Department of Food Hygiene

Literature review of heavy metal levels in wild edible European mushrooms in relation to the risk to common livestock species

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1. **Introduction**:

Mushrooms are a reliable source of food and livestock feed across the world due to their nutritional value, palatable flavours, and medicinal properties. However, these fungi have been shown to accumulate and retain heavy metals through absorption from their environment. This can pose a significant health risk in some cases if improperly treated feed is given to livestock, for consumers and livestock alike. High levels of heavy metal in wild edible mushrooms have become a growing risk worldwide, particularly in areas contaminated with industrial pollutants, agricultural runoff, and mining activity by-products. The heavy metals researched in the review are: Manganese (Mn), Iron (Fe) , Copper (Cu), Zinc (Zn), Cadmium (Cd) , Mercury (Hg), Lead (Pb) and the metalloid Arsenic (As). According to the European Mycological Association and European Council for the conservation of Fungi, there are over 10,000 known species of macro-fungi within Europe, with around 700 of those being edible. Of those, 33 species have been under intensive commercial cultivation. Fungi that will be used in this review and are both edible and commonly available for livestock, such as cattle, sheep, swine or poultry, and are found within Europe: *Agaricus macrosporus, Agaricus campestris, Agaricus bisporus, Pleurotus ostreatus, Coprinus comatus, Lactarius deliciosus*. (Dowley A, 2021; Akgül, 2019; Nebojša Stilinović, 2020; Rodríguez-Barrera, 2021; Sohail Hassan Khan, 2019; Y. I. Kim, 2011)

It is well documented that, primarily, the fruiting bodies of mushrooms are prone to bioaccumulation of heavy metals alongside the stem and substrate. The absorption ability within mushrooms is due to the specific structure of the mycelium, as it is an uncovered surface of the vegetative cells, aided by the hyphae's large surface area (Michalak, 2013). Within the fruiting body of mushrooms, the storage of heavy metals is through binding with certain proteins, especially to low molecular weight proteins. It has been shown that the rate of uptake of metals from substrate is a species-specific characteristic and the level of concentration of individual microelements in the fungi is a genetic ability (Byrne, 1976).

This literature review aims to provide a general overview concerning the state of mushroom heavy metal toxicity across the EU in relation to the potential risk if used as feed to the stated species of livestock. A limited number of papers have detailed the heavy metal content in mushrooms in Europe alongside limited EU or national laws relating to mushrooms as feed despite its increasing market, availability and use. EU laws relating to limits of heavy metals in feed are also limited to certain heavy metals.

2. **Methodology**

To fulfil the objective of this literature review, I found previous research on heavy metal levels in the determined mushroom species, Google Scholar was used primarily as a search database. PubMed, Dublin Public Library, ScienceDirect were mainly used as most European based research was published there or accessible there. Search query key words were the species or heavy metal abbreviated names. Data of heavy metal levels in mushrooms tested in the EU were included. I excluded all data in papers that concerned heavy metal levels within mushrooms harvested outside of the EU. Included are peer-reviewed research papers on the benefits of mushrooms in feed, the absorption abilities or the effect of heavy metals on the stated livestock that were researched outside of the EU. English, Spanish, Polish and German papers were reviewed. Due to the lack of papers on this topic, the dates I searched for ranged from 1970s to recent years. To search for appropriate peer-reviewed literature, all papers that did not include mushrooms edible by common farm species were excluded. The relevance of papers was determined based on the relevant data and source of the data. Data was then collected for each species and presented in tables for each of the stated heavy metals. Maximum, minimum or the mean detected levels present in the papers were presented in a table to display the potential ability of absorption ability of each species in a readily understood manner. Median or mean levels were not possible as many papers did not include raw data, instead displaying ranges of data. All papers used were cited in the literature review. Due to the lack of testing concerning many of the heavy metals in edible mushrooms, especially within the EU, relevant data is lacking for a truly comprehensive view. Common heavy metals such as Arsenic, Iron or Manganese were often not researched**.**

3. Wild edible European mushrooms that can be used as feed for common farm animals

All images from (Volunteers, 2023; Ltd, 2023)

(1)*Pleurotus ostreatus* (Oyster Mushroom; Figure 1: This mushroom is an extensively cultivated edible mushroom with high nutritional value (Committee, 2023). Commonly used for human nutrition, it is edible for many farm animals, mass produced and is often found in deciduous farms across Europe which allows for its availability and use as animal feed. It contains a good balance of essential amino acids and has notable amounts of essential vitamins and minerals (Bal, 2018). Oyster mushrooms are often included in animal feed formulations to increase nutrient content and enhance palatability.

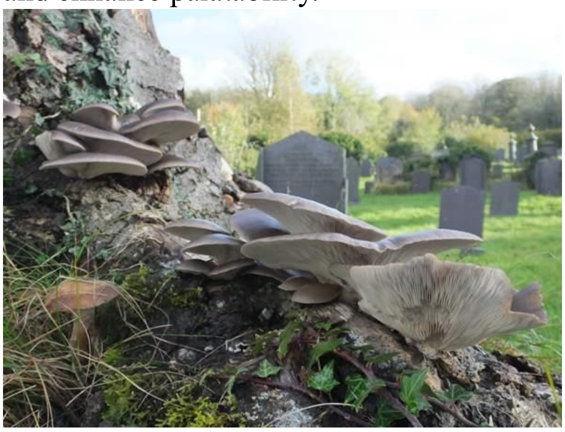

Figure 1. Pleurotus ostreatus (Volunteers, 2023)

(2*) Agaricus bisporus* (White button mushroom/ Brown mushroom; Figure 2): Its cultivation is common in Europe and across the world for its characteristics of low cost, taste and high nutritional value (Committee, 2023). Its mild flavour and wide availability make it a popular choice for farmers as an animal feed supplementation. Its use of a feed supplement has the characteristic as it increases crude protein digestibility when used as an additive. *A. bisporus* is known for its notable number of beta-glucans and other mushroom polysaccharides, which have been researched to show their effects as selected anticarcinogenic, antimicrobial, and antivirals (M. F. , 2016). These polysaccharides high antioxidant activity works alongside the antioxidant ergothioneine and phenolic acids (Öztürk, 2011). Due to this, *A.bisporus* is being used as a promising poultry feed additive today (Kelly Rutkowski, 2023).

