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Determination of anions in some wild grown edible different mushrooms species

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Abstract
Seven different species of wild grown edible mushrooms *Agaricus bitorquis, Auricularia auricula-judae, Cantharellus cibarius, Flammulina velutipes, Lactarius delicious, Pleurotus eryngii* and *Verpa bohemica* growing in the East Black Sea region were analyzed used ion chromatography with conductivity detector for their anions (floride, chloride, bromide, nitrite, nitrate, sulfate, chlorate, phosphate, chromate and oxalate) levels. In the mushrooms, the highest anion concentrations were measured as 0.52 mg/g F^-, 4.97 mg/g CI^-, 2.51 NO_2^- mg/g and 0.44 mg/g PO_4^{3-} in *Agaricus bitorquis*, 7.90 mg/g CrO_4^{2-}, 20.10 mg/g SO_4^{2-} and 0.042 mg/g $C_2O_4^{2-}$ in *Cantharellus cibarius* on a dry-weight-basis. Br^-, ClO_3^- and NO_3^- content were not determined in any mushrooms species. Retention times of the inorganic anions in standard solutions eluted between 3.34 and 18.29 min as shown in chromatograms. In all cases the retention order $F^- < CrO_4^{2-} < Cl^- < NO_2^- < Br^- < ClO_3^- < NO_3^- < PO_4^{3-} < SO_4^{2-} < C_2O_4^{2-}$ typical was observed.

Keywords: Ions, wild edible mushroom, Ion chromatography, Turkey

Introduction
The edible mushrooms consumed in Turkey are *Agaricus bitorquis, Auricularia auricula-judae, Cantharellus cibarius, Flammulina velutipes, Lactarius delicious, Pleurotus ostreatus* and *Verpa bohemica* are. *Pleurotus ostreatus* and *Agaricus bisporus*, oyster mushroom, and button mushroom are well known all over the world and therefore have been the subject of some investigations [1]. *Pleurotus ostreatus*, the king oyster mushroom is the one becoming increasingly popular [1, 2]. *Flammulina velutipes* is cultivated and found to be notable with abnormal features of small caps and long stripes [2]. Among these studies, heavy metal content of mushrooms has been the subject attracting considerable interest [4–7]. Commonly cultivated and wild-grown edible mushrooms are investigated for heavy metal contents [8–14]. However there is very little information about inorganic ion contents. Inorganic ions come from rocks, soil, plants, and animals in the environment. They can exist in different forms, such as in solution in water, insoluble salts or minerals in rock, sand, and soil, as well as in organic or inorganic molecules, and in particles in the air [15].
Human cancers are caused by environmental factors like food, water and air at 80% [16]. Especially high dietary intakes of nitrate and nitrite ions have been implicated in the etiology of human gastric cancer based on epidemiological and clinical investigations [17-19].
Mushrooms are traditionally food and medicines in many countries. Fruit bodies of mushrooms are important due to their texture and flavor. Therefore, in addition to mushrooms' nutritional value, it is necessary to examine their ion contents. As mushrooms contain highly toxic substances, considerable effort should be spend to determine the possible danger to human health from the ingestion of mushrooms.
Living cells produce nitrate and nitrite ions naturally and they are found in food and water. They are responsible for some important chemical reactions in the body. Besides, they react with other compounds in foods or in the body to form cancer-causing substances. The nitrite and nitrate ions detection in mushrooms is of vital importance because of their toxicity to infants and cause of methaemoglobinemia [20]. N-nitrosoamines highly canceregenic substance is formed with the reaction of nitrite and secondary amines or amides. The nitrite is admitted at the concentration value of 0.1 μg ml^{-1} [21] according to the European Union regulations.

Considering the effect of nitrite and nitrate ions, their accurate and precise determination should be kept significantly important.

Fluoride is a wide-spread pollutant and has severe effects on health. In humans, fluoride ions are firstly absorbed and ingested in the stomach and intestine into the general circulation according to their relative aqueous solubility of the form consumed. The acceptable limits of fluoride content of foods should be in the range of 0.2-0.3 ppm [22] and people are estimated to take in the limits from foodborne fluoride [22, 23].

