# THE SCIENTIFIC CHARACTERISTICS AND THE PRACTICAL SIGNIFICATION OF THE POSSIBLE ECOLOGICAL BIOHAZARDOUS FORMS IN EUROREGION BANAT

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**REZUMAT. Banatul este o parte componentă a regiunilor Dunărene. Strategia de dezvoltare și ridicare a competitivității economice a Banatului necesită o nouă dimensiune a cooperării bazată pe conlucrarea instituțiilor de cercetare, inovare, învățământ. In secolul 21 s-a catografiat harta genomica pentru oameni, taurine, porci si alte specii de animale. Cu ajutorul robotilor şi computerelor, laboratoarele de cercetări genetice au făcut un pas important. Agricultura Banatului, inclusiv utilizarea tehnologiilor respectoase cu mediul, nu se mai poate face fără existența laboratoarelor de cercetare performante, mai ales în genetică și biotehnologii. Biotehnologiile puse la punct cu ajutorul acestor cercetări ajută la descifrarea exactă a mecanismelor fundamentale ale ciclului vieții și limitarea biohazardului genetic. Un alt factor de hazard biologic este reprezentat de Genetically Modified Organisms (GMOs). Principala cale pentru diminuarea efectelor diferitelor tipuri de biohazard este doar prin aplicarea unor tehnologii de înaltă performanță care aparțin geneticii și nanotehnologiilor, iar pentru viitor, picotehnologiilor.**

**Cuvinte cheie:** bioluminiscenta, biohazard, genom, metabolomic, etnozootehnie

**ABSTRACT: The development strategy for raising economic competition in Banat requires a new dimension of cooperation based on the research and educational institutions working together. In the 21st century we already have genome mapping for humans, cattles, horse, pig and other animal species. The robots and computers in the genetic research labs are an important step ahead. Agriculture in Banat, including the nature-friendly technologies, cannot be done or utilized without certain well equipped research labs, especially in the case of genetics and biotechnologies. The biotechnologies used for this research help to decipher the fundamental mechanisms of the life cycle and to limit the genetic biohazard. Another factor of the biological hazards is represented by the Genetically Modified Organisms (GMOs). The main way to diminish the effects of the different types of biohazard can be accomplished only by applying some high performance technologies which belong to genetics and to nanotechnologies and, in the future, to picotechnologies.**

**Keywords:** bioluminescence, biohazards, genomics, metabolomics, ethnozootechny.

## 1. INTRODUCTION

Scientifically, the similarities and differences between ecology and biology are well known. This is why the authors of this paper consider that the ecological hazard has to be correlated with the biological hazard, i.e. the biohazard and the reason why a new expression referring to the ecological biohazard is introduced here in order to better delineate the ecological impact of the biohazard in the current and future context for the anthropic eco-bio-strategies for sustainable development in bio-systems in general and in agro-systems in particular.

One of the preoccupations of the Development Strategy in the Danubian Regions is the one related to environment pollution, its monitoring and finding solutions for preventing this to happen. The rich biodiversity in this region represents a way for rural development and for keeping the multicultural values. The development strategy in danubian regions implies land and water quality and security, preserving the forests, national parks and reservation.

## 2. METHODOLOGY

Banat is a part of the Danubian regions. The development strategy for raising economic competition in Banat requires a new dimension of cooperation based on the research and educational institutions working together. Networking is one of the most crucial elements in development. The Transilvanian Rare Breeds Association has elaborated a

strategy for Preserving and monitoring the environment quality in Banat, in full concordance with the Proposal and Draft Declaration on the Formation of Danube Basin Rural Development Network/Cluster (DBRDLN) [1] written in Budapest in 13 December 2012 by the International Organizing Committee. The Declaration was signed by the Transilvanian Rare Breeds Association which, according to the document, "agreed that creation of regional network is the only way to build social and economic cohesion in the Danube Basin".

The Tranilvanian Rare Breeds strategy is based on the results of the research conducted over the years, which have been presented at national and international scientific conferences and then published in highly-quoted journals. An important contribution is that of ethnozootechny research.