Figure 2. Agaricus bisporus (Volunteers, 2023)

(3) *Agaricus Macrosporus*: *(*Figure 3*)* This is a large white capped mushroom white similar attributes as *A.bisporus*, however it is considered uncommon and not intensively farmed despite its usefulness as a feed additive. (Biology, 2023) It is found in meadows or forest fringes during Summer and Autumn months. When used as feed, it has slightly slower growth rates and lower nutritional value, in comparison to other *Agaricus* species, however it is still a viable feed additive for use with livestock as its commonly found and is both edible and palatable to most species (Bal, 2018).

Figure 3. Agaricus macrosporus (Volunteers, 2023)

(4) <u>*Agaricus campestris*</u> (Meadow/Field mushroom; Figure 4): Often seen in fields grazed by ruminants, *A.campestris* can be consumed by grazing livestock and is notable as a good additive for the improvement of rumen digestibility. As in other agaricus species, *A. Campestris* is nutrient rich such as in carbohydrates, amino acids, fats, and minerals and has potential anticancer, antioxidant, anti-obesity, and anti-inflammation properties, while being cheap and readily available, especially in the Autumn months (Öztürk, 2011).

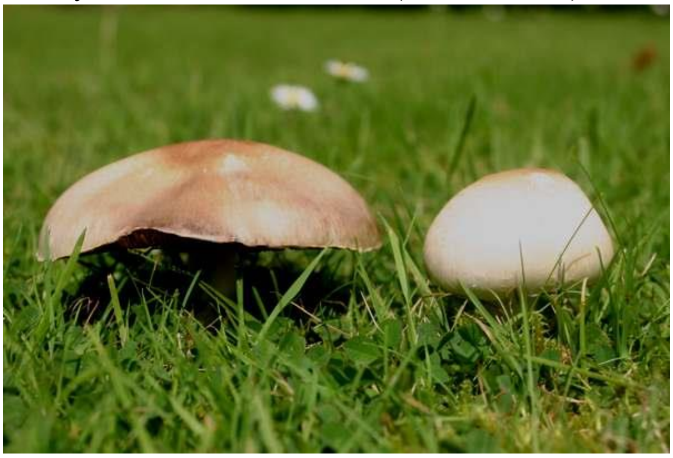

Figure 4. Agaricus campestris (Ltd, 2023)

(5*) Lacatarius deliciosus* (Saffron milkcap;Figure 5): While its primary benefit is its nutritional value and palatability, *L.deliciosus* acts as a notable source of beta-carotene, which is used to provide vitamin D (Committee, 2023). It is found in pine forests in the Autumn months, growing in large groups. Aside from the beta-carotene levels, antioxidant, antimicrobial, antihyperglycemic and anti-tumour effects have been shown in research (Bal, 2018).

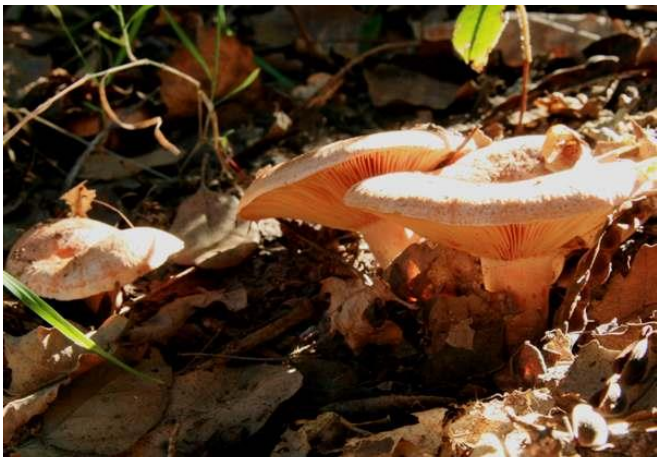

Figure 5. Lactarius deliciosus (Volunteers, 2023)

(6) Coprinus comatus (Shaggy mane; Figure 6): Harvested when young, this species is rich in carbohydrates, dietary fibres and proteins, and is also a valuable source of phenolics. Additionally, with low fat content, consisting mainly of polyunsaturated and omega-3 fatty acids and a high antioxidant capacity predominantly from the high phenolic acid content (Bal, 2018). Phenolic acids are the most prominent and effective contributors to the antioxidant activity of edible mushrooms. *C.comatus* also has been shown to have hepatoprotective effects. It is known as a swine and poultry feed additive.

Figure 6. Coprinus comatus (Volunteers, 2023)

3.1 Habitat and availability of wild edible mushrooms:

Wild edible fungi can be found in a variety of habitats, including deciduous and coniferous forests, meadows, and even urban environments across Europe (Fungi, 2021). They are often found in the months of Summer and Autumn. These fungi play a critical role in ecosystems, serving as decomposers of organic matter and providing a source of nutrition for other organisms. The availability of these wild mushrooms is dependent on the species and substrate suitability. From a financial perspective the availability reduces cost for feed and aids livestock in various manners, due to antioxidants, anti-inflammatory effects, anti-carcinogenic, high fibre content and high protein content (Bal, 2018) (Market, 2023). However, the risks from heavy metal toxins are an increasing risk. Industrialisation, improper use of fertilisers and improper disposal of waste has caused an increase in heavy metals within EU soils. In comparison to farmed mushrooms, there is an increased risk of harmful attributes as harvested wild edible mushrooms are not often tested. However farmed mushrooms can still have attached risks due to problems such as improperly sourced substrate with high levels of heavy metal contaminants.

3.2 Practicality of mushroom as dietary supplements in feed for livestock: Marketed as a source of dietary additive for feed, mushrooms (raw, processed or fermented) it has multiple advantages. They act as a cheap, high quality, sustainable protein source, in readily available quantity. Many species often have additional benefits, for example acting as an antioxidant. This is advantageous as, since 2006, the European Union has banned antibiotic use in animal feed (Commission Implementing Regulation (EU) 1831/2003 and 1463/2004, European Parliament). This means edible fungi can be a substantial economically viable alternative additive to farms, especially small-scale farms as wild edible mushrooms can be harvested in local environments or bought for a relatively cheap price. The production market for farmed mushrooms is expected to double worldwide by 2030 from $USD 54.9 billion (2022) to $USD115.8 billion with a growth rate of 9.7% predictions according to recent research (Market, 2023) (Research, 2021).