Chloride is very easily obtained from some foods together with potassium and other chemicals and substances like salt subsidies. Its deficiency is fatally important due to causing alkalosis [24].

Bromide is not of toxicological concern in nutrition as there are limited findings suggesting that bromide is nutritionally beneficial. Insomnia exhibited by some hemodialysis patients was associated with bromide deficiency [25]. Inorganic bromide was evaluated by FAO and WHO, and they recommended an acceptable daily intake (ADI) of 0–1 mg/kg body weight for humans. They based on a minimum pharmacologically effective dosage in humans at 900 mg of potassium bromide (600 mg of bromide ion) [26].

Phosphate in food was found harmful to the health of persons with normal renal function [27, 29]. The association between high phosphate concentrations and higher mortality is not attributed to persons with renal disease. The persons with cardiovascular disease with normal renal function were found to be under elevated mortality risks [27, 29].

A number of studies were conducted to determine the toxicity of sulfate in humans [29]. According to the literature survey and experimental findings, a sulfate dose that would induce dangerous health-based effects in adults was not possible to set as a standard in food and water [30, 31]. Children and the elderly are most probably sensitive to those effects of exposure at the same concentrations of sulfate [32, 33].

Chlorate ion works reversibly to inhibit the absorption of iodide in the thyroid. In case of overdose, sensitive groups of people such as children, pregnant women, and people with thyroid dysfunction or iodine deficiency may face with health hazards. Also chlorate ions are able to damage red blood cells. No acute reference doses for chlorate have been defined up to now. The Federal Institute for Risk Assessment (BfR) proposed a recommendation to apply for the acceptable daily intake (ADI) as 0.01 mg/kg body-weight according to the World Health Organization calculating both chronic and acute risks [34].

Turkey, an important exporter of wild grown edible mushrooms has a very large edible mushroom potential available to validate. Mushrooms are collected to make a substantial contribution to food intake primarily. For this reason, it is necessary to know the levels of inorganic ions in edible mushrooms. Here is the statement of common inorganic ions in wild grown edible mushrooms in Tokat-Turkey.

Experimental

Materials

Standard anion mixtures were prepared from 1000 mg L^{-1} inorganic anions standards at lower analytic concentrations. The working standard anions were prepared daily in the range of $1x10^{-1}$ – $1x10^{-5}$ mg/mL. Deionised water was obtained using a Millipore (18.2 MΩ cm^{-1} resistivity) Milli-Q water purification system. All reagents used in the preparation of eluents were at least p.a. purity grade.

Mushroom species

Samples of 7 mushroom taxa were collected during field trips in the Tokat region of Turkey between 2010 and 2014. The samples were dried at 105 °C for 24 h and homogenized using an Agate homogenizer. They were stored in pre-cleaned polyethylene bottles till analysis. Samples comprised all the edible parts of the mushrooms. Then, the samples were stored in closed containers at room temperature. 1 g of each mushroom samples were used in separate beakers. 10 ml deionized water was added and heated carefully on a hot plate. The samples were homogenized with ultrasonic bath for about 15 – 20 minute. In this stage of sample preparation, the existence of protein matter in the extract makes it difficult to obtain a clear solution. To eliminate protein matter, mushroom sample extracts were boiled to denature the protein [35]. Subsequently a separation of particulate matter, filtration with a previous centrifugation step was performed by 0.45 µm syringe filters. After all samples were diluted volumes of 10 ml with distilled water. Samples were stored in clean polyethylene bottles until analysis. There were three blank samples treated in the same way.

Apparatus

Dionex ICS-1100 Basic Integrated IC System was used for the analysis of common anions (fluoride, chloride, bromide, nitrite, nitrate, phosphate, chlorate and sulfate) in wild-grown edible mushrooms. The Dionex ICS-1000 system is designed to perform isocratic ion chromatography (IC) separations using conductivity detection. Dionex Ion Pac AS 9-HC analytical and Ion Pac AG 9-HC guard columns, and an anion micro membrane suppressor system (AMMS III) were used for the separation. Injection volume was 25 µL with flow rate set at 1 mL/min as the mobile phase was consisted of 20 mM sodium carbonate. All measurements were carried out at room temperature (25±2 °C).

