMWW, 50 SMWW, 75% SMWW, and 100% SMWW. The wheat straw was soaked overnight in 25 L of liquid treatment inside 50-L capacity plastic sinks, so that, in addition to the water, macro- and micro-elements in the soaking water were absorbed by the substrate. After the soaking water was drained off, the wheat straw was filled in amounts of 1 kg to polypropylene bags and sterilized in an autoclave for 90 min at 121 °C.

2.3 Substrate inoculation, spawn running, and fruiting

The sterilized bags were inoculated with grain spawn at the rate of 3% of the wet substrate under the laminar flow hood. After the inoculation, the bags were moved into the incubation room and maintained at 24 ± 2 °C, $80\% \pm 5$ humidity, and in the dark. When the substrate was completely colonized by mushroom mycelium, the bags were transferred to a growing room under controlled conditions (17 ± 2 °C and $\geq 90\%$ relative humidity, $CO_2 < 1000$ ppm, and lighting with a fluorescent lamp for 12 h daily), and the mouths of the bags were opened to promote pinhead formation and fructification. When the in-rolled margins of the fruitbody began to flatten, mushrooms were harvested. The cultivation cycle was ended on day 60.

Cultivation parameters such as effects of spawn running time (SRT) (days), days to first primordia initiation (DPI) (days), days to first harvest (DFH) (days), yield (g/kg), and biological efficiency (BE%) were evaluated during the cultivation period of *P. ostreatus* strains grown on substrates moistening with different concentration of SMWW. The total yield is defined as the fresh weight of the mushrooms harvested. The biological efficiency (BE%) was calculated by using the formula BE (%) = (fresh weight of harvested fruit – body per bag/dry weight of substrate per bag) × 100.

The experiments were conducted in the Mushroom Cultivation Laboratory of the Department of Horticulture, Ahi Evran University, Kırşehir, following a completely randomized plot design, with 10 replications.

2.4 Macro–micro and heavy metal analyses

For mushroom analysis, the samples were taken out from the bags of each treatment in the first flush. The fruiting bodies of the harvested mushrooms were dried to a constant weight using an oven at 60 °C then were powdered using a grinder for further analysis. Water samples were directly subjected to analysis.

Ten milliliters of concentrated HNO_3 was added to the 50-mL water sample and digested at 80 °C until a clear liquid was obtained [30]. One gram of each dry mushroom sample was digested by adding 15 mL of an acid mixture ($H2SO_4$, $HClO_4$, and HNO_3, in a 1:1:5 ratio) at a temperature of 80 °C until the solution turned into a clear solution [30]. Filtration of digested samples was performed using Whatman (no. 1) filter paper. The filtrate was maintained up to 50 mL by adding distilled water. The samples were stored in a refrigerator at a temperature of 4 °C until analysis.

The total nitrogen was determined following Kjeldahl's method. Phosphorus (PO_4^{-3}) content was determined by a spectrophotometer at 690 nm. The mineral content (K, Na, Mg, and Ca) and heavy metal content (Fe, Mn, Cu, As, Cd, Pb) were analyzed using inductively coupled plasma mass spectrometry (Thermo Scientific X II Series ICP-MS) at the Central Laboratory, Van Yuzuncu Yıl University. Measurements of pH and electrical conductivity of the water samples were performed using a pH meter (Ohaus, Starter3100) and a conductivity meter (Mettler Toledo 7100) [31].

2.5 Calculation of daily oral intake and health risk

The daily oral intake (DIM) of heavy metals from mushroom fruitbodies was calculated following Eq. (1) [32].

$$\mathrm{DIM} = \frac{\mathrm{DMCxTEC}}{\mathrm{BW}} \quad (1)$$

DMC is the daily mushroom consumption. It was considered to be 14 g person/day [12] which is equivalent to 2.1 g (dry weight), considering there was on average 85% moisture content in *P. ostreatus* [33]. TEC is the content of trace elements in dry weight of the mushroom (mg kg^{-1}). BW (in kilograms per person) indicates the weight body for adults (70 kg) [27].