The aim of this paper is to promote the research in animal genetic industry in Euroregion Banat. The paper draws on existing literature and database Association Tranisilvanian Rare Breeds. Also on the results of experiments performed by the authors during the years. The geographical scope of this paper is to promote cross-border cooperation in Danube region Banat.

## 3. RESULTS AND DISCUSSIONS

**The genetic biohazard.** Fecundation is a complex biological process which consists of mutual assimilation of the two opposite gametes, male and female and the formation of the zygote cell, the beginning of the new product of life. After the ovocyte has been fecundated by the sperm the diploid set of chromosomes is restored, and in the case of gene interaction it is possible that combinations of recessive homozygote type to appear, which have negative effects due to genetic biohazard, where it can all start from an error in a few atoms of a cell. For these errors there are tests which identify the problems. New technology for genomics is an interdisciplinary effort, requiring contributions from a range of fields that previously have never been so closely interconnected, including input from molecular biologists, geneticists, chemists, physicists, mathematicians, computer scientists, and engineers [2]. The information produced as a result of applying this new technology to genome analysis will lead to a new, exciting age in genetic-medicine [3]. In the 21st century we already have genome mapping for humans, taurines, horse, pig and other animal species. In Figure 1 is represented the interdisciplinary effort by genomics technologies.

The robots and computers in the genetic research labs are an important step ahead. Agriculture in Banat, including the nature-friendly technologies, cannot be done or utilized without certain well equipped research labs, especially in the case of genetics and biotechnologies. One has to mention the CELERA project (Celerity – speed) of CeleraGenomics Company funded by USA in which great institutions of genetics from USA and UK take part. The importance of this research program is huge especially due to its victories in research such as the Human genome project. In this project they created a unique laboratory where researchers worked ceaselessly in order to speed up the deciphering of the DNA. The laser equipment reflects the light on each DNA fragment in colours – red, yellow etc, meaning the base for CATG (cytosine, adenine, thymine, guanine) in the DNA genetic sequence. The fact that they obtained data quickly and that they posted it on the Internet led to the creation of a true informational highway regarding chromosomes, and human and animal genome decoding. The aim of this research is to find cures for incurable diseases and to find new drugs because the pharamaceutical research is very expensive. The biotechnologies used for this research help to decipher the fundamental mechanisms of the life cycle and to limit the genetic biohazard. The tools for interpreting the genome are sold, among others, to pharmaceutical companies [4]. The academic community ethos for free information exchange is the base of the CELERAGenomics Company. Their research has led to the treatment or triggering of certain diseases. For example, they have identified the gene which encodes insulin. „Databases of gene products having comparable expression patterns will be available. Thus, information using any of several gene expression technologies available now, or in the near future, could be available for rapid analysis and correlations. Tissue expression data obtained from various cDNA libraries in the gene index databases could be readily integrated to design experiments (new drugs as applied variables) and select candidate new agents. Genes that are expressed in coordinated ways in response to defined applied variables could provide a valuable tool for identifying regulatory elements" [5].

Celera uses 3 methods for biomarker discovery:

– Genotyping looks at single letter substitutions in the genetic code. These genetic variations are called Single Nucleotide Polymorphisms (SNPs).

– Gene Expression analysis identifies patterns in gene activity, determining if a gene is "switched on or off" to produce RNA signals to manufacture proteins.

– Proteomics uses mass spectrometry to identify differentially expressed proteins on the surface of cancer cells. Then, serum from patients with cancer is tested to see if the informative proteins are present and predictive of disease. Protein biomarkers can be detected with simple immunoassays that can be used

in diagnostic products to monitor residual disease or the recurrence of cancer "[6].

The importance of the CELERA project reflects an essential aspect. Taking into consideration the fact that in general the society recognizes only the merits of the first mentioned and based on the laws regarding the intellectual property right there is the risk that certain firms or labs will appear and claim that parts of the human genome are their property. Thus the drugs made, meaning the molecules which can cure people and animals would become very expensive. The fact that starting with 1999 the research results have been posted on the Internet, updated quickly and monitored by technicians 24 hours a day has discouraged and made impossible for some patent requirements over the human genome to be taken into consideration.