Results and Discussion

Mushroom samples were analyzed after appropriate clean-up by IC with conductivity detection. The results of ion content determinations obtained were tabulated in Table 1. In aquatic systems, environmental factors such as temperature, salinity, pH, and the presence of organic matter influenced uptake of ions [36]. Mushrooms contained wide ranges of ion concentrations of 0.22±0.01-0.52±0.03 for F^-, 0.84±0.05-4.97±0.83 for Cl^-, 2.51±0.48 for NO_2^-, 0.026±0.01-0.44±0.03 for PO_4^{3-}, 3.13±0.52-7.90±0.17 for CrO_4^{2-}, 1.05±0.36-20.10±3.41 for SO_4^{2-}, and 0.013±0.002-0.042±0.01 for $C_2O_4^{2-}$ in mg/g (Table 1). Br^-, NO_3^- and ClO_3^- content could not be determined in all of the mushrooms species as the NO_2^- in only *Agaricus bitorquis* was found as 2.51±0.48 mg/g.

Ion chromatographic conditions

Ion chromatography is applicable for the determination of many of anions in some wild grown edible mushrooms species. Calibration curves were obtained in $1x10^{-1}$ – $1x10^{-5}$ mg/mL concentration range for F, Cl^-, Br^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, ClO_3^-, CrO_4^{2-} and $C_2O_4^{2-}$ ions.

Experiments ran at five concentration levels, using at least two replicate injections for each anion concentration level. For the separation of inorganic anions, anion-exchange chromatography with a conductivity detector was used after suppressor column. The mobile phase contained 20 mmol/L solution of Na_2CO_3 for the separation of inorganic anions. The injection volume, 25 µL and the flow rate, 1.0 mL/min were applied. Total run times were approximately 20 min.

Under the optimum condition described above, a mixed standard solution of inorganic anions was measured. The chromatogram of the standard solution of inorganic anions is shown in Fig. 1. The inorganic anions in mushroom samples were separated without interference peaks. Retention times of the inorganic anions in standard solutions were between 3.34 and 18.29 minutes as shown in the chromatograms. In all cases the typical retention order of F^-< CrO_4^{2-}< Cl^-< NO_2^-< Br^-< ClO_3^-< NO_3^-< PO_4^{3-}< SO_4^{2-}< $C_2O_4^{2-}$ was observed (Fig. 1).

Determination of ions in mushrooms

The current method was applied to 7 wild grown edible mushroom samples collected in Tokat region of Turkey between 2010 and 2014. For those samples with analyte concentrations beyond the highest point of the calibration curves, sample solutions were prepared at appropriate concentrations by dilution.

Extracted solutions obtained from the wild grown edible mushrooms are presented in Fig. 2-7. The results were based on a single sampling of the mushrooms; however, the method is robust and reliable and is applicable to real samples according to this short survey.

In this study *Agaricus bitorquis was* found with the highest concentrations of floride (0.52±0.03 mg/g) as the lowest floride content was 0.22±0.01 mg/g in *Verpa bohemica* (Table 1). In the wild mushroom samples, chloride concentrations ranged from 0.84±0.05 (*Verpa bohemica*) to 4.97±0.83 (*Agaricus bitorquis*) mg/g. The concentration of phosphate which is present in all mushrooms were generally low in any of mushroom samples, ranging from 0.026±0.01 to 0.44±0.03 mg/g, being lowest in *Auricularia auricular-judae* and highest in *Verpa bohemica* and *Agaricus bitorquis.* According to the analysis results chromate were found to be 3.13±0.52 mg/g for *Pleurotus eryngii*, and 7.90±0.17 mg/g for *Cantharellus cibarius* (Table 1). The sulfate content was the highest among the determined anions in all the mushrooms, which varied between 1.05±0.36 *Lactarius delicious* and 20.10±3.41 mg/g *Cantharellus cibarius*. The level of oxalate obtained in this study was low as compared to the other ions in the analyzed mushrooms. The oxalate concentrations were found to be 0.042±0.01 mg/g in *Cantharellus cibarius*, 0.013±0.005 mg/g *in Lactarius delicious*, and 0.013±0.002 in *Agaricus bitorquis* mg/g.

