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A.M.B.
Associazione Micologica Bresadola

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Centro Studi Micologici

5th INTERNATIONAL MEETING

MYCOTOXICOLOGY
(5° C.I.M.T.)

Mushrooms and health:
Public health, clinical, regulatory and control issues related to trade globalization

Abstract

Milan, Italy, 3-4 December 2012

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Provincia di Milano

Centro Antiveleni
Azienda Ospedaliera
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PREFACE

Emi Morroni,
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The "Wild Fungi Special Project" managed by Nature Defence Department of ISPRA promotes and carries out different studies on fungal species, with the major aim to understand their role as biological indicators of environmental quality.
The main thrust of this project is the development of an information system that collects mycotoxicology data, including information on bioaccumulation of heavy metals and xenobiotics in fungi. These data can then be used to plan bioremediation projects in polluted sites, on the one hand, and on the other to carry out studies on potential health-related problems linked to mushroom consumption.
The leading role of ISPRA in mycotoxicology has been evident in the 5th CIMT, during which the most recent and important, international, scientific mycotoxicological data have been presented. Contrary to previous CIMT events, however, the program of the 5th CIMT has included not only anthropocentric aspects of classical mycotoxicology but other mycological issues such as mycotherapy, environmental toxicology and the use of fungi in bioindication and bioremediation.
This is a new vision of mycotoxicological and mycological aspects that ISPRA has been continuously developing during the last ten years; it has been accepted and used with success in this last CIMT, in which it has shown to be a novel approach to mycotoxicology not only in Italy but also abroad. It is certainly a big step forward in a more rational and scientific approach of mycology, as discussed in more detail in the Press Release of the congress.
During the 5th CIMT participants have suggested to improve information on mycotoxicology by educating both scientists and the population on the most relevant mycotoxicological aspects, in the attempt to reduce drastically mushroom poisoning and to improve synergies in the monitoring and census activities carried out by the Operative Units of ISPRA's "Wild Fungi Special Project" that aim to provide a realistic picture of the health status of Italian ecosystems. This publication by ISPRA is the answer to this suggestion.

THE IMPORTANCE OF A CORRECT INFORMATION ON FUNGI AND THEIR PROPERTIES

Novo Umberto Maerna
Vice-president and Culture assessor
Province of Milano

The Milano Province Administration is very proud and honoured to have hosted on its premises the 5th International Congress of Mycotoxicology, having Fungi and Health as the main theme, organised in collaboration with the Associazione Micologica Bresadola, the Fondazione Centro Studi Micologici and the Centro Antiveleni Niguarda Cà Granda.
We believe it is crucial and very important that the state institutions support and foster the efforts by medical and scientific researchers aiming at providing the public with serious and reliable, yet clear and understandable information.
During this congress, physicians and mycologists from all over the world have given an important contribution to mycotoxicology research, thus increasing the scientific knowledge and information on the potential risks that toxic fungi may represent for the population and contributing to the prevention of mushroom poisoning.
A correct scientific information on mushrooms and their properties is crucial to protect consumers, be it when collecting or when buying and eventually consuming mushrooms. Health hazards are not linked only to toxic fungi: edible mushrooms may also cause health problems if they are collected in contaminated sites, are not properly conserved or cooked inadequately.
Fungi, on the other hand, are extremely important also for the ecosystem and its conservation. The Milano Province Administration, in a synergic collaboration with local associations, is fostering since several years projects aiming at increasing the knowledge of mushroom taxonomy and ecology, in an effort to prevent mushroom poisoning through a targeted information of the population.
A good knowledge of mycological issues is of paramount importance, however, also to protect and conserve the natural and agricultural environment that is so characteristic of the Lombardy; this knowledge should be a part of the general culture that is due to our population.

PRESS RELEASE

5th International Meeting of Mycotoxicology

The numerous and often fatal mushroom poisoning cases registered in 2012 in Italy must prompt national and regional health authorities, environmental research agencies and mycological societies to consider means to set up, coordinate, structure and enhance activities towards a better information among the population aiming at preventing mushroom poisoning.
This is the main message of the round table discussion held as a conclusion of the four scientific sessions of the 5th International Congress of Mycotoxicology (5th CIMT), which took place in Milan on 3-4 December 2012, in the Oberdan Facilities of the Milan Province Administration.
The event, titled "*Mushrooms and health: Public health, clinical, regulatory and control issues related to trade globalization*" and organised by the Mycotoxicology Committee of the Associazione Micologica Bresadola, with the collaboration of the Milan Province Administration and the Poison centre of the Niguarda Hospital (CAV) in Milan, was supported by the Health Ministry, the Environment Ministry (MATTM) and the Environmental Research Agency (ISPRA). This important congress, the fifth in a series of which the last was held in Trento in 2007, dealt with a number of topics, including clinical and modern aspects of mycotoxicology, prevention and control activities by Italian health agencies, consequences of globalization in commercial aspects of mycology, and activities and tasks of the food and health control agencies.
The congress was well attended and the survey carried out by the congress organisers "Alfa Quality" underlined the overall satisfaction of the attendees, among them many health operators, who were also able to earn up to 16 CME credits during the event.

The clinical **mycotoxicology session** dealt mainly with epidemiological and diagnostic issues.
The epidemiology of mushroom poisoning in Italy is still largely unknown, because no mushroom poisoning registry is available at the national level. The CAV Niguarda presented the data available at their hospital, cautioning the audience, however, that the data are limited to a very small part of Italy and not generalizable; in particular, nothing or little is known on the problems arising from the ingestion of mushrooms that have not been checked for their edibility by a professional. Nevertheless, the cases shown reveal the gravity of the situation, with a large number of fatalities and organ transplants needed after mushroom poisoning.
Mushroom poisoning requires the concerted action by the emergency room physician, the toxicologists, the mycologist and the lab technicians in order to provide the best diagnosis and treatment. The session highlighted the profile of these professionals and gave advice on how to improve their education, interaction and activities.
Diagnostic tools discussed included details on the biosynthesis of amanitin and the use of molecular biology methods to detect it, and screening methods that could be used to rapidly detect and identify amanitin in biological samples, thus allowing a rapid therapeutic treatment aiming at decreasing the number of transplant and eventually of fatalities after amanitin poisoning. Several talks by international specialists have highlighted the ongoing research activities on this topic, discussing poisoning cases after ingestion of species of *Amanita* and linking them conclusively with renal insufficiency.
As for the therapy, decontamination with carbon and fluid replacement are still the methods of choice to treat amanitin poisoning, because more effective therapies are still lacking.

The second session – **modern aspects of mycotoxicology** – discussed new aspects of mycological research that are tightly connected with mycotoxicology, i.e. mycotherapy, environmental toxicology and bioremediation.
It is important to approach mycotherapy carefully, evaluating its pros and cons and considering not only the activities described in vitro but also the clinical and toxicological development carried out for each product. Good pre-clinical studies, followed by a sound clinical development, must show the safety and efficacy of all products to be used for the treatment of human and animal diseases. The importance of fungi in medicine is beyond any doubt, but their use should be firmly supported by an evidence-based medical approach.
With regards to bioindication and bioremediation, research has now shown that mushrooms have a clear role in the environment. The human being, interacting closely with the environment and often altering the delicate balance between the different players and variables in it, needs to be aware of this and consider mushrooms in the planning and execution of activities that may influence the environment.
The results of this session are a significant contribution towards a new vision of mycology, and the organisers are proud to have reached this objective in the 5th CIMT. This success has been possible thanks to the great work and enthusiasm by the members of the "Progetto Speciale Funghi" (Fungi Special Project) by ISPRA, who in the last 10 years have worked synergistically with AMB and other research institutes, integrating taxonomy with ecology and other scientific disciplines to achieve a more holistic knowledge of mycotoxicology.

A session was also dedicated to **prevention and control activities of the Italian health agencies and the problems arising from the commercial globalisation**. This important session highlighted the problems linked to the safety of consumers and related to the consumption of mushrooms and mushroom containing products available on the market. Internationally known experts have described all kinds of contaminants present in mushroom products, from biologicals such as the larvae of diptera or arthropods, to bioactive molecules, including nicotin and botulin. With regards to the latter, recommendations on how to deal with mushroom containing products in order to avoid contaminations by *Clostridium botulinus* have been given. An important outcome of the discussion was the need for clear European guidelines on the maximum allowed levels of contaminants in all types of commercial food containing mushrooms.

The **activities of Italian control and inspection agencies** was the topic dealt with in the last session. Here representatives of several Italian agencies, including the Ufficio di Sanità Marittima ed Aerea (USMAF), the Protocollo Operativo del Corpo Forestale dello Stato (CFS) per la Regione Campania and the Nucleo Antisofisticazioni e Sanità (NAS) dell'Arma dei Carabinieri have presented their activities. The issues liked to the import of mushrooms and mushroom products, the collection and marketing of hypogeous and epigeous mushrooms on the territory, and the control of marketed products containing mushrooms have shown that a close collaboration of these agencies with a mycological association such as the AMB is crucial to improve the efficacy of the actions undertaken by the agencies, in particular also by helping in the continuous professional education of the officers active in this field.

Round table on "methods to collect data and to produce better and more complete reports on mushroom poisoning, by compiling information derived from different procedures and data sources".
The participants have unanimously acknowledged the progress made in several Italian regions by the mycological control agencies, the health authorities and other associations, including the certification of mushrooms to be commercialised, the prevention activities aiming at a better control of mushrooms used for private consumption, the role of the surveillance offices (USMAF) in the control of import products, as well as the establishment of expert mycological control centres. These activities, however, are not well established over the whole Italian territory, and each region (and not all!) has its own organisation. Clear procedures, protocols and guidelines are still lacking and each agency is having its own operational instructions. The interactions between emergency room physician and the other operators on the territory are not or at most ill-defined. Most

inspection and control offices do not regularly collect information on cases and prepare and distribute reports on the activities carried out and the information is rarely transmitted to the authorities.

Mushrooms are regularly consumed by people, therefore their safety is regulated by the Italian law (Reg. CE n.178/2002). All local and national agencies, including DGSAN (Health Ministry), should work together to create a common database on mushroom poisoning, similar to or integrated in the Rapid Alert System for Food and Feed (RASFF) system. The experts feel that a modification of the guidelines on the commercialisation of fresh and conserved mushrooms is needed, as is the revision of the DM 686/96 directive that defines the education needed for a mycologist to be accepted as a controller, to be compatible with the existing EU directives and to provide a better and more complete mycological education. As a conclusion, the experts voice the need for a better national information policy for the prevention of mushroom poisoning, by promoting collaborative projects among national and regional health authorities, the administration bodies of the regions, the poisoning centres, the veterinary and environmental institution (e.g. ISPRA), and all mycological associations and organisation, such as the Associazione Micologica Bresadola.

OFFICIAL REPORTS

1ST SESSION – CLINICAL TOXICOLOGY

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Session Scientific Committee: F. Assisi, A. Granziero, C. Papetti

RELATIONSHIP AMONG TOXICOLOGIST, MYCOLOGIST AND LABORATORY IN THE DIAGNOSIS OF POISONING BY MUSHROOMS

ASSISI F., BALESTRERI S., VERZOLLA M., FOLLESA P., MASARIN A., MORO P.A.

Clinical picture and severity of mushroom poisoning depends on the type of toxin ingested. Identification of species involved, through mycological examination and urinary amanitin analysis, is essential for an appropriate management of the poisoning. For these reason the toxicologist of the Poison Control Centre (PCC) cooperates together with the mycologist and the laboratorist to make a correct diagnosis, especially if an amatoxin intoxication is suspected. After having evaluated the clinical picture and the time elapsed between ingestion of mushrooms and onset of symptoms, the toxicologist suggests the first line treatment of the poisoned patients and gives advice to the emergency room physicians about mycological examination and urinary amanitin analysis. The proper use of the procedures for activation of these three professionals by the emergency room physician makes effective and efficient therapeutic action with the health of the patient as ultimate goal. In the present paper will be discussed the validity of interventions, based on the more than fifty years practical experience in mushrooms poisoning management of the PCC of Milan.