The non-carcinogen risk was calculated as the Healthy Risk Index (HRI) corresponding to the ratio of the DIM in the food crops to the oral reference dose (RfD) as shown in Eq. (2) [32].

$$\mathrm{HRI} = \frac{\mathrm{DIM}}{\mathrm{RfD}} \quad (2)$$

If HRI = 1 could create a health risk over multiple trace elements, HRI < 1 may indicate a safer product [34]. The values of RfD of heavy metals were shown in Table 1 [35].

2.6 Data analysis

The results were reported as mean ± S.E.M. The statistical significances of the differences between treatment means were evaluated by using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The values were considered statistically significant at $P \leq 0.05$. Data generated were calculated using SPSS original version 16.0.

3 Results and discussion

3.1 Chemical properties, macro–micro-element, and heavy metal contents of SMWW and TP

The collected SMWW was light brown in color and had a cloudy appearance and a slightly bad smell. The chemical compositions of SMWW and TP used in the study are presented in Table 2. The mean pH value of SMWW was almost neutral (equal to 6.80) and had a moderate electrical conductivity (EC) (2.27 dS m^{-1}). Sobal et al. [36] reported that the optimum pH for mycelial development was 5 to 7. The high salt content can affect mycelium growth, resulting in a significant reduction in mushroom yield. However, the EC value of SMWW was not high enough to harm the mushroom growth [37].

The macro-element and heavy metal contents of SMWW were much higher than TP. The availability of nitrogen and phosphorus in the growing media directly affects mycelial growth and productivity [38]. Moreover, mushrooms need minerals such as Mg, Ca, Fe, Cu, Mn, and Zn for their growth and vital activities. Calcium is one of the most important of these minerals [39]. SMWW containing high concentrations of macro- and micro-nutrients may affect positively the yield, BE (%), and nutritional content of *P. ostreatus* strains.

3.2 Effects of substrate moistening with SMWW on SRP, DPI, and DFH of *P. ostreatus* strains

The experiment conducted under different SMWW concentrations revealed that the SRP, DPI, and DFH of *P. ostreatus*

Table 1 Oral reference dose (RfD) for heavy metals

Heavy metals	Oral RfD (mg/kg/day)
Fe	7×10^{-3}
Mn	1.4×10^{-2}
Cu	4.2×10^{-2}
As	3×10^{-4}
Cd	5×10^{-4}
Pb	3.5×10^{-3}

Source: USEPA IRIS 2011

Table 2 Chemical properties, macro–micro element, and heavy metal contents of tap water and sugar mill wastewater used in the study

Parameters	Tap water (TW)	Sugar mill wastewater (SMWW)
Ph	7.4 ± 0.0.8	6.8 ± 0.08
EC (dS m^{-1})	0.9 ± 0.01	2.3 ± 0.16
TKN (mg L^{-1})	0.021 ± 0.001	0.201 ± 0.014
$PO4^{3-}$ (mg L^{-1})	0.002 ± 0.000	0.236 ± 0.017
K^{+} (mg L^{-1})	0.003 ± 0.000	0.279 ± 0.013
Ca^{2+} (mg L^{-1})	0.073 ± 0.004	0.504 ± 0.006
Mg^{2+} (mg L^{-1})	0.013 ± 0.000	0.176 ± 0.004
Na^{+} (mg L^{-1})	0.011 ± 0.001	0.336 ± 0.018
Fe^{3+} (mg L^{-1})	0.196 ± 0.014	6.698 ± 0.312
Mn^{2+} (mg L^{-1})	0.076 ± 0.002	5.707 ± 0.394
Cu^{2+} (mg L^{-1})	0.0027 ± 0.0003	0.7167 ± 0.039
Pb^{2+} (mg L^{-1})	0.0021 ± 0.0002	0.0970 ± 0.0045
Cd^{2+} (mg L^{-1})	0.0011 ± 0.0001	0.2940 ± 0.0114
As^{3-} (mg L^{-1})	0.0011 ± 0.0001	0.3053 ± 0.001

strains were significantly ($P < 0.01$) affected by the available SMWW in the growing substrate (Tables 3 and 4).

