Stage 3. Determining innovative solutions for on site or off site biological treatment of soil contaminated with heavy metals, sulfur dioxide and hydrocarbons

This is a very complex stage that involved activities oriented on five main directions.

Analysis of technical solutions for biological treatment of soils contaminated with sulfur dioxide, hydrocarbons, that is used in soil conservation is divided into two chapters: **A. Critical analysis of bioremediation methods** and **B. Identifying opportunities for the recovery of industrial waste dumps caused by metallurgical activities. A.** In order to apply biotreatments on contaminated areas we need to know the behavior and interaction between pollutants and soil. As part of the oxidation-reduction reactions occurring in soil, this is an essential component of metabolic and respiratory processes, which can be used successfully to reduce toxicity and to treat certain soil pollutants. Redox reactions are very important for the environment, as many biological degradation processes involve electron transfer reactions. In this case, oxygen is the electron acceptor (if the process is aerobic and there are enough oxygen intakes). In anaerobic microbial processes, nitrate, ferric ion, Fe^{3+}, sulphates or carbon dioxide can be electron acceptors. Variation, namely the dominance of aerobic microorganisms is evidenced by the change in the level of dissolved oxygen. At lower concentrations of dissolved oxygen, anaerobic bacteria will prevail in the degradation of organic contaminants. Electronic transfer will point out which of the microorganisms to receive energy from the source available, namely the organic contaminant. Many soil bacteria are heterotrophic, so they need an external food source for their own metabolism in order to increase number. The food source can be organic – consisting of vegetal mass that grows on soil, other damaged microorganisms and tissue, but may also derive from pollutants that reach the ground. The main methods of bioremediation of soils contaminated with heavy metals take into account their migration into the environment under conditions of increased acidity, and are mainly based on stabilization and solidification, on oxide reducing reactions, on the capacity of the metals to cumulate, namely by extraction. **B.** Data on soil composition and its chemical and physical characteristics are very important in soil research. Soil quality is determined by a series of physical, chemical and biological processes and the intensity of their development (such as alteration, leaching, vegetable soil, the substances' exchange potential, erosion, etc.). Specific activities in the metallurgical industry (be it ferrous or nonferrous metallurgy) lead to large amounts of industrial waste that are stored *in situ* or *ex situ* contaminated sites. These industrial dumps are part of the large industrial dump history and emerged as a result of the 70 years old activity of several units in the field of metallurgy. They are built on land owned by companies or local authorities, were there were not taken additional steps towards waterproof the soil surface, as the clay layer in the basement was considered sufficient in terms of natural barrier. There were determined the types and composition of waste in industrial dumps resulted from metallurgical activities. It was suggested a technology of extraction / exploitation / recovery of existing dumps with emphasis on: ♦ determination of height an tilt of the steps, ♦ relations were presented for estimating the height of operation steps, ♦ it was determined the width of the working and transport berms, ♦ the dimensions of safety berms. There were determined basic principles to explore ways of

processing slag and sand waste and it was proposed a technology to deposit waste – in a temporary warehouse.

Research devoted to finding possible usage of microorganisms in biological extraction of heavy metals and petroleum products from contaminated soils has two main parts**:** A. **Possibilities to use microorganisms in biological extraction of heavy metals** and B**. Possibilities to use microorganisms in biological extraction of petroleum products from contaminated soil.**

A. **Possibilities for the use of microorganisms in biological extraction of heavy metals.** This paper presents an evaluation of spatial and temporal variability of pedo-climatic key parameters and their evolution in the area contaminated with heavy metals and sulfur dioxide in the former mining area in the Calimani Massif. Research activities focused on issues regarding the evolution of palaeogeographic and geological composition. There have also been investigated morphometric and morphographical aspects, general types of terrain, general climate issues and hydrographic network.

Physically, except hydromorphic soil, Calimani soil appears to be light, dusty, inconsistent and well aired. From a chemical point of view, these soils display a variety of nutritional combinations, on the one hand due to mineral-rich substrate and the other due to harsh wet climate that favors leaching.

Mapping was done based on information from field in the sulph quarry area and the waste dumps in the former mining area.

Determinations of *pH* and *Eh* have been made in order to determine the physical parameters (texture, density, structure and hydro-indices), and the results of chemical analysis allowed characterization of representative samples of soil from mining and other exponential soils.