Calibration curve and statistical data

Table 2 shows the parameters for the calibration curves of the anions. The retention times of inorganic ions are also listed in the table. The accuracy of the calibration curves were evaluated by the correlation coefficient. It can be seen that the correlation coefficients (r^2) of inorganic ions were determined as 0.999. The calibration curves were linear in the range 0.01–100 mg/L for inorganic anions according to the regression equations (Table 2). By using the signal to noise ratio which is equal to 3 as a limiting requirement, experimental determination limits (LOD) and relative standard deviation (RSD) of the inorganic anions were tabulated in (Table 2).

Table 1: ions concentrations in mushroom samples analyzed. (mg/g, dry weight)(mean±SD),(n: 5)

Musroom Species	F^-	CI^-	NO_2^-	Br^-	PO_4^{3-}	ClO_3^-	NO_3^-	SO_4^{2-}	$C_2O_4^{2-}$	CrO_4^{2-}
Pleurotus eryngii	0.38±0.04	2.04±0.18	-	-	0.28±0.04	-	-	8.56±1.22	0.027±0,01	3.13±0.52
Verpa bohemica	0.22±0.01	0.84±0.05	-	-	0.44±0.03	-	-	2.32±0.70	-	-
Cantharellus cibarius	0.49±0.05	2.92±0.26	-	-	0.28±0.08	-	-	20.10±3.41	0.042±0.01	7.90±0.17
Lactarius delicious	0.35±0.03	0.98±0.03	-	-	0.12±0.02	-	-	1.05±0.36	0.013±0.005	-
Flammulina velutipes	0.28±0.04	4.02±0.95	-	-	0.14±0.02	-	-	1.21±0.42	0.033±0.01	-
Agaricus bitorquis	0.52±0.03	4.97±0.83	2.51±0.48	-	0.44±0.07	-	-	8.73±1.57	0.013±0.002	-
Auricularia auricular-judae	-	1.12±0.07	-	-	0.026±0.01	-	-	1.47±0.11	0.041±0.02	-

Table 2: Analytical parameters for inorganic ions analysis by ion chromatography

Anions	Retention times (t_R), (minutes)	Retention Time Precision, % RSD	Peak Height Precision, % RSD	Linearity range (mg/L)	Detection limits (LOD) (x10^{-6})	Linearity	*r*2
F^-	3.34	2.08	0.21	0.01-100	8.2	y = -36,55x + 2942,9	0,9868
CrO_4^{2-}	3.75	1.48	0.14	0.01-100	5.6	y = -57,70x + 2983,2	0,9071
CI^-	5.38	2.14	0.16	0.01-100	4.2	y = -65,63x + 3000,7	0,9228
NO_2^-	6.74	1.29	0.12	0.01-100	7.5	y = -41,26x + 2845,6	0,9808
Br^-	8.49	1.71	0.24	0.01-100	9.7	y = -26,48x + 2803,1	0,9936
ClO_3^-	9.15	1.53	0.11	0.01-100	6.3	y = -23,63x + 2805,9	0,9678
NO_3^-	9.79	2.52	0.17	0.01-100	3.8	y = -30,39x + 2797,1	0,9190
PO_4^{3-}	12.38	2.77	0.26	0.01-100	5.9	y = -12,57x + 2722,7	0,9473
SO_4^{2-}	13.94	2.61	0.23	0.01-100	2.7	y = -13,80x + 2699,9	0,9141
$C_2O_4^{2-}$	18.29	2.86	0.19	0.01-100	7.4	y = -48,63x + 2910,9	0,9216