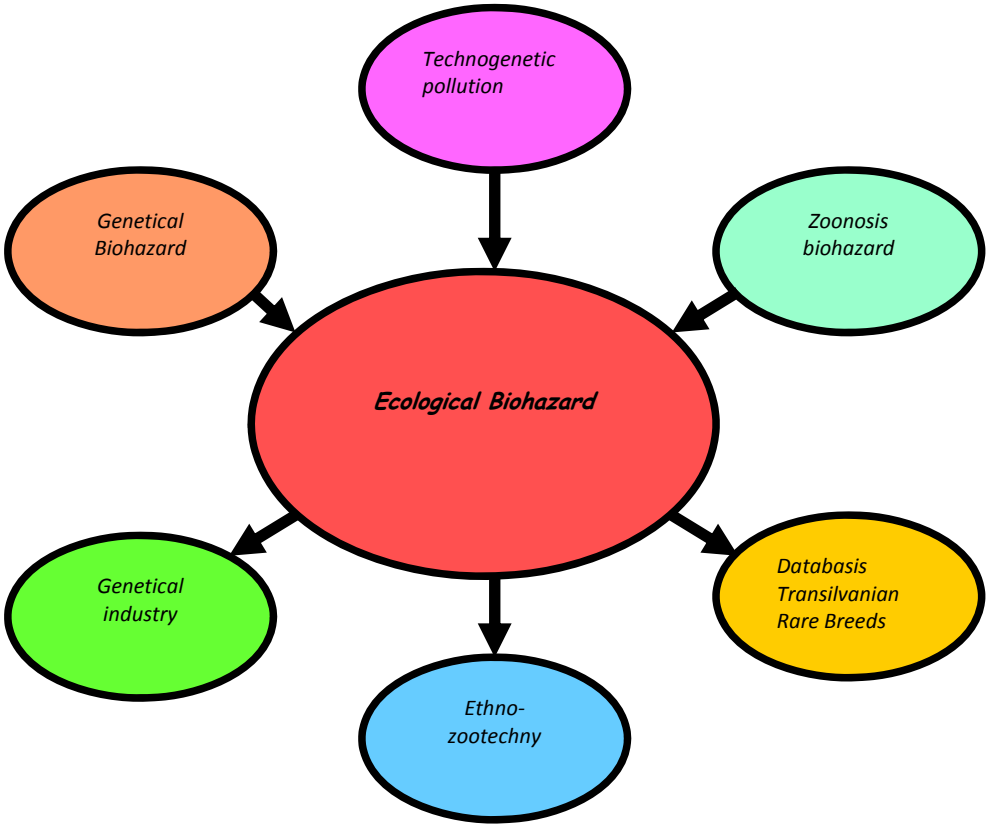


**Fig. 1.** The interdisciplinary effort by genomics technologies.

Another factor of the biological hazards is represented by the Genetically Modified Organisms (GMOs). As we have stated before, the animal genetics industry is dominated by several big companies and the majority of the GMOs belong to them [7]. By having the patent for these GMOs, the companies earn huge amounts of money, claiming that they have invested very much in research.

Genetically modified organisms should be carefully monitorised both in the case of plants and in that of animals since plants represent the food for animals and, in turn, the animals, being a source of food for people, can spread various allergies, cause high resistance to antibiotics and even have a role in cancer occurrence. The good part about GMOs is that the animal products are obtained in a much shorter time, the animal themselves have a higher resistance to diseases and, as some specialists prove, there is no difference in quality between the animal products obtained through GMO and the ones obtained from animals which have been traditionally raised. In the future, however, there might be a significant raise in the number of diseases spread from animals to humans, since both the human population and the animal population are under a continuous growth. Concentrating only on industrial animal production requires a good bio-security to monitor the diseases which might pose a risk for people's health and this means using the safety food strategy. A good example is the very pathogenic virus H5N1 which has migrated from poultry to pigs and to humans and has seriously affected the pig farms in the West of Romania in the recent years. The fact that this disease is not known well enough (the symptoms are not clear, the way in which the whole situation must be handled is not clear either) has led up to serious

consequences in Banat a few years ago when 40000 pigs had to be killed. By animal genetics industry the possibility of preventing diseases such as this one will be much higher and the appropriate management of the situation will be much more available.