The SRP, DPI, and DFH of PO-Holl strain grown on media moistening with different ratios of SMWW varied between 19.6 ± 1.02 and 21.9 ± 0.83 days, between 23.8 ± 1.08 and 26.1 ± 1.2 days, and between 28.7 ± 0.78 and 30.3 ± 0.9 days, respectively, depending on the growing medium, whereas the SRP, DPI, and DFH of PO-HK35 strain grown on the same media ranged from 15.4 ± 1.02 to 18.2 ± 0.75 days, from 19.8 ± 0.60 to 23.7 ± 0.64 days, and from 26.0 ± 0.77 to 29.9 ± 0.54 days, respectively. In applying undiluted SMWWs (%100 SMWW), a weak inhibition of the mycelial growth of *P. ostreatus* strains was observed. On the other hand, TP75:SMWW25 and TP50:SMWW50 treatments presented a shortening time in SRP and DPI. These findings are similar to those in the study conducted by Kalmış et al. [24] and Abou Fayssal et al. [40] who found that olive mill wastewater has a positive effect up to a certain ratio in shortening the cultivation cycle of *Pleurotus* species, while it prolongs the cultivation cycle at higher concentrations. Zervakis et al. [41] interpreted that fast

Table 3 *F* test of the two-way ANOVA and its statistical significance (*P*) for strains, treatment, and their interaction in yield parameters of *Pleurotus ostreatus* strains grown on different treatment

		Spawn running time (days)	Days to primordial initial (days)	Days to first harvest (days)	Yield (g/kg)	BE (%)
Strain	*F*	467.373	324.900	110.05	357.015	347.006
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment	*F*	32.434	41.822	25.950	346.299	388.812
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Strain × treatment	*F*	3.552	7.087	7.711	86.099	81.855
	P	0.010	< 0.05	< 0.001	< 0.001	< 0.001

Table 4 Effect of treatments on production stages, yield, and BE (%) of *Pleurotus ostreatus* strains examined in the study

Strain	Treatment	Spawn running time (days)	Days to primordial initial (days)	Days to first harvest (days)	Yield (g/kg)	BE (%)
PO-Holl	Control	20.1 ± 0.94** b	24.4 ± 0.8** b	29.1 ± 1.14**bc	118.1 ± 4.2 **d	32.8 ± 1.2**d
	75TP:25SWW	19.6 ± 1.02 b	23.8 ± 1.08 b	28.7 ± 1.27 c	183.9 ± 11.1 c	55.7 ± 3.3 c
	50TP:50SWW	19.8 ± 0.60 b	24.1 ± 0.54 b	28.7 ± 0.78 c	215.6 ± 12.5b	61.6 ± 3.6 b
	25TP:75SWW	21.5 ± 1.02 a	25.6 ± 1.11 a	30.1 ± 0.54 b	222.7 ± 7.0 b	63.6 ± 2.0 b
	100SWW	21.9 ± 0.83 a	26.1 ± 0.94 a	30.3 ± 0.9 a	235.0 ± 8.5 a	67.2 ± 2.4 a
PO-HK35	Control	15.4 ± 0.66*c	19.8 ± 0.60**c	26.0 ± 0.77**d	196.5 ± 4.8**c	54.6 ± 1. ** 3c
	75TP:25SWW	16.7 ± 0.64 b	21.3 ± 0.64 b	26.6 ± 0.80 cd	224.0 ± 5.5 b	64.0 ± 1.6 b
	50TP:50SWW	16.6 ± 0.66 b	21.0 ± 0.63b	27.2 ± 0.87 bc	222.8 ± 3.3b	63.6 ± 1.0 b
	25TP:75SWW	18.1 ± 0.54 a	23.7 ± 0.64 a	29.9 ± 0.54 a	239.2 ± 4.5 a	68.3 ± 1.3 a
	100SWW	18.2 ± 0.75 a	23.0 ± 0.77 a	27.6 ± 0.80 b	236.3 ± 4.1 a	67.5 ± 1.2 a

The mean values in the same column within each strain followed by the same letters are not significantly different by Tukey's tests

SMWW sugar mill wastewater, *TP* tap water, *n.s.* not significant

*Significant at $P < 0.05$

**Significant at $P < 0.01$

***Significant at $P < 0.001$

mycelial extension is an indicator of hyphae growth, usually in a nutritionally poor or unfavorable medium. Similarly, Naraian et al. [42] and Curvetto et al. [43] also reported that the growing media with low N concentrations gave better results in terms of mycelial growth. On the other hand, 75% and 100% concentrations of SMWW may have reduced mycelium growth due to high trace metal concentration of the substrate. Although trace metals including Cu, Fe, and Mn are essential elements that have positive effect in the growth of fungi [44], these elements may cause some negative effects at higher concentrations on mushrooms [45].

