


Article

# Quantification of Platinum in Edible Mushrooms Using Voltammetric Techniques

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**Abstract:** Edible mushrooms are a food source with interesting nutritional values. The chief objective of this research was to develop a consistent method for the quantitative ultra-trace analysis of Pt in mushrooms, which is complex because it cannot be readily quantified by common analytical procedures. This research is one of the first analytical methods to establish Pt amount in these vegetables. In this research, 28 different edible mushroom samples from Italy were investigated. Determination of Pt in mushrooms was completed using Differential Pulse Voltammetry (DPV). In this study, we applied the standard addition method because there are no certified reference mushrooms containing platinum group elements on the market. The platinum quantification limit was  $0.03 \mu\text{g kg}^{-1}$  d.w. In the analyzed samples, platinum amount was in the range of  $0.03\text{--}73 \mu\text{g kg}^{-1}$ . Our mushroom samples had a Pt content lower than the concentrations recommended by international establishments for other foodstuffs. In the future, the optimized method could be used for the analysis of plant and animal matrices intended for food supply.

**Keywords:** platinum; mushrooms; voltammetry; pollution; contamination; food



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## 1. Introduction

Mushrooms have a very efficient metabolism for accumulating elements from environmental matrices (atmosphere, soil, water). Therefore, these vegetables are widely employed as bioindicators or bioaccumulators to establish environment matrices quality [1–4]. Comestible mushrooms have previously been intensively investigated for their nutritive and botanical proprieties [5]. Considering their chemical compositions, several researchers have stated that mushrooms have salutary proprieties, and for this reason they are recommended for minimizing several disease risks (hypertension, hypercholesterolemia, and cancer) [6–8].

With the removal of tetra ethyl and tetra methyl lead compounds from automotive fuels, it is no longer possible to use lead as a chemical indicator of vehicular road traffic. Today, in several European states, lead free gasoline, diesel, and liquefied petrol gas and, more and more frequently, batteries are the common energy sources used in cars and industrial trucks. Consequently, attention is now being paid to new indicators involving other metals, although other compounds (CO, NO<sub>x</sub>, PAHs, etc.) persist in the environmental matrices and are significant concerns because of their acute and chronic toxicity. Their high environmental persistence could lead to bioaccumulation and biomagnification, with related consequences to the food chain.

In the last quarter of a century, in several states, vehicles have been furnished with catalytic converters to reduce the release of several gases (CO, NO, etc.) by transforming them to less hazardous substances [9]. The majority of converters consist of monoliths with large surface areas containing platinum group metals (PGM). These devices cause the emission of emerging pollutants (Pt, Rh, Pd, etc.) [9]. During their use, particulate containing emerging elements is emitted into the environment. Several researchers have quantified the average emission amounts of platinum produced by new cars as being

100  $\mu\text{g km}^{-1}$ , while, for older vehicles, the value reduced to approximately 6  $\mu\text{g km}^{-1}$  [10]. The platinum group metals, after being released into the air by vehicles, are transported by winds and end up accumulating in the vicinity of the emission zones, along roads, on surrounding agricultural areas, and on plants, so there is a possibility for them to enter the diet of animals and, consequently, the food chain.

