TIME VARIATION OF SOME CHEMICAL AND MICROBIOLOGICAL OIL POLLUTED SOIL PROPERTIES UNDER BIO-REMEDIATION TECHNOLOGY ACCELERATED BY BACTERIAL INOCULUM

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Abstract: *Researches have been carried on in order to elaborate a platform for oil pollution risk assessment, in the framework of the PN 2 Project No.11036/2007 BIOREGIS. a bio-remediation technology accelerated by bacterial inoculum has been applied on a former petroleum park, at Sfinţii Voievozi, Dâmboviţa County. The experiment mainly approaches bio-electro-kinetic remediation techniques to extract organic pollutants from soil by stimulating the direct movement of the contaminants and of the bacteria towards the carbon source pollutants. The technology is insufficiently investigated, especially as in situ real experiments, due to the complexity of the physical and chemical changes undergone by soil and by the contaminants.Some chemical and micro-biological changes are studied in the present paper, in order to asses the soil evolution under the bio-electro-kinetic remediation techniques impact. Laboratory analyses were performed to determine the soil properties, as follows: the organic carbon by the Walkley-Black method modified by Gogoaşă, total nitrogen by the Kjeldahl method, nitrates – potentiometric determination with electron-selective electrode, mobile phosphorus and potassium contents, extracted in ammonium acetate-lactate and colorimetric respectively flamphotometric determinations, soil reaction (pH), potentiometric determination in 1:2,5 aqueous suspension and soluble salts contents, conductometric determination in aqueous extract. Microbiological analyses were performed as well, namely quantitative and taxonomic determinations of bacterial micro flora, using Topping growing medium.The evolution of the soil chemical and microbiological properties has been studied, by sampling and laboratory analyses carried out at different time intervals. Under these circumstances the variation of the soil macro-elements contents had a generally increasing tendency and strong fluctuations, especially influenced by the groundwater level variation due to rainfall. The nitrates content is alarming as it reaches over 900 mg·kg^{-1}, more than 15 times higher than the normal content in vegetable growing soils. The reaction remained slightly alkaline throughout the experiment, with a slight decreasing tendency in the control samples due to ammonium nitrate fertilization. The pH values at the end of the experiment were smaller than the initial ones in all three sampling points.All the samples present slight and moderate salinisation; chlorides predominate. The salinisation intensity alternates between slight and moderate. Sodium chlorine clearly predominates in the soluble salts composition.An increase of the total microorganisms number is noticed, respectively bacteria, reported per gram of dry soil, towards the end of the monitoring period.*

Key words*: organic contaminants, bio-electro-kinetic remediation, soil chemical properties, soil micro-biological properties*

INTRODUCTION

Large areas have been contaminated with different pollutants, as a result of increasing industrial activity, both in Europe and in the whole world. So the interest in assessment, monitoring and, most of all, ecological restoration of these areas is also increasing in the last years. A large range of technologies have been developed , *ex situ* or *in situ*, based on different degradation processes and mechanisms. Choosing a certain polluted soil decontamination method depends on a series of factors, such as: risk management, technical feasibility and applicability, the cost/profit ratio, and the environment, social, and economic impact.

On a strategic level, the ecological restoration and remediation of contaminated sites supports the global durable development and contributes to land preservation as a resource and prevents pollution spreading to the other environment components.

In situ treatments have the advantage that they insignificantly expose the workers and the environment to further contamination, the costs are lower as compared to the *ex situ* technologies, which imply excavation, transport, depositing in places specially arranged, and the risk of an accidental pollution during transport is avoided.

In situ technologies based on biological processes, known as bioremediation technologies, have been more and more applied lately, as they are considered to be the most environment friendly and to have reasonable costs, especially in the case of mineral oil pollution.

Several experiments have been carried out in the frame of PN 2 Project No.11036/2007 BIOREGIS: electro-kinetic remediation, bio-remediation accelerated by bacterial inoculum (which the present paper deals with), and electro-kinetic and bio-remediation. The final outcome of the bioremediation method accelerated by bacterial inoculum was that the organic compound contents decreased by 70% a year after the beginning of the experiment.

MATERIALS AND METHODS

A dellocated technologic park was chosen for the experiment, at Răzvad – Valea Voievozilor, Dâmbovița County, near Târgoviște (Figure 1). The lot was contaminated with a significant oil quantity proceeded from accidental pollution: tanks leaking and cracked pipes.

