

Chemical elements in Ascomycetes and Basidiomycetes

The reference mushrooms as instruments for investigating bioindication and biodiversity

Roberto Cenci, Luigi Cocchi, Orlando Petrini, Fabrizio Sena, Carmine Siniscalco, Luciano Vescovi

Editors: R. M. Cenci and F. Sena









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Attached to this document is a CD containing:

- A PDF copy of this document
- Information regarding the soil and mushroom sampling site locations
- Analytical data (ca, 300,000) on total samples of soils and mushrooms analysed (ca, 10,000)
- The descriptive statistics for all genera and species analysed
- Maps showing the distribution of concentrations of inorganic elements in mushrooms
- Maps showing the distribution of concentrations of inorganic elements in soils

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Chemical elements in Ascomycetes and Basidiomycetes

The reference mushrooms as an instrument for investigating bioindication and biodiversity

R. M. Cenci, L. Cocchi, O. Petrini, F. Sena, C. Siniscalco e L. Vescovi

Fungi in the wild are among the principal agents in biogeochemical cycles; those cycles of matter and energy that enable ecosystems to work.

By investigating the biodiversity of Italian fungal species and concentration levels of chemical elements in them, it may be possible to use these fungi as biological indicators for the quality of forest, woodland and semi-natural environments.

The database of this EUR Report record the dry-material concentrations of 35 chemical elements, including heavy metals, in over 9,000 samples of higher mushrooms (Ascomycetes and Basidiomycetes). These samples represent approximately 200 genera and a thousand species. As the database has attained statistical stability it has been possible to define the concept of a "reference mushroom". The use of a "reference mushroom" may benefit — perhaps only as a methodological approach — various fields of mycological and

environmental research; from biodiversity and bioindication, through taxonomy right up to health and sanitation issues.

The sheer volume of the collected data may prove to be useful as a comparison for data collected in the future; such results would also allow a better and more exhaustive interpretation of the effects of environmental protection laws that have been in place over the years to reduce or remedy current climate change phenomena and the environmental damage caused by human activity.

Studies pertaining to the frequency of occurrence and the ecology of the various fungal species found on Italian soil have tended to link the reference *habitats* used to European classification guidelines (Natura 2000, CORINE Land Cover, *CORINE Biotopes* and EUNIS). Thereby the foundations have been laid for the use of mushrooms as biological indicators for the measurement of soil and ecosystem quality.

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A heartfelt thank you goes to members and friends who have worked for more than twenty years, in various ways and with different skills, on the work presented here.. It is therefore fitting and correct to offer collective thanks to the *Associazione Micologica Bresadola* and special thanks also to all members of the *Comitato Scientifico Nazionale* and the *Gruppo Micologico e Naturalistico "R. Franchi"* in Reggio Emilia.

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Index

IN	NDEX		9
1	PR	ESENTATIONS	11
•	1.1 1.2	ICMAMB	11
	1.3 1.4 1.5	ISPRA EU - CCR - IES ENIA	14
2	IN	TRODUCTION	17
_			
	2.1 2.2	MUSHROOM TAXONOMY BIOLOGICAL NOTES ON MUSHROOMS	
	2.3	MUSHROOMS AND ENVIRONMENTS FOR GROWTH	
	2.4	MUSHROOMS AS A SOIL-QUALITY BIOINDICATOR	
	2.5	THE REFERENCE MUSHROOM	46
	2.6	BIODIVERSITY AND BIOINDICATION: EC AND INTERNATIONAL LEGISLATION	53
3	DA	TA SYNTHESIS	55
	3.1 3.2	CONSIDERATION OF STATISTICS AND THE STATISTICAL METHODS EMPLOYED APPLIED GEOSTATISTICAL ANALYSIS	
4	MATERIALS AND METHODS		63
	4.1	METHODS FOR CHEMICAL ANALYSIS OF SOIL AND MACROMYCETES	63
	4.2	DISTRIBUTION MAP OF ELEMENTS IN SOIL	64
	4.3	DISTRIBUTION MAP OF ELEMENTS IN THE MUSHROOMS	
	4.4	SAMPLING: A DATA SHEET EXAMPLE	107
5	CO	CONCLUSIONS	
6	BII	BLIOGRAPHY	113
7	AP	PENDIX	121

Chapter I Presentations



1.1 ICM

Even if it is not always apparent, mushrooms affect our daily lives in many important ways. They are indispensible elements for food production, and yet many of them, as plant pathogens, cause great agricultural damage. Others, fortunately only a few – although their numbers are growing – are pathogenic agents for animals; including man. Mycorrhizae are symbiotic associations between fungi and plants that are important in agriculture and, last but not least, several species of basidiomycetes are valuable edible mushrooms. Therefore, it is important to know and understand mushrooms, and even more important to classify them accurately.

For years the taxonomy of basidiomycetes and ascomycetes was based almost entirely on morphological characters; we can see this today in the identification keys still used by amateur and professional mycologists alike. However. mycologists were quick to realise that morphology was not enough to build exhaustive and trustworthy classifications, especially when the organisms to be classified were of simple shape or when the morphological features were limited or varied little. Therefore attempts were made to classify these organisms not only by their morphology, but also physiological and their biochemical characteristics. Even at the beginning of the 20th Century, for example, staining and biochemical reactions were being studied in bacteriology: these same properties are now used in mycology.

Thus arose the problem of reconciling morphological classification with the type of classification established bv genetic biochemical methods. In fact, genetic analysis often leads us into creating taxonomic schemata which are not, certainly at first glance, entirely compatible existing classifications. A trustworthy classification must take into consideration not just phylogenetic properties (being connected to the evolution of organisms over time) and phenotype (the observable morphology and physiology of an organism), but also ecological peculiarities. Such an approach is commonly referred to as "polyphase taxonomy".

The work contained in this book provides a new and important piece of the puzzle which is the taxonomy of mushrooms. I hope that these data will be of help to taxonomists in completing their research and that they might become part of a taxonomic scheme which will go towards resolving the difficult issue of the definition of taxa. By their very nature, these data are also relevant from physiological and ecological viewpoints. I hope therefore that this book will also be of service to physiologists who aim to better understand the biochemical aspects connected to absorption and retention of chemical elements in mushrooms, and that it will serve the ecologists who are trying to achieve a complete picture of the biodiversity of these fascinating organisms.

Orlando Petrini

Director of the Istituto Cantonale di Microbiologia, Bellinzona, Switzerland



1.2 AMB

Research into the presence of chemical elements, including heavy metals and radioactive isotopes, in higher mushrooms began in 1986 at the "Renzo Franchi" mycological-naturalist group in Reggio Emilia, Italy and was then taken up by the National Scientific Committees of the Bresadola Mycological Association (AMB). This is not a mere "historical fact", but instead attests to an essential aspect of the scenario: given the size of the project and the level of skills it involved, this research could not have been achieved outside of the AMB and of its network of associated groups.

One of the most delicate issues here, as in all analogous studies into higher mushrooms, is that of having a positive identification of the fungal exemplars being studied and analysed: herein lies the skill and know-how that the AMB provides at a level which is widely recognised as the gold standard. We believe this characteristic is something over which we should be highly protective, in a spirit of militant volunteerism, fuelled by passion, respect and love for nature and its equilibrium.

Such equilibrium is increasingly necessary for the future of humankind; understanding it and its mosaic of different pieces, possibly thanks to collaboration and communication between various scientific disciplines, is becoming an essential task for all those who hold the future of the planet to heart

The piece that we bring to this mosaic is our knowledge of mycological classification and taxonomy, a science created by the great naturalists such as Linnaeus, and which, in Italy, culminated with the lofty heights of Giacomo Bresadola.

This knowledge is born of the intense work carried

out by our association, which consists of 130 groups from around the country and over 12,000 members. Our members are involved in capillary networks of activities, which include research, information and education programmes regarding mycology and the environment through hundreds of local initiatives, ranging from mycology courses and mycological exhibitions to mycological studies and investigations.

The AMB is also an important national and international mycological publisher, with two magazines to its name Rivista di Micologia (Mycology Magazine) and Pagine di Micologia (Mycology Pages) and various books that deal with basic mycology all the way up to highly-specialised tomes

Two National Scientific Committee meetings are held each year: one in spring, the other in autumn, and these involve hundreds of mycologists.

The work of the National Scientific Committee meetings is the main driver of the National Herbarium, which has now surpassed 10,000 dry specimens and has recently been admitted to the Index Herbariorum.

The recognition that the publication of this EU report constitutes for a research project that has lasted over 20 years is not only thanks to the authors and their patience and dedication, but should be extended to the whole AMB.

The fact that this research has led to fruitful collaboration with European Community institutions, the Italian state, with private companies and non-EC research institutes, such as the Istituto cantonale di Microbiologia, in Bellinzona, Switzerland has brought the statutory purposes of the AMB to the highest levels.

Luigi Villa

President of the Associazione Micologica Bresadola



1.3 ISPRA

The "Special Mushrooms Project" by the ISPRA's "Natural Protection" Department promotes and carries out studies on fungal species and is thereby well placed in the project using them as indicators of environmental quality.

Mushrooms are important diversity indicators in terms of population richness and abundance at a genetic level and can therefore be used in the study and monitoring of biodiversity in a particular environment or ecosystem.

One of the 16 research themes in the "Special Mushrooms Project" was aimed at developing an IT system for micotoxicological aspects, including phenomena of bioaccumulation and bioconcentration of heavy metals and xenobiotic substances in the mushrooms. The idea behind this was to facilitate bioremediation plans for degraded environments and also to promote studies regarding the health and hygiene aspects related to the human consumption of mushrooms.

The work described in this volume, edited by JRC-IES, is the fruit of collaborations between five different institutions that have together been able to create one of the first ever applications for bioindication using mushrooms.

The work springs from the results of an intense sampling campaign carried out in Italy by the

Associazione Micologica Bresadola which was started over 20 years ago and has conducted chemical analysis on over 9,000 mushroom samples and which has led through statistical inference to the definition of a "reference mushroom". These last two aspects will go on to play a fundamental role when the fungal diversity indicators for richness and abundance at the genetic level are defined. By linking fungal species to habitats it will, in fact, be possible to use mushrooms to study and monitor the biodiversity of an ecosystem or environment with numerous elements of evaluation. Those species occurring more frequently will act as the first sample of ecological value and as environmental quality indicators.

In the near future then, we will have a deeper understanding of both those mechanisms which maintain and regulate the evolution of ecosystems and a new knowledge of those mycotoxicological aspects which will be used to inform protective legislation regarding human mushroom consumption.

Andrea Todisco

Direttore Dipartimento Difesa della Natura di ISPRA, Roma, Italia





1.4 CCR - IES

It was a great pleasure to be asked to write a small preface to the volume edited by Roberto Cenci, Fabrizio Sena and colleagues. Through this work there flows a clear love for the environment and a strongly-motivated scientific interest in a little-understood or studied field: mushrooms.

The study of soil ecology and, more specifically, the use of mushrooms to evaluate the health of the soils in which they grow would, on the face of it, be a complex affair; both due to the limited knowledge we have about the field today and also to the objective difficulties presented by the formulation of a model by which we might "read" the soil. The book is a complete and exhaustive illustration of the characteristics of mushrooms and their role in the soil compartment. At the same time it lays out a solid base for the use of biodiversity and bioindication as diagnostic measures to better understand the health and quality of soil.

The impressive amount of detail given to trace elements in over 9,000 analyzed mushroom samples makes this book as a guide that covers many other fields, such as food and nutrition. We should bear in mind that fungi are used in many different foodstuffs, and so knowledge of heavy-

metal presence in certain types would allow the creation of healthier products for the consumer. The data collected here may aid the future formulation of EC food guidelines. The database has already been bought by the Italian Health Ministry (its acquisition taking place in 2008) for in debating and defining Community Regulation n. 628/2008. Another important aspect of this work is the vast sampling area covered and the high number of fungal species identified. These today are of fundamental importance but will be even more important in the future when further sampling campaigns will be carried out: at that stage we shall have a clear view of whether, and to what extent, the activities of man and climatic change influence the biodiversity of mushrooms. One final, but by no means unimportant effect of this book relates to the use of the information it contains as a database for the numerous experts in the field who will now be able to better describe, and so improve, their investigations.

I am sure that this book will be recognised for its merits and that it will be a great aid and step forward to both international and national experts and researchers in the field of mycology.

Leen Hordijk

Direttore dell'Istituto dell'Ambiente e della Sostenibilità, Ispra, Italia



1.5 ENIA

We are honoured to be among the list of authors of this report, which stemmed from the historical connection between the "Renzo Franchi" mycological-naturalist group in Reggio Emilia, Italy and the Bresadola Mycological Association – Mycological Study Centre, which has previously seen so many fruitful collaborations.

It may, on the face of it, seem rather odd that a multi-utility company such as ours should be working with groups so far away from our daily activity. In reality, however, the logic that moved and continues to move us is the goal of widening our vision and forging bonds with the most interesting partners in the territory so as to favour an exchange of knowledge to our mutual advantage. These choices have enabled Enia to establish a new concept of "territorial proximity" by going beyond the usual partners one would expect an energy and environmental services company to have and instead seeking out different, but like-minded companions to explore and travel the road ahead.

As always, in this relationship we have given our best; above all we have made our professionality and know-how available, in this case those belonging to our Reggio Emilia Laboratories. This project represents a very positive exchange of knowledge and also a serious means of supporting

research initiatives in Italy and it placed Enia among the authors of an instrument which extends far beyond Italy in its uniqueness.

Allow me then one last thought, not just as Chairman of a company, but also as a Mycologist – a passion which allows me to appreciate the findings of this book even more. Often, when one thinks of the instruments of innovation and research, habit leads us to think of modernized gadgets and sharp, complicated machines, as cold as only machinery can be. However, sometimes we only have to look around us and nature comes to the rescue. In this case it is mushrooms that we have to thank; "simple" mushrooms – and I ask mycologists to forgive me this definition.

These mushrooms lend us a help that should make us reflect upon and rethink our relationship with the world that surrounds us and of which we are part. It is a world which we ever more frequently know little about and often mistreat. We waste, dirty and even ruin a bounty which should be there for everybody.

If the research in this book sets us to thinking about this, we will have reached another goal and provided another significant result which can be added to the extraordinary value of this Report.

Andrea Allodi

Chairman of Enìa Spa, Parma, Italia

Chapter II Introduction

2.1 Mushroom taxonomy

2.1.1 Introduction

Mushrooms, as all living beings, are scientifically named according to their "binomial nomenclature", which indicates their "genus" (capitalised) and their "species" (not capitalised); both names are usually italicised. The binomial denomination of living creatures, which had previously been partially used by Theophrastus and Pliny, was given a scientific basis by Linnaeus, who established "taxonomy" (based on a binomial denomination using Latin or Latinised names) as a real and proper scientific discipline. Linnaeus (*Linnaeus*, 1753) described fungi for the first time, grouping them into just ten genera. Since then the taxonomy of mushrooms and fungi has made great leaps forward.

Later on, (1816 - 1817) Nees broadened and refined Linnaeus's work by enhancing taxonomy. First considered as vegetables and then being assigned to the realm of plants, mushrooms were regarded from early on as "abnormal" bodies, the taxonomic position of which remained very unclear. These organisms attracted the attention of biologists, and, after some initial important work in Italy, Saccardo's monumental efforts (1882-1931) culminated in a veritable encyclopaedia of mycology.

Initially, fungi were classified into four divisions. The Basidiomycetes were distinguished from the Ascomycetes and from the Deuteromycetes largely based on morphological characters such as the way their spores formed, the colour and shape of those spores and the appearance of their bodies. Clearly, the most studied fungi were Basidiomycetes; visible to the naked eye and of interest for their gastronomic properties and economic value.

2.1.2 Classification of fungi into kingdoms and phyla

Fungi are eukaryotes (therefore having a nucleus delimited by a nuclear membrane). Their cell walls contain chitin and glucans (and rarely cellulose) and their nucleus may be haploid or diploid, dikaryon, homokaryon or heterokaryon. The fructifications can be microscopic or macroscopic, differentiated or undifferentiated. Their ecological role is very varied and among themselves they may be symbionts, saprobes, parasites, commensals or hyperparasites.

Fungi are fairly cosmopolitan: around 70,000 species have been described, but Hawksworth (1991) estimates that there may be as many as 1.5 million. Only around 300 are known pathogens for humans, while many cause plant diseases and a considerable number play a fundamental role in the ecosystem, both as destroyers of vegetable detritus and as symbionts (Mycorrhizae and lichens).

Until 1980, taxonomists considered fungi a compact, though not necessarily homogenous, group. Müller and Löffler (1976), in their book on mycology (still considered a classic reference for mycologists) included not only the Ascomycetes, the Basidiomycetes, the Deuteromycetes and Zygomycetes, but also some groups of organisms that have since been transferred into the kingdoms of Protista and Chromista. In 1981, Cavalier-Smith (1981) proposed a separate kingdom for the "higher mushrooms" (the Ascomycetes, Basidiomycetes, Zygomycetes and their asexual forms, grouped together as Deuteromycetes) and transferred most of the "lower fungi" (single-celled organisms or hyphae, often with flagella spores) into the Protista and Chromista. Cavalier-Smith's work, which showed how mushrooms are more similar to animals than to plants (fig. 1) opened the door to a more detailed re-elaboration of the taxonomy and phylogeny of fungi.

Kendrick (1992) described the ideas of Cavalier-Smith very well in his book, "The Fifth Kingdom".



Fig. 1. The "Tree of Life", the position of fungi in the phylogenic tree . Source: "Tree of Life Project" (Maddison and Schulz, 2007)

It was the same Cavalier-Smith who, over the following years, proposed ever more complex and detailed models (Cavalier-Smith, 1993; 1998; 2004; 2006), which were then taken and formalised, at least as regards the kingdom of fungi, by Hibbett and Binder (2007) (fig. 2).

Currently, living organisms are divided into seven kingdoms (Eubacteria, Archaebacteria, Archaezoa, Protozoa, Plantae, Animalia, Fungi). To this list should be added the kingdom of Chromista, itself an area of considerable controversy.

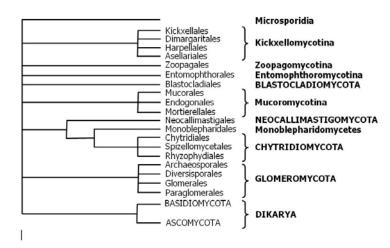


Fig. 2. The phylogeny and classification of fungi. Source: Hibbett (Hibbett and Binder, 2007), amended. The length of the branches of the cladogram is not proportional to the genetic distance between taxa.

Based on the work by Cavalier-Smith (1993) and Hibbett and Binder (2007) fungi, that until 1980 were grouped together in one kingdom, are now subdivided as follows:

 the kingdom of fungi includes the Ascomycota and Basidiomycota (Dikarya), the Glomeromycota (which includes the endotrophic mycorrhizae), the Chytridiomycota, the Neocallimastigomycota, the "Mucormycotina" (traditionally Zygomycota), the Blastocladiomycota, the Entomophthoromycotina, the Zoopagomycotina, the Kickxellomycotina and a group of unicellular human parasites Microsporidia.

- the myxomycetes have been transferred into the kingdom of Protozoa (Amoebozoa, Eumycetozoa);
- the Oomycetidae and Thraustochytridae are now assigned to the kingdom of Chromista.

This new classification is the result of intensive phylogenic work, based in particular on genetic analysis, but it is interesting to note that the results are in perfect concordance with previously stated hypothesis based on observations of morphology and physiology (Müller et al., 1976).

2.1.3 Problems with taxonomy in mycology: fungal variability

Within any wide taxon, not only molecular genetics, but also morphology and physiology are useful for reaching taxonomic conclusions.

Morphologically speaking, fungi are incredibly variable. Beyond the morphological variations present both at a macroscopic (just think of the difference between porcini mushrooms and an amanita muscaria, for example) and at a microscopic level, fungi create further difficulties by their ability to express two distinct phenotypes in their reproductive and vegetative forms.

Furthermore, often a fungus will produce just one form (sexual or vegetative) through having reduced or lost the capacity to reproduce either sexually or vegetatively (asexually).

From a phylogenetic point of view, morphological analysis can lead to organisms being erroneously identified as belonging to two distinct taxa when, in reality, there exists only one, either in its sexual or asexual form. Only in cases where both forms are found together can a morphological analysis give a trustworthy classification; in other cases genetic analysis becomes indispensible.

Mushrooms are pleomorphic and therefore assume a different shape depending not only on the type of reproductive organ they develop, but also on the environmental and physiological conditions influencing their growth.

Regarding pleomorphic growth in different physiological and ecological conditions, see the dimorphism of certain bodies (especially animal pathogens) in the form of yeast that grow in particular conditions (e.g. *Paracoccidioides brasiliensis*: yeast at temperatures above 37°C, hyphae at temperatures below 37°C).

Also important is the **pleomorphism of the sexual** and vegetative forms. Over the last years this led to a reconsideration of nomenclature in fungi. The fungus considered as a complete entity, or "the whole fungus" was named the "holomorph": this is the sum of the "anamorph" (from "anatomic morphology": the asexual form), and the "teleomorph". A holomorph is thus normally composed of a teleomorph and its anamorphic form. However, in some cases one of the forms will be unknown and so the holomorph will correspond either to the teleomorph or the anamorph. Not infrequently, a single holomorph may have several diverse anamorphic forms. One typical example of this is Aspergillus (an anamorphic form) and its teleomorphic form Eurotium (fig. 3).



Fig 3. Example of a holomorph: Eurotium and its anamorph, Aspergillus.

2.1.4 The phyla of fungi relevant to this work: Basidiomycota and Ascomycota

During our work we were able to examine a large number of samples belonging to the Phylum Basidiomycota and also many specimens belonging to the Ascomycota although fewer than those belonging to the former group.

We have followed the classification proposed by Hibbett and Binder (2007) for higher-level taxonomy, while inside each family we have kept to the classification used by CAB International (www.indexfungorum.org). This was not an "ideological choice" (in this period of continuous systematic and taxonomic upheaval that is only partly mitigated by the International Code of Botanical Nomenclature, "marrying" systematics to taxonomy is, indeed, often arbitrary), but rather an operative choice – made simply to render the work more easily accessible to all.

In any case, the systematics and taxonomy are proposed while fully taking into account the results that continuously arise from phylogenetic analysis. A greater difficulty from our point of view was the lack of a universally-accepted definition of "species" in mycology. This not only creates problems when one is attempting to attribute a name to a species, but also means that it is not

Cells that contain just one set of chromosomes (one chromosome of each type)

Basidiomycetes Fungi that produce sexual spores (i.e. those formed after

meiosis) exogenously on their basidia.

Classification Very broad term that denotes an organisational scheme. It is often the result of a taxonomic scheme, notwithstanding that classification does not necessarily

give names to the organisms classified.

(a non-taxonomic term) fungi that develop asexual

spores (i.e. formed after mitosis).

Diploid Cells that contain two copies of each chromosome.

Phylogeny The hierarchical structure that orders living organisms

according to their reciprocal evolution.

Lichens Forms of life resulting from a combination of autotrophic organisms (algae or cyanobacteria, mostly

chlorophyta) and a fungus, usually an ascomycete or basidiomycete mushroom. They are classified according to the taxonomic position of the fungus.

Homokaryon Cells that contain two or more identical nuclei, usually produced by the fusion of one or more cells of the same species.

Pleomorphism A characteristic of mushrooms that develop different forms at different stages of their lifecycles.

Saprobic An organism receiving nutrition from non-living organic material.

Systematics The process of classifying living creatures based on their phylogenetic position.

always easy to be sure that carpophores identified by different mycologists (or even by the same mycologist, but during a different period) belong to the same species.

The problem also arises, obviously, when we want to compare our data with those of other researchers, although fortunately for us this was only an issue in very few cases and has no bearing on data after the level of genus. More challenging (and surely requiring further study) is the attempt to define a species. For consolidated species the difficulties are only quantitative in nature (having data on a great number of samples) but species which are still being debated are far more complex and, beyond quantitative issues, present systematic and taxonomical questions to the resolution of wich our research may perhaps be able to contribute.

2.1.5 Glossary

Many terms used in mycology are interpreted differently by different researchers. For definitions of some of these and in particular those concepts such as classification, phylogeny, systematics and taxonomy, where there are several schools of thought we list here their simplified definitions.

Ascomycetes Fungi that exogenously produce sexual spores (i.e. those formed after meiosis) in containers called asci.

Chromista The kingdom that includes unicellular or multicellular living organisms called eukaryotes. These are mostly photosynthetic, but Chromista also encompasses organisms previously classified among the "lower fungi".

Commensal Organisms that live on or in other organisms, usually receiving some advantage from the relationship (food or protection) but without damaging the host organism.

Dikaryon A hypha or cell with two nuclei.

Heterokaryons A cell containing several genetically different nuclei. They are artificially generated by the fusion of two or more cells and in nature are only found in fungi.

Hyperparasites A parasite that lives on another parasite

Mycorrhiza A symbiotic association between a fungus and a higher plant, found in the root wall of the vegetable symbiont.

Parasite An organism that obtains nutrition and/or shelter from another organism without giving anything in return.

Protist The kingdom including unicellular eukaryote organisms, including protozoa, algae and fungi.

Symbiosis A close relationship between diverse organisms whereby each organism receives a reciprocal benefit. Lichens and

Mycorrhizae are examples.

Taxonomy The assegnation of names to organisms. A term frequently considered a synonym of systematics.

Haploid

Deuteromycetes

2.2 Biological notes on mushrooms

The word mushroom immediately gives one the idea of edible mushrooms, those we gather from the woods or we buy straight from the market, however, mushrooms are just one type of fungi along with many others. Other fungi include the moulds that sometimes attack cultivated plants or the walls of our houses, those that contaminate our food, produce damaging toxins; or those that are used industrially in the production of food, drinks pharmaceuticals. Fungi are employed commercially in the production of antibiotics, steroids, cyclosporin and enzymes for use in cooking and in the production of food and drink. Just try to imagine a world without fungi: we would have to say goodbye to wine, beer, bread, several types of fine cheese, antibiotics and other therapeutic chemical compounds.

Fungi, as all eukaryotic organisms, possess cells that contain a nucleus wrapped up in a membrane, more than one chromosome and organelles such as mitochondria.

They have many unique features in terms of their structure, cellular components and organisation. They are in fact filamentous, multicellular organisms made up of long, branching tubular cells called hyphae which are of varying lengths, but uniform diameter, of 2-30 $\mu m,$ and are together known as mycelium.

The hyphal walls are composed of polysaccharides (80-90%), proteins, lipids, polyphosphates and

organic ions, but their main constituent is chitin, a polymer of N-acetylglucosamine, which is derived from glucose (*Carlile et al.*, 2001).

The hyphae grow from the tip; the extension zone may be between 30 to 400 micrometres and the hyphal walls stiffen rapidly. The part of the hypha immediately below the extension zone ages progressively and its oldest parts can either be lysed by the organism's own enzymes (autolysis) or by other organisms (heterolysis). Protoplasm moves continuously from the old parts of the hypha towards the tip and so the hyphae continuously grow from one end and continuously age from the other; all the while the protoplasm shifts along from the aging part to the new. The hyphae of most fungi are divided at regular intervals by transversal septa, but these are not present, for example, in the hyphae of the Glomeromycota, except where they are used to isolate the hyphae's own dead or decaying regions. In any case, the functional subdivision of fungi into those with septa and those without is not so clear since fungal septa contain pores through which cytoplasm, and in some cases even nuclei, may pass. Therefore septate hyphae are composed of interconnecting compartments and function as integrated units. Inside the hyphae the cytoplasm and nuclei move and there may be varying numbers of them in each compartment separated by septa, from one to dozens of them, right up to tens of thousands of them in coenocytic fungi (without septa) (Gregory, 1984) (fig. 4).

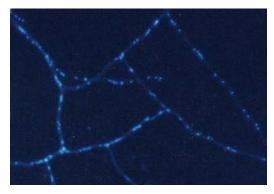


Fig. 4. Epifluorescent photo of the many nuclei present in the fungal hyphae of a coenocytic fungus, detected by staining with DAPI.

A distinctive aspect of fungi is the ability of their hyphae to fuse to form a closely inter-connected mycelium where the identities of the individual hyphae are lost in favour of the sharing of nutrients and genetic heritage: a fundamental characteristic allowing fungal colonies to resist environmental stresses (*Brasier*, 1992; Glass et al., 2004) (fig. 5).



Fig. 5. View of hyphal fusions that give rise to a closely interconnected fungal mycelium.

Fungi can reproduce both sexually and asexually. Asexual reproduction takes place by mitosis, with the production of spores that are dispersed in the air and then fall on an appropriate substrate where they germinate and the cycle may repeat. Sexual reproduction instead occurs after the fusion of two haploid cells, with the creation of one diploid cell. After meiosis and subsequent mitosis this cell develops single spores. In the Ascomycetes, these are formed inside closed receptacles called asci and are known as ascospores. In the Basidiomycetes they are found externally on structures called basidia and are called basidiospores (Carlile et al., 2001).

Fungi are chemoheterotrophic organisms and therefore need a source of organic nutrients from which to draw energy for their cellular metabolism. Given a simple energy source, such as glucose, many fungi can then absorb all the other cellular components they need from inorganic sources (ammonia or nitrate, phosphate and other minerals such as calcium, potassium, magnesium and iron). Their cell walls are composed of complex polysaccharides, such as chitin, and fungi can absorb simple soluble nutrients through their cell walls and membranes.

Thanks to their ability to produce extracellular enzymes, they can break down complex polymers such as cellulose and lignin so as to then reabsorb the simple sugars remaining. Fungi produce a wide range of enzymes that can degrade the most varied

and recalcitrant polymers such as lignin (Carlile et al., 2001).

Yeasts also belong to the kingdom of fungi; these are; mostly-unicellular organisms with globose or oval cells that measure 6-12 micrometres in diameter.

Yeasts may reproduce through budding, division or sexually by formation of asci or basidia and live in sugar-rich environments. The species most commonly used in the industrial fermentation process is *Saccharomyces cerevisiae*, the "brewer's yeast". Each industry will have its own selected strains, which are treated as genuine industrial secrets in the production of wine, beer, cider and bread

When yeasts reproduce asexually through the production of buds on the cell surface, each bud will grow until it reaches the same dimensions as the mother cell. At this stage the new cell will detach itself, leaving a scar that will be covered with chitin. Since chitin (the principal constituent of insects' and crustaceans' exoskeletons) is a polysaccharide with a rigid consistency. new buds cannot form at these chitin-covered scars and as a consequence, the mother cell, upon having produced as many budsto cover her whole surface with chitin, dies.

Yeasts have a special metabolism that allows them to exist in the presence of oxygen, by breathing, and also in oxygen's absence, by fermenting (Carlile et al., 2001).

Fungi are an important component of the ecosystem, instrumental in the continuation of biogeochemical cycles and represent the main agents in the decomposition of organic matter containing carbon, nitrogen, sulphur and phosphorus into mineral compounds that can be used again by plants.

In terms of the carbon cycle, the main components of organic matter to be decomposed are cellulose, hemicellulose and lignin, which make up around 70% of all material in plant cell walls. Fungi can completely break down cellulose by producing three principal enzymes: Endocellulase, which acts randomly inside the chain of cellulose, breaking up the molecules into smaller fragments; Exocellulase, which only acts at the ends of the cellulose chains. releasing cellobiose units: and the third enzyme: breaks cellobiase, which the disaccharide cellobiose into two molecules of glucose that can be absorbed by the fungus.

The three enzymes act synergistically and are carefully regulated to ensure that a fungus that degrades cellulose does not release sugars at higher rates than it can absorb them.

The regulation of cellulose degradation is achieved through a feedback system called catabolyte repression, in which genes that encode enzymes are repressed when readily usable substrates (including glucose) are available in the environment.

The most notable species of cellulolytic fungi are Chaetomium cellulolyticum, Humicola grisea and Trichoderma reesei. Fungi are also the only organisms capable of completely degrading lignin, a recalcitrant compound consisting of units of phenylpropane linked together by chemical bonds of different types. Among these, the most studied is the basidiomycete Phanerochaete chrysosporium, which is also capable of degrading other molecules which have a nature similar to lignin (Bosco et al., 2008a).

The mushrooms and truffles that we gather in the woods are none other than the sexual fructification of filamentous fungi that grow in soil, most of which live in close symbiosis with the roots of various forest plants such as chestnut, oak, beech, fir, larch, pine, hazel and linden.

To date we are unable to cultivate boletus, amanita, chantrelle, milkcap or russula mushrooms, or Caesar's mushrooms or white or black truffles: for these we must await until completion of their lifecycles inside the plant roots, which terminates with the production of their fruiting bodies and

frequently depends on the season and environmental conditions.

A series of studies have been using techniques to inoculate sterile plants with truffle spores, create symbiosis in the laboratory and then transplant the mycorrhizal plants into the field (Bosco et al., 2008b).

There are, of course, other mushrooms that do not live in symbiosis with plants and as such can be cultivated on an industrial scale and are available all year round, such as Agaricus bisporus (J. E. Lange) Imbach. These mushrooms are usually grown on inexpensive material such as straw and wood residues to which manure is added, and under well-controlled light conditions, temperature and humidity. After inoculation of the mycelium of the fungus obtained in pure culture invading hyphae start growing all over the substrate and after 3 weeks from sowing begin to produce fruiting bodies that can be collected, packaged and distributed to retailers. A mushroom mycelium obtained from an axenic culture is used to inoculate the substrate and the hyphae begin to grow. Three weeks after seeding, the fruiting bodies begin to grow and can be gathered, packaged and sent to resellers

Another mushroom produced on a large scale and particularly favoured in Japan and the Far East is *Lentinula edodes* (Berk.) Pegler ("Shetake"), which is able to degrade the cellulose content in trees. Small logs are hydrated by soaking in water and the fungal mycelium is put into pre-drilled holes in each log. After about a year the first batch of fruiting bodies appear (Carlile et al., 2001).

Fungi living in symbiosis with the roots of plants form associations called mycorrhizae (table 1). which can be found in around 90% of terrestrial plants: these symbioses involve over 6,000 species of fungi and 240,000 vegetable species. The two symbiotic organisms, the fungus and the plant, initiate a very close physiological, ecological and reproductive relationship that works to their mutual advantage. Fungi colonise the root without causing damage and get sugars, which they are unable to synthesise, and the plants receive mineral nutrients and water absorbed and translocated through the large hyphal network (known as the Wood Wide Web) that extends from the mycorrhizal roots to the surrounding ground and acts as a true auxiliary absorption system. Many types of mycorrhizae exist, with diverse morphological and physiological features, and they have colonised very diverse environments (Bosco et al., 2008b).

In our woodlands a large number of ectomycorrhiza can be found in forest plants such as the fir, larch, pine, birch, chestnut, beech, hazel and oak, and often their fruiting bodies (e.g. the well-known amanita, pinaroli, russula mushrooms and truffles) are visible to the naked eye.

In total ectomycorrhizal symbionts include over 500 species of mushrooms, among which we find members of the genera *Boletus*, *Lactarius*, *Russula*, *Suillus*, *Amanita*, *Paxillus*, *Morchella* and *Tuber*.

Previously, the number of fruiting bodies found in association with various plant species was considered indicative of the total number of fungal species in an ecosystem: in fact, molecular studies have shown that some species found in the roots produce few fruiting bodies, while other species producing many carpophores in the forest were rarely found among the roots of plants.

Table 1. Types of mycorrhiza, host plants and symbiont fungi.

Type of mycorrhiza	Host plant	Symbiont fungi
ectomycorrhiza	Evergreen forest plants and trees such as fir	About 5,000 species of fungi belonging to
	and pine and other forest trees such as beech,	Basidiomycota (Amanita, Boletus, Laccaria,
	chestnut and oak	Ascomycetes (<i>Tuber</i>) and Glomeromycota
		(Endogone)
Ecto- endomycorrhiza	Ectomycorrhizal plants (pine and larch),	Ascomycota, Basidiomycota, some
	Ericales (Arbutus, Arctostaphylos), and	ectomycorrhizae (Boletus, Laccaria)
	Monotropa and Pyrola	
Ericoid mycorrhiza	Some Ericales species such myrtle, heather,	Two species of Ascomycota, Hymenoscyphus
	Calluna, Rhododendron	ericae and Oiodendron maius
Orchid mycorrhiza	All species of orchids	Eight genera of Basidiomycota, belonging to the
•	•	genus Rhizoctonia
Arbuscular mycorrhiza	Bryophytes, Pteridophytes, Gymnosperms	Around 150 species of the phylum
-	and Angiosperms (around 80% of plant	Glomeromycota
	species)	

It seems then that just a few fungal species (fiveten) are alone able of colonizing around 50-70% of the roots of the plants on this Earth. Furthermore, some species form symbiosis with many species of forest plants (e.g. *Cenococcum geophylum* colonises approximately 150 host plant species), while others tend to associate with very few or only one type of host (for example, *Suillus luteus* is found only in black pine and *Suillus grevillei* in the larch tree) (*Bosco et al.*, 2008b).

In the same forests and woodlands ericoid mycorrhizae associate with such plants as myrtle, heather, Calluna and Rhododendron. Arbuscular mycorrhizae are the most commonly found mycorrhizal symbiosis in nature, associating with around 80% of plant species and most cultivated foodstuffs such as wheat, corn, barley, potatoes, tomatoes, vegetables, citrus fruits, grapes, olives, fruit trees, cotton, sugarcane, rubber tree and meadow flowers.

In this type of mutual relationship, the symbiont fungus forms characteristic branch-like structures called "arbuscules" inside the root cells of the host plant and it is through these structures that the exchange of nutrients between fungus and plant occurs.

Irrespective of the type of mycorrhiza, the plants that host the fungal symbionts in their roots demonstrate not only better growth, due to the improved absorption of minerals effected through the fungal hyphae that stretch between the root and surrounding ground, but also a higher tolerance of biotic and abiotic stresses, and therefore a general fitness far larger than plants devoid of these fungal symbionts (Giovannetti and Avio, 2002).

Recently, scientists have demonstrated that the sugars synthesised by a plant can be transported to other plants, even belonging to other species, if both plants share the same type of symbiotic fungus and if the two symbionts are joined by the same network of mycorrhizal fungal hyphae.

This demonstrates that mycorrhizal fungal symbionts, beyond absorbing and carrying mineral nutrients to the host plant, also have an important role to play in the redistribution of energy resources within plant communities: in fact, adult plants can transmit nutrients via the fungal network to younger plants, thus increasing their chances of survival (Simard et al., 1997).

The existence of networks of hyphae that explore the environment and act as a vehicle for nutrients seems to be of fundamental importance for plants. that must grow, develop and reproduce while anchored to the same spot. The importance of these subterranean fungal networks which link several plants together can be fully appreciated if we consider fungi's characteristic capacity for indefinite growth. Fungi can extend for hundreds of metres in every direction and in the most extraordinary case, reported by several North American researchers in 1992, one unique fungal individual was shown to colonise 15 hectares in a forest (Smith et al., 1992).

The mechanisms underlying the formation of these fungal networks are still little understood, despite recent data showing that hyphae originating from one individual are able to recognise and form anastomoses with the hyphae from another compatible individual and thus create networks of indefinite length (Giovannetti et al. 1999; 2001; 2004; 2006) (fig. 6).

In conclusion, studies on fungi have helped us to understand their importance in natural ecosystems and in agroecosystems: they are able to modify the availability, capture and use of soil resources, such as water and mineral nutrients, and to directly intervene in the trophic relationships of plant communities and in the regeneration of soil fertility.

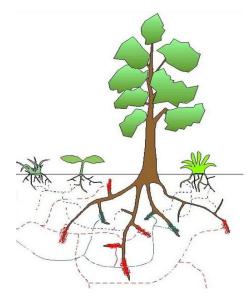


Fig 6. Graphic representation of the networks of fungal mycorrhizae connecting diverse plants.

2.3 Mushrooms and environments for growth

2.3.1 Introduction

The Italian Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) (Superior Institute for Environmental Protection and Research) inherited the role and responsibilities of the APAT (Agenzia per la Protezione dell'Ambiente e per i Servizi Tecnici) (Environmental Protection and Technical Services Agency), the 'ICRAM (Istituto Centrale

per la Ricerca Applicata al Mare) (Central Institute for Applied Maritimes Studies) and the INFS (Istituto Nazionale per la Fauna Selvatica) (National Wild Fauna Institute).

As part of the institutional activities of ISPRA's Dipartimento Difesa della Natura (Nature Defence Dept.) the "Special Mushrooms Project" was established to develop understanding and awareness of these ecosystem components, regarding which very few national-level studies had been previously carried out.

To date, one of the main studies carried out by ISPRA, in collaboration with, above all, the

Associazione Micologica Bresadola – Centro Studi Micologici (AMB-CSM) and other partners, constituted a census of Italian myxomycetes and macromycetes so as to compile a checklist of the national mycological flora which could then be used in developing mycological cartography.

A critical step in the acquisition of field data on comparable and acceptable national mycoflora is the classification of the habitats where fungi are found according to standardised systems recognised at a European level.

Thanks to their diffusion, different trophic forms and specific ecological characteristics, fungi can be used as indicators of biodiversity environmental quality (a series of monthly seminars on this subject has been taking place at ISPRA since 2007). Therefore it would be advantageous for fungi to be mentioned in environmental protection laws as soon as possible. As things currently stand, no fungal species are contained in the attachments to the Bern Convention (European Commission, 1982) or the Habitats Directive (European Commission, 1992) which detail the main acts of European legislation aimed at protecting wild species and their habitats. Furthermore, in Italy, unlike in the case of vascular flora, information regarding the links between fungal species and their habitat is sporadic and localised. Other difficulties have also arisen from attempts to link mycoflora to their Italian habitats:

- Great numbers of species
- Difficulties in understanding the taxonomy of many taxa.
- Carpophore phenology, the emergence cycles of which (from seasonal to multiannual) display an apparent absence of the species for years.

Such problems, given the overall importance this kingdom has within ecosystems can easily be overcome by increasing understanding of the role that mycoflora play in these same ecosystems. The acquisition of such understanding, especially in the light of the dearth of institutional studies on the matter, should be considered a national priority and, consequently, supported by adequate financial resources to allow further research in this field.

In recent years the role that fungi play as natural ecosystem regulators has been recognised at a European level and as such ever more attention is being paid to mycoflora. We have, in fact, seen Red Lists of mushrooms gaining greater diffusion in at least 35 EU member states. In August 2003, for

example, a report was presented by the Swedish Environmental Protection Agency (Naturvårdsverket) and by the European Council for the Conservation of Fungi (ECCF) to the Environment Directorate-General of the European Commission (Dahlberg et al., 2003). That document proposes the inclusion of 33 European fungal species into Appendix 1 of the Bern Convention and into the Habitats Directive. The species recommended for inclusion are rare in Europe and are already contained in some countries' Red Lists. These 33 species are only a fraction of the threatened varieties throughout Europe, but the document represents a first step towards official recognition of the importance of mycoflora and of its conservation by the European Commission.

In countries where research in this field is more developed it has been recognised that macromycetes are threatened to a far greater degree than vascular flora. In Switzerland, 32% of the macromycete species recorded in the country have been placed on the Red List (Senn-Irlet et al., 2007). The endangered species are concentrated mainly in dry grasslands and swamps.

These data, extrapolated to cover the Italian ecological situation, underline the necessity of taking rapid action to build awareness of mycoflora and to make focused interventions to protect it. With the aim of extending awareness and establishing monitoring systems across the national territory as part of an international network, the Special Mushrooms Project involved a systematic data-collection drive which specifically sought to associate the environments where mycoflora was found in Italy with European classification systems governing soil use [CORINE Land Cover (APAT, 2005)] and biotopes [CORINE Biotopes (AAVV, 1991), EUNIS (Davies et al., 2004), NATURA 2000 (European Commission, 2007)]. Beyond enabling us to learn more about the ecology of various environments of national and European Community interest, this laid the foundations for the use of fungal species as possible ecological indicators in varying thematic cartography projects. These in turn make possible global biodiversityevaluation and other nature conservation initiatives.

2.3.2 Materials and methods

Based on the mycological lists currently available from the Special Mushrooms Project and from those records which were comparable to our classification systems, it has been possible to construct a database which correlates fungal species to their habitats.

The lists used at this juncture were:

- Database of heavy metals in macromycete, edited by L. Cocchi and L. Vescovi, from the Gruppo Micologico e Naturalistico "R. Franchi" di Reggio Emilia - AMB (4.956 records).
- Database of species deposited in the Herbarium mycologicum at the Natural History Civic Museum of Venice, edited by G. Robich and M. Castoldi, Società Veneziana di Micologia - AMB, 21.823 records).
- ISPRA Database (edited by C. Siniscalco, ISPRA - Gruppo Micologico dell'Etruria Meridionale - AMB, 5.334 records).
- For the genus *Russula*, Sarnari's monograph (2000).
- For alpine fungi, works by Bizio, Campo, (1999) and Jamoni (2008) regarding alpine and sub-alpine flora.
- For dune environments, works by Monti et al. (2000) (Tuscany) and Lantieri (2003) (Sicily).

The fields currently in the database, always expandible, are:

- Nomenclature: Genus, Species, Variety.
- Ecology: trophic features, host plants, habitats
- Geography: area, municipality, province, altitude, latitude, longitude.
- Habitat: CORINE Land Cover third and fourth level, CORINE Biotopes third level, fourth level and fifth level, Natura 2000.
- Sample data: collector, identifier, date.

Statistical analyses regarding the percentage of occurrence for each type of habitat were carried out.

First, data regarding the occurrence of each species in different habitats of the same area were analysed while excluding those data pertaining to recordings of the same species in the same type of habitats in that area.

Cluster analysis was used to group the CORINE Biotopes – fourth level data – with regards to the distribution and frequency of mycological species'

occurrence. To this end, the Minitab software was used. We applied single-linkage, complete-linkage and Ward methods to the Manhattan and Euclidean distances calculated on standardised values. Bonds that continued to repeat themselves during analysis were identified. Finally, based on the ordered tables, "characteristic species" and "differential species" were derived for each category of habitat.

2.3.3 Results

We employed a process of upscaling that better enables mycological components to be classified at the various levels into which the Italian ecological environment may be separated. We present here the comparative and statistical analyses, following their hierarchical classification. The species have been ordered according to the frequency they occurred in the records regarding the diverse habitats analysed.

2.3.3.1 CORINE Land Cover, Third level

Using the CORINE Land Cover (Third level) categories, the species-habitat association provides macro-level information.

This type of classification made it possible to obtain useful information on the distribution of macromycetes even across densely anthropic areas, and has furthermore enabled a broad spectrum analysis of species common to the coniferous and deciduous forests. The species were ranked according to the frequency they occurred in the records regarding the diverse habitats analysed.

Land Cover code, third level: 1.4.1. Green urban areas

(508 records, 158 species)

Agaricus bitorquis (Quél.) Sacc.; Agaricus campestris L.; Agaricus bresadolanus Bohus; Agaricus xanthodermus Genev.; Coprinus comatus (O.F. Müll.) Pers.; Inocybe rimosa (Bull.) P. Kumm.; Lepista sordida (Schumach.) Singer; Lepiota subincarnata J. E. Lange; Leucoagaricus leucothites (Vittad.) Wasser; Lyophyllum decastes (Fr.) Singer; Mitrophora semilibera (DC.) Lév.; Morchella hortensis Boud.; Psathyrella candolleana (Fr.) Maire; Russula ochrospora (Nicolaj ex Quadr. & W. Rossi) Quadr.; Boletus rubellus Kromb.

Lignicolous species:

Agrocybe cylindracea (DC.) Gillet; Armillaria mellea (Vahl) P. Kumm.; Flammulina velutipes (Curtis) Singer; Pleurotus ostreatus (Jacq.) P. Kumm.; Polyporus squamosus (Huds.) Fr.

Land Cover code, third level: 3.2.1. Natural grasslands

(907 records, 281 species)

Marasmius oreades (Bolton) Fr.; Lycoperdon utriforme Bull.; Agaricus macrocarpus (F. H. Møller) F. H. Møller; Agaricus campestris L.; Amanita vittadini (Moretti) Sacc.; Leucoagaricus leucothites (Vittad.) Wasser; Volvariella gloiocephala (DC.) Boekhout & Enderle; Agaricus arvensis Schaeff.; Helvella crispa (Scop.) Fr.; Agaricus xanthodermus Genev.; Hygrocybe ingrata J. P. Jensen & F. H. Møller; Hygrocybe conica (Schaeff.) P. Kumm.; Hygrocybe quieta (Kühner) Singer; Hygrocybe calyptriformis (Berk.) Fayod; Hygrocybe pratensis (Fr.) Murrill; Macrolepiota procera (Scop.) Singer; Hygrocybe nitrata (Pers.) Wünsche; Hygrocybe psittacina (Schaeff.) P. Kumm.; Panaeolina foenisecii (Pers.) Maire; Hvgrocvbe ceracea (Wulfen) P. Kumm.: Hygrocybe punicea (Fr.) P. Kumm.; Hygrocybe irrigata (Pers.) Bon; Inocybe fraudans (Britzelm.) Sacc.; Calvatia gigantea (Batsch) Lloyd; Laccaria laccata (Scop.) Cooke; Coprinus comatus (O. F. Müll.) Pers.; Entoloma mougeotii (Fr.) Hesler; Hygrocybe citrinovirens (J. E. Lange) Jul. Schäff.; Hygrocybe coccinea (Schaeff.) P. Kumm.; rhacodes Chlorophyllum (Vittad.) Vellinga; Lycoperdon pratense Pers.

Land Cover code, third level: 3.1.1. Broad-leaved forest

(2,653 records, 590 species)

Boletus subtomentosus L.; Cantharellus cibarius Fr.; Russula vesca Fr.; Russula cyanoxantha (Schaeff.) Fr.; Boletus rhodopurpureus Smotl.; Amanita caesarea (Scop.) Pers.; Boletus reticulatus Schaeff.; Boletus calopus Pers.; Boletus luridus Schaeff.; Boletus edulis Bull.; Boletus pinophilus Pilát & Dermek; Amanita rubescens Pers.; Cortinarius caperatus (Pers.) Fr.; Amanita muscaria (L.) Lam; Mitrophora semilibera (DC.) Lév.; Verpa bohemica (Krombh.) J. Schröt.; Russula nigricans (Bull.) Fr.; Amanita phalloides (Vaill. ex Fr.) Link; Infundibulicybe geotropa (Bull.) Harmaja; Clitocybe nebularis (Batsch) P. Kumm.; Lactarius piperatus (L.) Pers.; Boletus

appendiculatus Schaeff.; Russula delica Fr.; Morchella esculenta (L.) Pers.; Russula acrifolia Romagn.; Amanita vaginata (Bull.) Lam.; Calocybe gambosa (Fr.) Donk; Russula chloroides (Krombh.) Bres.; Amanita pantherina (DC.) Krombh.: Boletus aereus Bull.: **Boletus** Alessio; pulchrotinctus Fistulina hepatica (Schaeff.) With.; Russula albonigra (Krombh.) Fr.; Agaricus silvicola var. silvicola (Vittad.) Peck; Armillaria tabescens (Scop.) Emel; Boletus queletii Schulzer; Boletus satanas Lenz; Gymnopus fusipes (Bull.) Gray; Entoloma sinuatum (Bull.) P. Kumm.; Boletus rubellus Krombh.

Land Cover code, third level: 3.1.2. Coniferous forest (including aforestation)

(1,040 records, 417 species)

Agaricus silvicola var. silvicola (Vittad.) Peck; Amanita muscaria (L.) Lam; Amanita rubescens Pers.; Boletus calopus Pers.; Boletus edulis Bull.; Boletus erythropus Pers.; Boletus pinophilus Pilát & Dermek; Craterellus lutescens (Fr.) Fr.; Chalciporus piperatus (Bull.) Bataille: Chroogomphus rutilus (Schaeff.) O. K. Mill.: Clitocybe gibba (Pers.) P. Kumm.; Clitocybe nebularis (Batsch) P. Kumm.: Clitopilus prunulus (Scop.) P. Kumm.; *Rhodocollybia butyracea* (Bull.) Lennox; Entoloma hirtipes (Schumach.) M. M. Moser; Geopora arenosa (Fuckel) S. Ahmad; Hebeloma laterinum (Batsch) Vesterh.: Hygrophorus agathosmus (Fr.) Fr.; Hygrophorus latitabundus Britzelm.; Hygrophorus marzuolus (Fr.) Bres.; Inocybe arenicola (R. Heim) Bon; Inocybe bongardii (Weinm.) Quél.; Inocybe dunensis P. D. Orton; Catathelasma imperiale (Fr.) Singer; *Inocybe geophylla* (Fr.) P. Kumm.; *Inocybe* mixtilis (Britzelm.) Sacc.; Inocybe nitidiuscula (Britzelm.) Lapl.; Inocybe cincinnata var. major (S. Petersen) Kuyper; Inocybe piceae Stangl & Schwöbel; Inocybe fraudans (Britzelm.) Sacc.; Inocybe rimosa (Bull.) P. Kumm.; Inocybe splendens R. Heim; Lactarius chrysorrheus Fr.: deliciosus (L.) Gray; Lactarius Lactarius deterrimus Gröger; Lactarius salmonicolor R. Heim & Leclair; Lactarius sanguifluus (Paulet) Fr.; Lactarius scrobiculatus (Scop.) Fr.; Lycoperdon perlatum Pers.; Mycena pura (Pers.) P. Kumm.; Rhizopogon roseolus (Corda) Th. Fr.; Cortinarius caperatus (Pers.) Fr.; Russula torulosa Bres.; Sarcosphaera coronaria (Jacq.) J. Schröt.; Suillus bellinii (Inzenga) Watling; Suillus collinitus (Fr.) Kuntze; Suillus granulatus (L.) Roussel; Suillus

luteus (L.) Roussel; Suillus mediterraneensis (Jacquet. & J. Blum) Redeuilh; Suillus variegatus (Sw.) Kuntze; Tricholoma myomyces (Pers.) J. E. Lange; Tricholoma scalpturatum (Fr.) Quél.; Tricholomopsis rutilans (Schaeff.) Singer; Boletus badius (Fr.) Fr.

The comparison between the lists at this level has allowed us to extrapolate a great deal of ubiquitous species listed below. Knowledge of these species has facilitated the interpretation of lists derived from the application of the Nature 2000, EUNIS and CORINE Biotopes classification systems, allowing a "cleaning up" of the species characteristics and differentials identification tables.