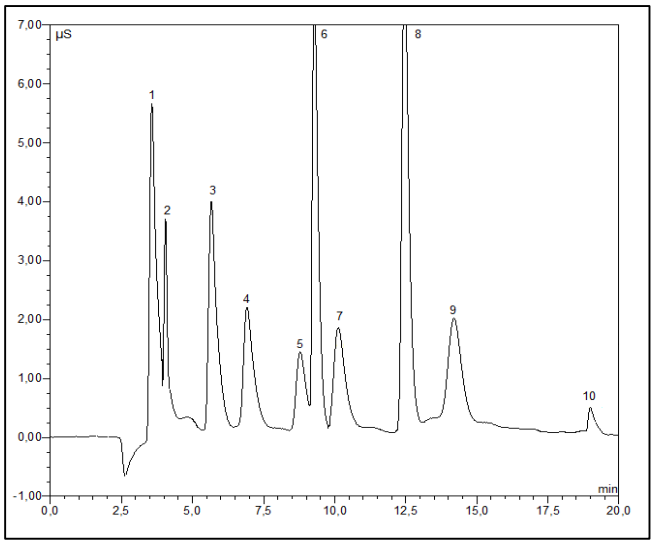

Fig 1: Suppressed ion chromatographic separation and detection of each ion. Eluent: 20 mmol/L solution of Na_2CO_3; Flow rate: 1 mL/min; injection volume: 25 µL; solutes: 1- fluoride, 2- Chromate, 3- chloride, 4- nitrite, 5- bromide, 6- chlorate, 7- nitrate, 8- phosphate, 9- sulfate, 10- oxalate.

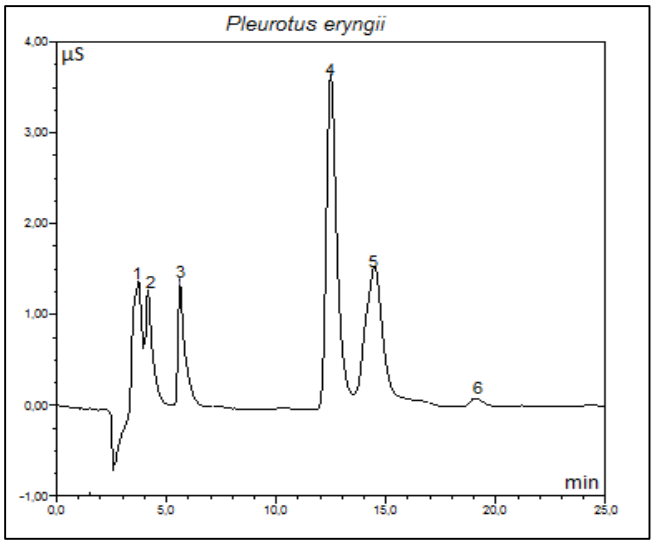

Fig 2: Chromatograms obtained from wild grown edible *Pleurotus eryngii;* 1- fluoride, 2- chromate, 3- chloride, 4- phosphate, 5- sulfate, 6- oxalate.

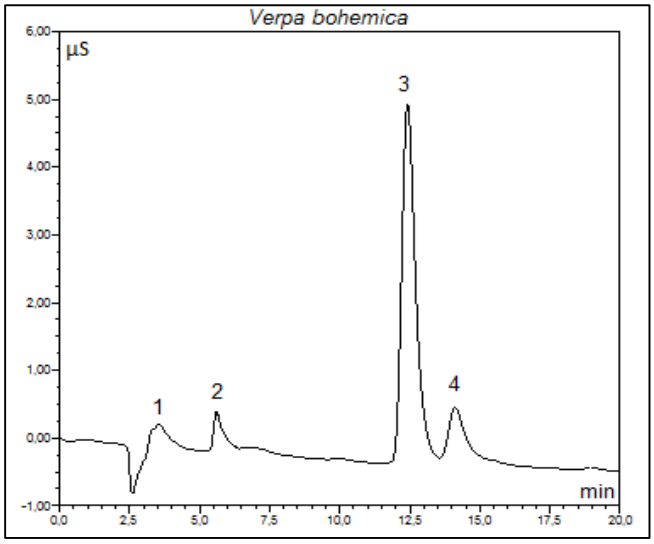

Fig 3: Chromatograms obtained from wild grown edible *Verpa bohemica;* 1- fluoride, 2- chloride, 3- phosphate, 4- sulfate.