The bacterial inoculum was first tested in the laboratory. The contaminating agent (crude oil hydrocarbons) degradation efficiency was tested.

An 196 m^2 area was delimited in the lot, from which three samples have been collected, at different time intervals (Figure 2).

The soil profile was homogenized on the 0-70 cm depth. The soil was sampled, in the three designated points, on the 0-25 cm depth. Mineral fertilization was applied, in order to feed the microorganisms. A 15-20% humidity was insured over the whole treatment period. The soil profile was again homogenized, after fertilization and before applying the bacterial inoculum.

Mineral fertilization comprised ammonium nitrate (NH_4NO_3) and N-P complex. The ammonium nitrate contains 20% nitrogen, out of which 10% ammonium nitrogen and 10% nitrate nitrogen. The initial fertilization used 141 kg ammonium nitrate and 33 kg N-P complex, with 20% phosphorus as pent oxide (P_2O_5), 16% active substance. In the coming weeks less fertilizers were used, namely 63 kg ammonium nitrate and 15 kg N-P complex. For an easier and more uniform application the fertilizers were dissolved in water and sprayed on the surface.

The bacterial inoculum is a species mix capable of degrading both aliphatic and aromatic hydrocarbons as well as phenol compounds, clorurated solvents, and different types of oils and fats. The bacteria are not pathogen and use the oil products present in soil as source of carbon and energy. Eventually, they transform the toxic substances in water, carbon dioxide and cellular mass. The bacterial inoculum contains *Mycobacter vaccae*, *Arthrobacter sp.*, and several *Streptomyces* and *Bacilus* stems. Initially 40 l inoculum was applied on a 500 m^2 surface, later reduced to 18 l a week.

Figure 1 The localization of the experimental field

The soil was aerated. The treatment was applied for 80 days. The agrochemical and microbiological soil parameters were monitored over a period of 120 days. Eight sampling – analyzing stages were carried on, on May 3, 15, and 27, June 12 and 26, July 9 and 16, and September 2, 2009.

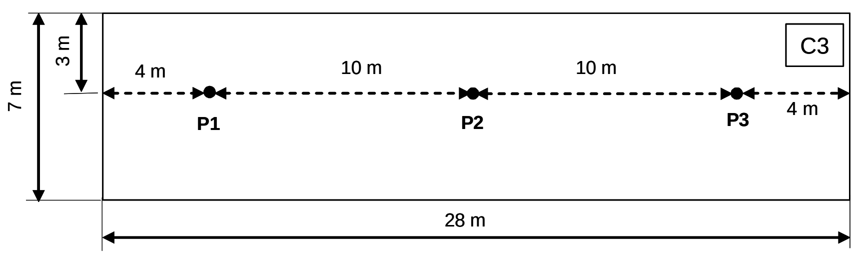

Figure 2 The detailed plan of the area undergoing *in situ* bio-remediation; sampling points are highlighted (P1, P2, P3)

During the treatment period the soil temperature values ranged within the optimum domain limits, 15-25°C. A growing tendency was registered, but with big fluctuations as well.

The soil humidity varied between 17 and 23%, over the minimum allowable value for microorganisms' growth, so no additional moistening was necessary during the treatment.

Laboratory analyses were performed to determine the soil properties, as follows: the organic carbon by the Walkley-Black method modified by Gogoaşă, total nitrogen by the Kjeldahl method, nitrates – potentiometric determination with electron-selective electrode, mobile phosphorus and potassium contents, extracted in ammonium acetate-lactate and colorimetric respectively flamphotometric determinations, soil reaction (pH), potentiometric determination in 1:2,5 aqueous suspension and soluble salts contents, conductometric determination in aqueous extract. Microbiological analyses were performed as well, namely quantitative and taxonomic determinations of bacterial micro flora, using Topping growing medium.

The standard analytical methodology was used, the same that is currently practiced in the RISSA Bucharest laboratories and in those of the County agrochemical an pedological studies Offices.

The analytical results were mostly graphically processed in order to highlight the changes that occurred during the first 120 days of the remediation process.