Strongly ubiquitous species (found both in forests and meadows)

(3,693 records, 950 species)

Lycoperdon utriforme Bull.; Inocybe fraudans (Britzelm.) Sacc.; Agaricus arvensis Schaeff.; Helvella crispa (Scop.) Fr.; Cantharellus cibarius Fr.; Macrolepiota procera (Scop.) Singer; Russula cvanoxantha (Schaeff.) Fr.; Hygrocybe conica (Schaeff.) P. Kumm.; Calocybe gambosa (Fr.) Donk; Hygrocybe quieta (Kühner) Singer; Clitocybe nebularis (Batsch) P. Kumm.; Boletus luridus Schaeff.; Lactarius deliciosus (L.) Gray; Infundibulicybe geotropa (Bull.) Harmaja; Boletus reticulatus Schaeff.; Agaricus silvicola var. silvicola (Vittad.) Peck; Lepista nuda (Bull.) Cooke; Laccaria laccata (Scop.) Cooke; Mycena pura (Pers.) P. Kumm.; Clitocybe gibba (Pers.) P. Kumm.; Lycoperdon perlatum Pers.; Amanita vaginata (Bull.) Lam.; Lepista flaccida (Sowerby) Pat.; Lepista sordida (Schumach.) Singer; Boletus ferrugineus Schaeff.; Inocybe splendens R. Heim; Phallus impudicus L.; Russula virescens (Schaeff.) Fr.; Bovista aestivalis (Bonord.) Demoulin; Paxillus involutus (Batsch) Fr.; Lyophyllum decastes (Fr.) Singer; Amanita crocea (Quél.) Singe; Morchella elata Fr.; Clitocybe phyllophila (Pers.) P. Kumm.; Clavulina coralloides (L.) J. Schröt.; Inocybe inodora Velen.; Schizophyllum commune Fr.; Hygrocybe persisitens (Britzelm.) Singer; Marasmius oreades (Bolton) Fr.; Agaricus macrocarpus (F. H. Møller) F. H. Møller; Agaricus campestris; Suillus collinitus (Fr.) Kuntze; Leucoagaricus leucothites (Vittad.) Wasser; Lactarius deterrimus Gröger; Agaricus xanthodermus Genev.; Inocybe mixtilis (Britzelm.) Sacc.; Cortinarius praestans Cordier; Hygrocybe

nitrata (Pers.) Wünsche; Boletus rubellus Krombh.; Morchella esculenta (L.) Pers.; Chlorophyllum rhacodes (Vittad.) Vellinga; Coprinus comatus (O. F. Müll.) Pers.; Calvatia gigantea (Batsch) Lloyd; Inocybe piceae Stangl & Schwöbel; Clavulina rugosa (Bull.) J. Schröt.; Russula delica Fr.; Entoloma mougeotii (Fr.) Hesler; Chroogomphus rutilus (Schaeff.) O. K. Mill.; Russula torulosa Bres.; Clitocybe rivulosa (Pers.) P. Kumm.; Leccinum duriusculum (Schulzer ex Kalchbr.) Singer; Leucoagaricus barssii (Zeller) Vellinga; Agaricus osecanus Pilát; Gymnopus fusipes (Bull.) Gray; Lycoperdon excipuliforme (Scop.) Pers.; Boletus dryophilus Thiers; Entoloma incanum (Fr.) Hesler; Agaricus augustus Fr.; Hygrophorus hypothejus (Fr.) Fr.

Species ubiquitous to forests

(3,693 records, 949 species)

Amanita muscaria (L.) Lam; Boletus pinophilus Pilát & Dermek; Boletus calopus Pers.; Amanita rubescens Pers.; Cortinarius caperatus (Pers.) Fr.; Russula vesca Fr.; Boletus erythropus; Chalciporus piperatus (Bull.) Bataille; Inocybe rimosa (Bull.) P. Kumm.; Amanita phalloides (Vaill. ex Fr.) Link;; Clitopilus prunulus (Scop.) P. Kumm.: Tricholoma scalpturatum (Fr.) Quél.; Russula foetens (Pers.) Pers.; Gyromitra gigas (Krombh.) Cooke; Lactarius piperatus (L.) Pers.; Inocybe geophylla (Fr.) P. Kumm.; Sarcosphaera coronaria (Jacq.) J. Schröt.; Tricholoma myomyces (Pers.) J.E. Lange; Pluteus cervinus (Schaeff.) P. Kumm.; Lactarius chrysorrheus Fr.; Amanita pantherina (DC.) Krombh.; Russula albonigra (Krombh.) Fr.; Hydnum repandum L.; Russula acrifolia Romagn.; Rhodocollybia butyracea (Bull.) Lennox; Tricholoma saponaceum (Fr.) P. Kumm.; Xerula radicata (Relhan) Dörfelt; Gyroporus castaneus (Bull.) Quél.; Rhodocybe gemina (Fr.) Kuyper & Noordel.; Amanita citrina (Pers.) Pers.; Amanita excelsa (Fr.) P. Kumm.; Clitocybe odora (Bull.) P. Kumm.; Hebeloma laterinum (Batsch) Vesterh.; Rhizopogon roseolus (Corda) Th. Fr.; Russula fragilis Fr.; Russula romellii Maire; Gymnopus dryophilus (Bull.) Murrill; Lactarius volemus (Fr.) Fr.; Tricholoma columbetta (Fr.) P. Kumm.; Boletus chrysenteron Bull.; Boletus pruinatus Fr. & Hök; Kuehneromyces mutabilis (Schaeff.) Singer & A. H. Sm.; Tricholoma sulphureum (Bull.) P. Kumm.; Hypholoma fasciculare (Huds.) P. Kumm.; Trametes versicolor (L.) Lloyd; Inocybe leucoblema Kühner; Suillus lakei (Murrill) A. H.

Sm. & Thiers; Tricholoma imbricatum (Fr.) P. Kumm.; Ramaria pallida (Schaeff.) Ricken: Boletus armeniacus Quél.; Tapinella atrotomentosa (Batsch) Šutara; Auricularia auricula-judae (Bull.) Berk.; Hebeloma sinapizans (Fr.) Sacc.; Russula risigallina (Batsch) Sacc.; Craterellus tubaeformis (Schaeff.) Ouél.: Helvella acetabulum (L.) Ouél.: Amanita submembranacea (Bon) Cortinarius laniger Fr.; Lactarius pallidus Pers.; Lepiota ignivolvata Bousset & Joss. ex Joss.; Stropharia aeruginosa (Curtis) Quél.; Trametes pubescens (Schumach.) Pilát; Tricholoma orirubens Quél.; Hydnum rufescens Pers.; Lactarius vellereus (Fr.) Fr.; Russula heterophylla (Fr.) Fr.; Hydnum albidum Peck; Inocybe oblectabilis (Britzelm.) Sacc.; Lepiota clypeolaria (Bull.) P. Kumm.; Tricholoma portentosum (Fr.) Ouél.; Amanita gemmata (Fr.) Bertill.; Coprinopsis picacea (Bull.) Redhead, Vilgalys & Moncalvo; Lyophyllum rhopalopodium Clémençon; Gymnopilus penetrans (Fr.) Murrill; Inocybe erubescens A. Blitt; Lactarius uvidus (Fr.) Fr.; Ramaria botrytis (Pers.) Ricken; Ramaria gracilis (Pers.) Ouél.; Russula amoena Ouél.; Russula parazurea Jul. Schäff.; Tricholoma batschii Gulden; Agaricus urinascens (Jul. Schäff. & F. H. Møller) Singer: Russula luteotacta Auriscalpium vulgare Gray; Hypholoma capnoides (Fr.) P. Kumm.; Limacella guttata (Pers.) Konrad & Maubl.; Paxillus filamentosus Fr.; Pholiota squarrosa (Bull.) P. Kumm.; Polyporus lepideus Fr.; Ramaria stricta (Pers.) Quél.; Russula persicina Krombh.

2.3.3.2 CORINE Biotopes Third level

Use of the third level of the CORRINE Biotopes classification system characterises several habitats of particular importance and interest for the European Community.

45.2 Cork (Habitat Natura 2000: 9330 Forests of *Quercus suber*)

(221 records, 100 species)

Characteristic and differential species:

Amanita ponderosa Malençon & R. Heim; Boletus pseudoregius (Heinr. Huber) Estadès; Gymnopilus suberis (Maire) Singer; Plectania platensis (Speg.) Rifai; Russula albonigra (Krombh.) Fr.; Trichaptum biforme (Fr.) Ryvarden.

45.3 Ilex (*Habitat* Natura 2000: 9340 Forests of *Quercus ilex* and *Quercus rotundifolia*)

(198 records, 75 species)

Characteristic and differential species:

Boletus aemilii Barbier; Boletus pulchrotinctus Alessio; Boletus rhodoxanthus (Krombh.) Kallenb.; Russula ilicis Romagn., Chevassut & Privat (Fig. 7 (Chiari et al., 2008)); Leccinellum lepidum (Bouchet ex Essette) Bresinsky & Manfr. Binder.



Fig. 7. Russula ilicis Romagnesi, Chevassut & Privat (Photo: Maurizio Chiari).



Fig. 8. Real distribution of *Russula ilicis* Romagnesi, Chevassut & Privat in the Province of Venice obtained by associating location data from mycological records and the polygons from the Carta della Natura (*Nature Map*) (ISPRA; 2008) of the relative habitats (ilex: CORINE Biotopes 45.3; Natura 2000).

41.9 Chestnut (Habitat Natura 2000: 9260 Forests of *Castanea sativa*)

Frequent species: Cantharellus cibarius Fr.; Russula vesca Fr.; Amanita caesarea (Scop.) Pers.; Boletus reticulatus Schaeff.; Boletus subtomentosus L.; Fistulina hepatica (Schaeff.) With.; Russula cyanoxantha (Schaeff.) Fr.; Lactarius piperatus (L.) Pers.; Gymnopus fusite (Bull.) Gray; Amanita phalloides (Vaill. ex Fr.) Link; Amanita rubescens Pers.; Boletus edulis Bull.; Lactarius volemus (Fr.) Fr.; Ramaria formosa (Pers.) Quél.

42.8 Mediterranean Pine forests (Habitat Natura 2000: 9540 Mediterranean pine forests with endemic Mesogean pines)

(143 records, 75 species)

Characteristic and differential species:

Buchwaldoboletus lignicola (Kallenb.) Pilát; Suillus mediterraneensis (Jacquet. & J. Blum) Redeuilh; Mycena seynesii Quél.; Rhizopogon roseolus (Corda) Th. Fr.; Boletus obscuratus (Singer) J. Blum.

Frequent species:

Lactarius sanguifluus (Paulet) Fr.; Cantharellus cibarius Fr.; Lactarius deliciosus (L.) Gray; Suillus collinitus (Fr.) Kuntze; Tricholoma saponaceum (Fr.) P. Kumm.

At this level by superimposing the Habitats Map and the Nature Map (Various Authors, 2004; AAVV, 2009th; AAVV, 2009b) with the maps of

the stations where the mycological recordings were made, one obtains the real and potential distribution maps (fig. 8 and fig. 10).

2.3.3.3 CORINE Biotopes Fourth level

At the fourth level of the CORINE Biotopes system it is possible to identify other leading species, especially in those environments considered as being of particular ecological importance at European level (Dir. 92/43 CEE).

16.27 Dune juniper thickets and woods (Habitat Natura 2000: 2250 Coastal dunes with *Juniperus* spp. – Priority)

(214 records, 94 species)

Characteristic and differential species:

Geastrum minimum Schwein.; Geastrum schmidelii Vittad.; Helvella juniperi M. Filippa & Baiano; Marcelleina atroviolacea (Delile ex De Seynes) Brumm.; Melanoleuca rasilis (Fr.) Singer; Pithya cupressi (Batsch) Fuckel.

Frequent species:

Geopora arenicola (Lév.) Kers; Greletia planchonis (Dunal ex Boud.) Donadini; Inocybe dulcamara (Alb. & Schwein.) P. Kumm.; Pustularia patavina (Cooke & Sacc.) Boud.; Octospora convexula (Pers.) L. R. Batra; Xerula mediterranea (Pacioni & Lalli) Quadr. & Lunghini.

16.29 Wooded dunes (Habitat Natura 2000: 2270 Dunes with forests of *Pinus pinea* and/or *Pinus pinaster* – **Priority)** (126 records, 73 species)

Characteristic and differential species: Inocybe pseudodestricta Stangl & J. Veselský; Inocybe psammobrunnea Bon (Fig. 9); Melanoleuca microcephala (P. Karst.) Singer; Rhizopogon luteolus Fr. & Nordholm.



Fig. 9. Inocybe psammobrunnea Bon (Photo: M. Marchetti).

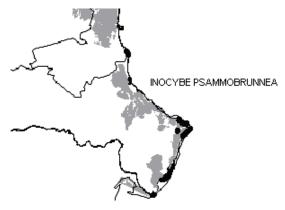


Fig. 10: Real distribution of *Inocybe psammobrunnea* Bon in the Province of Rovigo obtained by associating location data from mycological records and the polygons from the Carta della Natura (*Nature Map*) (ISPRA; 2008) of the relative habitats (wooded dunes: CORINE Biotopes 16.29, *Habitat* Natura 2000: 2270 - Priority).

Very common species:

Geopora arenosa (Fuckel) S. Ahmad; Inocybe arenicola (R. Heim) Bon; Inocybe dunensis P. D. Orton; Inocybe heimii Bon; Inocybe inodora Velen.; Inocybe dulcamara (Alb. & Schwein.) P.

Kumm.; *Melanoleuca rasilis* (Fr.) Singer; *Inocybe rufuloides* Bon.

36.11 Boreo-Alpic acid snow-patch communities (Habitat Natura 2000: 6150 Siliceous alpine and boreal grasslands)

(90 records, 88 species)

Characteristic and differential species:

Hebeloma bruchetii Bon; Octospora humosa (Fr.) Dennis; Naucoria tantilla J. Favre; Cortinarius cinnamomeoluteus P. D. Orton; Cortinarius anomalus (Pers.) Fr.; Entoloma papillatum (Bres.) Dennis; Galerina pseudotundrae Kühner; Helvella queletii Schulzer; Inocybe bulbosissima (Kühner) Bon; Inocybe giacomi O. K. Mill.; Laccaria montana Singer; Lactarius dryadophilus Kühner; Peziza alaskana E. K. Cash; Russula laccata Huijsman; Russula saliceti cola (Singer) Kühner ex Knudsen & T. Borgen; Scutellinia kerguelensis (Berk.) Kuntze; Scutellinia superba (Velen.) Le Gal.

Frequent species:

Cortinarius favrei D. M. Hend.; Helvella corium (O. Weberb.) Massee; Inocybe salicis-herbaceae Kühner; Mycena pura (Pers.) P. Kumm.; Helvella lacunosa Afzel.

36.12 Boreo-Alpic calcareous snow-patch communities (*Habitat* Natura 2000: 6170 Alpine and subalpine calcareous grasslands)

Characteristic and differential species:

Helvella alpestris Boud.; Cortinarius chamaesalicis Bon; Cortinarius phaeochrous J. Favre; Hebeloma alpinum (J. Favre) Bruchet; Helvella capucina Quél.; Helvella solitaria (P. Karst.) P. Karst.; Inocybe albovelutipes Stangl; Inocybe canescens J. Favre; Inocybe favrei Bon;

Inocybe geraniodora J. Favre; Inocybe lacera (Fr.) P. Kumm.; Inocybe splendens var. phaeoleuca (Kühner) Kuyper; Inocybe subbrunnea Kühner; Inocybe taxocystis (J. Favre & E. Horak) Senn-Irlet; Inocybe umbrinodisca Kühner; Lactarius salicis-reticulatae Kühner; Peziza saniosa Schrad.; Russula subrubens (J. E. Lange) Bon; Russula nana Killerm.

Other Frequent species:

Inocybe fraudans (Britzelm.) Sacc.; Inocybe godfrinioides Kühner; Inocybe calamistrata (Fr.) Gillet; Inocybe nitidiuscula (Britzelm.) Lapl.; Tricholoma scalpturatum (Fr.) Quél.

44.61 Mediterranean riparian poplar forests. (Habitat Natura 2000: 9240 Quercus faginea and Quercus canariensis Iberian woods)

Characteristic and differential species:

Helvella spadicea Schaeff.; Inocybe leucoblema Kühner; Lactarius controversus (Pers.) Pers.; Leccinum nigellum Redeuilh; Pholiota populnea (Pers.) Kuyper & Tjall.-Beuk.; Tricholoma populinum J. E. Lange.

Frequent species:

Leccinum duriusculum (Schulzer ex Kalchbr.) Singer; Morchella esculenta (L.) Pers.; Pluteus cervinus; Mitrophora semilibera (DC.) Lév.

Even at this level it is possible to match the mycological data with the Natura Map (AAVV, 2004; AAVV, 2009a; AAVV, 2009b) so as to obtain distribution data for each species in their relative habitats.

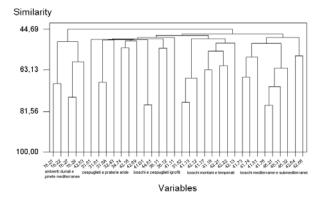


Fig. 11: Cluster analysis over 33 CORINE Biotopes categories, Fourth level and 975 species of macromycetes.

2.3.3.4 CORINE Biotopes Fifth level

Using the fifth level where possible, particularly relating to catenal ecological series, it was possible to achieve a greater level of detail, especially regarding the interpretation of dune environments, which, as also shown in figure 11, are significantly distinct from other habitats in terms of macromycete populations:

16.211 Embryonic dunes (Habitat Natura 2000: 2110 Embryonic shifting dunes)

Frequent species:

Cyathus stercoreus (Schwein.) De Toni; Diderma spumarioides (Fr.) Fr.; Geopora arenosa (Fuckel) S. Ahmad; Pustularia patavina (Cooke & Sacc.) Boud.; Psathyrella ammophila (Durieu & Lév.) P. D. Orton; Rhodocybe malençonii Pacioni & Lalli.

16.212 Biscay grey dunes (Habitat Natura 2000: 2120 Shifting dunes along the shoreline with Ammophila arenaria "white dunes")

Characteristic and differential species:

Agaricus menieri Bon; Agrocybe pediades (Fr.) Fayod; Gyrodon lividus (Bull.) Fr.; Agaricus aridicola Geml, Geiser & Royse; Gyrophragmium delilei Mont.; Hygrocybe persistens (Britzelm.) Singer; Lepiota brunneolilacea Bon & Boiffard; Marasmius oreades (Bolton) Fr.; Melanoleuca cinereifolia (Bon) Bon; Montagnea arenaria (DC.) Zeller; Panaeolus cinctulus (Bolton) Sacc.

Other frequent species:

Agrocybe pediades (Fr.) Fayod; Psathyrella ammophila (Durieu & Lév.) P. D. Orton; Peziza pseudoammophila Bon & Donadini.

16.221 Northern Atlantic grey dunes (Habitat Natura 2000: 2130 Fixed coastal dunes with herbaceous vegetation "grey dunes", Priority)

Characteristic and differential species:

Arrhenia spathulata (Fr.) Redhead; Xerula mediterranea (Pacioni & Lalli) Quadr. & Lunghini; Clitocybe barbularum (Romagn.) P. D. Orton; Rhizopogon roseolus (Corda) Th. Fr.; Tulostoma brumale Pers.

Frequent species:

Geopora arenosa (Fuckel) S. Ahmad; Hebeloma ammophilum Bohus.

16.223 Ibero-Mediterranean grey dunes (Habitat Natura 2000: 2210 Crucianellion maritimae fixed beach dunes)

Characteristic and differential species:

Marasmius anomalus Peck; Peziza boltonii Quél.; Galerina laevis (Pers.) Singer; Arrhenia retiruga (Bull.) Redhead; Clitocybe barbularum (Romagn.) P. D. Orton; Gymnopus aquosus (Bull.) Antonín & Noordel.; Conocybe blattaria (Fr.) Kühner; Conocybe leucopus Kühner ex Kühner & Watling; Conocybe rickeniana P. D. Orton; Coprinus xanthothrix Romagn.; Crinipellis scabella (Alb. & Schwein.) Murrill; Hydnocystis piligera Tul.; Hygrocybe conica (Schaeff.) P. Kumm.; Mucilago crustacea P. Micheli ex F. H. Wigg.; Octospora leucoloma Hedw.; Pachyella celtica (Boud.) Häffner; Peziza varia (Hedw.) Fr.

Frequent species:

Agrocybe pediades (Fr.) Fayod; Cyathus olla (Batsch) Pers.; Peziza pseudoammophila Bon & Donadini; Volvariella gloiocephala (DC.) Boekhout & Enderle.

2.3.4 Discussion

Mycological communities, as can be seen in fig. 11 are far more differentiated than the natural habitats in which they reside; this can also be seen in the specificity of the relationships between plants and fungi.

The CORINE Land Cover system, by using wider units to classify vegetation, simplifies linking species to habitats and enables the use of a greater volume of information; This renders ecological and biogeographical analyses less detailed, but it also allows us to create a map on a scale of 1:100,000 for the entire Italian territory which is comparable to analogous territorial analyses at a European level.

The CORINE Biotopes system, instead, makes possible a connection to the national project called "Carta della Natura" (Nature Map) (AAVV, 2004; AAVV, 2009a; AAVV, 2009b), which is itself useful for determining the real distribution of macromycetes but which could also be used to map out the potential distribution of fungal species.

The availability of data regarding mycological diversity in the CORINE Biotopes and EUNIS categories also allows us to gather more useful

information for a full evaluation of habitat diversity and the vulnerability of these habitats.

During the creation of the mycological datacards, available from ISPRA, several fundamental guidelines for their use in high-level CORINE and EUNIS classification schemes were established. Beyond the data normally contained in mycological datacards, it was found to be essential to:

- For mixed consortia, besides the dominant plant species, state the codominant ones also, both in the canopy, and the shrub layer and ground cover. Usually, three or four species are sufficient to identify the habitat.
- Increase as far as possible the site information with geological substrata information, height and geographic location (WGS 84 for the Carta della Natura and CORINE Land Cover).
- For garigue and mediterranean undergrowth formations, indicate the height of natural surface levels, as the CORINE Biotopes and EUNIS categories distinguish between high undergrowth (>3m) low undergrowth (1-3 m) and garigue (< 1 m).
- For conifers, it is essential to establish whether they are in a natural forest or a reforestation (in the latter case, check whether the installation was done with native or alien species). For this type of analysis prior knowledge of the natural formations in the analysed area is necessary.

2.4 Mushrooms as a soil quality bioindicator

2.4.1 Introduction

2.4.1.1 The quality of the soil-system

The environmental quality of an area or territory can be estimated by the use of effective indicators whereby these indicators are designated as instruments capable of representing particular environmental conditions.

The quality of a selected environmental system cannot be described by one indicator alone, but usually needs a combination of different indicators which work over different scales and which therefore each have a different weight in the overall analysis (Benedetti et al., 2006).

A good indicator needs to have certain characteristics that guarantee representativity, accessibility, trustworthiness and operability. Each indicator has to furthermore guarantee a certain level of political relevance and utility, analytical validity and measurability (OECD, 1999).

The need of turning to synthetic indicators to establish soil quality stems from the fact that, being a very complex system, often, through objective difficulties in its measurement, its importance tends to be overlooked. This has led to a decrease of over 10% in the productive capacity of cultivated land worldwide since the early '80s as soil erosion, pollution, aggressive modern farming methods, grazing, salification and, above all, desertification due to the loss of organic substances and biodiversity all take their toll.

Among the various definitions of soil quality, one of the most widely-accepted is that of Doran and Parkin (1994) who defined it as "the ability of soil to interact with the ecosystem to maintain biological productivity, environmental quality and to promote plant and animal health". In reality, many scientists link soil quality with a fundamental conceptual place in regards to territorial planning and company management, adding the vocational concept of "fit for" to it and then focusing primarily on what the soil will be used for.

From inspection of the scientific literature on the subject one quickly notes that there is no single parameter or universal indicator capable of defining every single situation or environmental pressure; instead, each time a situation arises it is necessary to determine the most appropriate parameters to measure that particular environment, which, afterwards may be used to monitor the state of the soil under its new use (*Doran et al.*, 1994).

In the Mediterranean area, which is at risk of desertification owing to the marked loss of organic substances and biodiversity of its soil, bioindicators – those living organisms that can be used as environmental indicators – are of particular importance.

Beyond the traditional existence of "index species", which underline the necessity of having systems of indicators at diverse trophic levels to obtain precise answers from such complex objects as the

Mediterranean soil, there is now the need to replace this with a concept of ecosystem functionality and to employ those organisms or indicators that can tell us something about how regularly an ecological system is carrying out its role or how much an ecological function is slowing down or accelerating in response to environmental or anthropic pressures.

Of particular usefulness to environmental scientists are biochemical indicators that describe the metabolic processes at work in the soil and, therefore, provide a summary of how well-working nutritional cycles are. We can discover this information by looking at the dosage of molecules or process-marking elements in them.

This is the case, for example, for the determination of soil respiration by measuring the CO₂ flows, which define all aerobic processes in the soil and therefore govern the organic-matter mineralization processes as well. Or, the determination of enzymatic levels, which in addition to metabolic functions can impact genetic diversity by representing the phenotypic expression of one producing organism rather than another.

2.4.1.2 Soil ecology

Soil is in a close relationship with the plants it supports and thereby forms a unique ecosystem with these and other microorganisms. Its fertility is in fact defined as the capacity of the soil to render crops productive. Normally we speak about chemical fertility (the sum of nutritional elements capable of being absorbed by crops), physical fertility (structure, terrain consistency, etc.) and biological fertility (Bloem et al., 2006).

The concept of biological fertility, however, has only really been established over the last 20 years; its aim has usually been to characterise soil metabolism and microbic turnover.

The function of microorganisms in the soil is multifaceted. It is expressed in both pedogenic processes and in the regulation of nutrient cycles, and thus in plant nutrition. Microorganisms are involved in the mineralisation of organic matter, in the synthesis of nitrogen, in the formation of humus and also impact the mobilisation of mineral elements (Lavelle et al., 2001).

The soil, however, is also an extremely vital entity and as such parallel studies have been carried out aiming to understand the synergistic and competitive relationships between microorganisms and mycorrhizae/carpophores in diverse pedological situations (Steinaker et al., 2008).

Recently soil fauna has been the object of careful research and correlations between their presence and the development of fungal fruiting bodies has started to emerge. For example, to guarantee the normal development of a Tuber ascocarp, that organism needs to absorb the nutritional substances - in particular, small organic molecules and mineral salts – from a land mass which is fairly rich in humus and which is equal to around double the average range of the truffle (Granetti et al., 2005). This volume of earth, unfortunately, cannot be explored by the strands of hyphae that are formed by the ascocarp because they are of insufficient length. Instead, to facilitate nutrition, truffles take advantage of the soil microfauna, the biological activity of which ensures a continuous supply of nutrients to the immediate vicinity of the carpophore. Even the relationships between ectomycorrhizal hyphae and soil fauna, including the relation between the various biotic components and their potential function as indicators, have been examined in detail during research into the most precious Tuber species (Callot et al., 1999). These studies analysed the roles and relationships between the various components and established new foundations for future experimentation on soil bioindication.

The term **microfauna** of the soil, in its widest sense, includes a complex series of animal species of varying shape, dimension and role. Species with individuals between 0.01 and 0.2 mm fall into this category: protozoa that mineralize nitrogen, phosphorus and sulphur, making easily absorbed nutritional substances available to hyphae; and nematodes that feed on bacteria, protozoa and fungal hyphae and are involved in the decomposition of underground carpophores (Lavelle et al., 2001; Callot et al., 1999).

The mesofauna consists of animal species measuring from 0.2 to 2 mm, mostly mites and ticks, other microarthropods (protura, diplura, symphyla, pauropoda, pseudoscorpions, etc.). and annelids (oligochaetes), enchytraeidic display some of the most diverse and specialised eating habits around (Lavelle et al., 2001; Siepel, 1994); many microfitofagous and detritivorous species feed on mycorrhizal hyphae and tufts of mycelium from carpophores. Their catabolites (rich in undigested fungal hyphae) are a food source for earthworms (annellids) and nutritional hyphae of fungal species (Callot et al., 1999), the spores of wich are inoculated in mesofauna fecal pellets, sometimes in organic and organo-mineral compounds (Lavelle et al., 2001; Siepel, 1994), which have an enormous positive effect on the growth rates of fungal hyphae.

Macrofauna includes all individuals larger than 2 mm; among others earthworms, snails, isopods, spiders, opiliones, chilopoda (centipedes), diplopoda (millipedes) and many insects, mostly larvae and adult beetles, flies, termites and ants. These organisms contribute to varying degrees towards decomposition and vertical and horizontal remixing of organic matter, which can be of vegetable, animal or fungal origin (Lavelle et al., 2001; Menta, 2008).

The function fulfilled by earthworms takes on particular relevancy, where, by feeding on organic fragments, the excretions of mites and ticks and mineral particles, they produce catabolites of around 1 mm in size, surrounded by a mucus-like intestinal material (Lavelle et al., 2001; Lavelle, 1997), which are easily and quickly colonised by the nutritional hyphae of the fungal species. The fungi also derive great benefits from the aeration produced by the tunnels these earthworms dig. which can be up to 2.5 metres deep. Even ants provide soil aeration with their tunnels, which can be at depths of 70-80 cm, but their most important function is the moving of material accumulated during the excavation of tunnels from lower strata towards the surface and thus eliminating the negative impact of rainwater runoff on nutrients. (Granetti et al., 2005).

2.4.2 Mushrooms as indicators

2.4.2.1 mushrooms as indicators of particular soil characteristics

Mushrooms may assume the role of bioindicators in a given ecosystem. In particular we should underline the enormous contribution to this situation made by the studies carried out over the last 40 years on valuable truffles (ectomycorrhizal species) to achieve a proper artificial cultivation and production of them.

Analysis of soil characteristics (profile, particle size, pH, content of minerals, including trace elements, organic component, macro and micro porosity) have provided a database that is helping to shed light on the complex relationships between soil and fungi. Investigations based on ecological study in artificial ecosystems have shown that some

fungi can be identified as indicators of undisturbed natural forests and can demonstrate the degree of trunk decomposition (Holmer, 1997). According to these authors, however, studies of fungal propagation should consider all the mycelium and not only the fruiting bodies, which constitute a minor part in the vegetative body of a fungus. In general, epigean and hypogean fungi and other microorganisms that colonise the humic layer of soil tend to prefer acid or sub-alkaline reactant substrates; alternatively they tend to be resistant to thermal stress and stresses deriving from water. It is not by chance that microbial taxonomy splits organisms into groups that demonstrate such characteristics as being thermophilic, cryophilic or alkaphilic, according to whether they need to exist at very high temperatures or in places with near eternal snowfall or in ground with sub-alkaline pH. Mushrooms tend to grow in soils with varying pH levels, from sub-alkaline to subacid, although many species prefer soil of around pH 7. Studies on the pH of natural soils conducted in different wooded areas showed a range of levels from 4.8 to 8 with an optimal value of 7.2 found in woods with green cover in excellent health with no loss of branches and/or leaves. The pH of these forest floors is affected by the number of trees per hectare and the type of vegetation cover of the soil (Bersan, com. pers., 2002).

Research carried out to increase knowledge and improve cultivation of the ecological characteristics of hypogeous fungi, especially the precious tuber varieties, showed soil pH levels ranging from 7 to 8.3 depending on the species studied (Granetti, 1994)

In general, Tuber ascocarps have improved growth when their hyphae live in micro-environments with a pH 6.0, while other ectomycorrhiza develop best in substrates that range from subalkaline to alkaline (from pH 7 to over 8) (Granetti et al., 2005). Various studies carried out on naturally occurring Tuber sites have demonstrated the following characteristics for the different species:

1. T. melanosporum Vittad. (Fig. 12) usually prefers richly skeletal soil with the remaining parts made up of fine soil (sandy-loam texture). The pH is very uniform and has an average of 8.0 ± 0.4 pH (having extremes of 5.7 and 8.25). Most of the soils studied in three regions of central Italy have a pH of around 8, and an average skeleton of 52% in



Fig. 12. Tuber melanosporum Vitt. (Prized black truffle from Norcia) (Photo: AMB - CSM Archives).

2. For *T. aestivum* Vittad. (Fig. 13) the soil is 19 cm deep on average, with a soil skeleton made of limestone (20%) and fine earth

(80%); of which 16% is sand, 56% silt and 28% clay. The pH is 7.7 on average (Bencivenga et al., 1996).2.



Fig. 13. Tuber aestivum Vitt. (Summer truffle or scorzone) (Photo: AMB – CSM Archives),

- 3. T. aestivum Vittad. f. uncinatum (Chatin) Montecchi & Borelli¹; prefers soil on average 28 cm deep, with a skeleton consisting of 10% limestone and the remaining 90% fine earth
- 4. T. mesentericum Vittad., on average, is found in soil deeper than 30 cm, soft or compacted, as in road cuttings where macadam limestone with a neutral or subalkaline pH accumulates (Palenzona et al., 1976). Other studies in Irpinia (Campania) found it in sandy-loam soil with low levels of

(28% sand, 56% silt and 47% clay). The pH varies from 7.0 to 7.8 with diverse amounts of organic matter (Bencivenga et al., 1996).

soil skeleton and greater percentages of limestone [which almost always ensures a pH close to neutral (7.7)] and in some cases, the pH drops to subacid levels (*Bencivenga et al.*, 1996).

This taxonomic entity is recorded as a variety (described in 1998 by Ian R. Hall, Peter Buchanan, Wang Yun and Anthony L.J. Cole), referred to and recorded in the CABI (Commonwealth Agricultural Bureaux International) "Index Fungorum" database (RecordID = 318194) (http://www.indexfungorum.org). After appropriate evaluation, we prefer to classify this entity as a form, as suggested by Montague & Borelli in 1995 and in (Montecchi et al., 2000).

5. T. magnatum Pico (Fig. 14) can be found in deep soil with a poor skeleton but rich in silt and clay, which make up a skeleton of 68.4%.

pH levels are close to 8 and vary little (Bencivenga et al., 1988).



Fig. 14. Tuber magnatum Pico (Prized white truffle from Alba) (Photo: AMB – CSM Archives).

6. *T. borchii* Vittad.; is found in soil with an average skeleton of 31.7%, the remainder being on average 66.3% sand, 23.2% silt, and 13.2% clay. The pH varies from 7.5 to 8.0 with average values of 7.6 (Giovagnotti et al., 1999).

2.4.2.2 Mushrooms as indicators of ongoing degradation

Some fungal species, by their mere presence and quantity, indicate a current imbalance in the ecosystem and can predict other detectable forms of degradation in advance. A fungal species present on the woody remains and an indicator of significant amounts of nitrogen in the substrate is *Megacollybia platyphylla* (Pers.) Kotl. & Pouzar, which, by its intrinsic characteristic of acting on large surfaces with its mycelial cords and producing basidiomycetes directly on these surfaces (Fig. 15), is considered a good indicator of ongoing forest degradation.

In these cases, the genetic and functional ranges of fungi provide a large number of species, which indicate large ecosystem suffering due to excessive dead biomass (necromass). We note, among others, Cerrena unicolor (Bull. : Fr.) Murr., Coriolopsis gallica Fr. and Trametes trogii Berk., the spores of which precede the hyphal entrance of Megacollybia platyphylla (Pers. : Fr.) Kotlaba & Pouzar (Bersan, com. pers., 2002). Even Clitocybe phaeophthalma (Pers.) Kuyper is an indicator species, suggesting excessive amounts of nitrogen in the substrate, but unlike Megacollybia platyphylla it has manifold functions and so its presence needs to be evaluated a case-by-case basis. For example, an accumulation of substrate in a place with stagnant water and low ventilation, related to a number of C. phaeophtalma fruiting bodies, may indicate the decay of plants in the area circumscribed by the basidiomycetes as the excess necromass inhibits the recycling processes related to other species of fungi (Bersan, com. pers., 2002).



Fig. 15. Rhizomorphs of Megacollybia platyphylla (Pers.: Fr.) Kotl. & Pouzar, with fruiting bodies highlighted (Photo: C. Siniscalco).

Other species of gasteroid fungi (Sarasini, 2005), belonging to diverse families (Phallaceae Corda, Lycoperdaceae Corda, Clathraceae E. Fisch.) are also indicators of ongoing decay processes: for

example, *Mutinus caninus* (Huds.: Pers.) Fr., *Lycoperdon pyriforme* Schaeff.: Pers., *Clathrus ruber* Micheli: Pers. (Fig. 16) (*Bersan, com. pers., 2002*).



Fig. 16. Clathrus ruber P. Micheli ex Pers. (Photo: C. Siniscalco).

2.4.2.3 Mushrooms as indicators of future decay activites

There are species which, with their fruiting bodies, by feeding on the by-products of other fungal species (primary degraders) indicate ecosystem changes that will only be perceivable to us in the distant future (when these primary degraders, with their long cycles, fruit).

Owing to characteristics connected to their biological cycle, several species of the genus *Mycena* (Pers.) Roussel have this predictive

capacity (Robich, 2003): Mycena rosea (Schumac.) Gramberg; Mycena pura (Pers.: Fr.) P. Kumm.; Mycena pelianthina (Fr.) Quél.; Mycena galericulata (Scop.: Fr.) Gray; Prunulus niveipes Murril [Sin. Mycena niveipes (Murrill) Murrill]; Mycena polygramma (Bull.: Fr.) Gray; Mycena amicta (Fr.) Quél.; Mycena flavoalba (Fr.) Quél. Mycena rosea has established itself as an excellent indicator of biodegradation caused by primary fungal agents (Fig. 17) (Bersan, com. pers., 2002).



Fig. 17. Mycena rosea (Schumac.) Gramberg (Photo: AMB - CSM Archives).

2.4.2.3 Mushrooms as indicators of habitat diversity

Organisms capable of indicating biological diversity in terms of richness and population abundance are very important for the understanding and conservation of ecosystems. Even mushrooms, then, can be used in the study and monitoring of biodiversity of an ecosystem or an environment (*Benedetti et al.*, 2006).

Regarding mycological elements, several studies were conducted by APAT (today the ISPRA) as of 2003 to build databases in collaboration with the Associazione Micologica Bresadola, Centro Studi Micologici (AMB. - CSM), who have the mandate to conduct the first stages of habitat-linking between the nationally-recognised habitats to their CORINE Biotopes and Natura 2000 equivalents (Siniscalco, 2008; 2009).

As an example of a similar activity, we can cite the preliminary analysis conducted on some habitats of European importance that has allowed for certain guide species to be identified for dune environments (*Bianco et al.*, 2009).

Species lists for each habitat have been created. These are based on available national data and are made according to the frequency of occurrence of each species.

Characteristic and differential species have emerged when comparing their presence and frequency with other habitats; frequent species are those with high levels of occurrence, but also present in other habitats. These species (n. =177) represent an initial sampling of precious ecological elements and environmental quality indicators (Bianco et al., 2009).

2.4.2.4 The trophic qualities of fungi as a key function of processes linked to soil fertility

Mushrooms are increasingly becoming a principal tool in land-quality monitoring thanks to their specialised trophic activities which guarantee them a spot in all the national habitats.

Mushrooms, along with bacteria and other microorganisms, ensure the catabolic degradation of organic substances so as to obtain simple molecules in the form of water, carbon dioxide and mineral salts and also ensure the metabolic synthesis of complex organic and organomineral molecules that are involved in the formation of humus (Zanella et al., 2001).

Therefore mushrooms and microorganisms play a fundamental role in guaranteeing soil fertility and in the absence of which the soil would be simply an inert mechanical support.

Recent observation (Papetti, pers. comm.) seem to lend weight to the hypothesis that in meadows and mountains, the presence of fruiting bodies of Hygrophoraceae (grass symbionts) is limited by excess nitrogen of mineral and organic origin. The reduction of anthropic nutrition seems then to allow a return to the soil's original condition, probably because the mycelium causing mycorrhizal activity is not, in this case, permanently affected by nitrogen pollution of the soil.

2.4.2.5 Mycorrhizal fungi as indicators of plant health

Ectomycorrhizae, besides being a physical barrier to the penetration of parasites and being able to qualitatively and quantitatively change the metabolites released in the plant rhizosphere, also tend to produce antibiotic compounds that represent a barrier against many toxic soil microorganisms (Montecchio, 2008). Understanding the mycorrhizal metabolism and its working mechanisms provides a number of bioindication keys, given that the roots of an adult forest plant, normally, can simultaneously bear mycorrhizal fungi from 30 to 50 different species, each able to best develop only under certain environmental, phenological and soil microclimate conditions (Koide et al. 2000).

These new bioindication resources allow us to say that mycorrhizae producing their fruiting bodies, enable us to monitor their beneficial effects on host plants. For example, *Rhizopogon vinicolor* A. H Sm. confers greater resistance to drought to seedlings of *Pseudotsuga menziesii* (Mirbel.) Franco, while *Laccaria lacquer* (Scop.) Cooke shows greater resilience than *Hebeloma crustuliniforme* (Bull.) Quélet (Fig. 18), staying alive far longer than the plant itself after cutting (*Parke et al.*, 1983).

H. crustuliniforme shows better efficiency in the nitrogen mobilization from proteinous substances in *Betula pendula* Roth than both *Amanita muscaria* (L.) Lam. (Fig. 19) and *Paxillus involutus* (Batsch) Fr (Abuzinadah et al., 1989).



Fig. 18. Hebeloma crustuliniforme (Bull.) Quél. (Photo: AMB - CSM Archives).



Fig. 19. Amanita muscaria (L.: Fr.) Hooker. (Photo: AMB - CSM Archives).

Mycorrhizal communities are complex and many different factors influence their dynamics in various ways, so that is not possible to speak of a single "mycorrhizal effect", but many associated effects. For example, there is a very complex syndrome that is usually designated with the generic term "deterioration". In recent years, "deterioration" has

been interpreted and evaluated according to different criteria owing to various studies on the mycorrhizal community. Research has demonstrated that the absorbing roots of deteriorating trees often show significant variations in the make-up of mycorrhizal communities (*Blaschke*, 1994; Causin et al. 1996, Mosca et al., 2007). Often, the

symptoms of deterioration are observably more markedly in conditions when there is little water, or where the water is heavily saline, which means that these environmental factors can play a more relevant role than others in determining the deterioration of the lesser resistant plant genotypes (Schütt et al., 1985; Shi et al., 2002; Manion et al., 1992). In these cases, it has been observed that the frequency of some ectomycorrhizae is associated with plant health, thus meaning that it may be possible to identify the level and intensity of plant deterioration, even in a preventative manner. through objective underground parameters. It has been observed, in fact, that trees deteriorate gradually, progressively losing their ability to select the most efficient mycorrhizal symbionts and allowing them to be replaced with those better adapted to the changing environmental conditions. The relative frequency of ectomycorrhizal communities typically varies significantly between healthy and slightly/heavily deterioration points, allowing these communities to be used as bioindicators of the presence and degree of deterioration (Lilleskov et al. 2001; Loreau et al., 2001).

2.4.2.6 The presence of heavy metals in mushrooms: a possible new instrument for soil bioindication

The ability of living beings to exchange elements and substances creates an ecological cycle that will continue until the sun's energy is no longer available, provided that no external factors with an intensity of disturbance exceeding the homeostatic capacity of the interested ecosystem are introduced. The presence of man on Earth has had a powerful impact on these natural cycles by artificially manipulating the chemical elements of life and dispersing in the environment synthetic and foreign (xenobiotic) substances that have already entered the metabolic cycle of organisms (*Ravera*, 1981). A survey conducted by the American Chemical Registry found that over fourteen million different chemicals are available on the world market and ten thousand new ones become available every week. The vast majority of these chemicals have been (and are still being) released into the environment and are interfering with the balance of terrestrial ecosystems (Sequi, 1981; Siniscalco et al., 2002).

For a long time it was considered that soil was able to retain pollutants and very quickly dampen their harmful effects. Therefore more attention was paid to those environmental compartments such as air or surface water resources where the effects of anthropogenic pollution are more immediately obvious. The capacity of soil to accumulate pollutants can in fact prevent immediate contamination of neighbouring environmental assets, but it may also lead to a sudden release of pollutants once retention limits are exceeded (Gallini, 2002).

Mycelium in the soil is in direct contact with the external environment and can absorbe and accumulate heavy ions. These, in turn, may be transferred within the cell. This phenomenon occurs in different ways across various fungal families and species. There have been numerous studies over the past twenty years, particularly in Europe, examining the presence of heavy metals in mushrooms and the results show heterogeneous behaviour between species (Siniscalco et al., 2001). Many metals that are present in trace amounts on earth are essential for growth and reproduction of microorganisms. Different concentrations of heavy metals in soil affect the composition of fungal communities present in the litter and soil (Onofri et al., 1999). In the last decade there has been a growing conviction that the results of these studies require a refinement of analysis so as to achieve the creation of a comparative tool, to be called the "reference mushroom" (Cocchi et al., 2006). When such a reference has been developed for each species, this could help to shed light on the physiological function of chemical elements in fungi; it will give us more information on bioindication and taxonomic assessment and no less importantly, it will provide an estimate of the volume of heavy metals that are ingested through diet by man and other living beings (Cocchi 2009). Reference mushrooms are probably a very valid instruments for evaluating soil biodiversity and the ecosystems which are connected to the soil (Petrini et al., 2009).

From a functional point of view the complex formed by hyphal emanations from ectotrophic mycorrhizae and its connected mycoclena mobilises various minerals, starting with proteins, in order to protect the apex from those toxic pollutants (including heavy metals) that are in the soil at mycotoxic concentration levels (*Rousseau et al.*, 1994).

Absorption of heavy metal not only inhibits fungal growth but also causes physiological and morphological changes. Their toxic effect seems to be mainly exerted on enzymes. Growth inhibition may be caused by catalytically active groups being masked; protein denaturation; steric conformation being modified; or by the activation of other sites involved in the formation of enzyme-substrate complexes competing with those normally present. These toxic actions vary from species to species and depend on metal concentrations and exposure time (Onofri et al., 1999; Tyler et al., 1989).

2.4.2.7 mycorrhizal fungi as indicators of the quality and health of the plant-soil complex

In mycorrhizal symbiosis, the positive effects of nutritional exchanges can be observed in the metabolisms of both partners. The efficiency of these associations varies according to a series of dynamic interactions which involve not only the plant and the fungus, but other environmental and pedological factors and the relationships that are established between these variables (Montecchio, 2008).

Mycorrhizal fungi can be used as soil-quality indicators because they carry out key functions within the soil. The identification of easily monitorable metabolic markers makes the observation and evaluation of changes which can affect soil environment functionality possible. A good example for this is glomalin, a hydrophobic glycoprotein produced by arbuscular mycorrhizal (AM) fungi (Wright et al., 1996). Glomalin accumulates in soil in the form of a proteinous substance known as Glomalin Related Soil Protein (GRSP). GRSP is an easily measurable marker that can determine the medium- to long-term activity of AM fungi. It has been demonstrated that this marker is not only sensitive to environmental changes such as the increase of atmospheric CO₂ (Rillig et al., 2000) and to various soil use and management systems (Bedini et al., 2007), but also bears a high correlation to the stability of particle aggregations within the soil (Bedini et al., 2009), an important parameter of the soil's own functionality.

2.4.2.8 Mushrooms and the health of the plantsoil complex

It is rather difficult to make use of the values supplied by mycological and microbiological parameters because soil microorganisms and fungi in the litter react very quickly to seasonal changes and rapidly adapt to different environmental needs. Thus it becomes difficult to distinguish natural fluctuations from changes caused by human activity, especially when the data are recorded without a control group, as is the case in natural systems.

Several authors have proposed solutions to this issue. Brookes (1994), for instance, states that no parameter can be used in isolation but rather a whole series of correlated parameters should be jointly considered so as to create a kind of "internal control group", as is the case when considering carbon in microbial biomass and the total organic carbon in soil. When a soil sample presents a significant variation from "normal" values (Cbiomass/Ctotal organic in soil) in a particular soil management system under particular climatic and soil type conditions, this value then becomes an indicator of the soil's changing ecosystem functionality.

There is actually an almost linear relationship between these two variables, even if large discrepancies may exist between soils with differing physical characteristics or which are managed differently (Bloem et al., 2006).

Many studies have been conducted on the possibility of using microbiological and biochemical parameters to characterize soil microbial diversity in both genetic and functional terms. Firstly, one must determine the presence of microbial life in soil and its order of magnitude; next it is essential to understand which functions the living population has and how active it is; then finally, it is important to determine the structure of the microbial and mycological communities therein and the relationships they establish with plants (ISPRA, 2009).

Microbiological and biochemical methods are now able to provide necessary information on soils. Recently Bloem et al. (2006) have divided these methods into four groups depending on the type of information they can provide:

 I. Measurement of biomass and microbial load: includes all methods that define the weight of soil and the number of microorganisms it contains, both in terms of total load and as nutritional or physiological groups, such as plate counts, colorimetric microscopy, and biochemical methods able to provide information on active populations; to this group one should add the study of mycorrhizae linked to the mapping and inventory of fruiting bodies of macromycetes.

- II. Measurement of microbial activity: includes all biochemical methods that provide information about the metabolic processes of microbial communities, both in their entirety and divided into functional groups. The biochemical methods can be divided into two subgroups: the first includes methods that count the active population in its entirety and that, depending on the outcome and the type of information it provides, should be included in the first group of methods, regarding weight and number, mentioned above. The second subgroup contains methods that define the current activity and the activity potential of individual organisms or metabolic groups, such as respirometric tests, nitrogen mineralisation, etc. methods can determine the maximum activity potential that can be reached in specific substrates (Benedetti, 2004).
- III. Microbial and structural diversity of a community: this includes the most up-to-date methods for acquiring ecological and molecular data. Traditionally, analysis of microbial communities in soil was carried out using cultivation techniques, but only a small fraction (<1%) of the microbial communities in soil could characterised using this approach. There are currently several methods for studying soil microbial communities. The use of molecular techniques continues to provide new knowledge on distribution and diversity of organisms in soil habitats. Among these methods the most useful are those in which small subunits of rRNA are amplified from nucleic acids extracted from soil. With these techniques it is possible to distinguish and study soil microorganisms that cannot currently be cultivated. The microbial ribosomal subunits can be detected directly from

soil samples and sequenced. These sequences can then be compared with those of other known microorganisms. It is also possible for oligonucleotide probes, for specific groups and taxa to be constructed from these sequences, thus enabling the visualisation of soil microorganisms directly in their habitat.

• IV. Plant-microorganism interactions: this is based on the rhizosphere being recognised as a zone of influence by all the roots on surrounding biota and soil. Many ecophysiological studies give a description of the region in which these interactions occur. The key areas of study look at the influence of nutrients on plants, including those mediated by free microorganisms and by symbionts (ecto-and endo-mycorrhizae) as well as photosynthesis efflux, or products of rhizospheric deposition, which provide substrates for the associated biota.

2.4.3 Conclusions

The biological indicators of soil represent a synthetic and relatively economical instrument to study such complex systems and multiple functions that soil fulfils. This ranges from plant nutrition to the preservation of fertility, biomass degradation and filtering of xenobiotic and unwanted substances.

Furthermore, their use makes various assessments possible, such as: environmental impact assessment measures, strategic environmental assessment, recovery treatment effectiveness evaluation, testing to establish the speed of functionality recovery in certain sites and also measures to determine the potential strength and resistance of a site to anthropogenic and natural pressures.

2.5 The reference mushroom: a useful instrument for defining the capacity for bioindication of superior fungi

2.5.1 Introduction

The first hurdle to be overcome, in order to be able to interpret and evaluate the significance of the presence of chemical elements, especially heavy metals in higher fungi, was to have internal and external standards, previously absent in scientific literature. The kingdom of fungi is very complex and holds more species than the animal and plant kingdoms, and it is estimated that the still undescribed fungal species are still hundreds of thousands (Hawksworth, 1991). Fungi are thus one of the most significant components of biodiversity and the scientific potential for the development of studies of these living beings is great. These considerations led us to further our work in trying to identify an object of comparison for fungi; the "reference mushroom", (Cocchi et al., 2006). The fungal metabolism, however, is still far from being fully understood and it is therefore hard to find an example in nature which, with reference to the concentrations of chemical elements it contains, can be described as "pure". The variables involved are many, as is shown by the high number of deviations from standard concentrations measures (as found in data from all other authors) for almost all chemical elements (the few exceptions taking on especial importance) and not all the variables are known. In this situation statistical analysis becomes essential.

The idea for the definition of a "reference mushroom" came from an article by Markert (1992), who stated that "Two thirds of naturally occurring chemical elements in eco-systems are not investigated since they are viewed as nonessential or nontoxic to biota. In view of the important role plants play in most ecosystems, their inorganic chemical characterization, according to modern multi-elements techniques, instrumental establishment of a "Reference plant", comparable to the "Reference man" by the International Commission on Radiological Protection (ICRP), can be a useful tool for this type of chemical "fingerprinting" [...] In the future, more attention should focus on establishing baseline values for "normal" elemental concentrations in ecosystem components [...]"

The idea of extending the same concept to macromycetes, based on the statistical stability reached by our database, was thus evident (Cocchi et al., 2006). As our database contains information only on selected ascomycetes and basidiomycetes, it only allows us to establish a "reference mushroom" of initial approximation; the following objective will be to refine analyses (and therefore gather more data for those species for which it is necessary) to arrive at the definition of "reference mushrooms" for different taxa, up to the level of species.

In summary, the concept of a "reference mushroom" aids us in understanding whether the concentrations of chemical elements in higher mushrooms might play a role in:

- Bioindication.
- Taxonomic evaluation
- Estimation of the consumption of heavy metals by different species of edible mushrooms

It should be noted that when speaking of taxonomic assessments, we refer to a species paradigm conceptually different from the morphological species concept defined by both macroscopical and microscopical characters used so far almost exclusively in basidiomycete taxonomy, but widely and historically considered obsolete. Indeed in the animal and plant kingdoms, the concept of species is essentially biological and historically based on both classical and molecular phylogenetics. Molecular methods are now increasingly being applied to fungal taxonomy and the concept of species to which we refer in our analyses is analogous to that of a "biological species", because the concentrations of the various chemical elements depend heavily on the different metabolisms of each species. It is obvious, however, that it would be absurd to completely disregard the systematics and taxonomy currently used in mycology; these being the sole criteria available for the taxonomic determination of the fungal samples analysed. For this reason, identifications were all controlled by expert mycologists from the Associazione Micologica Bresadola (many of our samples came from the National Scientific Committee of the AMB) and from the Gruppo Micologico e Naturalistico "Renzo Franchi" di Reggio Emilia (AMB). In fact, the comparison and exchange of information with other researchers must be underpinned by the "certainty" that the samples each researcher is studying come from the same species – because systematics and morphological taxonomy, even under the rules of the International Code for Botanical Nomenclature, still leave ample room for manoeuvre in naming species and in the use of synonyms.

