SUMMARY

PHYTOREMEDIATION OF PETROLEUM-POLLUTED AREAS

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KEY WORDS: soil pollution, crude oil, salts, bioremediation, phytoremediation, perlite

ENVIRONMENTAL POLLUTION

The main factor that almost totally or irreversibly transforms natural renewable resources into non-renewable resources is pollution. As a result of human activity, serious pollution problems have arisen in the last few centuries over the entire ecosystem of the Earth. The increasingly common pollution of our day is the pollution of the soil, the flowing waters, the seas and the oceans with crude oil or petroleum products. The negative effects of soil pollution with petroleum products may be less noticeable as it is less frequently mentioned in the media, but they are long-lasting, with a great negative impact upon agricultural areas. Since the transport or extraction of crude oil is carried out in the presence of salts, along with pollution with petroleum residues, there is waste, salty water pollution, capable of causing a strong salinization of petroleum-polluted soils.

In Europe, petroleum product and salt pollution represent about 11% of areas contaminated with different pollutants. Salinisation affects around 3.8 Mha in Europe. In Romania, saline or sodic soils are concentrated in Prahova County, a county with oil fields and refineries, as well as in the southern part of the country, in areas crossed by oil pipelines. There are also strong saline areas, unfit for agriculture, in the vicinity of the Apuseni Mountains.

In order to remedy the polluted areas in the European Union, a series of laws and strategies have been developed that guide the European environment policy pane in 2020 and beyond. In this context, fertile soil and productive land are considered part of the "Natural Capital", to be managed sustainably and adequately protected, while action to remediate contaminated areas is encouraged.

Conventional methods for depolluting soils contaminated with petroleum products apply on an international scale, but most of them have inconveniencies: the generation of liquid or gaseous effluents requiring additional treatment or storage, longer operating times, monitoring and control difficulties, high capital costs and operation.

Environmental biotechnology is an alternative for the remediation of soils contaminated by both microorganisms and/ or plants capable of reducing or even eliminating the presence of soil pollutants. The main remedy ways by using living agents is *bioremediation* and *phytoremediation*.
Bioremediation is the use of living organisms, especially microorganisms, in order to remove contaminants from the environment.
Phytoremediation involves the use of green plants for the decontamination of polluted areas. The capacity of plants to assimilate contaminants depends to a large extent on the cultivated species and the environmental conditions. Also, in nature, plants, together with the microorganisms, forman assembly of connections in the soil, in symbiosis relationships so as to develop and contribute, if necessary, to the removal of contaminants.

MAIN OBJECTIVES
In the current world context, to remedy areas polluted by natural methods, the main purpose of this thesis was *to establish a process for the remediation of polluted soils with petroleum products and salts through environmental-friendly methods*.
Thus, the researches carried out in this PhD theses aimed at the following major objectives:

- ❖ Evaluation of the bioremediation process by assessing the capacity to consume petroleum products by selected microorganisms in the polluted area.
- ❖ Greenhouse and field experiments in phytoremediation methods and the determination of biochemical properties and the capacity of cultivated plants to absorb sodium, potassium, calcium and magnesium ions from polluted soils.
- ❖ Elaboration and development of a method for improving the phytoremediation capacity of the plants selected by the addition of a chemically inert product (perlite) in the soil.
- ❖ Evaluation of the perlite intake in the phytoremediation process.
- ❖ Evaluation of the phytoremediation process on the soil by determining the concentrations of pollutant ions (sodium, calcium, magnesium) in the polluted soil.

The polluted soil used for experiments is part of the agricultural areas of Icoana Village, the Icoana Commune in Olt County, an area with frequent pollution accidents with petroleum products and salts.