RESULTS AND DISCUSSIONS

The macro-elements contents dynamics in the soil samples during the monitoring period (May 3 – September 2, 2009)

The organic carbon contents of the samples taken from the experimental field varied very much, from 1.53 to 5.95%, with an average value of 3.16%. These values range within the small up to big content classes, for a medium texture soil (ICPA Bucureşti, 1987). An increasing tendency is noticed, in all three sampling points (Figure 3), with frequent oscillations, due to the unevenness of the oil pollution as well as to the weather variation (especially rain, which modifies water soil regime which, in its turn, drives the polluting residues). The finding is puzzling, as organic carbon contents decrease is the main goal if the remediation process. But the monitoring activity was carried on for only 120 days and the final analyses, performed after 360 days, revealed a 70% decrease of the organic pollutants content.

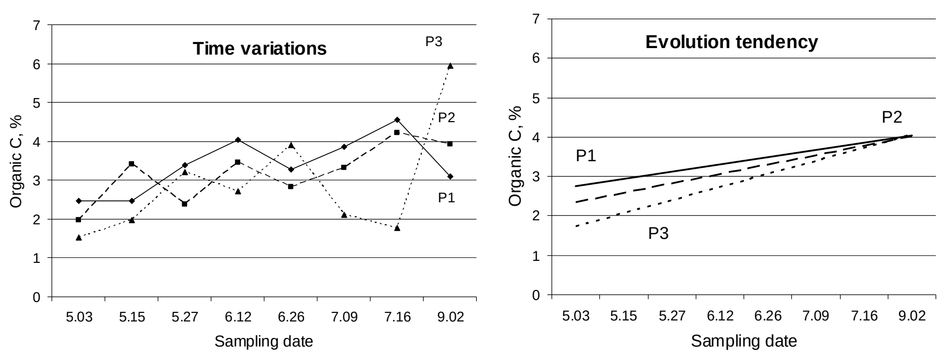

Figure 3 Organic carbon contents changes of the soil samples along the monitoring period and their evolution tendency

The total nitrogen contents vary from 0.108 to 0.243%, with an average value of 0.180%, corresponding to low and average content classes. The values have an increasing tendency in time (Figure 4), due to fertilization, with oscillations due to weather variations (especially rain) which influence the groundwater. The fact can also be noticed that the total nitrogen content values changes are not concordant with those of the organic matter, due both to fertilization and organic carbon belonging not only to the soil organic matter but also to the polluting substances.

Nitrate nitrogen ($N\text{-}NO_3$) contents dramatically vary, from 6.2 to 946.1 $mg \cdot kg^{-1}$. The increasing tendency (Figure 5) is due to fertilization. Nitrate contents variations are not concordant with the total nitrogen ones; that means that nitrates are not entirely proceeded from total nitrogen mineralization. The strong variations are due to the mineralization and leaching processes, influenced by temperature and humidity and by the frequent oscillations of the groundwater level[1]. The fact must be highlighted that most of the nitrate contents are excessive, even toxic, taking into account that the soils have a normal nitrate nitrogen content of 20 mg·kg^{-1}, this content vary between 20 and 40 mg·kg^{-1} in fertilized soils, and reaches 60 mg·kg^{-1} in vegetable growing ones (LĂCĂTUȘU, 2006).

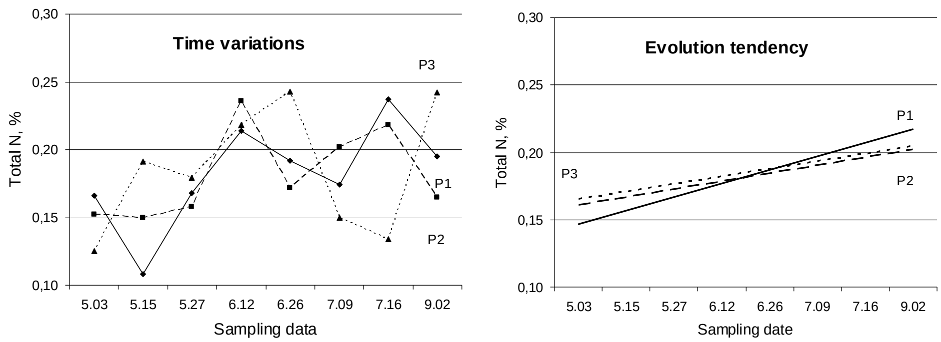

Figure 4 Total nitrogen contents changes of the soil samples along the monitoring period and their evolution tendency