Clearly, the "reference mushroom" is strongly influenced by the size and composition of the global sample used. Despite this, but by employing a large sample, the use of this concept allows us to determine to a good degree the average values of the variables under consideration.

Our work has shown that macromycetes can accumulate large quantities of various chemical elements in their mycelia. Some authors have suggested that this accumulation may be specific to species and genera, but certainly the composition of substrate may affect concentrations in the mycelium and then in the fruiting bodies. During this study, which lasted more than 20 years, we analysed the distribution of over 30 chemical elements in the fruiting bodies of more than 9,000 samples of ascomycetes and basidiomycetes collected in Italy and, to a lesser extent, in other European regions. The data are presented in this work, both in extended form in the Appendix and in summarised form in Table 2 in paragraph 3.1.7. This type of presentation should enable interested parties to analyse the information contained in the database in detail, potentially even starting from the raw data included in the accompanying CD.

2.5.2 Elaboration of the "reference mushroom": an example

We herein aim to clarify the procedure we used to define our "reference mushroom". We identified the species for which we had data from at least 20 samples, and from these (about 60 species), we selected some at random.

This is a list of the selected species. We have indicated, for each of the species, the symbol that represents it on the graph in Fig. 20 and the number of samples analysed:

- Agaricus arvensis Schaeff. : (AA). Nr. 58
- Agaricus bisporus (J. E. Lange) Imbach: (AB). Nr. 43

- Agaricus bitorquis (Quél.) Sacc.: (AC).
 Nr. 37
- Agaricus urinascens (Jul. Schäff. & F. H. Møller) Singer [sin. A. alberti Bon; A. macrosporus (F.H. Møller & Jul. Schäff.) Pilát]: (AM). Nr. 51
- Amanita caesarea (Scop.) Pers. : (AI).
 Nr. 27
- Amanita muscaria (L.) Lam. : (AF). Nr. 197
- Amanita phalloides (Vaill. ex Fr.) Link: (AP). Nr. 24
- Armillaria mellea (Vahl) P. Kumm. : (AR). Nr. 26
- Boletus edulis Bull. : (BE). Nr. 115
- Boletus luridus Schaeff. : (BL). Nr. 37
- Boletus pinophilus Pilát & Dermek: (BP).
 Nr. 78
- Calocybe gambosa (Fr.) Donk: (CG). Nr. 20
- <u>Lycoperdon</u> utriforme Bull. [sin. Calvatia utriformis (Bull.) Jaap]: (CU). Nr. 49
- Cantharellus cibarius Fr. : (CC). Nr. 42
- Craterellus lutescens (Fr.) Fr. [sin. Cantharellus lutescens Fr.]: (CL). Nr. 39
- Infundibulicybe geotropa (Bull.) Harmaja [sin. Clitocybe geotropa (Bull.) Quél.]: (CA). Nr. 23
- Entoloma saundersii (Fr.) Sacc. : (ES). Nr. 41
- Helvella crispa (Scop.) Fr. : (HC). Nr. 29
- Hydnum repandum L.: (HR). Nr. 37
- Marasmius oreades (Bolton) Fr.: (MO). Nr. 66
- *Mitrophora semilibera* (DC.) Lév. [sin. *Morchella semilibera* DC.]: (MS). Nr. 27
- Cortinarius caperatus (Pers.) Fr. [sin. Rozites caperatus (Pers.) P. Karst.]:
- (RC). Nr. 52
- Russula cyanoxantha (Schaeff.) Fr. : (RA). Nr. 33

- Russula vesca Fr: (RV). Nr. 39
- Boletus rubellus Krombh. [sin. Xerocomus rubellus (Krombh.) Quél.]: (XR). Nr. 77
- Boletus subtomentosus L. [sin. Xerocomus subtomentosus (L.) Quél.]: (XS). Nr. 31

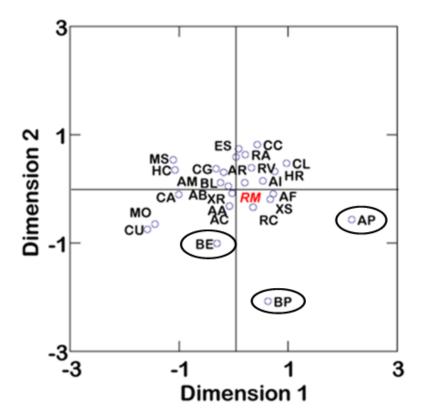


Fig. 20. Results of a "multidimensional scaling" analysis applied to the species listed in the preceding text. Abbreviations: see text. RM: calculated values of the reference mushroom (stress coefficient = 0.129).

Statistical analysis and multi-dimensional scaling (MDS; a powerful multivariate data analysis technique that helps to identify key dimensions among a large set of variables) were applied to the concentration data of the selected species. MDS, others, does not imply particular mathematical assumptions about the distribution type (e.g. data linearity or distribution normality) and is therefore a general procedure which is not, in practice, subject to significant mathematical distortions. The stress coefficient of the final configuration is an index that measures the quality of reduction and indicates if the model is

applicable to the sampled data: data range from 0 (the "ideal value" in a practical sense) to 1 (a poor value). Two coordinates were calculated for each species and these helped to identify one point, a depiction of the species, in the two-dimensional graph in Fig. 20.

The quality of reduction is normally considered good when the stress coefficient is less than 0.2. The point that represents the "reference mushroom (RM) was measured on all samples using the mean values. From Fig. 20 one can note that the RM point, as expected, is very near to zero. From the graph one can also see that points BE (*Boletus*

edulis), BP (Boletus pinophilus), AP (Amanita phalloides) differ significantly from RM. This difference is characteristic of the species. What we must do then, is to check to see what the causes of these respective positions are.

To this end we may refer to the tables containing the specific average data and their confidence intervals as determined in our research. From these tables it is clear that factors influencing distance from the "reference mushroom" for *A. phalloides* include high chlorine concentrations (Cl); for *B. edulis* and *B. pinophilus* (and to a lesser extent, for *Agaricus bitorquis*) high concentrations of Se are responsible.

In summary we indicate below the key features of some species on which we have carried out the same statistical analysis:

- Mitrophora semilibera (DC.) Lév. [sin. Morchella semilibera DC.] (MS): high levels of aluminium (Al), barium (Ba), calcium (Ca), cobalt (Co), iron (Fe), nickel (Ni), phosphorus (P), strontium (Sr).
- Lycoperdon utriforme Bull. Isin. Calvatia utriformis (Bull.) Jaap] (CU): high levels of copper (Cu), potassium (K), lead (Pb) (the fact that this species, always collected in high-altitude grasslands, presents relatively high concentrations of Pb as compared to species, requires further investigation), sulphur (S), zinc (Zn).
- Agaricus arvensis Schaeff. (AA): high levels of silver (Ag), cadmium (Cd), cobalt (Co), copper (Cu), phosphorus (P);
- Agaricus urinascens (Jul. Schäff. & F. H. Møller) Singer [sin. A. alberti Bon; A. macrosporus (F. H. Møller & Jul. Schäff.) Pilát] (AM): high levels of silver

- (Ag), cadmium (Cd), cobalt (Co), copper (Cu), phosphorus (P).
- Amanita muscaria (L.) Lam. (AM): high levels of vanadium (V), zirconium (Zr).
- Boletus edulis Bull. (BE): high levels of mercury (Hg), selenium (Se), sulphur (S) and low potassium (K).
- Boletus pinophilus Pilát & Dermek (BP): high levels of mercury (Hg), selenium (Se), sulphur (S).

2.5.2.1 Procedures to follow

The methodology described above can be applied to varying types of both ecological and taxonomic data so long as care is taken to establish control structures each time it is used. In general the procedure to follow will be:

- Checking that the data represent a "statistically stable" set.
- Using in a first step descriptive/ exploratory statistics methods (mean, median, confidence intervals, maximum, minimum, and standard deviation).
- If the descriptive methods indicate possible differences, moving on to multivariate analysis. Here one can either use summary data or the raw data, depending on the purpose of each analysis.

Below, we present a concrete example of how the data gathered can be analysed. The graphs in Figs. 21, 22, and 24 are largely similar to those given in Petrini *et al.* (2009).

2.5.2.2 Univariate analysis

Control of sample group homogeneity:

• By geographic region.

• By matrix or support.

• By elevation

When confidence intervals overlap one cannot postulate significant statistical differences between the samples.

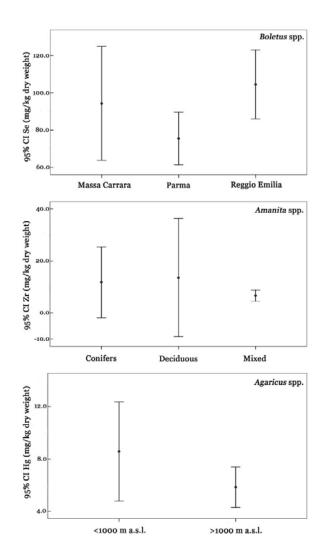


Fig. 21. Univariate analysis: the first step.

Second step:

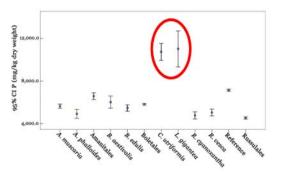
Choice of reference mushroom (Reference)

For example, if one would aim to study *Amanitales*, *Boletales* and *Russulales*):

- Reference calculated for all samples
- Reference for the *Amanitales* order
- Reference for the Boletales order
- Reference for the Russulales order

Explorative analysis – comparison of groups:

The P content in samples of Lycoperdon utriforme Bull. [sin. Calvatia utriformis (Bull.) Jaap] and Calvatia gigantea (Batsch) Lloyd [sin. Langermannia gigantea (Batsch) Rostk.] is much higher than in other studied samples.



The Pb content in samples of *Lycoperdon utriforme* Bull. [sin. *Calvatia utriformis* (Bull.) Jaap] is much higher than in other studied samples.

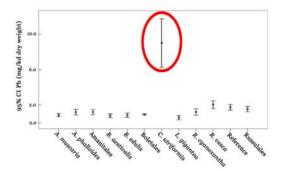


Fig. 22. Univariate analysis: the third step.

Applicability to other samples:

The Se content in samples of the *Boletus edulis* group gathered in Calabria and in the Province of Massa are different from those found in other Italian, European and worldwide regions.

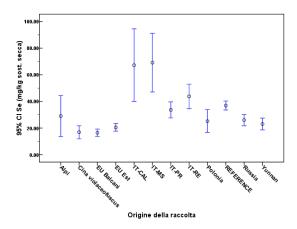


Fig. 23. Univariate analysis: the fourth step.

2.5.2.3 Multivariate analysis

Example: classification of several Boletus species. The use of MDS (Figure 24) to classify certain species of Boletus – also investigated by Vizzini et

al. (2008) – by using their content of certain chemical elements has produced results that are in complete agreement with those produced by molecular biology (details in Petrini *et al.*, 2009).

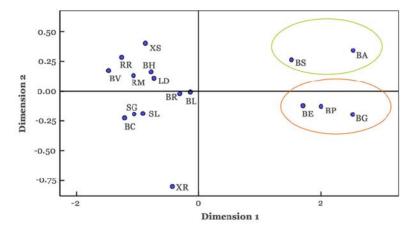


Fig. 24. Results of analysis on several species from the genus Boletus using MDS (Petrini et al., 2009). Final configuration stress: <0,005.

Key: BA: Boletus aereus Bull.; BC: Boletus calopus Pers.; BE: Boletus edulis Bull.; BG: Boletus edulis Group; BH: Boletus rhodopurpureus Smotl.; BL: Boletus luridus Schaeff.; BP: Boletus pinophilus Pilát & Dermek; BR: order Boletales; BS: Boletus reticulatus Schaeff. [sin. B. aestivalis (Paulet) Fr.]; LD: Leccinum duriusculum (Schulzer ex Kalchbr.) Singer; RM: Reference Mushroom; RR: order Russulales; SG: Suillus granulatus (L.) Roussel; SL: Suillus luteus (L.) Roussel; XR: Boletus rubellus Krombh. [sin. Xerocomus rubellus (Krombh.) Quél.]; XS: Boletus subtomentosus L. [sin. Xerocomus subtomentosus (L.) Quél.]

2.5.3 Conclusions

The "reference mushroom" (Cocchi et al., 2006) could be used to detect differences and anomalies in the samples studied and is therefore useful to identify variations of the chemical elements in the same organisms in different ecosystems. Its use in projects that involve bioindication could be important, as it may help to detect outliers in homogeneous groups. Even a polyphasic taxonomy based on the reference mushroom could be helpful in solving taxonomic problems.

2.6 EC legislation and mushrooms: biodiversity and bioindicators

The soil is of strategic importance for life on our planet and must therefore be protected. This

statement has been made not only by the European Union, but by international organisations as well. The Strategy for Sustainable Development of the European Union and the 6th European Community Programme in 1991, enshrined as a target the protection of the soil from erosion and pollution due to declining soil fertility; noted as the main cause of falling European agricultural productivity in many areas. In 1992, in connection with the summit in Rio de Janeiro, several statements were made concerning the protection and preservation of soil, and even went so far as raising the issue of biodiversity loss. In 1994, the United Nations Convention aimed at combating desertification and established the need to prevent and reduce land degradation, and to rehabilitate lands that had been degraded or affected by desertification.

In 2002 the European Commission adopted Communication COM 179 detailing the Thematic Strategy for the Protection of Soil. The document recognises the importance of soil in the fulfilment of vital environmental functions, i.e. the production

of biomass as well as the storing and transformation of energy and mineral/organic elements; the soil also acts as a filter protecting subterranean waters and exchanges gas with the atmosphere; it constitutes a support for life and whole ecosystems. Furthermore, soil is a reserve of genetic resources and raw materials, the custodian of lost civilisations, and a pillar of the landscape.

To allow the soil to carry out these functions, it must be defended from degradation and from other threats to its well-functioning. The Communication lists the main threats such as erosion, organic matter decline, local and diffuse contamination, sealing, compaction, salinisation, landslides and flooding as well as adding the loss of biodiversity as a full problem in its own right. This last point assumes strategic importance as it was one of the first times the word "biodiversity" featured clearly in any official EC documents.

Two other directives are believed to be fundamental to safeguarding the environment and its biodiversity: Directive 79/409/EEC, better known as the "Birds Directive" and Directive 92/43/EEC, the "Habitats Directive". With these two, signatory countries were asked to make efforts to conserve biodiversity through the conservation of natural habitats and of wild fauna and flora, through the establishment and maintenance of a coherent ecological network of special areas of conservation. The message sent out was to preserve and restore plant and animal biodiversity.

So as to better implement the strategy, in September 2006, the European Commission adopted a series of tools. These were: the SFD Soil Framework Directive, COM 232 (2006).Commission Communication, COM 231 (2006) and the Impact Assessment SEC 620 (2006). These tools established soil as having a central role to play and consequently saw biodiversity as a key feature in its preservation and restoration. The United Nations Framework Convention on Climate Change (UNFCCC), and the subsequent Kyoto Protocol defined strategies for containing emissions of greenhouse gases. They also recognised that the terrestrial biosphere plays a fundamental role in the conservation of ecosystems, plants and the creation of new forests which are all important steps for combating the greenhouse effect and restoring biodiversity.

The documents required signatory countries to quantify the spatial distribution of six different categories of land use (forests, wetlands, meadows, farmland, urban areas and other). Furthermore, for each land category, information regarding the type of management it requires, the biomass associated with it, the changes that occur there over time and an evaluation of the type of transformation there should be provided. In relation to these aspects the concepts of biodiversity and bioindication take on an ever-greater relevance.

Regarding forests, the Forest Principles, adopted during the Earth Summit on Sustainable Development, called on States to maintain or increase the extent of forest cover; an essential strategy to protect and increase biodiversity.

The European Landscape Convention, signed in Florence in 2000, acknowledges that, "the quality and diversity of European landscapes constitute a common resource, and that it is important to cooperate towards their protection, management and planning". Actions aimed at guiding and harmonising the transformation of the area, such transformation being caused by processes linked to social, economic or environmental development, are a valid means for the sustainable management of the "landscape resource". To provide an idea of the importance attached to maintaining the extension of natural and semi-natural areas for sustainable development, it should be mentioned that the "Land Use Change" indicator is part of a number of indicators proposed by the United Commission on Sustainable Development. More recently, the European Environment Agency, through the IRENA project (Indicator Reporting on the Integration of Environmental Concerns into Agriculture Policy) has selected Land Use Change as one of the 35 agri-environmental indicators for monitoring the integration of environmental needs with the Common Agricultural Policy.

Throughout the Italian national territory, the Sustainable Use of Natural Resources Sector of the Natural Resources and Parks Service - APAT, (ISPRA, today), launched a study of changes in land use and vegetation cover that took place in Italy between 1990 and 2000 using the CORINE Land Cover Database for each year. It is evident biodiversity, its maintenance and its restoration play an important role in this work, often with aspects related to the concept of bioindication going hand in hand with biodiversity and almost assuming a single, interchangeable identity. Unfortunately at present not enough is known about soil biodiversity. This will be addressed in the Seventh Framework Programme, aimed at gathering a better understanding of biodiversity as an environmental function. This process of knowledge building will also be supported by ongoing initiatives connected to the Convention on Biological Diversity and the "Forest Focus" programme.

In conclusion, the data relating to mushrooms presented in this book augment and complete the information requested by the various international agencies.

The biodiversity of the Italian fungal species we have studied and the use of the chemical concentration levels in them, single out fungi as potential biological indicators for the quality of forest, woodland and semi-natural habitats.

In addition, the very broad range of data included here can be used over the next few decades to enable a comparison with future data, which could then make possible a better and more comprehensive interpretation of the effectiveness of current environmental protection legislation in minimising or negating the effects of climate change.

Chapter III Data Synthesis

3.1 Consideration on statistics and statistical methods employed

One of the fundamental problems in taxonomy in general is the selection of internal and external control groups for the samples analysed. Phylogenetics researchers have found a solution with external samples (outgroups), but classical taxonomy does not yet have trustworthy models in this field.

Therefore, we have recently suggested making use of centroids to establish internal and external references in cases where significant amounts of data are available that can be summarised with parametric and nonparametric descriptive statistics (Petrini et al. 2009).

3.1.1 Sample choice

During our work we collected data for about 9,000 samples (carpophores) of basidiomycetes and ascomycetes. Our work can be described as a nonrandom semi-quantitative census, because the samples tested were provided in most cases by colleagues and friends belonging to the AMB. In general, the exact origin of each carpophore was recorded for all those mushrooms provided by Italian and foreign mycologists. However, we also examined samples from exhibitions of mycology in Italy, and the origin of these was not always traceable. The majority of samples were collected in the province of Reggio Emilia (50% of mushrooms were from exhibitions). Therefore, the representativeness of our sample is somewhat reduced, because the data collected are especially representative of Reggio Emilia and we cannot exclude the possibility that analysis of a different set of sample data may provide a different set of

results. Descriptive analysis of subsamples, however, showed that at least for Italian mushrooms, our results are representative.

Furthermore. our sample particularly representative of basidiomycetes, because only relatively few ascomycetes were examined. We note that our tables, which include the mean values and 95% confidence intervals for the various chemical elements studied, summarise all the data we collected and most of these tables are included in the CD attached to this document. Please note that only samples of taxa for which at least 20-30 carpophores (originating from different sites) were examined provide reliable values. When only a few carpophores of a given species were studied, the values are to be regarded as indicative only.

3.1.2 Statistical measurements

In our work we mainly used descriptive statistics, particularly mean, median and confidence intervals. Of the different types of mean (such as arithmetic, geometric, and harmonic), we used the arithmetic mean, which together with the median proved best suited to describe the datasets we collected.

The 95% confidence interval (95% CI) is used to estimate "real" values. By definition, the actual value of the entire population can only be estimated, because one never has access to the entire population. The CI therefore describes a set of values inside which most likely lies the "real" value, calculated using observed results from the sample, given a certain margin of error.

The calculated data can then be plotted using different techniques. An example is shown in Fig. 25.

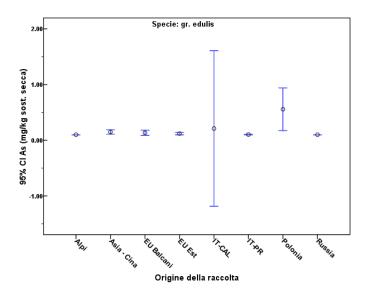


Fig. 25. Arsenic content in specimens of *Boletus edulis* collected in different geographical regions. The circle represents the average, while the vertical bars enclose all values within the CI of 95%.

3.1.3 Statistical stability

To perform reliable statistical analyses, it is important that the variation of data within taxonomic (or ecological) groups to be examined be stable and remain constant over random samples during resampling procedures and when new elements are introduced (*Cocchi et al.*, 2006). Our database has reached statistical stability, as shown in Figure 26.



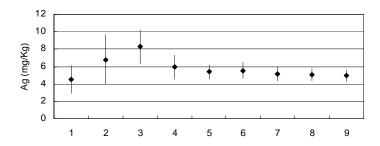


Fig. 26. Content (average and 95% CI) of Ag in the genus *Boletus*. Results of nine resamplings with the introduction of new elements in the samples. The final sample contains 504 elements.

3.1.4 The reference mushroom

A great aid in statistical analysis is the use of a "reference mushroom". A propos this we hereby

reproduce a part of a document we published in 2006 (Cocchi et al. 2006).

Markert (1992), has proposed the adoption of a "reference plant", an ideal plant that would have the anatomical and physiological characteristics of the average plant in the sample. This idea is intimately linked to that of the "reference man" proposed by the ICRP and which corresponds to a person with the anatomical and physiological characteristics of the average individual. Similarly, the "reference mushroom" is a fungus that has the anatomical and physiological characteristics of the average fungus in the sample group studied (Cocchi et al., 2006).

In summary, the concept of the "reference mushroom" is used to determine whether the concentrations of chemical elements in higher mushrooms may have a role in determining the possibility of their use in bioindication, taxonomic evaluation and estimating the dietary intake of heavy metals through the consumption of edible mushroom species.

Obviously the "reference mushroom" is strongly influenced by the size and composition of the global sample group used. Nevertheless, with a large sample group, the use of this concept allows us to gain a good approximation of the average value of the variables under consideration. Therefore one must not forget that any average will depend greatly on the size and composition of the sample group and that consequently a "reference mushroom" needs to be identified on a case-by-case basis.

3.1.5 Data analysis

During this study, which lasted more than 20 years, we analysed the distribution of over 30 chemical elements in the fruiting bodies of more than 9,000 samples of ascomycetes and basidiomycetes collected in Italy and, to a lesser extent, in other European regions.

3.1.5.1 Procedures followed

The above-described methodology can be applied to various data-types, including both ecological and taxonomic data.

3.1.5.2 Multivariate analysis

The purpose of multivariate analysis is often to detect clusters (taxonomic, ecological, etc..) that can lead to a classification which can be used for identification purposes. In this work, we have not used any ordering or classification techniques, since the purpose of this document is purely descriptive, and instead we aim to very briefly describe some methods that could be applied to the data presented in this work.

There are various methods available for grouping ("ordering") data, and include, to mention only the most popular, cluster analysis, factor analysis, multidimensional scaling (MDS) and multiple correspondence analysis. Each of them has advantages and disadvantages, but all result in a reduction of a model from "n" to just a few values, this being mostly accomplished by optimising/reducing system variance. A deeper but simple description of the process, written for non statisticians and with more-detailed bibliographical references can be found in Petrini and Sieber (2000) and Sieber et al. (1998).

As regards the identification of samples, canonical discriminant analysis is perhaps the best known technique. Here we refer to more specialised texts, also cited in Petrini and Sieber (2000).

3.1.6 Software used

The data were gathered using a Microsoft® Access® database and analysed with SPSS, version 17 (SPSS Inc., Chicago, IL, USA).

3.1.7 Results

The CD that accompanies this report contains descriptive statistics describing the reference mushrooms for each of the families, genera and species of fungi studied. It also contains the number of samples, the average values and the relative 95% CI for the taxa studied. As a representative example of the analyzed data we present here the levels of all chemical elements considered in determining the universal "reference mushroom" (the centroid of the total sample of about 9,000 carpophores).

Table 2. Total samples, average values and 95% CI for all samples (N= 9328) (in mg/kg dry weight or bq/kg dry material by Cs^{134} , Cs^{137} and K^{40}).

Element	N	Average	95% CI
Al	9074	346	333 - 360
Ag	9326	3.44	3.27 - 3.61
As	9327	15.4	11.6 - 19.2
В	8881	9.64	9.06 - 10.2
Ba	9279	3.84	3.59 - 4.08
Ве	7222	0.014	0.01 - 0.01
Ca	9326	914	848 - 980
Cd	9328	4.20	3.92 - 4.49
Cl	845	3670	3290 - 4040
Co	9240	0.40	0.38 - 0.42
Cr	9327	1.49	1.40 - 1.58
Cs	7852	2.28	2.04 - 2.52
Cs ¹³⁴	328	91.8	44.7 - 139
Cs ¹³⁷	328	2590	1740 - 3435
Cu	9327	58.8	56.4 - 61.3
Fe	9323	330	318 - 343
Ge	1182	0.033	0.03 - 0.04
Hg	9296	1.19	1.11 - 1.28
K	9327	39630	39310 - 39950
K^{40}	328	1350	1290 - 1410
La	6534	0.34	0.29 - 0.39
Li	9248	0.36	0.35 - 0.38
Mg	9327	1310	1300 - 1330
Mn	9327	34.7	33.0 - 36.4
Mo	9216	0.20	0.19 - 0.21
Na	9327	328	314 - 342
Ni	9327	1.87	1.79 - 1.96
P	9300	7195	710 - 7286
Pb	9320	1.61	1.51 - 1.72
Rb	9327	138	133 - 144
S	9317	3364	3314 - 3415
Sc	5623	0.27	0.25 - 0.3
Se	9327	4.13	3.87 - 4.39
Sr	9307	3.22	2.97 - 3.48
Ti	8102	10.2	9.8 - 10.6
V	9327	3.22	2.83 - 3.61
Y	5620	0.20	0.18 - 0.22
Zn	9327	117	115 - 119
Zr	6633	0.42	0.37 - 0.47

3.2 Applied geostatistical analysis

3.2.1 Introduction

Geostatistics is the branch of statistics that deals with the analysis of spatial data derived from sampling. In environmental analysis and modelling it is an essential tool for the management, understanding, and correct use of data from environmental surveys and measurements (such as meteorological data, pollutant concentrations, piezometers, etc.).

Geostatistical analysis consists in modelling the phenomenon one wishes to investigate with a random variable characterized by a spatial, temporal or spatiotemporal law. This approach allows one to highlight and describe the regional or temporal variability (qualitative and quantitative) of the data analysed and to map out the results. It measures the effect of the position of the measuring point on the variability of the data observed.

Geostatistical methods are valid for all fields of applied science in which the phenomena to be studied are spatial in nature. Over the past three decades, it has been used, for instance, in soil science, hydrology, hydrogeology, geochemistry, meteorology, oceanography, environmental health, agronomy, and imaging analysis.

Let us take a spatial phenomenon, for example the heavy metal pollution of a site. In general, by indicating the concentration of the pollutant with z, and the generic coordinate point of the field $(x_{lat}, x_{long})_i$ with x_i , Z(x) is a variable that represents the concentration of pollution in certain points of the site

If one property varies in a more or less continuous way through space, it can be taken as a regionalised variable *(Goovaerts, 1997)* and analysed with geostatistical instruments.

Estimating by kriging interpolation enables a more detailed local spatial variation of the properties under study to be achieved. This type of interpolation is appropriate only where the property varies in a continuous manner and the data are spatially dependent or correlated. The model of spatial variation for geostatistical estimation is as follows:

$$Z(x) = \mu_v + \varepsilon(x)$$

where Z(x) is the random variable in location x, μ_v is the local average of Z in the predefined limits of

location x, and $\varepsilon(x)$ is a random term with an expectation of zero, and a variance equal to:

$$\operatorname{var}[\varepsilon(x) - \varepsilon(x+h)] = \operatorname{E}[\varepsilon(x) - \varepsilon(x+h)^2] = 2\gamma(h)$$

variance is calculated for all couples of locations x+x+h, where h is a distance vector (lag) for all distances and directions. γ is the semivariance between two locations, which, where this is stationary (μ_v is locally constant) will be equivalent to:

$$\gamma(h) = \frac{1}{2} \text{ var } [Z(x) - Z(x+h)] = \frac{1}{2} E [Z(x) - Z(x+h)^2]$$

and defines the variogram of Z. The variogram provides an unbiased description of the scale and pattern of spatial variation, the spatial model needed for kriging, and a basis for designing optimal sampling schemes (McBratney et al., 1981). Theoretical variogram modelling, starting from experimental variogram modelling, can indicate which approach to take in predictive investigation.

3.2.2 Ordinary kriging

At this point values can be estimated in points or in blocks through kriging, a shifting mean weighted to observed values based on the variogram inside determined limits defined by an area N (inside which the stationary values of the variable are assumed to hold). For a regionalised variable Z, with values measured as being $z(x_i)$ at site (x_i) , $I=1,\,2,\,\ldots,\,n$, the ordinary kriging algorithm will be:

$$Z(B) = \sum_{i=1}^{N} \lambda \cdot z(x_i)$$

where Z(B) represents the value estimated for block B and λ_i the weights assigned to internal points at N. The kriging estimator can be defined as non-distorted (the weights add up to one) and optimal (the weights are selected so as to reduce variance to a minimum). Unlike with classical interpolation methods (inverse square of the distance, triangulations, variable mean ...), this interpolation allows not only obtaining an estimation map of the parameter but also a map of the estimation variance (the kriging error), allowing evaluation of the reliability of prediction.

One of the most common tasks in the processing of spatial data is the construction of thematic maps, i.e. geo-referenced maps relating to selected geographic areas, in which, by an appropriate method of representation, the trend of a variable under study – in our case the concentration of metals – is given.

These maps are normally made by starting from the values of the variable as measured within the area. For example, from the initial situation shown in Fig. 27a, which represents the location of the samples and the measured values of a variable, we aim to build an iso-value contour map such as that shown in Fig. 27b. Note the non-uniform distribution of the samples in the example.

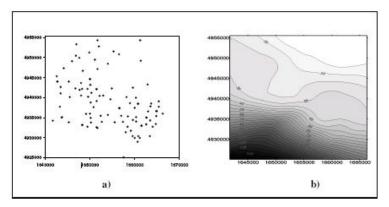


Fig. 27. Construction of a map, starting from measurement points.

Iso-value contour maps, which in cartographic jargon are also known as vector maps, are just one of many ways of representing a geographic variable. They are not obtained directly, but through creating a regular grid to represent the variable, itself obtained by an estimation

calculation (Fig. 28a). The contour lines are created by interpolating the values on the mesh axes (Fig. 28b). It is thus clear that the quality of the map is wholly dependent on the equation that produced the grid values.

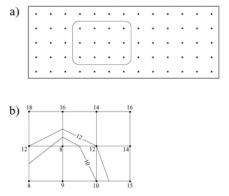


Fig. 28. Construction of a vector map:

- a) reconstruction of the variable to a regular grid;
- b) interpolation tracing of the iso-value lines.

3.2.3 Geostatistical analysis applied to the distribution of inorganic elements in soil and in fungi

For this book, our geostatistical analysis was focused on the following elements or physiochemical parameters found in the surface soil and in mushrooms gathered in the Province of Reggio Emilia: pH, aluminium, arsenic, cadmium, chromium, mercury, nickel, lead, copper, selenium, vanadium, zinc and zirconium. Irregular gridpattern sampling was carried out over a large area in the southern part of the province of Reggio Emilia

An exploratory analysis of experimental data using basic statistics was carried out, along with data frequency distributions and experimental variograms. All elements tested showed a tendency to lognormal distribution, characterised by the presence of a small number of sampling points with values of between one to two orders of magnitude greater than the database. Analysis of the spatial correlation of the data was conducted by

calculating the experimental variogram, which was then used to develop a model of interpolation according to the Kriging method (Isaaks and Srivastava, 1989) for the methodological framework and Carlon et al. (2000), for specific application to contaminated soils.

The spatial correlation of the data were generally good and proved sufficient to furnish an interpolation function. In a few cases however, the correlation was very weak, returning a spatial representation of the data which was too low to allow an estimate of the distribution of the chemical elements by interpolation. In these cases, the distribution of elements cannot be represented by a vector map, but instead by maps of sampling points divided by different classes of concentration.

The experimental variograms were modelled with spherical functions characterised by the range, sill and nugget values shown in Table 3, a value of zero intercept was imposed at the outset (nugget = 0) in order to bonour the measured values

Table 3. Classification	of	variograms	used.

Sample	Nr	Model	Range (mt)	Sill	Nugget	
Soil	181	Spherical	24300	0.46	0	
Tricholomatales	121	Spherical	18800	0.61	0	
Clitocybe	36	Spherical	29400	0.20	0	
Amanita	231	Spherical	20350	11.20	0	
Am. Muscaria	45	Spherical	31200	0.26	0	
Russula	322	Spherical	21700	0.61	0	
Agaricus	29	Spherical	21700	0.70	0	
Bitorques	51	Spherical	38900	0.62	0	
Arvenses	162	Spherical	16400	0.38	0	
Boletus E.	103	Spherical	28350	1.23	0	
Cantharellus	74	Spherical	22550	0.66	0	
Ramaria	28	Spherical	41000	4.21	0	
Morchella	61	Spherical	17800	0.51	0	

In some cases, a logarithmic transformation of data was applied to normalise distributions and to clarify the spatial correlation, by inserting a new transformation in the original scale of the estimate data.

The figures, relating to the spatial distribution of concentration parameters, were generated with the program Surfer 8.0, while the data grid was

obtained by Kriging (Isaaks and Srivastava, 1989; Clark and Harper, 2004). The variographic model was tested by cross-validation. The data provided relate to the sampling and chemical analysis procedures as described in previous chapters, and they are all listed in the attachments to this document.

Chapter IV Materials and Methods

4.1 Methods for chemical analysis: soil and macromycete carpophores

4.1.1 Carpophores

For a meaningful comparison of analytical results it is important to consider the degree of maturity at harvest of the carpophores that were used to determine the concentration of inorganic elements. Based on experience and the standard practice followed by many mycologists, maturity can only be estimated empirically as there are no analytical methods to measure this parameter. Nevertheless, the empirical method that assigns "numbers" to the various stages of carpophore maturity combined with the experience of the mycologist works quite well and certainly contributes to reducing the errors that could arise through variations of chemical concentrations due to the varying age of carpophores. The scale used is as follows:

- 1. Primordial (carpophore is still in embryonic form)
- Young (carpophore already formed, with hymenium not exposed to air in carpophores having a secondary veil like a cloak or a ring)
- Almost mature (spores begin to mature and the hymenium is completely exposed to the air)
- Mature (the spores are ripe and in most species the hymenium takes on the colour of a spore)
- 5. Old (the first signs of deterioration or putrefaction have set in)

It is clear that a more-refined evaluation (by use of half numbers) is the highest degree of sensitivity that this empirical scale of carpophore maturity could attain.

Most of the carpophores analysed during this study had a maturity-level of 4, therefore mature carpophores formed the basis of the study.

After being gathered, the carpophores were very carefully cleaned with special, soft brushes to remove any soil or sand particles, plant debris, insects, larvae or other material. After cleaning, the carpophores were roughly cut into slices to verify that there was no foreign material on the inside either

At this stage the amount of fungal material to be dried was weighed out: 20 grams can usually be considered sufficient. During this operation it was important to maintain the real weight proportions between the different parts of each carpophore (stem, cap, hymenium). The cut material was then set in a crystallizer and placed in a ventilated oven at a temperature of 45°C for 48 hours. At the same time, for a small number of samples, drying was carried out at 105°C to measure water loss.

Mushrooms were not kept for longer than necessary in contact with metal objects to avoid contaminating the samples, especially when damp. Instead we made use of other tools and treatment methods that involved plastic or glass tools.

After being dried at 45°C the samples were ground with an agate pestle and mortar and the ground part was then put into a pre-washed polyethylene container with two stoppers. Into this receptacle we put a 10 mm-diameter sphere of Teflon or glass to homogenise each sample before weighing and before following treatments.

The acid mineralisation of all carpophores was performed by placing a quantity of sample weighing 0.5-0.7 grams in a microwave oven with *aqua regia*. After mineralisation, the sample was increased to 50 ml in a volumetric flask with ultrapure H₂O (Cenci et al., 2008).

The quantitative determination of all elements (except Hg) was performed with a ICP-AES Perkin Elmer Optima 3000 XL spectrophotometer. The determination of mercury was performed using an Perkin Elmer FIMS 100 atomic absorption spectrophotometer designed for cold-vapour mercury determination.

The preparation of calibration standards was carried out starting from certified ultrapure monoelement solutions of 1000 mg/l by ICP instruments.

Certified Reference Materials (NBS and SRM) with a matrix similar to that of fungi were mineralised and subsequently analysed. The concentration levels obtained were within tolerance intervals.

4.1.2 Soil

At the base of the carpophore stems 4-5 cubes of soil, each measuring 5x5x5 cm, were collected and all grass, stones, leaves and other matter were immediately removed.

The soil samples were subsequently placed in plastic bags and mixed manually to form a single sample. In the laboratory each sample was dried and then passed through a 2 mm sieve. The particles of the sample which were equal to or smaller than 2mm were then ground with a mortar and pestle and then placed in polyethylene bags, as was the case for the mushroom samples; likewise,

the mineralisation process was also carried out in the same way as for the mushrooms.

Determination of the concentrations of the various elements under investigation was carried out using the same analytical instruments as for the mushroom carpophores.

The Certified Reference Materials for soil and sediment (CRM 141 R, Calcareous Loam Soil e CRM 277, Estuarine Sediment) were mineralised and subsequently analysed. The concentration levels obtained were within tolerance intervals.

4.1.3 Criteria for data collection

Table four indicates the element-by-element detection limits (d.l.) obtained by measuring each sample ten times . The d.l. represents the triple of the standard deviation. This criterion is widely used in analytical laboratories.

	d.l.										
Al	1	Cd	0.05	Cr	0.1	Hg	0.05	Cu	0.2	Ti	0.05
Ag	0.05	Ca	2	Fe	0.1	Мо	0.2	Rb	0.5	V	0.1
As	1	Cs	0.1	P	5	Ni	0.2	Se	2	Zn	0.2
Ва	0.1	CI	5	Mg	0.2	Pb	0.5	Na	3	Zr	0.05
В	0.2	Co	0.1	Mn	0.05	K	500	Sr	0.3	S	10

Table 4. detection limits (d.l.) expressed in mg/kg.

When a measurement was less than the detection limit, a value equal to one tenth of the d.l. was entered in the archive: this is because otherwise, in our statistical analysis, it would not have been possible to distinguish a cell with no value (where a measurement for that element in that sample was not made) from a cell in which the measurement was less than the d.l..

Although the tables show values for samples and/or elements for which also very few measurements were taken, only samples and/or elements having at least 30 measurements are to be considered reliable in this statistical analysis.

4.2 Distribution map of elements in soil

An exploration of the thematic maps resulting from our studies will now follow, aiming to interpret the spatial distribution of several inorganic trace elements, also known as persistent inorganic contaminants. Furthermore, both aluminium and the pH levels of surface soil will be taken into consideration.

After appropriate treatment, around 180 samples of surface soil, taken from depths of between 0 to 5cm, were used to assess concentration levels of aluminium, arsenic, cadmium, chromium, copper, mercury, nickel, lead, vanadium, selenium, zinc, zirconium, and pH levels.

The area of investigation is represented by the province of Reggio Emilia (Fig. 29), more precisely, starting from State Road 9, (known as

Via Aemilia) to the Apennine border with Tuscany, including a small area of the Tuscan-Emilian National Park.

This area can be divided into five sections starting from the city of Reggio Emilia. In this segment of lowlands the towns Bibbiano, Cavriago and Montecchio are situated. Farther to the south is a narrow strip of land with hills spread out between the plains and the northern footpath. The towns which in part make up this area are: San Polo,

Quattro Castella, Albinea, Scandiano and Casalgrande.

Continuing south, one comes across a wide hilly area. The towns it encompasses are Vezzano, Baiso, Viano and Canossa. The towns of Carpineti, Toano, Vetto and Castelnovo nei Monti lie between the Enza and Dolo rivers. Finally in the area with a purely mountainous morphology are the resort towns of Villa Minozzo, Ligonchio Besana, Ramiseto and Collagna.

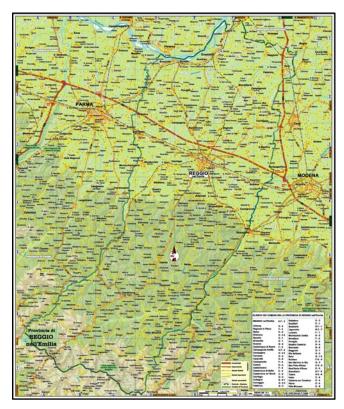


Fig. 29. Geographical map of the Province of Reggio Emilia.

Before commencing with a description of the thematic maps pertaining to the elements under study, it is important to emphasise that what is proposed and illustrated in the figures represents concentration distributions. This aim to demonstrate the real concentration base levels. These are the sum of geochemical contents, understood as purely natural phenomena, and concentration values due to anthropogenic activity.

For all twelve elements under study, general remarks can be made stating that the concentration distribution throughout Reggio Emilia is mostly monotonic and qualitatively comparable.

In general, the many human activities that have acted and are acting on the Reggio Emilia area, have not left significant signs of contamination, furthermore, the data we have been obtained are very similar to findings reported by Marks *et al.* (2009) in their study of the soil of Emilia Romagna.

Further confirmation of this was provided by the chemical data obtained by analysing the surface soils collected in Scandiano, Carpineti, and their large surrounding areas. For most of the elements, the concentration levels found during this

monitoring study yielded very similar results again (Cenci et al., 2005).

Aluminium (Fig. 30) can be seen to have a rather homogeneous concentration distribution. Lower levels can be found in the hills.

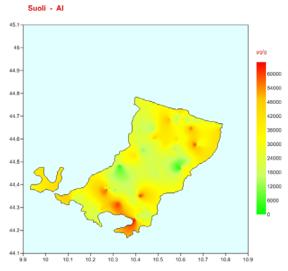


Fig. 30. Spatial distribution of the concentration levels of aluminium (mg/kg dry weight) in surface soils.

In the plains and mountains concentrations are higher; this is mainly due to the geology of the area. Arsenic (Fig. 31) mirrors the findings for aluminium, while for cadmium (Fig. 32), there are two areas, San Polo d'Enza and Reggio Emilia,

where one could reasonably expect to see significant man-made effects. For the remaining part of the area, levels remain rather uniform.

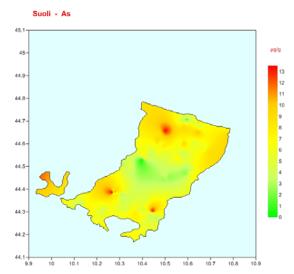


Fig. 31. Spatial distribution of the concentration levels of arsenic (mg/kg dry weight) in surface soils.

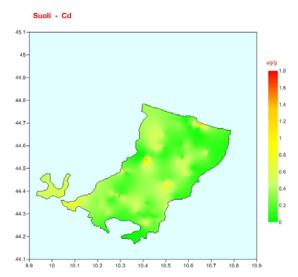


Fig. 32. Spatial distribution of the concentration levels of cadmium (mg/kg dry weight) in surface soils.

Chromium and nickel (Fig. 33) have a very similar spatial distribution of concentration levels, as one would expect, and both are present in the western zone, in two areas lying congruent to ophiolitic ultramafic outcrops of the Enza basin: the levels found here were quite high. The small area near State Road 9, which is already known for its high

cadmium levels, should also be considered a point of contamination for chromium. Often chromium is found to be enriched with many elements, but not nickel, which, in cases where there has been a geogenic malfunction, grows in proportion to the chromium.

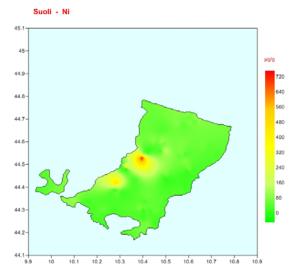


Fig. 33. Spatial distribution of the concentration levels of nickel (mg/kg dry weight) in surface soils.

The elements copper and mercury (Figs. 34 and 35) show a fairly uniform distribution of concentration levels which, as a whole, are fairly low. Only two

localized areas in the mountains showed higherthan-expected levels, presumably due to local contamination.

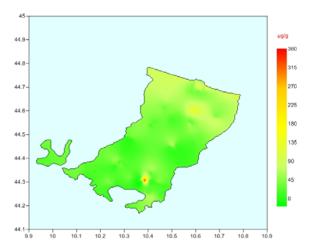


Fig. 34. Spatial distribution of the concentration levels of copper (mg/kg dry weight) in surface soils.

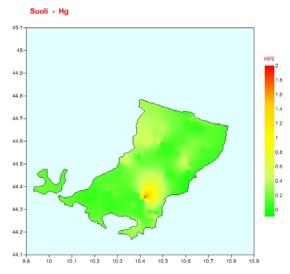


Fig. 35. Spatial distribution of the concentration levels of mercury (mg/kg dry weight) in surface soils.

The spatial distribution of the concentration levels of lead were also fairly homogeneous throughout the territory (Fig. 36); such values suggest a general enrichment due to contribution by man. There are two areas that have certainly been "contaminated": one near State Road 9 and the other in the Baiso area.

For vanadium and selenium, the Apennine area appears to have lower concentration levels, the

latter also being confined to the mountainous areas. The largest concentration levels are diametrically opposed: high values for selenium were found in the plains, while for vanadium, high levels were observed in the mountains. Regarding the latter, there appears to be also a localised contamination in this area (near Reggio Emilia and State Road 9) of cadmium, chromium and lead.

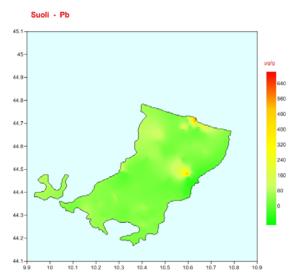


Fig. 36. Spatial distribution of the concentration levels of lead (mg/kg dry weight) in surface soils.

For zinc, as with cadmium, chromium, lead and vanadium; soil contamination is found near State Road 9. The remaining area contains practically uniform concentration levels, except for the higher levels found in the mountainous ranges to the east. Low concentration levels were found consistently for zirconium and it was only in the Apennines that higher concentrations of it were registered. The zirconium levels observed in this study are on

average one order of magnitude lower than those obtained by Marchi et al. (2009).

Lastly, the acidity levels of Reggio Emilia soils measured spatially (Fig. 37) are considered. Throughout most of the territory pH levels of around 6.5 are the norm. In two areas, one located in the mountains and the other in the south, there are more acidic soils, while those in the Apennines tend toward neutral-alkaline pH.

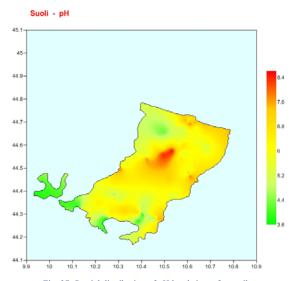


Fig. 37. Spatial distribution of pH levels in surface soils.

4.3 Distribution map of elements in mushrooms

Heavy metals can be regarded as one of the main sources of environmental pollution. For several decades the output of heavy metals into the environment due to human activity exceeded those of natural origin such as volcanic eruptions and large forest fires. A large part of these persistent inorganic contaminants ends up in the soil, increasing chemical element concentration levels. The soil, thus enriched with heavy metals, may, as a result of leaching processes, allow some of these metals to leak into groundwater tables. The fact that mushrooms are bioaccumulators means that their fruiting bodies may take on these contaminants and pass them on to animal biota – with potentially hazardous results for humans.

Regarding the heavy-metal and macro-element concentration levels which accumulate in mushrooms, they can depend on and be influenced by factors such as soil type and the concentration levels of the metals. It should be noted that concentrations of heavy metals in soils, in this study area of the province of Reggio Emilia, are completely consistent with the geology of the area (Marchi, pers. comm., 2010).

Other factors that may affect this accumulation are the chemical forms of the heavy metals involved, the content of organic matter and the concentration of hydrogen ions in the soil, and many other factors (Garcia et al., 2009) that, together, may play a decisive role in the process of bioaccumulation.

Other aspects relate to the genera and species of mushrooms, and which are able to bioaccumulate heavy metals, and to what degree. It should also be remembered that aspects such as density, depth and age of the fungal mycelia; their lifecycles that can last for months to years – all these factors affect the growth of fungal fruiting bodies (carpophores), affecting and conditioning the processes of bioaccumulation. Knowledge about the mechanisms which transport heavy metals from the soil-substrate to the mycelium and from thence to the carpophores, however, are as yet poorly understood (Svoboda and Kalač, 2000).

The data collection in this book is really quite vast, with more than 9,000 carpophore samples collected from all over Italy, corresponding to a universe of about 1,000 species of fungi. For each sample the concentrations of 32 elements were quantified. In

addition, approximately 350 surface soil samples were examined so as to obtain a better interpretation of bioaccumulation processes. For the surface soil samples, in addition to the 32 elements, their respective concentrations of hydrogen ions were evaluated.

The graphic representation of over 300,000 sample data cannot be shown and discussed in a comprehensive way here; therefore it was decided to present, evaluate and interpret data only for areas in the province of Reggio Emilia where carpophore collection was carried out with greatest intensity. These zones extend from State Road 9 up to the southern ridge of the Tosco Emiliano Apennines and include areas of the Tuscan mountainside (Alta Garfagnana and Lunigiana).

We selected for examination the elements which, based on the scientific data and literature to hand, were the most significant and highly-studied: aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). Selenium (Se) was only examined for *Boletus edulis*, as it is not found to a significant degree in other taxa; Vanadium (V) and zirconium (Zr) that were investigated only for *Amanita muscaria* (L.) Lam., as they were not found to a significant degree in any other taxa.

Also included in the study were those taxa that had an "abundance" of sufficiently large sample groups (a total of 24,000 concentration level values) in order to create distribution maps for the average, minimum and maximum concentration values, thereby forming a complete overview, which is summarised in Table 5.

Subdivision Basidiomycotina - Subclass Agaricomycetideae

- Order *Tricholomatales* and its Genus *Clitocybe*.
- Genus *Amanita* and the species *Am. Muscaria*.
- Genus Russula.
- Genus Agaricus and the sections Bitorques [Subgenus Agaricus (L. : Fr.) Heinem.] and Arvenses [SottoGenus Flavoagaricus Wasser].
- Group of *Boletus edulis (B. aereus, B. reticulatus, B. edulis, B. Pinophilus).*

Subdivision Basidiomycotina - Subclass Aphyllophoromycetideae

- Genus Cantharellus.
- Genus *Ramaria*.

Subdivision Ascomycotina - Subclass Pezizomycetideae

Genus *Morchella*.

Table 5. average, minimum and maximum values for the elements analysed relative to taxa included in the study.

		Al	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Se	٧	Zr
Order Tricholomatales	Average	217	1.0	2.37	1.94	36.4	0.35	1.37	0.94	157	-	-	-
	min Value	16	0.1	0.09	0.10	4.0	0.01	0.14	0.05	50.0	-	-	-
	Max Value	2400	59	14.5	63.5	93.0	4.0	24.5	18.8	340	-	-	-
	Average	178	0.4	1.08	1.26	93.7	2.24	1.44	1.97	98.2	-	-	-
Order <i>Tricholomatales</i> Genus <i>Clitocybe</i>	min Value	6	0.1	0.30	0.10	33.0	0.37	0.20	0.05	65.0	-	-	-
Condo Cindoyao	Max Value	1520	9.0	3.88	5.30	319	6.00	5.00	21.5	165	-	-	-
	Average	334	0.2	5.02	2.03	63.6	1.19	1.37	1.32	131	-	-	
Genus Amanita	min Value	13	0.1	0.12	0.01	6.0	0.01	0.02	0.05	20.0	-	-	
	Max Value	3410	7.0	33.4	29.9	740	24.3	10.1	64.3	328	-	-	-
	Average	225	0.4	12.2	1.01	31.7	0.73	0.84	0.91	138	-	99.0	4.66
Amanita muscaria	min Value	33	0.1	2.74	0.10	6.0	0.20	0.02	0.05	58.0	-	12.5	0.05
	Max value	1500	2.0	33.4	4.90	69.0	2.55	3.30	19.1	280	-	195	19.4
	Average	288	0.2	3.33	1.06	53.7	0.52	1.69	1.83	91.0	-	-	-
Genus <i>Russula</i>	min Value	11	0.1	0.08	0.01	10.0	0.01	0.20	0.05	21.0	-	-	
	Max Value	2940	7.0	24.6	6.10	189	4.06	13.5	53.6	864	-	-	-
	Average	488	0.1	1.58	6.07	161	2.29	3.15	1.09	110	-	-	
Genus Agaricus	min Value	113	0.1	0.12	0.20	33.0	0.19	0.20	0.05	47.0	-	-	
	Max Value	1290	1.0	5.83	125	934	7.26	27.0	3.70	203	-	-	-
	Average	539	0.3	1.99	1.90	132	5.25	2.89	3.94	100	-	-	-
Genus Agaricus Section Bitorques	min Value	61	0.1	0.25	0.10	20.0	0.12	0.20	0.05	46.0	-	-	-
Conon Enorques	Max Value	2770	4.0	9.20	7.40	812	25.3	14.1	29.5	273	-	-	-
	Average	119	1.5	40.9	0.74	188	4.99	2.39	2.52	156	-	-	-
Genus Agaricus Section Arvenses	min Value	9	0.1	0.05	0.01	27.0	0.43	0.20	0.05	50.0	-	-	-
	Max Value	797	21	391	10.9	1410	19.3	14.3	22.7	361	-	-	-
	Average	252	0.1	3.52	1.52	37.3	3.52	2.49	0.80	138	51.2	-	-
Genus Boletus Gruppo B. edulis	min Value	3	0.1	0.34	0.10	5.0	0.13	0.75	0.05	28.0	5.0	-	-
Crappo Di Caano	Max Value	2490	3.0	15.7	22.1	98.0	28.9	16.6	9.10	447	223	-	-
	Average	270	0.1	0.49	2.91	42.9	0.23	2.33	1.97	71.3	-	-	-
Genus Cantharellus	min Value	26	0.1	0.09	0.10	15.0	0.01	0.20	0.05	28.0	-	-	-
	Max Value	1360	0.1	2.87	57.3	111	1.44	28.6	5.50	143	-	-	-
Genus <i>Ramaria</i>	Average	327	8.7	5.74	3.54	51.4	0.99	10.19	0.98	71.1	-	-	-
	min Value	10	0.1	0.52	0.30	19.0	0.01	1.10	0.05	31.0	-	-	-
	Max Value	970	40	32.3	28.6	244	8.23	48.3	3.20	167	-	-	-
Genus <i>Morchella</i>	Average	709	0.1	0.94	3.27	53.6	0.09	2.40	1.19	144	-	-	-
	min Value	21	0.1	0.19	0.20	11.0	0.01	0.40	0.05	60.0	-	-	-
	Max Value	6100	3.0	4.12	30.7	123	0.25	12.2	11.0	281	-	-	-

The discussion deals separately with each of the various taxa considered. Below, there are distribution maps of certain elements in conjunction with evaluations regarding different enrichment factors and with data obtained from analysis of the principal components of this study.