Recently, researchers published a review containing the results of the amounts of hazardous heavy metals, not including PGMs, in edible mushrooms in relation to the period January 1970–June 2020 [11]. Five macro elements and 31 minor metals were measured in several samples produced in China and Poland [12]. The content of essential and toxic deleterious elements was found to be in a wide concentration range. During these investigations, in one sample, platinum concentration exceeded 7  $\text{mg kg}^{-1}$  dry matter. Consuming foods containing high concentrations of heavy metals can damage the kidneys and heart, and it can also compromise the digestive system, immune system, bone, and nervous apparatus [13,14]. In one study, researchers [15] established that the high concentrations of heavy metal in mushrooms consumed for food constitute a serious problem in the Yunnan Province (China). In other research [16], heavy metal amounts in the mushroom samples of Turkey's Black Sea area were quantified. Some research on PGMs relating to environmental matrices and food [17–22] has been carried out since catalytic converters came into use, but there is no information regarding platinum in mushrooms intended for human consumption. Ours is the first method to measure platinum in edible mushroom samples. A total of 28 different samples produced in Italy were analyzed. The principal intent of our study was to develop a consistent analytical method to quantify ultra-trace platinum in mushrooms, which is complex because it cannot be readily analyzed by common analytical techniques. A direct analysis of Pt at the ultra-trace level by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is problematic due to the difficulty of separating the analyte from the sample, along with interferences that cannot be removed [23–25]. Voltammetry is often employed for several analytes (organic and inorganic), particularly for the analysis of traces of one or more heavy metals [26], but in the literature there are very few documents regarding the quantification of ultra-traces of platinum [27–29] in comestible mushrooms. We have used Differential Pulse Voltammetry (DPV) to carry out this study, and mushrooms have been utilized because they are common, well known, and consumed all over the world.

Another scope of the study is to compare the concentrations of platinum in mushrooms sampled in Italy, where catalytic converters have been used for decades, with those determined in other geographical areas, or in Italy at different times.

## 2. Materials and Methods

Experimental details have been described in previous research [17–22].

### 2.1. Quality Assurance

Every fifth sample, or standard procedural blanks, were analyzed. Considering that certification for platinum mushroom samples does not exist, the analytical method was tested for accuracy by analyzing improved samples created by the authors; a known volume of platinum standard solution was added to a blank sample, and the enriched sample was analyzed. The average recovery of enriched samples was in the range of 90–98%. The relative standard deviations on the platinum recovery amount were approximately 10%. The detection (LOD) and quantification (LOQ) limits of the optimized method were established as a three- and ten-fold standard deviation of concentrations relative to 10 procedural blanks, which were similar to the mushroom samples.

### 2.2. Samples

The 28 investigated edible mushroom samples all had Italian origins; some were acquired from local farmers between 2018 and 2019, while others were acquired from

the market. Specifically, 10 samples were mushrooms from diverse Sicilian areas and 18 samples were from northern Italy.

Three to four mushrooms were cleaned, first mechanically with a brush to remove traces of earth, and then with tap water, before being rinsed with bi-distilled water. Each sample was reduced into several small pieces using a metal knife. A blender with a glass beaker was used to obtain representative samples.

### 2.3. Mineralization Procedure

In a muffle at 600 °C (5 h) three to four grams of representative sample were incinerated and then desiccated in an oven for 24 h at 105 °C. To the ashes were added 5 mL of Ultrapure concentrated HCl. The obtained solution was filtered through 0.45 µm filters. The clear, colorless solution was transferred into a volumetric flask and brought to volume with Milli-Q water.

### 2.4. Analytical Method

By loss at 105 °C, the water content was established and used to correlate the Pt content to the dry sample. Differential Pulse Voltammetry (DPV) [17–22]. The blanks were obtained as described in our previous papers [17–22].

The high sensitivity of the method is provided by the catalytic action of platinum on the water reduction. In this study, we applied the standard addition method because there are no certified reference mushrooms containing platinum group elements on the market [30,31]. The used analytical process was validated by the standard addition method because the composition of mushrooms or of similar vegetables can also affect the analytical signal (current) [17–22]. This difficulty is chemically known as the matrix effect. In these cases, it is difficult to relate, in our case, the current between that obtained from unknown samples and those obtained from voltammetric analysis of solutions at known concentrations. By the intercept of the line on the abscise axes (negative), the volumes of the solutions in the voltametric cell, and the quantity of the samples, it is possible calculate the concentrations of analita in the different unknown edible mushroom samples. Calibration graphs were valued by the least-square linear regression method. In Figure 1, we show the calibration curve for platinum. The trend of the calibration curve indicates that the method for the platinum is a very good linear; in fact, the  $R^2$  values obtained during all the measures ranged from 0.991 to 0.999.