LIGHTS AND SHADOWS OF URINARY AMANITIN DOSAGE IN LABORATORY PRACTICE

MASARIN A., GECHTMAN C.

Until some years ago there were very few laboratories (in Lombardy, but more in general in Italy) able to execute the dosage of Amanitine in urine samples. The only company, that was able to supply the material needed to perform such analysis, used a radioactive element, the Iodine 125, as a marker. In order to use such radioimmunologic method, the laboratory had to present very particular structural requirements. Furthermore the laboratory had to identify a "Zone under surveillance" where to execute such a dosage, perform a dosimetric control in the room where the reagents were used, and it was indispensable to have a dedicated instrumentation. Also the time of decay of this radioisotope was a limiting factor in the use of this method, which implied a constant monitoring of the stocks availability. With the introduction of the ELISA test, the number of laboratories able to perform such dosage is grown. Another positive aspect was the longer lifetime of the marker. Contextually new problems appeared in relation to the use of this new method, those questions will be discussed during the report.

LIGHTS AND SHADOWS OF URINARY AMANITIN DOSAGE IN CLINICAL PRACTICE

ASSISI F., MUSELLA G., GAVIRAGHI S., MASARIN A., SEVERGNINI P., MORO P.A.

Identification of ingested species is essential in the management of mushroom poisoning, expecially if consumption of an amatoxin-containing species is suspected. In this case, urinary amanitin ELISA analysis appears as an essential diagnostic tool; nevertheless, predictive and diagnostic value of the level of amanitin detected is still uncertain. The producer declares 0,22 ng/ml as analytical sensitivity and 1,5 ng/ml as functional sensitivity of the assay. In the year 2010, the Milan Poison Control Centre (MPCC) carried out a study to accountability of urinary amanitin analysis. Urine samples were collected from 45 patients out of 60 diagnosed as amatoxin poisoning. It is known that test reliability depends on time elapsed between consumption of mushrooms and urine collection; the test is considered unreliable for samples collected after 36 hours since ingestion. On the other hand, the report of patients (six cases in our study) showing amanitin urinary level > 10 ng/ml and no liver enzyme increase, puts into question the specificity of the test. For these reasons, the dosage of amatoxin must always be interpreted in the light of the clinical history of the subject and supported by the advice of an expert toxicologist.

Poisoning by mushrooms: ASL Milano report and mycological problems

Verzolla M., Casa R., Gentili G., Callegari L., Vivarelli S., Giometti A.

In the report "Poisoning by mushrooms: ASL Milano report and mycological problems", are described the main problems encountered daily by mycologists in-call service in the management of episodes of intoxication caused by the consumption of mushrooms. The presentation, expresses the real operational difficulties in carrying out the intervention in the emergency room (called Level 1) and subsequent analysis, if necessary, in the laboratory (level 2). It is also highlighted the complexity of the organization of an "on-call service" covering a very long period, from 1 August to 30 November - 24 h 24, but unfortunately guaranteed by a small number of operators, often called to work in emergency situations. Then the data of poisoning from 2004 to 2012 are briefly analyzed with short comment on the data presented. The cornerstone of the report is the projection of photographs taken during surgery, describing, as a demonstration, the difficulties and problems that most frequently are encountered during the course of the intervention (emergency access, collection of epidemiological data, sample management - from 'gastric aspirates residues of cleaning mushrooms, subsequent analysis in the laboratory). Then suggestions for improving the service are proposed (eg.: to indicate in the record, the referent emergency doctor with his contacts data, to actively look for residues of cleaning mushrooms, to deliver in the shortest possible time the sample, etc..) Finally to improve collaboration among those who work in the emergency department and who perform the analysis. The presentation has a practical, positive, point of view, based on the experience of speakers for several years ealing of poisoning by mushrooms and their problems.

THE LIVER AND POISONING AMATOXINS

LANGER M., ASSISI F.

Poisoning by cytotoxic mushrooms (*Amanita phalloides* and related species) is associated with severe morbidity and a high mortality rate. Due to the difficulty of performing controlled studies, there is considerable debate about appropriate treatment, particularly the feasibility and the efficacy of detoxification. Amtoxins produce necrosis of eukaryotic cells by binding to nuclear RNA polymerase B and inhibiting enzymatic activity. Although amatoxins are equally toxic to all cells, in human poisoning they exhibit an apparent specific toxicity to intestinal epithelium, hepatocytes, and possibly to the kidneys. Amatoxins have been evaluated by radioimmunoassay in the serum of poisoned patients up to 36 hours from ingestion (1975) with values of few ng/mL. Because the toxins are eliminated through bile and urine, higher concentrations than in serum have been found in gastric juice and urine. The clinical course with amatoxin poisoning is characterized by intense vomiting and diarrhea leading to severe dehydration after a latency period of 6 to 18 hours. Dehydration may cause by itself early renal failure. Liver cell necrosis becomes evident about 36 hours after intoxication and transaminases reach peak values at about 60 hours. The prolongation of the prothrombin time and the decrease of the liver-dependent clotting factors is slightly delayed in time. (from: Therapy of cytotoxic mushroom intoxication. Vesconi et al, 1985)

AMANITA POISONINGS RESULTING IN ACUTE, REVERSIBLE RENAL FAILURE: NEW CASES, NEW TOXIC *AMANITA* MUSHROOMS

KIRCHMAIR M., CARRILHO P., PFAB R., HABERL B., FELGUEIRAS J., CARVALHO F., CARDOSO J., MELO I., VINHAS J., NEUHAUSER S.

Background.
Renal failure as a consequence of eating mushrooms has been reported repeatedly after ingestion of webcaps of the *Cortinarius orellanus* group. But mushrooms of the genus *Amanita* can also cause renal failure: *Amanita smithiana* (NorthAmerica) and *Amanita proxima* (Mediterranean area). Here we discuss poisonings caused by otherwhite amanitas. A German and – independently - two Portuguese patients reported the ingestion of completely white mushrooms with ring. Similar to intoxications with *A. smithiana* or *A. proxima*, the clinical icturewas characterized by nausea and vomiting 10-12 h after ingestion, severe acute renal failure and mild hepatitis. Renal biopsy showed acute interstitial nephritis and tubular necrosis. Two patients were given temporary haemodialysis. All have fully recovered their renal function. Poisonings caused by mushrooms containing the toxin of *A. smithiana* were suspected. We tested 20 *Amanita* species for the presence of this toxin. Methods. Thin layer chromatography was applied to detect *A. smithiana* nephrotoxin in herbarium specimens using authentic material of *A. smithiana* as reference.
Results.
A. smithiana toxin could be detected in *Amanita boudieri*, *Amanita gracilior* and in *Amanita echinocephala*. *A. boudieri* was collected by the Portuguese patients. *A. echinocephala* is the only nephrotoxic *Amanita* growing North of the Alps and is suspected to be the cause of renal failure in the German patient. No *A. smithiana* toxin was detectable in the nephrotoxic *A. proxima*.
Conclusions.
A. boudieri, *A. gracilior* and *A. echinocephala* are nephrotoxic. These intoxications are clinically similar to that of *A. smithiana*, with acute reversible renal failure and mild hepatitis but are different in their clinical picture from Orellanus syndrome characterized by a delayed onset of severe and often irreversible renal failure.
2012 are briefly analyzed with short comment on the data presented. The cornerstone of the report is the projection of photographs taken during surgery, describing, as a demonstration, the difficulties and problems that most frequently are encountered during the course of the intervention (emergency access, collection of epidemiological data, sample management - from 'gastric aspirates residues of cleaning mushrooms, subsequent analysis in the laboratory). Then suggestions for improving the service are proposed (eg.: to indicate in the record, the referent emergency doctor with his contacts data, to actively look for residues of cleaning mushrooms, to deliver in the shortest possible time the sample, etc..) Finally to improve collaboration among those who work in the emergency department and who perform the analysis. The presentation has a practical, positive, point of view, based on the experience of speakers for several yearsealing of poisoning by mushrooms and their problems.

BIOSYNTHESIS OF CYCLIC PEPTIDE TOXINS OF LETHAL *AMANITA* MUSHROOMS

JONATHAN D. WALTON, HONG LUO, SUNG YONG HONG

The vast majority of fatal mushroom poisonings are due to the amatoxins such as α-amanitin. Amanitins are found in species in section Phalloideae of the genus *Amanita* and also in some species in the genera *Galerina* and *Lepiota*. The amatoxins are bicyclic octapeptides. Many of the *Amanita* species also make the chemically related phallotoxins, which are bicyclic heptapeptides. These cyclic peptides are synthesized on ribosomes as proproteins and not by nonribosomal peptide synthetases, like all other known fungal cyclic peptides. The proproteins are 34 (for amatoxins) or 35 (for phallotoxins) amino acids in length. The first step in processing is cleavage at flanking conserved pro residues to release a linear octa-or heptapeptide. The enzyme that cleaves the proproteins is a prolyl oligopeptidase (POP), a type of serine protease. Toxin-producing species of the *Amanita* species have two POP genes. Whereas POPA is present in all species of *Amanita*, POPB is present only in toxin-producing species (sect. Phalloideae). *Amanita bisporigera*, which grows in North America and produces amatoxins and phallotoxins, has a large family of genes related to those for α-amanitin (AMA1) and phallacidin (PHA1).This "MSDIN" family is characterized by highly conserved amino acid sequences flanking a hypervariable "toxin" region. In addition to potential toxins of 7 or 8 amino acids, others are predicted to encode nonapeptides and decapeptides. It appears that *Amanita* mushrooms have evolved a combinatorial mechanism that can theoretically generate millions of different cyclic peptides. Some nontoxic cyclic peptides have been previously described from *A. phalloides*, including antamanide (a cyclic decapeptide). The small brown mushroom *Galerina marginata*, which grows in Europe and North America, also makes α-amanitin (but not phallotoxins). The full genome of *G. marginata* was sequenced by the U.S. Department of Energy Joint Genome Institute (http://genome.jgi-psf.org/Galma1/Galma1.home.html). G. marginata contains two genes for α-amanitin and no extended "MSDIN" family. The α-amanitin proprotein from *G. marginata* is only weakly conserved compared to that of A. bisporigera. Whereas the proprotein for α-amanitin in A. bisporigera has the sequence MSDINATRLPIWGIGCNPCVGDDVTTLLTRGEALC, the proprotein for α-amanitin from *G. marginata* is MFDTNSTRLPIWGIGCNPWTAEHVDQTLVSGNDIC. (Conserved amino acids are single-underlined; toxin regions corresponding to the amino acid sequence of α-amantin are double-underlined).