4.3.1 Order Tricholomatales (Subdivision Basidiomycotina – Subclass Agaricomycetideae)

The distribution maps for the elements arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc, were far from uniform. Aluminium,

chromium and nickel tend to have a similar concentration distribution and are generally consistent throughout, but it is interesting to note that for chromium and nickel there is a strong overlap with the distribution maps of soil. Figs. 38 and 39, that show copper and zinc, highlight

common areas where concentrations are similar in proportion. In the Albinea and Quattro Castella areas, maximum concentration levels for arsenic were observed. Regarding pH, areas with more highly-acidic soils display correspondingly high levels of copper and zinc.

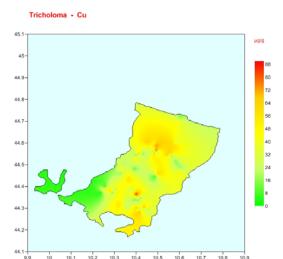


Fig. 38. Spatial distribution of copper for the order Tricholomatales.

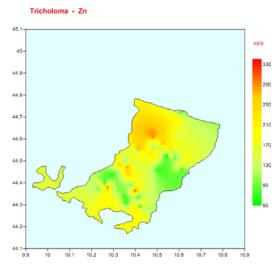


Fig. 39. Spatial distribution of zinc for the order Tricholomatales.

Cadmium, mercury and lead (figs. 40, 41 and 42) have higher levels in hilly areas and, only for cadmium, even in the mountainous border with Tuscany.

Cadmium: the average concentration level was 2.37 mg/kg. Slightly lower levels (1.67 mg/kg) were found east of the Black Sea (*Demirbaş*, 2001), while also in Turkey (Soylak et al., 2005) in *Ma*.

oreades and *T. argyraceum* 0.63 and 0.91 mg/kg were found. In the same area Yamaç *et al.* (2007) repeatedly found 0.58 and 1.99 mg/kg in *I. geotropa* and *T. equestre*. In the area of Epirus and

Macedonia, Ouzoun *et al.* (2009) found, respectively, concentrations of 1.8, 1.67 and 0.25 mg/kg in three species, *Ar. tabescens, Ar. mellea* and *T. rutilans*.

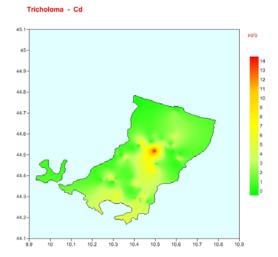


Fig. 40. Spatial distribution of cadmium for the order Tricholomatales.

Mercury: the average level of mercury was 0.35mg/kg; this is far lower than in the Czech Republic in the proximity of silver mines, where Svoboda *et al.* (2006) found levels of 10.5 and 8.9

mg/kg in *Le. nuda* and *Ar. mellea* respectively. On the other hand, in Turkey, levels of 0.07 and 0.09 mg/kg (*Demirbaş*, 2000) were recorded in *La. laccata* and *T. terreum*.

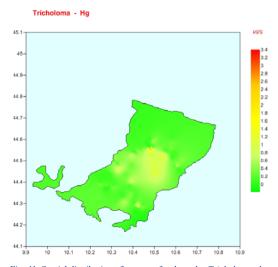


Fig. 41. Spatial distribution of mercury for the order *Tricholomatales*.

Lead: the average concentration level was 0.94 mg/kg. In an area east of the Black Sea, a level of 0.06 mg/kg was observed in the species *T. terreum*

(Demirbaş, 2001). In a recent study, significantly higher values were observed in central Spain by Campos *et al.* (2009).

During analysis of *I. geotropa*, *T. ustaloides* and *T. rutilans*, the authors found levels of 4.73; 3.33 and 3.23 mg/kg respectively (although the method of measurement used should be considered here). In the Black Sea area, Sesli *et al.* (2008) found a concentration of 2.6 mg/kg in *Le. nuda*. Demirbaş (2001) in the same area, found levels of 2.43 mg/kg in the species *T. terreum*, while a level of 0.86 mg/kg was found in *La. laccata* (Demirbaş, 2000). In Turkey, Yamaç *et al.* (2007) found 1.22 and 1.59 mg/kg in *I. geotropa* and *T. equestre* respectively.

In accordance with our own findings, in Macedonia and in the area of Epirus, Ouzouni *et al.* (2009), found levels of 0.79; 0.49 and 1.16 mg/kg in *Ar. tabescens*, *Ar. mellea* and *Le. nuda* respectively, while in Turkey (Soylak *et al.*, 2005) levels of 1.05 and 1.89 mg/kg were recorded in *Ma. oreades* and *T. argyraceum*. In France; in the area surrounding Paris, *C. nebularis*, *Ma. oreades* and *T. terreum* were found to have levels equal to 42.5; 33.6 and 24.3 mg/kg.

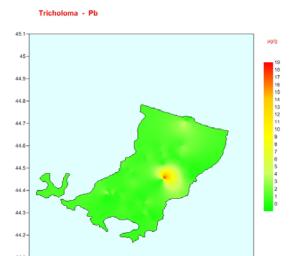


Fig. 42. Spatial distribution of lead for the order Tricholomatales.

10.2

The enrichment-factor levels show that the elements tend to accumulate in carpophores and their accumulation usually varies with fungal species. In general it was observed that arsenic, chromium, nickel and lead do not tend to accumulate in the fungi of this order, despite the soil being rich in them (Kalač and Svobova, 2000; García et al., 2009). In contrast, cadmium, copper, mercury and zinc can accumulate in carpophores, with above average concentration factors, even when the soil only holds low concentrations. This has been further confirmed for mercury (Falandysz et al., 2002).

In addition, Kalač and Svobova (2000), found enrichment factors equal to 50-300 and 30-500 for

cadmium and mercury and 0.1-0.2 for lead, as previously observed by Kalač *et al.* (1989b).

The results obtained from principal component analysis are presented in Table 6. The first three components describe 63% of the total variance. In the first component, soil acidity does not seem to affect the increase in cadmium, chromium, copper and zinc; this is in contrast to the behaviour of lead as described by Kalač and Svobova (2000). Main component 2 describes the relationship between nickel, cadmium, mercury and lead that tend to build up under moderately acidic conditions. Cadmium, mercury and lead are all typically affected by human activities. The third component describes the relationship between alkalinity of the soil and high levels of arsenic bioaccumulation.

Table 6	Results of	principal	component	analysis.

	Component			
	1	2	3	
pH_s	042	060	.803	
As_FA	.006	.093	.721	
Cd_FA	.437	.304	.003	
Cr_FA	.839	.004	037	
Cu_FA	.897	.191	055	
Hg_FA	.209	.788	.054	
Ni_FA	.242	.629	350	
Pb_FA	102	.692	.131	
Zn_FA	.932	013	019	

4.3.2 Genus Clitocybe (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Tricholomatales)

The genus Clitocybe, as a whole and also for selected species, will be examined here in comparison with species from the order Tricholomatales. In the genus Clitocybe, spatial distribution of element concentrations displays two types of associations. The first is represented by chromium, nickel and zinc with maximum levels of 5.3, 6 and 165 mg/kg, respectively which were observed in the areas of Toano and Reggio Emilia. The total remaining area, with the exception of zinc in Collagna bore concentrations concentration values. A second association is represented by aluminium and copper and it is the area of Reggio Emilia which provided the highest levels.

The maps representing arsenic, mercury, cadmium and lead are very different both among themselves and compared to other distribution maps. They confirm that the only overlap with the soil acidity map is the mercury content in mushrooms. The Spatial distribution of element concentrations shows a few types of associations. The first is represented by chromium and nickel with the highest levels, 125 and 27 mg/kg, respectively, found in Montecchio; other areas displaying uniform concentration levels. A second association is represented by copper and lead with the area of Carpineti which registered the highest values for those elements.

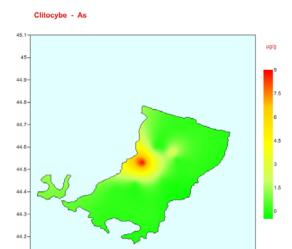
Aluminium, zinc and mercury partly overlap, while arsenic and cadmium are quite different both

between themselves and with other distribution maps. The map showing soil acidity has a resemblance to that displaying the zinc and mercury content in mushrooms and also a quantitative overlap between the aluminium in the soil and the concentrations of aluminium in mushrooms has been observed.

The maps in Figs. 43, 44 and 45 illustrate the concentration distribution of arsenic, copper and mercury, the average levels of which are, respectively: 0.43, 93.7 and 2.24 mg/kg.

Arsenic: the areas of highest concentration were found between Casina and Canossa, where the maximum is 9 mg/kg, while for the rest of the territory, the distribution is mostly uniform. Other authors found higher values (1.76 mg/kg) in *La. laccata (Demirbaş, 2001)* at the Black Sea. In samples from the various European nations and Brazil Slejkovec *et al. (1977)* found a value of 0.66 mg/kg in *La. laccata*, while their highest concentration was found in *La. fraterna*, having 30 mg/kg. Konuk *et al. (2007)* reported a level of 0.44 mg/kg in a sample of *Ar. mellea* collected in Turkey.

Copper: Fig. 44 shows the distribution of copper. High values are present in the plains around the city of Reggio and area to the north of the Pedemontana road with a maximum level of 319 mg/kg. In a previous study, different values were reported in *La. laccata*; respectively 12.9 and 92.5 mg/kg (*Demirbaş*, 2000, 2001).



10.4 Fig. 43. Spatial distribution of arsenic for the genus Clitocybe.

10.5

10.7 10.B

10.2 10.3

In Macedonia and the area of Epirus, Ouzoun et al. (2009) have found levels of 17.38 and 17.47 mg/kg in samples of Ar.mellea and Ar. tabescens respectively. The same authors, Ouzoun et al (2007), found concentrations of 16 and 4.65 mg/kg in Hy. eburneus and Hy. chrysodon. In Turkey Yamachiche et al. (2007) found 144.2 and 82.4

mg/kg in Le. nuda and I. geotropa respectively. In an area of the Black Sea, Sesli et al (2008) found concentrations of 32.8, 52.4 and 20.1 mg/kg in the species La. amethystina, Cl. gibba and Le. nuda. In France there was a concentration of 56.9 mg/kg in samples of Cl. nebularis (Michelot et al., 1998).

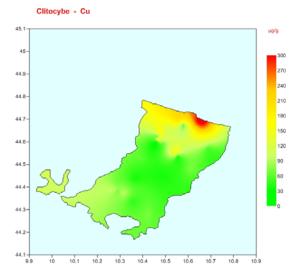


Fig. 44. Spatial distribution of copper for the genus Clitocybe.

Mercury: the Mercury map is shown in Fig. 45. The Quattro Castella site displays the highest levels, equal to 6 mg/kg. Moderately high values were also observed on the Apennine border. In literature there are relatively few data on mercury. Demirbaş (2000, 2001), in two studies carried out east of the Black Sea, obtained levels which were different (0.39 and 0.072 mg/kg) and much lower than those observed in Reggio Emilia, by using and analyzing samples of *La. laccata*. Cocchi *et al.* (2006), in the province of Reggio Emilia have reported levels of 6.25, 0.12, 0.22, 0.77, 1.74 and 1.78 mg/kg in *Le. nuda*, *La. laccata*, *Hy. penarius*, *Hy. russula*, *Ly. decastes* and *Ma. oreades*

respectively. In *Cl. nebularis* there was a significantly higher concentration, equivalent to 62.9 mg/kg (*Michelot et al., 1998*). In the Czech Republic, in an area adjoining silver mines, Svoboda *et al.* (2006) found levels of 12.9 and 4.2 mg/kg in *Ar. mellea* and *Le. nuda*. These values are considerably higher than both the average concentration level and the maximum value obtained in the province of Reggio Emilia.

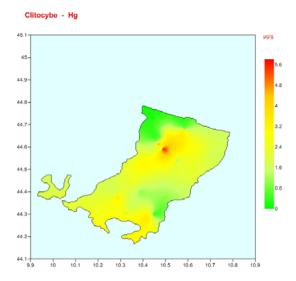


Fig. 45. Spatial distribution of mercury for the genus Clitocybe.

As has already been observed for other genera and species of fungi, arsenic, chromium, nickel and lead do not tend to accumulate in carpophores. This is evidenced by the enrichment factors for lead and confirmed by the studies of Garcia *et al.* (2009). In contrast the elements mercury (especially in *Le. nuda*) (*Kalač et al., 1989b*), cadmium, copper and

zinc tend to accumulate, but not in such a marked manner in *Clitocybe* carpophores, even though the soil is low in concentration levels of these. All this is confirmed for mercury by Falandysz *et al.* (2002).

The results obtained from principal component analysis are presented in Table 7.

	(Component			
	1	2	3		
pH_s	128	096	.961		
As_FA	.975	.175	090		
Cd_FA	.948	.256	123		
Cr_FA	.701	.304	.267		
Cu_FA	.980	.097	128		
Hg_FA	.215	.964	105		
Ni_FA	.988	.040	132		
Pb_FA	.911	.312	085		
Zn FA	.977	.159	113		

Table 7. Result of principal component analysis.

The first three components describe and explain 94% of the total variance. It is noted that mercury is in contrast with other elements and is dissociated by the acidity of the soil. The second component reinforces what has been described, mercury being separated from the other elements, and the third constituent describes the "weak" link between acidity and chrome. The remaining elements are not related to pH.

4.3.3 Genus Amanita (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Amanitales)

The chart of the concentration levels for arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc, was quite uniform. The exceptions are the pairs of aluminium and nickel and also chromium and cadmium that are qualitatively similar. As for the comparison between the concentration distributions in the mushrooms with those of the soil, the map does not appear related to the pH distribution, while the content of chromium, nickel and aluminium in the soil displays a correspondence with the mushrooms.

Cadmium: Fig 46 shows the distribution of cadmium, for which the average value is 2.5 mg/kg with a maximum of 33.4 mg/kg located in the Apennines. High levels are also found in the air between Vezzano and Viano. In Turkey, Tüzen (2003) found a similar level in Am. solitaria, equal to 7.5 mg/kg, while in Macedonia and in the area of Epirus, Ouzoun et al. (2009) found a rather modest level of 1.3 mg/kg in Am. caesarea. Similar values were found in the Black Sea in M. muscaria, Am. rubescens and Am. vaginata, with values of 1.6, 0.79 and 0:56 mg/kg, respectively (Demirbas, 2001). In Turkey, Yamaç et al. (2007) measured a level of 2.46 mg/kg in Am. Caesarea, which is in the middle between what is stated in our investigation and that of the other authors cited. A significant number of samples from species in the genus of Amanita was analysed by Michellot et al. (1998) in the Paris region. These included Am. Excelsa var. excelsa, Am. gemmata, Am. muscaria, Am. ovoidea, Am. pantherina, Am. phalloides, Am. rubescens, Am. solitaria, Am. excelsa var. spissa and Am. vaginata. The values obtained were similar to those observed by us, being, respectively: 6; 14.9; 13.9; 2.9; 10.3; 1.5; 2.4; 2.6; 2.5 and 7.7 mg/kg.

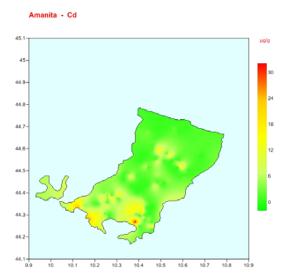


Fig. 46. Spatial distribution of cadmium for the genus Amanita.

Copper: the distribution of copper is shown in Fig. 47. The average level is 63.59 mg/kg. The highest values are found in the plains of the province while

other areas are very uniform and quite close to the average level. A similar value of 50.8 mg/kg was found in Turkey in Am. Caesarea (Yamachiche et

al., 2007), while Tüzen (2003) found 96.2 mg/kg in Am. solitaria. Demirbaş (2001) recorded slightly erratic values of 23.5, 51.2 and 5.1 mg/kg in Am. muscaria, Am. rubescens and Am. vaginata in the Black Sea area. In Macedonia and Epirus, Ouzoun et al. (2009) recorded 19.3 mg/kg in samples of Am. Caesarea. Also regarding copper, Michellot et al. (1998) observed similar levels in

Am. excelsa var. excelsa (75.6 mg/kg), Am. gemmata (44.4 mg/kg), Am. muscaria (28.4 mg/kg), Am. ovoidea (21.7 mg/kg), Am. pantherina (38.5 mg/kg), Am. phalloides (29.7 mg/kg), Am. rubescens (41.9 mg/kg), Am. solitaria (24 mg/kg), Am. excelsa var. spissa (29.2 mg/kg) and Am. vaginata (65.9 mg/kg).

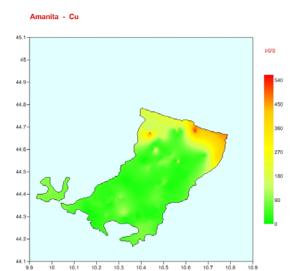


Fig. 47. Spatial distribution of copper for the genus Amanita.

Mercury: The highest values of mercury were found in the area of Casalgrande (24.3 mg/kg being the maximum level) (Fig. 48).

The average value of 1.19 mg/kg is representative of a substantial part of the Reggio Emilia area. In the Czech Republic, in an area close to silver mines, Svoboda *et al.* (2006) found 1.55 mg/kg in the *Am. rubescens*. Values one order of magnitude lower were found in the Black Sea in the *Am. muscaria* (0.18 mg/kg), *Am. rubescens* (0.23 mg/kg) and *Am. vaginata* (0.32 mg/kg) (*Demirbas*, 2001). In Poland, Falandysz *et al.* (2002) found

levels between 0.07 and 1.5 mg/kg in the caps and between 0.021 and 1.3 mg/kg in the stalks of Am. muscaria. The mercury levels found by Michellot et al. (1998) appear excessively high in: Am. excelsa var. excelsa (61.3 mg/kg), Am. Gemmata (37.4 mg/kg), Am. muscaria (61.3 mg/kg), Am. ovoidea (61.4 mg/kg), Am. pantherina (64.9 mg/kg), Am. phalloides (40.3 mg/kg), Am. rubescens (57 mg/kg), Am. solitaria (48.8 mg/kg), Am. excelsa var. spissa (58.4 mg/kg) and Am. vaginata (54.3 mg/kg).

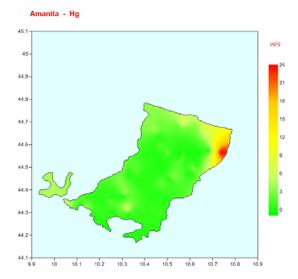


Fig. 48. Spatial distribution of mercury for the genus Amanita.

4.3.4 Amanita muscaria (L.) Lam. (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Amanitales -Genus Amanita)

For Am. muscaria, a medium-high to high frequency species, distribution maps of vanadium and zirconium (Figs. 49 and 50) have been drawn because the concentrations of these elements in this species are by all means exceptional. The areas

with the greatest concentrations are located in the Apennines, where vanadium and zirconium occur in their highest levels of 195 and 19.4 mg/kg respectively. From the concentration levels of the two elements and the soil pH levels, no direct relationship is evident, as was the case with samples of *Am. muscaria*. The two elements are bioaccumulated regardless of the concentration present in soils and their acidity.

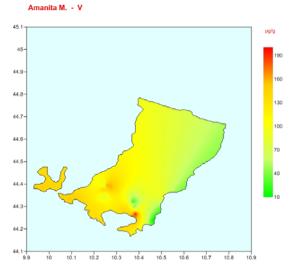


Fig. 49. Spatial distribution of vanadium for Am. muscaria.

Amanita M. - Zr

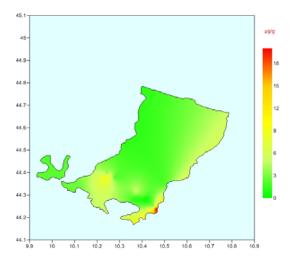


Fig. 50. Spatial distribution of zirconium for Am. muscaria.

It has been noted that arsenic, chromium, nickel and lead do not tend to accumulate in the genus *Amanita*, even if the soil is rich in these elements (*Kalac and Svobova, 2000, García et al., 2009*). The elements copper and zinc tend to accumulate but to a lesser extent, while cadmium and mercury accumulate abundantly (*Sova et al., 1991; Vetter*,

1994). This has been confirmed by Falandysz et al. (2002) for the element mercury. Kalač and Svobova (2000) examined enrichment factors equal to 50-300 and 30-500 for cadmium and mercury. The results obtained from principal components analysis are presented in Table 8. The first five components explained 59% of the total variance.

Table 8. Results of principal components analysis.

	Component				
	1	2	3	4	5
Al_a	096	.017	.832	035	.054
As_a	038	.139	221	104	.099
Cd_a	084	.611	138	004	.077
Cr_a	.091	026	.721	058	054
Cu_a	065	.025	.069	.802	.137
Hg_a	.129	043	.057	.612	.065
Ni_a	.006	104	.884	031	.009
Pb_a	091	.186	.272	.020	052
Zn_a	104	.432	048	.664	.026
Al_s	.009	.776	.040	093	.457
As_s	.293	.627	.011	.171	049
Cd_s	.730	083	056	.106	.010
Cr_s	.872	.227	.027	.075	.086
Cu_s	.026	.056	056	.259	.917
Hg_s	.086	599	032	.077	098
Ni_s	.913	163	.004	031	003
Pb_s	.211	263	181	.613	036
Zn_s	.070	.277	088	.002	.913

In component one, nickel and chromium in particular and also cadmium are linked together and fall marginally below the other elements in soils and fungi. In component two, cadmium and zinc tend to increase in mushrooms with increasing aluminium and arsenic in soils, while the mercury in the soil decreases. The third component describes the link between aluminium, chromium and nickel in mushrooms of terrigenous origin, in contrast again with falling arsenic levels in

mushrooms. The fourth component shows an increase of copper and mercury in mushrooms with lead, while the remaining elements do not change. The fifth component concerns aluminium, copper and zinc in soils.

As for the enrichment factor, the results obtained from the principal components analysis are presented in Table 9. These are related to enrichment factors, and the first three components describe and explain 93% of the total variance.

Table 0 Reco	ults of principal	Componente	analycic in	Amanita m	niccaria (I.)	[am
Table 9. Kesi	uits of brinciba	Components	anaivsis in	Amaniia m	iuscaria (L.)	Laiii

	Component			
	1	2	3	
As_FA	.996	013	050	
Cd_FA	.867	.451	044	
Cr_FA	.926	.154	.170	
Cu_FA	.112	.973	113	
Hg_FA	.941	.063	.304	
Ni_FA	265	.922	.018	
Pb_FA	.070	088	.954	
Zn_FA	.279	.923	187	
V_FA	.587	.759	126	
Zr_FA	.984	123	.110	
pH_s	.416	460	.549	

Base levels in soils are directly correlated with the bioaccumulation of almost all elements except for copper and lead, while nickel is predominantly bioaccumulated in acidic soils. Component two explains how acidic soils are in particular opposition to vanadium, cadmium, copper, nickel and zinc. The third component describes a strong relationship between pH and lead, an increase in one also increases the other and vice versa.

4.3.5 Genus Russula (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Russulales)

An overview and comparison of the maps showing arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc reveal a rather heterogeneous distribution of concentrations.

Arsenic: for this element (Fig. 51), the distribution is constant over the entire investigated area. The

higher values of 7 and 5 mg/kg were found near Ligonchio and Viano. The average level is 0.2 mg/kg.

Higher concentration values in four species of Russula were found by Demirbaş (2001) to the east of the Black Sea. In central Finland concentrations ranging from 0.1 to 0.5 mg/kg were found (Nikkarinen and Mertanen, 2004).

Lead and Zinc: Figs. 52 and 53 illustrate the spatial distribution of lead and zinc concentrations that appear to be qualitatively similar, except for a small area represented by the area of the city of Reggio Emilia, where the levels of lead appear to be significantly higher. These levels are due to motor vehicle fuel containing tetraethyl lead as an antiknock additive *(Cenci et al., 2008)*. The average level is 1.8 mg/kg, in agreement with that found in Finland (1.4 ÷ 2 mg/kg; *Demirbaş, 2001*) and Turkey (2.3 mg/kg; *Tüzen, 2003*).

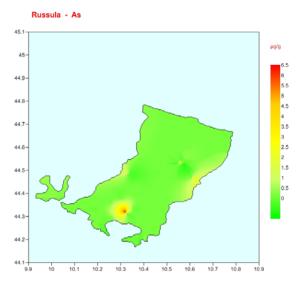


Fig. 51. Spatial distribution of arsenic for the genus Russula.

Still focusing on Turkey, the lead concentration in *Ru. delica* was 3.89 mg/kg (Demirbaş, 2000), while Konuk *et al.* (2007) found the rather small level of 0.03 mg/kg. This value, judging by observations of other elements in different fungi, seems somewhat underestimated. For zinc the average concentration

of 91mg/kg was higher than average levels, that ranged between 19 and 32 mg/kg (*Demirbaş*, 2001), whereas the value of 78 mg/kg repored from Turkey (*Tüzen*, 2003) looks quite similar to the one observed in our study.

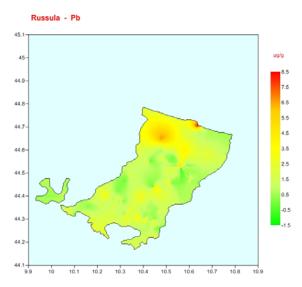


Fig. 52. Spatial distribution of lead for the genus Russula.



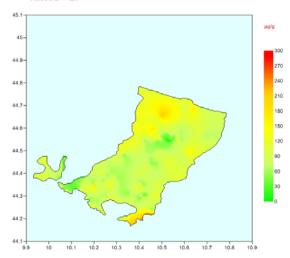


Fig. 53. Spatial distribution of zinc for the genus Russula.

The values of enrichment factors showed that arsenic, chromium, nickel and lead do not tend to accumulate in mushrooms even if the soil has high concentrations of these elements. This phenomenon has been further confirmed by Garcia *et al.* (2009). By contrast, cadmium, copper, zinc and mercury tend to accumulate in the fruiting bodies of fungi even when these elements are at low concentrations

in the soil (Kalac and Svoboda, 2000). This has been confirmed for mercury in Ru. emetica (Falandysz et al., 2002) and for cadmium in Ru. cyanoxantha (Vetter, 1994).

The results obtained from principal component analysis are presented in Table 10. The first five components describe and explain 60% of the total variance.

Table 10. Results of principal component analysis.

	Components				
	1	2	3	4	5
pH_s	.325	.123	.282	662	403
Al_r	083	.850	.022	.059	.136
As_r	.075	.139	096	144	.365
Cd_r	011	162	332	127	.018
Cr_r	.058	.904	.101	.017	.101
Cu_r	.110	204	472	.165	.129
Hg_r	.188	.090	148	031	258
Ni_r	030	.767	.073	143	235
Pb_r	.203	.383	358	005	.476
Zn_r	.239	.181	071	.046	.289
Al_s	.065	.146	004	.719	491
As_s	.196	068	.114	.893	083
Cd_s	.196	211	.574	.051	.471
Cr_s	.267	033	.851	.064	183
Cu_s	.939	018	.047	072	.116
Hg_s	.027	080	.055	043	.681
Ni_s	.094	061	.884	102	.166
Pb_s	.834	086	.213	.116	060
Zn_s	.910	.009	.060	.067	.124

In component 1, only copper, lead and zinc are associated with soil pH. With increasing pH the three elements increase, therefore lower soil acidity directly related to an increase in the concentration levels of those three elements. The association of chromium and nickel in the genus Russula is affected by aluminium. The same association, along with cadmium, can be found in soil and it represents a countertrend to copper and lead in mushrooms. Increases in the aluminium concentration in soil are associated with increases in arsenic and run counter to pH levels. In the fifth component we can see a divergence between the pH level and aluminium against arsenic and lead in soil and cadmium and mercury in mushrooms. For these last two items the increase in concentration inside carpophores is independent of concentration levels of the substrate (Kalač et al., 1989b; Jorhem and Sundström, 1995; Falandysz et al., 2002).

4.3.6 Genus Agaricus (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Agaricales)

Spatial distribution of the element concentrations highlights a few types of "associations". The first is represented by chromium and nickel with the highest values, 125 and 27 mg/kg respectively. They are characteristic of the area of Montecchio, while the remaining area displays mainly uniform

concentration values. A second association is represented by copper and lead: we registered the maximum levels in the area of Carpineti. The levels of aluminium, zinc and mercury partly overlap, while arsenic and cadmium are quite different both from each other and from other concentration distribution maps. The map showing soil acidity has a resemblance to that displaying zinc and mercury in mushrooms: one even notes a quantitative overlap between the aluminium in the soil; apparently, aluminium accumulates in fungi. Maps 54, 55 and 56 illustrate the concentration distribution of cadmium, mercury and lead. The average levels are respectively 1.58, 2.29 and 1.09 mg/kg.

Cadmium: the areas of highest concentration were found near Carpineti, Vezzano and Reggio Emilia. Demirbaş (2001) reported similar values, of 3.84 and 1.04 mg/kg in Ag. bisporus and Ag. Silvicola near the Black Sea. The same author in Turkey (Demirbaş, 2000) reported 2.17 mg/kg by analysing the species Ag. bitorquis. In Greece Ouzoun et al. (2007) found 0.15 mg/kg of cadmium in Ag. cupreobruneus.

Mendil *et al.* (2004) reported a value of 0.1 mg/kg in *Ag. bisporus* in an area with high vehicular traffic in Turkey.

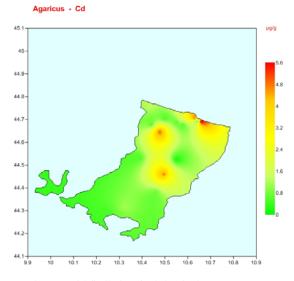


Fig. 54. Spatial distribution of cadmium for the genus Agaricus.

Mercury: the distribution of mercury concentrations over the territory was not uniform (Fig. 55). The highest values were seen in the area of Carpineti. Also, those areas comprising the towns of Castelnovo Monti, Vetto and Ramiseto have comparatively high values.

in the Black Sea Demirbaş (2001) recorded lower levels, 0.6 and 0.15 mg/kg, in Ag. bisporus and Ag.

silvicola respectively. The same author (Demirbas, 2000) reported 0.14 mg/kg in Ag. bitorquis in Turkey. These levels are significantly lower than those observed in this study. In the Czech Republic, in an area close to silver mines the average concentration levels reported for Ch. rhacodes were 2.59 mg/kg (Svoboda et al., 2006).

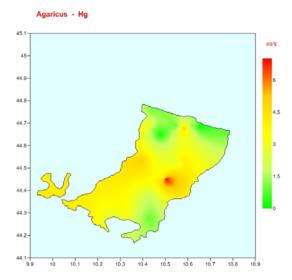


Fig. 55. Spatial distribution of mercury for the genus Agaricus.

Lead: Fig. 56 shows that the highest levels are those in the areas of Carpineti and the town of

Reggio Emilia. The latter probably due to its high volume of vehicular traffic.

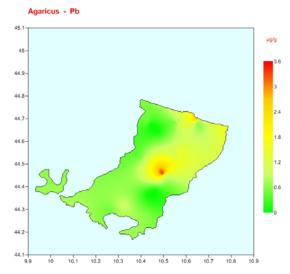


Fig. 56. Spatial distribution of lead for the genus Agaricus.

Different levels were reported by *Demirbas* (2000, 2001): in samples of *Ag. bitorquis*, *Ag. bisporus* and *Ag. Silvicola* they found 1.34, 2.41 and 0.92 mg/kg respectively. Mendil *et al.* (2004), reported a level of 6.9 mg/kg in *Ag. bisporus* in an area with high traffic levels in Turkey. Campos *et al.* (2009) found a 2.53 mg/kg in an *Ag. campestris* sample in central Spain. García *et al.* (2009), in the province of Lugo (Spain) analyzed *Ag. bisporus*, *Ag. campestris*, *Ag. urinascens* and *Ag. silvicola*, and found concentrations in the mushroom caps of 0.35, 3, 1.4 and 1.4 mg/kg. The same authors evaluated enrichment factors, concluding that lead does not tend to accumulate in fungi, even if it is present in high concentrations in the soil.

Arsenic, chromium, nickel and lead do not tend to accumulate in fungi. This aspect has been shown by enrichment factors, and convincingly confirmed for lead by García *et al.* (2009). In contrast the elements mercury (in particular), cadmium, copper,

and zinc tend to accumulate in the genus *Agaricus*, even where the soil has low concentrations, however this accumulation is not particularly strong or marked. This was confirmed for the element mercury by Falandysz *et al.* (2002).

The results obtained from principal component analysis are presented in Table 11. The first four components describe and explain 75% of the total variance.

The first component shows that the mercury in mushrooms goes against the majority of elements in the soil and is not affected by the mercury content of the soil itself. The second component supports this idea and links pH levels to cadmium, lead and copper in mushrooms in contrast to aluminium and arsenic in the soil. The third component describes the link between nickel and chromium in fungi with the soil pH level. The fourth component explains how the concentration of arsenic in mushrooms is independent and detached from soil.

Table 11. Results of principal component analysis.

	Components				
	1	2	3	4	
pH_s	.486	.462	.621	.086	
Al_s	.203	562	.284	.727	
As_s	.551	592	.187	.538	
Cd_s	.961	180	.078	062	
Cr_s	.898	.056	.286	.274	
Cu_s	.944	.242	.186	028	
Hg_s	.102	.684	102	266	
Ni_s	.979	.120	.144	032	
Pb_s	.918	019	.256	.269	
Zn_s	.944	264	.123	.083	
AL_a	.101	756	.305	074	
As_a	.014	440	.159	755	
Cd_a	003	.835	.253	.055	
Cr_a	.420	219	.810	.017	
Cu_a	190	.710	133	017	
Hg_a	495	.359	068	486	
Ni_a	.301	.214	.828	107	
Pb_a	.177	.811	.186	.032	
Zn_a	.002	.256	838	144	

4.3.7 Section Bitorques (Subdivision Basidiomycotina - Subclass Agaricomycetideae - Order Agaricales - Genus Agaricus - Subgenus Agaricus)

Only one pattern adequately summarises the spatial distribution of the concentration levels of arsenic, chromium, mercury and nickel. For arsenic and other more-strongly "associated" elements, the areas of highest concentration are between Canossa and the northern border of the Apennines, for chromium and mercury we can also include the area of Reggio Emilia and Castellarano. As regards the other elements, aluminium reaches its highest levels (2,771 mg/kg) in the Apennine zone of Ramiseto; cadmium (9.2 mg/kg) in the area of Villa Minozzo, copper (812 mg/kg) in Casalgrande and Castellarano, lead (29.5 mg/kg) in certain areas

near the city of Reggio Emilia and zinc (273 mg/kg) in Montecchio.

Overlap with the values in soils is possible with nickel and to a lesser extent for chromium and lead. The remaining elements and pH levels have apparently no direct relationship to the concentration levels in mushrooms.

Arsenic: the distribution of the concentration levels of arsenic is shown in Fig. 57. An average level of 0.3 mg/kg was found over most of the territory. The maximum levels were found in a vast area ranging from Canossa to Ligonchio. Slejkovec *et al.* (1977), analysed dozens of mushroom samples from European countries and Brazil and found a level of 1 mg/kg in the species *Ag. bisporus*, which is three times higher than that we found. Always in the Black Sea, Demirbaş (2001) reported a concentration level of 0.76 mg/kg in *Ag. bisporus*.

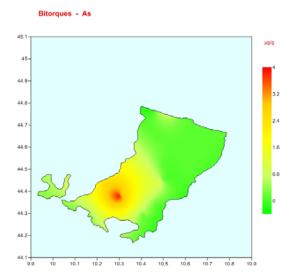


Fig. 57. Spatial distribution of arsenic for the section Bitorques of the genus Agaricus.

Cadmium: Fig 58 shows the distribution of cadmium. The highest levels were recorded in the Apennine area and the whole southern zone that runs from Casalgrande to Villa Minozzo. The average level, equal to 1.99 mg/kg, is characteristic of a good part of the territory. Values higher than those we encountered were reported by Demirbaş (2000, 2001) to the east of the Black Sea and in Turkey respectively in Ag. bisporus (3.48 mg/kg) and Ag. bitorquis (2.17 mg/kg). Mendil et al. (2004) in an area of high traffic in Turkey reported an extremely low level of 0.1 mg/kg in the species

Ag. bisporus, a fact not easily explained, because from the environmental context in which it was found one would expect far higher concentration levels.

Lead: lead is shown in Fig. 59. The average level of 3.94 mg/kg was found in almost every part of the territory investigated, the highest values for this species were in the vicinity of the city of Reggio Emilia. Mendil *et al.* (2004) reported a level of 6.9 mg/kg in *Ag. bisporus* in an area with high vehicular traffic.

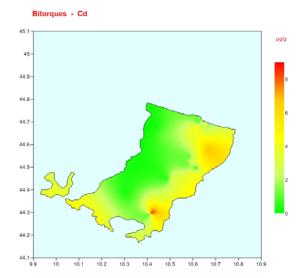


Fig. 58. Spatial distribution of cadmium for the section Bitorques of the genus Agaricus.

Demirbaş (2000, 2001) found a level slightly below the average level of 2.41 mg/kg in Ag. bisporus east of the Black Sea, while in Turkey on Ag. bitorquis the concentrations were significantly lower (1.34 mg/kg). García et al. (2009), in the province of Lugo (Spain), analysed Ag. bisporus

and observed levels of 0.35 mg/kg in the caps and 0.54 mg/kg in the stems which represent fairly low concentration levels.

In the Paris region, Michellot *et al.* (1998) observed 31 mg/kg in *Ag. maleolens* and 32 mg/kg in *Ag. silvaticus*.

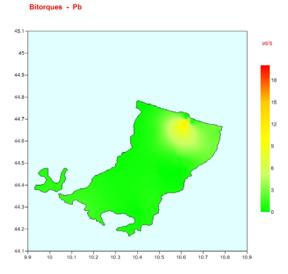


Fig. 59. Spatial distribution of lead for the section Bitorques of the genus Agaricus.

Arsenic, chromium, nickel and lead do not tend to accumulate in the fruiting bodies, while mercury (Vetter, 1994), cadmium, copper, and zinc, in

descending order tend to accumulate in the fruiting bodies of section *Bitorques*, but not in a particularly marked manner.

The results of the principal component analysis are presented in Table 12. The first four components

	Components				
	1	2	3	4	
Al_b	013	.667	165	.210	
As_b	425	.375	.051	369	
Cd_b	125	108	027	.825	
Cr_b	.002	.939	.069	012	
Cu_b	.037	.197	.101	.812	
Hg_b	.470	.607	071	.035	
Ni_b	.013	.858	.138	191	
Pb_b	.516	.195	.353	.080	
Zn_b	.370	.522	.202	.395	
Al_s	.315	007	.761	.215	
As_s	.234	.220	.856	.041	
Cd_s	.849	.202	.033	.113	
Cr_s	.728	.015	.525	192	
Cu_s	.050	232	.568	084	
Hg_s	.464	417	650	111	
Ni_s	.709	.040	.529	208	
Pb_s	.930	.030	034	061	
Zn_s	.702	164	.431	.047	

Table 12. Results of the principal component analysis.

Component one describes how a great deal of the elements in soils are themselves linked and present a countertrend to arsenic and, to a lesser extent with cadmium, in mushrooms. Component two in particular describes the conflict that exists between the concentrations of mercury, zinc, cadmium and copper in soils in relation to those found in fungi. It also describes the lack of any link between chromium, nickel, and to a lesser extent for arsenic and lead in mushrooms with the elemental contents of the soil. Component three groups several elements in soil which have trends contrary to mercury, while the fourth constituent explains how cadmium and zinc in fungi are not related to the concentration levels in the soil for the same elements and are both opposed to arsenic in soil.

4.3.8 Section Arvenses (Subdivision
Basidiomycotina - Subclass Agaricomycetideae - Order Agaricales - Genus
Agaricus - Subgenus Flavoagaricus)
Detailed observation rules out significant
similarities between the concentration distributions

of the elements investigated. A kind of association can only be seen for aluminium and lead. The remaining elements generally show far from uniform distributions. For cadmium the highest levels (390 mg/kg) were found between Ligonchio and Collagna, while in two areas (Casalgrande and Ligonchio-Collagna) the highest concentration level (19.2 mg/kg) was recorded for mercury.. For chromium, the distribution was fairly uniform throughout the area: the highest levels (10.9 mg/kg) were seen in the central Apennines. The maximum concentration level for copper (1.41 mg/kg), was recorded in the city of Reggio Emilia, where anthropogenic activity is of considerable importance, and to a lesser extent, on the border of the Apennines. Zinc and nickel were recorded as having their maximum levels, respectively, in Montecchio-Cavriago (361 mg/kg) and Quattro Castella (14.3 mg/kg).

Arsenic: Fig 60 shows the distribution of arsenic in the Reggio Emilia area. The maximum concentration of 21 mg/kg was reported in Villa Minozzo. Other areas of high concentration were

observed, while the average level of 1.49 mg/kg was recorded over the majority of the territory. Cocchi *et al.* (2006) reported levels of 1.06 and 1.52 mg/kg in *Ag. arvensis* and *Ag. silvicola* respectively. In samples from some European

countries and Brazil, Slejkovec *et al.* (1977) found 6.24 and 3.32 mg/kg in *Ag. silvicola* and *Ag. Macro-carpus*, levels significantly higher than those observed in our study.

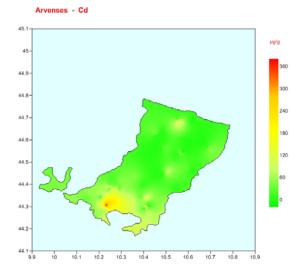


Fig. 60. Spatial distribution of cadmium for section Arvenses of genus Agaricus.

Lead: Fig. 61 shows that the maximum level (22.7 mg/kg), can be found in the vicinity of the city of

Reggio Emilia. The average concentration level of 2.52 mg/kg was found across most of the territory.

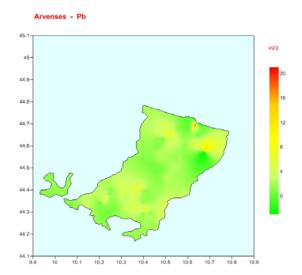


Fig. 61. Spatial distribution of lead for section Arvenses of genus Agaricus.

In the region of Paris (France), Michelot et al. (1998) analysed 92 species of mushroom including

Ag. arvensis, Ag. silvicola, Ag. altipes, finding levels of 22, 31 and 33.4 mg/kg respectively.

Demirbaş (2001) reported an average level of 0.92 mg/kg in Ag. silvicola, to the east of the Black Sea. In the province of Lugo (Spain), Garcia et al. (2009) found the same level (1.4 mg/kg) in both the cap and the stem of Ag. silvicola. Cocchi et al. (2006) reported 1.78 and 3.08 mg/kg in Ag. arvensis and Ag. silvicola respectively in Reggio Emilia

For the section *Arvenses*, as well as for the other taxa presented here, the elements arsenic, chromium, nickel and lead tend not to accumulate in the fruiting bodies, while cadmium, mercury, copper and zinc, in descending order, do, despite low concentrations of these elements in the soil.

The results of the principal component analysis are presented in Table 13. The first three components describe and explain 44% of the total variance.

The first component comprehensively describes the aggregation of cadmium, mercury, copper and zinc which run in countertendency to the other elements in the soil and soil acidity. The second component associates with arsenic and lead, while the third is influenced by chromium and nickel, and is opposed to soil acidity and other elements. It seems clear how these two groups of elements, having a totally different behaviour in bioaccumulation, were separated during the analysis of the main constituents.

Table 13. Rresults	of the principal component analysis.

	Components				
	1	2	3		
As_FA	.168	.703	.022		
Cd_FA	.978	.048	.026		
Cr_FA	.271	.033	.427		
Cu_FA	.937	.225	.160		
Hg_FA	.928	.093	.074		
Ni_FA	121	.204	.827		
Pb_FA	.137	.891	047		
Zn_FA	.784	.512	.107		
pH_s	119	.314	<mark>741</mark>		

4.3.9 Group Boletus edulis (Subdivision Basidiomycotina – Subclass Agaricomycetideae - Order Boletales)

For the group *Boletus edulis* (*B. aereus, B. reticulatus, B. edulis, B. pinophilus*), in addition to arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc, selenium was also taken into consideration. An overview and a comparison of the spatial maps showing the distribution of the

element concentrations, highlights four types of "associations." The first is represented by aluminium, chromium, nickel and lead (figs. $62 \rightarrow 65$). The maximum concentration levels are located in the hilly areas of Casina and Canossa. As for the similarities with the soil, there are only affinities for chromium and nickel, as also evinced by other species of fungi.



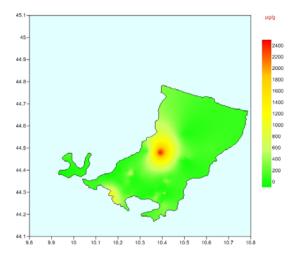


Fig. 62. Spatial distribution of aluminium for the group Boletus edulis.

Boletus E. - Cr

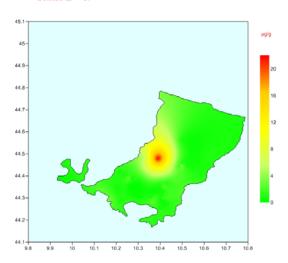


Fig. 63. Spatial distribution of chromium for the group Boletus edulis.

Average concentration levels are respectively 252 mg/kg for aluminium; 1.52 for chromium, 2.49 for nickel and 0.8 mg/kg for lead. A second group is composed of copper, mercury and zinc, the average levels of which are respectively 37, 3.52 and 138 mg/kg. The third group is composed of cadmium and selenium, with maximum levels (15.7 and 223 mg/kg, respectively) found at the border with Tuscany. The fourth group is formed by arsenic

only: its maximum level of 3 mg/kg was observed near Ouattro Castella.

Similar concentration levels of almost all elements analysed were found in samples of *B. edulis* in Finland (*Nikkarinen and Mertanen, 2004*). We found lead, selenium and zinc to have lower levels, however at: 0.18, 18.5 and 91 mg/kg. Falandysz *et al. (2008)* found selenium at lower amounts still, ranging between 8.7 and 32 mg/kg in the mountainous regions of Poland.

Boletus E. - Ni

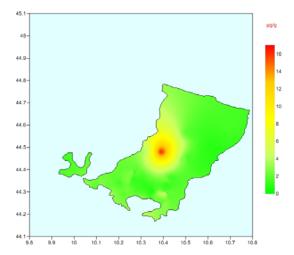


Fig. 64. Spatial distribution of nickel for the group Boletus edulis.

Boletus E. - Pb

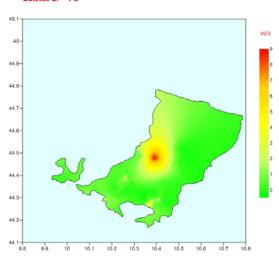


Fig. 65. Spatial distribution of lead for the group Boletus edulis.

In Greece Ouzoun *et al.* (2009) observed lower concentration levels for cadmium (0.23 mg/kg), chromium (0.86 mg/kg), nickel (1.61 mg/kg), lead 0.09 mg/kg) and zinc (89 mg/kg), and an almost identical level to copper (41 mg/kg) in *B. aereus*. Higher concentration values in the *Boletaceae* were found by Demirbaş (2001) in an area east of the Black Sea for arsenic (1.41 mg/kg), nickel (65 mg/kg) and lead (6.9 mg/kg). Lower levels were found by the same author for cadmium (1.36

mg/kg), chromium (0.86 mg/kg), copper (11.5 mg/kg), mercury (0.48 mg/kg) and zinc (19.6 mg/kg).

In the region of Paris (France), Michelot *et al.* (1998) analysed 92 species of mushroom including *B. edulis* and found 5.35 mg/kg for nickel and 21.2 mg/kg for lead. Smaller amounts were found for cadmium (1.39 mg/kg), chromium (1.34 mg/kg), copper (14.9 mg/kg), mercury (40.6 mg/kg) and zinc (55.4 mg/kg). In a recent paper by Frankowski

et al. (2010), the concentration levels of heavy metals in specimens of *B. edulis* collected in Poland are reported. The concentrations in the mushroom caps are significantly higher (Cd 5.5; Cu 47; Hg 4.9 and Zn 190 mg/kg). Similar levels were observed in the mountains in Poland (*Falandysz et al., 2008*); the following concentration intervals have been proposed: Cd (4-18 mg/kg), Cu (26-57 mg/kg), Hg (0.95-2.39 mg/kg) and Zn (150-210 mg/kg).

Enrichment factors confirm the tendency of arsenic, chromium, nickel and lead to *not* bioaccumulate, even in soils with high concentrations of the same elements. This is in strong contrast to copper, zinc and especially cadmium, mercury and selenium, which have a pronounced ability to bioaccumulate in the fruiting

bodies of fungi even at low concentrations of metals in the soil. This has been confirmed by García et al. (2009); Falandysz et al. (2002) Jorhem and Sundström (1995), Kalač et al. (1989b) and Cocchi et al. (2006). In particular, the last group of researchers has shown that the species of the Boletus edulis are able to accumulate large quantities of selenium. A further confirmation comes from a study by Frankowski et al. (2010) who also analysed the soil collected in the vicinity of the fungi. The enrichment factors were higher for Zn, Cd, Cu and Hg, in ascending order, demonstrating the ability of Boletus edulis to bioaccumulate these four elements, even if the soil has low concentrations of them (Zn 22; Cd 0.35, Cu 2.8 and Hg 0.04 mg/kg).

Table 14. Results of principal component analysis.

	Components			
	1	2	3	4
Al_b	.918	.020	042	202
Cd_b	153	086	.394	.436
Cr_b	.952	.064	.054	222
Cu_b	037	048	.731	.160
Hg_b	.195	.007	.418	.141
Ni_b	.936	052	005	199
Pb_b	.939	001	069	171
Se_b	022	251	.157	.483
Zn_b	321	.113	.692	030
pH_s	.130	.398	401	621
Al_s	353	056	027	.682
As_s	200	.211	281	.751
Cd_s	.140	.476	689	.169
Cr_s	.790	.322	029	.204
Cu_s	.070	.876	037	.041
Hg_s	.229	095	.808	237
Ni_s	.825	.339	.037	132
Pb_s	.080	.667	157	369
Se_s	.076	.576	423	.640
Zn_s	.166	.917	.062	078

The results of the principal component analysis, which relate the concentrations of heavy metals in mushrooms and the soil as well as pH levels, are presented in Table 14. The first four components describe and explain 84% of the total variance.

In component one, it is clear that the pH does not affect and is not linked to any elements either in the fungi or in the soil, while chromium and nickel in soil have a similar qualitative behaviour as they do in fungi in addition to lead and aluminium. Zinc in

species of *Boletus edulis* and aluminium in soils exist in countertendency.

Component two shows that, as Cocchi et al. (2006) observed, selenium in the species of the Boletus edulis is not related to the concentration of the same element in the soil. Copper, lead and zinc are linked and tend to increase regardless of the levels in the soil. The third constituent demonstrates that soil pH, selenium and cadmium in the soil are linked together and in countertendency to copper, zinc and mercury in fungi, and to the latter element in the soil. This highlights the bioaccumulating properties of mercury (Falandysz et al., 2002). The fourth constituent binds cadmium and selenium in

the species of the *Boletus edulis* to arsenic and selenium in the soils and in countertendency to lead in soils, thereby confirming the strong capacity for bioaccumulation of selenium (*Cocchi et al., 2006*). Enrichment factors (Table 15) show that the pH is unconnected to most elements. Component two clarifies that high acidity levels are inversely proportional to high concentration levels of chromium, mercury, and selenium, themselves running in countertendency to lead. This is illustrated by the values of main component three.

	Components			
	1	3		
pH_s	.055	428	769	
As_FA	.915	.361	042	
Cd_FA	.686	.003	.212	
Cr_FA	.419	.869	047	
Cu_FA	.922	.317	012	
Hg_FA	.179	.946	.019	
Ni_FA	.833	.306	.236	
Pb_FA	.301	244	.677	
Se FA	436	835	183	

435

.099

Zn FA

Table 15. Rresults of principal component analysis.

4.3.10 Genus Cantharellus (Subdivision Basidiomycotina – Subclass Aphyllophoromycetideae – Order Cantharellales)

The maps show a similarity between the couples of cadmium/mercury and chromium/nickel (Figs. 66 → 69). The remaining elements (arsenic, copper, lead and zinc) have very heterogeneous concentration distributions.

Cadmium and mercury: these have average levels of 0.49 and 12.23 mg/kg respectively, while their highest levels are found in hilly areas of Casina and

Baiso and in the mountain range between Busana and Villa Minozzo. In the area of Epirus and Macedonia, Ouzoun *et al.* (2009) recently reported cadmium concentrations of 0.38 mg/kg, in perfect agreement with those observed in this study. Also in Greece (Ouzoun et al. (2007) 0.41 mg/kg of cadmium was found in Ca. cibarius. For mercury, in the Czech Republic, in an area with many silver mines, the average level was 0.25 mg/kg (Svoboda et al., 2006). In Svoboda et al. (2000), reported mercury concentrations were similar to those observed by us in the Reggio Emilia Apennines.



