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MATHEMATICAL OPTIMIZATION BY GENETIC ALGORITHMS OF BIOSTIMULATION IN A DIESEL-GASOLINE CONTAMINATED SOIL

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ABSTRACT

This work aimed to determine the optimal concentrations of inorganic fertilizers and the treatment duration in biostimulation of a diesel-gasoline contaminated soil by evaluating the soil biological quality, in an approach of sustainable development. The biological parameters considered in this study are the mineral carbon of the released CO_2 by the microbial respiration (C-CO_2), the microbial biomass and the germination of maize seeds. The optimization using genetic algorithms gave optimal concentrations of fertilizers which permit to restore the biological quality of the soil and to economize 0.627 kg and 0.361 kg of nitrogenous and phosphorous fertilizers respectively, in 1m^2 of soil over 20 cm depth, in only 6-6.5 weeks of the treatment. Laboratory and field experiments yielded results consistent with the optimal solutions given by the genetic algorithms optimization.

KEY WORDS : Hydrocarbons, Soil, Biostimulation, Optimization, Genetic algorithms

INTRODUCTION

The petroleum derived hydrocarbons represent the main energy source for the humanity but they are in turn, an important environmental polluting source (Romaniuk *et al.,* 2007; Doley and Barthakur, 2020). Remediation of contaminated sites can be achieved through physical (e.g. disposal in landfill, incineration), chemical (use of chemical oxidants), thermal and biological processes. The selection of suitable techniques for soil remediation must consider the impact on soil quality, which is of paramount importance for soil development. Bioremediation of soil and groundwater sites contaminated by petroleum hydrocarbons is known as a technically viable, cost-effective, and environmentally sustainable technology (Bento *et al.,* 2005; Silva-Castro *et al.,* 2013; Pucci *et al.,* 2013, Yuniati, 2018; Safdari *et al.,* 2018; Sist *et al.,* 2020). Low nutrient levels limited natural attenuation yields (Mendez-Vega *et al.,* 2007). Nutrient additions can stimulate petroleum hydrocarbon degradation (Wu *et al.,* 2016; Calvo *et al.,* 2019). Several studies have documented positive effects of biostimulation in the attenuation of total petroleum hydrocarbons (Coulon and Dellile, 2003; Bento *et al.,* 2005; Silva-Castro *et al.,* 2013; Ron and Rosenberg, 2014; Bosco *et al.,* 2019; Brzeszcz, 2020). The application of biostimulation to decompose petroleum pollutants has been shown to be a promising technological alternative (Lin *et al.,* 2010; Chikere, 2012; Fukuhara *et al.,* 2013; Simarro *et al.,* 2013). But the use of excessive amounts of fertilizers can lead to surface and ground water contamination, air pollution and have toxicity on worms and microorganisms in soils (Good and Beatty, 2011; Savci, 2012). The fertilizers must be applied at the amounts really needed in the bioremediation process. Thus, the determination of the optimal concentrations of inorganic fertilizers is an important step to avoid excess which may cause other pollutions. This can be done by utilizing mathematical algorithms to optimize a multiple regression model (Huang *et al.,* 2008). For optimum biodegradation conditions, it is important to know the characteristics of the contaminated site before beginning the treatments. Basic information such as concentration of nutrients and treatment duration are needed. That is why, the optimal concentrations

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of fertilizers and the treatment time must be determined. Genetic algorithms (GAs) are nowadays considered as powerful tools of optimization, even for complicated problems (Leardi, 2003; McCall, 2005; Ghaheri, 2015). They are based on the mechanisms of natural selection and genetics. They combine a strategy of "survival of the strongest" with a random but structured exchange of information. For a problem for which a solution is unknown, a set of possible solutions is created randomly. This group is called "population". The characteristics (or variables to be determined) are then used in gene sequences that will be combined with other genes to form chromosomes and afterwards individuals. Each solution is associated with an individual, and this individual is evaluated and classified according to his resemblance to the best, but still unknown, solution to the problem. We can therefore hope, by combining the characteristics of powerful solutions, obtain even more efficient solutions (Cerf, 1996; Bäck *et al.*, 1997; McCall, 2005). They have been successfully applied to a wide range of real-world problems of significant complexity. GAs are a heuristic solution-search or optimisation technique, originally motivated by the Darwinian principle of evolution through (genetic) selection (McCall, 2005). The experiment aimed to determine the optimal concentration of fertilizers and the optimal time needed, in a biostimulation treatment, to restore the biological quality of a soil contaminated with gasoline and diesel, using GA optimization. The biological restoration of the soil was measured by the improvement of seed germination rate, microbial biomass and biological activity which expressed the biodisponibility of hydrocarbons in the soil. The results given by the mathematical optimization were confronted with laboratory and field results obtained after application of the optimal solution.