Experimental modeling of the process of acid drainage was performed using laboratory experiments. The results of these experiments are detailed in the report.

Determination of chemical parameters focus on metal concentration (Cu, Fe, Cr, Pb, Zn, Ni, Mn), using spectroscopy, *pH*, total and variable content of N, K, Ca, Mg.

In June and September 2010, sample analysis revealed a maximum of microorganisms in the mining dump Dumitrel (29 x 10^6 UFC / g soil) at a depth of 0-6 cm, while the minimum was noticed in dump Puturosul (172 x 10^3 UFC / g soil) at a depth of 0-10 cm.

The study on fungi interaction reveals that in the care of samples *Salix caprea+Descampisa cespitosa* (Dumitrel dump) and *Picea abies* (Pin dump) the number of rhizosphere microorganisms is higher compared to those present in regular soil. The difference between the two populations of microorganisms can be explained by the positive effect of plant roots on rhizosphere microorganisms, due to root exudates. Dumps are investigated in the early period of colonization by soil organisms, therefore we believe that installation of biota decisive in the early stages of pedogenesis is very long, under a natural evolution.

B. Possibilities of using microorganisms in biological extraction of petroleum components from contaminated soil. Research has been performed both on field and green house of I.C.P.A. In order to study technical solutions for biological treatment of soils contaminated with hydrocarbons, to rehabilitate the land and regain its previous fertility level and to explore possibilities for using microorganisms in soil remediation.

Contaminated site investigation was conducted in three stages: ♦ field phase, ♦ laboratory, ♦ office stage. Research in the green house will also have three stages: ♦ identification, collection and characterization of the soil sampling site, ♦ structuring the experiment, ♦ the ongoing experiment and interpretation of results. In order to find the best scientific and economic solution for remediation of petroleum contaminated soil, research was conducted on a real case: an accidental crude oil pollution in the area near Perisoru, Braila county. This area was chosen because of massive petroleum pollution caused by the cracking of an crude oil pipeline from CONPET. Spilled oil contaminated the soil in different proportions at a depth of 80-100 cm, forming a 30 cm thick flowing crude oil stratum.

The methodology for the *field phase*: The soil has been investigated immediately after pollution: a profile and soil surveys in the area were made, the samples collected were analyzed according to the methodology of characterization of soil contaminated with oil and were studied ecopedological factors in the areas were the soil was formed. Meanwhile, a profile of the same soil type and subtype was made in the unpolluted area adjacent the contaminated area. A pedological characterization of the area was realized: it details profiles and polls, the analysis results were interpreted, comparisons were made between polluted soils and unpolluted areas. After three months, new samples were collected on two new depths in order to evaluate changes in the chemical composition of the soil and the level of oil. In addition, observations were performed over the territory.

Laboratory stage: According to SR ISO 11464: 1998 each sample was conditioned, dried, and prepared for analysis; structure and organic debris were removed. In order to determine the main mechanical characteristics of the soil, size analysis was performed on soil samples, at least seven fractions without the oxidation of organic matter: sieving and sedimentation method, according to SR ISO 11277. Granule fractions were determined. The textural classification into classes and subclasses was realized according to the system *"Methodology of Soil Elaboration",* I.C.P.A. 1987, used in our country. **Chemical analysis**: soil samples were subjected to the following chemical investigations, according to I.C.P.A. methodology: pH, organic carbon, total nitrogen (Nt %), C/N ratio, available phosphorus (P mobile), available potassium, total petroleum hydrocarbons, total salt content. An experiment was conducted to investigate the possibility of using microorganisms in biological extraction of petroleum from contaminated soil. The experiment consists of installation of 19 variations, each repeated three times. In order to elucidate the ecopedological conditions in which soil type and subtype were formed within the studied area, research was carried out on the following factors: geology, lithology, relief, climate, hydrology, vegetation, fauna.

These factors have been studied based on both bibliography and field observations on the relief (microrelief), soil, vegetation and native fauna. Two soil profiles were distinguished in the control area. For studying the soil cover, particularly its upper level, samples were collected on two levels (0-20 cm and 20-40 cm), mainly corresponding to the upper level of the soil Am (table 5). Psychochemical investigations were carried out for a chemical and physical research.