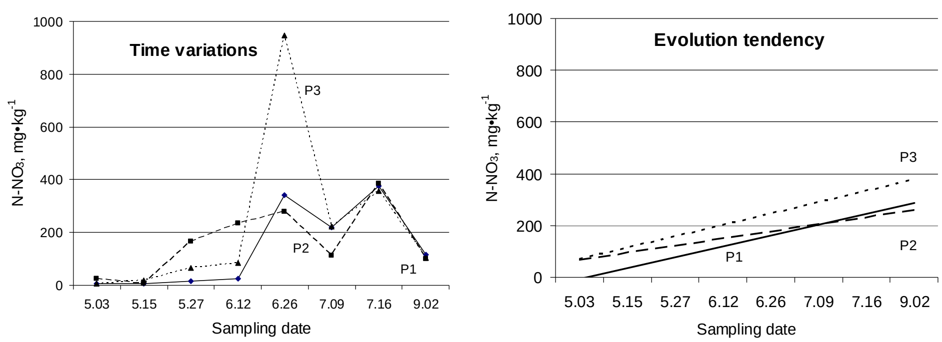

Figure 5 Nitrate contents changes of the soil samples along the monitoring period and their evolution tendency

The mobile phosphorus contents, soluble in ammonium acetate lactate (P_{AL}), vary from 120 to 528 mg·kg^{-1}, with a 285 mg·kg^{-1} average value. They range in the very high content class. Taking into account that this class' inferior limit is 72 mg·kg^{-1}, the contents can be considered even excessive for plant growing. The increasing tendencies (Figure 6) are due to fertilization. In the P2 sampling point, the increasing tendency is very slight, due to a very strong decreasing tendency in the second part of the monitoring. In this case too the values variation has an oscillating character.

[1] The groundwater level was not measured during the tratment period, its fluctuations were only noticed.

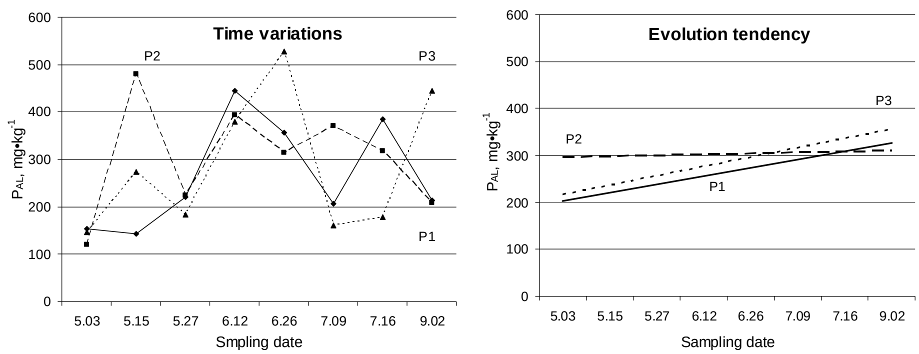

Figure 6 Mobile phosphorus contents changes of the soil samples along the monitoring period and their evolution tendency

The mobile potassium contents, soluble in ammonium acetate lactate (K_{AL}), vary from 202 to 358 mg·kg^{-1}, belonging to the high and very high content classes. The average value is 264 mg·kg^{-1}. There is no obvious increasing or decreasing tendency (Figure 7), and the variations amplitude from one sampling series to another is smaller than for the other analyzed macro elements.

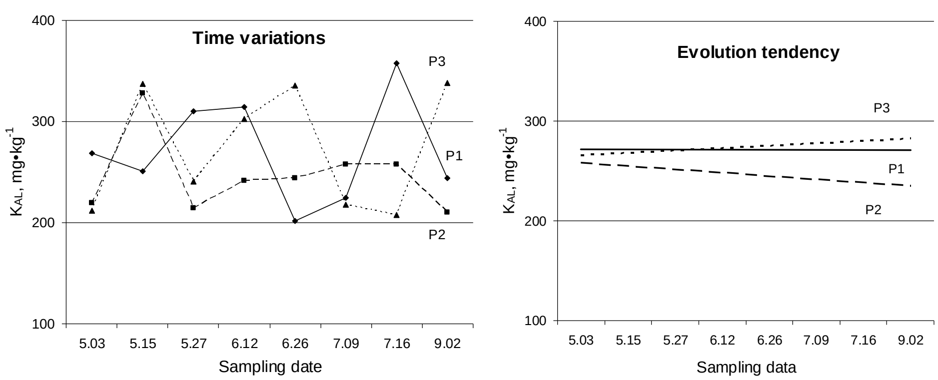

Figure 7 Mobile potassium contents changes of the soil samples along the monitoring period and their evolution tendency

