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Ordinary least squared linear regression model for estimation of zinc in wild edible mushroom (*Suillus luteus* (L.) Roussel)

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Abstract

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Suillus luteus (L.) Roussel is a basidial fungus, and the type species of the genus *Suillus*. A common fungus native to Eurasia, from the British Isles to Korea, it has been introduced widely elsewhere, including North and South America, southern Africa, Australia and New Zealand.The aims of this work were to determine trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) contents in the wild edible mushroom *S. luteus* growing in the Batak Mountain, Bulgaria and to identify the relationship between Zn and the other elements using ordinary least squares multiple linear regression model. Quantitative determination of the concentration of the studied trace elements was carried out in mineralized samples by Perkin Elmer A Analyst 800 atomic absorption spectrometer with deuterium background corrector. All statistical computing, analysis and all charts were performed with the statistical software R program. The ordinary least squares linear regression model was obtained for Zn. Based on the obtained model, the following interpretations for Zn contents in the wild edible mushroom *S. luteus* growing in the Batak Mountain, Bulgaria could be made: if Fe and Cd increase by 1%, the effect of this increase would result in an increase in Zn by 15.245% on the average; if Mn, Cu, Co and Pb decreases by 1%, the effect of this decrease would result in an decrease in Zn by 3.582% on the average.

Keywords: atomic absorption; spectrometry; trace elements; linear regression model; ordinary; least squares; *Suillus luteus*

Introduction

Mushroom fruiting bodies, as well as their spores have the highest ability to accumulate micro- and macronutrients. The most important mechanism of accumulation of elements in mushrooms is based on binding by metallothionein – a low molecular-weight protein, which has an affinity especially formetals. The accumulation of elements in fruiting bodies of mushrooms is influenced by fungal structure, ontogenetic development of the fungus, biochemical composition, decomposition activity, environmental factors, for example metal concentration in air and soil and its pH, organic matter andthe bioavailability of metals (Garcia et al., 1998; Demirbas, 2001b, 2002; Ivanova et al., 2019). Increased concentration of these micronutrients in the soil, and then in the mushroom is associated with its place of location (Ouzuni et al., 2007; Demirbas, 2001a; Dospatliev et al., 2018).

Mushrooms growing in a natural environment have always been consumed by humans due to their unique taste and aroma (Turkekul et al., 2004). The medicinal use of mushrooms has a very long tradition (Wasser & Weis, 1999; Kalač, 2009). Fruiting bodies of edible mushrooms are a source ofmany important organic and inorganic substances, such asproteins, polysaccharides, phenolic compounds, terpenoids, indole compounds, vitamins and bioelements, which exhibit akey physiological role in the human body (Barros et al., 2008; Dospatliev & Ivanova, 2017; Muszynska et al., 2011; Elmastas et al., 2007). Some of the metabolites are used to treat such serious diseases ascardiovascular diseases, diabetes, atherosclerosis, cancer andexhibit antioxidant, antiviral and antibacterial activity (Zembron-Lacny et al., 2013; Zaidman et al., 2005; Muszynska et al., 2015; Sulkowska-Ziaja et al., 2005; Kalac, 2010; Dospatliev et al., 2019).

Zinc concentrations in fruiting bodies of edible mushrooms are considerably higher than those in plants (herbs,-fruits, vegetables, crops) (Dogan, 2006). Only sea foods (oyster) are abetter source of this element than mushrooms (WHO/FAO).

Zinc is also one of the antagonists of the glutamate system,which is involved in antidepressant activity. This elementcrosses the blood-brain barrier as well as the blood-cerebrospinal fluid barrier, thanks to histidine and divalent metal transporter 1(DMT1). In the brain, thanks to the appropriate transporters, zincis moved into the cytoplasm, and its concentration is regulated by metallothioneins (Opoka et al., 2008; Sowa-Kucma et al., 2013). Zinc is also important in theprevention of Alzheimer‘s disease, involved in immuneprocesses affecting the immune system and increases maintenance of normal levels of vitamin A in plasma (Brewer et al., 2010). The proper functioning of skin and mucous membranes and theprocess of heavy metal detoxification, e.g. cadmium and lead, are also zinc-dependent.