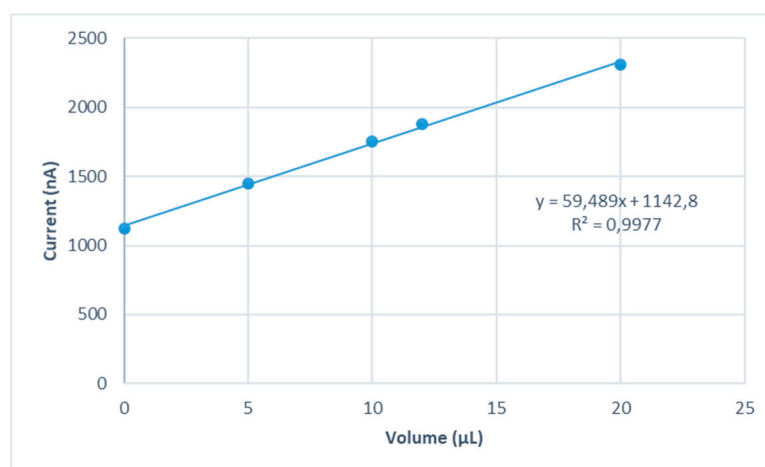


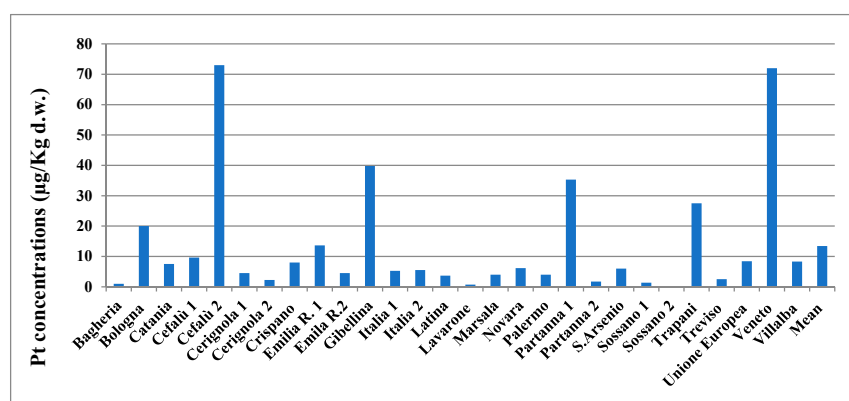
Figure 1. An example of calibration curve using the addition standard method.

## 3. Results and Discussion

The concentrations of platinum on the dry sample, obtained for all the samples, are shown in Table 1 and Figure 2.

**Table 1.** Platinum concentrations ( $\mu\text{g kg}^{-1}$ ), Enrichment factors (EF), and Geoaccumulation index ( $I_{\text{geo}}$ ), measured on mushroom samples.

Sample Origin	Pt ( $\mu\text{g kg}^{-1}$ )	EF	$I_{\text{geo}}$
Bagheria	0.96	0.096	−4.0
Bologna	20	2.0	0.41
Catania	7.5	0.75	−1.0
Cefalù 1	9.6	0.96	−0.64
Cefalù 2	73	7.3	2.3
Cerignola 1	4.5	0.45	−1.7
Cerignola 2	2.3	0.23	−2.7
Crispano	8.0	0.80	−0.91
Emilia R. 1	14	1.4	−0.14
Emilia R. 2	4.5	0.45	−1.7
Gibellina	40	4.0	1.4
Italia 1	5.2	0.53	−1.5
Italia 2	5.5	0.55	−1.4
Latina	3.7	0.37	−2.0
Lavarone	0.70	0.070	−4.4
Marsala	4.0	0.40	−1.9
Novara	6.1	0.61	−1.3
Palermo	4.0	0.40	−1.9
Partanna 1	35	3.5	1.2
Partanna 2	1.7	0.17	−3.1
S. Arsenio	6.0	0.60	−1.3
Sossano 1	1.4	0.14	−3.5
Sossano 2	0.030	0.0030	−9.0
Trapani	27	2.8	0.87
Treviso	2.5	0.25	−2.6
Europa	8.4	0.84	−0.83
Veneto	72	7.2	2.3
Villalba	8.3	0.83	−0.85