3.3 Effects of substrate moistening with SMWW on yield and BE of *P. ostretaus* strain

In the present study, the effectiveness of using SMWW in place of tap water in preparation of mushroom growing substrate was studied by comparing the yields and BE obtained from the treatments. There were significant differences observed between the strains, treatments, and strain × treatment interaction ($P < 0.01$) (Tables 3 and 4).

The yield of PO-Holl increased from 118.01 ± 4.2 g kg^{-1} (control) to 235.0 ± 8.5 g kg^{-1} in 100SMW treatment, whereas that of PO-HK-35 increased from 196.5 ± 4.8 g kg^{-1} (control) to 239.2 ± 4.5 g kg^{-1} and 236.3 ± 4.1 g kg^{-1} in 25TP:75SMW and 100SMW treatments, respectively. It was determined that the total yield of harvested mushrooms from the media prepared using 75 and 100% SMWW was 58.69% and 20.3% ratios higher than the yield produced on the control substrate, respectively. Carrasco et al. [38] reported that nitrogen and phosphorus are essential elements for mushroom cells, and *A. bisporus* growth and productivity were directly affected by the availability of the elements in the substrate. The high N and P concentrations of SMWW may have also had a positive effect on the yield of *P. ostreatus* mushroom.

The BEs (%) of *P. ostreatus* strains also increased with increasing concentrations of SMWW for both strains used in the present study. The BEs (%) were between 32.8 ± 1.2 and 67.2 ± 2.4% for PO-Holl and between 54.6 ± 1.3% and 67.5 ± 1.2 for PO-HK35 in the different treatments. The use of various wastewaters in mushroom production has been evaluated in previous studies. Kalmış et al. [24] reported that increasing volumes of olive mill effluent (OME) in the mixture had negative effects on the yield and BE of *P. ostreatus*. Although BE of the mushroom was 53.6% for the control group, it was found to be 13.8% for substrates wetted with 100% OME. Kalmis and Sargin [25] observed that OME at 25 and 50% concentrations was favorable for cultivation of *Pleurotus sajor-caju* and *Pleurotus cornucopiae*. Loss et al. [46] have recommended using the maize wastewater at concentrations of 50% to moisten and supplement the growing substrate for a maximum mushroom yield of *P. ostreatus*. Pant et al. [47] reported that *Pleurotus* spp. reached maximum yield by using a 50% concentration of distillery wastewater. Kumar et al. [48] cultivated milky mushrooms (*Calocybe indica*) on a substrate prepared by mixing rice straw and sugar mill effluent. They reached maximum mushroom BE(%) (63.99%) using a 75% concentration of sugar mill effluent, but the yield and BE were decreased at 100% concentration. Nevertheless, *P. ostreatus* strains exhibited a remarkable increment in the yields and BEs (%) when using a 100% ratio of SMWW supplementation in the present study. SMWW is rich in nitrogen and contains considerable phosphates, potassium, calcium, and other minerals [27]. The enhanced yield and BE (%) of *P. ostreatus* might be correlated with the macro–micro-element contents of SMWW. Yıldız et al. [49] reported that the addition of different minerals such as supplements could help the fructification in some mushroom strains. Although wheat straw is already rich in lignocellulosic content, a supply of extra nutrients was ensured through SMWW supplementation [27].

The potential of *P. ostreatus* mushroom to benefit from SMWW also differed according to strains ($P < 0.01$). Although both strains exhibited significant higher BE(%) on all treatments as compared to control at the end of the cultivation period, the increase in yield with supplementation with SMWW for the PO-Holl strain was significantly higher when compared to the PO-HK35 strain. The PO-Holl strain demonstrated higher BE on the 75TP:25SMWW, 50TP:50SMWW, 25TP:75SMWW, and 100SMWW, by 55.7%,82.5%, 88.6%, and 99%, respectively, when compared with control substrate.