MOLECULAR METHOD FOR ASSESSING THE PRESENCE OF ALPHA AMANITIN IN THE GENUS *AMANITA*

EPIS S., C. MATINATO, D. SASSERA

The continued and increased use of mushrooms in the culinary field is often cause of human intoxications. Every year numerous cases of poisoning or human death are reported, due mainly to the complete superficiality of amateur seekers of fungi who, unfortunately, consume them without serious mycological control not realizing the real danger. This common problem is preventable or anyway containable if diagnosed as early as possible. Here, we present an overview of the possible application of molecular methods in order to support the work of mycologists. In recent years the use of wild mushrooms in culinary is constantly growing, thanks to the nutritional and commercial value. In Europe, most mushroom species are edible, while less than 100 are known to be poisonous, and approximately 35 species contain amatoxins. For this reason, ingestion of mushrooms of unknown origin and of dubious edibility, can be a serious health risk: some of them are not only toxic but very poisonous if eaten and can be lethal. For example, amatoxin poisoning is a worldwide problem; approximately 50· 100 fatal cases are reported every year, most in Europe and North America but also in Africa, Asia and Australia. In general, the identification of fungi is a very laborious and knowledge intensive task; in fact, the morphological identification of mushrooms requires specialized mycologists. Furthermore, the rapid identification of poisonous mushroom species is required for proper medical treatment, but the morphological characteristics essential for the identification are often not well preserved and thus unclear for a rapid identification (spores are few and their morphology generally is altered due to the environmental conditions). If a morphological analysis of mushrooms is inconclusive, a tool not dependent on morphology may be required to verify the cause of mushroom poisoning. Real-time PCR is a useful method for identifying fungi independently from morphology. Mushrooms are highly appreciated in culinary and they are also used in the production of a wide variety of food products. Often it happens that the more expensive mushrooms, such as white truffles, are subjected to fraud; in particular, Chinese truffles tend to be milder and less fragrant than Europe truffles and are the most commonly used in truffle fraud. Real-time PCR can thus be used for food traceability and fraud discovery relative to human consume of edible mushrooms. The present review paper reports the recent applications of molecular techniques (DNA-based identification, which do not depend on morphology) in the identification of cooked mushroom species in different matrices such as food products or gastric aspirates in order to offer a tool to integrate the work of the mycologists. In order to detect the mushrooms in the processed products, different authors set up specific quantitative PCR protocols using the amplification of the rDNA ITS region (the internal transcribed spacer region in the nuclear ribosomal repeat unit). These real-time PCR approaches represent fast, effective methods enabling the quantification of a gene based on the detection of fluorescent signals emitted during the exponential phase of DNA amplification. Omphalotus japonicus,

Entoloma rhodopolius, *Clitocybe* acromelalga and Tricholoma ustale are poisonous mushrooms responsible for approximately 85% of the mushroom poisoning events in Japan. Maeta and colleagues (2008) propose species-specific identification of these mushrooms, cooked and fresh, using a rapid system of detection consisting of four primer pairs, one of each of the target species. To validate their method, they used fresh fruiting bodies and 3-cm square pieces of baked, stir-fried, tempura, deep-fried mushrooms. They obtained, for each primer set, species-specific detection in all analysed samples with a real-time PCR detection in less than 1 h. This was the first study able to obtain detection of fungal DNA in various cooked preparations using a molecular protocol based on DNA analysis; the real-time PCR has proven to be most suitable method for species identification in all types of products independently from the processes to which these have been subjected. Epis and colleagues (2010) demonstrated that th real-time PCR technique can be used also for the identification of poisonous mushrooms in cases of clinical poisoning, supporting and integrating the morphological work of the mycologist. In particular, they proposed a method to detect the DNA of fungi species not only in cooked mushrooms, but also in mixed food preparations and gastric aspirates. This approach considers that the mycologist can often have difficulty in identifying the morphological structure of the spores in cooked food or gastric aspirates, where they are generally altered. In Italy, during the decade of 1996· 2006, about 10,000 cases of mushroom poisoning were reported and 15% of these cases were caused by mushrooms of the genera *Lepiota*, *Amanita* and *Inocybe*, often mistaken for edible fungi (data recorded at the Mycology Section, Laboratorio di Sanita Pubblica of Milano, Italy). Epis and colleagues propose four primer sets, each specific for one of the following toxic mushroom species: *Amanita* phalloides, Lepiota cristata, Lepiota brunneoincarnata and Inocybe asterospora. All the proposed protocols were highly specific and sensitive enough to detect as low as 32 ng of dried samples of the four species; this paper thus confirms that the molecular detection of fungal DNA integrated with the mycologist work can be a potential tool for the poisoning diagnosis. One other application of molecular detection trough specific real-time PCR is the use of molecular techniques to identify truffle species in intense processed commercial products. Recently, Rizzello et al. (2012) proposed two real-time PCR protocols to identify and quantify the prized white and black truffles, in order to protect the consumer, opening the way towards automated monitoring of these products on the market. *Tuber magnatum* Pico, the Iba white truffle, and *Tuber melanosporum* Vittad, the igord black truffle, are in fact highly appreciated despite the prices, which are tipically around $4000 per kilogram. Due to this high price, whit and black truffles are often replaced with less aromatic species. For this reason, the authors propose these molecular methods to detect fraudulent practices and to protect the consumers of truffle delicacies. Specifically, they propose two specific real-time PCR primer sets to authenticate and quantify the two truffles *T. magnatum* Pico and *T. melanosporum* Vittad in different food matrices, such as cream, oil, and butter-based products. This is another application for the traceability of mushrooms in food in order to help the work of the mycologists and to authenticate the quality of the foods. Real-time PCR methods have proved to be useful for identifying mushroom species independently from the morphological approach. Real-time PCR is a method characterized by quick turnaround time, efficiency, specificity, sensitivity and is applicable to different

matrices. Here, we presented an overview of potential applications of this technique to the mycological world, however, many other important applications could be developed to support the mycologists' work on poisoning and frauds. In particular, mainly in the poisoning intoxications field, it is essential to be fast, specific and sensitive in order to interact with the hospital for proper medical treatment. Future developments of the molecular approaches applied to mushrooms identification can now be envisioned. Here we propose the use of a recent technique, the BioMark dynamic array (Fluidigm, South San Francisco); this innovative real-time PCR instrument. To develop an experiment, 48 samples and 48 assays are loaded into the connections of the Dynamic Array input frame and pressure loaded into 2,304 reaction chambers. The BioMark dynamic array can be used to detect 48 poisoning mushrooms in up to 48 DNA samples in a single run (e.g. unknown mushrooms, foods or gastric aspirate DNAs) using only few nanoliters of extracted DNA for real-time PCR amplification. This application could represent a novel standard to investigate the potential presence of fungi responsible for intoxications, evaluating different target species in a very short timespan. In addition, we can focus our analysis on amatoxin identification using specific real-time PCR protocol in order to identify the potential lethal intoxication of patients. The specific PCR product of AMA1 gene, the gene that encode alpha-amanitin, was ligated into the pGEM-T Easy vector and cloned into *Escherichia coli* DH-5α competent cells according to the manufacturer instructions.Threshold cycle (Tc) obtained from real time PCR were plotted in order to obtain an estimation of gene copy numbers for each sample. This real time PCR protocol exhibits a number of features that make it a useful diagnostic tool. Both these novel approaches are currently in progress.

2ND SESSION – MYCOTOXICOLOGY: NEW PERSPECTIVES

Chairmen: L. Cocchi, O. Petrini

Session Scientific Committee: L. Cocchi, G. Consiglio, O. Petrini

MYCOTHERAPY BETWEEN REALITY AND PHANTASY: WHAT CAN FUNGI DO FOR OUR HEALTH?

PETRINI O.

Since Fleming's discovery in 1928 of penicillin, an antibiotic produced mainly by *Penicillium* species, fungi have taken up an important role in medicine. The pharmaceutical industry has immediately started screening fungi for drug production and particularly after 1960 substances of fungal origin have been investigated for their pharmacological activities. Screening programs have soon shown that fungi are excellent producers of pharmacologically active compounds. Noteworthy examples are lovastatin, isolated from *Monascus purpureus* and *Aspergillus terreus*, cyclosporin A, produced by *Tolypocladium inflatum*, the cephalosporin antibiotics derived from some *Acremonium* species, or paclitaxel, a substance produced by some fungal endophytes of *Taxus*, most notably *T. brevifolia*, and widely used in the treatment of some cancer forms. Almost daily the interested reader can found a large number of papers on the pharmacological activity of substances of fungal origin. The activity of compounds isolated from *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida* and *Pleurotus pulmonarius* against cancerous cells are worth mentioning. But this does not mean, unfortunately, that these substances will soon be available for use in conventional medicine. The new drug development is long and difficult and most often its results are negative. Only a small percentage of new, sometimes very promising drugs end with a marketable product. In fact, many drugs that show in vitro activities fail to do so in vivo as well; in addition, many active compounds are simply too toxic for human or animal use. Last but not least, their galenical formulation can be plainly impossible and preclude any pharmaceutical development. The traditional Chinese medicine (TCM) and Ayurveda are prime examples of the use of fungal products in the treatment of ailments. For example, the ascomycete Cordyceps sinensis is widely used in TCM and in the Tibetan medicine to treat almost 20 different diseases, from asthenia to tuberculosis and prostate cancer. The basidiomycete Ganoderma lucidum ("Reishi" or "Lingzhi" in TCM) is considered to be active in the treatment of cardiovascular problems: its use, however, is by no means restricted to this, but the fungus is considered to be beneficial in the treatment of pain, psychic disorders and sleep problems, as well as an immunostimulant (mainly because of the terpenoids and polysaccharides it contains) or even anticancer agent. The clinical evidence for all these activities, unfortunately, is scant if not completely lacking – and this applies also to most of the other fungal uses described in the Oriental medicine. It is also almost impossible to transfer the knowledge collected in the Asian traditional medicine to the Western medical practice, because the medical approach to diagnose or treat a disease is completely different in the two cultures. The use of "mycotherapy" in the western medicine is possible and appropriate only after a careful clinical and toxicological development. The safety profile of a drug needs to be thoroughly investigated before a product can be approved for human or animal use. Beneficial drug effects are mostly accompanied by adverse effects, as shown by the "red rice", obtained by the fermentation of rice by the ascomycete

Monascus purpureus and used as a nutraceutical as a replacement of (or adjunct to) statins to control hypercholesterolemia. The lovastatin contained in the red rice may, in fact, cause severe adverse effects such as rhabdomyolysis, as known for other statins. It is thus important to approach mycotherapy carefully, evaluating its pros and cons and considering not only the activities described in vitro but also the clinical and toxicological development carried out for each drug. Good pre-clinical studies, followed by a sound clinical development, must show the safety and efficacy of all products to be used for the treatment of human and animal diseases. The pharmaceutical industry has since a long time recognised the importance of fungi in medicine, but their use should be firmly supported by an evidence-based medical approach.

MICOTHERAPY: CLINICAL RESEARCH

CALAPAI G.

Interest for pharmacological effects and consequently for potential therapeutic properties of mushrooms is increasing. This is interest supported by the discovering that several compounds originating from mushrooms show to have different biological activities such as immunomodulatory, antitumoral and antimicrobial action. On these basis it has become urgent the need to confirm the potential beneficial effects of mushrooms through the evaluation of their effects on human. Clinical studies are the main instrument that we have to demonstrate that pharmacological activities of substances can be transformed in successful therapies. Clinical studies are designed to collect data on efficacy and safety of natural or synthetic substances that we want to use as medicinal. The main aim of clinical research is to obtain valid results showing efficacy against human pathologies affecting general population. First of all, on the basis of the experience with clinical studies on medicinal plants, is fundamental the characterization of the product investigated for clinical effects. In consequence, it is necessary to know what is the part used if it is not the whole mushroom, method of extraction and solvent used and its concentration. These are examples derived from herbal medicines but more peculiar issues could be necessary to investigate on micotherapy. Different degrees of evidence can be obtained according to the methodology used in clinical studies. The form of clinical research that is more suitable to have valid data representing the highest degree of evidence is certainly the randomized and controlled clinical study in which the results obtained with the studied substance are compared with those obtained with placebo and in which substance or placebo are given through the double blind administration. According to this methodology, patients are divided in two groups by randomization (casual distribution of patients in the groups), one receiving the substance examined and the other with a formulation apparently similar but not containing the substance examined (placebo). To increase the value of results, treatments)substance and placebo) can be successively exchanged (crossover). The fact that mushrooms are natural substance does not to have induce to short or simplify the hard road map of clinical research. So, it is important to avoid common errors that limit the value of results such as: reduced number of patients, not randomization, do not use placebo, not adequate statistics or endpoints or outcomes having poor clinical value (for example, excessive use of patients self-evaluation scales). In conclusion, if we want results from clinical research on potential therapeutic effects of mushrooms we should have in mind that insufficient methodology produce consequently not useful results. The consequence of conducting clinical studies without the necessary rigorous scientific approach are generally over or under-evaluation of effects of the treatments. It should be not a good service for micotherapy.