3.3 Key aspects of using mushrooms in farm animal feed:

(1) Nutritional Value: Mushrooms are rich in essential nutrients such as proteins, vitamins (B-complex vitamins etc), minerals (potassium, phosphorus especially), and dietary fibres (Bal, 2018). These nutrients are essential for aiding the growth, development, and overall health of farm animals.

(2) Protein Source: These species contain significant amounts of high-quality proteins, making them a viable protein source for livestock (Committee, 2023). Including mushrooms in animal feed can help reduce the dependence on traditional protein sources like soybean meal or fishmeal, which may have environmental and sustainability concerns. For example: in poultry, additives were added directly to the diet, or a portion of an ingredient was replaced with either pure probiotic powder, the fermented product, or mushroom waste compost (Kelly Rutkowski, 2023).

(3) Prebiotic properties: Certain species of edible mushrooms contain compounds like beta-glucans, which have prebiotic properties (F.M.N.A. Aida, 2009). Prebiotics promote the growth of the gut microbiome in animals, leading to improved digestive health, nutrient absorption and an overall improved immunity. This can be shown in swine or ruminant digestion especially, when fed mushroom additives.

(4) Antioxidant and Immune-Boosting Effects: Known for their antioxidant properties, which is attributed to compounds such as polyphenols and flavonoids act by neutralizing harmful free radicals, reducing oxidative stress, and strengthening the immune system of farm animals. An anti-inflammatory response in animals can be seen also, through the polysaccharides, triterpenes, polyphenols, ergosterol, and adenosine (Öztürk, 2011). Mushrooms have also been shown to be rich in beta-glucans, enzymes, polysaccharides, polyphenols, triterpenes, ergosterol, and adenosine which are functional proteins which play important roles as bioactive agents, acting as antioxidants, anti-inflammatories and immune system boosting. (Chuang W. Y., 2020).

(5) Improved Feed Conversion: The inclusion of mushrooms as an additive in animal feed has recently been shown to have improved feed conversion rates. This can lead to improved growth rates, productivity and reduced feed costs for farmers (Chuang W. Y., 2020). As such, fungal feed additives have a significant benefit in livestock, especially breeding stock. In previous studies, fungal feed additives enhanced body weight and egg production in poultry and improved the feed conversion rate (Hsieh, 2021).

4. Sources of heavy metal contamination:

Current literature shows modern industrial activities, such as poor waste management of mining, smelting, and manufacturing by-products are known to release a significant quantity of heavy metals into the environment, leading to soil contamination (Brzezicha-Cirocka, 2016). Additionally, agricultural practices involving the improper use of fertilizers, pesticides, and irrigation with wastewater can contribute to metal accumulation in the soil. Atmospheric deposition from emissions from industrial zones and vehicular exhaust is another major source of heavy metal contamination. Mushrooms absorb heavy metals from a substrate via spacious mycelium or fruiting bodies (Trust, 2023). Age and the size of the fruiting body are of less importance when considering the ability of the mycelium absorption abilities. The proportion of the metal contents originating from the atmospheric depositions seems to be also of less importance due to the short lifetime of a fruiting body, which is usually 10-14 days (Blum, 2007). Mushrooms, compared to green plants, can bio-accumulate more heavy metals in their fruit bodies due to specialised proteins and mycelial structures they possess (Demirbaş, 2000) which are used in small amounts for the mushrooms biochemistry.

4.1 Detecting heavy metals: Commonly used laboratory techniques to detect heavy metals used in literature are:

- *Atomic Absorption Spectrometry (AAS):* AAS is the most widely used method for quantifying the concentration of individual heavy metals in mushrooms. The sample is first digested using acid or microwave digestion to convert the heavy metals into a solution suitable for analysis. The concentration of each metal is then measured based on its characteristic absorption of light at specific wavelengths. In the analysis of heavy metals in mushroom with Atomic Absorption Spectrometer (AAS)Atomic fluorescence spectrometry (Fernández, 2018), inductively coupled plasma optical emission spectrometry.
- *Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)*: ICP-MS is a more sensitive than specific technique, capable of analysing multiple heavy metals simultaneously. It can detect a wide range of elements at very low concentrations. Like AAS, sample digestion is often required before analysis to ensure accurate measurements.
- *Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES):* ICP-AES is similar to ICP-MS but measures the emission of light from the excited atoms rather than mass spectrometry. It can also analyse multiple elements simultaneously, making it a useful tool for heavy metal analysis in mushrooms.
- *X-ray Fluorescence (XRF) Spectroscopy*: XRF spectroscopy is a non-destructive technique that measures the fluorescent X-rays emitted by the sample when exposed to high-energy X-rays. It can determine the elemental composition of the mushrooms, including heavy metals, without the need for sample digestion.
- *Voltammetry*: Voltammetry is an electrochemical method that measures the current produced when heavy metal ions undergo redox reactions at an electrode surface. It is relatively simple and can be used for rapid screening of heavy metal content in mushrooms.
- *Colorimetric Methods*: Colorimetric methods involve the use of specific chemical reagents that react with heavy metals to produce a colour change. The intensity of the colour change is proportional to the metal concentration, and this can be measured using a spectrophotometer or colorimeter.

- *Enzyme-Based Assays*: Enzyme-based assays use specific enzymes that react with heavy metal ions to produce measurable signals, such as fluorescence or luminescence. These assays are often used in combination with other detection methods for enhanced sensitivity and specificity.

4.2 Uptake ability and bioavailability of Mushrooms with Heavy metals: Biosorption is used as a process of rapid and reversible binding of ions from aqueous solutions onto functional groups that are present on the surface of biomass of the mycelium (Michalak, 2013). This process is a method of bioremediation, working by absorption and adsorption. It has been shown to be an efficient process in literature (Castanho, 2021). Biosorption is evident in other species such as bacteria, algae, fungi and yeasts. The biosorption of heavy metal ions from substrate within mushroom directly correlates to the substrate contamination and the bioaccumulation factor depicts the capacity of the mushrooms to accumulate the metals from the substrate. Biosorption is a species-dependant process along with the concentration capacity of the fruiting body (Barh, 2019). Other edaphic factors, such as substrate pH, soil type or organic matter play a role in the translocation and concentration ability of the fungi.