Problems such as climate change, high economic competition, shortage of fresh water, possible natural disasters and even wars and terrorism have led to new concepts as solutions: safety food and defense food. The first one refers to the fact that the population must be ensured with enough food throughout time and the second one to the prevention of terrorist attacks upon sources of food [8].

The GMOs are useful in agriculture and can become biological agents against diseases and pests in crops. Sustainable and nature-friendly agriculture implies knowledge of the alternative methods of fight against pests. The microbial bio-technologies which are labeled as 'green' in the colors' catalogue are very important in agriculture, environmental protection, bio-remedies, geo-micro-biology.

The fundamental role of the biohazard in various life dynamics levels is well represented. GMOs can sometimes escape human control having unknown consequences on animals, plants and humans. It is important that specialized personnel permanently monitors them and thus genetic labs must be created. Research in what GMOs are concerned are so advanced that nowadays we have cloned animals and transgenic animals (Gnotobiotics animals) used for xenotransplantation.

Something topical and to be used even more in the future is bioluminescence which has a degree of precision of $10^{-12}$. Starting from the $10^{-6}$ ppm precision tests (parts per million) used in the biosafety food methods, defense food, biosecurity tests – for example how much aflatoxin or heavy metals are allowed in foods, today research has acquired nanotechnologies which allow tests of $10^{-9}$. For zootechny, the main direction of nanotechnologies are towards biomedical equipments, genetic therapy and new types of drugs. Nanobiotechnology can be used as nanomedicine in diagnosis, treatment and tissue/organ repair [9]. Econanotechnology is nanotechnology in environmental protection and pollution, environmental filtering, being new solutions of renewable energy. By developing the biosensors, new labs of molecular diagnosis and imagistic have been created. The bioluminescence methods introduce the picotechnologies where the precision degree is of $10^{-12}$ this representing the methods of analysis and control for environmental pollution to be used in the future.

Bioluminiscent analysis, conform with Svetlana Medvedova [10], is one of the most promising express methods for biologically monitoring the environment because the luminiscent system is highly sensitive to ever micro quantities of pollutants. The enzime of bacterial luminiscent system are used in developing highly sensitive analytical methods. Bioluminiscence of bacteria is an efficient tool to determine of various inhibitors of biological activity [11]. The presence of toxic compounds and ecological biohazards can be determined by using bioassays and toxicity tests, where using alive organisms as indicators, because toxicants directly affect the luminiscent system. Luminiscent genetically – modified microorganisms with lux-genes from marine are used as test objects among bioluminiscent bioassays to determine the presure of different substances in water and soil samples [12]. Correlation of a lux-label with the PCR – analysis have shown, that put cloned marker lux-genes in common live with other modern methods of identification [13]. Bioluminiscent bioassays can be used to controlling ecological biohazards with negative effect like polluants or toxicity from soil land water.

The animal genetic industry can lead to the treatment of many illnesses genetically transmited in animals. For example, there is a lab near Munchen, in Germany, called Agrobiogen, which is unique in Europe for the identification of disease-carrying genes. It is run by professor Brem Gotfried Ph.D. The method of DNA swabbing called Typifix was first used in Romania on the 22nd July 2008 by Matiuti M. in a sheep farm near Arad. The results of the tests have proven the existence of some specimens that had or were carrying scrapie. This method can be used to take samples from cattles, horses, pigs, game or from meat. It is safe, efficient, cheap, accurate and it simplifies some standard procedures in DNA determination [14]. Zoldag L. [15] in 2008 published several names of genetic diseases which can occur in animals. Example of genetic abnormalities:

– Stress sensitivity in swine – autosomal, ryanoid receptor gene localized on chromosone 6, with complete penetrance;

– muscular dystrophy is a heterogeneous group of hereditary diseases affecting humans and other mammals that causes progressive muscle weakness duet o defects in the biochemistry of muscle tissue;

– achondroplasia synonim dyschondroplasia is a form of hereditary dwarfism due to retarded grouth of the long bones;

– resistance to diseases e.g. Chinese pig breeds to K88 E. Coli strain.

