

Stage 1. Hypothesis formulation regarding the development of conceptual models and theories applicable in the case of bioremediation of contaminated soils with heavy metals, sulphure dioxide, hydrocarbons.

Large scale production, processing and use of chemicals led to serious pollution of surface soil and subsoil with a wide range of dangerous and toxic hydrocarbons.

Hydrocarbons are a generic group who contain only H and C. It is the most important group of chemicals because of their abundance, industrial importance, their use as a primary source of energy, but also because of toxicity.

A chemical compound which, through accumulation in nature, becomes dangerous for the environment is considered to be POLUTANT and its removal with the help of microorganisms may be considered BIOREMEDIATION.

Among the various technologies available today for decontamination and detoxification of hydrocarbons polluted soil, it appears that bioremediation is one of the safest regarding the environmental impact and implementation costs. Understanding the biochemical and physiological aspects of the overall process of bioremediation it can provide us, in the same time with the necessary knowledge, tools for optimizing these processes to control the basic parameters and to make processes more efficient.

The ability to degrade hydrocarbons in the world of microorganisms is widespread, being found at bacteria (including actinomycetes), yeasts, filamentous fungi, but also at algae. Present in soil, in marine and freshwater and in some sediments, in a wide range of environmental conditions, these microorganisms are able to synthesize an large spectrum of enzymes which provides degradation of individual hydrocarbons and the removal potential or conversion of oil from the environment.

Microorganisms have developed a variety of biochemical pathways to degrade or detoxify hydrocarbons. Oxygenolases and hydrolases are the most important class of enzymes, which are responsible for catalyzing the biotransformation reactions. Hydrolases (helidohydrolases, esterases, amidases) does not require factors and are stable at high pH and temperature variation.

(Activity A.1.1.) Work has focused on the formulation of hypotheses on the biodegradation of hydrocarbon pollutants under the action of microorganisms and on the biological extraction of heavy metals, occasioned a review of the latest information regarding project RESOLMET domain. Thus was examined the mode how biodegradation of hydrocarbons pollutants it can be realized under the action of microorganisms, and how bioremediation using microbial communities and condition for application of *in situ* bioremediation defining the role played by the chemical structure of pollutants, soil nutrients and soil characteristics. All were analyzed in terms of bioavailability of pollutants.

Starting from general considerations on methods for determining biological toxicity of soils were made assumptions about the biological extraction of metals, especially considering:

- ▶ how to estimate the degree of soil pollution and the effectiveness of remedial technologies;
- ▶ how to ensure the isolation of microorganisms with high de-polluted and bioremediation potential;
- ▶ has identified the role of microorganisms in mobilization or fixation of metals in soil. It was analyzed the mode how can be assured bioremediation of contaminated soils with heavy metals:

- ▶ most frequently used experimental models of bioremediation of contaminated soils and
- ▶ *in-situ* bioremediation of soils contaminated with heavy metals.

(Activity A.1.2.) Work has focused on developing conceptual models and theories applicable to bioremediation of soils contaminated with heavy metals, sulfur dioxide, hydrocarbons. Were analyzed and developed conceptual models and theories applicable to the remediation of soils contaminated by metallurgical industry specific activities, were identified methods for remediation of soil and suitable biotechnologies for remediation of polluted environment by metallurgical activities. Were elaborated conceptual models and theories applicable to bioremediation of soils contaminated with sulfur dioxide, analyzing sulfur dioxide emissions and deposition of them on the soil and vegetation, sources of sulfur dioxide and the formation of acids and deposition of SO₂ on soil and vegetation. It was made an analysis regarding the pollution with sulfur dioxide in our country, starting from the presence of sulfur in soil and his transformation, in other words forms of sulfur founded in soil (organic and inorganic form), sulfur transformations in soil and factors which affect the reduction of sulphates in soil. Based on analyzing mode how volatile compounds are generated and mode how sulfur is oxidized were identified the sulfur dioxide pollution effects (effects on human and animals health; on the plants; on the environment). The realized analysis led us to the elaboration of bioremediation models for soils contaminated with sulfur dioxide due to involvement of heterotrophic organisms in sulfur oxidation that occurs in soil.

Conceptual models were analyzed and theories applicable in bioremediation of soils contaminated with heavy metals, namely: remedial measures, the assessment on soil eco-toxicity starting from soil as a substrate for microorganisms and as substrate for plant growth.

It was elaborated a method which will be applied in RESOLMET for determination of soil eco-toxicity. In elaboration of its standards were used IDT ISO 11269-1:1993 (determination of the effects of pollutants on soil flora – Method for measuring roots growth); ISO 11269 (Determination of the effect of substances added).

Also it was elaborated some conceptual models and theories applicable to bioremediation of soils contaminated with hydrocarbons: monitored natural attenuation; phytoremediation; treatment using agricultural land; composting. Have been identified principles of bioremediation processes of soils contaminated with hydrocarbons and the factors which affect biodegradation of soil polluted with hydrocarbons (microbial population density; soil reaction; nutrient concentration; water content of the soil).

The analysis on the ecological reconstruction technologies of degraded or polluted industrial sites has helped at identification of plants used in recultivation of degraded land, trees and shrubs used in recultivation of degraded land and also species which can ameliorate the soil (nitrogen fixing). Afforestation of stock-piles and the operation done to improve the conditions for installation and development of forest vegetation are methods applicable in areas polluted due to human activities.

At implementation of a technology which lend itself to the bioremediation of the contaminated soil due to the metallurgical specific activities, are required to be completed following stages:

- polluted site description;
- environmental characterization (physical, chemical, geological, etc.);
- asses the environmental risk arising
 - identifying the pollution sources;
 - identifying the pollution ways and pollution targets;
 - probability assessment of consequences;
- determining objectives and methods for remediation;

- performing the remediation work itself;
- monitoring and enforcing restrictions on land use.

Soil bioremediation technologies which can be applied in situ are following:

- bioaugmentation;
- land treatment;
- co-metabolism;
- bioventing;
- biosparging;
- biofilters;
- stimulation by surfactants;
- phytoremediation.

Soil bioremediation technologies which can be applied ex situ are following:

- treatment in bioreactors;
- land farming ;
- biosurry;
- biopile;
- static composting and mechanical agitation.