Oil pollution significantly damages soil chemical composition, fauna, flora and microorganisms, decreasing soil fertility – the most important property, which supports plant and animal life, and thus human. In order to evaluate pollution, a profile was isolated at a depth of 120 cm of which 5 samples were collected, depending on the

sensory aspect of the collection surface, 0-20 cm, 20-40 cm, 55-75 cm, 75-95 cm and 100-120 cm. Soil profile was described in terms of pollution. Samples were analyzed according to STAS for oil contaminated soil, and concentration degrees classification was made according to given parameters.

Oil from transportation pipeline covered the soil and formed a crust, while other hydrocarbons with a lower molecular mass blocked soil pores and air flow. Lack of oxygen involves stopping the process of biodegradation of oil hydrocarbons. This phenomenon, similar to animal bodies hypoxia (lack of oxygen in the cells) led to an anaerobic system in the soil, which causes death of aerobic microorganisms, with the consequent inability of the roots to take sap and support plant metabolism.

In order to create the experiment, field research were made that showed the appropriate testing soil. It was used an aluviosol eutric with a sandy-loam texture in Ciorogarla-Sabar Meadow. The experiment was made according to this methodology. After oil pollution, treatment was applied as mentioned in the methodology (differentiated doses of NPK) and after 7 days was used the bacterial inoculum I.C.P.A.. After another 7 days sampling was done for microbiological investigations in order to observe how oil pollution affected soil microorganisms population. This evaluation will be the starting point for further experimentation.

There have been developed innovative solutions for biological treatment of soils contaminated with heavy metals, sulfur dioxide and hydrocarbon (such as historically contaminated areas due to metallurgical industry or garages of large transport fleets). Studies sought to identify bacterial consortium to be applied to ensure complex bioremediation.

The innovative solution for treating soils contaminated with heavy metals is based on documentary research conducted in the first two phases of the project and preliminary experiments conducted in the partnership project RESOLMET. Thus, it was concluded that ***bio-leaching*** (or bio-extraction) is a treatment method that allows extraction of the target biological inorganic pollutants initially present in insoluble forms. If applied under controlled conditions this can be an **innovative solution for treatment of soil contaminated with heavy metals due to metallurgical activities.**

Also, during this phase were established quantitative and qualitative analysis for soils contaminated with sulfur dioxide and hydrocarbons. For this purpose, this paper presents the basic principles of soil sampling and the general steps involved in soil sampling, such as sampling of gas from soil, tools for this process, sample preparation for the laboratory. We have established methods for determining soil properties: the method for determining moisture in the soil, the method for determining soil granulation, method for determining soil density, method for determining soil plasticity parameters, the method for determining soil permeability and mineralization methods/ separation of the soil samples. We presented the principles underlying the choice of a certain method for measuring the physicochemical characteristics and the advantages and disadvantages of chemical and instrumental methods. We established quantitative and qualitative analysis of soils contaminated with sulfur dioxide; determination of sulfur dioxide in the air; determination the mass concentration of sulfur dioxide using the spectrometric method; the determination of mass concentration of sulfur dioxide by ultraviolet fluorescence; determination of sulfur concentration in soil and determination of sulfates in the soil. We established qualitative and quantitative methods of analysis of soils contaminated with

hydrocarbons: determination of mineral oils by FTIR analysis under standardized method ISO/TR 11046:1997 and determination of total hydrocarbon content in soil. Gravimetric method according to standard SR 13511:2007.

The results so far have been classified as follows:

1. articles/studies published in journals of international interest (listed/indexed in ISI or indexed in international database): 5
2. articles/studies published in accredited national journals CNCSIS:4
3. papers published in international scientific events' journals abroad: 2
4. papers published in international scientific events' journals recognized in the country: 6
5. organizing an international conference (International Conference "Environmental Legislation, Safety Engineering and Disaster Management" ELSEDIMA, 21 to 23 October 2010, Cluj-Napoca): 1
6. organizing a national seminar (SEMINAR PROGRESS IN MANAGING CONTAMINATED SITES IN ROMANIA, Babes-Bolyai University, Faculty of Environmental Science and Engineering, Lecture Justinian Petrescu, 24 to 25 June 2010, Cluj-Napoca):1.