WORKING METHODS
The practical experiments were carried out in the field, in Icoana commune on polluted soil (strip ground 71) and unpolluted soil, as well as in the Bucharest USAMV Greenhouse, using polluted and unpolluted soil samples. The microbiology, biochemistry and mineral analyzes were carried out in the laboratories of the Faculty of Biotechnology - University of Agronomic Sciences and Veterinary Medicine of Bucharest.
The biological materials used to study the phytoremediation process were: lettuce (*Lactuca sativa L.*) *Butterhead* variety soil, potatoes (*Solanum tuberosum*), Colette variety soil, tomato (*Lycopersicon esculentum L*), *hybrid Cindel F1 variety soil*, pepper (*Capsicum*

annuum) Albaregia variety soil, beans (*Phaseolus vulgaris*), *Verdana* variety soil, wheet (*Triticum aestivum*), *Glosa* variety soil, maize (*Zea mays*), *Olt* variety soil and sea buckthorn (*Hippophae rhamnoides L.*). For experiments with soil quality improvement 5 mm *Perlit Horticol* was used.
The varieties of soil used in the greenhouse experiments were: V0 – unpolluted soil; V1 – polluted soil; V2- polluted soil plus 25% perlite; V3-polluted soil plus 50% perlite; V4 – polluted soil plus 75% perlite (2014) as well as soil varieties; V0-unpolluted soil; V1- polluted soil and V2-polluted soil plus 50% perlite. (In the field and in the greenhouse) (2015, 2016, 2017).
Soil, microorganism and plant analyzes were performed according to the laws in force, respectively with methods validated by international laboratories or ISO, as follows:*thedetermination of dry substance* was performed according to ISO 751, 1998; *the determination of reducing sugars* was performed by the 3,5-dinitrosalicylic acid (DNS) method; *the determination of proteins*was performed using the Lowry method; *the determination of chlorophyll* was carried out by extraction of chlorophylls with N, N-dimethylformamide; for miner*alization by calcination at 450º* the method Răuță and Chiriac was used, 1980; *thedetermination of sodium concentration* was performed by flame photometry; *the determination of potassium concentration* was

performed by the Egner-Riehm-Domingo method; *the determination of calcium and magnesium concentration* was accomplished by the method described by El Mahi et al. in 1987; *the determination of superoxide dismutase activity* by the method of Winterbourn et al. such as lordachescu and Dumitru; *the determination of catalase activity* was described by lordachescu and Dumitru; *the determination of peroxidase activity* by the Brad method. Soil sampling was carried out according to Order no. 184/1997 and the determination of petroleum products in the soil was carried out according to the method 1664 of the US Environmental Protection Agency.
Isolation, identification and assessment of microorganisms to consume petroleum products were performed by standard microbiological methods of cultivation in test tubes and petri dishes. The assessment of the degradation capacity of the petroleum products was made by determining the content of petroleum products at different time intervals after the polluted soil and environments with known content of diesel, petrol or crude oil were inoculated with the selected microorganisms.

OBTAINED RESULTS

The assessment of the level of pollution with crude oil. In July 2012, an agricultural area in the Icoana village of about 5 hectares was polluted with crude oil following the breaking of a pipeline. For the extraction of the dispersed petroleum product from the soil samples, a mixture of hexane and petroleum ether in a ratio of 1: 1 was optimally used as solvent. The results obtained for the soil samples collected in November showed much greater values of the petroleum products, about 300 times up to 65 times depending on the distance to the area of the accident, as compared to the Maximum Allowed Limit for agricultural lands (MAL = 500 ppm).
Use of microorganisms specific to the polluted area in the bioremediation process. From polluted soil samples taken in autumn 2012, ten complexes of microorganisms were