3.4 Effects of substrate moistening with SMWW on macro- and micro-element contents of *P. ostreatus* fruitbody

The effect of the strain, the treatments, and their interaction on the macro-element content of the fruitbody was found to be statistically significant ($P < 0.01$) (Table 5 and Fig. 1).

It is reported that the variations in the element composition of the fruitbody are directly related to the element compositions of the substrate and the accumulation of these elements in the fruitbody [50]. However, the results in Table 5 suggest that the uptake of macro- and micro-elements by the PO-HK35 strain was not significantly influenced by the varying levels of SMWW supplementation, with the exceptions of P, whereas macro- and micro-element contents of PO-Holl varied depending on the treatments, except for K and Na. These results are in accordance with Atila et al. [51] who reported that the chemical composition of the growing media influenced the chemical composition of the mushrooms, but their macro- and micro-element composition did not correspond in all the cases to the chemical

Table 5 *F* test of the two-way ANOVA and its statistical significance (*P*) for strains, treatment, and their interaction in macro element and heavy metal contents of *Pleurotus ostreatus* strains grown on different treatment

		N	P	K	Ca	Mg	Na	Fe	Mn	Cu	As	Cd	Pb
Strain	*F*	190.613	31.580	286.997	43.199	109.459	15.390	0.551	6.730	562.228	527.919	225.080	233.117
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	> 0.05	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001
Treatment	*F*	397.874	134.468	3.387	17.372	10.402	3.420	0.937	1.731	29.191	54.695	77.713	1.451
	P	< 0.001	< 0.001	> 0.05	< 0.001	< 0.001	> 0.05	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	> 0.05
Strain × treatment	*F*	450.535	49.402	2.451	4.925	9.013	1.982	0.719	2.580	7.586	30.858	34.062	0.581
	P	< 0.001	< 0.001	> 0.05	< 0.01	< 0.001	> 0.05	< 0.001	> 0.05	< 0.01	< 0.001	< 0.001	> 0.05

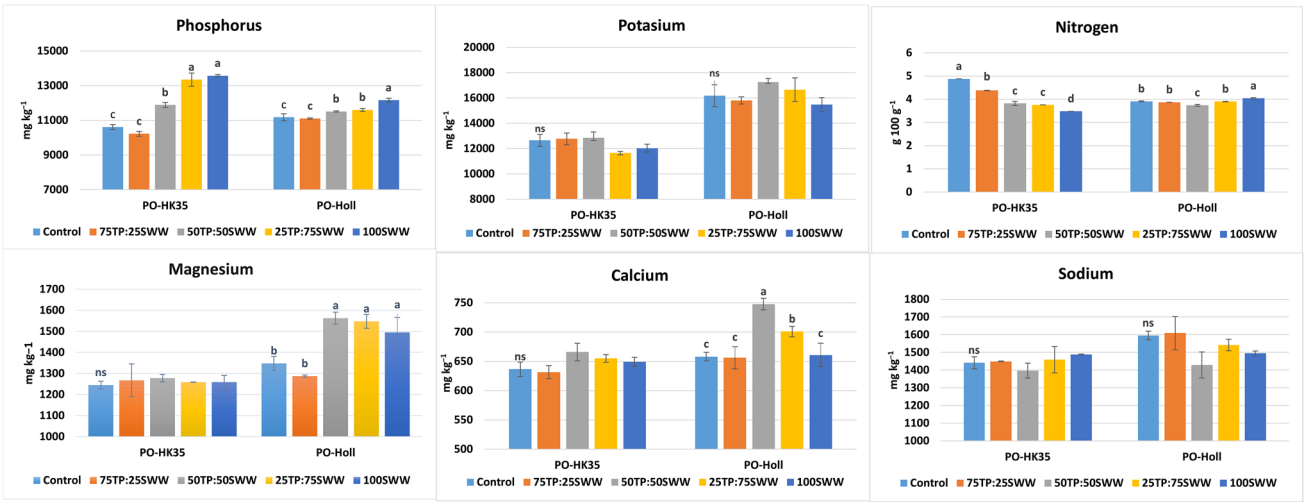

Fig. 1 Effects of different treatments on macro element content of the fruiting body of *P. ostreatus* strains examined in the study

composition of the substrate, although in many cases it was well correlated.