FUNGI AS BIOINDICATORS OF XENOBIOTICS IN THE ENVIRONMENT

SINISCALCO C., COCCHI L., BENEDETTI A., JACOMINI C., PETRINI O., VESCOVI L. & BARBI L.

During the last twenty years bio-indicators, namely living organisms, have been increasingly used for environmental monitoring, especially to detect xenobiotic substances potentially harmful to human, animal and environmental health, such as mosses for air pollution, some molluscs for water pollution, earthworms or micro-organisms for soil pollution. On the other hand, the use of higher fungi for environmental monitoring is much more recent, it's based on the fundamental role of fungi as major agents of biogeochemical cycles and of cycles of matter and energy that regulate the functioning of ecosystems. Many studies, in fact, show the correspondence of methodologies based on the ability of mycorrhiza to infect as a determining factor in the diagnosis of environmental pollution, but much more recent studies demonstrate their ability in bioaccumulating xenobiotics elements in carpophore, thereby making them ineffective against plants. In this context, it has been developed a monitoring and data collection project that produced a European Report based on more than 9000 samples of higher fungi representing over 200 genera and that shows the concentrations of 35 chemical elements. This study permitted to identify criteria and methodologies for the identification of indicators of environmental toxicology. In particular, it was possible to define the concept of "mushroom of reference", only for basidiomycetes and ascomycetes, because of the statistical stability achieved with a large number of observations and a subsequent statistical analysis made using "multi-dimensional-scaling ". The mushroom of reference can be used in environmental toxicology to define differences and anomalies in the samples analyzed and it can therefore be useful to detect variations for the same xenobiota accumulated in different environments, as well as for taxonomical studies. In the present work some examples will be provided.

FUNGI AND BIOREMEDIATION: EXPERIENCE IN THE FIELD

SINISCALCO C.TA, LAZZARI A., FAVERO-LONGO S.E., PEROTTO S. , GIRLANDA M.

Despite their potential in bioremediation, mycelial fungi are rarely the biological agents of choice for the decontamination of polluted soils. A rhizoremediation field experiment was set up within an area featuring a mixed soil contamination (by heavy metals and aliphatic and aromatic hydrocarbons). Poplar plants were introduced alone or in combination with a fungal consortium consisting of autochthonous strains. The fungal treatment induced significant reductions in soil levels of both organic and metal pollutants. Decrease in pollutant concentrations correlated with bioaugmentation of the reintroduced fungal species and shifts in the diversity of non-reintroduced, indigenous fungal assemblages. These findings suggest that effective bioremediation may result from direct and/or indirect effects of the fungal inoculum.

SPECIEGRAPHY OF FUNGI CONSIDERED IN THE 1ST AND 2ND SESSION AND CONSIDERATIONS ON ALIEN SPECIES

CONSIGLIO G.

Not available.

3RD SESSION – FUNGI AND HUMAN FEED

Chairmen: C. Siniscalco, S. Borrello

Session Scientific Committee: C. Papetti, C. Siniscalco, O. Tani

FOOD SECURITY OF MUSHROOMS IN TRADE, PROCESSING AND CONSUMPTION

BORRELLO S.

The European Regulations 852 and 882/2004 laid down general rules for all food, including mushrooms.
In addition to the European regulations, general and specific provisions apply to the marketing and processing of mushrooms: Law 283/62, Decree 327/80, Decree 376/95, Ministerial Decree 686/96 and Law 352/93.
Horizontal legislation applies to all stages of production (cultivation or harvesting of mushrooms), processing (drying, cutting, packaging, etc. …) distribution and export. The primary production for the mycological sector is represented by: Mushrooms cultivation or harvesting of wild mushrooms - Operations in the farm or in the site of collection that do not substantially modify the features of the raw mushroom - transport to a processing plant. The Ministry of Health with two specific notes in 2011 and 2012 gave clarifications to improve the activity of official control on mushroom sector and presented the leaflet "The Mushrooms: A Guide to the prevention of poisoning" that aims to inform clearly the healthcare professionals and the consumers of the dangers that toxic, poisonous or fatal species of mushrooms may cause.

ARE THE MYCETOPHILIDAE MAGGOTS A HAZARD/RISK FOR CONSUMER'S HEALTH?

FERRINI A.M., BARLETTA B., DI FELICE G., BIANCHI R., KHOUY C., AURELI P.

In this work, some aspects of the presence of dead larvae of mycetophilidae in dried edible mushrooms are considered with respect to the relevance of the assessment as "hazard".
Risk characterization is discussed in the perspective of a possible establishment of a tolerable level of contamination for dead larvae in this product.

FUNGICOLOUS ARTHROPODS: PRESENCE, FREQUENCE AND IMPACT IN WILD MUSHROOMS (FRESH, DRIED AND OTHERWISE PRESERVED) DESTINED FOR HUMAN CONSUMPTION

SÜSS L., SITTA N.

Mycophagy, among the many interactions between arthropods and fungi, has definitely the greatest impact on the commerce and food consumption of edible mushrooms. Insects are the most important taxon among fungicolous arthropods, in particular for the order Diptera which feed, only at the larval stage, on fleshy, edible (and also economically important) mushrooms species. A natural hierarchy of preferences or palatability exists, by which certain fungal species are much more likely to be consumed by arthropods than others. Nearly all mushroom species which are economically important (including truffles) have spore dispersal mechanisms involving mycophagous insects and, except for Cantharellus cibarius s.l., are remarkably prone to be attacked by fungivorous arthropods. The presence of some species of fungicolous arthropods which are not strictly fungivorous, but are predators of other arthropods, can occur in edible mushrooms and in some cases it can be quite frequent. In dried and otherwise preserved mushrooms destined for human consumption, all the arthropods that are present in fresh mushrooms are dead at the end of preservation procedures; a different phenomenon is the entomofauna that lives in dried foodstuff (i.e., post-drying infestations, which in dried mushrooms are due mainly to Lepidoptera). Thousands of tons of wild edible mushrooms imported in Italy for the domestic market and for re-exportation are mainly porcini. Preservation processes are performed in the supplying countries, and consequently mushrooms destined for the Italian market (except for fresh mushrooms trade) are imported already dried, frozen, brined and orherwise preserved. Mushroom batches are built up in the supplying countries, after a first phase of processing and sorting which aims to reach merceological uniformity. On the contrary, uniformity of geographical origin or season is usually lacking within batches. In Italy, processing operations, which currently occur on already preserved mushrooms, include several controls, and a manual selection, based on characteristics that can be detected macroscopically. Such a selection allows to eliminate foreign bodies, foreign species and deteriorated mushooms units. Examination of parasitological data available in the literature on overall 260 mushroom samples, reveals that only one sample (0,4%) was found without dipteran larvae, and 37,3% of the samples contained a number of fungivorous dipteran larvae between 51 and 150 (referred to the standard quantity of mushrooms of 10 g dry weight or 100 g fresh weight). A separate parasitological analysis carried out on a higher number of porcini mushroom samples show similar data, with the most significant number of samples containing between 51 and 150 fungivorous dipteran larvae. Interestingly, there is a similarity between the number of dipteran larvae found in samples of dried porcini in commerce (in compliance with mycological control and after manual selection) and those found in samples of production waste from the very same selection. Evaluating the presence of arthropods in mushrooms by counting the number of insects per

unit weight is feasible only by close inspection of relatively small quantities of mushrooms under a stereomicroscope, after defreezing or rehydrating mushrooms, with the mere purpose of checking the extent of insect contamination. The methodology is a filth-test adapted to mushrooms and does not consider macroscopic parameters such as the different modifications caused by arthropods in the fungal tissues, which on the contrary are duly assessed during mushroom control and processing. For example, mushroom units which present only holes caused by the passage of dipteran larvae in fresh fungal tissue are judged fit for human consumption and tolerated by some regulations, included the Italian regulation currently in force (DPR 376/95 art. 5: "tramiti di larve di ditteri micetofilidi", i.e. "mushroom units riddled with dipteran larvae holes"). On the contrary, deteriorated mushroom units, which show evident alterations in colour and/or tissue structure by macroscopic control, are unfit for human consumption and represent the most frequent reason for the dried porcini mushrooms to be non-compliant. The presence of deteriorated units is more rare in frozen and otherwise preserved mushrooms, but other arthropods that usually leave the sliced fruiting bodies during the drying process (for example springtails, ants and larvae of Elaterid beetles) are more common. Clearly, mushrooms species destined for human consumption are a foodstuff that always includes in itself the presence of fungicolous arthropods, of different typologies and in various numbers, even without traces visible at the naked eye. It is also evident that fungicolous arthropods (at least dipteran larvae, springtails and the other most frequent taxa) are completely "edible". They do not carry of pathogenic microorganisms, because usually they do not move from mushrooms or in any case they do not feed on dung or decomposing material (except for Anoplotrupes stercorosus). They do not produce benzoquinones and other toxic substances as darkling beetles (Coleoptera, Tenebrionidae) do. When considering allergic phenomena that are sometimes associated with mushroom ingestion, they are probably due more to mushrooms themselves than to their content in dipteran larvae and other "parasites". It is worth noting that the shiitake (Lentinula edodes), despite being reported as responsible for allergy in consumers more frequently than other mushrooms, is by far one of the species that is less attacked by arthropods. However, the presence of arthropods in foodstuff can create a sense of disgust in consumers in western countries, where traditions of entomophagy are lacking. Taking into consideration only the parameter "presence of arthropods", which is constant in porcini and nearly all the other edible and valuable mushroom species, makes all these wild edible mushrooms "unsafe foodstuffs" (Reg. CE 178/02) because they are considered to be unfit for human consumption. On the contrary, considering other parameters, such as size and visibility of the arthropods, or the presence of deterioration of fungal tissues caused by the "parasites", the whole category of wild mushrooms can continue to be destined for human consumption. In this case, only wild mushroom samples or batches that can cause a problem to the consumer (i.e., sense of disgust in seeing the "worms" or use of a deteriorated foodstuff) will have to be declared unfit and unsafe foodstuffs. The definition of these cases, requested since 2003 from the Italian Ministry of Health, could be clarified with the researche that is currently being carried out at the National Institute of Health.

EPIDEMIOLOGY OF DISEASES POTENTIALLY RELATED TO MYCOTOXINS [1]

BRERA C.

The distribution of mycotoxins in food products may be as high as 25%; therefore, these toxins may contaminate the diet of a large part of the population and thus become a rather widespread health problem, which is, however, still largely underestimated and not solved. The impact of mycotoxins on human health is probably not the same in different world regions, in some of which it can be particularly severe. In many developing, low-income countries, mycotoxins are consumed in high amounts and continuously in the general diet, which is often composed prevalently by cereals. In industrialised countriesconsumers may also be exposed chronically to mycotoxins, but thanks to rather restrictive health regulations to a minor extent and in general to low amounts. In the last few years, epidemiological studies have been increasingly carried out also in developing countries, thanks to the widespread use of now available biomarkers. Among the most important results, one should mention the proven role of aflatoxin B1 (AFB1) on the development of liver cancer, in particular in hepatitis B or C infected individuals. An interesting correlation has been observed between AFB1 and growth as well as AFB1 and immunomodulation. With regards to fumonisin B1, several epidemiological studies carried out in South Africa have shown a striking correlation between fumonisin B1 contaminated maize and oesophageal cancer (EC). This correlation is now being considered in Italy as well. Several biomarkers have been developed for fumonisin B1, but the techniques still need to be improved: the use of the recently discovered 1-deoxyfinganin seems to hold some promises. Epidemiological studies have also been carried out for other mycotoxins such as ochratoxin A and deoxynivalenol. In the industrialised countries the laws regulating the maximum tolerable levels, which are based on strict toxicology studies and safety margins, should help in setting up prevention measures. On the other hand, no epidemiological studies have so far been carried out to demonstrate the real impact of mycotoxins present in foodstuff on human health. For instance, only recently have studies been done to show the impact of mycotoxins on the foetus: there are first indications on the effects of the consumption of high amounts of fumonisin B1 contaminated maize by the mother on the formation of neural tube defects and other studies have shown that AF1 may pass the placental barrier. No studies, however, are available on effects of mycotoxins on the immune system or on the growth.