isolated. Purification and exact identification of strains required specific and long-term assessments of microbiologists specialized in this field. As a result, 10 pure strains, seven strains of Aspergillus, one strain of Penicillium and two strains of Trichoderma were obtained.
Their testing was carried out under similar conditions to a usual field culture and, in order to give the microorganisms the optimum development capacity, each soil sample was administered a corresponding amount of nutrient solution {25 g (NH_4) 3 PO_4, 25 g K_2HPO_4, 2 g $MgSO_4$ and 100 g NaCl and 1 L water}. In soil samples so prepared petroleum products (crude oil, diesel,gasoline) were added and they were inoculated with 10 mg of mycelium. The most active microorganisms selected by us as regards fuel consumption were one strain of *Aspergillus niger* and two strains of *Aspergillus ochraceus* (An, Ao6, Ao7). It has been thus determined that the higher the concentration of hydrocarbons with big molecules is higher, the lower the microorganisms' ability to destroy them. None of the microorganisms we tested failed to completely decompose even gasoline, the lightest of the fuels tested, in the 40-day incubation period at room temperature. The most active strain was *Aspergillus niger*, which manages to decompose gasoline and diesel up to 78%, diesel up to 60% and crude oil up to 50% after 40 days of incubation.
Each strain was tested separately on polluted soil. Our experiences showed that in the year following pollution, that is in the spring of 2013, the contaminated soil contained only very few petroleum products, represented by asphaltenes.
The biodegradation of petroleum products in the soil is influenced by a number of factors such as the composition of these compounds, the soil structure, temperature, precipitation and work taking place on the polluted land. All these factors, plus the synergism of the consortia of microorganisms in the soil, allowed the petroleum products to be practically totally degraded after about 7 months of soil sampling.
The enrichment of the soil with fertilizers in particular organic ones determines the increase in the efficiency of applied or soil microorganisms.
As a result of the results obtained, it should be emphasized that by the bioremediation process, the addition of one or more specific microorganisms with a high capacity to consume petroleum products to a polluted soil, the degradation period is significantly reduced.
In conclusion, in the areas where oil pollution may occur, it is advisable to create a bank of specific, high-activity microorganisms to be inoculated into the polluted soil to enrich the soil microorganisms and to rush the bioremediation process.

Greenhouse experiments in phytoremediation methods

Although in the spring of 2013, asphaltenes are the only ones remained of the polluting petroleum products, the salts that accompanied the oil eruption turned the polluted land into a salt field, inappropriate for the usual agricultural crops in the Icoana Commune. The various studies have shown that the period of remediation of such soils is quite long, depending on the degree of pollution (most of them over 10-13 years from the date of the

pollution), the nature of the soil, cultivated plants, natural physicochemical conditions and the effectiveness of applied treatments (e.g. the nature of the fertilization).
In the greenhouse, experiments were carried out to track the capacity of plants with different tolerances to the electric conductivity to develop and to absorb sodium, potassium, calcium and magnesium ions from polluted soils. To improve the phytoremediation process, to synergistically combine the effect of cultivated plants, an appropriate treatment on soil quality was also initiated by adding a chemically inert material. In this context, besides the unpolluted soil and polluted soil variants, soil variants with added perlite of 25%, 50% and 75% respectively were also achieved.

Experiments with lettuce (EC = 1.3 dS/ m) and potatoes (EC = 1.7 dS/ m).

On the polluted soil, only one of three plants has visibly grown both in lettuce and in potatoes. When lettuce was harvested, only lettuce cultivated on the normal soil formed specific heads. Plants on the polluted soil were small and without inflorescence. Plants grown on soil variants with perlite added did not develop heads as the leaves grew on a high stem;however the leaves of the samples cultivated on polluted soil with 50% and 75% perlite addition had colors close to normal lettuce and a growing number of leaves, in relation to the increase in perlite concentration.

In the case of potatoes, as the added perlite concentration in the soil increased, the appearance of the potato plants approached the appearance of the plants grown on unpolluted soil, that with addition of 50% and 75% perlite respectively, the plants were similar to the ones grown on unpolluted soil. Potato crops obtained per plant were 16.77% on polluted soil, 41.3% on soil with 25% perlite addition, 72.1% on soil with 50% perlite addition and 95.94% on 75% perlite addition versus unpolluted soil production. Also, the potatoes harvested from the polluted soil had a green color corresponding to a high content of solanine, a toxic alkaloid for humans.

From the biochemical point of view, we analyzed the protein concentration in lettuce leaves, potato leaves and potato tubers because high protein concentrations above the average values of plants grown on healthy soil mean mainly high concentrations of enzymes involved in the photosynthesis process and especially of antioxidant ones as a response to toxic stress caused by high concentrations of salts, but low concentrations can also indicate the inability of plants to respond to toxic stress.

In case of lettuce cultivated on unpolluted soil, the protein concentration was similar to the data from the literature (1.62 g/ 100 g leaves). Plants grown on the variants of the polluted soil with perlite added protein content is smaller and grows with the added perlite concentration. This fact reduces the ability of lettuce to adapt to saline stress and to synthesize antioxidant enzymes.