The content of N was the highest among all elements in *P. ostreatus* and ranged from 3.48 to4.88 g 100 g^{-1} in PO-HK35 and from 3.74 to 4.04 g 100 g^{-1} dw in the PO-Holl fruitbody. These results were around the same range or lower than the one reported by Mendez et al. [52]. The highest N content of PO-HK35 was obtained in the control substrate, while the lowest N content was determined in the 100% SMWW substrate. In the PO-Holl strain, on the contrary, the highest N content was observed in fruitbodies grown on 100% SMWW substrate. Although Khan et al. [53] reported that in most cases the high protein content of mushrooms is correlated with the high protein content in substrates, our results showed that there was no correlation between the N content of the fruitbody and the N content of the substrates. The second most dominating element was potassium which ranged from 11,644.1 ± 139.1 to 17,249.1 ± 89.7 mg kg^{-1} dw in different treatments. Hoa et al. [54] reported that the K content of *P. ostreatus* ranged from between 1424.37 and 2624.16 mg 100 g^{-1} dw, although Jin et al. [55] found that this element could rise to 19.496–20.388 mg kg^{-1} dw. The phosphorus values recorded in the PO-HK35 fruitbody were between 10,222.8 ± 143.1 and 13,572.4 ± 70.9 mg kg^{-1} dw and in the PO-Holl between 11,107.7 ± 36.9 and 12,160.6 ± 110.4 mg kg^{-1} dw. The highest P content was obtained on SMWW100 treatment in both strains. These results are slightly higher than those obtained in previous studies [56, 57]. Mg represented the third major element, ranging from 1245.2 ± 18.2 to 1562.7 ± 28.7 mg kg^{-1} dw. The Mg content obtained was generally in accordance with those given in previous publications [55, 58]. The concentrations of Ca ranged from 631.6 ± 11.2 to 747.8 ± 9.8 g kg^{-1} dw, and these were higher than those obtained by Sopanrao et al. [57] and Hoa et al. [54]. Na content of the fruitbodies varied from 1397.3 ± 41.9 to 1609.1 ± 93.3 mg kg^{-1} dw. These values were lower than those obtained by Sopanrao et al. [57] but higher than those obtained by Jin et al. [55].

3.5 Effects of substrate moistening with SMWW on heavy metal contents of *P. ostreatus* fruitbody

The strain, the treatments, and the strain × treatments interaction were significant for the heavy metal content of the fruitbody as shown in Table 5 and Fig. 2.

It was found that the heavy metal accumulation in all SMWW treatments was significantly ($P < 0.01$) increased as compared with the substrate treated with tap water supply, except for Fe and Mn. Moreover, it was observed that the accumulation of heavy metals in the fruitbodies was closely related to the heavy metal content of the substrates. *P. ostreatus* strains grown on substrates supplemented with a high ratio of SMWW invoked significant Cu, As, Cd, and Pb accumulation; heavy metal accumulation ability of mushrooms was previously also reported for some mushroom species examined [59–61].

In the present study, Fe was found to owe the highest concentration among micro-elements which was in accordance with the findings of Hoa et al. [54] who reported that Fe is the major microelement in *P. ostreatus* fruitbody. Although Almeida ve ark [62] found that the iron bioaccumulation in *P. ostreatus* mycelial bio-mass was affected from iron content in the culture medium, the Fe and Mn concentrations of the fruitbodies were not affected by the different treatments used in the study ($P > 0.05$). The Fe values in the dry weight of mushrooms have been recorded as 45.428 ± 2.32 to 49.236 ± 3.52 mg kg^{-1}, and the Mn values ranged between 5.547 0.08 and 6.946 0.43 mg kg^{-1} dw. The Mn and Fe values in this study are in agreement with the results found in the literature [54].