[1] The English version of the abstract has been prepared by the Scientific Committee

BIOACTIVE MOLECULES IN FUNGI ALSO IN RELATION TO ENVIRONMENTAL AND CONSERVATION FACTORS [2]

GUERZONI M.E.

The medicinal properties of many species of mushrooms and their extracts have been known for millennia, particularly in Asia and the Americas, and many species have become essential components of traditional medicine in many countries. In fact, at least 270 species of fungi have been shown to have therapeutic properties. The names under which some mushrooms have been called in different cultures in all continents attests an ancient and unbroken knowledge of functional properties. The extraordinary development of instrumental chemistry allowed the identification of numerous active compounds, belonging to different chemical families, with multiple nutritional and functional activities. In particular have been identified polysaccharides as beta glucans, peptidoglucani, antioxidant molecules such as carotenoids, lycopene, ascorbic acid together with phenols and flavonoids with also antimicrobial activities. Therefore many edible mushrooms have become an increasingly attractive source of molecole with specific activity on human health demonstrated through the determination of specific metabolic markers such as the level of cholesterol, triglycerides and markers of oxidative stress.

[2] The English version of the abstract has been prepared by the editors

STORAGE OF MUSHROOMS: SANITARY PROBLEMS (BOTOX AND BACTERIOLOGICAL CONTAMINATION)

FINAZZI G., DAMINELLI P., BERTASI B., LOSIO M.N.

Botulism is a severe foodborne disease that can lead to the death of those affected. In this paper are described the main characteristics of the causative agent, the epidemiology, the pathogenesis and symptoms of intoxication. In the paper are also described the analytical methods used for the laboratories diagnosis applied on food samples and on biological samples from suffering patients.

NICOTINE IN WILD MUSHROOMS. RAPID DETERMINATION BY QUECHERS AND LC/MS/MS TECHNIQUE

SANTILIO A.

(S)-3-(1-methylpyrrolidin-2-yl)pyridine (nicotine) is the predominant component of the crude alkaloid extract. It is used as insecticide to control of aphids, thrips, whitefly and other insects on glasshouse ornamentals and on crops including fruit, vines and vegetables. Nicotine has a wide distribution in various edible vegetables included the nightshades family (Solanaceae) among them are some common vegetables such as potatoes, tomatoes or eggplants (aubergines). Several authors have described analytical methods concerning the determination of nicotine in food material. These methods include liquid-liquid extraction and clean up step to eliminate interfering compounds followed gas chromatography /mass Spectrometry and Liquid Chromatography /mass spectrometry. In European Countries, the use of plant protection product containing Nicotine has been phased out by June 2010 but its use in Third Countries may continue and may lead to residues of Nicotine in food. During the years 2008/2009, nicotine was detected in dried wild mushrooms. The levels found in wild mushrooms (mainly Boletus edulis) were higher than 0.01 mg/kg on a fresh weight basis (maximum residue level, MRL, set by Article 18.1.b of Regulation (EC) No 396/2005). Residue levels was founded up to 9.9 mg/kg on the dried product. The European Commission launched a monitoring and testing programme at the start of the forthcoming 2009 harvest season. According to the EFSA opinion, a MRL of 0.04 mg/kg for fresh wild mushrooms has been established. In addition, a value of 2.3 mg/kg for dried wild mushrooms (Boletus edulis) was established as safe value for consumers and for other dried wild ceps a level of 1.2 mg/kg was considered safe for human health. As a result of the European Commission request for monitoring the level of nicotine on mushrooms, the European Reference Laboratory for single residue methods (EURL-SRM) developed a single method for the determination of nicotine on mushrooms by LC/MS/MS. As for the determination of Nicotine in mushrooms no papers have been reported, the Italian National Reference Laboratory for Single Residues (NRL-SRM) studied the performance of the method proposed by CRL-SRM for the determination of nicotine levels in both dried and fresh mushrooms. A procedure based on the QuEChERS methodology and on Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) has been adopted, using Nicotine–d3 as internal standard. The performance of the method was studied according to the Document SANCO/10684/2009. Limit of quantification was 0.01 mg/kg for both fresh and dried mushrooms. Calibration curve was linear over the concentration range of 0.01-2.3 mg/mL, with r2 >0.99. As for recoveries in dried mushrooms, spiking levels of 0.01 mg/kg, 0.02 mg/kg, 0.32 mg/kg and 2 mg/kg were considered, whereas for fresh mushrooms recoveries were determined at 0.01 mg/kg, 0.02 mg/kg, 0.036 mg/kg and 0.36 mg/kg. Satisfactory results were obtained in either matrix: recoveries proved to range from 97 to 178%, with a relative standard deviation (%RSD) of 7 - 29%. The technique LC/MS/MS is suitable for the

determination of Nicotine in mushrooms at 0.01 mg/kg level for both fresh and dried mushrooms. The method was applied to the analysis of Nicotine to assess the levels of nicotine in fresh and dried mushrooms.

4TH SESSION – CONTROLS AND RELATED NORMATIVE

Chairmen: D. Monteleone, O. Tani

Session Scientific Committee: E. Borghi, K. Kob, D. Monteleone

THE MUSHROOMS: LEGAL INTERESTS PROTECTED, REAL BULWARKS FOR THE PROTECTION OF FOREST BIODIVERSITY. THE OPERATING PROTOCOL ON MYCOLOGY DRAWN UP BY THE ITALIAN FOREST RANGER

CURTO M.D., TREZZA G., OTTAVIANO N.

Today, especially in Italy, the mushroom industry is more important than some years ago, because the culture, the customer sensibility, the knowledge and the approach toward this particular food have changed. Many studies have deepened the knowledge of mushrooms, underlying above all how the mycology world, the macromycetes in particular, is always more resourceful, more suitable to new fields of application and more surprising. Over the years, the "sensiblerie" toward the mushroom world has changed, thanks to the well-known and valued ecosystem role, which considers mushrooms as authentic goods protected by law and bulwarks for the protection of forest biodiversity. In fact, when we often think about forests, we refer to several vegetable species that characterize it, but we don't pay attention to other living organisms that are essential for the ecosystem balance: the mushrooms. As a result, the ecological role of mushrooms is very important for life on earth: without them, the higher plants could not feed themselves and grow up. For this reason, the life of living beings would be in danger, because without plants, the process of photosynthesis, which is necessary for the production of oxygen for life on earth, would fail. Therefore, the mycodiversity, such as the mushroom complex typology, form and ecosystem, is very important. In some way, epigeal and hypogeal (truffles) fungi are a poor resource, because they are irreplaceable by ecological, food, economic and tourist valence. So, the legal guardianship of mycodiversity requires the application of industry standards that necessarily lead to a related control action through the supervision of the Italian Forest Ranger. Mycology has always caught attention, charm and curiosity for remarkable and different interdisciplinary fields it concerns. The mycological information and education have been often born out by close examinations about biology, morphology, systematics and some aspects related to toxicity and edibility of mushrooms. There was not a book which deepened in detail the perspective aspects linked to inspections on mycology, especially locally for the Region of Campania. The Operating Protocol titled "The supervision in the field of gathering and marketing of epigeal and hypogeal mushrooms in the Region of Campania" has the claim and the aim to fill in this gap, suiting the needs of both gatherers and controllers, in order to clarify interpretative doubts arose by general, local and regional rules. It provides a practical and functional manual which can be interesting for all the different Italian regional realities. Moreover, this Operating Protocol takes the opportunity to underline how the inspective controls on mycology, related to the marketing and the distribution of epigeous and hypogeous fungi, are set in a widen and more complex scenario of tests for food security. Developed by the Mycology Inspectorate in the AA.SS.LL, besides the common functions of police force, these tests locally provides the centrality of their role in range of preventive

and repressive controls and public health. Therefore, the purpose of this manual, made by the shared synergies of the institutions and non-institutions involved, is the elaboration of a valuable means of work for responsible of mycological controls. It includes some functional forms (patterns of minutes and a breach handbook), synoptic tables of procedural research and mycological profiles to let the topic interesting giving an excellent vademecum for mushroom pickers, as well. Among the operative and interpretative in-depth analysis, the Operating Protocol deals with specific contents, such as: how to gather and where (private or public place), the differences between qualification and authorization to gather, forfeiture and administrative and penal seizure, stable or itinerant fungi marketing, drawing a check-list for truffle gathering controls comparing it to other rising scenarios of control. Moreover, the synoptic tables summarise the type of fungi marketing and define for each of them an illustrative modus operandi for inspective tests with a clear reference to the various rules which regulate this field. From its publication on intranet, the Operating Protocol has given to the Italian Forest Ranger a valuable mean of work that gets easier and nationally more homogeneous the controls, thanks to suitable forms. The Italian Forest Ranger keeps controlling the epigeous and hypogeous fungi selling and marketing in the national country. Concerning this contest, it's important to cite the complex inspective research of November 2011 to March 2012 called Operation Por-Cina: it interested different regions and different departments of the Italian Forest Ranger that sometimes were inspired by this Operating Protocol.

BEST PRACTICES OF CONTROLS OF FEED AND FOOD OF FON ANIMAL ORIGIN [3]

MONTELEONE D.

The health checks at the border for food of non-animal origin and materials in contact with food are carried out by the Offices for Sea, Air and Border Healthcare (Uffici di Sanità Marittima, Aerea e di Frontiera - USMAF). USMAF are offices of the Ministry of Health.

[3] The English version of the abstract has been prepared by the editors

PROBLEMS ARISING IN THE VERIFICATION STAGE OF THE PRODUCTION, MARKETING AND SALE OF MUSHROOMS [4]

BELGI P.

The Units of Carabinieri for the Health Protection (Carabinieri NAS) are the functional dependance of the Minister of Health: these military departments have qualifications and powers of Sanitary Inspectors for prevention regarding Health, Pharmacy, Doping, Food Safety, Safety products, during day and night, in all those places where there is production, administration, storage or sale of products intended for human consumption.

[4] The English version of the abstract has been prepared by the editors

FREE REPORTS

1ST SESSION – CLINICAL TOXICOLOGY

NEUROTOXICITY CAUSED BY MUSHROOMS CLASSIFIED AS EDIBLE

ASSISI F., GILIOTTI B., DAVANZO F., BISSOLI M., PANZAVOLTA G., REBUTTI I., STELLA A., MUSELLA G., MORO P.A.

Mushrooms' ingestion causes mainly gastroenteric symptoms but in a large number of patients were founded central and peripheral neurological diseases. Poison Control Center of Milan performed a retrospective study (2004-2011) checking 756 patients that, after ingestion of edible and not edible mushrooms, showed neurological symptoms with different seriousness, ranging from simple headache and weakness, to seizure and coma. Most of cases with neurological symptoms, analyzed by a toxicologist, were caused by ingestion of unclassified mushrooms consistents with species liables of the typical syndromes. Neurological diseases, such as lipothymia, drowsiness, paresthesia, headache, dizziness etc etc, happened in 19% of intoxication in patients that ate only mushrooms classified as edible and no other causes were recognized.