At the same time, however, the sodium content is 1.5 higher in the lettuce obtained on the polluted soil than on the unpolluted soil. This content decreases with the increase of perlite content in the soil.

In the case of potatoes, the high concentration of proteins in the potato tubers cultivated on the different substrates decreases in the order of 2.47g / 100g (unpolluted soil), 2,2 g/ 100 g (polluted soil plus 75% perlite), 2,1 g/100 g (polluted soil plus 50% perlite), 1,9 g/ 100 g (polluted soil plus 25% perlite) and 1,8 g/ 100 g (polluted soil). The protein content of the leaves was higher than in the tubers and decreased in the order of 4.20 g/ 100 g of vegetal material (polluted soil plus 75% perlite), 4.2g/ 100g (unpolluted soil), 3.5 g/ 100g (polluted soil plus 50% perlite), 2.5g/ 100g (polluted soil plus 25% perlite) and 2.2g/ 100g (polluted soil).

The Na content in the tubers exceeded the one in the leaves. Thus, potato tubers contained: unpolluted soil - 28 ppm, polluted soil - 47 ppm, polluted soil +25% perlite - 42% ppm, polluted soil +50% perlite - 35% ppm and polluted soil +75% perlite - 30% ppm. At the same time, in the leaves we obtained: unpolluted soil-51ppm, polluted soil-62ppm, polluted soil + 25% perlite-63% ppm, polluted soil + 50% perlite-55% ppm and polluted soil + 75% perlite-50% ppm.

From the comparative analysis of the data for lettuce and potatoes, we can say that the response of potatos to the polluted soil and to the one with perlite addition is better than with the lettuce, but both vegetables only confirm their low tolerance to electrical conductivity.

However, we must mention that such studies using lettuce and potatoes are extremely scarce, most of them using different herbs with high tolerance to salt or sometimes cereals.

Experiments in the greenhouse in 2016 and 2017 were done with tomatoes. They were chosen because they have a higher tolerance to salt (EC = 2.5 dS/ m), which allows it to behave better compared to the lettuce and potatoes, being often cultivated on extended areas in Icoana Commune.

Experiments with tomatoes (EC = 2.5 dS/ m)

Experiments with tomatoes were carried out by cultivating tomato seedling on unpolluted soil (V0), on polluted soil (V1) (average EC = 2.82 dS/ m), and on polluted soil with 50% perlite added (V2). Tomato yields on the plant compared to the ones on unpolluted soil were 30.2% in 2016 and 36.8% in 2017 on polluted soil, and perlite-enhanced soil yields were 92% and 94.4% respectively.

Tomatoes are the most consumed vegetables, under different forms, all over the world. That is why we need to know how the salinity influences the quality of tomatoes. In this context, we analyzed, in fruits, leaves, strains and roots: dry matter, chlorophyll, sugars, proteins, some minerals specific to saline soils (potassium, sodium, calcium, magnesium) and some enzymes important in the response of plants to stress (catalase, peroxidase and superoxide dismutase).

Literature shows that salted tomatoes grown under saline conditions are of superior quality by the fact that some properties such as dry matter and sugars give a better taste to fruit and have an important role for the market and processing. But increasing the concentration of reducing sugars leads to the lack of enough fiber and starch, causing deficiencies in structure, fruit resistance and the cracking capacity is enhanced.

Data obtained in relation to the entire plant show a higher dry matter, sugar and protein content for plants grown on polluted soil (dry matter -maximum 46%; sugars– maximum

129%; proteins - maximum 53%), followed by the plants grown on polluted soil plus perlite (dry matter - maximum 32%, sugars - maximum 66%, proteins - maximum 24%;), as compared to the values obtained for the plants harvested from the unpolluted soil. The results obtained show that the exposure of plants to salt stress begins with the exposure of the roots to this stress and the whole plant is affected. In the case of tomatoes, the plants' response is the adaptation to produce osmotically active substances, mainly amino acids and sugars, which help to alleviate salinity caused by osmotic stress.

In the case of total chlorophyll the plant response is different. The total chlorophyll concentration is higher in plants harvested from unpolluted soil. The samples collected from the polluted soil had a 26.3% lower chlorophyll concentration and, in the case of samples collected from the polluted soil plus perlite, they were 19.3% lower compared to the concentrations obtained for the plants harvested from the unpolluted soil.