Mutations are changes to the nucleotide sequence and can caused by copying errors in the genetic material during cell division by action of biological hazards. More than one mutation and the same clinical signs is genetical heterogeneity like dermatos-

paraxis or cutaneous asthenia, Klinefelter's syndrome (trisomy XXY) or hemy-feathering in rooster chickens.

Many gene mutations in domestic animals are lethal factors exemple:

– Manx factor in cats (Mx Mx lethal);

– Silver fox platinium colour mutant (W $W^p$ ------ $W^p$ $W^p$ embryonic lethal);

– Horse white colour ($W_w$ -------WW embryonic lethal).

Epigenetics is changes in gene expession and transcription, it is a heritable changes in gene function whitout a change in the sequence of nuclear DNA. Is posible to appear genomic, disorders associated with genomic imprinting,when e.g. the bull and the cow, contribute different epigenetic patterns for specific genomic loci in their germ cells. It all starts with an error of a few atoms in a cell.

An example of epigenetic regulation enabling unicellular organisms to respond rapidly to environmental stress ist o make widespread use of post-replicative DNA methylation for the epigenetic control of DNA – protein interactions.

One way in selection for reduced genetic's biohazard is assotative mating or assortative pairing, betwen animals with similar characteristics. Assortative mating have the effect of increasing or reducing the range of variation or trait variance, with effect to elimined indesiderables genes. Heterosis or hybrid vigour descrides the incresed streght of different characteristics in crossbred progeny from the average of the parental breeds is another way for selection to reduce genetic abnornalities. For the elimined undesiderable genes is preferable directional selection, when the advantageous allele will increase in frequency indepently of its relative dominance to other alleles, in favour of the advantageous heterozygote.

The expected response to selection can be estimated by the realized average difference between the parent generation and the next generation.

An indirect selection process is Marker Assisted Selection (MAS) used for determinants of a trait interest like disease resistance, abiotic stress tolerance etc.

Nowadays there is a great amount of nebulosity regarding the application of bio-technologies in the genetic progress. The difference between marker-assisted selection (MAS) and genetically modified organisms (GMOs) is not explained well enough. The two are different bio-technologies. In the case of MAS selected genes are used, which are either markers or targets selected by the reproducer. The complicated legislative process of obtaining approvals in the case of GMOs is not met in the case of MAS, but the costs for GMOs are relatively lower than those for MAS. In order for the MAS bio-technology to develop, sophisticated infrastructure is needed and large investments have to be made in equipments, laboratories and specialists [16].

One example in west of Romania is the Research and Development Station for Cattle Arad belong to the Academy of Agricultural Sciences and Forestry "Gheorghe Ionescu Şisesti" – Bucharest. The unit is located 5 km west of the city of Arad, near the river Mures and Ceala forest. The unit has two breeds: Romanian Spotted – Fleckvieh type and Romanian Brown. The station aims is to conduct scientific research and technological development in cattle breeding and improvement. Dissemination of research and valuable biological material obtained in the unit is done in the counties of western and central regions, the main beneficiaries being farmers. Basic concern of the elite farm of the unit is improvement the genetic structure of cattle through implementation of the breeding plan of Romanian Spotted and Brown breeds. The aim of this systematic breeding plan is to achieve a larger number of cows "mothers bull" with high productive performance, and dissemination of high-performance biological material in the area of influence and beyond. Thus, the station has the main objects: improving the cattle breed, creating lines of high productivity in milk production and also transfer of marker assisted selection in order to improve economic traits. The Research and Development Station for Cattle Arad holds one laboratory in area of molecular biology. The laboratory was established through a project funded by the World Bank in 2008. Research in the laboratory are led by Daniela Elena Ilie, PhD. In frame of the laboratory are conducted DNA tests in order to increase in population the frequency of favorable alleles of genes involved in milk synthesis (Photo 1). The core of the cattle owns by the Research and Development Station for Bovine Arad, present a higher genetic potential in terms of milk production. For this reason, station has been awarded three years consecutive with "Genetic bell", by president of the European Federation of Simmental breed.