THE MYCOLOGICAL DEPARTMENTS IN THE VENETO REGION

PAOLO DI PIAZZA

This research was realized in 2008 as a graduation thesis about "The mycological departments in the city of Padua and in the Veneto region" in which we intended to underline the competence of the Mycologist working in the Public Agency in the Veneto Region. For this purpose a questionnaire was given to all the representatives of the Departments and Mycological Services of the U.S.S.L. Agency of the Region. The purpose of the questionnaire, made up of a logical series of questions, was to collect all the basic data to give a profile of the mycological service in the different U.S.S.L. Agencies of the Veneto Region as the micologists ways of working vary from Agency to Agency.
The results of the questionnaire were developped and analyzed to provide new ideas and operating suggestions for the improvement of the mycological services in the Region. Nowadays the Mycologist requires more and more specialistic competences. They have showed a training need, generic as well as specialistic, inside the Region. They have also showed the need to get regional laws with operating procedures to address the organisation and the purposes of the Mycological Departments and the duties assigned to the Mycologists.

First experience of mycological advice service for hospitals in the district of Pesaro Urbino province

Falasconi M.

In 2009 ASUR Marche approved a two-year plan for the years 2009-2010 introducing a round-the-clock mycological advice service for hospitals of the region to be carried out by mycological inspectors on call-in shifts.
In the District No. 1, corresponding with Pesaro Urbino province, this service was carried out by 6 mycologists who worked shifts for the seven hospitals of the district. A total of 21 actions were performed – 1 in 2009 and 20 in 2010 and more specifically 7 in the hospital of Pesaro and 7 in the hospital of Urbino, 4 in the hospital of Sassocorvaro, 2 in the hospital of Fano and 1 at ARPAM in Pesaro (this one requested by NAS, the Carabinieri office against the adulteration of foodstuffs). Tests were mainly carried out on the available remnants (whole sporocarps; parts of sporocarp; cooked and frozen mushrooms; prepared foods; cleaning remnants). Failing these, spores were searched for in vomit and/or gastric aspirate. Nearly all actions were performed in a short period of time, namely the first 15 days of October 2010, in which period an exceptional mushroom growth took place. In all cases the mushrooms responsible for actual or presumed poisoning were identified. In only one case was the object not achieved, because material was totally lacking and the patient was hospitalized after 4 days (gastric aspirate unusable). In this case, the diagnosis was made only on a clinical basis. The identified mushrooms turned out to belong to both to poisonous and edible mushrooms, though eaten in a improper way. The following species and/or genera were identified: 5 *Russula olivacea*, 3 *Omphalotus olearius* and *Agaricus* sp., 2 *Agrocybe aegerita*, 1 *Entoloma sinuatum*, *Hygrocybe conica*, *Armillaria mellea*, *Macrolepiota procera*, *Polyporus varius*, *Xerocomus* sp., *Boletus* edulis. The cases examined show that poisoning mostly resulted from mushrooms picked by private individuals (85%), while only few cases resulted from mushrooms given as a present (10%) or from those purchased (5%).

MUSHROOM POISONING: NOVEL OR RECENT SYNDROMES REPORTED IN THE INTERNATIONAL LITERATURE

FRANCHINA P., BERNARDELLO F., FRANCHINA G.

Recently a new syndrome related to *Podostroma cornu damae* mushroom consumption has been reported in China. 13 cases of poisoning related to this mushroom have been observed with 2 death. The initial symptoms included diarrhea, vomiting and dehydration; 3 days after anuria, low blood pressure, polypnea and consciousness disturbances arose. In the described cases, if not adequately treated, a multiple organ failure led to death. Typical clinical symptoms of *Podostroma cornu damae* poisoning were palms desquamation, alopecia, leukopenia, and thrombocytopenia. Another syndrome has been reported in Japan with 20 cases and 8 deaths related to the *Russula subnigricans* ingestion. At an early stage the patients complained nausea, vomiting, abdominal pain and diarrhea; after 2 or 3 days the patients developed severe rhabdomyolysis and myoglobinuria followed by renal failure and multiple organ failure. A new syndrome, defined as "Yunnan sudden death syndrome", has been recently reported in China; the syndrome is related to the consumption of the Trogia venenata, a little mushroom belonging to the Marasmiaceae family. The syndrome is characterized by sudden cardiac arrest and 400 deaths have been reported. In the hours before the death the patients developed nausea, seizures, tachycardia, asthenia. It is believed that the toxins responsible for the "Yunnan syndrome" could be 2 extremely toxic amino acids, but some Chinese and American toxicologists believe that the syndrome may be related to a barium poisoning, found in large amounts in drinking water in the Yunnan province and in the soil where this mushroom grows. A further toxic syndrome, described in Japan and Europe, is a toxicoderma linked to the consumption of raw or undercooked mushroom *Lentinula edodes*; the poisoning, called "flagellar dermatitis", is characterized by the appearance of skin linear lesions in individuals who had eaten the mushroom in large quantities or raw. These new syndromes are in addition to the recent ones reported in the last decade: rhabdomyolysis following consumption of the *Tricholoma equestre*, reported in France and Poland, acromelalgic syndrome due to the ingestion of the *Clitocybe amoenolens*, described in France and Italy, and cryptogenic encephalopathy linked to the *Pleurocybella porrigens* observed in Japan.

THE MYSTERIOUS *AMANITA OVOIDEA*

MARTELLI L., NICOLARDI V., MIRALDI E., BIAGI M., PERINI C.

It is widely known that the genus *Amanita* includes various species: edible, inedible, poisonous and sometimes even deadly. Despite the number of studies carried out on the genus *Amanita*, the toxicity of some species, like the group of white *Amanita*, remains uncertain. The current study examines a case of *Amanita ovoidea* poisoning, maybe due to allenic norleucine. In 2000 a patient was hospitalized in Siena after ingesting this mushroom with gastrointestinal problems at the beginning, followed by an acute renal failure. The experimental steps carried out are the following: preliminary phytochemical screening, extraction, isolation, identification and quantification of the toxin, toxicology samples on human kidney cell cultures, analyses of soil pollution and mushroom pollution by heavy metals, analysis of possible interactions between toxins, heavy metals and human physiology.

TRAINING: USEFUL TOOL FOR REDUCING RISK OF POISONING FROM MUSHROOMS

DURANTE A., RIZZI R., SCHIFONE C., TRIA M., PALMISANO M., PERNIOLA G., ALTAVILLA G., D'ORIA G.

Objectives: the objective of this study was to evaluate if after the institution of the Mycological Centre ASL Taranto there has been a decline in the epidemiological data of poisoning by mushrooms.
Materials and Methods: we took into consideration the number of poisonings observed from 1999 to 2011 as well as the number of people trained for the harvest and for the sale. Mushroom species related to intoxication were identified and the annual incidence of epidemiological data referred to poisoning in relation to trainees was studied.
Results: in 99% of cases, mushroom poisoning is caused not only by toxic mushrooms but also by edible mushrooms, with symptoms affecting the gastrointestinal system - nausea, vomiting, abdominal cramps, diarrhea, solved thanks to the expert advice of mycologists intervened on request of the PP.OO. and the symptomatic treatment administered by different hospitals; only in the 1% of cases, caused by mushrooms containing lethal substances (in this case the mycological center was not called for advice) resulted in the death of the subject.
Cases of poisoning: year 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011,
Nr. of cases 38, 20, 16, 12, 10, 4, 6, 6, 3, 5, 3, 4, 4.
Trained subjects: from 1999 to 2011 nr. 3700
Advice to the population: from 1999 to 2011 nr. 4250
Conclusion: analysing the data, the cases of poisoning showed a significant decrease over the time in the cases of hospitalization in parallel to the increase in trained subjects. The species responsible for the poisoning resulting from advisory mycological boards were: *Entoloma sinuatum*, *Omphalotus olearius*, *Russula emetica*, *Boletus satanas*, *Agaricus xantoderma*, *Inocybe* sp. In 2011 there was a serious case with admission to intensive care. The macroscopic examination, confirmed by the microscopic, had led to the determination that the consumed mushroom was a Lactarius tesquorum that had been preserved in oil. Toxicology tests were carried out to see if it could be Botox. They were positive, confirming a botulinum intoxication and excluding a mushroom intoxication.

NEW COLLECTIONS OF *CLITOCYBE AMOENOLENS* (AGARICALES, TRICHOLOMATACEAE) FROM ITALY AND NOTES ON ITS DISTRIBUTION

VIZZINI A.

Clitocybe amoenolens is a rare species known only from Morocco (Malençon & Bertault 1975), southern France (Moreau et al. 2001), central Italy (Contu et al. 1999, Leonardi & Maggi 2007), and northern and central Spain (Martínez et al. 2010). It was responsible, first in France (Fourré 1997, Charignon & Garcin 1998, Moreau et al. 2001, Saviuc et al. 2001) and then in Italy (Leonardi et al. 2002, Marinetti & Recchia 2005), for induced erythromelalgia (= acromelalgia syndrome sensu Saviuc et al. 2001), a poisoning syndrome caused by the ingestion of *Clitocybe acromelalga* in Japan (Nakamura et al. 1987). *C. amoenolens* was confused with edible mushrooms in the *Lepista flaccida* complex (e.g., *L. flaccida*, *L.. lentiginosa*, *L. gilva*) and with *Infundibulicybe gibba* (Fourré 1997, Moreau et al. 2001). In this communication, records of this poisonous species are reported for the first time from northern Italy (Vizzini & Ercole 2012), and its geographical distribution is discussed.

2ND SESSION – MYCOTOXICOLOGY: NEW PERSPECTIVES

EPIDEMIOLOGY AND USE OF EPIGEAL MUSHROOMS WITH MEDICINAL PROPERTIES IN ONCOLOGY: THE ROLE AND FUNCTIONS OF THE MYCOLOGICAL INSPECTORATE

BAGNATO M.

Mushrooms had been always used by rural populations like food integration, often as meat substitution and like nutrient elated by culinary art, but often criticized for frequent intoxications phenomenons. This is the cultural italian context, where the mushrooms as seen as a food in two different way: from people is elated the gastronomy, from health authority is advised against the use as a food for risk intoxication. This is ,also, the context in which the Mycological Inspectorates was born in Italy , only nation in all over the world where is a health figure, the mycologist, insert in National Health Services to prevention mushrooms intoxication with a quick scan of species and consultant in poisoned cases. To the public mycologist is requested just to recognize species, but not to recognise nutrient or pharmacological properties, despite Inspectorates asserts on technical scientifical and epidemiological. This is the italian mycological paradox. Instead these aspects are well known from Otzi's time on western mountain community or in siberian countries and, like even in CTM on eastern regions, the use as a medicinal drug was common and researched and sometimes exclusive of kings for the power of therapeutical effects. Above all, in recent times in Japan, epidemiological researches on mushrooms vs cancers, were the being of the hospital mushroom extracts administration and to the international modern research, with a movement that usually and all over the world, brought to important discovers and medical/clinical application of mushroom extracts especially on cancer and, in this study, the author shows a vast international panorama. However in Italy, arrived food supplements with mushrooms, often publicized as a remedy for cancer and administrated by no medical doctors, really because they are only foods. In a very short time, it could be a serious public health problem, and Mycological Inspectorates, should play a very important role through a serious. epidemiological analysis of the phenomenon.

PLEUROTUS OSTREATUS IN THE DEGRADATION OF XENOBIOTIC COMPOUNDS

GALLI E.