Bioaccumulation of major soil minerals (Na, K, Ca, Mg) by tomatoes shows the degree of different accumulation for each tissue. Data on ionic absorption per overall plant in the two years 2016/2017, in the order V0, V1 and V2 are as follows: Na-63,3/ 72,5 ppm, 628,8/ 542 ppm, 484,5/ 433, 3 ppm; K-237,5/ 185 ppm, 472.3/ 401 ppm, 434/353 ppm; Ca-194,5/ 148,3 ppm, 389,3/ 330,5 ppm, 280/ 234,8 ppm; Mg-258.8/ 183.5 ppm, 548.3/ 452.8 ppm, 455.5/ 342.5 ppm. For all the elements, one can notice the clear difference between the amount of minerals absorbed in unpolluted soil and the quantities absorbed in the variants with polluted soil. In the samples taken from the tomatoes developed on the polluted soil and on the one with perlite, the sodium ion is predominant compared to the other ions.

The abiotic stress, caused in our case by crude oil pollution followed by salt pollution, is responsible for the production of reactive oxygen species (ROS) (H_2O_2, OH*, RO*, ROOH, etc.) species that are toxic to any organism. In this context and depending on the endowment, we determined in the tomatoes harvested on the three types of soil the following activities: peroxidase, catalase and superoxide dismutase. The average values obtained per overall plant in the two years 2016/2017 in the order V0, V1 and V2 are as follows: SOD, U/ mg prot. - 0.74/ 0.69, 1.93/ 1.84, 1.35/ 0.90; CAT, μmolH_2O_2/ min/ mg prot/ ml – 280,43/ 241,49, 407,42/ 390,43, 336,13/ 274,85; POD, μmol ac. asc/ mg protein/ min – 29,24 / 24,74, 39,52/ 29,70, 30,29/ 22,39. Higher activity of catalase, superoxide dismutase and peroxidase in the samples collected from the polluted or polluted plus perlite soil compared to those collected from the unpolluted soil, is a response of the plants to the increase of the oxidative level in plants, stress due to the presence of high concentrations of ion, sodium and calcium.

Field experiments of phytoremediation methods

In 2014, on the experimental areas, delimited on the polluted soil: *tomatoes* (EC = 2,5dS/ m), *peppers* (EC = 1,5 dS/ m) and *green beans(EC=1,0* dS/ m) were cultivated). On the remaining area of about 3.5 ha of polluted land, wheat (*Triticuma estivum*) (EC = 6.0 dS/ m) was cultivated.

On the edge of the field cultivated with wheat, the sea buckthorn shrubs with a maximum relative tolerance of EC = 8.0 dS/ m were cultivated for testing purposes.

However, this year, the endeavour led to a failure, on the one hand the cumulative effect of the salt content in the soil (average EC = 2.82 dS/ m), the basic pH value (7.8), and on the other hand the effect of repeated floods (5 until July). Thus, the tomatoes have a dried-up appearance, the leaves are scarce and small, the stems are thin and dry, and the fruits are small, few and very little in the normal maturation stage. The tomato harvest on the polluted land was 5 less than on the unpolluted land. The peppers had the appearance of normally developed plants but did not have any flowers or fruit. Bean crops looks like a field of weed among which some plants appeared, only leaves, mostly yellow, no fruit. Although the sea buckthorn has a high toleranceto salt, the floods that occurred in the area led to the complete destruction of the shrubs.

In the aforementioned weather conditions only the wheat has grown to maturity. However, the wheat yield on the polluted land wasonly 56.25% compared to the one on the unpolluted land. Evaluating the profit, for the polluted land it is less 780 RON per hectare. As a result, the profit on strip ground 71 (11.45 ha) including the polluted area is practically insignificant, with a corresponding amount of about **31.4 lei per hectare**.

As a result of the many damagesdue to flood, a dam was built on the Vedea River. Thus, successive experiments could be carried out in 2015, 2016 and 2017, under repeatability conditions.