This element is involved in the activation of over 300 enzymes, among others, alcohol dehydrogenase involved in the metabolism of alcohol, carbonic anhydrase, which is involved inthe production of bicarbonate (HCO_3^-) ions, and histidinedeaminase, which catalyzes the deamination reaction (Andreini & Bertini, 2012). Zinc exhibits antioxidant properties, because it is present in superoxide dismutase responsible for the elimination of free radicals (Szynkowska et al., 2008; Powell, 2000). Studies have shown that zinc supplementation causes a reduction of T cell activation and a decrease in tumornecrosis factor (TNF-α) release. In the future, it may affect the treatment of autoimmune diseases (Shankar and Prasad, 1998). This element is also required to treat ulcers, because it promotes the healing of wounds (Opoka et al., 2010) and the normalization of serum Zn/Cu ratios may be useful in the treatment of depression (Mlyniec et al., 2014). The daily requirement for zinc in a healthy adult human is dependent on age and isabout 15 mg.

The aims of this work were to determine trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) contents in the wild edible mushroom *S. luteus* growing in the Batak Mountain, Bulgaria and to identify the relationship between Zn and the other elements using ordinary least squares multiple linear regression model. This type *S. luteus* was chosen because of its popularity among consumers and documented healing properties.

Materials and Methods

Mushroom samples

Mushroom samples were collected in 2018 from the Batak Mountain, Bulgaria by the authors themselves. The Batak Mountain is located in western Rhodopes. Its western border is defined by the Chepinska river, the southern border – by Dospatska river and Dospat dam, the eastern border – by Vacha river and the northern border – by the Thracian Plane (GPS41°46′02.6″N 24°08′48.4″E). The region is industry-free and is characterized with forests, land and low buildings.

Chemical analysis methods

Quantitative determination of the concentration of the studied trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) was carried out in the mineralized samples by Perkin Elmer A Analyst 800 atomic absorption spectrometer with deuterium background corrector.

Digestion procedures

Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and the maximum pressure in Teflon vessels – 40 bar) was used in this study mushroom samples (0.25 g) were digested with 6 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%) in microwave digestion system for 23 min and diluted to 25 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear.

Statistical analysis

All statistical computing, analysis and all charts were performed with the Statistical software R program version 3.5.1.

The Shapiro-Wilk test is a test for normal distribution exhibiting high power, leading to good results even with a small number of observations. In contrast to other comparison tests the Shapiro-Wilk test is only applicable to check for normality. The null-hypothesis (H_0): the population is normally distributed. The test statistic is (Shapiro & Wilk, 1965):

$$W = \frac{(\sum_{i=2}^{n} a_i y_i)^2}{\sum_{i=1}^{n} (x_i - \overline{y})^2},$$

where n – number of observations, y_i – values of the ordered sample, a_i – tabulated coefficient.

The Durbin-Watson test statistic tests the null hypothesis that the residuals from an ordinary least squares regression are not autocorrelated. The Durbin-Watson statistic ranges in value from 0 to 4. A value near 2 indicates non-autocorrelation; a value toward 0 indicates positive autocorrelation; a value toward 4 indicates negative autocorrelation. The test statistic is (Gujarati, 2003):

$$d = \frac{\sum_{t=2}^{n} (e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^2},$$

where e_t is the residual associated with the observation at time t and n is the number of observations.

Because of the dependence of any computed Durbin-Watson value on the associated data matrix, exact critical values of the Durbin-Watson statistic are not tabulated for all possible cases. Instead, Durbin and Watson established upper (DU) and lower bounds (DL) for the critical values. Typically, tabulated bounds are used to test the hypothesis of zero autocorrelation against the alternative of positive first-order autocorrelation, since positive autocorrelation is seen much more frequently in practice than negative autocorrelation

(https://www3.nd.edu/~wevans1/econ30331/Durbin_Watson_tables.pdf).

The F-test for linear regression tests whether any of the independent variables in a multiple linear regression model are significant. The formula for F-test statistic is:

$$F = \frac{\textit{explained variance}}{\textit{unexplained variance}},$$

F-test is greater as:

● distance between groups is greater or dispersion media groups around the general average is greater;

● groups are more homogeneous or error represented by scattering within the groups is less.