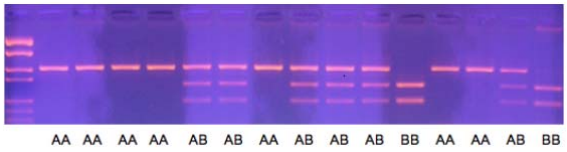


**Photo 1.** The PCR-RFLP analysis for the bovine CSN3, determined by digestion with HindIII on 3.5% agarose gel electrophoresis stained with ethidium bromide [Daniela E. Ilie, 2013].

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes [17]. One of the challenges of systems

biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms [18].

Specialists from France are publishing studies about Metabolomique d'Environment [19] where they describe an environmental metabolism, meaning an application for characterizing the metabolic answer of a wild or domestic animal in conditions of natural stress (temperature, light, UV radiations) or stress related to human activities (toxic waste, chemicals, drugs, sanitary products). For example in the poultry raising technologies, light is an important factor. There are light programmes for various bird species and age. If these conditions are overlooked the technological process can go wrong and diseases or abnormal behaviours (including cannibalism) may occur. Modern technologies require that in animal farms there be sophisticated control and monitoring equipment for the environment, especially since in these farms there can be high quantities of fumes that negatively impact the health of animals and humans.

This environment metabolism allows a simultaneous and quick characterization of numerous problems that might appear and an analysis of the answer regarding the breeding practice. The nature-friendly animal breeding technologies lead to bio-animal husbandry based on production and efficiency which takes into consideration animal health, the quality and security of the environment, well-being and sustainability. All these, according to G. Pedro [20] in his "Rapport sur les travaux de l'Academie d'Agriculture de France en l'an 2004", lead to the connection between animal organisms in various stages, taking into consideration the following:

- Upstream – organs – cells – genes;
- Downstream – animal – herd – production systems.

**The Biohazard in zoonosis occurrence and spreading.** Biological hazards or Biohazards refer to biological substances or other living organisms which can affect human or animal health. The sources of biohazards include bacteria, viruses, insects, plants, birds, animals and humans. The effect on people and animals can be skin rash, allergies or worse, infections, cancer, tuberculosis [21]. The most exposed to biohazards are the ones working in veterinary services, animal breeders, people working in fish farms, the employees that work in closed spaces, hotels, restaurants with air conditioning or the ones in wool and skin processing industry. Wrong handling of animals and biological products or unidentifying the diseases in them can lead to serious consequences on people's health. For example, brucellosis, a disease caught from infected animals or biological products, affected a relatively large number of the veterinary services personnel up until the 70's due to the transrectal check-up in taurines to see whether is animal is pregnant or not done without gloves and without subsequent proper cleaning or due to castrating infected pigs without the above mentioned hygiene requirements. The sheep farms from the former IAS Liebling was considered until 1992 to be an elite farm in the field. But one of the factor that led to the extinction of the animals there was the fact that brucellosis was identified too late. The disease had been caught from some imported rams which had not been tested for their health. Toxoplasmosis, a disease which mainly affects women's health, is transmitted especially by dogs and cats. Other diseases caught from animals are: babesiosis, Lyme disease or tularemia.

Fungi can produce serious allergies in humans. The insufficiently dried hay stacks or cereals kept in high humidity conditions can lead to the occurrence of Aspergillus. The waste from slaughterhouses or from food industry can be biological hazard factors, in the same way as the biological products which are incorrectly kept or wrongly handled by those who work in labs – for example the students of veterinary medicine and animal husbandry.

**The ecologic biohazard by technogenetic pollution.** Ethnozootechny is part of the zootechnical sciences. There are many presentations of the concept of ethnozootechny. The founder of the Ethnozootechny Society in Toul (France) in 1971 and of the term of ethnozootechny, Raymond Laurans presented a very clear conception upon this science, frequently insisting on the relationship between household – animal – environment [22].