Experiments with maize (EC = 1.7 dS/ m)

The soil of the plots delimited for experiments (plots of 0.3 ha) (three variations) was homogenized with 5 mmperlite, 50%, so in the field we had the three variants of soil: unpolluted soil (V0), polluted soil (V1) and polluted soil with 50% addition of perlite (V2) added. Unlike experiments in the greenhouse, variables occur in the field, especially due to meteorological phenomena, namely precipitation and temperatures, phenomena to be taken into account to have a fair overview.

Although maize grain yields have increased significantly each year, the profits made on the polluted soil have been negative. This was due, on the one hand, to low yields on the polluting soil compared to unpolluted soil (2015-14,3%, 2016-16,1%, 2017-17,7%), and on the other hand, to the significant growth of the yield costs and the decrease of the selling price. At the same time, the yields on polluted soil plus perlite compared to those on unpolluted soil were: 2015-88,4%, 2016-89,5%, 2017-85,3%. Under these conditions, the profits, although less, has positive values compared to those obtained on unpolluted soil: 2015-71,0%, 2016-71,8%, 2017-64,3%.

Besides the economic analysis, we analysed some aspects regarding the biochemical quality of maize grains (dry matter, protein concentration).

The average content of dry matter and protein in maize harvested on the three variants of saline over the period 2015-2017 was:V0-dry matter 87.3%, g prot/ 100 g, dry matter 8.03; V1-dry matter 82.3%, g prot/ 100 g, dry matter 9.1; V2- dry matter 85.3%, g prot/ 100 g dry matter 8.4.

The data show small differences between the values obtained for the maize grains harvested from the unpolluted soil, polluted soil and from the soil with perlite additions. However, relative to the dry substance the protein concentration is higher in the maize cultivated on the polluted soil.

Average values of the sodium, potassium, calcium and magnesium concentrations of maize grain harvested over the three years on the three soil types are: Na - V0 -75 ppm, V1-118 ppm, V2-93 ppm; K-V0-82 ppm, V1-98 ppm, V2-89 ppm; Ca-V0-37.7 ppm, V1-175.7 ppm, V2-119 ppm; Mg-V0-130.3 ppm, V1-110 ppm, V2-119.7 ppm. As it can be seen from the data, the largest absorption of ions is in plants grown on the polluted soil, followed by that of plants grown on the soil with perlite additions. Although the values obtained exceed those commonly considered nutritional, the concentration ranges for these ions are extremely wide, so that the obtained concentrations do not influence the nutritional value of the maize too much, but they help to remedy the polluted area.

The effect of the phytoremediation process on the polluted soil.

Between 2015 and 2017, we watched the effect of maize crop on polluted soil and perlite-enriched soil on Na, K, Ca and Mg concentrations. Pollutant ion concentrations (sodium, potassium, calcium, magnesium) in unpolluted, polluted and polluted with perlite additionsoil for a fair comparative assessment have been relatedto the dry matter of the respective soil variants. Determination of ion concentrations was performed before and after the crop. Thus, the decrease of ion concentrations was: **Na** -2015: polluted soil from 336 ppm to 313 ppm, polluted soil plus perlite from 351 ppm to 265 ppm, 2016: polluted soil from 318 ppm to 296 ppm, polluted soil plus perlite from 258 ppm to 187 ppm; 2017: polluted soil from 290 ppm to 269 ppm, polluted soil plus perlite from 182 ppm to 147 ppm; **K** - 2015: polluted soil from 171 ppm to 158 ppm, polluted soil plus perlite from 174 ppm to 137 ppm, 2016: polluted soil from 253 ppm to 225 ppm, polluted soil plus perlite from 223 ppm to 181 ppm, 2017: polluted soil from 215 ppm to 192 ppm, polluted soil plus perlite from 181 ppm to 145 ppm; **Ca** - 2015: polluted soil from 534 ppm to 510 ppm, polluted soil plus perlite soil from 511 ppm to 459 ppm, 2016: polluted soil from 506 ppm to 468 ppm, polluted soil plus perlite from 453 ppm to 379 ppm, 2017: polluted soil from 464 ppm to 423 ppm, polluted soil plus perlite from 370 ppm to 321 ppm; **Mg** - 2015: polluted soil from 195 ppm to 179 ppm, polluted soil plus perlite from 235 ppm to 211 ppm,2016: polluted soil from 180 ppm to 161 ppm, polluted soil plus perlite from 182 ppm to 150 ppm, 2017: soil polluted from 165 ppm to 153 ppm, polluted soil plus perlite from 154 ppm to 125 ppm. The data show that through the process of phytoremediation there has, clearly, successively, beena decrease in the concentrations of pollutants in the soil.