White-rot fungi possess ligninolytic enzymes with low substrate specificity; that permit them the degradation of organic xenobiotic compounds with different origin, scarcely susceptible to bacterial attack. In the Institute of Agro-environmental and Forest Biology of CNR, fungi belonging to *Pleurotus* genus have been used for the treatment of polluted waste for many years. In particular, olive mill waste waters were used as growth medium for *P. ostreatus*, with the result of an abundant mycelium growth and the decrease of COD and phenols in the waste waters. Experiments were performed to study the remediation of wood treated with creosote, a product used as wood preservative and containing phenols and polycyclic aromatic compounds. *P. ostreatus* was able to grow in a mixture containing creosote treated wood + wheat straw, totally degrading phenols and reducing the 65-70% of polycyclic aromatic hydrocarbons and heterocyclic compounds. To clarify the degradation of fluoranthene, one of the principal polycyclic aromatic compounds contained in creosote, a study was performed in batch. The results pointed out that before the degradation began fluoranthene was absorbed by the mycelium (15 days) and then degraded by 50% in 40 days. Another study focused the problem of pollution by the antibiotic oxytetracycline, that is administered in high doses to livestocks and enters the environmental compartments as a consequence of animal waste disposal. Oxytetracycline disappearance in *P. ostreatus* culture medium was clearly evident as early as the third day of exposure onwards, with an almost complete removal after 14 days. Further experiments focused on the activity of ligninolytic enzymes, in particular the laccases, to understand their involvement in the degradation process.

ACTIVE PRINCIPLES IN HEALING MUSHROOMS

LORENZI M.

After years of low academic activity, when the research mostly considered ß-glucans and sterol-like substances, starting from the early 2000 a progressive extension of studies occurred, based on a more systematic approach and a more extended analysis spectrum. ß-glucans(D-glucose unit based polymers) have been deeply studied and their structure, at least concerning principal aspects, are now well known. Nevertheless they are complex molecules, variously conformed and characterized by very different molecular weight. Besides their ramification and structure differences they present complexation with heterogenic proteinic material, and on this aspect still remains a large margin for in-depth examinations. The other main investigation field concerned, as already said, triterpenic based substances, inherently very changeable, of which more than 200 different molecules have been identified. In the last decade, following a not so linear course, Research finally has extended to other substances typology: a substantial quantity of research activities concerns lipid substances, proteinic, lecitins, generic AO power potential molecules and elements like Va and Ge. At present, after a quite confused phase, scientific publications finally seem to improve, following more organized and structured outlines. Unfortunately the investigation concerning symbiotic species still appears very defective, but this aspect is constantly characterized by criticisms very hard to overcome, at least in the short period. Nevertheless, the data quantity we gain is now so vast that it needs to be constantly updated and catalogued in classes.

3RD SESSION – FUNGI AND HUMAN FEED

NICOTINE CONTAMINATION IN WILD MUSHROOMS

DAVOLI P., SITTA N.

The recent and most unexpected detection of nicotine – a plant alkaloid endowed with insecticidal activity which occurs naturally in tobacco and other members of the Solanaceae – in commercial samples of dried porcini (and other wild mushroom species as well) has urged the European Food Safety Authority (EFSA) to assess potential risks for consumers' health and has resulted ultimately in new maximum residue levels that were issued for EU countries, albeit on a temporary basis. The highest level was set at 2.3 mg nicotine per kg dry weight for dried porcini, i.e. *Boletus* edulis and allied species, which represent the most frequently contaminated wild species; porcini also account for the highest amounts of traded wild mushrooms and originate mainly from China and East Europe. Nicotine concentrations in the 0.5–1 mg/kg range were rather common in commercial samples of dried porcini of different origin, but values above 10 mg/kg were also detected, especially in dried porcini from China. Samples of European origin (Germany, Spain) were found contaminated only in trace amounts (< 0.1 mg/kg) or were not contaminated at all. The reasons for the presence of nicotine in wild mushrooms still need to be clarified at present. Post-harvest cross contamination during drying and processing is most likely, as in mushroom-producing areas (e.g. Yunnan in south western China) part of the harvest is dried in the very same drying ovens where tobacco leaves are also processed, and sometimes tobacco plant waste might be employed as fuel for heating; lower levels of nicotine contamination in dried mushrooms might also result from skin contact during processing and sorting by local workers who are smokers. The utilization of EU-banned nicotine-containing pesticides to control post-harvest attacks by arthropods has been also invoked, as the use of nicotine as pesticide is still permitted in Third World countries and also in China. By contrast, the hypothesis of endogenous formation of nicotine in wild mushrooms, perhaps as a response to biotic or abiotic stress factors such as dehydration, must be regarded as awkward and rather unlikely in the absence of any sound biochemical evidence. In fact, the ability of mushrooms to produce ornithine and nicotinic acid, which represent the biochemical precursors in nicotine biosynthesis, does not imply necessarily that mushrooms are also capable to synthesize nicotine endogenously, as the key steps of nicotine biosynthesis have never been demonstrated outside the plant kingdom so far; in addition, it has been clarified that standard drying did not result in increased nicotine levels in mushrooms. For purposes of inspection and enforcement, care must be taken in evaluating the origin of mushroom samples used for analysis, as for dried and preserved mushrooms the real geographic origin cannot usually be inferred from commercial labels; in many instances, moreover, samples of dried mushrooms sold on the market are obtained by blending raw materials of different origins. Such 'misleading' labels have been encountered frequentely during enforcement actions by official control bodies (e.g. Corpo Forestale dello Stato in Italy); therefore, it might well be that nicotine contamination is detected in samples of dried porcini which are labelled as 'from Europe' but originate actually from extra-European countries. In addition, the analytical

determination of nicotine, especially at levels close to the limit of quantification/detection, does not represent a trivial task due to the high volatility of nicotine itself and to the relative unease of complete extraction from the matrix under examination; worryingly, the whole analytical process is also most prone to environmental contaminations before and during analysis, due to the ubiquity of cigarette smoke.

POSTERS

1ST SESSION – CLINICAL TOXICOLOGY

MUSHROOMS OF GARGANO BETWEEN FOLK CUSTOMS AND CONSUMPTION. PREVENTION PROJECT OF FOGGIA

LEPORE A., PENNISI L., TORCHETTI I.

The Gargano called the spur of Italy has in it the heart of the National Park. To act as a master in the first autumn and spring are the mushrooms that, because of poor eating habits, are making record both true and false cases of poisoning cases of poisoning. The mushroom hunters are not as experienced as they could be years ago, becoming exposed to a real risk of poisoning by fungi. This period is all the rage, during happy hour, tasting raw mushrooms, soaked in vinegar, oil and spices, in this way exposes the consumer to potential poisoning that food poisoning. In many countries garganici are collected and consumed mushrooms defined potentially toxic, as *Lactarius controversus* or *Morchella esculenta*, the latter is defined not only potentially toxic, but can also be confused with *Gyromitra esculenta* which is highly toxic and causes the "gyromitric" syndrome with long latency. The Poison Control Centre of Foggia is being done promoter of the implementation of an information brochure that will be useful in the first instance of recognition of those species are edible Gargano.

PATHOGENICITY AND TOXINS OF *ALTERNARIA* SPECIES-GROUPS ON WHEAT IN ARGENTINA

PERELLÓ A., LABUDA R., SULYOK M.

A total of 32 isolates of *Alternaria* sp., *Pithomyces* and *Ulocladium* obtained from grains and leaves of wheat plants (*Triticum aestivum* L.) in Argentina were grouped according the results of mycological and mycotoxicological analysis. For identification, isolates were grown under standardized conditions on Potato carrot agar (PCA) under alternating daylight and compared with standard patterns of the fungal collection Romer Labs (Tulln, Austria), where they are stored. Strains were categorized according to the pattern of sporulation 3D, ranked by the morphology of the conidium / conidiophore, and according to the colony color on PCA. Four *Alternaria* species-groups were identified, 13 isolates belonging to group of A. infectoria, 4 to the group of *A. alternata*, 6 to group *A. tenuissima* and 9 were determined as members of *A. arborescens* group. This is the first report of *A. arborescens* on wheat in Argentina. Morphological characterization was confirmed by molecular analysis of isolates (DNA extraction and sequencing ITS). Necrotic symptoms such as leaf spots, stained grains, root necrosis and weakening of seedlings were observed as results of the inoculations on five wheat cultivars tested under lab and greenhouse conditions. To assess the associated mycotoxicological risk of the fungi, the toxins involved were analyzed by liquid chromatography with mass spectrometry (LC / MS-MS). Alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxine I and II (ATX-I, ATX-II), tenuazonic acid (TEA) and tentoxina (TEN) were produced in a relatively high quantity. AOM, AME, ALT, ATX-I, ATX-II, TEA and TEN were detected mostly in the taxonomic members of the *arborescens*, *alternata* and *tenuissima* groups at concentrations that reached to 19.33, 8.05, 8.45, 10.36, 8.90 and 5.57 mg. L-1 raw extract, respectively, following 14 days incubation on Yeast extract agar (YES) at 25°C, in darkness. *Pithomyces* produced only AOH and AME. Interestingly, *Ulocladium* and members of the *A. infectoria* group produced mostly ATX-I and ATX-II, and eventually TEN at low concentrations. In addition to the virulence on wheat plants, argentinian grains are contaminated with a group of fungi being able to produce toxic substances. Considering the high ability of producing these mycotoxins, even in the group of *A. infectoria* population deserves attention due to the lack of regulations on food in Argentina and other regions of the world.

2ND SESSION – MYCOTOXICOLOGY: NEW PERSPECTIVES

ACCUMULATION OF TOTAL MERCURY AND CADMIUM BY *XEROCOMUS CHRYSENTERON*

DRYŻAŁOWSKA A., FALANDYSZ J.

Aim of study was to examine status of contamination and potential to accumulate mercury and cadmium in fruiting bodies by *Xerocomus chrysenteron* collected at several regions of Poland. Total mercury was determined by cold-vapor atomic absorption spectroscopy (CV-AAS) after a direct thermal decomposition of materials. Cadmium was determined by inductively coupled – atomic emission spectroscopy (ICP-AES) after wet digestion of mushrooms with concentrated solution (65%) nitric acid and cold extraction of soils with 20% solution of nitric acid. Mercury occurred in caps and stipes of fruiting bodies and soils at seven sites across of Poland at concentrations ranging (in mg/kg dry weight) from 0.08±0.01 to 0.36±0.09 (caps), from 0.06±0.04 to 0.27±0.08 (stipes) and from 0.03±0.02 to 0.10±0.10 (soils). Cadmium, compared to mercury, was more abundant and was more efficiently accumulated by X. chrysenteron in fruiting bodies (caps), which contained from 4.9±4.1 to 41±20 mg/kg dw, while in soils were from 0.02±0.02 to 0.37±0.20 mg/kg dw, on the average. A degree of contamination with cadmium of fruiting bodies of X. chrysenteron collected at some sites (especially from Włocławek forests) that are commonly used by mushroom fanciers was relatively high and can cause a health concern. Reviewed are also data available from literature on mercury and cadmium in *X. chrysenteron*.

STUDYING TRACE ELEMENTS (SE) IN MUSHROOMS

FALANDYSZ J.

Quality of analytical chemistry data highly matters in the society and this begins from a quality of results of chemical analyses in life sciences or material sciences. Mushrooms are an object of interest by numerous researchers as well as for consumers and depending on their specialist education and knowledge, the paradigm of quality of results of chemical analyses, e.g. trace element composition of edible flesh usually is to them not a matter of concern. In case of trace elements determined in mushrooms and other foods an accurate and precise data on their multi-element composition become more and more expensive to obtain. Within this communication is discussed a problem of emerging but also of earlier examples of incorrect data produced on Se content of mushrooms. Selected data on Se in several species of mushrooms are outlined and discussed. We show that some data published seem either dubious or concentrations too high to be credible and valid when compared to data reported by other authors. The examples of methods and specifically, the measurement techniques of Se as reported by authors examining mushrooms are outlined. A valid database available for Se in mushrooms could be helpful for the authors to consider if their data are correct. Nevertheless, high precautions need to be taken if presenting data for new species (for which no previous data are available). This is because certain analytical methods if used for Se measurement lead to highly incorrect results. Selection of improper analytical method for Se determination is considered a cause of incorrect data reported.

WHAT IS DOMINANT SOURCE OF MERCURY TO HONEY FUNGUS?

FALANDYSZ J., WOBALIS A., JARZYNSKA G.