Minimum and maximum Cd contents of PO-HK-35fruitbody were 0.0296 ± 0.004 mg kg^{-1} (control) and 0.0760 ± 0.001 mg kg^{-1} (100 SMWW) and 0.0748 ± 0.001 mg kg^{-1} and 0.0857 ± 0.006 mg kg^{-1} (50TP:50SMWW) in PO-Holl. The copper content was 6.492 ± 0.516 mg kg^{-1} in the fruitbodies of PO-HK35 from the control substrate and 9.318 ± 0.352 mg kg^{-1} in the samples from 100SMWW treatment. Similarly, the Cu content of the PO-Holl strain ranged from 4.415 ± 0.981 to 5.646 ± 0.469 mg kg^{-1}. The content of As in PO-HK35 and PO-Holl strains ranged from 0.463 ± 0.001 to 0.760 ± 0.003 mg kg^{-1} and from 0.775 ± 0.059 to 0.969 ± 0.010 mg kg^{-1}, respectively. The Pb values ranged from 0.6097 ± 0.010 to 0.668 ± 0.003 mg kg^{-1} (in PO-HK35) and from 0.9997 ± 0.040 to 1.2361 ± 0.026 mg kg^{-1} (in PO-Holl).

A two-way ANOVA showed significant differences between species ($P < 0.001$). The comparative evaluation of PO strains revealed that their ability to accumulate macro- and micro-elements appears to be strain- and not species-dependent. Overall, the use of a 75 and 100% ratio of SMW showed comparatively high contents of As, Cu, Cd, and Pb as compared to other treatments. The As, Cd, and Pb contents of the PO-Holl strain were considerably higher than those of the PO-HK35 strain, while the Cu content of the PO-HK35 strain was determined to be much higher. The highest determined contents of As, Cd, and Pb contents for the PO-Holl strain were 0.969 ± 0.010 mg kg^{-1}, 0.0846 ± 0.002 mg kg^{-1}, and 1.2361 ± 0.026 mg kg^{-1}, respectively. Also, the highest Cu content of PO-HK35 fruitbody grown on TP75:SBW25 and 100SBW was determined as 9.012 ± 0.157 mg kg^{-1} and 9.318 ± 0.352 mg kg^{-1}, respectively. PO-Holl and PO-HK35 demonstrated distinct accumulation patterns. The results showed that PO-Holl showed a greater amount of K, Mg, Na, As, Cd, and Pb, whereas PO-HK-35 fruitbody showed relatively more P and Cu.