Biological accumulation factor (BAF) is used to determine the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil [BAF = Cc/ Cs] where *Cc (in this case)* represents the heavy metal contents (in DM) in the plants and *Cs* shows the soil/substrate heavy metal concentration, and indicate the ability of plants to tolerate and accumulate heavy metals (Babar Hussain, 2022). The coefficient of accumulation of heavy metals (Ka) can be calculated using the equation: [Ka=Cm/Cs] where (Cm) is the concentration of heavy metal in mushroom and (Cs) is the concentration of heavy metal in mushroom substrate (Stihi, 2011). This accumulation coefficient primarily differs based on species and substrate contamination.

4.3 Uptake Mechanisms: Mushrooms, in comparison to plants, have a relatively high capacity to accumulate and store heavy metals from their surrounding environment, especially within the substrate they grow in. This ability is due to transport mechanisms and metal-binding proteins within the fungi. However, it's important to note that the storage capacity of mushrooms for heavy metals can vary significantly depending on several factors, such as the mushroom species, the type and concentration of heavy metals in the environment, and the environmental conditions. Certain mushroom species are known to have a higher affinity for specific heavy metals, while others may have the ability to accumulate a broader range of metals, such as the *Agaricus* species. Higher metal concentrations in the substrate result in greater metal uptake by the mushroom (Damodaran, 2013). Uptake occurs through the following mechanisms:

(1) Absorption from the Substrate: Fungi grow and develop by absorbing nutrients from the substrate they inhabit. If the substrate contains heavy metals, the mushrooms can also take up these metals along with other nutrients. Heavy metals present in the soil or other growth media can be transported through the mycelium and subsequently accumulate in the fruiting bodies.

(2) *Binding and Complexation*: Heavy metals can form complexes with organic compounds present in the substrate, such as humic acids or other organic matter. These complexes can be accumulated by the mushroom.

(3) *Ion Exchange*: In the rhizosphere (the region near the mushroom's root-like structures called mycorrhizae), the exchange of ions takes place. Heavy metal ions in the soil can be taken up by the mushroom through this mainly via passive processes.

(4) *Passive Uptake*: Passive uptake occurs when heavy metals are present in a dissolved form in the soil water or the substrate. Passive absorption of these metals as they take in water and nutrients from their surroundings via biosorption, osmosis or other natural processes.

4.4 Factors influencing the uptake of heavy metals in mushrooms include: (a) Species: Different species have varying capacities to absorb and accumulate heavy metals. Some have been shown to accumulate higher levels of certain metals than others, depending on genetic factors. (b) Environmental Conditions: The concentration of heavy metals in the environment plays a crucial role in their uptake by mushrooms. Higher metal concentrations in wet soil or substrate can lead to increased uptake. (c) pH and Soil Properties: The pH of the soil and its properties, such as organic matter content and mineral composition, can influence the availability and uptake of heavy metals by mushrooms. This depends on the species optimum substrate pH. (d) Growth Stage: The stage of growth of the mushroom can impact its metal uptake efficiency. Generally, younger mushrooms tend to accumulate more heavy metals than older ones. (e) Competition: The presence of other microorganisms in the substrate may compete with mushrooms for heavy metals, affecting their uptake and availability.

5. Heavy Metal Concentrations:

Within mushrooms, heavy metal concentration depends on the contamination of the substrate where mushrooms grow, bioavailability and absorption ability of the fungal species (Byrne, 1976). The following is recorded statistics found in literature of common heavy metals within wild edible mushrooms taken and examined across the European continent. The maximum and minimum recorded values are presented, displaying the accumulation abilities of each species, and therefore the suitability of the species for animal feed additive if improperly harvested from contaminated substrate. A high heavy metal level causes animal welfare issues and economic loss to the farmer (Falandysz, 2013).

5.1 Lead (Pb): A common soil contamination, usually arising from industrial mining, paint production or various vehicle emissions. It is commonly found in most harvested fungi at low levels (Muszyńska, 2017; Allen, 1978; Brzezicha-Cirocka, 2016; Byrne, 1976; García M. A., 2009; García M. A., 1998; Kalač, 2010; Marta Barea-Sepúlveda, Metal concentrations in Lactarius mushroom species collected from Southern Spain and Northern Morocco: Evaluation of health risks and benefits, 2021; Svoboda Lubomir, 2007; Weeks, 2006)

Table 1. Lead maximum and minimum concentrations.

Lead(Pb) mg/kg DM	*A.bisporus*	*A.campestris*	*A.macrosporus*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	6.24	3.0	1.4	0.91	14.0	1.31
Minimum	0.028	0.59	1.25	0.56	0.53	0.46

The lowest detected and recorded value for lead contamination was within *Agaricus bisporus [0.028mg/kg DM]* (Muszyńska, 2017), while the highest recorded value was detected in *Coprinus comatus [14.0mg/kg DM]* (Kuusi, 1981). This exceeds the Regulation (EU) No. 2019/1869 limit for animal feed with lead content at [*10mg/kg relative to a feed with a moisture content of 12 %*]. These high levels were in papers where mushrooms had been harvested in areas near cities or industrial areas. This is a heavy metal which accumulates in the tissues, damages the endocrine and renal system, causing malnutrition, notable muscle loss, foetal damage, abortion, moderate anaemia, lowers antioxidant levels and in neurological terms causes ataxia, hypoglossal nerve paresis alongside severe depression and coma can occur. In poultry lead has a comparatively higher potential to deactivate antibodies, compared to ruminants and swine, thus impairing the resistance to infectious illness. In severe cases, lead toxicity can lead to death in cattle, sheep, swine or poultry (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

5.2 Cadmium (Cd): A very toxic heavy metal, Cadmium contamination is limited in the modern world. It is mainly an emission from smelting or mining, however soil contaminated with it is not rare (Stihi, 2011; Svoboda Lubomir, 2007; Melgar M. J., 2007; Brzezicha-Cirocka, 2016; Byrne, 1976; J., 1994; Kuusi, 1981; Marta Barea-Sepúlveda, 2021; Melgar M. &.-D., 2016).