In Poland have been identified five species of *Armillaria* mushrooms: A. solidipes that is also called *A. ostoyae* and is a most common and other are *A. cepistipes* and *A. gallica*, and the rarest is *A. borealis* and *A. mellea*. *A. solidipes* is popular edible mushroom in Poland. In earlier study a relatively elevated concentration of Hg was found in some consignments of A. solidipes collected from 12 sites across of Poland. The mean values of Hg concentration ranged from 0.020±0.008 to 0.30±0.07 μg/g dry weight (d.w.) in caps, from 20±6 to 160±40 ng/g dw in stipes and in soils were from 0.020±0.002 to 0.10±0.13 μg/g dw (Falandysz et al., 2012). Mercury content of *A. solidipes* from other countries was even greater compared to mentioned study, i.e. on average was ~ 0.30 □g/g dw in Bohemia (Czech Republic), ~ 0.59 μg /g dw in Italy and ~ 0.90 μg /g in Turkey (cited from Falandysz et al., 2012). Honey Fungus utilize plant substrates (alive trees, dead trees, stumps) as source of carbon. The plant biomass compared to fruiting bodies of many mushrooms is known as poor in mercury. To get knowledge on what is dominant source of mercury to A. ostoyae we collected and examined 15 sets of mushrooms and corresponding samples of wooden substrate (stumps) and nearby soils (surface layer; 0-10 cm), which were collected from forest (mainly Scots Pine) in northern part of Poland in 2011. Based on mercury concentrations noted in mushrooms and wooden and soil substrates in this study, it seems possible that mode of life and space colonization by A. solidipes, which spreads by means of root-like rhizomorphs (tentacles, bootlaces) and a direct mycelial contact, enables to it to take-up mercury from degraded wooden substratum (minor source) and largely with water from soils (major source) via rhizomorphs.

SHAGGY INK CAP (*COPRINUS COMATUS*) MUSHROOM – CAN IT TOLD TO US ABOUT HG CONTAMINATED URBAN SITES?

FALANDYSZ J, LENZ E., JARZYŃSKA G.

Coprinus comatus is popular mushroom species and its common name is the Shaggy Ink Cap (also is called Shaggy Mane, Shaggy Parasol or Lawyer's wing). This saprophytic species can be found along roadsides, lawns and pastures. Aim of this study was to examine if the Shaggy Ink Cap has any potential as possible boindiactor of urban soils (roadside, barren lands, lawns) pollution with mercury. Also estimated was intake rate of total mercury by consumers of edible fruiting bodies (young specimens) of C. comatus at the region investigated. The fruiting bodies and beneath to them topsoils (0-10 cm layer) samples were collected at several sites from area of a small town of Kartuzy (Kaszuby region) in Pomorskie Voivodeship in northern part of Poland in 2011. Mercury content of mushroom and soils was determined by cold-vapor – atomic absorption spectroscopy (CV-AAS). Intake rate of total mercury was estimated based on median values of mercury concentrations noted in fruiting bodies, possible intake rates of mushroom and a provisionally tolerable intake limits of mercury to adult human. The Shaggy Ink Cap seems to be a sensitive boindicator of urban soils pollution with mercury that is efficiently sequestered by this species in fruiting bodies – both caps and stipes. Eating fruiting bodies of Shaggy Ink Cap collected from the urban environment and especially when emerged at the barren land or "industrial" sites can provide to consumer mercury at relatively high doses.

MERCURY, CADMIUM AND LEAD ACCUMULATION CAPACITY OF *XEROCOMUS BADIUS* (BAY BOLETE) FRUITING BODIES COLLECTED FROM TWO GEOGRAPHICALLY DISTANT SITES

KOJTA A.K., G. JARZYŃSKA, J. FALANDYSZ

Many edible mushrooms are known as heavy metals accumulators. Bay Bolete is popular edible wild-grown mushroom widely collected in Poland. This study was designed to evaluate the degree of mercury, cadmium and lead pollution and potential to accumulate these trace elements by specimens of Bay Bolete emerged at two spatially distant sites in Poland. The sites selected were outskirts of Złotoryja in southern part and the Bory Tucholskie forest in northern region of Poland. The method of Cd and Pb determination was inductively coupled plasma-optical emission spectroscopy (ICP-OES), while of Hg was cold vapor-atomic absorption spectroscopy (CV-AAS). Mercury and lead content of Bay Bolete varied highly between the sites. The specimens collected from Bory Tucholskie contained toxic to human mercury, cadmium and lead at concentration of 0.13±0.04, 1.2±1.2 and 0.14±0.07 μg g-1 and dry weight, respectively and from Złotoryja site mercury, cadmium and lead concentrations were 1.0±1.1, 0.84±0.48 and 0.52±0.32 μg g-1 dw, respectively. Data obtained were subjected to statistical evaluation including multifunction analysis.

MINERAL COMPOSITION OF POPULAR EDIBLE MUSHROOM COMMON CHANTERELLE (*CANTHARELLUS CIBARIUS*)

DREWNOWSKA. M., FALANDYSZ J.

Although the fact that wild-grown edible mushrooms can accumulate both essential and toxic metals and metalloids has been proven, there is still not enough information available about the toxicological risk and nutritional benefits of such substances in edible wild-grown mushrooms. We examined Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, P, Rb, Sr and Zn in Common Chanterelle fruitbodies and topsoils substratum collected from two sites in Poland: Augustowska Forest (2006) in north-eastern and Zagórów (2007) in central parts of Poland. mercury was determined by cold-vapor atomic absorption spectroscopy (CV-AAS) and other elements by inductively coupled plasma - atomic emission spectroscopy (ICP-AES). The Common Chanterelle at the sites investigated had some potential to bioconcentrate in fruiting bodies trace elements such as K, Mg, P, Rb, Ag, Ca, Cd, Cu, Na, Ni and Zn. Mushrooms from both sites could be considered as relatively abundant in essential elements such as: K (with median concentration range from 39,900 to 60,500 μg/g dw), P (4,300- 5,300) Mg (1000–1200), Na (56–158), Ca (226–516), Fe (69–99), Zn (73–85), Cu (39–57), Mn (26–42) and Co (0.19–0.27 μg/g dw). In light of the European Commission regulations on Cd and Pb and a value of provisionally available weekly intake (PTWI) for Hg by World Health Organization, the Common Chanterelle mushrooms at the sites studied were safe for consumption. The values of Cd, Pb and Hg in Common Chanterelles in this study were well below maximum permitted levels.

MERCURY IN *BOLETUS AESTIVALIS* AND BENEATH SOILS FROM TWO SITES IN BELARUS

PANKAVEC S., JARZYŃSKA G., FALANDYSZ J.

Boletus aestivalis (Summer Bolete) fruiting bodies and samples of surface layer (0-10 cm) were collected from two spatially distant sites in Belarus in 2010 and examined to assess contamination with mercury. Mercury was determined by cold-vapor – atomic absorption spectroscopy (CV-AAS) after thermal decomposition of sample material and amalgamation of released Hg vapors by gold trap and its further thermal desorption and measurement (MA-2000 Mercury analyzer). Total mercury content of mushrooms was from 1.3±0.4 to 2.8±0.6 mg/kg dw (caps) and from 0.56±0.17 to 1.5±0.5 mg/kg dw (stipes). Solis contained mercury in surface layer at 0.038±0.015 and 0.17±0.10 mg/kg dw, respectively. *Boletus aestivalis* efficiently accumulated mercury in caps (BCF from 25±23 to 40±21) and stipes (BCF from 13±11 to 18±15) on average. Data available from literature on mercury in B. aestivalis and some other *Boletus* mushrooms are reviewed and discussed.

Concentrations of chemical elements in mushrooms as an aspect of the interaction mushroom-soil

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

The role mushrooms play in nature and in soil ecosystems is not yet completely understood. Our study aimed at investigating the differential uptake of chemical elements by mushrooms. Finally, we have observed that some mushrooms concentrate high amounts of chemical elements even if they are present at low concentrations in the substrate. High concentrations of some elements in the substrate did not correspond to high concentrations in other species of mushrooms.

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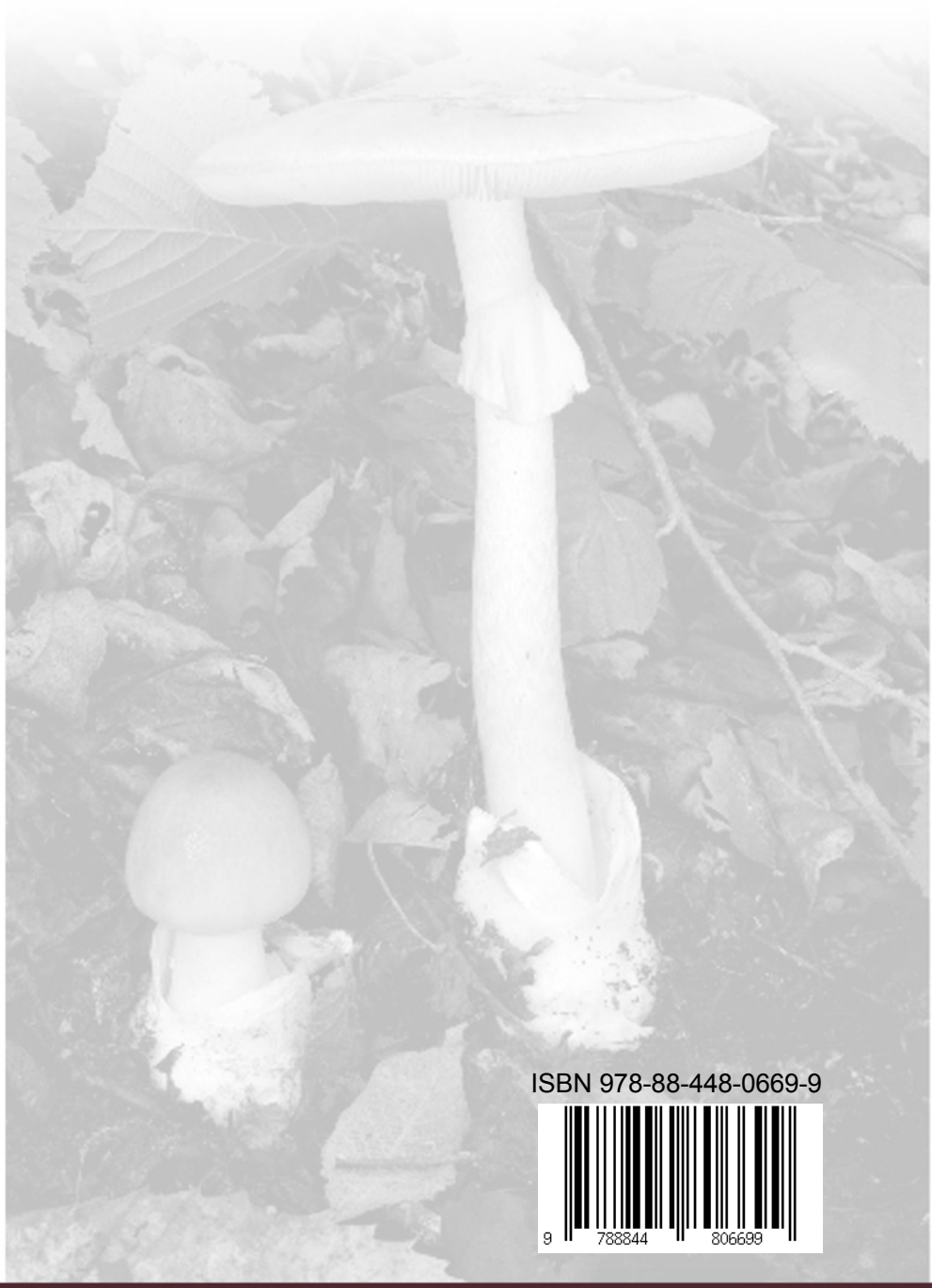

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