**Figure 2.** Platinum concentrations ( $\mu\text{g kg}^{-1}$ ) d.w measured on mushroom samples.

The platinum concentrations shown in this paper, calculated as the mean of three analyses, are corrected for blanks. The concentration of Pt ranged from 0.030 to  $72 \mu\text{g kg}^{-1}$  (d.w.). The great range of analita amounts (relative standard deviations, 145%) demonstrate heterogeneous levels of distribution of platinum in the mushroom samples taken into consideration in this research, and is principally attributable to the different geographical distributions of the analita in the environment and, to a lesser extent, to the uncertainty of the analysis, because the standard deviation, analyzing the same mushroom sample, is approximately 10%. The excessive variability of the results does not allow, by means of statistical methods, the establishment of the geographical origin of mushrooms. The lowest Pt concentrations were measured in a sample grown in a small town of about

4000 inhabitants of the Italian province of Vicenza. Considering all the samples, the mean Pt concentration was  $13 \mu\text{g kg}^{-1}$ . The higher concentration of Pt was detected in a sample of Cefalù. In general, the higher concentrations were determined in the samples collected directly by the authors (Cefalù 1, Cefalù 2, Gibellina, Palermo, Via Libertà) and grown in uncontrolled conditions, while the lower concentrations were found in cultivated mushrooms that were designated as edible. We can hypothesize that this can be determined by the fact that the cultivation of mushrooms for food use takes place in greenhouses far from anthropogenic sources of contaminants. It is impossible to compare our results with others from the literature because no data exist on the platinum content in mushrooms. As stated in previous studies, at different concentrations, platinum is present in all the environmental and food matrices. As a comparison, some data relating to other vegetable matrices are reported here. Considering 38 potato samples [20], platinum concentrations are in the range  $0.007\text{--}109 \mu\text{g kg}^{-1}$  (Sicilian sample). The Pt concentrations of 42 different alcoholic beverages (white and red wines, vodka, and brandy) produced in Italy, Malta, and Gozo in 2017 have been investigated previously [17]. Platinum concentrations ranged from 3 to  $470 \mu\text{g L}^{-1}$ . A study on platinum amounts in the Australian diet was carried out several years ago on market-basket samples [29]. In this case, Pt concentrations ranged between  $8.1 \mu\text{g kg}^{-1}$  for liver and  $0.13 \mu\text{g kg}^{-1}$  for full-cream milk. Particularly, Pt contents were highest in eggs and offal, with a mean concentration of  $5.8 \mu\text{g kg}^{-1}$ , followed by meat ( $3.2 \mu\text{g kg}^{-1}$ ), cereals ( $3.2 \mu\text{g kg}^{-1}$ ), fish ( $1.8 \mu\text{g kg}^{-1}$ ), fruit and vegetables ( $0.82 \mu\text{g kg}^{-1}$ ), and dairy products ( $0.27 \mu\text{g kg}^{-1}$ ) [29]. In Oleander leaves, Pt concentrations were in the range of  $0.33\text{--}25 \mu\text{g kg}^{-1}$  [18] and were of the same order of magnitude (Pt =  $6\text{--}30 \mu\text{g kg}^{-1}$ ) than that detected by other researchers on vegetables sampled in Germany [30] and those (Pt =  $1\text{--}102 \mu\text{g kg}^{-1}$ ) that Dongarrà [31] measured in Pinus needles sampled at Palermo (Sicily). In the soft tissue of mussels, mean Pt concentrations resulted lower, at  $20 \mu\text{g kg}^{-1}$  [32].

Moreover, we calculated the Enrichment Factors (EF) as:

$$\text{EF} = \frac{\text{Metal concentration in the mushroom sample}}{\text{Metal concentration in the earth crust. Pt in earth crust} = 10 \mu\text{g kg}^{-1} \text{ [33].}}$$

We used the enrichment factor (EF) [34,35] to differentiate between the platinum originating from human activities and those from natural processes and to value the degree of anthropogenic influence. EF values near to 1 indicate a natural origin of pollutants, while values higher than 10 are considered to be related to human activities. Based on the enrichment factors, five contamination categories are acknowledged (Table 2).