Thus, relatively high F's are strong arguments against H_0 (null hypothesis). P-value is the probability of obtaining a value of F which is at least as great as that observed by us if H_0 were true. Therefore the smaller the p-value – the chance, that H_0 to be fair is lower. For p-value < 0.05 to reject H_0 it is necessary the following condition:

$$F_{calculated} > F_{tabular}$$

Variance inflation factors (VIF) measure how much the variance of the estimated regression coefficients are inflated as compared to when the predictor variables are not linearly related. If VIF > 10, there is an indication for multicollinearity (https://www.statisticssolutions.com/wp-content/uploads/wp-post-to-pdf-enhanced-cache/1/assumptions-of-multiple-linear-regression.pdf).

Model specification

In econometrics, ordinary least squares (OLS) method is widely used to estimate the parameter of a linear regression model. OLS estimators minimize the sum of the squared errors (a difference between observed values and predicted values). The OLS estimator is consistent when the regresses are exogenous and optimal in the class of linear unbiased estimators when the errors are homoscedastic and serially uncorrelated. Under these conditions, the method of OLS provides minimum-variance mean-unbiased estimation when the errors have finite variances. Under the additional assumption that the errors are normally distributed, OLS is the maximum likelihood estimator.

In this article we was used OLS multiple linear regression model to identify the dependences of traceelements in wild edible mushroom *Suillus luteus*. The long-run functions are specified as follows:

Zn = *f* (Fe, Mn, Cu, Co, Cd, Pb)

From the point of view of multiple linear regressions, the constructed model must satisfy the following assumptions: linear relationship, multivariate normality, no or little multicollinearity, no autocorrelation and homoscedasticity (https://www.statisticssolutions.com/wp-content/uploads/wp-post-to-pdf-enhanced-cache/1/assumptions-of-multiple-linear-regression.pdf).

Results and Discussion

Concentrations of seven trace elements (Pb, Cd, Co, Cu, Mn, Zn and Fe) have been determined in this study (Table 1). The trace element contents of the species depend on the ability of the species to extract elements from the substrate, and on the selective uptake and deposition of elements in tissues. The results obtained, in the current study, indicated that Pb, Cd, Co, Cu, Mn, Zn and Fe content of the investigated mushroom samples were found to be comparable with those reported in the literature (Baros et al., 2008; Dospatliev & Ivanova; Muszynska et al., 2011; Elmastas et al., 2007; Zembron-Lacny et al., 2013; Zaidman et al., 2005; Muszynska et al., 2015; Sulkowska-Ziaja et al., 2005; Kalac, 2010; Dospatliev et al., 2019). The averages of Pb, Cd, Co, Cu, Mn, Zn and Fe present in the studied wild mushrooms were far below the limit sets

Table 1. Descriptive statistics of the results for Pb, Cd, Co, Cu, Mn, Zn and Fe concentrations (mg kg^{-1}, dry weight basis) in *S. luteus* mushroom (n = 15)

	Pb	Cd	Co	Cu	Mn	Zn	Fe
Mean	7.957	0.973	1.032	6.121	14.457	78.563	84.947
Std. Error	0.379	0.027	0.022	0.486	0.437	1.088	1.818
Median	7.910	0.950	1.028	6.751	14.084	77.901	86.844
Std. Dev.	1.468	0.106	0.083	1.881	1.692	4.215	7.040
Variance	2.155	0.011	0.007	3.537	2.863	17.769	49.566
Kurtosis	-1.148	-0.817	-1.227	-0.580	-1.213	-0.918	-0.341
Skewness	0.015	0.245	-0.389	-0.574	0.034	0.398	-0.778
Range	4.851	0.361	0.237	5.858	5.232	12.057	22.030
Minimum	5.545	0.788	0.889	3.032	11.624	73.156	71.963
Maximum	10.396	1.150	1.126	8.890	16.856	85.213	93.993
Sum	119.357	14.589	15.483	91.819	216.854	1178.452	1274.216
Count	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Conf. Level (95.0%)	0.813	0.059	0.046	1.041	0.937	2.334	3.899
Homogeneous coefficient (%)	18.450	10.905	8.069	30.723	11.705	5.366	8.288
Shapiro-Wilk Test	0.950	0.949	0.892	0.897	0.938	0.912	0.896
p-value	0.532	0.518	0.072	0.085	0.359	0.144	0.0813

by the WHO (1982). Descriptive statistics was used to make a preliminary analysis of the database (Table 1). It was concluded that data were fairly symmetrical because the skewness was between -0.5 and 0.5, except Fe and Cu which were negatively skewed (skewness between -1 and -0.5). Co and Fe can be considered homogeneous data. From the Shapiro-Wilk Test, the p-value > 0.05 suggested that the distribution of the data was not significantly different from normal distribution. In other words, normality could be assumed.