Studies in Ethnozootechny (or ethno-animal husbandry) have clarified animal behavior as efficient biosensors. Unfortunately, we consider that the perfected breeds which need special conditions to be raised have gone extinct from Banat due to maladjustment to the new environmental conditions, for example the sheep breeds brought from Australia. The maladjustment was shown mainly through the decrease in the reproduction function. There are reproductive mechanisms of isolation or of battle. According to the studies published by the authors over the years, the local breeds are the best adapted and resistant to the environmental conditions specific to Banat. P. Quemere [23] stated that a breed is not only a gene ensemble, but also a specific growing system, in a specific territory, which can give the products a certain identity, original techniques and which integrates in the general regional patrimony. For a long time, the domestic animal population dynamism was connected strictly to the human-animal-environment balance [22].

Game is another important biosensor, for example the grey partridge. The little birds eat only insects during their first month of life because they don not have yet the necessary enzymes to divide polysaccharides. If in agriculture insecticides are used, the entomo-fauna is destroyed, thus the populations of partridge reduced.

The waste from mine exploitation are a serious problem for the locals in the respective areas or, in some cases, they are even a transnational issue – for example the mine dumps in Moldova Noua (Caras Severin). They tried to use the waste in various ways – for example as feeding supplement for hens that laid eggs, but the experiments carried out by Matiuti M. in 1994 – 1996 proved that the waster could not be used for hens or for any other animal [24, 25]. When the mine was closed they also tried to cover the waste dumps with energetic vegetation but there were no significant results. One of the possible solutions for this waste could be the microbial technology. In the mini lakes created by the exploitation one can use for example reducing sulphate bacteria which can decrease Al, Cu and Fe by up to 60%.

This solution could also be used for the waste in thermoelectric plants near big cities.

In order to intervene in an ecological hazard, there are the so called biosensors. Together with instruments, apparatus and measurement equipment specific to environmental industry, we consider that there has to be an integrative approach, i.e. a holistic one, by adding the methods that use biosensors for environment, water or soil pollution. Solution is the Eco-bio-economy way.

Eco-bio-economy refers to the use of every bio-resources from a certai narea, thus ensuring the rural development according to the European Union requests. Eco-Bio-Economy may be considered an attempt for a new eco-economic and bio-economic vision, which reunites in an integrated pattern: the economy, the ecology, the biodiversity, the eco-economy and the bio-economy focusing the integrated smart sustainable development of the world. To this valuable areas, the Eco-Bio-Economy may address possible Eco-Bio-Policies and Eco-Bio-Strategies and allows the contribution of the social economy, of excellence and of the "*all in one quality*", of the welfare economy, of solidarity, social corporate responsibility, as elements which may be utilized in an integrated practical future platform in a multipolar world for a healthy and ecological environment, to ensure and to promote a smart, creative, innovative, economic sustainable development. The use of the decisions-making process at the highest level and the modern diplomatic tools are the expected and needed catalytic agent for a global eco-bio-policy and eco-bio-economic successful equation. Eco-Bio-Diplomacy is a smart diplomacy of the future, dedicated to a Smart Sustainable Integrated Development of the Earth planet and of the Humankind, through international cooperation among nations, by promoting Eco-Bio-Economy, the global welfare and the quality of live, through the innovative and rational use of the environmental resources [26, 27].

## 4. CONCLUSIONS

The main way to diminish the effects of the different types of biohazard can be accomplished only by applying some high performance technologies which belong to genetics and to nanotechnologies and, in the future, to picotechnologies. These technologies can be applied only by young and well trained researchers and in order for that to happen the connection between education-research-innovation must be further developed. Practicing these concepts and paradigm of Eco-bio-economy in the Banat area can be done if certain steps are taken. It is very important to clarify the huge role of animal raising in all the country. Recently is launched the concept Bioecoeconomy, which means an economy based on biological sciences, economic sciences and humanities [28].

Animal genetics industry is the future for zootechny in Banat. It's time to give up all kinds of "strategies" and "sterile" publication to make way to research with immediate applicability in fields.

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