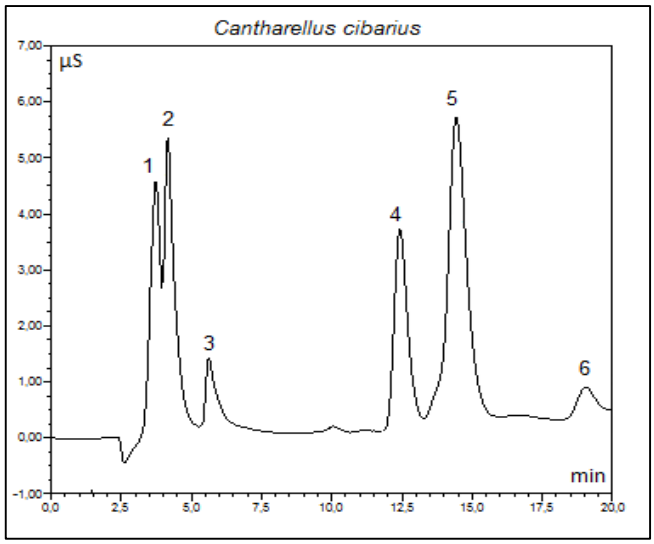

Fig 4: Chromatograms obtained from wild grown edible *Cantharellus cibarius;* 1- fluoride, 2- chromate, 3- chloride, 4- phosphate, 5- sulfate, 6- oxalate**.**

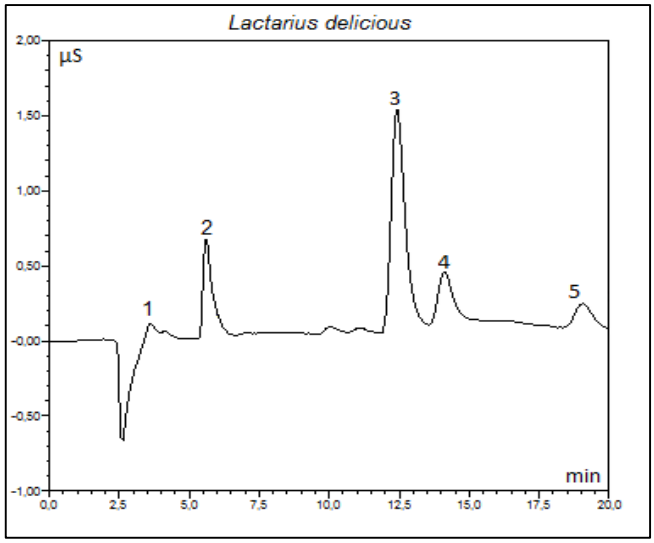

Fig 5: Chromatograms obtained from wild grown edible *Lactarius delicious;* 1- fluoride, 2- chloride, 3- phosphate, 4- sulfate, 5- oxalate.

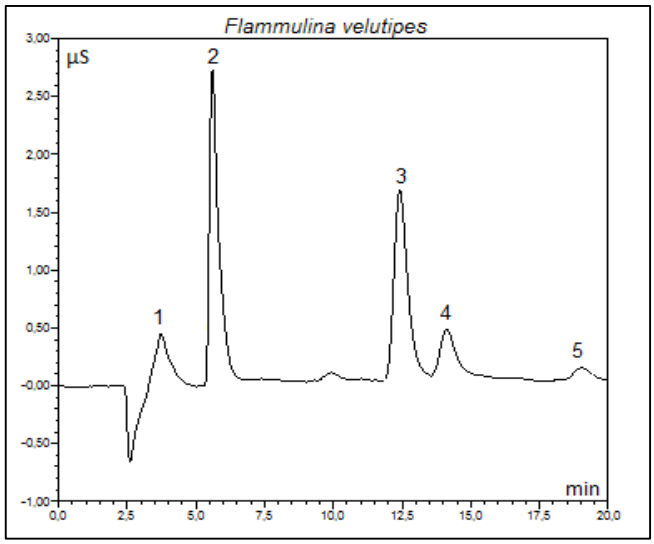

Fig 6: Chromatograms obtained from wild grown edible *Flammulina velutipes;* 1- fluoride, 2- chloride, 3- phosphate, 4- sulfate, 5- oxalate.