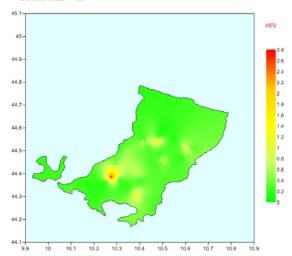


Fig. 66. Spatial distribution of cadmium for the genus Cantharellus.

Cantharellus - Hg

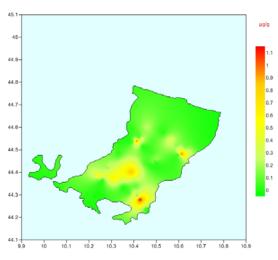


Fig. 67. Spatial distribution of mercury for the genus Cantharellus.

Chromium and nickel: these elements (Figs. 68 and 69) have average levels of 2.9 and 2.3 mg/kg respectively, while their maximum levels are 57 and 29 mg/kg. These levels were found in the mountains between Vetto and Ramiseto. There seems to be no link between these and soil acidity, although concentration levels in the soil do correlate to mushroom concentration levels. In Greece, lower concentration levels of chromium

(1.6 mg/kg) and nickel (1.1 mg/kg) were observed (Ouzoun et al., 2009).

Enrichment Factor values showed, like the genus *Russula*, little tendency for arsenic, chromium, nickel and lead to bioaccumulate in these mushrooms despite the soil being rich in the same elements. On the other hand, cadmium, copper, mercury and zinc were able to bioaccumulate in carpophores even where lower concentrations of these metals were found in the soil.



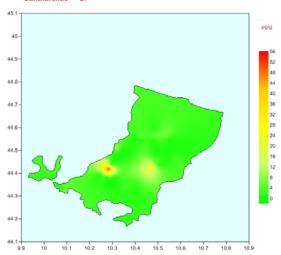


Fig. 68. Spatial distribution of chromium for the genus Cantharellus.

Cantharellus - Ni

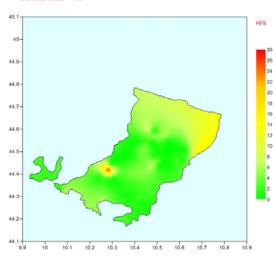


Fig. 69. Spatial distribution of nickel for the genus Cantharellus.

The results of the principal component analysis are presented in Table 16. The first three components describe and explain 79% of the total variance. In component one the acidity of the soil is disconnected from all the elements. While amounts of arsenic, copper, mercury, lead, zinc and, to a lesser extent, cadmium increase, those of nickel and chromium tend to decrease. In component two, as nickel and chromium increase, a decrease in

mercury and lead can be seen; this fact could be linked to the origin of these two elements, both notoriously influenced by human activities. The third constituent shows that basic soil corresponds to a reduction in cadmium concentrations, while other elements remain unchanged. This aspect of reduced bioaccumulation for cadmium has been shown in *Ca. cibarius (Jorhem and Sundström, 1995)*.

Table 16. Results of principal components analysis.

	Constituents			
	1	2	3	
pH_s	.076	.072	.860	
As_FA	.963	.040	037	
Cd_FA	.421	.149	708	
Cr_FA	.227	.811	.034	
Cu_FA	.935	.081	244	
Hg_FA	.499	652	.177	
Ni_FA	119	.760	.020	
Pb_FA	.796	468	.215	
Zn_FA	.919	093	153	

4.3.11 Genus Ramaria (Subdivision Basidiomycotina - Subclass Aphyllophoromycetideae - Order Clavariales)

Comparison of the maps showing the spatial distribution of element concentrations reveals three

types of "associations." The first is represented by four elements, namely aluminium, lead, zinc and, to a lesser extent, copper (Figs. $70 \rightarrow 72$). The maximum concentration levels are observed in flat areas and those in the Apennine border.

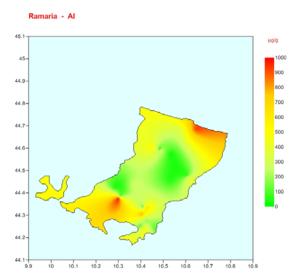


Fig. 70. Spatial distribution of aluminium for the genus Ramaria.

Ramaria - Zn

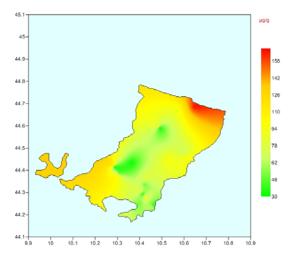


Fig. 71. Spatial distribution of zinc for the genus Ramaria.

Ramaria - Cu

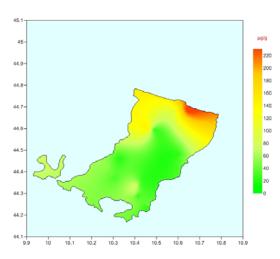


Fig. 72. Spatial distribution of copper for the genus Ramaria.

The second group includes arsenic, nickel and chromium (Fig. 73.) The areas with the highest concentrations are found in Ramiseto, Vetto and in Castelnovo nei Monti. The third group is composed of cadmium and mercury: the areas with the greatest concentrations were located between San

Polo, Bibbiano and Montecchio. The concentrations of aluminium and chromium in the soil overlap with their distribution maps in mushrooms, while the remaining elements only display a superposition of levels in some areas.

Ramaria - Cr

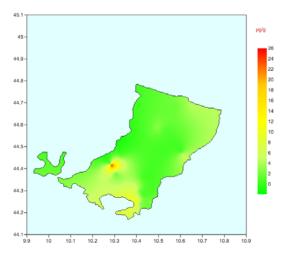


Fig. 73. Spatial distribution of chromium for the genus Ramaria.

Figs. 74, 75 and 76 illustrate the distribution of the concentration levels of arsenic, cadmium and lead. The average levels were 8.72, 5.74 and 0.98 mg/kg respectively. In Turkey, in *Ra. Flava*, arsenic concentrations of 0.02 mg/kg were observed (Konuk et al., 2007); this level is quite different to

that seen in the province of Reggio Emilia. A more comparable level (3.7 mg/kg) was reported by Slejkovec *et al.* (1977) who analysed samples of mushrooms from several European countries and Brazil.

Ramaria - As

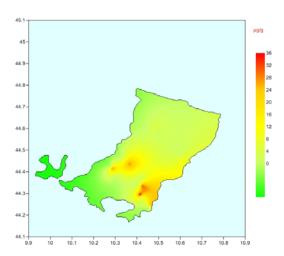


Fig. 74. Spatial distribution of arsenic for the genus Ramaria.

Cadmium: as for cadmium, very low levels (1.13 mg/kg) were reported in the area of Epirus and Macedonia in samples of *Ra. Largentii* by Ouzoun

et al. (2009). The value observed by Konuk et al. (2007) in samples of Ra. flava collected in Turkey (0.01 mg/kg) was 50 times lower than the

minimum we recorded (0.52 mg/kg). In the region of Paris (France), Michelot et al. (1998) analyzed

92 species of fungi, including *Ramaria* sp., and found levels of 4.32 mg/kg.



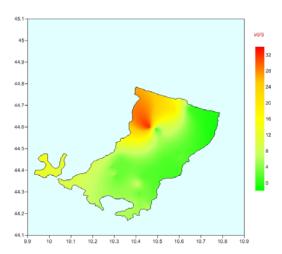


Fig. 75. Spatial distribution of cadmium for the genus Ramaria.

Lead: the average concentration levels we observed were significantly higher than those reported in previous literature [0.12 mg/kg

(Ouzouni et al., 2009) and 0.018 mg/kg (Konuk et al., 2007)].

Ramaria - Pb

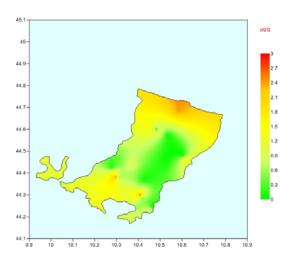


Fig. 76. Spatial distribution of lead for the genus Ramaria.

Enrichment Factor values showed that cadmium, mercury, copper, zinc, arsenic and nickel, in descending order, accumulate in *Ramaria*

carpophores in the presence of small concentrations of these metals in the soil, while lead and, to a lesser degree, chromium, do not tend to accumulate in mushrooms even if the soil is rich in these elements.

The results of the principal component analysis are presented in Table 17. The first three components describe and explain 92% of the total variance.

The first component explains how the acidity of the soil is not related to bioaccumulation of the elements we tested: all elements except lead tend to increase their concentration in the fruiting bodies of fungi.

The second component confirms no relation to other elements or pH levels for the bioaccumulation of lead and chromium. The third confirms the independence of the acidity of the soil from all elements considered.

Tabella17. results of the principal component analysis.

	Component			
	1	2	3	
pH_s	.129	.011	.985	
As_FA	.909	.187	.090	
Cd_FA	.960	.040	.060	
Cr_FA	.757	.549	.216	
Cu_FA	.927	.047	.061	
Hg_FA	.819	.380	.308	
Ni_FA	.952	.280	.073	
Pb_FA	.063	.956	020	
Zn_FA	.950	079	.141	

4.3.12 Genus Morchella (Subdivision Ascomycotina - Subclass Pezizomycetideae - Order Pezizales)

One type of distribution is able to represent the concentration levels of aluminium, mercury, nickel, zinc and to a lesser extent chromium and lead. The remaining elements arsenic, cadmium and copper show different types of "associations" for each of the six metals listed above. For aluminium and other, more "associated", elements, the areas with the greatest concentrations were Casalgrande and San Polo, and excluding chromium and lead, also the hilly areas. Arsenic values reach their maximum near Reggio Emilia, cadmium in the areas of Toano, Villa Minozzo and Quattro Castella, while copper is found in high concentrations near Castellarano and Castelnovo Monti.

Considering a quantitative overlap with soil concentrations, lead showed the greatest affinity,

but to a lesser extent also chromium and copper. Cadmium: the distribution of cadmium concentrations is shown in Fig. 77, the average level is 0.94 mg/kg and covers much of the territory. The highest levels (4.12 mg/kg) were observed between Toano and Villa Minozzo and in the area of Canossa. In the same area Cocchi et al. (2006) observed 12.55 mg/kg in Mo. esculenta. In Turkey (Tüzen, 2003) Mo. esculenta contained 1.43 mg/kg. Also in Turkey, in Mo. esculenta, Mo. esculenta var. umbrina, Mo. vulgaris, Mo. costata, Mo. deliciosa, and Mo. rigida cadmium concentrations were 0.031, 0.002, 0.036, 0.024, 0.029, 0.007 mg/kg respectively (Konuk et al., 2007). These levels are 20-200 times lower than those reported in this paper and given in literature. In France, in samples of Mo. esculenta, a concentration of 3.6 mg/kg was observed (Michelot et al., 1998).



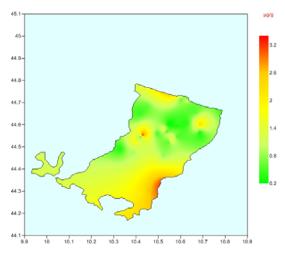


Fig. 77. Spatial distribution of cadmium for the genus Morchella.

Nickel: Fig. 78 represents the concentration of nickel in the Reggio Emilia area. The maximum level of 12.2 mg/kg was observed in the lowlands near Scandiano. The average level of 2.4 mg/kg is commonly found all over the area. In France a concentration of 15.4 mg/kg, higher than the maximum level we measured, was found in a sample of *Mo. esculenta (Michelot et al., 1998)*. Tüzen (2003) in Turkey recorded 1.18 mg/kg in

Morchella - Ni

Mo. esculenta; a value in line with the data presented here. In Turkey Mo. esculenta, Mo. esculenta var. umbrina, Mo. vulgaris, Mo costata, Mo. deliciosa, and Mo. rigida were found to contain nickel concentrations of 0.07; 0.68; 0.04; 0.4; 0.23; 0.41 mg/kg, respecitvely (Konuk et al., 2007); these levels are at least an order of magnitude lower than those we recorded.

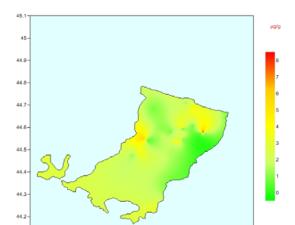


Fig. 78. Spatial distribution of nickel for the genus Morchella.

10.4

For completeness, we provide here several data for two species of the genus *Helvella* (order *Pezizales*): Cadmium: 1.97 mg/kg in *Helvella crispa* (Scop.) Fr. (Cocchi et al., 2006) and 0.033 mg/kg in *Helvella leucopus* Pers. (Konuk et al., 2007);

Nickel: 0.3 mg/kg in Helvella leucopus Pers. (Konuk et al., 2007)

For the genus Morchella as it was for other taxa, arsenic, chromium, nickel and lead do not tend to

accumulate in the fruiting bodies. In contrast, and in descending order, the elements zinc, cadmium, mercury and copper (Falandysz et al., 2002) tend to accumulate in the fruiting bodies (though not in a marked manner) even if the soil has low concentrations.

The results of the principal component analysis are presented in Table 18. The first three components describe and explain 94% of the total variance.

Table	18.	Ana	lysis	of	main	constituents.

	Constituents			
	1	2	3	
pH_s	043	.120	.964	
As_FA	.934	.307	029	
Cd_FA	.976	.142	014	
Cr_FA	.966	154	.032	
Cu_FA	.140	.954	.174	
Hg_FA	.816	.161	.329	
Ni_FA	.957	.140	131	
Pb_FA	.802	.377	206	
Zn_FA	.797	.502	091	

In the first component soil acidity and copper are linked but at the same time independent of other elements, instead, in the second copper and zinc move in the same direction and are differentiated from both the pH and from almost all other elements. The third reinforces the neutrality towards soil acidity for all elements, except, in part, for mercury that tends to increase with increasing pH, while the lead does the opposite and tends to decrease as soil pH rises.

4.3.13 Conclusions

The analytical results obtained by analysing thousands of different fungi belonging to hundreds of species have yielded distribution maps of heavy metals in an area with diverse geomorphological features ranging from lowland areas to the Apennine peaks. The land use includes residential zones such as the city of Reggio Emilia and other major urban centres, busy main roads, industrial areas, agricultural areas and other intensively farmed pasture, and woods and forests. There is a rich diversity of flora, fauna and countryside where many daily activities are carried out, leaving "footprints" that combine to change the very nature of the environment.

The results obtained from analysing mushrooms cannot tell us how we should behave as regards the chemical elements contained inside them, in particular heavy metals. The results of the spatial concentration distributions are quite different from each other and "dominated" by species-specific factors for each fungus such as the length, depth and age of the mycelium, the type of element under investigation, its chemical form and its availability in the soil substrate, the type of soil-substrate, the organic matter in the soil substrate, vegetation, the degree of humidity, and many other unknown factors, some of which are still unknown.

One important aspect that should perhaps be investigated further is the ability of fungi to accumulate heavy metals, even if the soil has no high concentrations of these. In this study we have seen that some elements, such as cadmium, copper, mercury and zinc tend to accumulate in different fungal species regardless of the nature and concentrations of elements in the soil-substrate. Such has been confirmed for mercury (Falandysz et al., 2002) and for mercury, and cadmium (Vetter, 1994, Kalac and Svobova, 2000). It has also been observed that, in general, arsenic, chromium, nickel and lead do not tend to accumulate in fungi, even if the soil is rich in these elements, a fact confirmed

for lead (Kalac and Svobova, 2000, García et al., 2009).

Giving "guidelines" at present is rather difficult and premature. In the light of current knowledge, the use of mushrooms as bioindicators of soil quality and the environment, is still in the embryonic stages and not yet viable, even if some fungal species could be used as "warning mushrooms". Future investigations in the same area could expand the information available for one or more species of mushroom, allowing some to be defined and used as environmental bioindicators.

Based on the wealth of data and considerations presented here, we think it is feasible to claim that, given the concentration levels of chemical elements in mushrooms in conjunction with the concentrations of these metals in soils, this study could facilitate the identification of "threshold limits" for these heavy metal concentrations, bearing in mind their impact on human health, which would apply to the sale of certain species of edible mushrooms.

The names of the fungal species considered in this chapter have been written in an abbreviated form to make the text more readable,. The species are now listed here alphabetically, alongside with their full names, in accordance with the taxonomy specified in paragraph 2.1.4.

- Ag. altipes = Agaricus altipes (F. H. Møller) F. H. Møller
- *Ag. arvensis* = *Agaricus arvensis* Schaeff.
- Ag. bernardii = Agaricus bernardii Quél.
- Ag. bisporus = Agaricus bisporus (J.E. Lange) Imbach
- Ag. bitorquis = Agaricus bitorquis (Quél.) Sacc.
- Ag. campestris = Agaricus campestris L.
- Ag. cupreobrunneus = Agaricus cupreobruneus (Jul. Schäff. & Steer) Pilát.
- Ag. macrocarpus = Agaricus macrocarpus (F.H. Møller) F.H. Møller
- *Ag. silvaticus = Agaricus silvaticus* Schaeff.
- Ag. silvicola = Agaricus silvicola (Vittad.) Peck.
- Ag. urinascens = Agaricus urinascens (Jul. Schäff. & F.H. Møller) Singer
- Am. caesarea = Amanita caesarea (Scop.)
 Pers.

- Am. excelsa var. excelsa = Amanita excelsa var. excelsa (Fr.) P. Kumm.
- Am. excelsa var. spissa = Amanita excelsa var. spissa (Fr.) Neville & Poumarat
- Am. gemmata = Amanita gemmata (Fr.) Bertill.
- Am. muscaria = Amanita muscaria (L.) Lam.
- *Am. ovoidea* = *Amanita ovoidea* (Bull.) Link
- Am. pantherina = Amanita pantherina (DC.) Krombh.
- Am. phalloides = Amanita phalloides (Vaill. ex Fr.) Link
- Am. rubescens = Amanita rubescens var. rubescens Pers.
- *Am. solitaria = Amanita solitaria* (Bull.) Fr.
- Am. vaginata = Amanita vaginata (Bull.) Lam.
- Ar. mellea = Armillaria mellea (Vahl) P. Kumm.
- Ar. tabescens = Armillaria tabescens (Scop.) Emel
- B. aereus = Boletus aereus Bull.
- *B. reticulatus = Boletus reticulatus* Schaeff.
- B. edulis = Boletus edulis Bull.
- B. pinophilus = Boletus pinophilus Pilát & Dermek
- *Ca. cibarius = Cantharellus cibarius* Fr.
- *Cl. gibba* = *Clitocybe gibba* (Pers.) P. Kumm.
- *Cl. nebularis* = *Clitocybe nebularis* (Batsch) P. Kumm.
- *He. crispa = Helvella crispa* (Scop.) Fr.
- *He. leucopus = Helvella leucopus* Pers.
- *Hy. chrysodon* = *Hygrophorus chrysodon* (Batsch) Fr.
- Hy. eburneus = Hygrophorus eburneus (Bull.) Fr.
- Hy. penarius = Hygrophorus penarius Fr.
- Hy. russula = Hygrophorus russula (Schaeff.)
 Kauffman
- geotropa = Infundibulicybe geotropa (Bull.) Harmaja
- La. amethystina = Laccaria amethystina Cooke
- La. fraterna = Laccaria fraterna (Sacc.)
 Pegler
- *La. laccata = Laccaria laccata* (Scop.) Cooke
- *Le. nuda = Lepista nuda* (Bull.) Cooke
- Ly. decastes = Lyophyllum decastes (Fr.) Singer
- *Ma. oreades* = *Marasmius oreades* (Bolton) Fr.

- *Mo. costata = Morchella costata* (Vent.) Pers.
- *Mo. deliciosa = Morchella deliciosa* Fr.
- Mo. esculenta = Morchella esculenta (L.) Pers.
- Mo. esculenta var. umbrina = Morchella esculenta var. umbrina (Boud.) S. Imai
- Mo. rigida = Morchella rigida (Krombh.) Boud.
- Mo. vulgaris = Morchella vulgaris (Pers.) Boud.
- Ru. cyanoxantha = Russula cyanoxantha (Schaeff.) Fr.
- Ru. delica = Russula delica Fr.
- Ru. emetica = Russula emetica (Schaeff.)
 Pers.

- Ra. flava = Ramaria flava (Schaeff.)
- Ra. largentii = Ramaria largentii Marr & D.E. Stuntz
- T. argyraceum = Tricholoma argyraceum (Bull.) Gillet
- T. equestre = Tricholoma equestre (L.) P. Kumm.
- T. terreum = Tricholoma terreum var terreum (Schaeff.) P. Kumm.
- T. rutilans = Tricholomopsis rutilans (Schaeff.) Singer
- T. ustaloides = Tricholoma ustaloides Romagn.

4.4 Sampling: a data sheet example

Each sampling site, where both soil and fungal samples were collected, is described in detail and correctly georeferenced, so that the analytical data of the samples can then be used to create geostatistical maps.

Here follows an example of one sample data sheet used in this study to describe the sample sites.

Sheet 1

Area and sample description





Map 1 Map 2





Photo nr.1 Photo nr.2

ID Toponym - Location: Fonte dell'Anatella

Municipality: Rocca di Mezzo

Geographic area: Abruzzo, Provincia dell'Aquila, Parco del Sirente, Velino

Sampling date: 7 Settembre 2005

Coordinates: Geographic coordinate system: UTM/UPS Map Datum: WGS 84

33T Long. 0379918 Lat. 4671322

Altitude and inclination: 1400 m; 5%

Area description: Specimen found in the city of Fonte Anatella, on the basal portion of a beech

(Fagus sylvatica L.) in the municipality of Rocca di Mezzo, in a beech forest on limestone matrix on the slopes of Mount Sirente. Wooded slopes, dense

vegetation, steep terrain

Habitat: natural woodlands

Substrate: Woody matrix

Fungus

Phylum: Basidiomycota

Class: Basidiomycetes

Order: Polyporales

Family: Meripilaceae

Specimen name: basidiocarp of *Meripilus giganteus* (Pers. : Fr.) P. Karsten

leg. Fabio Siniscalco, det. Carmine Siniscalco

Height: 94.5 cm (highest point of basidiocarp complex)

Width: 139.5 cm (widest point of basidiocarp complex)

Weight: 142.68 kg (total basidiocarp weight)

Note: basidioma growing at the base of a

beech tree between the collar and large

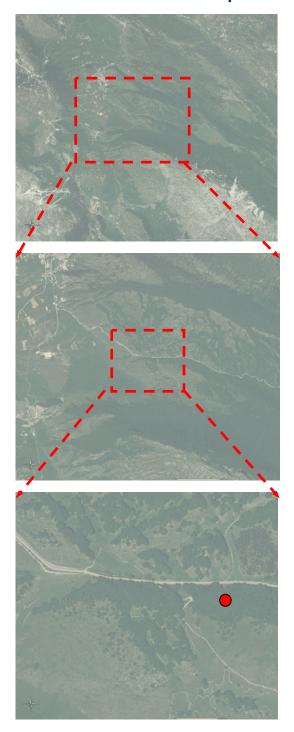
emerging roots.



Analytical results

element	concentration mg/kg	element	concentration mg/kg	element	concentration mg/kg
Ag	1.11	Ge	0.001	Se	0.1
Al	115	Hg	0.05	Sr	0.62
As	0.2	K	27900	Ti	9.37
Ва	1.18	La	n.d.	V	0.11
Ве	0.01	Li	0.13	Y	n.d.
В	10.4	Mg	1910	Zn	50.8
Cd	2.25	Mn	4.67	Zr	0.18
Ca	185	Мо	0.11	CI	n.d.
Cs	0.06	Na	80.0	Р	6270
Cr	3.34	Ni	2.02	S	1310
Со	n.d.	Pb	9.13	¹³⁴ Cs	n.d.
Cu	107	Rb	23.4	¹³⁷ Cs	n.d.
Fe	216	Sc	n.d.	⁴⁰ K	n.d.

Location of sample area



Starting from the macroarea, the three figures on the left describe the exact spot where the discovery and the collection of the fungus took place.

Chapter V Conclusions

Bioindication allows evaluating the effects of anthropic activity on the environment through the observation of living organisms. The widespread use of macromycetes for bioindication is often limited by taxonomic difficulties, by incomplete knowledge of fungal metabolism and physiology, and by the lack of data on the quality and characteristics of the substrate.

Further obstacles to the correct interpretation of environmental data through fungi are the lack of precision and accuracy in describing their habitat.

This EUR report aims to help develop a working methodology and to show applicable concrete examples of the paths to take in the future.

The first aspect which was studied herein was the measure of chemical substances contained in the carpophores of spontaneous macromycetes.

The second aspect considered was the "reference mushroom", a tool already in use for other organisms, and which could prove to be a key element in unravelling the issues surrounding chemical elements in macromycete carpophores.

We arrived at this by attempting to overcome the initial difficulties of interpreting, with no available parameters, the significance of the presence of different chemical elements (heavy metals in particular) in fungi. The presence of these elements was often surprising due to unexpected concentration levels and large differences between very closely-related taxonomic species.

The third aspect dealt with in this work was linking fungal species with their habitats. This allowed us to observe important biodiversities which might be further investigated and studied in future work, with the aid of new technologies and with the help of environmental coding carried out by the CORINE programme and the European system of natural information, EUNIS. Following this it will

be possible to be guided by objective criteria when describing habitats with specific macromycetes growth.

We hope that our work may sparkle new ideas for study and research that could help support the use of these organisms in environmental assessments. Such a feature will be important when considering the mycological components of terrestrial ecosystems, which is becoming an increasingly important and relevant factor in the evaluation of global ecological balances.

This work aims not to close but to open a new vision with new opportunities for scientific research on the world of higher mushrooms, which is little understood and too often undervalued. By making available to everyone this volume of data, that, in itself, constitutes an important contribution to the documentation of fungal biodiversity, we have above all aimed to describe a particular working methodology and some practical applications. We are convinced that it can be used to produce new research in this field.

For example, we are convinced that taxonomic research should be based on a polyphasic approach that includes the collection and analysis of macro and microscopic data, including morphological, physiological, and biochemical features; further, the most accurate possible definition of each macromycete's habitat; we believe that in this context the measurement of concentrations of different chemicals in and molecular characteristics of that habitat should be analysed.

Last but not least we hope that our work will prove useful to all those who have different levels of political and administrative responsibility of the territories, especially regarding the use of mushrooms as food.

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VII Appendix

For a better understanding of the book, we have decided to include descriptions and pictures of some fungal species studied, as well as some of the most interesting and commonly known fungal species. Pictures and descriptions are taken from Volumes I (1999), II (2001) and III (2009) of the series "Atlante Photografico dei Funghi d'Italia" ("Photographic Atlas of Italian Mushrooms"), published by the Fondazione Centro Studi Micologici of the Associazione Micologica Bresadola, edited by Giovanni Consiglio, Carlo Papetti and, only for Vol. 1, Giampaolo Simonini. In some casees the names of the species used by these authors are different from those used here, for the reasons described in paragraph 2.1.4(page 20). As already explained, the nomenclature follows www.indexfungorum.org; synonyms are given in square brackets.

The descriptions also provide information on the edibility of each species (please note: "edible" is appropriate only if the mushroom is thoroughly cooked!)..

The number of images presented here is limited, compared to the number of fungal species occurring in nature; we believe, however, that it represents appropriately the considerable potential for biodiversity possible in the fungal kingdom.

Species are listed in alphabetical order for purely practical reasons and not according to rigorous systematic criteria.

The photographers are credited in "Atlante Photografico dei Funghi d'Italia", with the sole exception of the image showing Agaricus urinascens, which was taken by Ennio Carassai.

1	Agarieus amousis	T EE	Unananhamia miasula
1 2	Agaricus arvensis Agaricus bernardii		Hygrophorus russula Laccaria amethystina
3	Agaricus bisporus	1000	Laccaria fraterna
4	Agaricus bitorquis	1000	Laccaria Jaccata
5	Agaricus ottorquis Agaricus campestris		Lactarius deliciosus
6	Agaricus cupreobrunneus	10000	Lactarius deterrimus
7	Agaricus macrocarpus	1000	Lactarius giperatus
8	Agaricus silvaticus	1000000	Lactarius sanguifluus
9	Agaricus silvicola	1000	Lactarius sangujitus Lactarius torminosus
10	Agaricus urinascens	1000	Lactarius volemus
0.000	Agaricus xanthodermus	100000	Langermannia gigantea [Calvatia gigantea (Batsch) Lloyd]
12	Agrocybe aegerita [A. cylindracea (DC.) Maire]	200	Leccinum aurantiacum
2.2	Amanita caesarea	37.00	Lepista nuda
	Amanita echinocephala	100	Lycoperdon echinatum
2000	Amanita excelsa var. excelsa	100000	Lycoperdon perlatum
1000	Amanita junquillea [A. gemmata (Fr.) Bertill.]		Lycoperdon periatani Lycoperdon pyriforme
400	Amanita muscaria		Lyophyllum decastes
100	Amanita ovoidea		Macrolepiota procera
	Amanita pantherina		Macrolepiota rhacodes [Chlorophyllum rhacodes (Vittad.) Vellinga]
0.000	Amanita phalloides	100000	Marasmius oreades
21	Amanita rubescens	1.00	Meripilus giganteus
1000	Amanita vaginata		Morchella conica var. costata [M. costata (Vent.) Pers.]
5-215	Armillaria mellea	100000	Morchella esculenta
100	Armillaria ostoyae		Morchella esculenta var. vulgaris [M. vulgaris (Pers.) Boud.]
2010	Armillaria tabescens	-3,5000	Morchella semilibera [Mitrophora semilibera (DC.) Lév.]
	Boletus aereus	80	Mycena pura
27	Boletus aestivalis [B. reticulatus Schaeff.]	81	Mycena rosea
120.5	Boletus edulis	1000000	Phallus impudicus
2552	Boletus pinophilus	100	Pleurotus ostreatus
1202	Boletus regius	84	Ramaria botrytis
100	Boletus satanas	77.00	Ramaria flavescens
32	Calocybe gambosa	C 1000 1000	Ramaria formosa
33	Calvatia utriformis [Lycoperdon utriforme Bull.]	87	Ramaria largentii
10000	Camarophyllus pratensis [Hygrocybe pratensis (Fr.) Murrill]	88	Ramaria pallida
1250323	Cantharellus cibarius	89	Russula aurea
36	Cantharellus lutescens [Craterellus lutescens (Fr.) Fr.]	90	Russula cyanoxantha
37	Clathrus ruber	91	Russula delica
38	Clitocybe cerussata [C. phyllophila (Pers.) P. Kumm.]	92	Russula emetica
39	Clitocybe geotropa [Infundibulicybe geotropa (Bull.) Harmaja]	93	Russula foetens
40	Clitocybe gibba	94	Russula mustelina
41	Clitocybe nebularis	95	Russula queletii
42	Coprinus comatus	96	Russula sanguinea [R. sanguinaria (Schumach.) Rauschert]
43	Cortinarius orellanus	97	Russula vesca
44	Cortinarius praestans	98	Sarcosphaera crassa [S. coronaria (Jacq.) J. Schröt.]
45	Entoloma bloxamii	99	Suillus granulatus
46	Entoloma sinuatum		Suillus luteus
0.000	Helvella crispa		Tricholoma argyraceum
48	Helvella pityophila	102	Tricholoma equestre
10.0000	Hygrocybe coccinea		Tricholoma portentosum
50	Hygrocybe psittacina	104	Tricholoma terreum [T. myomyces (Pers.) J.E. Lange]
	Hygrophorus chrysodon	100000	Tricholoma ustaloides
	Hygrophorus eburneus	1010000	Tricholomospis rutilans
	Hygrophorus latitabundus	107	Xerocomus subtomentosus [Boletus subtomentosus L.]
54	Hygrophorus penarius		



Agaricus arvensis Schaeff.: Fr.

CAP 80-140 mm, hemispheric, then convex-flat, hairless, white, bordering on a silky shine, at maturity yellowish on circumference, finally, from yellow-citrina to vivid ochre all over; edges hanging with residues of veil.

GILLS at the extremes from whitish to pale grey-pink, then pink-greying and only with age from brown-purple to brown-black, crowded, free, width of 6-8 mm, with pale gill edge.

STIPE $80-120 \times 12-25$ mm, cylindrical, widening towards base but not bulbous, white, to the touch yellow-citrina, annulus white, persistent, high, broad, flaring, yellow when handled, in two layers, lower layer has coarse white-ochre-ish scales , with appearance of a gear wheel. FLESH white, with age light hint of ochre, thick in the centre, strong smell of aniseed, flavour of hazelnut.

MICROSCOPY: ellipsoidal spores, smooth, under microscope dark brown, dimensions 6.5-8 \times 4.2-5.4 μ m; tetrasporophyte basidia; marginal cells from clavate to vescicle. Brown-black spores.

HABITAT: often gregarious, rarely isolated, from the spring to autumn, in meadows, in pastures, around the edges of woods. Infrequent.

EDIBILITY: edible

NOTE - Good to eat. The species of the subsection Flavescentes are however, natural concentrators of silver, cadmium and mercury. The Flavescentes produce carpophores with an anise or hazelnut odour and are weakly yellow at the extremities. The caps tend to be quite large, the gills at the extremes are a very pale fleshy grey, there will usually be evident yellowing, the base of the stipe is large but not bulbous, all factors which facilitate the identification of this lovely species. It could be confused with Agaricus nivescens, which, however differs by having a more modest yellowing, and smaller and rounder spores. Another similar species is Agaricus xanthodermus, which has a slight odour of phenol, marginally bulbous stipe and on scratching shows a golden yellow colour which is particularly evident at the base of the stipe and to a lesser degree in the flesh.



Agaricus bernardii Quél. in Cooke & Quél.

CAP 60-160 mm, fleshy, initially globular, then hemispheric or hemispheric-truncated, then flat-convex with slight central depression; edges convoluted even in mature examples, striated on lower parts; cap covering white, white-greying, ochre-ish or even brownish, in the wide central part, often dissociated in large irregular scales on a light background.

GILLS free, crowded, wide, initially whitish, then progressively dirty pink, brown, dark brown, blackish-brown; sterile surface, light, minutely denticulate.

STIPE $40-80 \times 20-35$ mm, cylindrical or ventricular with pointed base, robust, has a small annulus, simple and fine towards the middle; above the annulus white and smooth, under the annulus whitish or grey-ochre-ish, smooth or with transversal bands concolour with the cap.

FLESH firm white, reddish to gill edge. Odour normally strong and unpleasant of fish or of fish-eating birds. Cross reaction with Schäffer's reagent, negative.

MICROSCOPY: spore mostly ellipsoidal to subglobose, mono- or biguttulate, more rarely multiguttulate, 6-8 \times 4.5-6.5 μ m. basidia clavate, tetrasporic. Cheilocystidia very numerous, multiform, usually cylindrical, fusiform or clavate. Spores blackish-brown.

HABITAT: gregarious, in small groupings or in "witches' circles", both in dune or in coastal areas, also in urban areas (for example under *Cedrus*) or in mountainous zones, but never inside woods; from the end of spring to autumn.

EDIBILITY: of no value

NOTE - Is often confused, due to its similar appearance, with *A. litoralis* (Wakef. & A. Pearson) Pilát, which, however has a flaring annulus, or with *A. bitorquis* (Quél.) Sacc., which, however has two lower annuli.



Agaricus bisporus (J.E. Lange) Imbach

CAP 50-130 mm, fleshy, initially hemispheric, then flatconvex, finally flat; edges curving at the extremes; cap covering variable from white to ochre, up until brownish, outside the disc a little dissociated in triangulated fibrillated scales, adpressed, on a lighter background.

GILLS free, fairly crowded, wide, initially whitish, but soon pink then brown-reddish, finally brown-blackish, with light surface.

STIPE 40-90 × 10-25 mm, cylindrical or progressively widened towards base, which is often a little bulbous, straight, tightly fistular, white, under the annulus finely fibrillated often with white mycelial cords. Annulus thick, normally triangular at division; due to its complex structure, to be considered intermediate (neither growing upwards nor downwards), even though it can be pendant (drooping or skirt-like) and more rarely, sheathing (opening upwards around the stipe).

FLESH firm white, weakly pink-reddish to gill edge. Odour weak, fungal. Cross reaction with Schäffer's reagent, negative.

MICROSCOPY: spores mostly ellipsoidal, generally multiguttulate, 6.5- 7.5×5 - $6 \, \mu m$. basidia bisporic, but also monosporic (often very numerous) and more rarely trisporic or tetrasporic (these last most common in var. *eurotetrasporus*). Very numerous Cheilocystidia, clavate or a little fusiform, spores brown-blackish .

HABITAT: gregarious, in small groupings with isolated or clustered examples; common to fertilised ground or close to livestock enclosures, rare in "wild" areas, generally under *Cupressaceae*. Spring and late autumn. EDIBILITY: edible

NOTE – This is the only *Agaricus* with a great deal of bisporic basidia. It is good to eat and particularly suited to cultivation. It is produced industrially and sold almost all around the world.



Agaricus bitorquis (Quélet) Saccardo

CAP 50-120 mm, first hemispheric, then convex-flat, fleshy, firm, from pure white to dirty whitish, sometimes pale ochre-ish, smooth or with some under developed fibrils often covered by earth; edges convoluted and only with age distended.

GILLS from free to lightly adnate, close (4-6 mm), crowded, initially pale pink, soon becoming full and dirty flesh pink, and with age purple-black; gill edge whitish. STIPE 40-80 × 20-40 mm, cylindrical, attenuated at base, full, rigid, from whitish to dirty pink, at tip smooth or white and flocculent; bearing two membranous, white annuli, situated in the medial and basal zones of the stipe. FLESH thick, almost hard, gill edge, pale hazelnut, then taking tones of pink, red wine; sweet flavour, odour pronounced and pleasant.

MICROSCOPY: spore mostly ellipsoidal, almost rounded, smooth, under microscope brown to yellowish

shades; dimensions 4.5-6 \times 4-5.5 μm ; tetrasporic basidia; clavate cells. Brown-purple spores.

HABITAT: often gregarious on bare, sandy or compact soil at roadsides, in parks, riverside areas and streams, even under asphalt, from late spring to late autumn. EDIBILITY: edible

NOTE - Good to eat. The species is recognisable among other taxa from the section *Bitorques* by the clear set of double annuli, pleasant odour and the short stipe when compared to the diameter of the cap. The *validus* variety grows in clusters, has more compact and more heavily reddened flesh. It can be confused with the more fragile *A. campestris*, which has an ephemeral annulus and more brightly-coloured gills, and *A. bisporus*, which has a simple annulus and bisporic basidia.



Agaricus campestris L.: Fr.

CAP 50-120 mm, initially globular, soon convex-flat, fleshy, white to whitish, sometimes suffused with pink, silky-fibrillated, under developed fibrils more or less concentric, scales turn brown with age; at the extremes convoluted, rarely distended often bearing remnants of veil.

GILLS free, close, with lamellule, pink while still young concolour with gill edge, then purple-black in maturity. STIPE $50-80\times10-18$ mm, more or less cylindrical, but often attenuated at base, full, rigid white, bearing fine white dirty fibrils, browning with age; annulus tight and fragile, flaring, of cottony consistency, white, just reddening especially in the stipe-cap join, thick centrally, with pleasant odour and sweet flavour.

MICROSCOPY: oval spores, under microscope pale grey-brown, with just visible germinating pores, dimensions $6.5\text{-}8\times4.5\text{-}5.5~\mu\text{m}$; tetrasporophyte basidia. HABITAT: in fertilised meadows, in wheel tracks, in city parks, in small groupings or in circles of many individuals; from the start of summer to late autumn.

EDIBILITY: edible

NOTE - Good to eat. It is one of most renown and gathered edible *Agaricus* species, and is well known everywhere. It is easily recognisable by its shape, its odour, which is neither anise nor of almonds, the flesh, which when bruised assumes a shade of red, the fugacious annulus and the stipe, which is often pointed at its base. It belongs to the genus *Agaricus* characterised by their fragile carpophores, flaring annuli and gills which tend to red at the extremes. There are many forms and varieties about which the Authors do not agree; the var. *squamulosus*, in particular, which displays brownish concentric scales on its cap. Confusable with the *Agaricus* species in the section *Xanthodermatei*, which are poisonous, but which have yellowing flesh and an inky smell.



Agaricus cupreobrunneus (Jul. Schäff. & Steer ex F.H. Möller) Pilát

CAP 30-90 mm, initially globular, then hemispheric with truncated edges and with flattened centre, finally flat-convex with slight central depression; cap covering brownish copper, or even of varying tones of brown, in adulthood whitish silky-fibrillated edges; often the cap can be completely white with brown scales towards the centre.

GILLS free, fairly crowded, wide, with lamellule, from light pink to vivid pink up until brown-reddish, at maturity brown-blackish; edges concolour with surface.

STIPE $20-70 \times 8-20$ mm, clavate-ventricular often with pointed base, but also cylindrical, from full to tightly fistular, white, under the annulus smooth or finely fibrillated but bearing several more or less complete labra of the same colour as the cap.

FLESH white, uniform to gill edge, or just pink in the top part of the stipe and close to the gills. Light odour, fungal. Cross reaction with Schäffer's reagent, negative.

MICROSCOPY: ellipsoidal spores, generally mono- and biguttulate or with granular content, $7-9 \times 4.5-6$ µm. basidia clavate, tetrasporic. Cheilocystidia isolated, almost absent. Spores brown-dark purple.

HABITAT: gregarious in small groupings or in "witches' circles" in large grassy areas, both in the mountains and near coastal and urban areas; autumn.

EDIBILITY: edible

NOTE - Fairly rare but quite widespread, in its place of growth it is prized as a delicacy, considered superior to *A. campestris* L.: Fr. for its firmer flesh. A similar species is *A. porphyrocephalus* F.H. Møller, and it is often difficult to distinguish them macroscopically, but the latter has decidedly smaller spores. Its shape, with a white cap and brown scales in the centre, is similar to *A. campestris* var. *squamulosus* (Rea) Pilát, which is today considered synonymous with *A. cupreobrunneus*.



Agaricus macrocarpus (F.H. Møller) F.H. Møller

CAP 80-150 (180) mm, hemispheric, then for a long while convex, finally distended, dry and silky, fairly regular lip, with hanging-floccules from remnants of veil; white, then irregularly suffused with yellowish tones, more ochre-ish and intense around circumference.

GILLS highly crowded, free from stipe, intercalated with lamellule; initially whitish, then grey-pink, pink, and finally brown- dark purple-ish, keeping a (sterile) whitish surface.

STIPE 80-130 (150) × 20-30 mm, robust, cylindrical, sometimes a little attenuated at tip, with a very squashed basal bulb, typically in the shape of elephant feet; white, stained yellow-ochre-ish at base, in some adult specimens, more or less flocular fibrils under the annulus. ANNULUS broad, thick, fairly persistent, flaring, coarsely decorated with the shape of a gear wheel on the lower surface; white.

FLESH white, nearly uniform in cap, tendency to turn yellow on stipe, compact, tender; weak odour of bitter almonds, mild, pleasant flavour.

MICROSCOPY: ellipsoidal spores, smooth, 7.2-8.6 \times 4.6-5.2 $\mu m.$ Cheilocystidia clavate-obese, often with apical appendage.

HABITAT: grows in meadows and in pastures or in sparse, grassy conifer woods; first appears in late spring, and then fruits in autumn; infrequent.

EDIBILITY: edible

NOTE - A large mushroom which morphologically evokes the better-known and more common *A. arvensis* Schaeff.: Fr. from which, however, it differs mainly in its larger size, stipe base with a squashed bulb, its coarse irregular cog-like pattern on the surface just under the annulus and for its slightly smaller spores.



Agaricus silvaticus Schaeff.: Fr.

CAP 50-100 mm, initially hemispheric or campanulate, then convex or a little flattened, sometimes umbonate, with the edges convoluted, then straight, the cap covering brown-ochre-ish, more or less reddish, dissociated outside disc in fibrillated scales on lighter background.

GILLS free, crowded, close, greying-brownish, hint of pink, eventually brown dark, with the sterile surface, more or less pale.

STIPE $60-150 \times 7-15$ mm, cylindrical, sometimes a little bent, with bulbous base, firm then fistular, initially white then greying, bearing fine flakes or scales under the annulus. Annulus flaring, simple, fine, the upper part whitish, the lower concoloured with the cap, sometimes evanescent.

FLESH white, to gill edge turning to fairly intense blood red, with a sour odour and sweetish flavour. Cross reaction with Schäffer's reagent, negative.

MICROSCOPY: spore from oval to ovoid-ellipsoidal, monoguttulate, 5.4-6 \times 3.6-4 μm . clavate tetrasporic basidia. Cheilocystidia very numerous, from clavate to mostly clavate, with brownish content. Brown-purple spores.

HABITAT: isolated or gregarious, in hardwood and coniferous areas; summer-autumn.

EDIBILITY: edible

NOTE - A. silvaticus belongs to a group of species characterised by their slightly reddening flesh towards their gill edges and by their predominantly woodland habitat. It is distinguishable from other, similar species by the colour, shape and ornamentation of its cap, its flesh, which strongly reddens towards the gill edge and by its pleasant odour. According to several authors, A. silvaticus is a very variable, collective species,, and includes, among others, A. haemorrhoidarius and A. langei.



Agaricus silvicola (Vittadini) Saccardo

CAP 50-90 mm, ovoid or hemispheric, then convex-flat, with the edges convoluted, then straight and wavy, white cap covering, whitish cream, hairless, fibrillated, yellow on rubbing.

GILLS free, fairly crowded, pale at the extremes, with a slight reddish shade, finally brown dark, with pale sterile surface.

STIPE $60-90 \times 10-15$ mm, cylindrical, widened at base, fistular, white, silky under the annulus, often lightly pink then blackened above the annulus, yellowed by rubbing. Annulus flaring, simple, broad, white, upper part smooth, lower part with a flaky yellow bloom towards the edges . FLESH white, pink or reddish when exposed to air, with odour of anise and sour flavour. Cross reaction with

MICROSCOPY: spore oval with one or two guttules, (5.4) 5.8-6.6 × 3.8-4.2 (4.4) µm. clavate basidia, tetrasporic. Cheilocystidia subglobular, obpiriform,

Schäffer's reagent, positive.

ellipsoidal, even in cheilocatenule, with brownish content. Spores purplish-brown in mass.

HABITAT: gregarious, in large groups, in conifer woods. Summer to autumn, fairly frequent and common.

EDIBILITY: edible

NOTE - A. sylvicola belongs to a group of species which also includes A. essettei, A. tenuivolvatus and A. macrocarpus, where one can often find "family resemblances" between one species and another. The differences between them lie in characteristics such as size, degree of robustness, development of a universal veil and spore size, but in the absence of reliable studies on phenotypic variability such distinctions are only relatively valid. For example, A. syilvicola, of medium size and typically slender, has a stipe with a bulbous and well-defined base as, indeed have A. macrocarpus and A. essettei, but it has the smallest spore size of the group.



Agaricus urinascens var. urinascens

(Jul. Schäff. & F.H. Möller) Singer

[= A. macrosporus (Jul. Schäff. & F.H. Möller) Pilát; A. alberti Bon; A. stramineus (Jul. Schäff. & F.H. Möller) Singer]

CAP 100-200 mm (sometimes up to 350-400 mm), fleshy, hemispheric or campanulate, then convex often with flattened centre, finally flat; curving edge at the extremes; white cap covering, alutaceous or ochre-ish, usually dissociated outside disc, adpressed or even clearly cracked scales.

GILLS free, crowded, close, light at the extremes, then flesh coloured, finally brown-blackish with light surface. STIPE 50-100 \times 25-35 mm, usually short and thick, frequently with pointed rooting base, but often elongated, tight, medullar or even a little hollow, white or whitish, completely flocculent-scaly under the annulus; annulus flaring, broad, smooth above, irregularly serrated to edges on the lower face and flocculent-scaly elsewhere, white. FLESH firm white, flesh coloured on stem to gill edge. Odour of bitter almonds when fresh, then of mouldy straw or urine.

MICROSCOPY: spores ovoid-ellipsoidal, multiguttulate, $8.5-12\times5.5-6.5~\mu m$. Tetrasporophyte basidia, clavate. Cheilocystidia very numerous, mostly clavate. Spores brown-blackish.

HABITAT: gregarious or in "witches' circles" in meadows and hilly pastures and mountains, at high altitude, rarely in woods; start of summer-autumn.

EDIBILITY: edible

NOTE - A. excellens (F.H. Møller) F.H. Møller and A. stramineus (Jul. Schäff. & F.H. Møller) Singer, were originally recorded as two distinct species, varying only in stipe length and the straw yellow colour of the surface respectively. They are now unanimously considered as varieties of A. urinascens.



Agaricus xanthodermus Genevier

CAP 80-150 mm, from hemispheric to convex, sometimes conical typically flattened at tip, finally flat; convoluted edges, then curving towards the bottom, distends acutely late on, with partial remnants of veil; white colour, smooth and hairless, rarely lightly scaled, sometimes brown in the centre, intense chrome yellow on surface after minimal contact or rubbing.

GILLS free, close, quite crowded, with lamellule; initially whitish, then pink, pink becoming darker until chocolate brown; sterile gill edge.

STIPE:50-150 \times 8-25 mm, slim and cylindrical, often curving, with tendency to broaden at base forming a roundish and sometimes non-marginated bulb, up to 35 mm in width; white, yellowing if touched or upset; clear and broad annulus, fairly thick, separates late from edges of cap, flaring, sometimes appears dissociated from teeth in the lower face, yellowing if touched.

FLESH whitish, stained chrome yellow at base of stipe to gill edge. Odour characteristic of phenol or ink.

MICROSCOPY: spore ovoid, dimensions 5-6.5 \times 3.5-4 μ m; spores brown with violet shades.

HABÍTAT: grassy areas, parks, roadsides, meadows, under trees. Summer to late autumn.

EDIBILITY: toxic

NOTE - May cause gastrointestinal disturbances. The clear yellowing of the flesh and inky or phenol odour are important features clearly distinguishing it from other Agaricus species of the arvenses (e.g. A. arvensis, A. essettei). A. campestris can also be similar, but has slightly red flesh near gill edge. A. xanthodermus due to the variability in the appearance of its cap covering, should be distinguished from the following varieties: var. griseus, from its scaly grey ochre-ish cap, var. lepiotoides, from its cracked cap with large grey-brown scales. A. praeclaresquamosus has a cap covered with concentric dark grey scales, while its centre is of a blackish colour.



Agrocybe aegerita (Briganti) Fayod [= Agrocybe cylindracea (De Cand. : Fr.) Maire]

CAP up until 150 mm, from hemispheric to convex, then almost flat, sometimes fairly umbonate, smooth or more often minutely corrugated surface, dry and tends to crack coarsely in dry weather or becomes slightly greasy in wet weather; colour white cream, chamois, breadcrust, darker in the centre, sometimes, uniformly dark brown especially in smaller specimens.

GILLS adnate or just starting to form a tooth, curved, very tall (up to 13 mm) and crowded, with numerous lamellule; from milk white to grey-ochre-ish, finally tobacco colour at spore maturation.

STIPE full, dimensions extremely variable in both length and diameter depending on growing conditions: often thick and short, more often fine and slim, longer than diameter of cap, supple, curving, white then ochrebrownish. Surface smooth or fibrillated lengthwise sometimes finely scaled in dry weather. Annulus high, broad, membranous, persistent, white and then brown tobacco due to deposition of spores.

FLESH white, a little brown at base of stipe in mature examples, tenacious, elastic. Odour characteristic and indefinable, acidic, like wine.

MICROSCOPY: ellipsoidal spores, light brown under microscope, dimensions 8.4-9.5 \times 5.0-6.0 μ m; tetrasporophyte basidia. Brown spores.

HABITAT: in numerous groups, cespitose, on live or dead trunks of broad-leaved trees, with preference for *Populus, Ulmus, Acer.* Fruits several times a year. From spring to late autumn.

EDIBILITY: edible

NOTE - This unmistakable mushroom is common to plains and flatlands, is much sought after and is safe and very good to eat. When given the right substrate it grows very well and is a commonly sold species. It, however, rapidly kills any plant or tree which hosts it, and will continue to grow on the dead tree for many years afterwards.



Amanita caesarea (Scop. : Fr.) Persoon

CAP 80-120 (180) mm, initially hemispheric, then convex, eventually flat-distended, hairless, occasionally bearing patches of membrane from veil, finely striated at hem; vivid orange colour, a little lighter at edges.

GILLS free, crowded, a little ventricular; uniformly chrome-yellow colour.

STIPE 80-140 (200) \times 18-25 (35) mm; subcylindrical, attenuated at tip, normally straight, hairless; concolour with gills or of lighter tones; fairly hard and fibrous, full-medulla, then fistular. Base area covered with a wide VOLVA SACK, attenuated at base, free and laciniate at edge, white, soft consistency, but fairly tenacious, thick up to 3 mm.

ANNULUS membranous, positioned above median zone; long and densely striated; yellow.

FLESH abundant and compact on cap, more granularfibrous on stipe; white or lightly yellowing, more yellow in peripheral area. Little or no significant odour and pleasant taste. MICROSCOPY: spore from suboval to fairly regularly ellipsoidal, 9.4-11 \times 6.2-6.8 μm ; not amyloid.

HABITAT: loves temperate climates and fruits in hardwood areas not rare even though not widespread. EDIBILITY: edible

NOTE - Popularly known as Ovolo Buono (the tasty button), this is certainly one of the most popular edible species; appreciated since Roman times. It has recently been subjected to unrestricted gathering and moreover, this has often included specimens which are still closed. Because of this, in several areas A.Caesarea is in serious danger of extinction. In order to ensure its continued ability to sporulate, the law governing the collection of epigeal fungi in Italy prohibits the collection of these mushrooms during their button stage. This restriction can also be considered a safety measure because it prevents confusion with button mushrooms of the (very similar at this stage) deadly Amanita (see A. phalloides and similar species).



Amanita echinocephala (Vittad.) Quél.

CAP 50-90 mm, hemispheric then flat-convex, bearing fairly weak pyramidal warts. cuticle completely separable, viscid then dry, smooth, bright, whitish then with green tinges; smooth edges, displaying hanging remnants of veil.

GILLS sub-free, slightly crowded, whitish with green shades, with floccose surface.