Also the ability to absorb ions from polluted soil plus perlite compared to the one in the polluted soil was 2,7 – 4,0 higher for the Na ion, between 1,5 – 2,3 higher for the Ca ion and 1,2 – 2,6 higher for the Mg ion.

An exception is the presence of potassium ions, for which the evolution of concentrations could not be traced, depending only on the crop and the addition of perlite in the soil, because during the three years the soil was fertilized with NPK fertilizers.

From these data, the influence of perlite addition is obvious.

The effect of the phytoremediation processes experienced by us on the pH and EC values shows their gradual approach to normal values for agricultural areas. Thus the pH value decreased for the polluted soil from 7,88 to 6,74 and from 7,75 to 6,42 for the soil with perlite additions.

Also, the electrical conductivity (EC) values decreased from 3,84 to 3,38 for the polluted soil and from 3,67 to 2,63 for the perlite-added soil.
By using the perlite, an improvement in overallcontentof salt in the soil, plant uniformity in field maize crops, a soil structure build up, a better development of the soil and a proper drainage of water are achieved.

GENERAL CONCLUSIONS

The experience of assessing the degree of polluting with oil or saltan agricultural area in the Icoana Village Icoana Commune, as well as the capacity of some microorganisms/ plants to absorb the pollutants, alone or helped by the enhancement of the soil quality by the addition of perlite, we can present the following general conclusions:

- ❖ For the extraction of petroleum products, for the determination of their concentrations in the soil is recommended to establish the best solvent for the relevant soil.
- ❖ Ten strains of microorganisms were isolated, purified and identified from the polluted soil. Of these, three complexes (An, Ao6, Ao7) showed high capacities of consuming petroleum products. As a result of microbial activity at about 7 months after the date of pollution, there were only 0,51 and 2,26 g/ kg of asphaltenes in the soil.
- ❖ The application of fertilizers improves the bioremediation process.
- ❖ Polluted soils with high salt concentrations showed high electrical conductivity values (an average of 3.9 dS/ m) in 2015.
- ❖ Lettuce with low relative tolerance (EC = 1.3 dS/ m) could not develop properly. Potatoes (EC = 1.7 dS/ m), on soil with concentrations of 50% and 75% perlite in soil, developed similarly to those cultivated on unpolluted soil. The Na content of lettuce and potatoes was high in crops on polluted soil compared to those on unpolluted soil.
- ❖ Tomato yieldreached an average of 33.5% in polluted soil and 93.1% in polluted soil plus perlite compared to unpolluted soil.
- ❖ Concentrations of dry matter, proteins, sugars, Na, K, Ca, Mg and the enzymatic activities of catalase, superoxide dismutase and peroxidase, relative to the whole plant, are higher for plants grown on polluted soil, followed by tomatoes cultivated on polluted soil plus perlite versus values obtained on unpolluted soil.
- ❖ Concentration of totalchlorophyll in plants decreases in the order of crops on unpolluted soil> polluted soil plus perlite> polluted soil.

- ❖ Average maize yields over the three years are about 16% on the polluted land and 87.7% on polluted soil plus perlite compared to those on unpolluted land.
- ❖ Ion concentrations are higher in maize grains harvested from polluted soil compared tothe other variants. In culinary tests, the products (polenta) obtained with the three categories of maize were virtually not distinguished by the consumers, who found them as being very tasty.
- ❖ Concentrations of pollutant ions in the soil, as well as pH and EC values have decreased significantly in the soil.
- ❖ The presence of the perlite allows drainage of the water in the soil to be much more efficient and rebuilds the soil structure allowing proper development of the soil. Perlite was not used to date for improving the polluted soil

As a result of the obtained results, we consider that over time the polluted soil to which the perlite is added will be remedied in a shorter time lapse than the period of 10-13 mentioned in the literature.