Table 2. Cadmium maximum and minimum concentrations.

Cadmium(Cd) mg/kg DM	*A.bisporus*	*A.campestris*	*A.macrosporus*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	7.5	1.38	86.6	5.39	14.0	4.6
Minimum	0.01	8.7	20.0	0.06	0.9	0.11

The lowest detected and recorded value for cadmium contamination was within *Agaricus bisporus* [0.01mg/kg DM] *(Bosiacki, 2018)*, while the highest recorded value was detected in *Agaricus macrosporus* [86.6mg/kg DM] *(Melgar M. &.-D., 2016)*. These very high values were detected near industrial smelting plants. Many of the recorded values exceeded (EU) No. 1275/2013 limit of [0.5mg/kg relative to a feed with a moisture content of 12 %]. Cadmium effects almost every system within the bodies of livestock, causing cardiorespiratory damage, anaemia, hypertension, hepatitis, renal damage, reproductive issues, reduced appetite and an increased stress sensitivity. This heavy metal primarily accumulates in the kidneys, muscles, bones and liver. In poultry the Cd transfers to eggs. *(Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021)*.

5.3 Mercury (Hg):

A common contaminant prevalent in many foodstuffs, especially marine food, mercury is a toxic heavy metal which has become a growing concern in consumers and farmers alike (Allen, 1978; Bosiacki, 2018; Brzezicha-Cirocka, 2016; J, 2016; Kuusi, 1981; Marta Barea-Sepúlveda, 2021; Vetter, 1997; Zurera, 1986; Zurera-Cosano, 1988).

Table 3. Mercury maximum and minimum concentrations.

Mercury (Hg) mg/kg DM	*A.bisporus*	*A.campestris*	*A.macrosporus*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	9.21	14.1	7.2	3.47	144.0	0.77
Minimum	0.002	2.9	3.0	0.5	0.68	0.13

The lowest detected and recorded value for mercury contamination was within *Agaricus bisporus [0.002mg/kg DM]* (Weeks, 2006), while the highest recorded value was detected in *Coprinus comatus [144.0mg/kg DM]* (J, 2016). Many of the recorded values exceeded the regulation (EU) 2019/1869 at *[0.1mg/kg relative to a feed with a moisture content of 12 %]*. In livestock, clinical signs of mercury poisoning vary greatly depending on dosages and species. It accumulates in tissues such as the brain, kidney, and the foetus or egg. Mercury is mutagenic, nephrotoxic, carcinogenic, embryotoxic, and highly teratogenic. Mercury, which accumulates in mushrooms as methylmercury and mercury, are lipid soluble and has high oral absorption rates. Low concentrations of Hg are dangerous for poultry, with symptoms including development of anaemia and depressed growth rate to neurological signs and death. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

5.4 <u>Arsenic (As)</u>: As a very toxic heavy metal, Arsenic is not commonly used in the modern world. It is mainly found in some pesticides or improperly handled industrial waste (Brzezicha-Cirocka, 2016; J., 1994; Kalač, 2010; Marta Barea-Sepúlveda, 2021; Melgar M. J., 2014; Nowakowski, 2020; Patryk Nowakowski, 2021; Svoboda Lubomir, 2007).

Table 4. Arsenic maximum and minimum concentrations.

Arsenic (As) mg/kg DM	*A.bisporus*	*A.campestris*	*A.macrosporus*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	0.36	4.24	2.76	0.34	2.52	9.0
Minimum	0.07	0.35	0.82	0.04	0.75	0.1

The lowest detected and recorded value for arsenic contamination was within Agaricus bisporus [0.07mg/kg DM] (J., 1994), while the highest recorded value was detected in Lactarius deliciosus [9.0mg/kg DM] (Marta Barea-Sepúlveda, 2021). The Regulation (EU) 2019/1869 [2mg/kg relative to a feed with a moisture content of 12 %]. Cattle are more susceptible to arsenic poisoning than other ruminants, swine or poultry. The toxicity symptoms range from carcinomas, gastrointestinal, nervous system symptoms and skin discolouration such as weight loss, loss in appetite, conjunctivitis, mucosal erythematous lesions, excessive salivation and decreased productivity, notably milk production in cows or sows. The role of arsenic in poultry nutrition is heavily disputed but it is highly dangerous to their health even in very low quantities in food. Poultry symptoms can display as watery diarrhoea, abdominal pain and hypothermia leading to death. Animals can tolerate low levels of arsenic; the normal level in cattle tissues is <0.5 mg/kg. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

<u>5.5 Manganese (Mn):</u> As a necessary micronutrient, manganese contamination is of a lesser concern when compared to other heavy metals and commonly found in all kinds of substrate (Mirończuk-Chodakowska I, 2019; Weeks, 2006; Kalač, 2010; Byrne, 1976; Bosiacki, 2018; Brzezicha-Cirocka, 2016). It is often used as supplements of feed with other minerals such as Copper and Zinc When overfed to pregnant or young, it can be toxic despite being a mandatory supplement.

Table 5. Manganese maximum and minimum concentrations.

Manganese (Mn) mg/kg DM	*A.bisporus*	*A.macrosporus*	*A.campestris*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	387.43	Not Available*	9.8	31.4	21.7	9.16
Minimum	0.2	Not Available*	0.1	1.0	0.1	7.5

Values not available due to lack of available data in peer-reviewed literature

The lowest detected and recorded value for Manganese contamination was within Agaricus bisporus [0.2mg/kg DM] (Bosiacki, 2018), while the highest recorded value was detected in Agaricus bisporus [387.43mg/kg DM] (Bosiacki, 2018). There is no current EU legislation for manganese limits in animal feed. Manganese is a necessary mineral in many animals and its ability to accumulate is less pronounced than other heavy metals. An essential metal, manganese can still cause toxicity in high concentrations over long periods of time. This toxicity displays significant weight loss, poor productivity, and anorexia. in other heavy metals, this causes a productivity and economic loss. In cattle feed containing 1 000 mg/kg, sheep feed containing concentration 2000 mg/kg, pigs feed containing concentration of 500 mg/kg and in poultry feed with over 2000mg/kg causes toxicity. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

5.6 Iron (Fe): A very common heavy metal, Iron is widespread in large amounts in the substrate's fungi grow in (Borovička, 2007; Bosiacki, 2018; Brzezicha-Cirocka, 2016; Kalač, 2010; Öztürk, 2011; Muszyńska, 2017; Stihi, 2011). Classified as corrosive or cellular toxicity, iron toxicity from consumption can be extremely damaging. Iron supplements are occasionally given to ruminants, poultry and swine.