STIPE 90-120 \times 15-30 mm, cylindrical, with bulbous-rooting base, white, sometimes hint of green, bearing small sections of more or less erect warts. Annulus: pendant, membranous, persistent, white, striated, with the edge bearing small flakes.

FLESH hard white, hints of green. Fairly strong, unpleasant odour and flavour.

MICROSCOPY: spore 10-11 \times 7-8 μ m, ellipsoidal, smooth, hyaline, amyloid. basidia 45-60 \times 11-13 μ m, tightly clavate, tetrasporic, with joints at hinge.

HABITAT: solitary or gregarious, under wide leaved trees, mainly beech and oak. Summer-autumn.

EDIBILITY: edible

NOTE - This species is characterised by its whitish colour, with greenish tinges here and there, the small, pointed warts which confer an echinulate appearance to the cap (hence the name), and its membraneous annulus and bulbous rooting stipe.



Amanita excelsa var. excelsa (Fr. : Fr.) P. Kumm.

CAP 80-120 mm, hemispheric then convex, eventually flat; smooth edges. Viscous cuticle in wet weather, greybrownish or lead-grey, bearing floury plates of greybrown or dirty white veil.

GILLS free, crowded, ventricular, white, sometimes shaded grey.

STIPE 100-160 × 15-30 mm, robust, cylindrical, sometimes attenuated at tip, very fixed in ground, straight, full and dense, white-greying. VOLVA whitish, dissociated into plates at the base of the stipe, evanescent on cap, which usually appears bare.

ANNULUS broad and persistent, striated on upper part, white-greying.

FLESH firm and compact on cap, fibrous on stipe, whitegreying, a little reddening-browning. Root-like odour, similar flavour.

MICROSCOPY: spore 8.5-9.0 \times 5.5-7.5 μ m, ovoid or mostly ellipsoidal, smooth, hyaline, amyloid. basidia 30-

 50×9.5 -11 µm, tetrasporic, tightly clavate, without joints at hinge.

HABITAT: single or in small groups, in hardwood and coniferous areas. Spring-autumn.

EDIBILITY: edible

NOTE - the separation of *A. excelsa* var. *excelsa* from *A. excelsa* var. *spissa* (Fr.) Neville & Poumarat [in this series, *A. spissa* (Fr.) P. Kumm.] has caused gallons of ink to be spilt, since these are two extremely similar species whose differentiating characteristics often "cross over". *A. spissa* typically has a more robust, "boletoid" form, an obese-clavate stipe, a veil that breaks down into powdery plates and adheres to the cap, while *A. excelsa* is more slender (hence its name) has a nearly cylindrical rooting stipe which holds tightly to the ground and a volva that dissociates into fairly large flaps, which do not adhere to the cap.



Amanita junquillea Quélet

CAP 50-70 (100) mm, hemispheric-glandiform, then convex, only distended late on, hairless, regularly bearing white membranous plates, residues of veil; warm to pale yellow, primrose yellow, lighter at edge which is finely striated.

GILLS FROM free to subfree, ventricular, crowded and thin; white.

STIPE 55-85 \times 10-20 mm, progressively attenuated at tip, bulbous-napiform at base where it is covered by a membranous, fine, adherent VOLVA normally clear-cut at edge, sometimes also dissociated in rings that persist in the lower part of the stipe; white, hairless, full, fistular at base.

ANNULUS positioned above the medial zone, very fine, soon becoming dissociated-evanescent, slightly persistent, sometimes completely absent in adult examples.

FLESH tender, white, just yellowish under the cuticle; almost odourless, pleasant taste.

MICROSCOPIA: spore from subglobose to short ellipsoidal, $9.5-11 \times 7.5-8.5 \mu m$; not amyloid.

HABITAT: early, from April to May, in hilly woods, a little later in June, in mountains. Common and widespread both in hardwoods and coniferous areas.

EDIBILITY: suspect

NOTE - Thanks to its sloping shape and its decidedly tender consistency, *A. junquillea* reminds one of the *Amanitopsis*, from which it can be distinguished by its annulus, which is actually rather ephemeral. Its edibility is a controversial topic. It must always be cooked, but even then is not tolerated well by all individuals. Among related species one should be careful of the decoloured form of the toxic *A. pantherina* (see below), which is morphologically similar, but has a brown cap.



Amanita muscaria (L.: Fr.) Hooker

EDIBILITY: toxic

CAP 100-150 (250) mm initially hemispheric then convex, eventually flat-distended, with the centre often depressed, with smooth cuticle, sticky in humid weather, almost always covered with white pyramidal warts, raised, concentric, residues of the veil, whitish, finely striated rim, reddish-orange, red, dark red.

GILLS free, dense, ventricular, pure white or slightly yellow.

STIPE 80-140 $(200) \times 10\text{-}20$ (30) mm; cylindrical, attenuated at the apex, straight, hairless or slightly flocculent, white, rather tough and fibrous, then fistular and eventually hollow. The basal zone widens into a bulb covered with a thin VOLVA, dissociated with concentric whitish warts.

ANNULUS membranous, large, located in the upper middle of the stem, striated on the top, remains of the veil found at the edge.

FLESH firm in the cap, more granular-fibrous on the stipe; white and faded on the cuticle immediately below. No odour and a sweet, pleasant taste.

MICROSCOPY: spore subovoid and roughly ellipsoidal, 9.0-11.2 \times 6.5-7.5 μ m; not amyloid; white in mass. HABITAT: in mountains, acidic soil, with hardwood and coniferous trees. Summer-autumn. Widespread.

NOTE - This is without doubt the mushroom which has most stimulated the imaginations of illustrators over the years. In fact there are countless representations of this splendid species, both realistic and less so, which are employed when depicting the toadstools of mythical and fantasy worlds. In popular belief, *A. muscaria* is often confused with the lethal *A. phalloides*, a fact which brings no shortage of consequent danger to the inexpert. From a taxonomic point of view, we feel it appropriate to note a variant with a more brittle volva and, rarely, warts on the cap: the form *aureola*.



Amanita ovoidea (Bull.: Fr.) Link

CAP 70-130 (200) mm, hemispherical, then convex, convex-flattened for a long time, even revolute in old age; edge quite often joined, non-striated, exceedingly appendicular; silky-shining, white, ivory, cream and ochre with weak shade in the centre, hairless, sometimes with a few dissociated strips, remnants of veil.

GILLS free, dense and thin, white, creamy pink shade in adults, minutely flocculent edge, alternating with truncated lamellule.

STIPE 80-170 \times 15-25 (35) mm, robust, yet slender, gradually expanding towards the sub-bulbous base, rooting at the base, full, firm, white, with concolour, or coloured, and highly transient flocules.

VOLVA membranous, thick, fairly high, sheathing at the base, free at edge; ochre-ish externally, ochre-ish-pale orange, light clay-coloured, whitish inside.

ANNULUS often situated fairly very high, typically softtender consistency (reminiscent of whipped cream), dissociated-evanescent, white, striated top. FLESH highly abundant, softened, white on cap, more compact on stipe, with ochre-ish shades; salty odour, fairly pleasant, sweet flavour.

MICROSCOPY: spore lengthwise ellipsoidal-ovoid, smooth, $9.5-11 \times 5.5-7 \mu m$; amyloid.

HABITAT: regarded as a thermophilic species, but also spreads to subalpine zones, although more rarely. In oak and coastal pine woods it is very abundant and under broad leaved trees in woods in the hills it is rare.

EDIBILITY: edible

NOTE - the size, colours and the downy-tender consistency of the annulus of this mushroom constitute excellent diagnostic elements which should hopefully avoid any confusion between this species and the similar lethal white *Amanita* varieties. In literature we can often find it compared to *A. proxima*, which has a more colourful (even tawny) volva, but which may also be toxic; therefore we recommend caution be taken in its culinary use.



Amanita pantherina (De Cand. : Fr.) Krombholz

CAP 80-100 (150) mm, initially hemispheric, then convex, eventually flat-distended sometimes depressed in the centre, smooth, covered with minute pure white floury warts, often concentric, remnants of veil; finely striated to edge (but smooth in var. *abietum*, which is heavier); brown-ochre, brown, brown dark.

GILLS free to just non-marginated, crowded, slightly ventricular; pure white.

STIPE 10-25 (30) × 80-150 (180) mm; cylindrical, attenuated at tip, straight, hairless; white; firm fibrous, then fistular and finally hollow, with base widening into a broad bulb. Basal zone is covered in an adherent VOLVA and dissociated from annulus, white.

ANNULUS more or less broad, typically low on stipe; white and striated on the upper part.

FLESH firm on cap, more fibrous on stipe; white. Odour almost nonexistent or slightly earthy, sweet flavour. MICROSCOPY: ellipsoidal spores, 9.5-11.5 \times 6.5-7.5 μm ; not amyloid; white in mass.

HABITAT: in mountains, in coniferous and hardwood areas, often around the edges of woods. Summer-autumn. EDIBILITY: toxic

NOTE – Here is another toxic *Amanita*, whose dangerousness is without a doubt greater than that of *A. muscaria*, of which, at first sight, this might be considered a brown variant. In reality, *A. pantherina* has an evident and sturdy volva and its annulus is distinctly lower. *A. junquillea*, whose edibility is shrouded by serious doubts, has a similar shape, but its colour is a strong cowslip yellow. *A. franchetii* (which is edible cooked) has colourful forms and these could appear similar were it not for its yellowish veil, while *A. rubescens* (also edible when cooked) is of a brown-reddish colour and its flesh can easily be seen to redden when exposed to the air or in the tunnels created by larvae.



Amanita phalloides (Vaill. : Fr.) Link

CAP 60-120 (150) mm, hemispheric, then convex, finally distended radially from crowded and fine fibrils, often covered with white membranous strips, residues of veil; greenish, green-olivish, yellow-brown-greenish, lighter at edge which is smooth.

GILLS free, ventricular, crowded and thin, fairly wide; white.

STIPE 75-120 (150) \times 10-22 mm, progressively attenuated towards apex, typically decorated with yellow-olivish zig-zag bands on a white background; full, then medullar. Bulbous at base where it is covered with a membranous, fairly fine VOLVA sack, adherent to bulb but free towards edge where it is usually splits into white petal shapes.

ANNULUS positioned fairly high, pendant, fairly fine, white, persistent.

FLESH initially firm soon becoming soft, white, just faint green shades under the cuticle; from odourless to slightly smelly; older examples smell of putrid water (water from old flowers); no significant flavour.

MICROSCOPY: spore from mostly ellipsoidal to subglobose, $9-11.3 \times 7-9 \mu m$; amyloid.

HABITAT: found in woods of deciduous trees in summer (rarely in conifers?) it seems to prefer oak, chestnut and beech trees, is also able to grow at high altitudes where it is regularly associated with hazelnut trees. Very common and widespread.

EDIBILITY: deadly

NOTE - This mushroom is the primary cause of all deaths which occur through mushroom poisoning. It is (unbelievably) confused with green Russule, from which it differs through a series of primary characteristics: it has a volva at the base of its stipe, it has an annulus, free gills, heterogeneity between cap and stipe, changing bands of colour on the stipe, non-chalky flesh. In nature one encounters numerous colour variations, including one which is completely white, the *fo. alba*.



Amanita rubescens Pers. : Fr.

CAP 80-150 (180) mm, initially hemispheric, then convex, sometimes distended, smooth, viscous in humid weather, with minute remnants of veil in the form of crowded and prominent small warts, dirty white, greying or sometimes also ochre; with smooth brown, reddish edges, paling with age or heavy rainfall with characteristic vinous patches.

GILLS rounded to stipe, crowded, ventricular and large, strong; white, stained red wine with age or to the touch. STIPE 12-25 (35) \times 90-140 (200) mm; cylindrical, attenuated at tip, normally straight, hairless or sometimes minutely floccose under the annulus; from whitish to white pinkish and finally vinous brown; more or less full; firm then fibrous and fragile. The base widens into a non marginated ovoid bulb covered with an adherent VOLVA, soon becoming dissociated in floccose residues the same colour as stipe with a tendency to disperse.

ANNULUS positioned in the middle to upper stipe, membranous, pendant, whitish or pinkish, sometimes even a little yellow, striated on the upper part.

FLESH abundant and compact in cap more fibrous and fragile in stipe; white then pinkish to gill edge; typically vinous red in veiled area, which is almost always visible. Odour irrelevant, pleasant, slightly salty-sour flavour.

MICROSCOPY: ellipsoidal spores, 7.5-9.6 \times 5.6-6.5 μ m; amyloid; white in mass

HABITAT: ubiquitous and widespread in conifer and hardwood areas.

EDIBILITY: edible

NOTE - Good to eat; though should be cooked first, as the flesh contains thermolabile toxins, and are best used in mixes. Confusion with the toxic *A. pantherina* is possible for the inexpert; the latter, however, has a very individualised volva and always has uniformly white flesh. The species which it most resembles morphochromatically is *A. franchetii*, which has more uniform flesh and a yellowish veil.



Amanita vaginata (Bull. : Fr.) Vittadini

CAP (30) 45-70 (95) mm, initially campanulate-parabolic, then convex, finally distended with broad slightly emerging umbo; hairless, rarely with residues of veil in shape of large plates, finely striated to hem; of ash grey, pearl grey colour, sometimes with a hint of brownish colour (white in var. *alba*; lead grey in var. *plumbea*), sometimes hint of ochre in disc area.

GILLS free, crowded and thin; white, intercalated from lamellule trunk.

STIPE 65-120 \times 10-22 mm, slim, attenuated at tip, white, smooth, or becoming flocculent adnate and coloured; fully-medullar, finally fistular.

VOLVA membranous, not very fine but fragile, adherent in basal area, free to hem; from white to whitish.

ANNULUS apparently absent (reduced to shreds that remain at the base of the stipe, inside the volva, as in other "Amanitopsis" specimens).

FLESH not very abundant, tender, white or whitish; no particular odour, pleasant flavour.

MICROSCOPY: spore from subglobular to globular, 10- 12.2×9.6 - $11.6 \mu m$; not amyloid.

HABITAT: ubiquitous; found especially in hardwood or coniferous areas, common, from summer to late autumn. EDIBILITY: edible

NOTE - As is the case for its relatives, this mushroom is considered an edible delicacy, ideal for frying (it must be cooked prior to consumption). The group, A. vaginata is certainly the most complex of those inside the subgenus Amanitopsis, which includes many species without annulus, at least at maturity; and in fact, there are many entities (species, varieties or forms) that are cited in literature, with nearly all having a greyish colouration in common. A. Mairei is a little more stocky and it has no umbo on its cap; A. nivalis, which is paler and smaller, can be found on the microsilve Alps. A. lividopallescens has more grey tones on its cream-brownish cap. The varieties plumbea, argentea, and alba differ only in their colour.



Armillaria mellea (Vahl: Fr.) Kummer

CAP 25-60 (100) mm, hemispheric, then distended finally flat-umbonate with slight prediscal depression, scattered with fine erect brownish transient scales, with fine edge, initially fringed with residues of veil, then bare and slightly striated, becoming undulating-sinuous in adults; variable colour, from yellowing brown to amber, dark brown, sometimes with olivish hints remaining at the extremes of the edges, pale, whitish.

GILLS adnate and slightly decurrent lengthwise, fairly crowded and fairly close; whitish, then weak beige, finally bearing brown reddish spots.

STIPE 60-120 (160) \times 10-22 (35) mm, cylindrical, often tapered at base, other times a little dilated, grows cespitosely, fibrous-woody, full, soon becoming filled with an evanescent medulla, exterior is scattered with flocculent dissociated membrane disorderly arranged under the annulus; rather transient, fibrillated at tip; from cream-flesh coloured to concolour with cap, clearly browning at base.

ANNULUS membranous, persistent, striated on lower face, floccose-cottony at edge; white, sometimes yellow at extreme edges .

FLESH fairly thick on circumference, whitish, highly leathery in stipe, rather bitter and astringent to taste, fungal odour.

MICROSCOPY: spore from ellipsoid to ovoid, smooth, 7-8.6 \times 5.4-6 μ m; basidia without joints on hinge.

HABITAT: parasitic on wood of deciduous trees, often late, abundant, very common.

EDIBILITY: edible

NOTE - Edible with care (see note to *A. ostoyae*). *Armillaria tabescens*, which also grows cespitosely on hardwoods, is very similar but can be recognised by its lack of a membraneous annulus; *A. ostoyae* (= *A. obdark*) is darker, with persistent erect scales, annulus bordered with brown and prefers to grow on conifers; *A. cepistipes* is more frail and pallid and pallida, fairly hygrophanous; *A. gallica* (= *A. bulbous*, *A. lutea*) is less cespitose, usually grows as fairly-isolated specimens, happily on the ground (on underground woody deposits) and has residues of veil, as does the border of its annulus, which are largely yellow.



Armillaria ostoyae (Romagnesi) Herink
[= A. obdark (Schaeffer) Herink]

CAP 30-80 (110) mm, hemispheric, then convex or convex and mostly umbonate, maintaining a bent edge at the extremes, finally distended with fine peripheral area, striated in maturity, initially fimbriated due to floccose remnants of veil, which is brown reddish, brown-tawny, sometimes paler, becoming brown-grey, paler at edge, up to cream whitish; scattered with erect scales, denser on circumference and dark pink in colour.

GILLS adnate, then fairly decurrent, bent, not particularly close, attenuated at edge and join; whitish, pale yellow, often with brownish spots on surface, which eventually turns brownish.

STIPE $60-140 \times 10-22$ mm, slim, cylindrical or a little tapered at base, up to sub bulbous, clustered-cespitose growth (generally few connate specimens), striated at tip, fibrillated-floccose under the annulus creating a white fluff, subcoloured near cap or darker at base, tendency to take on brown-olive-blackish tones.

ANNULUS membranous-cottony, fairly persistent, striated, white, often brown at extreme edges.

FLESH fairly consistent on circumference, very fine elsewhere, fibrous-leathery in stipe, firm when young, then soft, whitish then suffused with flesh colour.

MICROSCOPY: ellipsoidal spores, sometimes slightly compressed, smooth, to thick side, $8\text{-}10 \times 5\text{-}6.6~\mu m$. basidia with joints at hinge.

HABITAT: prefers coniferous woods, but a hardwood form seems to exist; autumn, abundant and widespread. EDIBILITY: edible

NOTE - For the differences from similar species, see the notes to *A. mellea* and *A. tabescens*. Edible, but as with the other *Armillaria* with annuli, a long cooking time and eliminating the stipe are necessary to avoid fairly unpleasant gastro-intestinal poisoning.



Armillaria tabescens (Scop.) Emeland

CAP 30-70 mm, initially campanulate-convex, then distended, finally flat with broad obtuse umbo, sometimes rather depressed in prediscal area, superimposed with erect fairly persistent scales; edges curving at the extremes, fine, distended in maturity and a little striated; brown reddish, brown-beige, paling to cream ochre-ish.

GILLS bent and decurrent, thinned at the front, not very crowded, close, fairly thick, whitish, then pale-flesh coloured, browning on surface in maturity.

STIPE 35-70 \times 8-15 mm, slim, cylindrical or subfusiform fairly bent, densely connate with other examples, white at tip, whitish flesh with splashes of colour, then decidedly and extensively browning in a smooth continuum away from darker fibrils; full-medullar, finally fistular.

ANNULUS nearly absent or reduced to transient residues, coniform, only visible in very young examples.

FLESH compact in cap, more fibrous in stipe, white, whitish-beige pale; fungal odour, sweet flavour with slightly bitter aftertaste.

MICROSCOPY: ellipsoidal spores with thick wall, 9-10.2 \times 5.4-6.6 μ m. basidia without joints at hinge.

HABITAT: cespitose, in large groups connate, above all on logs or at the base of oak trees, then spreading to the ground. Not very widespread but abundant in growth areas.

EDIBILITY: edible

NOTE - the tender consistency of the flesh, even the stipe, combined with a "more traditionally fungal" flavour make this species an excellent and sought after edible mushroom, doubtlessly superior to the annuli-bearing *Armillaria*. It can sometimes seem like a twin of *A. mellea* s.l., but the absence of a membraneous annulus makes for a fairly easy distinction. *A. ectypa* has no annulus either, but grows in beds of sphagnum moss or in mountain bogs; its size brings to mind the *Laccaria*.



Boletus aereus Bull. : Fr.

CAP 80-160 (250) mm, fleshy, from hemispheric to convex-pulvinate; edge curving at extremes, then regularly distended with white evanescent bloom; matt cuticle, finely tomentose, then hairless, not viscous, even in humid weather; fairly dark, generally brown-blackish, typically discoloured to brown-ochre yellowish.

TUBES length up to 25 mm, non-marginated-adnate, whitish and persistent until maturation is incipient; greenish-yellow late on, finally olive, uniform to gill edge, very small pores, concolour with tubes.

STIPE 60-130 \times 40-85 (110) mm, often rounded in young examples, then slimmer, ventricular, or even cylindrical, sometimes curving; ochre-ish, brown-ochre-ish, honey coloured, light hazelnut, towards the upper part (from 1/3 to 2/3) covered with a fine lattice, generally the same colour as the skin of the stipe.

FLESH firm and compact when young and this remains in adults, later soft; pure white, uniform, uncoloured under cuticle of cap. Weak but very pleasant odour, sweet, hazelnut flavour.

MICROSCOPY: spore fusiform dimensions 13.5-16 \times 4.0-5.0 μ m, pale yellow under microscope; olive-brown in mass.

HABITAT: the most thermophilic of porcini mushrooms, it prefers sparse oak or chestnut woods, in which it can be found from summer's start till the end of autumn, solitary or gregarious, not very common in the north, the species is fairly widespread in southern areas.

EDIBILITY: edible

NOTE - Commonly known as the "Porcino nero", after the colouration of its cap. Its particular biological needs mean that it is widespread particularly in southern areas, but can also be found in northern Italy (though not higher than 800 m above sea level) in hotter periods of the year. Sometimes the distinction from darker forms of *B. aestivalis* can prove challenging. In that case the colour of the flesh, pure white only in *B. aereus*, and the decolouration of the pileic cuticle are determining factors.



Boletus aestivalis (Paulet) Fries [= Boletus reticulatus auct. non Schaeff.]

CAP 100-250 mm, fleshy, from hemispheric to convex, finally pulvinate-flat; cuticle finely velvety, never viscous, often finely cracked especially at edges or, in very dry weather, evenly tessellated in large areolas which are barely visible beneath the flesh; uniformly pale brown, coffee, hazelnut, reddish brown, often also dark brown.

TUBES up to 30 mm, depressed-adnate, from white milk to straw yellow, then yellow-greenish, finally olivish, uniform to gill edge; has very small pores, the same colour as tubes, unchanging to the touch.

STIPE up to 150×80 mm, initially obese then slimmer, cylindrical, often curving, rarely ventricular, rounded at base and sometimes rooting a little; bark coloured when young, with tones which become intensified and match those of the cap, or a little paler. Fine mesh lattice the same colour as background, which covers the surface of the stipe right to the base.

FLESH firm and compact in young, soon becoming soft in cap and a little stringy in stipe, often eroded by small larvae; milk white, just brownish under cuticle; peculiar, intense and sweet odour; sweet, pleasant hazelnut flavour. MICROSCOPY: ellipsoidal fusiform spores with weak elevated depression, $12.8\text{-}15.1\times3.8\text{-}4.4~\mu\text{m}$, pale yellow under microscope. Olive brown in mass.

HABITAT: warm open woody grassland areas, often associated with *Quercus, Fagus, Castanea*, but also to conifers (*Picea*) from late spring to early autumn EDIBILITY: edible

NOTE - This is the "summer porcine" which appears in late spring in the clearings and glades of woodlands. The characteristics which can help to distinguish it from other porcine mushrooms are: a dry, often cracked cuticle; a stipe which is coloured even in young specimens, the flesh of the cap, which is usually pliable or saggy to the touch in mature examples.



Boletus edulis Bull. : Fr.

CAP 100-250 mm, fleshy, hemispheric then convexpulvinate, finally flat; undulating edge in mature examples, covered with a whitish bloom when young which tends to disappear. Cuticle initially finely velvety, soon becoming viscous, wrinkled surface especially in marginal zone; chestnut brown, hazelnut, brown dark, except at the extreme edges which have a permanently white surface, discoloured to pale ochre towards the edge. TUBES up to 30 mm, depressed-adnate, milk white then yellowish, finally olivish, uniform to gill edge; pores very small, concolour with the tubes, unchanging to the touch. STIPE up to 150 × 100 mm, obese, then ventricular or cylindrical, with rounded base; milk white in young, later hazelnut or pale brown; fine mesh lattice, concolour with background, spread over most of the surface.

FLESH firm and compact when young, then a little soft in maturation; white, slightly brown-reddish for a few millimetres under the cuticle; with typical pleasant odour, very pleasant flavour, sweet, like hazelnut.

MICROSCOPY: ellipsoidal fusiform-spores with weak elevated depression, 13.2-15.9 × 4.5-5.1 μm, pale yellow

under microscope; olive brown in mass; basidia mostly bi- and trisporic.

HABITAT: ubiquitous in the woods, often associated with *Fagus*, *Picea*, *Abies*, but also with many other species (chestnut, pine, birch, hazel, etc.) Found in temperate or cool periods, from late summer to late autumn, Widespread and very common.

EDIBILITY: edible

NOTE - This is the "autumnal porcino", common and recognised in almost all the continents, where it manifests a substantial stability in its characteristics and it associates with a great number of beings. It is recognisable by its viscous cap, which tends to lose its colour, especially at the edges. *B. aereus* and *B. aestivalis* are similar, but these have dry caps and more-richly coloured stipes from the start; *B. pinophilus*, which also has a viscous cuticle, but shows winey-brown tones on its cap and an obclavate stipe.



Boletus pinophilus Pilát & Dermek

[= *B. pinicola* Vittadini, non Swartz]

CAP 80-220 (300) mm, very fleshy, from hemispheric to pulvinate-convex, finally flat; edge curving at extreme edges, finally distended, scattered with whitish bloom which tends to dissolve at maturation; cuticle initially finely velvety, soon becoming viscous, rugulous; vinous brown, garnet, *reddish*-brown, copper red, with sporadic discoloured areas

TUBES width up to 30 mm, depressed-adnate, milk white to straw yellow, then yellow-green, finally uniform olivish to gill edge; pores are very small, concolour with the tubes, often slightly rusty in maturation, unchanging to the touch.

STIPE up to $100\text{-}200 \times 50\text{-}120$ (150) mm, typically obese, then a little longer, but relatively short and stocky and almost always dilated-rounded at base; whitish, then soon showing hints of reddish brown. Displays a fine lattice, concolour with background, which covers most of the stipe.

FLESH firm and compact, then a little soft and watery; white, purplish for a few millimetres under the cuticle;

typically pleasant, but weak odour, very pleasant sweet taste.

MICROSCOPY: ellipsoidal fusiform spores with weak elevated depression, $13.6\text{-}16.3 \times 4.5\text{-}5.2 \mu m$, pale yellow under microscope; olivish brown in mass.

HABITAT mainly associated with *Picea*, *Pinus*, *Fagus*, *Castanea*. Fruits typically two times a year: in late spring, at lower altitudes, and from the late summer to late autumn in the mountains and at high altitudes. Recurrent. EDIBILITY: edible

NOTE - Usually better known under the name *B. pinicola* Vitt. (an invalid name as it has already been used to designate a lignicole fungus, today known as *Fomitopsis pinicola*), it is popularly called the "red porcine". Its name would seem to suggest it is found only in affinity with pine trees, however *B. pinophilus* is, much like *edulis*, a widely-occurring species and is more usually found under beech or fir trees



Boletus regius Krombholz

CAP up to 150 (200) mm, from hemispheric to convex, then flat-pulvinate; regular edge, margins slightly larger. Plical, finely felted surface, initially coral pink-red, to raspberry pink, discoloured with age, gaining yellowish tones, often also with olive tones; cracks at areole in dry weather.

TUBES similar thickness to flesh of the cap, when carpophore matures, stipe is rounded; from gold yellow to olive green; quickly turning blue if cut lengthwise; small pores, round then a little angular; from gold yellow to olive green, uniform or turning blue if bruised

STIPE usually narrower in diameter compared to cap on maturation of carpophore, up to 40 (50) mm in diameter; stocky, with thick base, more often than not cylindrical; pale chrome yellow, often with innate raspberry red spots towards base; surface unchanging to the touch. Lattice usually limited to upper half, often slightly raised, concolour with background.

FLESH initially very firm in cap and also in adult carpophore, while stipe tends to become fibrous; pale

yellow, more intense yellow above tubes and under the bark of stipe, often reddish only at extreme base of stipe, rarely or never turning blue at gill edge in zone above tubes. Weak, slightly fruity odour; sweet flavour.

MICROSCOPY: spore pale yellow under microscope, fusiform dimensions $11.3\text{-}14.5 \times 3.5\text{-}4.5 \ \mu m$. Spores olive brown

HABITAT: isolated or in small groups under hardwood trees, mainly *Fagus sylvatica* and *Castanea sativa* in acidic ground; from early summer to autumn

EDIBILITY: edible

NOTE - A beautiful species which is only confusable with the *Boletus pseudoregius*, which however has more slanting proportions and flesh which manifests fairly intense tones. This species has been implicated in several cases of poisoning after undercooked carpophores were consumed.



Boletus satanas Lenz

CAP 120-300 mm, very fleshy, from hemispheric to convex; cuticle finely velvety when young, soon becoming hairless, dry, milk white, pale grey, cream, often with olivish hues, finally brownish olive, often just pinkish at edges in examples that have grown in humid weather.

TUBES up to 25 mm, rounded near stipe, from yellow, to yellow-greenish, finally olivish, blue at gill edge; pores very small, initially yellow but soon red, carmine red or red-orange; rarely remaining yellow or weakly orange even at maturity; blue when touched.

STIPE 60-150 × 50-100 mm, narrower in diameter than cap, very stocky, obese, pear shaped, more often than not cylindrical; usually yellow at insertion to cap, red or pink-purple, fuchsia at lower part. Lattice generally concolour with background, of fine mesh, isodiametric, limited to upper half of stipe; the surface turns blue to the touch.

FLESH very firm and compact when young, soft when old, pale yellow, turning pale blue at gill edge; weak particular odour when young, cadaverous or of decaying substances when mature, sweet flavour. MICROSCOPY:

ellipsoidal spores, with weak elevated depression, 11.4-13.4 \times 5.1-5.9 μm , yellow under microscope; olive brown in mass.

HABITAT: warm and chalky woods, associated with *Quercus*, commonly with *Castanea* or *Fagus*, not at high altitudes. Summer-autumn, not very common.

EDIBILITY: toxic

NOTE - This is one of the largest boletes, characteristic for its short and paunchy stipe and for the cadaverous odour that it emits when ripe. Its light cap, red pores and the latex in its stipe help to distinguish it from other species of *Luridi* that might seem similar. In many cases, it can be found with completely yellow pores and stipe, and as such it can even resemble certain species of *Appendiculati* (*Boletus fechtneri*). Notwithstanding, its identification. Toxic when raw, suspect (and certainly poorly tolerated by many people) even when cooked thoroughly.



Calocybe gambosa (Fr. : Fr.) Donk

[= Lyophyllum georgii (L. : Fr.) Kühner & Romagnesi; Tricholoma georgii (L. : Fr.) Quélet]

CAP 40-80 mm, from convex to flat, convoluted edges, often undulated, smooth, silky, matt, white, dirty white or dirty cream, often stained ochre or cracked.

GILLS crowded, close, adnate or non marginated, from whitish to pale cream, with the surface undulated or crenulated, coloured.

STIPE 40-70 \times 10-20 mm, cylindrical, almost clavate or attenuated towards the base, blooming or fully fibrillated, then filled, whitish or dirty cream.

FLESH thick, hard or a little spongy, white, with strong smell of flour and sweet, floury flavour.

MICROSCOPY: spore $5-6 \times 2.5-3.5 \mu m$, ellipsoidal, smooth, hyaline; basidia $20-25 \times 3-5 \mu m$, tetrasporic, tightly clavate, filled with siderophile granules; epicyte formed from interwoven hyphae, width of $3-5 \mu m$, hyphae of subgelled stratum surface, with membranous pigmentation; usually with hinges.

HABITAT: grows isolated or in groups, more often in circles or semicircles (witches' circles) in grassy areas in meadows, or in clearings in coniferous or hardwood areas, particularly close to *Rosaceae*.

EDIBILITY: edible

NOTE - This is a much sought after mushroom with many pseudonyms, the most famous of which is probably St. George's mushroom (so named because the period in which it grows in near that saint's day). In literature several varieties have been identified based on the diverse colours that the cap can assume and even sometimes the different odours it gives off. For example, *C. graveolens* (= *Tricholoma georgii* fo. *flavida*) has a darker cap and a rather unpleasant odour.



Calvatia utriformis (Bull. : Pers.) Jaap

[= Lycoperdon caelatum Bull. : Pers.]

BASIDIOCARP up to 100 (150) mm, composed of a fertile glebe protected by two cortical layers (endo - and exoperidia) supported by spongy sterile base; subglobular subellipsoidal or with short fairly differentiated pseudostipe; initially mostly convex on upper part then truncated-flat, external surface of exoperidium smooth, ruffled-rugulous and then separated into aureola; from milk white to pale grey ochre-ish. Across cracks produced by one sees that the endoperidium aureola in maturity is grey-brown and finally dark brown, in adults the skin tears so extensively and irregularly as to allow the dispersal of spores which remain for a long time on the ground; very spongy base, slightly marcescible, uniformly brown.

GLEBE white, then yellowish, greenish, olive, finally brown-olivish or chocolate brown; fairly soft consistency, then dusty.

MICROSCOPY: spore globular, with warty surface, of diameter 4-5 µm, brownish to microscope. Spores olive brown in mass.

HABITAT: isolated or in groups of several individuals, often with many examples joined at base, in meadows and in mountain pasture, from summer to autumn; recurrent EDIBILITY: edible

NOTE - From summer's end, walking through mountain meadows, one can often come across the seemingly indestructible brownish remnants of this large "puffball". These residues can in fact persist on the ground for over a year. It may be confused with some *Lycoperdaceae*, which are also edible, such as *Langermannia gigantea*, the largest, with its near-smooth exoperidium, free of pseudostipe. *C. utriformis* is also morphologically very similar to *Vascellum pratense* of which, at first glance, it seems to be a giant form.



Camarophyllus pratensis (Pers. : Fr.) Kummer

[= Hygrocybe pratensis var. pratensis (Fr.) Murril; = Cuphophyllus pratensis (Pers. : Fr.) Bon]

CAP 25-80 mm, hemispheric then convex, often with mostly obtuse umbo, fine margin; hairless, radially dry away from fairly fine fibrils; pale apricot orange or fleshy ochre, sometimes covered with white bloom; cuticle detachable for 3/4 of radius.

GILLS curved, deeply decurrent, concolour with cap or paler, highly spaced, noticeably thick, often spoked, intercalated with numerous lamellule.

STIPE 40-100 × 5-12 mm, normally cylindrical or attenuated at base, more rarely obclavate, from whitish to pale orange, visibly fibrous, fully dry, often invaded by larvae or small insects, soon becoming filled.

FLESH thick and firm fibrous in stipe; whitish or faded yellow in cap, white elsewhere, odourless, pleasant flavour.

MICROSCOPY: spore mostly ellipsoidal, 5-6.7 \times 4.2-5 μ m; tetrasporophyte basidia, sometimes bisporie, and so produce larger spores, up to 8.6 μ m.

HABITAT: grassy areas, meadows, clearings in hardwood areas; gregarious; widespread and common from summer to late autumn.

EDIBILITY: edible

NOTE - This is a good species of edible mushroom, which has seen fair commercial success, despite its fibrous flesh. Due to its orange colour, it can be confused with *Hygrophorus nemoreus*, but the latter has an even more fibrous cap, flesh which both smells and tastes of flour, pale gills, which range from creamy-whitish to flesh coloured. It also has a smooth sub-bilateral lamellar trama and grows in the woods (or, at least in the vicinity of trees). Several varieties are reported as different in literature due to their differing size and colour (var. *robustus*, of larger size, var. *Donadini* with denticulate edges, var. *Vitulinus*, more fragile and pale).



Cantharellus cibarius (Fr. : Fr.) Fries

CAP 30-70 (120) mm, initially highly differentiated from stipe, then convex, then distended with edge convoluted at the extremes, eventually irregularly flat-gibbous up to fairly deeply depressed-infundibulform, marginal zone very thin, with progressively rough, sinuous-lobed, wrinkled in parts, shiny appearance; with humidity minutely velvety, smooth; when dry, orange, apricot, paling yolk-yellow.

HYMENOPHORE pseudolamellar, very forked from compound-branched folds, abundantly spored, anastomosed, highly decurrent in adults, close, thick, with fairly dull surface, coloured or subconcolour with cap, Sometimes with pink shades.

STIPE 30-60 (90) \times 12-25 mm, cylindrical, normally progressively flared towards gill insertion, often even a little dilated at base, full, firm in maturity softer-rubbery, hairless, subcoloured.

FLESH white, with yellow-pinkish stains on outer zone, abundant on circumference, firm and compact in cap, fibrous and almost leathery in stipe; pleasant odour, like

apricot or white peach skin or sweet fruit. Initially mild flavoured, then sour-astringent-spicy.

MICROSCOPY: spore from ovoid to ellipsoidal, sometimes sublarmiform, smooth or fairly evidently grainy, 7.5-9.6 \times 4.6-5.6 μm .

HABITAT: very common and widespread all over, from hilly hardwood areas to coniferous mountains; from start of summer to late autumn.

EDIBILITY: edible

NOTE - A much sought and valued edible mushroom, this is one of the few which are also eaten in both southern and northern Europe. Literature is full of variants of this fungus which are sometimes considered separate species, sometimes mere variants. The var. *bicolor* is more precocious and displays a very pale cap and stipe which are whitish, in stark contrast to its yolk-yellow gills; the var. *ferruginascens* has tones of olive green and a strong tendency to assume a rusty colour when handled; the var. *amethysteus* has violet adnate scales on its cap, and can typically be found under beech trees.



Cantharellus lutescens (Pers. : Fr.) Fries

[= C. aurora (Batsch) Kuyper]

CAP 15-45 (65) mm, convex-umbillicate, then flat with fairly wide central depression, infundibulform, edge bent and fine at the extremes, progressively multi-lobed, fuzzy in parts, fissile, revolute in old examples, sinuous; fibrillous-scaly orange brown or reddish brown.

HYMENOPHORE reduced to low venucole with numerous branches and anastomoses, mostly rounded at margin, rugulous, not infrequently subsmooth especially in stipe where it is, somewhat blurred, decurrent lengthwise, yellow-orange, yellow or greying with a hint of salmon pink in paler forms.

STIPE 35-70 \times 8-12 mm, normally highly irregular, dilated at tip and progressively attenuated towards base, often bent, canalicular-compressed, gibbous and wrinkled, hairless, hollow-tubular, highly characteristic bright orange colour or hint of pink salmon,.

FLESH exiguous all over, of fibrous and fairly tenaciouselastic consistency, cream or pale yellowish; fruity odour, like of plums or of flowers of the *Muscari* family, sweet flavour. MICROSCOPY: spore from ellipsoidal to ovoid, smooth, guttulate, 9.6-11.4 \times 6.5-7.8 μm .

HABITAT: summer-autumn, in hardwood or coniferous woods, often in grassy or mossy areas, more rarely on bare ground, widespread all over and abundant in growing area

EDIBILITY: edible

NOTE - Had the name *C. aurora* not been synonymised, this would have the nomenclatorial priority, however, since *C. lutescens* is widely used, we adhere to this binomial and hope it lasts. Good to eat, Especially after drying and then steeping in water and milk. Its fine and slightly fibrous-elastic flesh make it appear like another species of similar colour: *C. tubaeformis*, but this latter has a hymenophore with fairly distinct pseudogills; less vivid colours, ranging from shades of gray to grey-brown, and is less odorous.



Clathrus ruber Mich. ex Pers. : Pers.

BASIDIOCARP appearance in young specimens of a rounded oval, internally gelatinous, while external surface is fragile and waxy at branches. When cut, the parts ready for growth are clearly visible.

EXOPERIDUM is similar to a railing round the elongated polygonal meshes, vivid colour, grows up to 70 mm in diameter at full development. On the lower part, connected to the remnants of the primordial oval, a volva is formed, the thin elongated hyphal rhizomorphs are clearly visible and whitish.

GLEBE formed from small granules of green-blackish mucilage containing spores, gives off a strong unpleasant odour similar to that of *Phallus impudicus*.

MICROSCOPY: ellipsoidal spores, 5 \pm 6 μm . Spores of white-brownish colour.

HABITAT: grows fairly isolated in the humid parts of woods, from summer to autumn.

EDIBILITY: non edible

NOTE - This is a rare species, but which grows abundantly in its chosen areas. It has the same cadaverous odour of *Phallus impudicus*; an odour which appears even before the mushroom does. The same repellent odour is often responsible for this species being invaded by flies.



Clitocybe cerussata (Fr. : Fr.) Kummer [= *C. phyllophila* (Pers. Fr.) Kummer; = *C. pytiophila* (Fries) Gillet]

CAP 40-90 mm, convex then flat, typically umbonate. pure white cuticle, smooth, covered with sericeous fibrils, blooming, matt; at most slightly cream colour under fibrils.

GILLS from adnate to subdecurrent, very crowded, pure white, sometimes a little cream, concolour with the whole surface

STIPE $40-70 \times 10-15$ mm, from cylindrical to subclavate, full, white, slightly fibrillated with base covered with white flakes.

FLESH whitish, firm with no significant odour or flavour. SPORES white.

MICROSCOPY: ellipsoidal spores, $5.0\text{-}6.0\times3.0\text{-}4.0~\mu\text{m}$, smooth, cyanophilia, prevalent in tetrads in dry conditions, obtuse base. basidia $16\text{-}25\times4\text{-}6~\mu\text{m}$, tetrasporic. hymenophore almost regular. very loose Pileipellis interwoven with structure of hyphal cutis.

HABITAT: gregarious, in coniferous woods; fairly common and widespread, from end of summer.

EDIBILITY: toxic

NOTE - C. phyllophila belongs to a group of organisms which are very similar to each other, and which maybe represent different aspects of one, single species. When trying to distinguish between C. cerussata (Fr. : Fr.) Kummer and C. phyllophila (Pers.: Fr.) Kummer, one would mention the habitat of conifers for the former and of hardwood trees for the latter, and also their different spore colours (pink-cream in C. phyllophila). It is not so simple to distinguish C. phyllophila from C. candicans (Pers.: Fr.) Kummer or C. rivulosa (Pers.: Fr.) Kummer. Normally C. phyllophila is slightly larger than these two latter species and has characteristic adnate, slightly decurrent gills, and spores arranged in tetrads in dried specimens. C. candicans, with its slanting gait, has more decurrent gills and single spores. C. rivulosa (= C. dealbata sensu auct. plur.) normally grows in meadows.



Clitocybe geotropa (Bull.) Quélet

CAP 60-200 mm, quite deeply infundibulform, with central umbo emerging from cavity, not hygrophanous, not striated for transparency, pale ochre-ish, alutaceous, smooth, matt, silky, Finely felted.

GILLS fairly deeply decurrent, fairly crowded, whitish, stained pinkish-brown, concolour with the whole surface. STIPE $60-150 \times 13-20$ mm, from cylindrical to lightly clavate, filled, subcoloured to cap, bearing white fibrils lengthwise, tomentum white at base.

FLESH white, with particular aromatic odour, and insignificant flavour.

SPORES white.

MICROSCOPY: ellipsoidal spores, 6.0- 7.0×5.2 - $6.0 \mu m$, smooth, single in dry conditions, sublacrimoid base. basidia 40- 45×7 - $9 \mu m$, tetrasporic. Fairly regular hymenophore texture, colourless hyphae, width 3-8 μm . Pileipellis interwoven with structure of hyphae cutis,

width 3-7 µm, with membranous and minutely encrusting. pigment

HABITAT: terrestrial, gregarious, in hardwood and coniferous woods or in meadows and pastures, in the shape of "witches' circles", not widespread but with plenty of areas of growth. Autumn and late autumn. EDIBILITY: edible

NOTE - Unmistakeable thanks to its typical shape, characterised by a very long stipe compared to cap diameter and to its fairly large size, this late-appearing species is a much sought edible.

With its basidia, which are fairly long for a clitocybe, *C. geotropa* is grouped in the subgenus Hygroclitocybe Bon, inside which it is unique for its subregular lamellar trama, its sublacrimoid spores and its mixed pigmentation.



Clitocybe gibba (Pers. : Fr.) Kummer [= *Clitocybe infundibuliformis* (Schaeff.) Quélet]

CAP 30-80 mm, fairly deeply infundibulform, with or without umbo, with the edges convoluted, then straight, often ribbed, not hygrophanous, not striated by transparency, ochre-ish pale or alutaceous light reddish, finely felted, matt.

GILLS decurrent, fairly crowded, bent, light yellowish brown, often with pink stains, with the whole surface, coloured.

STIPE 20-50 \times 5-8 mm, from cylindrical to lightly clavate, filled, then fistular, from whitish to light yellow, generally lighter than cap, smooth or with white fibrils lengthwise, with tomentum white at base.

FLESH whitish, with pleasant "cyanide" odour and sweet flavour.

SPORES white.

MICROSCOPY: ellipsoidal spores, $5.2-6.6 \times 4-4.6 \mu m$, smooth, not cyanophilous, single in dry conditions, with confluent base, lacrimoid. Basidia $22-30 \times 5-7 \mu m$,

tetrasporic. Hymenophore texture regular with colourless hyphae. Pileipellis interwoven horizontally with structure of hyphae cutis, with finely encrusted pigment.

HABITAT: in groups, also numerous, in hardwood and coniferous woods

EDIBILITY: edible

NOTE - It is known and sought after in Italy as the "imbutino" (funnel mushroom), and a much-appreciated edible, despite its fairly tough flesh. C. gibba belongs to the subgenus Clitocybe whose species are characterised by their typically funnel-like caps which are often opaque and almost velvety, by their decurrent gills, the (sub)regular hymenophoral trama and by the colouring in their stipe walls. It is not always easy to distinguish it from C. costata, though it can normally be identified by its stipe being lighter than its cap and its cap covering having a negative reaction to treatment with KOH.



Clitocybe nebularis (Batsch: Fr.) Kummer

CAP 80-150 mm, convex, with convoluted edges, not hygrophanous, from ash grey to grey-brown, smooth, finely felted, fibrillated from adnate to lightly decurrent, fairly crowded, pale cream, with the whole surface coloured.

STIPE $60-90 \times 15-30$ mm, clavate, filled, subcoloured to cap, striated lengthwise with fine fibrils, base covered with white mycelial felt.

FLESH white, with strong aromatic, slightly unpleasant smell and unpleasant flavour.

SPORES yellowish cream.

MICROSCOPY: ellipsoidal spores, 6.0- $7.5 \times 3.4.5 \mu m$, smooth, cyanophilous, prevalent in tetrads in dry conditions, obtuse base. basidia 20- 25×5 - $7 \mu m$, tetrasporic. Hymenophore texture regular with colourless hyphae. Pileipellis with structure of hyphal cutis more or less parallel, with intracellular pigmentation.

HABITAT: ubiquitous, in large groups; very common in autumn and late autumn.

EDIBILITY: suspect

NOTE - This is a well known species, which in many areas is gathered and eaten with impunity. In any case, recent studies (based on nutritional casuistics) seem to demonstrate the toxicity of the species, or at least, the ill-tolerance of it by several individuals. Based on the spore colour, the cyanophylla of the spore wall (which is smooth to optical microscopes, but warty to electronic ones), *C. nebularis* was considered by several authors, such as Moser and Bon, as belonging to the genus *Lepista*. We prefer, in accordance with Kuyper, to keep it in the genus *Clitocybe*, along with numerous other similar species which display cyanophylla and coloured spores which are smooth under an optical microscope.



Coprinus comatus (Müll.: Fr.) S.F. Gray

CAP 60-150 (220) \times 30-70 mm, initially glandiform to more or less cylindrical then, in maturity, from campanulate to conical, more expanded with age, finally deliquescent starting at the edges; surface initially silky and white, soon becoming covered in overlapping scales from whitish to light brown on white background; cap often joined and ochre-ish.

GILLS very crowded, unequal, very wide, free to stipe, initially white, then, pinkish at edges, eventually black, deliquescent.

STIPE 100-200-(300) × 10-25-(35) mm, separable from cap, slim in maturity, attenuated at tip and fairly bulbous at base, white, bearing fine white fibrils, empty with age; medial or basal annulus, which is membranous, minute, white, sometimes black due to spores.

FLESH slightly thick, tender in cap and soon becoming fibrous in stipe, white; weak and pleasant odour and flavour.

MICROSCOPY: spore from ellipsoidal to ovoid, smooth, with centrally germinating pores, brown-black under

microscope, $11-14.5 \times 6.5-8$ mm; tetrasporophyte basidia. Black spores.

HABITAT: from the spring to late autumn in grassy and fertile areas in gardens, at the edge of wheel tracks, in flood plains, in large groups; frequent.

EDIBILITY: edible

NOTE - This is, most probably, the only *Coprinus* which is considered edible and, as such, it deserves a certain level of attention; several authors, in fact, consider it excellent or, even, «the best edible species»; the exiguity of its flesh, however, means that long cooking times should be avoided and therefore would best be eaten fried or even raw. In any case one should only eat young specimens, which still have perfectly white gills. This species is unlikely to be confused with similar species: *Coprinus sterquilinus* grows in dung, is solitary or found in small groupings, it is also more slender and has larger pores; *Coprinus vosoustii*, is far rarer, has a non-deliquescent star shape covering on its cap and much larger spores.



Cortinarius orellanus Fries

CAP 40-80 mm, initially fleshy, campanulate-convex, then flat-convex, eventually distended gibbous or with wide central umbo, edges convoluted then straight, slightly lobed, dry cuticle, non-hygrophanous, matt, silky, densely fibrillated or scaled, reddish brown orange, brown auburn.

GILLS adnate-non-marginated, fairly spaced, wide, bulging, brown ochre-ish, brown reddish orange then rusty reddish due to spores, with eroded surface, yellowish on face.

STIPE $40-90 \times 10-15$ mm, fairly slim, cylindrical but often attenuated at base, more or less supple, full, firm citrina yellow, reddish orange in the centre, decorated lengthwise with coloured fibrils.

FLESH firm non hygrophanous, ochre-ish, reddish hint, with radish-like odour and acidic flavour.

MICROSCOPY: ellipsoidal amygdalform spores, 10-12.5 \times 5.5-6.5 $\mu m,\,$ densely covered with fine warts. Filamentous epicyte, with encrusted pigment.

HABITAT: isolated or gregarious, mostly under oak trees, but also under beech and hazelnut; not widespread, but constant in growing areas; from end of summer to all of autumn.

EDIBILITY: deadly

NOTE - Characterised by its fairly robust shape, its scaly orange-brown cap, subdistant coloured gills, yellowish stipe and root-like odour, *C. orellanus* is responsible for some of the worst cases cytotoxic poisoning, which have a particularly grave effect on the renal system. Considering its long latency period, the so called *Orellanus syndrome* is extremely dangerous. Of equal toxicity is the *C. orellanoides* (= *C. speciosissimus*), which has similar colouring, but which is found in conifer woods. This latter typically has a conical-umbonate cap and a stipe which is decorated with changeable, yellowish zig-zag stripes.



Cortinarius praestans (Cordier) Gillet

CAP 50-200 mm, initially subhemispheric, then convex, eventually flat-convex, with fine edges, convoluted at the extremes, then straight, very thick and tenacious cuticle, viscid in humid weather, shiny, chocolate brown, becoming a little reddish, brown purple, more or less blended with grey-violet or lilac tones, scattered with large silky silver purple strips, residues of veil, marked at the edge with visible wrinkles or radial grooves.

GILLS adnate-non-marginated, fairly crowded, bulging, greying or dirty whitish, hint of violet, then clay brown, finally rusty due to spores, with jagged surface, whitish. STIPE 70-150 (200) \times 20-40 mm, very robust, progressively dilated to a large non-marginated bulb, a little rooting; full, firm and almost bare at tip, hairless, whitish then cream, marked lengthwise with silky fibrils, the lower two thirds visibly decorated with thick residues from veil, violet-blue silver in colour

FLESH thick, very firm white or cream, hints of violet at top part of stipe, with weak, slightly fruity and pleasant odour and sweet flavour.

MICROSCOPY: spore from amygdalform to subcitriform, $14.0\text{-}17.0 \times 8.0\text{-}10.0 \mu m$, covered with coarse evident warts. Filamentous gelled epicyte, made from frail hyphae. Membranous pigment.

HABITAT: infrequent but abundant in growing area, found in hardwood areas.

EDIBILITY: edible

NOTE - By far and away the largest of the *Cortinari*, a real giant, plus it has organoleptic properties which make it a choice edible in great demand by enthusiasts. Beyond its size and masterly shape, the morphochromatic details which assist in its identification are the absence of a marginated bulb, the whitish colour of the gills which contrast with the reddish-brown of the cap, an abundant universal veil, at least in young specimens, which leaves clear traces on the cap but above all on the stipe, and its large spores. *C. cumatilis* is fairly similar, but is far smaller and grows under conifers.



Entoloma bloxamii (Berk. & Br.) Saccardo

[= *E. madidum* (Fries) Gillet]

CAP 35-75 (90) mm conical-campanulate then distended with broad and slightly accentuated obtuse umbo, fairly fleshy for the genus *Entoloma*; steel blue with hints of grey, tendency to take on a less obvious grey-brownviolet colour with age, often, with whitish bursts, dry, hairless and joint at opening, soon becoming dissociated from gathered fibrils, eventually fairly split; often rugulous-wrinkled in maturity. Fine Cuticle, separable, and only with small stripes.

GILLS non-marginated-adnate, ventricular, fairly wide, with surface irregularly undulated; white ivory, sometimes (when seen flat) almost pale cream, soon becoming pinkish due to maturing of spores, eventually dirty pink.

STIPE 30-70 \times 12-25 mm, normally widening in the middle and fairly tapered at base, not infrequently slightly canalicular, firm, full, fibrillated lengthwise, concolour with cap, often with violet tinges especially at

tip, always lighter or white at base; loses a lot of tone intensity with age, becoming slightly grey-blueish.

FLESH white, firm slightly fibrous in stipe flavourful, floury odour.