Soil reaction and soluble salts contents dynamics in the experimental field

The soil reaction remained slightly alkaline (pH = 7.25-8.11; RISSA, 1987) through the whole monitoring period, with a slight decreasing tendency of the pH values (Figure 8), due to fertilization with ammonium nitrate.

For a medium texture soil, the salinisation intensity, expressed as total soluble salts content (mineral residue value, mg per 100 g soil, Figure 9), is slight and moderate, chlorides are predominant; no particular tendency is registered and the two salinisation intensities alternate.

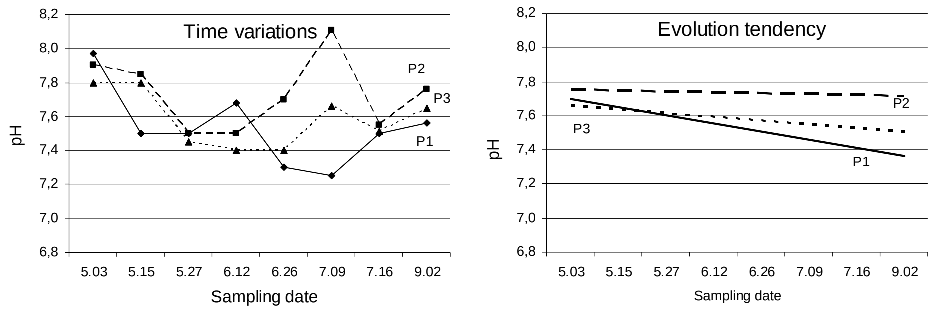

Figure 8 Reaction (pH) changes of the soil samples along the monitoring period and its evolution tendency

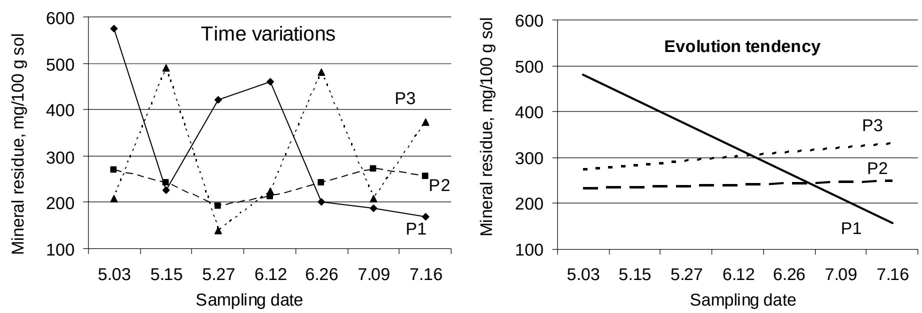

Figure 9 Soluble salts contents changes of the soil samples along the monitoring period and its evolution tendency

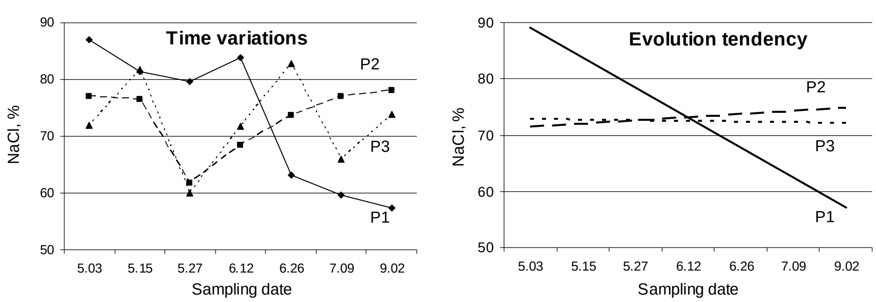

Figure 10 Sodium chloride contents changes of the soil samples along the monitoring period and its evolution tendency

Table 1

Quantitative determinations and taxonomic variety of the three samples of the experimental field