Ordinary least squares linear regression model for Zn

In an explicit and multiple linear regression form, the long-run function of Zn can be stated as:

$$Zn = a_0 + a_1 Fe + a_2 Mn + a_3 Cu + a_4 Co + a_5 Cd + a_6 Pb$$

For multiple linear regression modeling of the variables included in this study factor Zn we considered as a resultant factor and Fe, Mn, Cu, Co, Cd and Pb: as independent factors (Table 2). At the significance level, one out of seven regression coefficients, was statistically significant. The regression analysis of the model highlighted the fact that the relation between dependent and independent factors was rather strong (Table 2): the correlation coefficient was R = 0.887 and the determination coefficient (R-squared) were 0.786 or roughly 79% of the variance found in the response variable (Zn) can be explained by predictor variables. The constructed model satisfy the assumptions of multiple linear regression: the Durbin-Watson test indicated non-autocorrelation (test statistic value was near 2, then the null hypothesis was

Table 2. The components of the ordinary least squares multiple linear regression model for Zn

Coefficient	Estimate	Std. Error	t-Statistic	Pr (> |t|)
a_0	-16.015	38.183	-0.419	0.686
a_1	1.236	0.381	3.247	0.012*
a_2	-1.364	0.782	-1.745	0.119
a_3	-0.525	0.506	-1.037	0.330
a_4	-11.995	9.925	-1.209	0.261
a_5	29.253	18.227	1.605	0.147
a_6	-0.442	0.655	-0.675	0.519
Multiple R-squared	0.786	F-statistic		34.897 (p-value = 2.165e^{-05})
Adjusted R-squared	0.626	Durbin-Watson stat		1.976 (p-value = 0.072)
Residual standard error	2.581			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 not rejected); the calculated values of the Fisher's F-test of the econometric model indicated the relevance of the model ($F = 34.897$, p-value = $2.165e^{-05}$); tabular value given by test was 4.48 for a probability of 0.05 (36), which means that the resulting equation was: F calculated > F tabular, consequently the null hypothesis (H_0) was rejected and the variances included in the study differed significantly between them; the VIF values for the explanatory variables Fe, Mn, Cu, Co, Cd and Pb were: 2.218, 3.385, 1.987, 1.457, 5.393 and 2.055, respectively – the model is free from multicollinearity.

Thus, we have the following equation for the dependent variable Zn:

$$Zn = -16.015 + 1.236Fe - 1.364Mn - 0.525Cu - 11.995Co + 29.253Cd - 0.442Pb \quad (1)$$

According to (1), a direct influence, increasing the resultant factor Zn, was found out for the factors Fe and Cd. Classification ranges given by model coefficients are presented in Table 3. Calculations were performed with a probability of 90% and respectively 95%. Cu and Co variables ranges were relevant in both cases of the evaluation.

Table 3. Coefficient confidence intervals

Coefficient	Estimate	90%		95%	
		lower	upper	lower	upper
a_0	-16.015	-87.019	54.989	-104.067	72.037
a_1	1.236	0.528	1.943	0.358	2.113
a_2	-1.364	-2.818	0.089	-3.167	0.439
a_3	-0.525	-1.465	0.416	-1.691	0.642
a_4	-11.995	-30.451	6.462	-34.882	10.893
a_5	29.253	-4.640	63.146	-12.778	71.284
a_6	-0.442	-1.660	0.776	-1.953	1.069