MICROSCOPY: polygonal, subisometric spores, with evident apex 7.5-8.7 (9.5) \times 6.2-8.2 (9.5) μ m; joint at hinge.

HABITAT: in meadows and in grassy and open areas of woods; autumnal species, present from plains to mountains, not common, gregarious.

EDIBILITY: edible

NOTE - This is one of the tricholomatoid *Entoloma* with the most beautiful colour and shape; similar to *E. bloxamii* for its shape and habitat is *E. porphyrophaeum*, which, however, does not have the blueish colouration but is grey-violet or grey-purple-ish and has longer spores of $8.6\text{-}11.6 \times 6\text{-}7.8 \,\mu\text{m}$.



Entoloma sinuatum (Bull. : Fr.) Kummer

[= *Entoloma lividum* (Bull. □) Quélet]

CAP 50-200 mm, initially campanulate, then convex, eventually flat, with or without a low umbo, edges undulated, convoluted, lobed, the cuticle more or less light grey with silky tinges, metallic or even lead grey, grey brownish, blooming, lead or silverish from fine fibrils.

GILLS deeply non-marginated, almost free, fairly spaced, bent, then more or less ventricular, typically yellow, soon becoming yellowish salmon pink or pink ochre-ish, with the surface serrated, coloured.

STIPE $50-140 \times 15-35$ mm, slim, cylindrical or supple, often widened but often also attenuated at base, firm full then filled, white, pruinose at tip, marked lengthwise with silky fibrils.

FLESH white, firm non hygrophanous, with strong, unpleasant floury smell, and unpleasant flavour, bordering on disgusting.

MICROSCOPY: spore subisodiametric, with 6 sides, 8.5- 11.0×7.5 -8.5 μ m. Tetrasporophyte basidia, with joints to hinge. Cheilocystidia absent. epicyte framed from (ixo)cutis. Intracellular pigment. Joints at hinge.

HABITAT: in hardwood areas, preferably *Quercus* and *Fagus*; from end of summer to late autumn; fairly common.

EDIBILITY: toxic

NOTE - Despite its fleshy, inviting appearance and its often-pleasant, floury odour, this species is a dangerous one, the cause of potentially severe gastrointestinal illness. An added risk comes from its similarity with *Clitocybe nebularis*, a mushroom commonly consumed in certain parts of Italy, and with which *E. sinuatum* often shares its habitat and growing period. The latter has a non-fibrous cap, fairly decurrent, separable gills, a whitish cream colour and a very particular, strong odour.



Helvella crispa (Scop. : Fr.) Fries

ASCOCARP stipitate and pileate.

MITRA up to 35 mm, irregular shape vaguely resembling a horse saddle, with two-three lobes stretching towards the outside, many take on a curly appearance, whitish, ivory, up to fairly dark cream. Hymenophore covered with visible part of mitra, smooth, undulated. Lower surface slightly lighter, tomentose-floccose. Sinuous-lobed edge, jagged.

STIPE height up to 120 mm, cylindrical-clavate, widened towards at base, deeply ploughed lengthwise, incomplete both internally and externally, white or whitish.

FLESH leathery, but fairly elastic, sub-brittle; whitish, no distinctive odour or flavour.

MICROSCOPY: ellipsoidal or mostly ellipsoidal spores, smooth, $18\text{-}19 \times 10\text{-}12~\mu\text{m}$, hyaline under microscope, monoguttulate, uniseriat in asco; cylindrical asci, non amyloid, octasporic; cylindrical thin paraphyses, with clavate apex.

HABITAT: on the ground in hardwood or mixed woods or, also in grass, single or in small groupings of several specimens. Common in summer-late autumn. Sometimes even in late spring.

EDIBILITY: edible

NOTE - Helvella lactea has a similar shape, but is smaller (just 30-40 mm tall), and is completely ice white, has a smooth lower surface and fruits on the ground or on decaying plant matter (Fraxinus) in autumn. H. lacunosa has a much darker, blackish mitra a brownish-grey stipe, and is ubiquitous. H. sulcata is considered an extreme form of H. lacunose by some authors; it is rarer, bigger, and has a regular "saddle-shaped" it is almost completely grey in colour. Furthermore, it has a yellowish-ochre-ish or yellowish grey mitra, dimensions rather smaller size, and prefers southern, sandy ground near H. pityophila.



Helvella pityophila Boud.

ASCOMA pileate and stipitate, formed from apothecia (cap) and from stipe; total height up to 100 mm.

APOTHECIA irregularly lobed-curled, rarely subselliform, composed of two, three or more variously joined lobes, height up to 15-20 mm and of around 30 mm in diameter. External surface (hymenial) irregularly rippled-undulated, yellowish; lower surface (sterile) smooth or lightly rough, whitish. Edge irregularly undulated or sinuous.

STIPE up to $80 \times 10\text{-}20$ mm, subcylindrical or significantly widened at base, pleated-ribbed, lacunous internally, concolour with cap towards top, but with lilac or grey-lilac stains, more marked at base.

FLESH elastic and fairly tenacious, but fragile, brittle, whitish-yellowing; insignificant odour and flavour.

MICROSCOPY: spores mostly ellipsoidal, $18-20 \times 11-12.5 \mu m$, guttulate, with smooth wall, hyaline, uniseriat in asco; whitish in mass. Cylindrical octasporic, not amyloid

asci. Cylindrical paraphyses, dilatate at tip, sometimes forked, with several septums.

HABITAT: species not widespread all over, fruits alone or in small groups generally on sandy ground in humid hardwood coniferous woods (mainly *Pinus* sp.) In both alpine and subalpine mediterranean areas; summerautumn.

EDIBILITY: edible

NOTE - Very similar to *H. crispa* (Scop. : Fr.) Fr., it can be distinguished only by its colouring, which tends to be grey with cream reflections at the apothecia and have an absence of grey-lilac tones on its stipe. *H. crispa* can also reach larger dimensions (up to 120 mm in height) and is generally found only in hardwood forests. *H. lactea* Boud. is smaller (growing to a height of 40 mm), is completely white, becoming ochre-ish-brown in dry conditions; it has smaller spores (16-18 × 11-12 μ m), and fruits in autumn in woods rich in decaying and decomposing plant matter.



Hygrocybe coccinea (Schaeff. : Fr.) Kummer

CAP 15-50 mm, initially mostly campanulate or subhemispheric, eventually convex or convex-flat, hairless, shiny-lubricated appearance in humid conditions, shiny-dry in dry conditions; in these latter conditions shows very adpressed radial fibrils (slowly!). Margin fairly regular, initially inflected, fine, tendency to crack radially; intense red-cherry or red-carmine colour, paling until white to from disc but always with a characteristic red annulus in the peripheral area. Fine cuticle, separable until circumference.

GILLS adnate or briefly decurrent, sinuous-ventricular, rarely bent, wide, thick, unequal, fairly sparse, intercalated by numerous lamellule of different lengths; yellow initially, soon becoming red-orange or cherry red, the surface remains yellow.

STIPE $30-80 \times 5-8$ mm, irregularly cylindrical, sometimes compressed-canalicular, often bent, fragile, dry, coloured cap at tip, more shades elsewhere, white base; hollow.

FLESH fine, from yellow orange to red orange, also red cherry on periphery, watery, odourless, mild insignificant flavour.

MICROSCOPY: elliptical spores at prosurface or amygdalform, 8-10 × 4.2-5.2 μ m; tetrasporophyte basidia. HABITAT: in humid and mossy grassy areas, pastures, clearings, gregarious and abundant in growing area, frequent; end of summer-autumn even late autumn. EDIBILITY: edible

NOTE - Among other small, red *Hygrocybes* this one's cap, which is absolutely hairless and never scalytomentose sets it apart from *H. miniata*. Less simple however is its distinction from other hairless redcap species. *H. reae* is smaller, has a striated margin, bitter flesh and is abundantly viscous-glutinous; *H. insipida* is a near replica of the previously-described mushroom, but has sweet flesh and a size closer to *H. coccinea*; *H. punicea* is noticeably larger, has a campanulate cap, yellow stipe and white flesh with clear red fibrils.



Hygrocybe psittacina (Schaeff.: Fr.) Kummer

CAP 10-30 mm, campanulate or hemispheric-umbonate, then distended to a fairly prominent and acute papilla or distended and mostly umbonate, abundantly viscousglutinous, fragile, with the margin fine and striated with transparency; highly polychrome, yellow ochre-ish olivish, grass green with sulphur yellow tones, yellow orange with green stains, sometimes the yellow pigment is absent thus the green tones evolve towards blue-blueish ones. Fine cuticle, yet separable at circumference.

GILLS non-marginated-adnate, spaced, ventricular, with regular and joint surface; golden yellow, saffron yellow, sometimes with greenish or tawny stains (nearly white in absence of yellow pigment); intercalated by numerous lamellule of different lengths.

STIPE 40-80 \times 3-6 mm, cylindrical or progressively widened at base, often supple-curving, highly viscous due to a persistent and thick layer of hyaline gluten; yellow greenish, green or green blueish at tip, sometimes with tawny or brick coloured veins, soon becoming hollow.

FLESH fine, white, yet coloured in cap, also fairly deeply, on periphery, very watery; no odour and slightly earthy flavour, like moss.

MICROSCOPY: ovoidal spores, smooth, more rarely ellipsoidal, (6.5) 7.4-9 \times 4.5-5.6 μm .

HABITAT: in meadows, among grass and moss, highly camouflaged, in autumn; gregarious, fairly frequent. EDIBILITY: of no value

NOTE - This species is made unmistakeable by its colouring and its marked glutinous properties; in any case, there are often anomalies in its pigment-distribution (see description), which can make it appear with unexpected colours. H. perplexa (= H. sciophana) is a lookalike, though with wider gills, is a brick-red colour with slight green tinge and is fairly rare. Even H. laeta can seem similar due to its many colours and viscosity, while H. psittacina, on the other hand, has a convex cap, and more importantly, very decurrent gills, which are not non-marginated or adnate.



Hygrophorus chrysodon (Batsch: Fr.) Fries

CAP 30-60 mm, hemispheric, soon becoming convex, viscous, initially white, with tempo takes on fairly extensive yellowish; edge with fairly regular flow, finely hairy, adorned with floccose-cottony granules soon becoming yellow; fine separable cuticle,

GILLS decurrent, fairly wide of and fairly spaced, thick, intercalated from numerous lamellule of different lengths; white, in maturity with vague flesh coloured tinges, then tendency to turn yellow like the rest of the carpophore to the edge.

STIPE 30-70 \times 8-14 mm, slim, cylindrical, normally attenuated at base; cortiniform residues visible at tip which, like the edge of cap, bears the same grainy flocculence, characteristic, tendency to turn yellow, slightly viscous due to humidity, soon becoming dry initially full fibrils, soon becoming medullar.

FLESH very fine towards the edges, compact on circumference, white, due to imbibition takes on a citrina

tinge; indistinctive flavour, odour not especially pronounced, but clearly of the "cossus" type.

MICROSCOPY: spores lengthwise ellipsoidal, smooth, 8-9.2 \times 4.2-5 μm .

HABITAT: in the mountainous hardwood areas, with preference for beech but also in the coniferous and mixed woods; gregarious, from end of summer to all of autumn, fairly common and widespread.

NOTE - This beautiful hygrophore reaches the peak of its loveliness when the granulation which adorns its cap margin and the tip of its stipe assume their intense yellow colouration. It is liable to be confused with species of *eburneus-cossus*, which however, is highly viscous, non flocculent and does not visibly yellow. An exception to this last rule however is *H. discoxanthus* (= *H. chrysaspis*), which, when it dehydrates, becomes a distinct rusty fawn colour (the entire carpophore is rusty brown when dried).



Hygrophorus eburneus (Bull. : Fr.) Fries

CAP 15-45 mm, convex, then flat with broad obtuse umbo, finally also depressed in prediscal area, with the edge bent at the extremes, excess; pure white, only in aged specimens vague pale ochre-ish or flesh colour stains are visible on circumference, highly viscousglutinous, thick, elastic and separable cuticle.

GILLS adnate or slightly decurrent, sinuous, slightly ventricular, then bent, highly thick, not very spaced, white, finally lightly flesh coloured; intercalated from lamellule of different lengths.

STIPE 40-100 \times 8-15 mm, slim, cylindrical, sometimes supple, or a little dilated, attenuated at base, dry and floccose at tip, glutinous in the lower 3/4; white, with age often with ochre-ish shades or pinkish at base.

FLESH white, thicker on circumference, not unpleasant flavour, while on the contrary odour is pronounced and nauseating, with fruity, sweet and sour hints of the "cossus" family, similar to shellfish cooking, and defined in literature as similar to the odour of woodworm.

CHEMICAL REACTION: potassium hydrate (KOH) hot yellow, yellow orange, base of stipe.

MICROSCOPY: spores lengthwise ellipsoidal, sometimes subcylindrical, smooth, $7.4-8.5 \times 4.5-5.6 \mu m$.

HABITAT: in hardwood areas with preference for beech, but not exclusively; from end of summer to all of autumn, non very common.

EDIBILITY: non edible

NOTE – *H. eburneus* is likely to be a collective species in which other entities may be grouped which are currently considered as independent species by some authors. Thus *H. quercetorum* is a variant which is just slightly more robust; we consider *H. cossus* to be a simple variety with a yellow pale reaction to potassium. *H. discoxanthus* (= *H. chrysapsis*), despite being similar morphologically, we believe merits the dignity of its own species thanks to its all-over colouration of rusty-fawn to brown reddish, in dry conditions, which is even more evident in dried samples.



Hygrophorus latitabundus Britzelmayr

(= Hygrophorus limacinus Scop. ex Fr. ss. Auct.)

CAP 50-120 (160) mm, convex with broad and obtuse central umbo, then distended and also depressed around umbo, very fleshy, firm glutinous, grey-sooty or greygreenish, often with vaguely lilac stains (similar to *Gomphidius glutinosus*); generally the disc peripheral zone are darker, wide areas decorated in ivory white present elsewhere. Thick, stocky, very adherent cuticle.

GILLS bent-decurrent, whitish, then stained ochre- pale flesh colour; thick, wide, venous on background, not very spaced, intercalated from numerous lamellule, generally small and short in length.

STIPE 60-150 x 20-50 mm, full, bulky, fusoid, obese in middle then subrooting, white, covered by hyaline glutine (only in maturity or drying more brown-olivish coloured in parts), floccose decorations at tip.

FLESH thick, compact, firm white; odourless, no particular flavour.

HABITAT: exclusively in *Pinus* woods, in mountainous and coastal areas fairly late; rare, but abundant in growing area.

MICROSCOPY: spores fairly lengthwise ellipsoidal 8.5-11.5 x 4.7-7 μm . Tetrasporophyte basidia.

EDIBILITY: edible

NOTE - This is a very heavily-set *Hygrophorus*. It belongs to the *Olivaceoumbrini* with *Hygrophorus* olivaceoalbus, which is decidedly frailer, with a slender stipe and associated with spruce and with *H. persoonii* (= *H. dichrous*), a mushroom which can reach a considerable size, but which fruits associated with oak and has a particular green reaction to ammonia fumes. Other species of the genus are easily recognisable by their different morphological features.



Hygrophorus penarius Fries

CAP 35-100 (150) mm, convex-hemispheric, soon becoming distended with broad obtuse umbo, finally also depressed in prediscal area, with edge bent at the extremes, thinned; initially white or whitish ivory, shades of cream or yellow-pinkish on circumference in adults some excoriation is also present; slightly viscous due to humidity, soon becoming dry generally maintaining a fairly shiny look.

GILLS adnate, fairly decurrent in adults, bent, thick and spaced, concolour with cap or fairly uniformly pale cream; intercalated from lamellule.

STIPE 30-70 (90) \times 12-25 (30) mm, typically tapered at base, firm and fleshy, barely reaches in length the same dimensions as the diameter of cap; dry or slightly viscous with humidity, white, eventually ochre-ish at base in adults; full.

FLESH abundant on circumference, firm and compact, more fibrous in stipe, white; flavourful, sometimes with slightly bitter aftertaste, odour is not very clear but characteristic and unmistakable, like boiled milk.

CHEMICAL REACTION: flesh of stipe reacts yellow with KOH.

MICROSCOPY: ellipsoidal spores, sometimes briefly ellipsoidal, smooth, $6-7.4 \times 4.5-5 \mu m$.

HABITAT: in autumn, in hardwood areas, especially in the oak; abundant in growing area.

EDIBILITY: edible

NOTE - Much sought after due to its good organoleptic characteristics, and inexpensive thanks to its fleshiness, *H. penarius* has earned a reputation as the best edible hygrophore. Other similar white and dry hygrophores are: *H. Karstenii*, found on mountain spruce, with apricot gills, *H. poetarum*, found in beech forests, which has a matt cap, pink tinge and a balsamic odour; *H. phages*, grows on beech, is slightly shorter, with a pinkish reflection on its gills; finally *H. barbatulus*, a rare species with a pale cream-ochre-ish cap, is fairly hairy on its margin and has ochre-cream-ish gills.



Hygrophorus russula (Schaeff. : Fr.) Quélet [= *Tricholoma russula* (Schaeff. : Fr.) Gillet]

CAP: 35-100 (135) mm, convex with edge convoluted and excessive at the extreme edges, then from pulvinate to fairly distended sometimes lobed, very fleshy; initially whitish, soon becoming fairly sporadically spotted with wine red patches or frills, more crowded on circumference, then with zone fairly wide of delicate raspberry pink, sometimes also completely coloured with the same tone, sometimes yellowing with age; lightly viscous to humid weather, soon becoming dry very fine cuticle, separable for 1/2-2/3 of radius.

GILLS: from horizontal-adnate to lightly bent-adnate, sometimes non-marginated; very crowded for the *Hygrophorus* genus, thick, of irregular width but always fairly scarce; white or whitish-flesh coloured, in maturity fairly densely spotted with winy red colour, intercalated from lamellule in report of ca. 1:1.

STIPE: $35-70 \times 10^{-25}$ mm, irregularly cylindrical, often slightly attenuated at base, floccose at tip, white then

fairly extensively marked with red-purpling spots; dry, firm, full.

FLESH: white, very firm can take on light pinkish stains especially towards the foot of stipe; highly variable flavour also contained in the same report, from mild (ca. 50%) to bitter up to clearly bitter, no significant odour. MICROSCOPY: ovoid or ellipsoidal spores (6) 7-9.3 \times 4.5-5.2 μ m.

HABITAT: in hardwood areas with preference for oak, in autumn and also late autumn, gregarious in large groups, rarely solitary.

EDIBILITY: edible

NOTE - In some zones it is highly prized and is stored in oil. The most trustworthy characteristic in distinguishing it from the similar species, *H. erubescens*, is the particular crowdedness of its gills; other valid distinguishing features are its habitat, its indisposition to yellowing, and its slightly-smaller pores.



Laccaria amethystina (Huds.) Cooke

CAP 20-50 mm, initially convex, then from flat to depressed in centre, with the edges convoluted at the extremes, then distended, a little undulated, scalloped and furrowed; completely vivid purple-amethyst fairly dark, paling, with dry conditions, towards grey-blueish, ochreceam or dirty white with lilac-ish tinges; cap covering matt, smooth or felted, slightly scaled in the centre; hygrophanous.

GILLS mostly adnate to stipe or a little decurrent, fairly spaced, width of and thick, anastomosate at base; vivid violet, pruinose; with irregular surface. STIPE 40-60 (100) × 4-8 mm, cylindrical, often undulated, often wider at tip, striated lengthwise with white fibrils on dark violet-amethyst background; whitish towards base, strongly paling in dry conditions and when cut lengthwise reveals whitish flesh.

FLESH fine in cap, pale violet; flesh of stipe is fibrous, elastic, white and firm with sweet odour and flavour. MICROSCOPY: spores $9.0\text{-}10.0\times8.5\text{-}10.0~\mu\text{m}$, globular, with tall spines up to 2 mm; tetrasporophyte basidia. Cheilocystidia abundant, $30\text{-}80\times6.0\text{-}10.0~\mu\text{m}$, wiry, irregular, sometimes branched, hyaline. Pileipellis hyphae cutis cylindrical arranged radially.

HABITAT: grows humus rich soil, from hills to mountains, gregarious or in groups, in hardwood and coniferous woods.

EDIBILITY: of no value

NOTE - This species cannot be confused with other *Laccaria*. Only very old and particularly dry specimens, which have lost their characteristic violet colour, run any risk of confusing one as to their identity.



Laccaria fraterna (Sacc.) Pegler

CAP 10-35 mm, initially convex, then flat and depressed, striated for transparency almost up to centre; edges from round to crenulated. hygrophanous cuticle, ochre-beige, finely scaly with dry weather with almost marginal area, brown-tawny, finely warty-grainy in humid weather.

GILLS mostly adnate or lightly decurrent, fairly spaced, wide, salmon pink or red-brownish, with the surface whole coloured.

STIPE $30-60 \times 1-3.5$ mm, cylindrical, widening at base, from full to hollow, covered with fine white fibrils lengthwise on brown-red background, pruinose to apex, with mycelium white at base.

 $\ensuremath{\mathsf{FLESH}}\xspace$ fine, red-brownish, watery. Pleasant odour, sweet fungus flavour.

MICROSCOPY: spores $8.5\text{-}10.5 \times 7.0\text{-}9.5 \mu m$, from subglobose to mostly ellipsoidal, echinulate, hyaline, with spines reaching 1 μm in length; bisporic and monosporic basidia.

HABITAT: basidiomata, gregarious or cespitose, near *Eucalyptus*, *Pinus*, *Cupressus* sp. pl., in Mediterranean environment. Summer-autumn.

EDIBILITY: of no value

NOTE - It is almost impossible to determine a *Laccaria* with only the morphological data such as that evidenced by *L. fraterna*, to go on without the aid of a microscope. This species can be recognized by its mostly bisporic basidia, its spore size, the length of its spines and its typical Mediterranean habitat.



Laccaria laccata (Scop. : Fr.) Berkeley & Broome

CAP 10-35 (45) mm, initially hemispheric, then convex ed finally flat and depressed in the centre; edges striated for transparency, undulated and denticulate; from brown-orange, brown reddish, to pinkish red in humid weather, paler to beige or faded ochre-ish with the dry; matt surface, smooth, very fine radial fibrils, a little dandruff in centre, hygrophanous.

GILLS mostly adnate or lightly decurrent, wide, spaced; light flesh colour light at first, then brown-pink, on the whole surface.

STIPE 35-100 \times 3-5 mm, cylindrical, often slightly widened at base, full then hollow, elastic; surface from brown-red to brown dirty, smooth or just striated, covered longitudinal with whitish fibrils.

FLESH watery, from brown-grey to whitish, fine; weak herbal odour; sweet fungus flavour.

MICROSCOPY: spores from subglobose to ellipsoidal, hyaline, $8.0\text{-}10.0 \times 6.5\text{-}8.0 \mu m$; spines 1-2 μm in length. basidia clavate, $28\text{-}45 \times 8\text{-}15 \mu m$, tetrasporic, hinged; length of sterigmata up to $10 \mu m$. regular lamellar web. Cheilocystidia cylindrical, fairly supple, $25\text{-}60 \times 3\text{-}7 \mu m$. Cuticle formed from parallel hyphae, interwoven, width of 7-15 μm , with some hyphae scattered perpendicularly. Hinges present.

HABITAT: isolated or gregarious, in hardwood or coniferous woods or on their outskirts, in uncovered areas, on carpets of needles, among moss

EDIBILITY: of no value

NOTE - *L. laccata* var. *laccata* with its small and delicate carpophores this can be distinguished from other varieties above all due to its ellipsoidal spores, which have a length/width ratio of over 1-2.



Lactarius deliciosus (L.: Fr.) S.F. Gray

CAP 50-150 mm at start, convex, then flattened, finally depressed-infundibulform, yellow-orange, orange-ochreish, with more saturated concentric zone, edges convoluted, then more or less undulated-lobed, hairless surface, a little viscous in humid weather, then dry, pruinose, more clear at edges.

GILLS lightly decurrent, crowded, rigid, forked, pale orange then carrot red in fractures, finally, very slowly, dirty greenish.

STIPE 30-60 × 15-25 mm, filled then hollow, pruinose-felted, pale orange, more or less bearing red-orange dimples.

FLESH pale, carrot red in fractures to the latex, then very slowly becoming greenish (in 24 ore), with fruity odour and mild flavour.

LATEX: carrot red, uniform or paling, with mild flavour.

MICROSCOPY: spores from mostly ellipsoidal to ellipsoidal, $8.1\text{-}10.3 \times 6.5\text{-}8.0~\mu\text{m}$, with warts joint with fairly thick ridges, forming an almost complete lattice; tetrasporophyte basidia, subclavate; Pileipellis, an ixocutis is present

HABITAT: exclusively associated with *Pinus*; frequent in pine. From summer to the first signs of winter. EDIBILITY: **edible**

NOTE - Of those species which associate with conifers and have orange or red latex, *L. deliciosus* is easily recognisable by its cap with orange-ochre-ish patches, its stipe covered with red-orange dimples, its unchanging latex and its habitat of pine trees. Of the large, edible milk-caps this one is fairly well known, and, probably, the one best suited to the dinner table.



Lactarius deterrimus Gröger

CAP 30-100 mm, initially convex then flat, finally depressed in the centre, vivid orange, then rusty orange, greening all over with age, not zoned or with crowded zoning at edges, slightly evident, rounded edges then open, the surface slightly viscous in humid weather, then dry, pruinose-rugulous.

GILLS adnate-decurrent, very crowded, forked, ochreorange-ish, stained green-brownish in fractures.

STIPE $30-60 \times 10-25$ mm, filled, soon becoming hollow, concolour with cap, generally not scrobitulate, pruinose, bearing a white rim at tip, eventually greening all over.

FLESH cream-orange, slowly turning to winey red, then dark green, with fruity or carrot like odour and mild or lightly acrid and bitter flavour. Guaiac, winy grey.

LATEX: slightly abundant, orange, turning red-winey in 15 minutes, with mild flavour, then a little acrid and bitter.

MICROSCOPY: spores from subglobose to mostly ellipsoidal, $8.0\text{-}11.4\times6.6\text{-}8.7~\mu\text{m},$ with warts joined with thin ridges, forming a very incomplete lattice; tetrasporophyte basidia, subclavate; Pileipellis, it has an ixocutis.

HABITAT: symbiont of *Picea abies*; highly widespread and common in fir abetaie and red fir; from summer to late autumn.

EDIBILITY: edible

NOTE - The peculiar characteristics of this species are the non patchy cap and stipe, though which soon become stained green. The stipe is not dimpled, though has a typical white circle at its tip; the latex moves slowly from orange to winey red and its habitat is near *Picea abies*. In spite of its name, which means "the worst"... it is considered a good edible, even if less well-esteemed than *L. deliciosus*.



Lactarius piperatus (Scop. : Fr.) S.F. Gray

CAP 50-150 mm, initially convex, then flat-depressed, finally infundibulform, white-cream, soon becoming dotted with brown-ochre ones, eventually with some rusty shades, the edges are fine and convoluted when young, then lobed-undulated, the cuticle is adnate, dry, hairless, a little rugulous, with the tendency to crack.

GILLS from adnate-decurrent to slightly decurrent, very crowded and close, thin, with numerous lamellule, white with pale cream and flesh colour tinges, brownish in fractures.

STIPE 60-10 \times 15-25 mm, short or slim, cylindrical or attenuated at base, often eccentric, full, firm smooth, white, with dirty cream or brown-ochre-ish stains from the base.

FLESH thick, firm, white to gill edge, but soon turning cream, with a very acrid flavour and no significant odour. LATEX: uniformly white if isolated, but lightly yellow olivish, drying on the gills, with very peppery flavour.

MICROSCOPY: spores from subglobose to oblong, 7.2-10.4 \times 5.2-7.5 μ m, with warts joined in ridges and forming un incomplete lattice; bisporic or tetrasporic basidia, subclavate; Pileipellis, epithelium.

HABITAT: in hardwood and coniferous woods, very common, grows in groups, often early; summerautumn.

EDIBILITY: non edible

NOTE - *L. piperatus* can be told apart from *L. pergamenus* by the negative reaction of its latex with potassium hydroxide and by its gills and flesh which do not assume grey-greenish stains. *L. vellereus* and similar species are usually more robust and stubby, and have spaced out gills; *L. controversus*, a symbiont of poplar trees, has decidedly pinkish gills.



Lactarius sanguifluus (Paulet) Fr.

CAP 40-80 mm, from convex to flat-depressed, firm fleshy, with the edges convoluted at the extremes, then rounded, undulated-lobed, finely felted. Cuticle lightly viscous in humid weather, then dry, pruinose, slightly zoned at most, light orange, orange-ochre-ish, orange-greying, finally with non uniform greenish stains.

GILLS crowded, thin, from adnate to lightly decurrent, forked, lilac-pinkish, grey-lilac-orange, grey- vinous red, red-brownish in fractures then stained dark green.

STIPE $20\text{-}40 \times 10\text{-}20$ mm, firm filled, soon becoming hollow, cylindrical or a little attenuated at the base, pruinose, white-greying at the summit, pink-violet, pink-greyish, grey-violet in the lower part, smooth or with small darker dimples

FLESH firm whitish, then brick pink, red-brick. Fruity odour, mild then lightly bitter flavour.

LATEX slightly abundant, red-vinous, uniform, mild then bitter flavour.

MICROSCOPY: spores 7-9 × 6-7 µm, mostly ellipsoidal, crested-reticular, with little mesh completely closed.

HABITAT: thermophilic species, grows exclusively under *Pinus*, Summer-autumn.

EDIBILITY: edible

NOTE - A thermophilic species which is fairly common to maritime pines. It can be recognised by its orangetoned cap and its lilac-pink gills. It is confusable with *Lactarius vinosus* Quél., which, however, has a cap with patches of reddish-violet tones, and gills which are initially winey-red to violet, often with almost total greening.



Lactarius torminosus (Schaeff. : Fr) S.F. Gray

CAP 40-110, initially convex then flattened, finally depressed, more or less umbillicate, viscous if humid, dry and rough in dry weather due to fine, short innate fibrils, from flesh-pink to pink-orange-reddish, with dark pink-reddish concentric zoning, the edges are convoluted, bearing tangled woolly hairs.

GILLS adnate-decurrent, crowded, close, forked, cream pinkish.

STIPE 25-50 (80) \times 10-20 mm, full, then hollow, whitishcream, often with a pink labrum at tip, often with some pinkish dimples.

FLESH thick, hard whitish, sometimes with flesh colour stains, with fruity or pelargonium odour and very acrid flavour.

LATEX white, uniform, but slowly yellowing on a tissue or sheet of white paper.

MICROSCOPY: spores from mostly ellipsoidal to ellipsoidal, 8.3-9.8 × 6.2-7.5 µm, with warts connecting to form several closed meshes; tetrasporophyte basidia, subclavate; pileipellis, an ixocutis is present.

HABITAT: in hardwood areas near birch; fruits from the end of summer to the end of autumn; fairly common.

NOTE - This can be distinguished from similar species by its white, uniform latex, its fairly red-pinkish and hairy-felt cap, and by its symbiosis with *Betula*. *L. Pubescens* is a smaller and nearly white or white-pinkish replica. *L. tesquorum and L. mairei*, have a more yellowish colour, and tend to grow in more southern climes and are not linked to birch trees. Even if some guidebooks, a little recklessly perhaps, nominate this as an edible species, which it may be after a long cooking time, it is **certainly poisonous**, and causes gastrointestinal distress. This characteristic, which is rapidly discovered by those unfortunate enough to confuse it with the saffron milk-caps (edible milk-caps with a red or carrot-orange latex), is the origin of its well-deserved Latin name, meaning "causing colic".



Lactarius volemus (Fr. : Fr.) Fries

CAP 50-120 mm, initially convex, then flat, finally depressed, from yellow-reddish to tawny-orange, with the centre darker. Adnate, dry, matt, pruinose surface initially, from smooth to lightly rugulous, velvety convoluted edges, then distended often cracked radially when dry.

GILLS from adnate to a little decurrent, crowded, forked to stipe, cream, pale yellow-ochre colour, stained dark brown in fractures.

STIPE 30-80 (100) \times 15-30 mm, firm, full then filled, pruinose, cylindrical or a little attenuated at base, concolour with cap, but lighter at tip, browning in the manipulated points.

FLESH thick, firm compact, whitish-cream at gill edge, browning in cap, with an odour characteristic of fish (herring).

LATEX: very abundant, dense, white, brownish drying when exposed to air, with a sweet flavour.

MICROSCOPY: spores from globose to subglobose, 9.2-11.2 \times 8.4-10.6 μ m, with clearly joint warts in ridges forming an almost complete lattice; bisporic or tetrasporic basidia, subclavate; pileipellis, epithelium.

HABITAT: mostly hardwood areas, more rarely in coniferous areas; not widespread, faithful to its growing areas; not very common.

EDIBILITY: edible

NOTE - Apart from its dry cap, *L. volemus* can be identified by its flesh, which has a greenish reaction to ferrous sulphate solution, its voluminous latex which seeps from any cracks in its skin, and its characteristic odour of herrings (very similar to that evinced by the *Russula* in the *amoena* group). It is edible, though appreciated by all, and is best cooked on the grill so as to preserve a vaguely smoky aftertaste.



Langermannia gigantea (Batsch : Pers.) Rostkovius

BASIDIOCARP prosurface is globular, to irregularly roundish, with a circumference ranging 50 to 600 mm and, in exceptional cases, can reach even more remarkable dimensions. Sessile, with sterile radiciform base.

EXOPERIDIUM formed from a single layer to a white and velvety, smooth then yellowish-cream bark; at maturity tears into irregular strips, fairly coarse, leaving the fine endoperidium free, whitish, then greying-sooty or grey-brown-ochre-ish, of papyrus consistency, friable, gradually dehiscent due to erosion, starting from the summit.

GLEBE white, firm and compact when young, with weak, fungal odour and pleasant flavour, then soggy, from yellow-ochre to brown-olivish, powdery in maturity. MICROSCOPY: spores spherical or mostly ellipsoidal, 3.6-5.6 µm, with short, finely warty peduncle. Brownish spores.

HABITAT: in grassy areas, in pastures, grown in parks and gardens, from the end of summer throughout autumn. Solitary, gregarious. Infrequent.

EDIBILITY: edible

NOTE - This mushroom is spectacular for the sizes it can attain and there are reports of specimens over a metre in diameter and which have achieved the considerable weight of 20-25 kg. Species in the genus *Calvatia*, which have an exoperidium in two layers, can also achieve considerable size, among them, *C. lilacina*, which is coloured pale brownish-purple, prefers cultivated soil or footpaths, while *C. utriformis*, a white species whose exoperidium is ornamented with pyramidal warts, tends to grow in alpine pastures.



Leccinum aurantiacum (Bulliard) S.F. Gray ss. Pilát

CAP 40-120 (150) mm, subglobular, then tightly parabolic, margin finally distended regularly, visibly hanging from excess of cuticle; finely velvety-felted, sometimes with adnate scales, just greasy in humid weather; uniformly red, red-orange, red-tawny, decolouring to brick or to yellow orange at maturity, dry. TUBES rounded-depressed or almost free to stipe, tall, even beyond 30 mm; grey-whitish, then grey, grey greenish dirty due to the maturation of spores, dark grey at gill edge, then through a temporary violet colour. Very small, round pores,, concolour with tubes; staining grey brownish to the touch.

STIPE 60-130 (150) \times 15-30 (45) mm, progressively attenuated towards tip; whitish, thickly covered with scales becoming more crowded and coarse towards the base, initially white, then becoming darker brown-reddish,, almost black in old age or to the touch; basal area is often stained with a green-blue colour. Quickly becoming more woody-fibrous in consistency, full.

FLESH fairly firm in cap, very fibrous in stipe; whitish, slowly turning grey-lilac, then dirty violet, finally orange

subtle, insignificant odour and flavourful. MICROSCOPY: fusiform spores 13.5-16 \times 3.8-5 $\mu m,$ light brown under microscope; grey brownish olive in mass

HABITAT: in humid woods, associated with *Populus tremula*, in summer-autumn, recurrent.

EDIBILITY: edible

tendencies.

NOTE - This is the most common of the "porcinelli rossi", ("little red porcines"). It is very easy, however, to confuse it with the other red *Leccinums*: *L. quercinum*, which is bulkier, has more precociously coloured reddish scales, and associates with other hardwoods than *Populus*; *L. vulpinum* and *L. piceinum* are symbionts of conifers. *L. versipelle* is orange and is a symbiont of *Betula*. In our own gathering fieldwork we often had the impression that the correlations between carpophore characteristics as described in literature and symbiosis with host plants does not always follow precise rules, but rather preferential



Lepista nuda (Bull. : Fr.) Cooke

EDIBILITY: edible

CAP 50-125 mm, initially convex, then obtusely conical and finally flat, sometimes with large umbo; convoluted margin, sometimes a little affected, curving towards the base at the extremes, distended sinuated-undulated late on; smooth, matt surface, slightly greasy in humid weather; violet, lilac, blueish colour, tendency to become brown tawny mostly towards the centre of the cap, decolouring to ochre-ish-violet or ochre-ish-pinkish with age.

GILLS adnate-uncinate or just decurrent, crowded and with numerous wide lamellule; grey-lilac, lilac blueish colour, then with brownish tones.

STIPE 50 -90×15 -30 mm, cylindrical or widening towards the base, clavate, sometimes bulbous; base is rich with mycelial residues which typically encompass the substrate when the carpophore is picked; fibrous and elastic; lilac, lilac-grey-violet, violet colour, covered with a white fluff especially at the tip.

FLESH firm, soon becoming soft and a little watery; light grey colour, with violet tones; odour is characteristically strong, aromatic, hot and indefinable.

MICROSCOPY: ellipsoidal, lightly warty spores, dimensions 7-8.5 \times 3.5-5 μ m; salmon pink in mass. HABITAT: ubiquitous, on the substrate humus of plants, in large groups, often in lines or circles; from autumn to winter, often even in spring.

NOTE - This is a sought-after edible with a delicate aroma. It can be confused with *L. sordida*, which is slightly thinner and usually has a more intense and "dirty" colour, bordering on violet-dark grey. A *L. nuda* var. *lilacea* has been described, bearing intense violet colouring all over, with a slightly smaller body. *L. glaucocana* has a much paler cap, tending toward lilacgrey, and has a weaker and less-pleasant odour. *L. personata* is very similar to *L. nuda*, but combines a greying, white-coffee coloured cap with a lovely violet sipe. Several other mushrooms can display a general blue-violet colouration, (e.g.: *Cortinarius violaceus* and *Entoloma bloxamii*) but also have varying generic characteristics; (see their respective genus descriptions).



Lycoperdon echinatum Pers. : Pers.

BASIDIOCARP high 30-80 mm, 20-60 mm in diameter, subglobular, obvoid or piriform. Exoperidium formed from dense converging spines and composites, width of 3-6 mm, initially white, then dark brown, which can be removed fairly easily leaving a reticulated endoperidium. Endoperidium is, light brown papyrus.

GLEBE from grey-brown violet to chocolate brown, with an indistinct pseudocolumella. Areolated subglebe, with various brown and lilac stains.

MICROSCOPY: globose spores, 4.0-5.0 µm, warty. Light brown, elastic scalp, with small rounded pores, fairly numerous. Para-capitulum is absent. Exoperidium has large irregularly shaped thick walled spherocytes, dark

brown. Spore powder is chocolate brown or with lilac

HABITAT: solitary or in small groups, in calcerous soil, mainly in beech woods.

EDIBILITY: edible

NOTE - This is a very-easily recognisable species thanks to the entirety of its morphological features. When found in forests of chestnut trees it is even likely to be taken for a chestnut shell! *Lycoperdon foetidum* also possesses a brownish exoperidium and a reticulated endoperidium after its spines have dropped, but these are significantly shorter; *L. umbrinum* and *L. molle* too display a brownish exoperidium but their endoperidia are smooth.



Lycoperdon perlatum Pers. : Pers. [= L. gemmatum Batsch]

BASIDIOCARP height from 30-90 mm, 20-40 mm in diameter, subglobose, piriform, subcylindrical or almost pestle shaped, white, from cream to light brown. Exoperidium is formed from conical spines, length of 1-2 mm, fragile, whitish or cream, then light brown, surrounded with a circular row of more persistent spines. When the spines fall, they move apart to form a characteristic polygonal grid pattern on the endoperidium. Endoperidium is, grey-brown papyrus.

GLEBE white, then brown or olivish brown, with a well developed pseudocolumella. Subglebe is strongly developed, cellular, from olivish brown to grey-brown. MICROSCOPY: globose spores, 3.5-4.5 µm, warty. Scalp is yellow-brown, formed from hyphae sized 3-8 µm, with relatively thin walls; fairly numerous pores. Paracapitulum, usually abundant. Exoperidium has fine

walled spherocytes of 20-30 μm,. Spore powder is yellow-brown, olivish brown or grey-brown.

HABITAT: ubiquitous, in hardwood and coniferous areas. EDIBILITY: edible

NOTE - On a macroscopic level, *L. perlatum* can be identified by the shape of its basidiocarp and by the characteristics of its exoperidium, and microscopically, by its small spores. Occasionally it can be found on dead wood but confusion with *Lycoperdon piriforme* is highly unlikely. This latter displays, in fact, white glebae even at maturity and after shedding its exoperidium, its endoperidium has a smooth appearance. *L. nigrescens* has darker and more-persistent spines plus larger and lessonnamented spores.



Lycoperdon pyriforme Schaeff.: Pers.

BASIDIOCARP height 15-60 mm, piriform, clavate, rarely subglobose, connected by thick white rhizoids. Dehiscence comes through a fairly wide, open operculum. Exoperidium is white, then fairly dark brown, wartygranular, with adpressed scales, soon becoming hairless at tip. Endoperidium is papyrus colour and matt.

GLEBE with distinctive pseudocolumella, white then olivish, finally grey-brown, with a strong unpleasant odour and sweet flavour. Subglebe is firm and areolate which remains white.

MICROSCOPY: globose spores, 3.5-4.0 μm , almost smooth. Capitulum is brown, elastic, not pored, largo 3-

7.5 µm, with thick walls 0.7-1.0 µm. Paracapitulum is abundant. Exoperidium has large spinyspherocytes, walls thick, irregular shape. Spore powder is olive brown. HABITAT: in large groups on decaying wood, often on burnt wood, in woods, parks and gardens. EDIBILITY: edible

NOTE – Very easy to recognise, by its lignicole habits, floury, inconsistent exoperidium, its cespitose growth, and its uniform, white subgleba.



Lyophyllum decastes (Fr. : Fr.) Sing. [= *Lyophyllum aggregatum* (Schaeffer) Kühner]

CAP 50-70 mm, hemispheric, convex, then flat, often with large obtuse umbo, the margin is often lobed and undulated, fairly convoluted, then distended. Cuticle is smooth or fibrillated radially, shiny, lardaceous to the touch in humid weather, brown-grey, dark brown-ochre, with the edge lighter, often almost whitish.

GILLS adnate or uncinate, fairly crowded, relatively close, from whitish to cream, often stained pink.

STIPE $30-120 \times 8-20$ mm, from cylindrical to clavate, full, elastic-fibrous, whitish, fibrillated with the tip covered with a white bloom.

FLESH elastic, whitish, with fungal odour and sweet flavour.

MICROSCOPY: spores $5.5-7 \times 5.0$ - $6.5 \mu m$, subglobose, smooth, hyaline; tetrasporophyte basidia, clavate, with siderophile granulation; epicyte formed from fairly

parallel, braided hyphae, with pigment partially brownish, hinged.

HABITAT: grows generally collated and in groups or in circles in hardwood and conifer woods, especially in open areas, at the edges of paths, in parks or in gardens among grass.

EDIBILITY: edible

NOTE - The species in the section *Difformia* with tricholomatoid silhouette, brownish-grey colours and globose spores, constitute a fairly homogenous group of species, sometimes difficult to categorise. When the carpophores are connate at the base, but not branching, and their growth is not cespitose then we are looking at either *L*. decastes or *L. loricatum*. The former has a finely fibrillated, relatively thin cuticle, the while the latter has a thick, tenacious, glabrous cuticle.



Macrolepiota procera (Scop. : Fr.) Singer

CAP 100-250 mm, initially spherical, ovoid, then hemispheric-campanulate, finally flat, with large obtuse umbo, the cuticle is decorated with concentric plical scales, hazelnut ochre-ish in colour, often fairly brownish or reddish, on a light background, the margin is large and fringed.

GILLS free, crowded, white then ochre.

STIPE up to $200-350~(500) \times 10-20~\text{mm}$, slim, cylindrical, with base dilated to form a clear bulb, filled, then hollow, bearing mottled brownish bands which leave hints of the cream flesh below, subsmooth above the annulus. Double annulus, mobile, whitish externally, with the lower leaf brownish.

FLESH white, uniform, with pleasant fungal odour,, and hazelnut flavour.

MICROSCOPY: ellipsoidal smooth, hyaline spores, with germinating pores, 12.5-17.8 × 8.5-11 μm. clavate, tetrasporic basidia. Polycystidea is absent. Cheilocystidia clavate. Epicyte formed from trichoderma. Partially dominant pigment. Rarely joint at hinges.

HABITAT: isolated or gregarious in hardwood and conifer woods or in meadows; very widespread and common, from summer throughout autumn.

EDIBILITY: edible

NOTE - By its antonomasia, this is the "drumstick", a high-quality, much-sought edible. It is easy to identify, just look for its large size and outline which explain its German name, meaning "parasol", its stripy stipe and its characteristic, movable double annulus.



Macrolepiota rhacodes (Vittadini) Singer

CAP 80-150 mm, initially campanulate, then convex, finally flat, the cuticle is excoriated up to the circumference with wide, coarse, crowded, overlapping scales, fairly light brownish in colour, with a fairly tight central cap, of colour brown reddish.

GILLS free, whitish, cream, then dirty pink, reddening when rubbed, with a floccose surface.

STIPE 100-160 \times 10-15 mm, stocky, cylindrical, progressively dilated to form a bulbous submarginated bulb, hollow, smooth, white, gradually brown-reddish with time or when rubbed. Annulus is membranous, robust, whitish, mobile.

FLESH white, turning orange vivid at gill edge, then to red-vinous, with an odour of raw potato and sweet hazelnut flavour.

MICROSCOPY: ellipsoidal or ovoid, smooth, hyaline, with germinating pores, $8.8\text{-}11.2 \times 6.8\text{-}8.0 \ \mu m.$ clavate, tetrasporic basidia. Polycystidea is absent. Cheilocystidia clavate or piriform. Epicyte formed from a trichoderma of

fairly erect hyphae. Membranous brown pigment. Joint at hinges.

HABITAT: isolated or gregarious, in parks, gardens, and also in the woods, mostly of mixed conifers. from the end of summer to autumn, recurrent.

EDIBILITY: edible

NOTE - *M. rhacodes* is characterised by its peculiar pileic scaling, which is formed of tortoiseshell scales, concolour with the cap background, and above all for its reddening flesh. The variety, *bohemica* differs by having scales of a different colour to those of its background and for its habitat on the periphery of woodlands. *M. venenata*, heavily toxic, can be distinguished by the radial arrangement of its pileic scales and by the total absence of joint hinges, a characteristic which is incredibly difficult to verify. Therefore, in case of doubt, one would be best advised to avoid consuming any *Macrolepiota* with reddening flesh.



Marasmius oreades (Bolt.: Fr.) Fries

CAP 20-50 mm in diameter, initially hemispheric, campanulate, then flat, umbonate in the centre, the margin is acute, smooth, often lightly crenulated, the surface is smooth, hygrophanous, from orange-ochre to brownish with humid weather, but light cream-hazelnut when dry, spaced, interspersed from lamellule, sinuous, wide, from whitish to cream.

STIPE 30-70 $(100) \times 3$ -5 mm, full, cylindrical, often a little widened at the two extremities, slim, tenacious-elastic, whitish dirty-cream at tip, brownish on the lower part, from finely pruinose to velvety for the whole length, with brownish mycelium.

FLESH whitish, elastic, fine, hygrophanous, with a characteristic odour, like almonds, and sweet hazelnut flavour.

MICROSCOPY: spores from ellipsoidal, fairly elongated, to amygdalform, (7.0) 8.0-10.5 (11.5) μ m; tetrasporophyte basidia, tightly clavate; pileipellis hymeniform. Hinges present.

HABITAT: in meadows, in large groups found in lines or circles; from the spring throughout autumn.

EDIBILITY: edible

NOTE - This, fairly common mushroom, grows abundantly, from the spring to autumn, in meadows, forming "witches' circles". It is a choice edible, much sought after by collectors and can be stored and eaten dry. *M. collinus* (regarding whose edibility there are some doubts), is very similar, though can be told apart by its slender, smooth stipe and crowded gills.



Meripilus giganteus (Pers. : Fr.) P. Karsten

[= Polyporus giganteus Pers. : Fr.]

BASIDIOCARP pileate, devoid of or with rudimentary stipe, reduced to a point of attachment on the cap.

CAP of width up to 300 mm, depth of 10-20 mm and projection of around 100-150 mm; irregularly circular shape to gill edge, with several individuals around a common base. Surface plicea is undulated, brown or brown-reddish, markedly zoned, rough due to the presence of adpressed flakes. undulated margin, complete. HYMENOPHORE tubes and pores, follow the trend of the undulated surface plicea. Tubes about 15 in thickness mm, monolayer, from whitish to dark ochre-ish, blackening if handled. pores 0.2-0.3 mm in diameter, round.

FLESH fibrous and tenacious, but not hard (in the context of a monomorphic constitution), cream white, with fungal odour and sweetish flavour.

MICROSCOPY: spores subglobose, in the shape of chestnuts, monoguttulate, not amyloid, smooth and hyaline, dimensions 5.5-6.5 \times 4.5-5.5 $\mu m;$ basidia 20-25 (45) \times 5-10 $\mu m,$ cylindrical-claviform, tetrasporic, without joints at basal hinge; cystidia absent.

HABITAT: saprophyte or parasitic to conifer or *Fagus* trees, in thick groups with specimens overlapping, from summer to autumn; not widespread.

NOTE - At first sight this is liable to be confused with *Polyporus squamosus*, as it grows exclusively on hardwood trunks, the latter, however, has a very scaly cap surface and larger, angular pores; it is also marked out by its strong odour of cucumber, especially in the younger specimens.

The patchy appearance of the cap surface *Meripilus giganteus* is common also to several species of *Trametes*, as is their leathery consistency due to its trimeric hyphal structure; of these the ones which spring to mind are, in particular, *T. zonatella* and *T. versicolor*, the former having a creamy ochre-ish-brown colour, the latter being instead grey-brown, or with decidedly blueish tones. Both produce cylindrical-allantois spores and prefer to live on broken hardwood substrates.



Morchella conica Persoon var. costata Ventenat

ASCOCARP pileate and stipitate, of 120-130 mm in height and 40-45 mm in diameter.

MITRA elongated-conical, with slightly pointed tip, with alveoli clearly separated from intersection of lengthwise and transversal ribs arranged parallel to them.

HYMENOPHORE found on all exposed parts of the mitra, smooth, brick-brown colour, which blackens on the ribs with age. Edge is regular, separated from the stipe by a vallecula.

STIPE subcylindrical, sometimes swollen at base, rarely furrowed, rough, white suffused with pinkish tones, hollow or filled internally.

FLESH leathery-elastic, whitish, with light spermatic odour and sweetish flavour.

MICROSCOPY: ellipsoidal spores, $19-25 \times 12-13 \mu m$, smooth, with some small guttules arranged on the external surface of the polar zone, uniseriate in asco; asci are cylindrical, not amyloid, octasporic; slightly cylindrical paraphyses extended at tip, septate and sometimes forked. HABITAT: single or in small groups in conifer woods, in spring; not common.

EDIBILITY: edible

NOTE - *M. conica* var. *costata* can be distinguished from similar species by its characteristic darkened parallel ribs, and also the pinkish colouration of its stipe. Its habitat and microscopic features are not particularly useful in its identification.



Morchella esculenta (Linnaeus) Persoon var. esculenta

ASCOCARP pileate and stipitate, up to 100 to 250 mm in height and 50-80 mm in diameter.

MITRA irregularly ovoid, subspherical, globular, with longitudinal and transversal ribs in relief, variably carved between them forming irregularly polygonal alveoli. Hymenophore is smooth, from yellow cream to light ochre; edges of the ribs are coloured. insertion to stipe is without vallecula.

STIPE widened at base, sometimes semibulbous, often partially ruffled, irregularly cylindrical, cream ochre-ish, granular, internally cavernous.

FLESH elastic, ochre, with light spermatic odour.

MICROSCOPY: smooth ellipsoidal spores, $18\text{-}25 \times 14\text{-}16~\mu\text{m}$, hyaline under microscope, thick walls, with some small guttules on the extreme surfaces, monoseriat in asco; asci are cylindrical, not amyloid, octasporic; cylindrical clavate paraphyses at tip, septate, simple or branched

HABITAT: single or in small groups on mouldy, sandy ground to sandy or sandy-clay ground in fresh hardwood

areas, under *Fraxinus, Ulmus, Alnus*, in humid places. Fairly common, in spring.

EDIBILITY: edible

NOTE - There are some other varieties of M. esculenta, which can be distinguished by their morphological features; var. rigid which has a visibly more globular mitra and is larger (up to 300 mm in height); var. vulgaris which has a conic-elongated mitra, (sometimes) smaller dimensions (from a minimum of 50 mm in height); var. rotunda which has an oval, subglobular mitra, which, in size, can match var. rigid (which is difficult to distinguish from the latter). Morchella crassipes is very similar to M. esculenta (of which it may be just a giant variety), has a conical-rounded ochre-greyish mitra, clavate stipe which can be as large as 70 mm in diameter; Morchella esculenta var. umbrina, instead, diverges from the usual appearance by the brown colour of its mitra, which, however has rib edges which are light ochre-ish. Grows close to hardwoods such as Fagus or Fraxinus.



Morchella esculenta (L.) Persoon var. vulgaris Persoon

ASCOCARP pileate and stipitate, from 80 to 150 mm of height and 50-60 mm of diameter.

MITRA ovoid-conica, with rounded or subpointed apex, with irregular, polygonal or roundish alveoli, deep.

HYMENOPHORE found on the entire visible part of the mitra, smooth, light brown, whitish on the ribs. Edge is regular, separated from the stipe by a vallecula.

STIPE subcylindrical, but swollen at base where it is often deeply ribbed or furrowed, rough, white-ochre-ish, filled internally.