Table 6. Iron maximum and minimum concentrations.

Iron (Fe) mg/kg DM	*A.bisporus*	*A.macrosporus*	*A.campestris*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	207.3	Not Available*	752.0	775.0	584.0	1190.0
Minimum	37.7	Not Available*	50.3	18.0	64.0	22.0

Values not available due to lack of available data in peer-reviewed literature

The lowest detected and recorded value for iron content was within *Pleurotus ostreatus[18.0mg/kg DM]* (Stihi, 2011), while the highest recorded value was detected in *Lactarius deliciosus [1190.0mg/kg DM]* (López AR, 2022). There is no current EU legislation for iron limits in animal feed. Iron toxicity often occurs with a Vitamin E deficiency. It accumulates within the liver, heart, and brain of poultry and mammals. Iron toxicity usually displays anorexia, depression, orange-yellow discoloration of mucosa, abdominal pain and dyspnea. Chronic toxicosis occurs may lead to the development of hemochromatosis, a pathologic accumulation of iron in the tissues and parenchymal organs causing organ damage, lameness, anaemia eventually occur with a decreased appetite and depressed behaviour. As in other heavy metals, this causes a productivity and economic loss. In ruminants 2000mg/kg Fe, pigs and poultry 4000mg/kg Fe causes chronic toxicity. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

5.7. Copper (Cu): As a common heavy metal throughout most substrates, copper accumulates within most fungi. It is often used as supplements of feed with other minerals such as Manganese and Zinc (Alonso, 2003; Kuziemska, 2019; Bosiacki, 2018; Byrne, 1976; Kalač, 2010; Mirończuk-Chodakowska I, 2019; Svoboda Lubomir, 2007; Stihi, 2011; Weeks, 2006; Marta Barea-Sepúlveda, 2021).

Table 7. Copper maximum and minimum concentrations.

Copper (Cu) mg/kg DM	*A.bisporus*	*A.macrosporus*	*A.campestris*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	72.81	242.4	453.0	26.28	147.3	32.62
Minimum	2.6	217.7	35.5	1.0	69.0	0.5

The lowest detected and recorded value for copper content was within *Lactarius deliciosus [0.5mg/kg DM]* (Marta Barea-Sepúlveda, 2021), while the highest recorded value was detected in *Agaricus campestris [453.0mg/kg DM]* (Alonso, 2003). There is no current EU legislation for copper limits in animal feed. In comparison to other livestock, sheep are notably affected by high copper levels. In chronic copper poisoning, the sudden onset of clinical signs is due to a haemolytic crisis. Behaviour changes occur initially before gastrointestinal stasis, generalised weakness, liver failure and respiratory depression. Icterus and methaemoglobin occur and cause discoloration in the tissues are characteristic of chronic poisoning. For chronic toxicity, by species; adult cattle 3-5 mg/kg/day BW**,** 1-2 g/day in calves, sheep 3-5 mg/kg/day, weaned pigs 175 mg/kg/day and poultry 500mg/kg. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

5.8. Zinc (Zn): Not as common as other heavy metals used as micronutrients, zinc is still commonly seen in fungal substrate and therefore readily accumulated and detected in many fungal species (Weeks, 2006; Svoboda Lubomir, 2007; Mirończuk-Chodakowska I, 2019; Marta Barea-Sepúlveda, 2021; Kuziemska, 2019; Kalač, 2010; Bosiacki, 2018; Borovička, 2007). It is often used as supplements of feed with other minerals such as Manganese and Copper.

Table 8. Zinc maximum and minimum concentrations.

Zinc (Zn) mg/kg DM	*A.bisporus*	*A.macrosporus*	*A.campestris*	*P.ostreatus*	*C.comatus*	*L.deliciosus*
Maximum	93.2	267.0	215.0	265.0	139.7	309.8
Minimum	3.5	221.3	58.6	5.5	70.9	48.7

The lowest detected and recorded value for zinc contamination was within *Agaricus Bisporus [3.5mg/kg DM]* (Bosiacki, 2018)*,* while the highest recorded value was detected in *Lactarius Deliciosus [309.8mg/kg DM]* (Marta Barea-Sepúlveda, 2021). There is no current EU legislation for zinc limits in animal feed. Zinc toxicosis mainly occurs when overfeeding the supplement. An essential trace element, this heavy metal is utilised in the synthesis of several hundred enzymes. Toxicity often shows as an allergic reaction with hepatomegaly, splenomegaly, pancreatic nodules, red-brown kidneys with inflammation, oedema, or ulceration of the gastrointestinal mucosa. Swine that have consumed an excess of zinc show reduced weight gain, lameness, decreased litter size, gastrointestinal inflammation. Ruminants with zinc toxicosis have weight loss, diarrhoea, a decreased appetite, decreased production, polyuria with secondary dehydration, and generalized ataxia. In poultry, especially laying hens, zinc toxicosis can cause decreased egg production, decreased appetite, and weight loss while also causing renal failure secondary to haemoglobinuria. In cattle 700 mg of Zn/Kg of diet shows toxic effects, sheep 1 000 mg of Zn/Kg of diet, pigs 4 000 mg of Zn/Kg of diet and poultry 1 000 mg of Zn/Kg of diet. (Tchounwou, 2012; M. Hejna, 2018; Raikwar, 2008; Balali-Mood Mahdi, 2021).