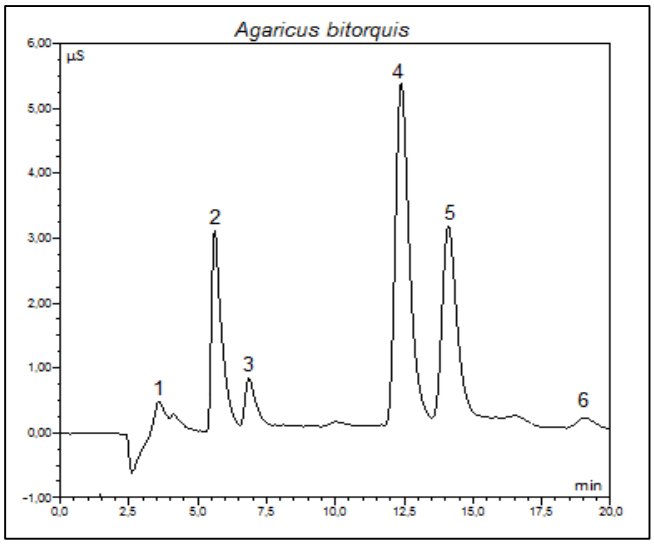

Fig 7: Chromatograms obtained from wild grown edible *Agaricus bitorquis;* 1- fluoride, 2- chloride, 3- nitrite, 4- phosphate, 5- sulfate, 6- oxalate.

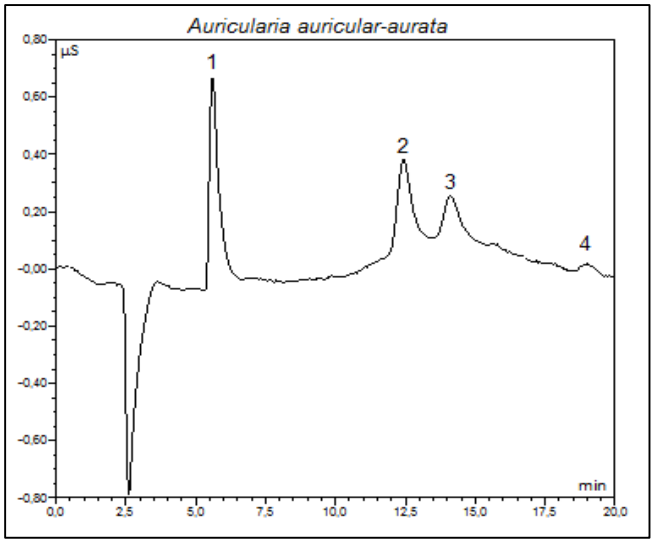

Fig 8: Chromatograms obtained from wild grown edible *Auricularia auricula-judae;* 1- chloride, 2- phosphate, 3- sulfate, 4- oxalate.

Conclusions

This paper describes a survey regarding to the ion chromatography method that was successfully applied to determine common inorganic anions in mushroom samples. Isocratic elution with sodium carbonate (20 mM) and conductometric detection enabled efficient separation of all ions within 20 minutes. Some inorganic anions (fluoride, nitrite, nitrate, bromate, chlorite, and chromate) with adverse human health effects were readily analyzed in wild grown edible mushrooms. The analysis performed on mushroom samples proved that chloride, phosphate were the most abundant followed by sulfate, bromide, chlorate, and nitrate. Also with this work; by detection of the concentration of inorganic ions in wild grown edible mushrooms has shown that fungi affect inorganic ions accumulate in the body of the fungus are able and that the impact of environmental pollution on wildlife.

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