FLESH elastic, whitish, with light spermatic odour and sweet flavour.

MICROSCOPY: ellipsoidal spores, 19-25 \times 12-13 μm , smooth, with some small guttules arranged on the external

surface of the polar zone, uniseriat in asco; asci are cylindrical, not amyloid, octasporic; paraphyses lightly cylindrical elongated in height.

HABITAT: single or in small groups near hardwood trees, especially *Ulmus*, in spring; not common.

EDIBILITY: edible

NOTE - This is confusable with other species of the same genus, both for its colour and appearance, which are not infrequently influenced by its growth conditions. One of its peculiar characteristics is the colour of its hymenophore which is light brown in contrast to its white ribs.



Morchella semilibera De Cand. : Fr.

ASCOCARP stipitate and pileate, up to 200 mm in height and around 30 in diameter.

MITRA conical-pointed, often rounded, sub-cerebriform, with longitudinal and transversal ribs slightly in relief forming the irregular alveoli. Hymenophore is smooth, of brown-brick colour, darker on the edges of the ribs. Lower surface is smooth or minutely rough, white cream. STIPE slim, subcylindrical, often ribbed lengthwise, rough, white cream.

FLESH tenacious, elastic, but to con fragile, sweet, with spermatic odour; white cream colour.

HABITAT: in humid places, on sandy or clay-sandy ground, under trees such as *Almus*, *Crataegus*, *Fraxinus* and *Populus*, but also in vineyards, more rarely under other hardwood; in spring, in groups of several specimens, recurrent.

MICROSCOPY: ellipsoidal spores, smooth, 20-25 \times 14-17.5 μ m, hyaline under microscope, often with small

guttules on the external surface at the ends, uniseriat in asco; asci are cylindrical, not amyloid, octasporie; cylindrical paraphyses with widened and often guttural apex, septate.

EDIBILITY: edible

NOTE - This has in the past been considered as part of another genus, the genus *Mitrophora*, distinct from the *Morchella* by the way the stipe was inserted into the mitra. In the *Mitrophora* the vallecula is lengthened, and joins onto the lower surface at around 1/3-2/3 of the length of the mitra itself. Today this difference is not held to be sufficient to warrant maintaining two diverse taxonomic genera.

 $M.\ fusca$, which is smaller (up to 80 mm in height) (and has spores just 8-9 μm wide) while $M.\ gigas$ can reach heights of 200 mm, with a clavate stipe which measures up to 50 mm at its wide base.



Mycena pura (Pers. : Fr.) Kummer

CAP 20-50 mm, from fairly campanulate to flat-convex, with or without an obtuse umbo, hygrophanous, striated to transparency, hairless; pale pink with light lilac stains.

GILLS 25-30, from ascending to fairly horizontal, from adnate to non-marginated, slight crowded, bulging, from smooth to venous, whitish, suffused with very light violet, concolour with the surface.

STIPE 40-70 \times 3-8 mm, cylindrical, a little widened at base, smooth, hollow, tenacious, pruinose at tip, dirty whitish, suffused with violet, lighter at tip, with base fairly densely covered with whitish fibrils.

FLESH fine, whitish, with raphanoid odour and flavour. MICROSCOPY: ellipsoidal spores, amyloid, $7.0-9.5 \times 3.5-4.5 \mu m$. Tetrasporophyte basidia, clavate, with hinges. Cheilocystidia is fusiform clavate, fairly elongated, generally smooth but covered with sparse diverticula at the edges of the cap. Pleurocystidia similar. Epicyte formed from filamentous smooth hyphae, very thin.

Hyphae of stipe is smooth, with fusiform or ellipsoidal caulocystides, smooth.

HABITAT: in hardwood and conifer woods, between fallen needles and leaves; very widespread and frequent at every latitude. From the end of spring.

EDIBILITY: suspect

NOTE - Of all the species belonging to the genus *Mycena*, *M. pura*, with its different varieties and forms, is probably the one which has the widest range of cap colouring. Following the guidelines proposed by Maas Geesteranus, any taxon's colour variations should be considered as mere forms rather than varieties. Among these we mention, for example, the completely white fo. *alba*, the blue-capped fo. *ianthina*, which has a hint of violet or grey, and the fo. *lutea* which can be identified by the yellow colour of its cap but also its pale stipe bearing just a hint of violet.



Mycena rosea (Bulliard) Gramberg

CAP 30-50 mm, from conical to campanulate, then convex or flat-convex, mamelon, pale pink, pink-lilac, sometimes vinous hints, with the edge lighter, hygrophanous, smooth, hairless, a little unctuous, striated to transparency.

GILLS 32-36, wide, ascending, bulging, adnate or non-marginated, fairly light pink, whitish towards the surface. STIPE 70-110 \times 5-10 mm, cylindrical, hollow, smooth or covered with very fine fibrils, white, hint of pink.

FLESH white pinkish, hygrophanous, with fairly clear raphanoid odour.

MICROSCOPY: spores mostly ellipsoidal, smooth, hyaline, amyloid, 7.5-10.2 × 3.8-4.5 µm. basidia clavate tetrasporic, hinged. Cheilocystidia and pleurocystidia fusiform, clavate or spherical-pendunculated. Epicyte

formed from parallel interwoven hyphae, the surface layer is geleld. Hinges present.

HABITAT: isolated or gregarious, on decomposing residues in hardwood and of conifer woods; non very common, fruits preferably in autumn.

EDIBILITY: suspect

NOTE - *M. rosea* belongs to the subsection *Purae* (Konr. & Maubl.) Maas G. of section *Calodontes* (Fr. ex Berk.) Quélet and differs from *M. pura*, of which it is often considered a variety, not only by the colour of its cap, which is a particular shade of pink, but also by its shape, which is conical-campanulate at the extremes or parabolic, and by its more-delicate stipe.



Phallus impudicus L.: Pers.

BASIDIOCARP initially content in a whitish peridium, globular, often and filled with a gelatinous substance, external surface is membranous, initially smooth, soon becoming percolated by elevated veins; the peridium has clear mycelial rootlets and the tip tears at maturity to allow the exit of a phallic receptacle, made from the stipe and cap.

STIPE $120-210 \times 20-35$ mm, cylindrical, progressively tapered at tip, white, fragile and spongy, often curving, to surface thickly dimpled.

CAP vaguely resembling a mitra, wider in diameter than stipe, honeycombed, shows a round opening at tip and margin; whitish colour but at the covered with a rotten green or olive green dark mucilage paste at extremes, which constitutes the glebe and contains its spores. It is not unusual for portions of gelatinous peridium to adorn the summit of the cap. After the dissolution of the greenish-whitish glebe, the cap eventually takes its cellular-ribbed form.

FLESH fragile in cap, fragile and spongy in stipe. Odour at first, until the carpophore is closed within the peridium

is not unpleasant, raphanoid, with a tendency to smell of flesh or faeces or with hints of gorgonzola cheese; very unpleasant. the odour is noticeable several feet away and clearly indicates the presence of the mushroom even before it is seen

MICROSCOPY: ellipsoidal spores, smooth, brownish under microscope, dimensions: 4-5 \times 1-2 $\mu m.$

HABITAT: solitary or in groups in humid recesses dei woods, often hidden among the undergrowth and close to decomposing vegetable residues; not rare.

EDIBILITY: non edible

NOTE - An unmistakeable mushroom with its odour which recalls fecal or strongly-rotten organic matter. An odour which attracts the flies which are necessary to the diffusion of its spores. *P. hadrianii*, which is rarer, is very similar but has a pink peridium, is slightly smaller and grows in sandy, arid earth. *Mutinus caninus* has a moreslanting stipe which is coloured orange-pink where it intersects with the cap, which is the same diameter as the stipe, and is pointed.



Pleurotus ostreatus (Jacq. : Fr.) Kummer

CAP: 50-100 (150) mm, convex, then more distended with convoluted margin at the extremes, finally flat, very eccentric, in the shape of a fan or a shell, thinned in the marginal zone which is sometimes a little striated; hairless, greasy-shiny, grey, grey steel blueish, greybrown, sometimes with fairly violet stains, cuticle is separable.

GILLS: very decurrent, not very crowded, with forks multiple and to various levels, thin, unequal, white or white-silver on whole surface, sometimes pale white-cream.

STIPE: $10-35 \times 10-20$ mm, sometimes nearly absent, very accentric or lateral, irregularly cylindrical, of pruinose appearance, white, or slight hint of grey; dry, firm, full. FLESH: white, abundant in correspondence with stipe insertion, soon becoming thinned, fairly elastic-tenacious, sub-leathery in adults, especially around the stipe; odour vaguely of mould in older specimens, sweet flavour.

MICROSCOPY: spores tightly ellipsoidal, subcylindrical, smooth, $8-11.6 \times 3.2-4.2 \mu m$.

HABITAT: on living or dead wood of various hardwood trees, in woods or in parks, numerous examples are found with overlapping caps; late autumn or winter, common in growing areas, but not widespread. Easily cultivated EDIBILITY: edible

NOTE - There is a variant of this which grows on conifer trees, the var. *columbinus*, which has a light blue tinge all over. This species is very well known for the ease with which they can be cultivated. Very commonly sold, it can be found on sale in all European and most international markets. It also used to be very readily found in the wild too, from meadowlands to the mountains, but it is becoming less and less easy to find them in their natural state. They are a choice edible and almost impossible to confuse with poisonous species.



Ramaria botrytis (Pers. : Fr.) Ricken

BASIDIOCARP coralloid, of 80-100 mm in width and 50-120 mm of height, verticillated, made from a base up 50 mm wide from which many small branches reach out, in various numbers. The principal branches create a pointed tip pink vinous in colour, which contrast with the rest of the branches which are white or whitish.

HYMENOPHORE indistinct, found on the smooth surface of branches and roughly half their height.

FLESH fairly compact but fragile (monomorphic hyphal structure), white, with pleasant odour and flavour.

MICROSCOPY: spores cylindrical-ellipsoidal, 14-17 \times 4.5-8 μ m, with fairly pronounced apiculture, irregularly furrowed lengthwise, not amyloid, hyaline under microscope; basidia 45-60 \times 9-11 μ m, cylindrical-claviform, tetrasporic, joint to hinges at base. Cystidia absent.

HABITAT: on the ground in hardwood and of conifer woods, fruits in groups which sometimes cover a large area, in summer-autumn; not widespread.

EDIBILITY: edible

NOTE - Ramaria botrytis has a winey pink colour which is commonly found in other species such as R. subbotrytis, in which, however one can see hues verging on corral pink; this latter has similar-looking carpophores, fruits in hardwood and conifer forests and has smaller, rough spores, from 8- 11×3 - $4 \mu m$. R. holorubella seems to be a variant found in conifer woods, and has a fairly-well rooted basal trunk which is firmly embedded in the ground. More common is R. rufescens, always white-reddish, with a tendency to yellow at the lower part of its branches. It has distinct basal trunk dimensions and can be found from summer to autumn in woodlands. It has amygdaliform spores, 8- 10×3 ,5- $4 \mu m$.

R. formosa has basidiocarps with an attenuated base with decreasing branches at the top; these tend to be of three colours: yellow on the uppermost extremities, salmon pink on the branches and white at the base. It has are 9-13 \times 5-6 μm , warty-crested spores, and lives on the ground in hardwood forests.



Ramaria flavescens (Schaeff.) Petersen

BASIDIOCARP initially in the shape of a cauliflower, then coralloid, width of 100-200 mm and height 100-150 mm, fairly verticillated, formed from an irregularly cylindrical, solid and fairly developed basal trunk from which numerous branches grow, eventually fairly long, and divide further, until ending in two short spikes at the upper end. The branches are yellow or apricot colour while the trunk is whitish

HYMENOPHORE not distinct, found on the surface on the upper half of the branches.

FLESH compact (monomorphic hyphal structure), white, sometimes marbled, no particular odour and weak, slightly sour flavour.

MICROSCOPY: spores cylindrical-ellipsoidal, 9-13 \times 4-5.5 μ m, with clear apiculture, with partially joined warts, not amyloid, hyaline under microscope; cylindrical-claviform, tetrasporic basidia, with basal joints at hinges. Cystidia absent.

HABITAT: on the ground in hardwood or mixed woods, in summer-autumn; occasional.

EDIBILITY: edible

NOTE - There are numerous yellow species in the genus Ramaria; some of these are fairly poisonous; among which R. formosa typically stands out due to three colours: white on its trunk, pink on its branches and yellow at the branch tips. Other species should be eaten with caution as they can have a laxative effect. R. flava, has least developed basal trunk and can also be identified by its large spores (10-15 \times 4-6 μ m) and the unpleasant odour of its flesh. R. aurea possesses a less-developed trunk, from which arise several columns, which in turn branch out into fairly-long, golden yellow appendages; it lives on the ground in woods of Fagus and has warty spores of the same shape, but smaller (9-11 \times 3,5-5 μ m). R. largentii too has several analagous features to R. flavescens but is a very vivid yellow-orange colour and possesses an unpleasant odour, similar to car tyres, and as such is usually considered inedible.



Ramaria formosa (Pers. : Fr.) Quélet

BASIDIOCARP coralloid, about 150 mm in width and 200 mm in height, usually appears robust, very branched, with wide base, usually wider than it is tall, very distinct, white or whitish, from which fairly thick, erect, cylindrical branches, vivid pink in colour when young, then, slowly becomes, pink-ochre-ish, salmon, ending with a lemon yellow pointed tip, finally concolour with branches. The bifurcation of the branches (saddles) very close to the structure of a U.

HYMENOPHORE not distinct, found on the surface of the branches.

FLESH fairly thick, white, soft, marbled with humidity, brittle when dry, often a little red-brownish at gill edge and when handled; odour is light or insignificant, slightly bitter, sour flavour.

MICROSCOPY: spores cylindrical-ellipsoidal, 9.0-13.0 x 5.0-6.0 μ m, warty, hyaline under microscope; tightly clavate, tetrasporic basidia, with joints at hinges; monomorphic structure, made from septate hyphae, with joints at hinges. Spores are yellow.

HABITAT: grows on the ground in hardwood areas, especially oak; summer-autumn, fairly common a little widespread.

EDIBILITY: toxic

NOTE – This is one of the largest *Ramaria* (carpophores over 300 mm in height have been found!) and can be easily recognised by its three colours and its parallel-shaped branches. It is toxic and provokes gastrointestinal disturbances including strong and continual diarrhoea. *R. neoformosa* has divergent branches, with prevalent bifurcations similar to Var; *R. flavescens*, with which it often shares the same habitat. Its colour makes it the closest species but it has divaricating, scattered branches, mixed, U- and V-shaped saddles and its branch tips are a corn-yellow colour; *R. fagetorum* has a longer, tighter base and prevalently V-shaped angles; *R. cettoi* can be told apart by the dark reddish colour of its branches and its sweet, pleasant odour and flavour.



Ramaria largentii Marr & Stuntz

BASIDIOCARP initially subglobular and with resembling a cauliflower due to the presence of branches, then coralloid, about 150-180 mm in width and in height, made from a solid, but not very developed basal trunk, white or whitish-yellow in colour, from which various columns grow which are then divided ending with one or two short points, yellow-orange, fire orange, sometimes scarlet red in colour.

HYMENOPHORE not individualised, found on the upper half of the branches.

FLESH compact (having a monomorphic hyphal structure), white, with a fairly pronounced odour of tyres or "dental surgery" and sweet-sour flavour

MICROSCOPY: spores ellipsoidal or cylindrical-ellipsoidal, 12-14.5 \times 3.5-5 μ m, with clear apiculture, warty, not amyloid, hyaline-yellow under microscope; claviform, tetrasporic basidia, with basal joint on hinges. Cystidia absent.

HABITAT: on the ground in conifer woods, in summerautumn; fairly common. EDIBILITY: **not edible**

NOTE - Ramaria aurea is similar but forms smaller carpophores with a golden-yellow colour and has branches which spread out from a rather undeveloped base. It fruits on the ground in Fagus woods during the summer-autumn period and produces small spores 9-11 × 3,5-5 μ m, which are covered with partially-united warts. R. flava too has yellowish colouration and has not-very differentiated branches which develop from its basal trunk; it grows in conifer woods and has 10-15 × 4-6 μ m spores which are mostly warty. Other species in this genus have similar colours; their morphological identification can mostly be based on the colour of their branches, but to be sure we must recourse to microscopic analysis, particularly of spore characteristics.



Ramaria pallida (Schaeff.) Ricken

BASIDIOCARP initially like a cauliflower, soon coralloid, up to 200 mm in width, height can reach more than 150 (200) mm, made from a basal trunk which is at most 40 mm in from which various branches grow and are subdivided again, ending in two short points. The trunk is whitish ivory towards the base, with ochre-ish shades elsewhere; branches are yellow or weakly yellowish, sometimes with flesh coloured stains, darker due to the maturation of the spores, fairly often bearing a sinuous pattern.

HYMENOPHORE not distinct, distributed over the upper half of the branches.

FLESH compact, then soft (in a monomorphic hyphal structure), white, with weak or insignificant odour of dry grass, and no distinct flavour.

MICROSCOPY: spores irregularly ellipsoidal, sometimes flattened on one side, 9-12 \times 4.5-5.5 $\mu m,$ with partially joined warts, not amyloid, hyaline-yellow under microscope; claviform, tetrasporic basidia, without basal joints at hinges. Cystidia absent.

HABITAT: on the ground in conifer and mixed woods, in summer to autumn; common. EDIBILITY: toxic

NOTE - The light colour of *R. pallida* is similar to several *Clavulina*, such as *C. rugosa*, which has far-less developed carpophores, with no defined basal trunk and featuring thick, sometimes rough and flattened branches. Furthermore, it has very diverse globose, round and smooth spores 9-13.5 × 7.5-10 μ m, and typically fruits on woodland floors. *R. gracilis* grows in conifer woods and has coralloid, white-ochre carpophores no larger than 60 mm, which have a pinkish colour to them. Its spores, which are ellipsoid and finely verrucose, are noticeably smaller and 5-7 × 3-4 μ m in size. With colours tending to grey-violet is *R. fumigata*, which produces arborescent-coralloid carpophores; fruits in hardwoods forests in summer and autumn and produces 9.5-11.5 × 4-5 μ m spores covered with partially-united warts.



Russula aurea (Persoon)

CAP 50-80 (100) mm, hemispheric or truncatedhemispheric, then convex, finally flat or flat depressed, with obtuse margin, slightly grooved in old age; initially of firm, hard, becoming more fragile during maturity of the carpophore. Surface appears greasy-shiny, viscous with humidity, fairly corrugated, from cinnabar red to fire red, vivid orange, often with extended sulphur yellow zone, sometimes completely yellow; fine cuticle, separable for 1/3 of the radius.

GILLS rounded, subfree, fairly wide, very thin and fragile, fairly crowded; whitish, then pale cream flat appearance, finally also extensively yellow, typically with a yellow surface (these features are rarely evident and often completely absent).

STIPE 35-80 × 12-25 mm, from cylindrical to subfusiform dry corrugated-rugulous, white, not infrequently evident and with fairly sulphuric yellow shades; full, soon becoming filled-medullar.

FLESH fairly hard in young specimens, soon becoming fragile and brittle, almost friable in adults; white,

sometimes yellow for a fairly extended subcuticular area, of flavourful and non distinctive odour.

CHEMICAL REACTION: Guaiac quickly blue greenish. FeSO₄ weak, pink pale ochre-ish.

MICROSCOPY: spore from mostly ellipsoidal to ovoid, 7.5-9.0 \times 6.2-7.5 μ m, warty, partially reticulated. Light yellow in mass.

HABITAT: ubiquitous and mostly widespread; present from summer and fruits until late autumn.

EDIBILITY: edible

NOTE - This species is easy to recognise when it shows its typical characteristics; its colouring, with a yellow surface surrounding its gills, and the fragility of adult specimens' flesh; however, it should be noted that this is a very "capricious" species, which sometimes appears completely yellow, and sometimes does not have the yellow marking around the gills. Among edible species it is, with good reason, considered one of the best, notwithstanding the frail flesh and gills of adult specimens.



Russula cyanoxantha (Schaeffer) Fries

CAP 50-140 mm, fleshy and compact, subglobular with a fairly pointed, gradually expanded summit, finally also depressed, margin is bent at the extremes, subacute; cuticle separable to half of the radius, lubricated-shiny, even greasy in humid weather, from violet lilac to violet blueish, fairly variegated of green, grey-green, sometimes completely cyclamen pink (fo. *lilacea*), completely green olive or pear green (fo. *peltereaui*), eventually dimpled towards the edge (fo. *cutefracta*).

GILLS bent-adnate or a little decurrent, sometimes biforked at insertion, close, fairly crowded, lardaceous, whitish, sometimes fairly clear cream, eventually also stained brown ochre.

STIPE 30-90 \times 15-35 mm, cylindrical, rugulous, completely white or suffused with lilac pink, vaguely greying due to imbibition, eventually a little stained of brown, filled with a compact medulla, then cavernous-filled

FLESH highly compact, almost hard normally lilac under the cuticle, white elsewhere; mild flavour, slightly sensitive odour, in mature specimens, after rubbing, it is possible to experience an unpleasant metallic note, like $\ensuremath{\mathsf{FeSO}_{\mbox{\tiny 4}}}.$

CHEMICAL REACTION: Guaiac strong and rapid. Aniline late on, orange on the gills. FeSO₄ negative, then slowly grey-green.

MICROSCOPY: spores ellipsoidal-oboval or reniform in parts, (6.4) 7.2-9 (9.5) \times 5.8-7 $\mu m,$ warty, hemispheric 0.4-0.5 $\mu m,$ isolated or grouped in part from slightly amyloid connections. Spores are pure white.

HABITAT: ubiquitous species, common from the start of the season under hardwood and conifer, from the mediterranean up to limited tree vegetation.

EDIBILITY: edible

NOTE - According to Bon, the types with cream, elastic instead of waxy gills, a dark violet cap and stipe usually featuring a lilac-pink colouring, belong to an independent species named *R. langei*. Specimens with a subuniform violet-lilac cap (fo. *lilacea*) can seem similar to *R. grisea*, differentiated only by the cream spores, fragile gills, spicy taste and red-orange reaction to FeSO₄.



Russula delica Fries

CAP 60-140 (185) mm, fleshy and firm, hemispheric, then convex, with obtuse umbilical dimple in the background, slowly expanding, eventually deeply infundibulform; margin bent at the extremes, thinned; semiadnate dry and matt cuticle, thorny or a little felted, covered with residues of soil and leaves difficult to separate, whitish at first, soon becoming stained ochre brown, then of rusty brown, starting with the most exposed parts.

GILLS a little decurrent on stipe, falciform-arcuate, subacute at the front, forked in parts, width of 6-9 mm, intercalated from lamellule of diverse lengths, fairly spaced, fragile although rigid, cream, with the whole surface coloured, soon becoming stained rusty brown on the most exposed parts.

STIPE 25-48 × 15-35 mm, hard, highly stocky and robust, flared at the top, or cylindrical subequal, subsmooth or finely corrugated surface, pruinose, white, then stained rusty brown, rarely with glucose belts under the gills, the medulla is compact, only eventually a little springy and wormy.

FLESH very thick, firm and fragile, white, clearly browning on the surface and washed with ochre tones to air after the gill edge; strong and unpleasant odour, like fish or salty, with fruity hints when young, peppery flavour, less on the gills.

CHEMICAL REACTION: Guaiac strong and rapid. ${\rm FeSO_4}$ pale pink.

MICROSCOPY: spores from obvoid to subglobose, 8.5-11.2 \times 7-9 μ m, echinulate, crested-catenulate, partly connected, subreticulate, with obtuse spines; creamwhitish in mass.

HABITAT: very common species under hardwood and conifer, with preference to dry, calcerous ground. EDIBILITY: of no value

NOTE - *R. chloroides* can be distinguished by its funnelshaped, fairly regular cap and its very close and crowded gills. *R. palespora* is a highly characteristic species for its refreshing, amarescent flavour which is never harsh. Its gills are ochre with an orange lustre at maturity, with full, cream spores.



Russula emetica (Schaeff. : Fr.) Persoon

CAP 50-100 (120) mm, fleshy, initially firm in the shape of a helmet, then convex, becoming flat, finally also lightly depressed; with obtuse and joint margin; cuticle is separable up to half of the radius, lubricated and shiny, full vivid red, apple red, arterial blood red, sometimes with blackish shades in the centre or with a sharply demarcated ivory-cream zone.

GILLS rounded or almost free at insertion, from lightly convex to straight, width of 6-9 mm, thin and fragile, finally spaced, sparsely forked, interveined, sometimes whitish, with cream straw tinges very clear view of gill edge; intercalated by sporadic lamellule.

STIPE 50-80 (100) × 10-20 mm, slim, slightly claviform when young, then fairly cylindrical, often lightly thinned at tip, visibly corrugated, white, slightly stained yellowbrown in certain conditions, finely striated and a little satin for the rest; medullar, soon becoming incomplete.

FLESH fragile, a little watery, white, slightly yellowing when handled; peppery flavour in every part, imperceptible or lightly fruity odour at gill edge.

CHEMICAL REACTION: Guaiac, subnul.

MICROSCOPY: spores mostly oboval $8.8\text{-}10.5 \times 7.4\text{-}8.8$ μm , echinulate, with large conical-obtuse warts, sometimes briefly crested, reticulated-connected. Spores are whitish.

HABITAT: under conifers in mountains, mostly *Picea*, but also under birch; with preference to sphagnum or other types of moss.

EDIBILITY: toxic

NOTE - Among similar species, we can distinguish: *R. grisescens* Sphagnicolous, around half the size of *R. emetica*, flesh greying with humidity, positive reaction to Guaiac, spores very much smaller and finely ornate; *R. nanat*ypical of Alpine microsilva. *R. mairei* found in fresh hardwood forests, with a predilection for the Beech has flesh that turns blue on treatment with Guaiac and gills that reveal a glaucous tinge which is inconsistent but characteristic. *R. silvestris* grows in dryer ground on mossy carpets at the bottom of oak, chestnut or pine trees.



Russula foetens Pers. : Fr.

CAP 55-140 (200) mm, fleshy and rigid subglobular, progressively expansive, then flat, grooved-tubercolate margin for 20-30 mm, acute; thorny surface, cuticle is separable for a third of the radius, viscous, persistently shiny when dry, yellow-brown colour, honey like, paler in peripheral area, then bearing brown tawny spots, also tawny blackish in the contused parts.

GILLS rounded-free, partly connate at insertion, width of 8-16 mm, unequal, fairly thick, sparsely forked, not very crowded, interveined, fragile, ivory cream, bearing watery drops in humid weather, with residual rusty stains on gill edge, browning with age.

STIPE $60-120 \times 20-40$ mm, cylindrical, fairly flared at tip, corrugated, rusty at tip, stained brown greying elsewhere, medullar-incomplete for three or four cells soon becoming confluent with wide caverns, the skin is fragile and thick, papered with brownish lumps and soft internally.

FLESH rigid and fragile, whitish on gill edge, not yellowing, very stained soon becoming rusty brown; very clear peppery flavour, unpleasant odour, complex and

difficult to define, with fruity hints in the second level and only to perceptible traits.

CHEMICAL REACTION: Guaiac positive. KOH negative.

MICROSCOPY: spore subglobose, $8-9.8 \times 7-8.2 \mu m$, with flat spines and apex, incompletely amyloid.

HABITAT: gregarious, widespread and abundant, under hardwood and conifers.

EDIBILITY: not edible

NOTE - *R. laurocerasi* can be identified by its average or small stature, the absence of gluten, a strong smell of bitter almonds; its rounded spores, embellished with spectacular winged crests. Also *R. illota* gives off effluence of bitter almonds, though to a lesser degree and mixed with rather less pleasant hints than *R. foetens*. The stipe and the gill edge of its gills display a characteristic blackish-brown series of markings. Its most similar lookalike, however is, *R. subfoetens*, recognizable by its slightly smaller stature, the surface of its cap which is usually without gluten, its yellowing flesh and its positive reaction to KOH.



Russula mustelina Fries

CAP 60-100 (130) mm, very fleshy and hard, globular or with lightly pointed summit, pulvinate, finally distended and a little depressed, with fleshy and obtuse rigid margin, curving at the extremes; cuticle is separable for a third of the radius, thick, tenacious-elastic, appears greasy, shiny, rarely matt or even pruinose, brown, brown honey or chestnut.

GILLS attenuated, then rounded, fairly crowded, with some forking, subacute at the front, nearly straight, width of 5-8 mm, interveined, sublardaceous, straw cream, then stained brown on the surface; some lamellule are present. STIPE 40-100 × 15-35 mm, bulky, from cylindrical to

STIPE 40-100 × 15-35 mm, bulky, from cylindrical to subclavate, clearly corrugated, stained brown and often plicate at base, ivory-cream, then with fairly extensive rusty stains; full, compact medulla, finally cavernous.

FLESH of notable thickness and hardness, firm, white, shades of yellow under the cuticle, clearly washed with ochre brown when exposed to air and with age; sweet flavour and no distinctive odour.

CHEMICAL REACTION: FeSO₄ vivid orange. aniline on the gills, slowly yellow. Guaiac fairly rapid and intense.

MICROSCOPY: spores oboval 7-9.7 × 5.8-7.8 µm, warty, crispate, weakly connected, subreticular. Pale cream ochre-ish in mass.

HABITAT: highly common alpine forests where it grows in abundance and fairly underground; end of summer-autumn.

EDIBILITY: edible

NOTE - An edible mushroom which is widely commercialised thanks to its firm flesh and pleasant flavour. It can be identified by its tawny-brown to honeybrown colouration, evocative of *R. foetens* or *Boletus edulis*, its pale cream spores, and a vivid orange reaction to FeSO₄. It loves to grow fairly buried in temperate periods, from the end of summer to autumn, until the first signs of winter. In some years it can be found abundantly while in others it can still be found, yet far less numerously.



Russula queletii Fries

CAP 40-70 (100) mm fleshy and firm then more fragile, at first convex-subhemispheric, progressively flat, finally mostly depressed, with thinned but obtuse margin, briefly grooved in maturity; cuticle separable to almost half of the radius, wet and shiny collectively, red purple, violet vinous, dark violet, often blackish towards the disc, other times brown violet, partly brownish, streaks of greengrey

GILLS attenuated or lightly rounded, obtuse at the front, interveined, sparsely forked, fairly crowded, width of 4-8 mm, fragile, whitish, then dirty cream, interspersed with infrequent lamellule.

STIPE $30-90 \times 10-20$ mm, cylindrical, sometimes a little fusiform, rugulous, white at base, with red shades elsewhere, carmine under a whitish bloom thickened and fugacious to the touch, often completely white, a little greying due to imbibition, the medulla is compact, then softened and partly incomplete.

FLESH violet under the cuticle, a little greying due to imbibition; intense fruity odour, like mature pears, clearly peppery flavour.

CHEMICAL REACTION: Guaiac positive, slow. FeSO₄ pale pink-orange.

MICROSCOPY: spores ellipsoidal-oboval 7.3-9 (9.8) \times 6-7.3 (8.2) μ m, from echinulate to spinulose, to warty conical-obtuse. Light cream in mass.

HABITAT: highly common in the subalpine area under *Picea* in calcerous non-humid ground, sometimes also under white fir and *Pinus*.

EDIBILITY: toxic

NOTE - *R. cavipes* is smaller and grows under *Abies alba*, and more rarely under Picea, it has a humid and bright cuticle, more widely-spaced gills which are whitish when young, its stipe is never red-violet and its spores are pale cream; Furthermore, it has a subnul reaction to Guaiac testing and a positive, reddening, reaction to ammonia. It has a peppery flavour and an intense, pleasant odour of geraniums. *R. sardonia*, can be identified by its pale, sulphuric gills, insignificant odour and its arenaceous pine habitat



Russula sanguinea (Bulliard) Fries

CAP 30-100 (120) mm, from convex to flat, finally depressed, fine firm margin, curving, regular, smooth, yet slightly striated, briefly, in adult specimens; vivid red, cherry red, carmine red, without violet tones, paler at margin, fading to pale pink, sometimes with white ivory patches; surface rough due to fine granules, dry and matt, lightly viscous and shiny with humidity.

GILLS adnate and fairly decurrent, initially bent, then horizontal or a little ventricular, width of 3 to 10 mm, thick, fairly crowded, sometimes biforked, joined on the background by fine veins, irregularly intercalated from some lamellule; whitish then cream-ochre-ish, fine edge, sometimes coloured red.

STIPE 30-80 \times 10-30 mm, fairly stocky, cylindrical or attenuated at base, rigid, full, eventually filled-cavernous; normally completely suffused with red or red-pinkish, up to almost concolour with cap, on white-ochre-ish background, fairly yellowing starting from the base; surface is finely rugulous-reticulated.

FLESH firm compact, firm, whitish, carmine red under the cuticle, yellowing fairly slowly; weak fruity odour; acrid, hot and also bitter flavour. Slow but positive reaction to Guaiac.

MICROSCOPY: spores pale ochre in mass, oboval with conical-obtuse warts about 1 μ m high, joined in parts with infrequent ridges, 7.8-9.4 \times 6.5-8.2 μ m.

HABİTAT: in conifer woods, principally under pine, fairly common, in summer-autumn.

EDIBILITY: toxic

NOTE - Russula sanguinea, due to its morphochromatic features, is similar to: R. persicina which, however, favours hardwood forests; R. helodes, typical of high mountain bogs and is linked to conifers; R. rhodopus, with its lacquered red cap, and habitat of acidic grounds in red fir forests. In the wild one also comes across some chromatic variants, generally ranked by shape or variety.



Russula vesca Fries

CAP 45-100 (140) mm, fleshy and firm, subglobular, then irregularly flat, eventually depressed, with thinned yet obtuse margin; cuticle is separable for two fifths of the radius, often a little retracted towards the margin, soon becoming dry and matt, pink lilac, brown vinous, sometimes with undefined pale areas, cream flesh colour, occasionally stained green-grey, with streaks darker than the background.

GILLS vaguely decurrent and biforked mostly at the insertion, subacute at the front, crowded and relatively close, delicately interveined, sublardaceous when young, whitish, with rusty stains and finally yellow when handled.

STIPE from subcylindrical to fairly progressively attenuated towards the base, corrugated, rare hints of pink on the side, rusty low down, with some yellow-brown stains, full, then a little filled with age.

FLESH compact, white, clearly yellowing when touched and partly stained brown; completely sweet flavour and indistinctive odour.

CHEMICAL REACTION: FeSO₄ red-orange. Guaiac positive. aniline, yellow.

MICROSCOPY: spores oboval or a little elongated, 6.4-8 × 5.3-5.8 μm, with isolated warts; any links between them are very thin and sporadic. characteristic needle-like forms protrude from rigid and thick cell walls.

HABITAT: highly common in mildly acidic or neutral ground under various types of hardwood and under conifers in mountains; from the late spring.

EDIBILITY: edible

NOTE - R. vesca can be recognised by its slightly lilac or winey-brown flesh, the tendency to turn yellow-brown on its deteriorating parts, its sweet flavour, white spores and a red-orange reaction to FeSO₄. One should guard against giving excessive importance to characteristics which are inconsistent and none too specific: the tendency of the cuticle to retract towards the margin ("habillé trop court" according to a metaphor given by French authors) for example. In the case of greening or partially discoloured samples, the completely sweet flavour, white spores and an energetic reaction to FeSO₄ should help to distinguish this against other, macroscopically similar species of Griseinae.



Sarcosphaera crassa (Santi ex Steudel) Pouzar

[= S. eximia Durieu & Léveillé; S. coronaria (Jacquin) Schroeter]

ASCOCARP made from a subspheric, sessile apothecium. APOTHECIUM initially semi-underground, globular, up to 160 mm in diameter, top is open only for a fairly small operculum (sometimes in a non apical position), then more and more open and protruding from the ground up to appear domed and epigean. Hymenophore is smooth, lightly undulated, initially violet, then darker, tending to turn brown violet. Smooth external surface, white greyish. edge soon becoming cracked, lacianated, with erratic points due to the lacerations on the carpophore during growth.

FLESH fragile, leathery, whitish, thick.

MICROSCOPY: regular ellipsoidal spores, with well rounded extremes, smooth,18-20 \times 7-8 μm , hyaline under microscope, biguttulate, uniseriat in asco; asci are cylindrical, amyloid, octosporic; cylindrical paraphyses with slightly widened tip, septate and forked.

HABITAT: ubiquitous, on the ground among needles, leaves, grass or moss, in humid places; rarely isolated, more often found in large groups, from the spring to summer, rarely in autumn. Quite common.

EDIBILITY: of no value

NOTE - S. crassa has amyloid asci such as the species in the genus Peziza; the genus Sarcosphaera is, however, distinguished from the genus Peziza by its semi-buried growing habits. This prerogative does not really seem a convincing justification for the creation and maintenance of this separation, not least of all because several "true" Peziza species, such as P. ammophila and P. pseudoammophila, fruit almost completely underground in the sand of coastal dunes in autumn.



Suillus granulatus (L.: Fr.) Roussel

CAP 40-120 mm, from hemispheric to convex, then flat; margin from convoluted to curving towards the base, then distended cuticle overflowing on the hymenophore; surface is viscous in humid weather, otherwise slimy, totally separable, smooth and shiny in dry weather; from brown reddish, to tawny, to brown yellowish.

TUBES up to 10 mm, from adnate to weakly decurrent; yellow, then golden yellow and finally yellow-olive at complete sporal maturation; pores are initially small and round, secretes opalescent yellow drops, a little angular in maturation, concolour with tubes, sometimes browning in patches.

STIPE $40-90 \times 10-25$ mm, cylindrical, a little widened at base, sometimes supple or curving; covered with a very minute, pale yellow granulation, sometimes milky, coloured on background and only browning later on; skin is chrome yellow, pale lemon yellow in colour, often brownish patches at base.

FLESH firm when young, then softer; whitish, yellow pale near the tubes and under the skin of the stipe; uniform to the area; weakly fenolic odour, sweetish flavour.

MICROSCOPY: ellipsoidal spores, $7.8-9.1 \times 2.8-3.5 \mu m$, pale yellow under microscope; cuticle of the cap is formed from un gelatinised trichoderma, made from cylindrical hyphae which soon transform becoming cutis. Spores are brown-ochre.

HABITAT: largely widespread species, considered to be highly associated with *Pinus* and to two needles. Fruits mainly in hills and mountains, from summer to late autumn; common.

EDIBILITY: edible

NOTE - This is perhaps the most well-known *Suillus*, very common, and grouped together with similar species by the common name of "pinarolo" in Italy. It might be confused with *S. collinitus*, due to its identical edible properties, but it can be told a part by its colour, which is normally more brown-red, the absence of radial fibrils on its cap cuticle and its more-minute stipe decorations. The spores of *S. granulatus* are also a fair degree smaller as well.



Suillus luteus (L.: Fr.) Roussel

CAP up until 120 (150) mm, initially hemispheric then convex, pulvinate, rarely; margin curving at the extremes towards the base, regular, acute, a little excessive, often decorated with whitish remnants of the partial veil; smooth cuticle, very viscous, separable, brown, yellow brown, brown violet, chocolate brown, often with darker radial fibrils when dry.

TUBES up to 12 mm, adnate or decurrent, 'yellow, then chrome yellow, finally yellow brownish; small, round pores, only angled in advanced maturation, concolour with tubes, uniform when pressed.

STIPE 40-70 \times 12-30 mm, longer than the diameter of the cap when young, then the same length as the diameter of the cap or shorter, cylindrical, often a little widened towards base, full; with a broad membranous annulus, whitish in colour, then violet brown, it is possible to find adherent volviform residues at the base, from whitish to white-grey-violet. A fine yellow lattice can be found above the annulus, below the annulus there are yellow points which then become concolour with cap.

FLESH initially firm soon becoming soft and watery in cap, more fibrous in stipe; white, then yellowing; uniform to gill edge. Pleasant, fruity odour, sweet flavour.

MICROSCOPY: ellipsoidal spores, 7.0-9.2 × 3.0-4.0 µm, pale yellow under microscope. Spores brown olive rusty in colour.

HABITAT: only in groups in *Pinus* woods, recurrent from late summer to late autumn.

EDIBILITY: edible

NOTE - This is a very common *Suillus*, the only one which has an annulus adorning its stipe and which grows with two-fascicle (*P. nigra*, *P. sylvestris*, more rarely anche *P. pinaster*) and three-fascicle (*P. radiata*) *Pinus* trees. Grows tendentially in hills and mountains and is not usually found under coastal pines. As it matures and ages, the cuticle has a tendency to become dehydrated and at that stage it often assumes an appearance very similar to that of *S. collinitus*. Several forms and varieties have been described: fo. *albus*, completely white, and fo. *volvaceus*, with a short stipe whose annulus has the appearance of a volva.



Tricholoma argyraceum (Bull. : Fr.) Gillet

CAP 30-60 mm, a little fleshy, convex campanulate, soon becoming flat, with an obtuse umbo, lightly convoluted, then distended and thinned margin, sometimes cracked. Cuticle is dry, separable, without remnants of veil at the edge, decorated with fine radial fibrils which take the appearance of small scales, grey-whitish when young, then grey.

GILLS non-marginated and decurrent with teeth, fairly crowded, thin and fragile, with numerous lamellule, white with greyish hints, not yellowing with age or when handled, the surface is undulated-crenulated, often a little serrated.

STIPE 35-70 \times 5-20 mm cylindrical, slightly curving, lightly widened at base, white, silky, often with remnants of veil, more visible in the apical part of young examples. FLESH compact then soft in cap, firm in stipe then fibrous, white not yellowing visibly after collection, with strong odour and flavour of fresh flour.

MICROSCOPY: spores 5-6 \times 2.5-3.5 μ m, ellipsoidal, guttulate. clavate, tetrasporic basidia. Epicyte made from parallel, collated hyphae, fairly erect.

HABITAT: rare and late species, grows in small numbers, on broken ground normally near hardwood (hornbeam and hazel).

EDIBILITY: edible

NOTE - This is a species belonging to the Section *Scalpturatum*, and is characterized by its odour and flavour, which recall fresh flour, and by the remnants of veil on its stipe. It differs from *T. scalpturatum* (Fr.) Quél. in its grey-silver cap and its non-yellowing flesh, and can be distinguished from the *Terreum* mushrooms, in that these do not have either a flavour or odour of flour.



Tricholoma equestre (L.: Fr.) Kummer

CAP 50-100 mm, convex or campanulate, then flat and with a large umbo, the cuticle is dry, lightly viscous and shiny with humidity, almost smooth or velvety on circumference in dry conditions, decorated with concentric brass brown scales or often with brown reddish fibrils darker in the centre, the margin is convoluted lengthwise, then distended, lobed and irregular, golden yellow with age.

GILLS non-marginated or subfree, fairly crowded, intense yellow or citrina yellow, tendency to darken with age, the whole surface is lightly undulated.

STIPE 60-90 \times 8-15 mm, subcylindrical or with lightly clavate base, sometimes short and bulging, stocky, bent, concolour with cap, with some sparse brown light reddish floccules towards base.

FLESH ochre-ish-yellowish or brass colour under the cuticle of the cap or in stipe, with a pleasant, lightly floury or a little aromatic odour and sweet floury flavour, bitter if chewed.

MICROSCOPY: spores mostly ellipsoidal or amygdalform, hyaline, 6.0-7.5 \times 3.5-4.5 μm . clavate,

tetrasporic basidia. Epicyte made from fairly erect, parallel interwoven hyphae,

HABITAT: in conifer and hardwood areas where it seems to prefer poplars.

EDIBILITY: suspect

NOTE – This species is fairly variable in its colour. Dry and completely yellow examples are confusable, at first glance, with *T. sulphureum*, which, however has spaced gills, a less intense yellow and unpleasant odour of coal gas. Known and valued as a choice edible up until a few years ago, they are today suspected of having been involved in several cases of poisoning (and even death) after abundant consumption and in undercooked meals; the episodes under investigation all occurred in a certain area in France and today we are awaiting further verification. In any case, as a precautionary measure, and in the absence of certainty, the gathering and consumption of this species (and of all the entities belonging to its immediate group – *Ed.*) is forbidden (by law) throughout the entire Italian and French territories.



Tricholoma portentosum (Fr. : Fr.) Quélet

CAP 60-150 mm, convex, campanulate, with large obtuse umbo, then flat, grey-ochre-ish, dark grey, slate grey, sooty blackish, with greenish or violet stains on a yellow background just visible towards the centre, but stands out at the margin which is often clearly grey- citrina yellow, the cuticle is fibrillated radially, a little viscous and shiny in humid weather, otherwise silky, the edges are slightly supple then lobed, cracked, often revoluted with age.

GILLS non-marginated, a little crowded, fairly wide, slightly thick, sinuate, white, then ash grey with yellowish stains, the edge is irregular and sometimes serrated. STIPE 50-110 \times 8-20 mm, robust, cylindrical or fusiform, satin-fibrillated, whitish, always with yellow stains especially towards the top, the apex is dandruff white and stains yellow-brownish-olive to the touch

FLESH firm in cap and fibrous in stipe, white, a little yellowish in the stipe, fairly greyish below the cuticle of the cap with a pleasant floury odour and taste.

MICROSCOPY: ellipsoidal, hyaline spores, 5.5- 7.0×4.0 - $5.0 \mu m$. Clavate, tetrasporic basidia. Epicyte made from an ixocutis of fairly erect, parallel interwoven hyphae. HABITAT: in conifer and hardwood areas, in autumn even late on.

EDIBILITY: edible

NOTE - When the cap fades, leaving just a glimpse of the yellowish-ochraceous colour, it can be confused with *T. sejunctum*, which, however, has a bitter, floury taste. Also *T. virgatum* has a fibrillated-virgated cap but its flavour no dodur are quite unpleasant. One should pay attention to avoid taking it for the toxic *T. josserandii*, which has a dry, velvety cap, and a characteristic insect-like odour.



Tricholoma terreum (Schaeff. : Fr.) Kummer

CAP 30-90 mm, campanulate, conical, flat, often irregular, obtusely umbonate, matt cuticle, almost smooth or slightly woolly-felted at first, then bearing thick fibrils, almost uniform, smoke grey, dark brown or almost black in colour, the margin is convoluted or curving for a long while, often with an overflowing edge.

GILLS non-marginated-adnate or hooked, slightly crowded, whitish or a little light greying, the surface lightly crenulated with age.

STIPE $30-70 \times 8-12$ mm, cylindrical, stocky, full and fibrous, fragile, hollow or a little fistular with age, smooth, silky, completely white or with light greying fibrils which darken slightly in young specimens.

FLESH fibrous, fragile, white, greyish under the cuticle, with a light fungal odour, and a herby or light floury flavour.

MICROSCOPY: spores mostly ellipsoidal, subglobulose, hyaline, guttulate 6.0-7.5 \times 4.5-5.5 μm . clavate,

tetrasporic basidia. Epicyte made from fairly erect, parallel interwoven, hyphae.

HABITAT: generally abundant in growing areas, found in large groups; in conifer woods (pine or fir), from the end of summer until the first frosts.

EDIBILITY: edible

NOTE - This *Terreum* species iso ne of the most sought-after mushrooms for human consumption and it is popularly known a "Moretta" (little brunette) in Italy. It is often confused with: *T. myomyces* which is smaller and more fragile with remnants of a silvery veil at the tip of its stipe and a floury odour; *T. triste* whose gill surface is darker and which has a stipe with brown-blackish shades; or else it is mixed up with grey-toned species of the *scalpturatum and atrosquamosum* groups, but which have almost no odour, non-wide, white or more or less grey gills, and whose stipe is whitish with no veil; in the field they can be told apart very easily.



Tricholoma ustaloides Romagnesi

CAP 50-120 mm, hemispheric, convex, then a little flat, finally depressed, vivid red-brown, the cuticle is very viscous or glutinous, in dry conditions it tends to stain and discolour towards the margin with shades of ochreorange, the margin is convoluted at the extremes, with very evident ribs.

GILLS hooked, sinuate-adnate, not very crowded, close, white, stained reddish in adult specimens, the surface is a little sinuous.

STIPE $50-120 \times 10-20$ mm, cylindrical or fairly clavate or fusiform full then hollow, white dandruff at tip, stained brown-reddish to from the annular line which is more evident in young specimens, with fibrils coloured or often lighter, a little viscous with humid weather.

FLESH firm white, with strong smell of flour or cucumber and floury flavour.

MICROSCOPY: spores subglobose, suboval hyaline, guttulate 5.5-6.5 \times 4.5-5.0 μm . clavate, tetrasporic

basidia. Epicyte made from an ixotrichoderma of fairly erect, interwoven, parallel hyphae.

HABITAT: under hardwood (oak, chestnut, beech, hornbeam), in autumn; fairly common.

EDIBILITY: not edible

NOTE - This species is characterised by a slimy cuticle that, on drying, tends to leave traces of mucus grouped around the edges. It is often confused with *T. ustale*, which, however has a smooth margin, a stipe devoid of a delimited annulus zone and the flesh of the base of the stipe right up to the gill edge is a reddish-brown. It can also be confused with *T. fracticum* and *T. striatum* with smooth caps bearing radial fibrils and which principally grow under mountain conifers. *T. ustaloides*, however, it can easily be recognised in the field by its flavour and odour.



Tricholomopsis rutilans (Schaeffer: Fr.) Singer

CAP 30-140 mm, conical, hemispheric, then flat, often with obtuse umbo, vinous red, pink-red intense purple on a vivid yellow or golden yellow background, the cuticle bearing adpressed woolly decorations in the centre and small scales which are adpressed gradually and radially towards the edges, the margin is convoluted for a long time.

GILLS from non-marginated to mostly adnate, partly anastomosed, wide, moderately crowded, intense sulphur yellow or golden yellow, the surface is finely floccose or fimbriated.

STIPE $50-120 \times 10-25$ mm, cylindrical or fusiform often sinuous, full, hollow with age, concolour with cap or lighter, decorated with scales, more fleeting towards the base which eventually becomes yellow, the apex is white-yellowish.

FLESH soft, thick in the centre, yellow-cream, with sour mould or light odour, like wood, and sweet light flavour, like hazelnut or a little bitter.

MICROSCOPY: spores mostly ellipsoidal, hyaline, guttulate 7.0-8.5 \times 5.5-6.5 μ m. clavate, tetrasporic basidia. Epicyte made from fairly erect, parallel, interwoven hyphae.

HABITAT: grows collated or in groups on rotting parts of conifers especially fir and pine.

EDIBILITY: of no value

NOTE - This is an unmistakeable species thanks to its lignicole habitat and its yellow gills which contrast with the wine colour of its cap. *T. decora* can be found in the same habitat, but has a lighter cap which bears no winey tones and is generally more slender. *T. flammula* is a very small species, with a cap diameter that reaches only up until 15 mm, purplish-brown fibrillated scales and a yellow stipe; *T. ornata* has a yellow-olivish cap with fairly sparse, intense reddish-brown scales, a pale yellow stipe with fibrils, and grows on woody debris.



Xerocomus subtomentosus (L.: Fr.) Quélet

CAP 50-120 (150) mm, fleshy, from hemispheric to convex, pulvinate, finally flat; edge very soon becoming regularly distended or a little undulated; cuticle is velvety when young, dry, sometimes dimpled; with very variable colourations, from pure yellow, to yellow with citrina tinges, to alutaceous brown with greenish hints, brown orange in dry weather, rusty brown, brown reddish, up to liver red in humid weather. Scratching the cuticle with the finger when it is in its dry state it is possible to observe a rusty Brown subcuticular layer.

TUBES up to 15 mm, adnate and sometimes subdecurrent, chrome yellow, then with greenish hints, finally olivish, slowly turning blue at gill edge; pores are concolour with tubes, round, soon becoming open, then large and angular, turning blue to the touch.

STIPE 50-80 $(100) \times 10$ -20 (25) mm, cylindrical, curving at base, supple, almost always dilated at tip and attenuated at base; very pale yellow, tendency to turn brown slowly during maturity; fine points or lengthwise ribs are often found which form a sort of lattice.

FLESH firm and compact, soon becoming soft in cap and fibrous in stipe, pale chrome yellow, typically ochre at

base of stipe, more evident in humid weather; slowly turns blue at gill edge; weak slightly acidic odour; sweet flavour.

MICROSCOPY: ellipsoidal fusiform spores with superior depression, $10.6\text{-}13.2 \times 4.3\text{-}5.0 \ \mu\text{m}$, pale yellow under microscope, olive brown in mass.

HABITAT: in relatively low numbers, fairly indifferent to substrate, isolated or in small groups; recurrently associated with oak and chestnut; summer-autumn. EDIBILITY: edible

NOTE - This is one of the most noted and common *Xerocomus* species. The chromatic variability of its cap is mainly due to climatic-environmental conditions: examples with a reddish-brown cap are common after it rains, even in the same places where one usually finds carpophores with olive-brown caps. The variants with yellow caps should probably be considered as intraspecial varieties. *X. ferrugineus* is very similar, but prefers to grow on siliceous ground and, generally, at higher altitudes.

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Title: Chemical elements in Ascomycetes and Basidiomycetes – The reference mushrooms as an instrument for investigating bioindication and biodiversity

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Abstract

Fungi in the wild are among the principal agents in biogeochemical cycles; those cycles of matter and energy which enable ecosystems to work.

By investigating the biodiversity of Italian fungal species and concentration levels of chemical elements in them, it may be possible to employ these fungi as biological indicators for the quality of forest, woodland and semi-natural environments. The data archives of EUR Reports record the dry-material concentrations, of 35 chemical elements, including heavy metals, in over 9000 samples of higher mushrooms. These samples represent around 200 genera and a thousand species. As the archive has attained statistical stability it has been possible to define the concept of a "reference mushroom". The use of a "reference mushroom" may bring benefits – perhaps only as a methodological approach – in various fields of mycological and environmental research; from biodiversity and bioindication, through taxonomy right up to health and sanitation issues.

The sheer volume of the collected data may prove to be useful as a comparison for data collected in the future; such results would also allow a better and more-exhaustive interpretation of the effects of environmental-protection laws which have been enacted over the years in order to reduce or remedy current climate-change phenomena and the environmental damage caused by human activity. Studies pertaining to the frequency of occurrence and the ecology of the various fungal species found on Italian soil have tended to link the reference habitats used to European classification guidelines (Natura 2000, CORINE Land Cover, CORINE Biotopes and EUNIS). Thereby the foundations have been lain for the use of mushrooms as biological indicators for the measurement of soil and ecosystem quality.

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