6. Risk assessment:

To do an example risk assessment, a Monte-Carlo simulation on a sample literature (Bosiacki, 2018) was done. Using this method, a risk estimate was calculated based on if the heavy metals exceeded either EU feed limits or the lowest toxicological limit, depending on the metal. All values in the Monte-Carlo simulation estimates that were listed as below the average detectable limit of 0.001mg/kg were changed to 0.001mg/kg. All values were listed in the unit's mg/kg. 500 trials were done for each metals data in the simulations. For the first simulation, Table 9 (Bosiacki, 2018) is of the literature data and Table 10 is the Monte-Carlo simulation results. In the second simulation, Table 10. Literature heavy metal values was the results seen in the literature (Marta Barea-Sepúlveda, 2021) was shown with a monte-carlo simulation results in Table 12.

To calculate the mean and standard deviation of all samples in the literature (Marta Barea-Sepúlveda, 2021) the formula $\Sigma x^2 = SD^2(n-1)+((\Sigma x)^2/n)$ before the total standard deviation SD $= \sqrt{}(txx-tx^2/tn) / (tn-1)$ is found. (Combined n = tn, Combined mean = tx / tn). With this the total mean and SD can be found (Kong, 2020).

Table 9. Literature heavy metal values (Bosiacki, 2018)

A.bisporus	mean	min	max	SD	Estimated Samples exceeding limit
Cd	1.513833	0.68	6.14	0.829819	1
Cu	26.832	1.9	101.71	12.81629	0.983333
Fe	66.73283	33.01	432.24	64.53146	0
Mn	12.14817	2.86	387.43	49.69151	0
Pb	7.424333	0.98	42.83	7.445979	0.133333
Zn	63.23717	31.87	124.84	15.61767	0

Table 10. Monte-Carlo simulation of heavy metal values found in literature (Bosiacki, 2018)

Monte-Carlo	Estimated mean	Estimated min	Estimated max	Estimated SD	Estimated risk
Cd	1.571585	0.001	3.755203	0.836083	0.892
Cu	26.91492	0.001	73.30183	12.77078	0.974
Fe	73.98785	0.001	240.6929	55.17314	0
Mn	27.51847	0.001	140.9401	32.56541	0
Pb	8.009539	0.001	34.85014	6.468061	0.372
Zn	63.54108	22.51776	107.6137	15.18452	0

*(min=minimum, max=maximum, SD=standard deviation) *

For the risk estimates, each metals limit was based on either their EU regulation or lowest toxicological limits. The toxicological limits for Manganese were set as 500mg/kg, Zinc as 700mg/kg, Copper as 3mg/kg and Iron as 2000mg/kg. The EU regulation limits for Lead is 10mg/kg, Arsenic 2mg/kg, Cadmium 0.5mg/kg and Mercury as 0.1mg/kg

Table 11. Literature heavy metal values (Marta Barea-Sepúlveda, 2021)

L.deliciosus	**mean**	**min**	**max**	**SD**	**% samples exceeding limit**
As	1.7508	0.352±0.0202	5.62±0.05	1.9144	<0.5
Cd	0.5461	0.11±0.004	2.05±0.06	0.4711	>0.5
Pb	0.3966	0.073±0.0001	1.31±0.06	0.3496	0
Hg	0.3025	0.19±0.01	0.44±0.02	0.0979	1
Cu	8.8163	3.90±0.14	43.7±1.19	7.4437	1
Zn	90.9745	48.7±0.20	147.5±4.75	29.134	0

Due to the literature only having several means and standard deviations, an accurate minimum, maximum and risk could not be calculated

Table 12. Monte-Carlo simulation of heavy metal values found in literature (Marta Barea-Sepúlveda, 2021)

Monte-carlo	**Estimated mean**	**Estimated min**	**Estimated max**	**Estimated SD**	**Estimated Risk**
As	1.997492	0.001	7.713819	1.614004	0.47
Cd	0.582384	0.001	2.137439	0.437495	0.56
Pb	0.429248	0.001	1.3566	0.304596	0
Hg	0.300169	0.040245	0.562884	0.092495	0.978
Cu	9.385652	0.001	33.84394	7.17798	0.748
Zn	91.59806	13.80746	187.8831	29.55619	0

*(min=minimum, max=maximum, SD=standard deviation) *

For the risk estimates, each metals limit was based on either their EU regulation or lowest toxicological limits. The toxicological limits for Manganese were set as 500mg/kg, Zinc as 700mg/kg, Copper as 3mg/kg and Iron as 2000mg/kg. The EU regulation limits for Lead is 10mg/kg, Arsenic 2mg/kg, Cadmium 0.5mg/kg and Mercury as 0.1mg/kg

Assessment by metal: From the results in both papers and simulations, it can be presumed that Manganese, Zinc and Iron in mushroom feed contain a minimal risk of toxicity, unless fed with alongside high levels of the metal in another supplement. As Mn, Zn and Fe are all essential metals that are often given as feed supplements, the use of the mushrooms as feed with mineral supplements needs to be further researched.

For Lead, in Table 9 & Table 10 that the risk estimates on *Agaricus bisporus* exceeded the levels set by the EU limit with an estimate 37.2%, while the literature only displayed a 13.33% risk when used as feed. For *lactarius deliciosus* in Table 11 and Table 12, the detected lead levels did not exceed the EU feed limit in any of the researched samples or estimated figures. For Cadmium, in Table 9 & Table 10, the reviewed literature had a high risk of 100%, while the risk estimate was 89.2%. The figures seen in Table 9 & 10 are different from Table 11 & Table 12 where *L.deliciosus* had just greater than 50% of recorded samples exceeding EU feed limits, while the simulated estimated risk being 56%. This means there is a considerable risk in the use of these species for feed as there is a high chance it will exceed EU limits. In Copper, Table 9 & Table 10 shows that a.bisporus samples had a very high rate exceeding 98.33%, while its estimated risk was also very high at 97.4%. In Table 11 & Table 12 the recorded samples in the literature all exceeded the EU feed limit, while when simulated, only an estimated 74.8% would exceed the limit. The difference is due to the literature only recording means and standard deviations for its data. Arsenic and Mercury were not recorded in the literature for *a.bisporus* however it was recorded for *l.deliciosus.* Arsenic in Table 10 could be seen in less than half of the samples and also in Table 12, where an estimated risk of 47% was seen. Mercury in Table 11 exceeded EU limits in all samples while it had a simulated estimated risk of 97.8%.