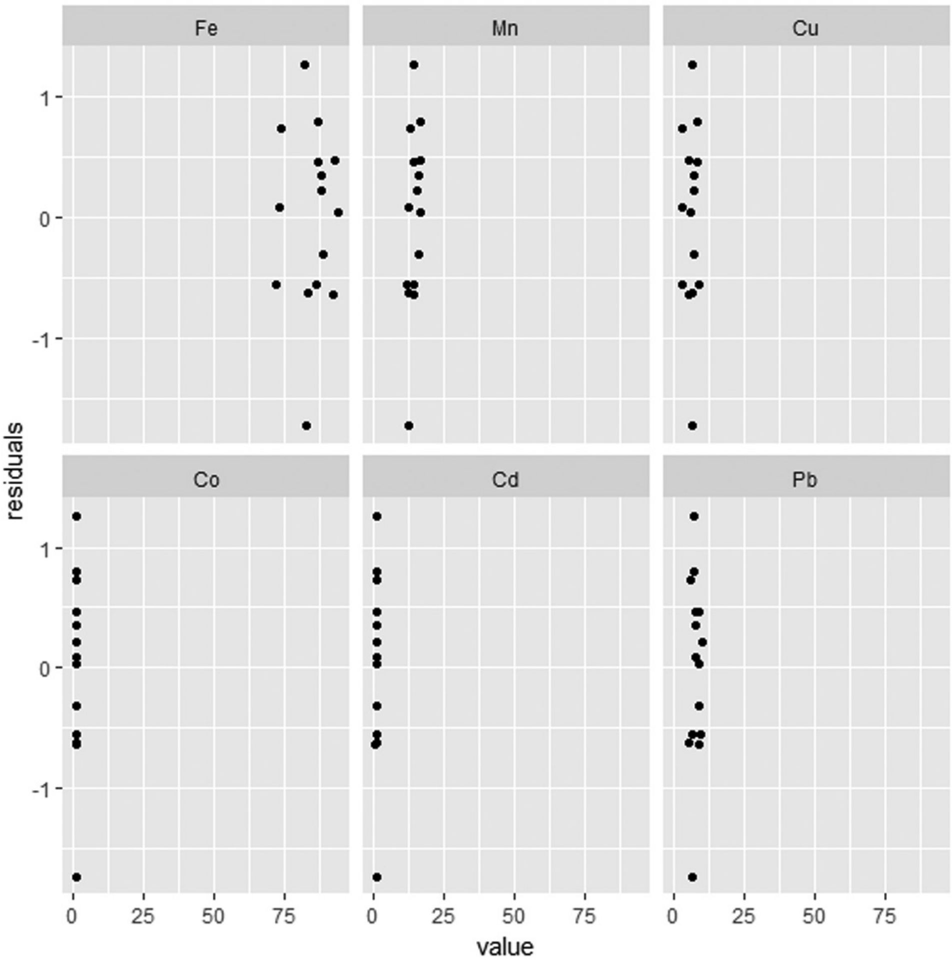

Fig. 1. Plots of the residuals versus each regressor

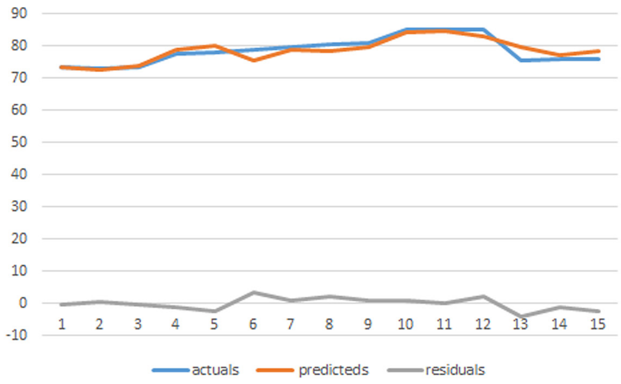

Fig. 2. Differences of actual data vs. predicted data from the model of Zn

Figure 1 depicts graphically the validity of regressors, i.e. variable plots and plots of residuals versus each regressor.

Figure 2 depicts graphically the differences in the calculation of actual data vs. predicted data from the model, which indicate the ability to perform some calculations for different values of the variable Zn of the factors included in the presented model.

Conclusions

Results of the studied area showed that the selected elements concentrations were below the safe limits of WHO/FAO set for edible mushrooms and for foodstuffs.

Based on the obtained ordinary least squares multiple linear regression model, the following interpretations for Zn contents in the wild edible mushroom *Suillus luteus* growing in the Batak Mountain, Bulgaria could be made:

If Fe and Cd increase by 1%, the effect of this increase would result in an increase in Zn by 15.245% on the average;

If Mn, Cu, Co and Pb decrease by 1%, the effect of this decrease would result in a decrease in Zn by 3.582%on the average.

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