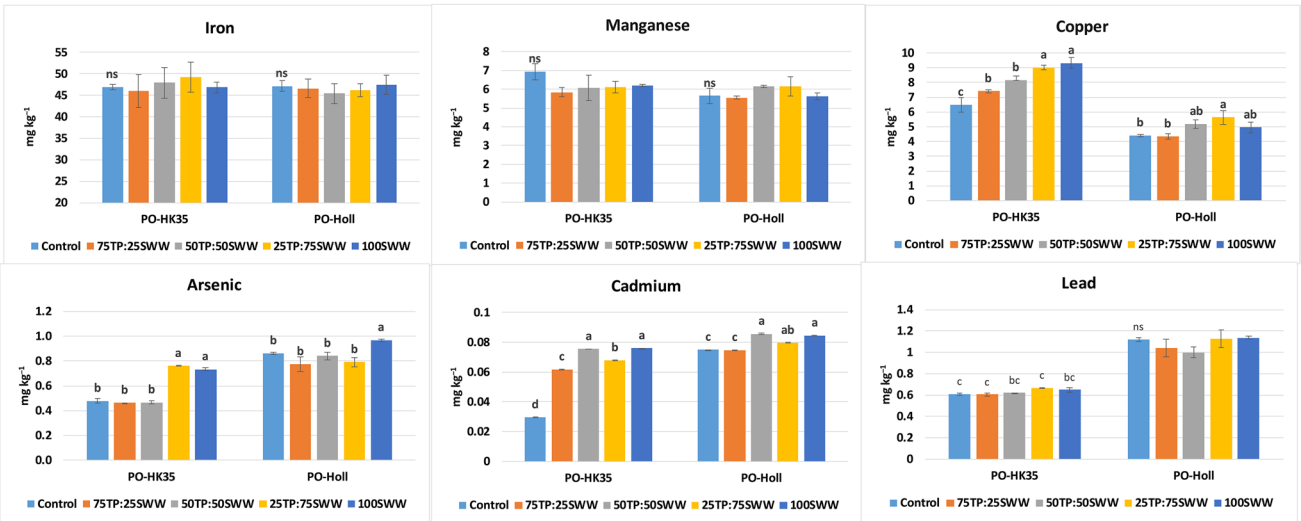

Fig. 2 Effects of different treatments on heavy metal content of the fruiting body of *P. ostreatus* strains examined in the study

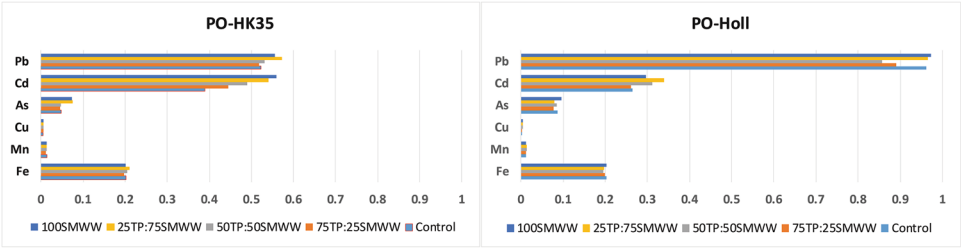

Fig. 3 Health risk index (HRI) of heavy metals in the *P. ostreatus* fruitbody grown on substrates wetted by SMWW (for adults)

3.6 Health risk assessment of heavy metals in the mushrooms

The calculated values of HRI of heavy metals for adults are shown in Fig. 3. The HRI of Fe, Mn, Cu, As, Cd, and Pb ranged from 0.19469 to 0.21101, 0.01189 to 0.01488, 0.00310 to 0.00666, 0.0463 to 0.0969, 0.26058 to 0.55908, and 0.51780 to 0.99600, respectively. The values of HRI of all heavy metals were less than 1, indicating that consumption of *P. ostreatus* fruitbody grown on substrates wetted by SMWW was assumed to be safe.

Moreover, the permissible limit of heavy metals in vegetables suggested by the FAO/WHO such as Cu, As, Pb, and Cd are 40, 2, 0.3, and 0.2 mg kg^{-1}, respectively [63]. Although the heavy metal concentrations of the fruitbodies grown on substrates moistening with different concentrations of SMWW were higher than those of the control substrate, these values were below the recommended daily intake (RDI) provided by the FAO/WHO [64].

4 Conclusion

The results clearly demonstrated that the use of different concentrations of SMWW resulted in a significant increase in yields and BEs (%) of *P. ostreatus* strains compared to the control medium. Even the use of the highest concentration of SMWW did not display negative effects on productivity of the *P. ostreatus*, in fact, the highest yields and BEs (%) of *P. ostreatus* strains were obtained using a 100% ratio of SMWW. The results revealed that SMWW may provide an economically acceptable production alternative for mushroom cultivation and reduce the disposal problems caused by this wastewater. Although the use of SMWW for moistening substrates resulted in significantly higher heavy metal accumulation in the fruitbody, the heavy metal accumulation in the fruitbody was found to be within safe limits. Briefly, the fruitbody grown on substrates moistening with SMWW can be consumed without any concerns for food safety.

According to the results of the present study, SMWW can be effectively utilized in the cultivation of *P. ostreatus* mushroom. However, additional studies are needed to understand the bioaccumulation of different macro- and micronutrients in the *P. ostreatus* fruitbody grown on substrates wetted by SMWW. Furthermore, the possibility of the use of SMWW which could be found abundantly in numerous countries should be tested for the cultivation of other mushroom species.

Author contribution F. Atila: formal analysis, conceptualization, validation, investigation, writing—original draft preparation, and visualization. A. Kazankaya: resources, methodology, review, and editing.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Ethical approval is not required.

Competing interests The authors declare no competing interests.

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