Other environmental factors need to be considered in further research, such as the *Mushroom Species* and their varying accumulation ability, *Environmental Conditions* such as pH levels, weather, pollutants and substrate organic matter and finally the different species *Growth Duration*, where young mushrooms tend to have higher absorption rates or accumulation ability. These factors could not be estimated in this review due to the lack of literature on them.

Regulatory Measures

To ensure food safety, EU and state regulatory agencies have set maximum permissible limits for heavy metal content in feed and foodstuff. These limits may vary across countries and depend on the specific metal content, if they were not determined by EU law. These limits only apply to Lead, Cadmium, Arsenic and Mercury. Magnesium, Copper, Zinc and Iron need to be regulated by the farmer or harvester based on toxicological limits. Regular monitoring and surveillance programs are implemented to assess the levels of heavy metals in feed and ensure compliance with the established standards. Additionally, there are various mitigation strategies which can be employed to reduce heavy metal contamination, including the implementation of sustainable agricultural practices, decontamination of soils, and identification and cultivation of low-accumulating mushroom species.

Regulations and guidelines exist in many countries to limit the maximum allowable concentration of heavy metals in food and feed, including mushrooms. These regulations aim to protect consumers and livestock from potential health risks associated with heavy metal consumption. Regular monitoring and testing of mushrooms for heavy metal content are essential to ensure food safety.

Within the EU, food law regulations applicable to "mushroom and mycelium products (MMP)" (EFSA, 2023) produced or marketed are the following: Products obtained from "mycelium of the same species" are regulated by the **Regulation (EU) 2015/2283**. When used as feed, MMP are required to comply with **Regulation (EU) No 767/2009** on the market and use of feed with all applicable hygiene related requirements. "**Regulation (EEC) No 2658/8743** clarifies the content of each chapter of the Brussel Nomenclature in relation to the currently used classification system, stating that Annex I Chapter 7 shall be read as covering edible mushrooms as well" (EFSA, 2023).

Heavy metal limitations: Commission **Regulation (EC) No. 1881/2006** sets the maximum levels for certain contaminants in foodstuffs, **Regulation (EC) No. 178/2002** is the legal aspects of the food law requirements and the establishing of the European Food Safety Authority (EFSA), alongside the Rapid Alert System for Food and Feed (RASFF) (Commission, 2023). **Council Regulation (EEC) No 315/93** establishes local procedures for contaminants in food. According to **Regulation (EC) 1881/2006** the permitted maximum levels in mushroom feed products. Some Heavy metals limits in concerning fungal feed are not yet stated. National regulations apply to these heavy metals. Commission regulation **(EU) No. 1275/2013** and **(EU) No. 2019/1869** supplement and provide an updated legislation laid down by the regulations **(EC) 1881/2006** and **(EC) 178/2002**.

Discussion and Results

Overall, incorporating mushrooms in farm animal feed offers various benefits, such as improved nutrition, reduced environmental impact, and enhanced animal health. However, it is necessary to properly check the mushroom feed for heavy metals levels. This implies proper feed formulation and quality control are necessary to maximise the advantages while mitigating the potential risk of heavy metal consumption. Various mushrooms are seen to have high levels of risk for Lead, Copper, Cadmium, Arsenic and Mercury. These metals often exceed the EU determined feed limit in the researched mushroom species, with an estimated high-risk. An estimated 312,500 possible farms regularly using mushrooms as animal feed, of these there is not enough geographical data and mushroom research to accurately create a map of potential high heavy metal substrate risk regions.

The other heavy metals that were reviewed in the literature, Manganese, Zinc and Iron, had a far lower risk in comparison to the other reviewed heavy metals. These heavy metals may have had a lower risk estimate alone but due to their necessary use as essential mineral supplements, they pose a risk if used with a mineral supplement. This risk needs to be further examined in future research.

It is also evident in literature that some species, for example *Agaricus bisporus*, have generally lower heavy metals in Lead or Arsenic but can have high levels of Cadmium in some samples. This indicates that some species have a prevalence to accumulate some heavy metals in higher levels than others. However, there is too few data in the current literature to provide an accurate picture, such as in *Agaricus macrosporus* which has very limited research done on them.

Additionally, educating farmers and consumers about the potential risks and safe consumption practices can raise awareness and prevent adverse health effects. Responsible farming practices, environmental monitoring, and adherence to food safety regulations can help minimize the risk of heavy metal contamination in mushrooms and other food sources. The stage of growth of the mushroom can also influence its storage capacity for heavy metals. In general, younger mushrooms tend to accumulate more heavy metals than older ones.

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I hereby confirm that I am familiar with the content of the thesis entitled

Literature review of heavy metal levels in wild edible European mushrooms in relation to the risk to common livestock species

……… written by Ruaidhrí Ó Murchadha

(student name) which I deem suitable for submission and defence.

Date: Budapest, 13 day 11 month 2023 year

DR. PLEVA DÁNIEL

Supervisor name and signature

Institute of Food Chain Science

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Thesis progress report for veterinary students

Name of student: Ruaidhrí O'Murchadha

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Thesis title: Literature review of heavy metal levels in wild edible European mushrooms in relation to the risk to common livestock species

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	7	6	Discussion of the exact topic	[signature]
2.	2023	3	1	Literature searching	[signature]
3.	2023	4	13	Thesis announcement	[signature]
4.	2023	4	27	First thesis draft	[signature]
5.	2023	6	28	Expanded thesis draft	[signature]

Grade achieved at the end of the first semester: 5

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	7	13	Tables and data from Excel	[signature]
2.	2023	8	28	Third thesis draft	[signature]
3.	2023	9	8	Possibilities of risk assessment	[signature]
4.	2023	9	29	Monte-Carlo	[signature]

5.	2023	11	6	Plagiarism and formatting	[illegible]

Grade achieved at the end of the second semester: 5 ....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

....

signature of the supervisor

Signature of the student: ....

Signature of the secretary of the department: ....

Date of handing the thesis in 15/11/2023 ....