**Table 2.** Contamination categories based on EF values.

EF	
<2	Deficiency to minimal enrichment
2 ÷ 5	Moderate enrichment
5 ÷ 20	Significant enrichment
20 ÷ 40	Very high enrichment
>40	Extremely high enrichment

Relating all the analyzed samples, Geoaccumulation index ( $I_{\text{geo}}$ ) values were calculated. Different classes of  $I_{\text{geo}}$  are given in the literature [34,35] (Table 3).

In our case, the enrichment factors ranged from 0.003 to 7.3. The EFs of Pt calculated for most of the samples are lower than 2, indicating either deficiency or minimal enrichment of the analyzed samples. Considering the EF values obtained, we can conclude that the Gibellina, Partanna 1, and Trapani samples can be considered as only moderately enriched, while the Cefalù 2 and Veneto samples are significantly enriched. In these last samples, the platinum certainly comes from anthropogenic contamination.

**Table 3.** Contamination categories based on  $I_{geo}$  values.

Class	Index	Significance
0	<0	Practically uncontaminated
1	$0 \div 1$	Uncontaminated to moderately contaminated
2	$1 \div 2$	Moderately contaminated
3	$2 \div 3$	Moderately contaminated to heavily contaminated
4	$3 \div 4$	Heavily contaminated
5	$4 \div 5$	Heavily contaminated to extremely contaminated
6	5	Extremel contaminated

$I_{geo}$  ranged from  $-9.0$  to  $2.3$ , with a mean of  $-1.4$ . Results show that approximately 79% of the analyzed mushroom samples could be classified as practically uncontaminated, two sample from uncontaminated to moderately contaminated, two samples moderately contaminated, and two from moderately to heavily contaminated. No sample resulted as heavily and extremely contaminated.

To our knowledge, no lowest-observed-adverse-effect level (LOAEL) data are available in the literature. Given that no data are reported regarding the platinum group element daily intake for people, it is very important and of great interest to obtain information regarding daily intake of Pt for edible mushroom consumers as reported in this study. To value the consequential health risks of the intake of platinum by eating mushrooms, our results are compared with the available toxicological values [36,37]. Two international organizations (Food and Nutrition Board, the Institute of Medicine), specify a tolerable higher intake amount of  $0.3 \mu\text{g}$  per day per kg of body weight in an adult, corresponding to about  $21 \mu\text{g}$  per day for a person of  $70 \text{ kg}$  [24]. The European Medicines Agency suggests  $100 \mu\text{g d}^{-1}$  as the permitted daily amount (PDE) for Pt residues in the drug daily intake (DIM) of platinum, which was calculated using the following Equation:

$$\text{DIM} = C_{\text{Pt}} \times D_{\text{food intake}}$$

$C_{\text{Pt}}$  and  $D_{\text{food}}$  intake are the metal concentrations and daily intake of food, respectively. We considered an edible mushroom consumption of  $100 \text{ g/person/day}$  corresponding to about  $20 \text{ g/person/day}$  dry food. Assuming the consumption of food previously considered, this supplies from  $0.0006$  to  $1.5 \mu\text{g}$  of platinum per person. For example, every day, an Australian adult consumes  $1.4 \mu\text{g}$  of platinum.

#### 4. Conclusions

The voltammetric analytical method, which we have optimized and used for the quantification of platinum in mushroom samples intended for human consumption, is very sensitive, but at the same time it shows excellent reproducibility; specifically, the quantification limit is  $0.03 \mu\text{g/kg d.w.}$  The concentrations of platinum in mushroom samples produced in the Italian areas shown in this article are of great environmental and public interest. Analyzed mushroom samples contain concentrations of Pt lower than those indicated by international authorities for other type of food. In our case, the calculated intake of Pt was lower than the reported values. It is important not to ignore the fact that many people in many countries consume daily amounts of mushrooms much greater than those we have assumed.

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