Sampling point	R1			R2			R3		
	Colonies number/ plate	Identified bacterial species and genera (in their frequency order))	TBN x 10^6 cfu/g dry soil	Colonies number/ plate	Identified bacterial species and genera (in their frequency order))	TBN x 10^6 cfu/g dry soil	Colonies number/ plate	Identified bacterial species and genera (in their frequency order))	TBN x 10^6 cfu/g dry soil
P1	50	*Pseudomonas, Arthrobacter globiformis, Arthrobacter citreus, Bacillus megaterium, Mycobacterium roseum*	59,24	35	*Pseudomonas, Bacillus megaterium, Arthrobacter citreu*	41,46	45	*Pseudomonas, Mycobacterium roseum, Bacillus megaterium, Arthrobacter citreus*	53,51
P2	55	*Pseudomonas, Arthrobacter citreus, Bacillus megaterium Mycobacterium roseum, Bacillus circulans, Bacillus cereus, Arthrobacter globiformis*	66,10	39	*Pseudomonas, Arthrobacter citreus, Bacillus megaterium, Bacillus cereus, Arthrobacter globiformis, Actinomicete*	46,87	25	*Pseudomonas, Arthrobacter citreus, Mycobacterium roseum, Bacillus circulans, Arthrobacter globiformis*	30,04
P3	42	*Arthrobacter citreus, Pseudomonas, Bacillus megaterium, Bacillus cereus, Mycobacterium roseum*	50,29	45	*Pseudomonas, Mycobacterium roseum, Bacillus megaterium, Bacillus cereus, Arthrobacter citreus*	53,89	21	*Pseudomonas, Mycobacterium roseum, Arthrobacter citreus, Bacillus megaterium*	25,14

Dilution 10^{-5}

Sodium chloride (NaCl) is predominant in the total soluble salts contents, determined in aqueous extract. The values range from 57.3 to 87.0% (Figure 10). Only one of the three sampled points registered a decreasing tendency, while in the two other ones no clear tendency was highlighted.

Small quantities of other salts were also determined, such as: calcium bicarbonate ($Ca(HCO_3)_2$, 4.6-25.7%); sodium bicarbonate ($NaHCO_3$, 0-17.7%); sodium sulphate (Na_2SO_4, 0-9.7%); magnesium bicarbonate ($Mg(HCO_3)_2$, 0-4.4%), and potassium chloride (KCl, 0.8-2,5%).

Time variations of the microbiological properties of the soil samples from the experimental filed

The total soil microorganisms (bacteria) number increases over the monitoring period, until 65 days, and decreases after other 55 days. Analysis performed on some samples taken later showed a return to the initial increasing tendency of the total number of soil microorganisms. Table 1 presents the analytical results for the samples taken at the end of the monitoring period (September 2).

CONCLUSIONS

Bioremediation accelerated by bacterial inoculum was applied on a dellocated technological park, contaminated with a significant oil quantity proceeded from accidental pollution: tanks leaking and cracked pipes. The agrochemical and microbiological soil parameters were monitored over a period of 120 days.

The macro elements content variation had a generally increasing tendency over the monitoring period and strong oscillations, especially influenced by the groundwater level variation due to frequent rain. The nitrate contents are alarming, as they reach 900 $mg \cdot kg^{-1}$, more than 15 times the normal contents of the vegetable growing soils.

The reaction remained slightly alkaline over the monitoring period, with a slight decreasing tendency due to the ammonium nitrate fertilization.

All the samples have a slight to moderate degree of salinisation, chlorides predominate, especially sodium chloride.

The total number of soil microorganisms (bacteria) steadily grows over the first 65 days and decrease at the end of the monitoring period. Analyses carried on later showed the return of the growing tendency.

Through its fertilization component, the bioremediation treatment accelerated by bacterial inoculum ensures a soil mineral elements supply which could allow its cultivation after the decontamination process.

Still, the excessive nitrate contents urge us to recommend the replacement of the ammonium nitrate (NH_4NO_3) used for fertilization with another nitrogen source, the diminution of the fertilizers quantities and the steady monitoring of this parameter.

Taking into account the initial very high mobile phosphorus and potassium contents, as well as the first's increasing tendency, the diminution of fertilizers quantities is recommended.

Salinisation is also a limitative factor, therefore at the decontamination process' end technologies to decrease salinisation are to be applied.

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