MATERIAL AND METHODS

Optimization experimental design : The contaminated soil was collected from the surface layer soil (0-20cm) of a gas station situated in Kabylia (Algeria), sifted to 2mm. A biostimulation treatment was applied to stimulate the indigenous bacteria activity, by adding different doses of N and P fertilizers calculated basing on a ratio C/N/P of 100/10/1 (Abid *et al.*, 2014) taking in the account the rate of nitrogen and phosphorus contained initially in the soil (Table 1).

Table 1. Soil properties

Soil properties	
pH	7.2
OC (%)	8.84
N (%)	0.3
C/N	29.47
P_2O_5 (mg/kg of soil)	5.46
CE (mmhos/cm)	0.74
Density (g/cm^3)	1.9
Clay (%)	21.4
Sand (%)	36.5
Silt (%)	42.1

The tested concentrations of fertilizers in 100g of soil were 0.125, 0.25, 0.375 and 0.5g of K_2HPO_4, corresponding respectively to the treatments P1, P2, P3 and P4 and 1.035, 2.07, 3.105 and 4.14g of $(NH_4)_2SO_4$ to the treatments noted N1, N2, N3 and N4 respectively. The fertilizers were added as solutions to 200 g of contaminated soil contained in glass bottles. The soils were kept under ambient temperature during 2, 4, 6 and 8 weeks corresponding respectively to T1, T2, T3 and T4. Each treatment was repeated in 6 bottles. The effectiveness of the treatments was evaluated in laboratory by assessing the soil microbial respiration and biomass and the toxicity on the seed germination of maize (*Zea mays*); a sensitive plant to hydrocarbon contamination (Chaineau *et al.*, 1996).

Biological activity: Soil respiration is commonly used as a relevant indicator of hydrocarbons biodegradation. Depressed biological activity signifies little involvement, while positive response indicates that indigenous microorganisms contribute in the hydrocarbons biodegradation (Margesin et Schinner, 2001), since the microbial respiration measures directly the microbial activity and reflects indirectly hydrocarbons degradation (Lin *et al.*, 2010). The soil treated with different fertilizers concentrations were used to assess the biological activity at the end of the incubation. The soil respiration was undertaken in glass bottles (as described previously). The released CO_2 was trapped in NaOH solution and measured by titration. Each bottle contained a pot filled with water to avoid the desiccation of the soil. This measurement served as fast and relatively easy determinant of the most efficient combination of fertilizer concentration and treatment duration.

Microbial biomass: The microbial biomass is a measure of the living fraction of the organic matter in the soil. It was determined by the fumigation method described by Jenkinson-Powlson (1976) modified by Chaussod and Nicolardot (1982).

Ecotoxicological test A germination test is conducted to evaluate the toxicity of the residual hydrocarbons in the soil using maize seeds. 40 seeds were placed in each Petri-dish containing 100 g of each soil after addition of water to achieve 85% of holding capacity. Seeds were then covered with 90 g of clean sand and incubated at 20 °C during 15d (Winquist *et al.,* 2014). At the end of the test, the number of emerged seedlings was counted. The result was calculated as a percentage of germination.

The contaminated soil was analyzed to determine the initial statute. It was characterized by mineralized carbon (C-CO_2) of 1.28 mg/100g of soil, germination rate of 18.33% and microbial biomass of 0.09 mg/100 g of soil.

Mathematical methods : Multiple regressions, correlation matrix, GA optimization and graphical analysis were performed with R software. A correlation matrix was calculated to determine the linear relationships between the maize seed germination rate, the C-CO_2 released and the microbial biomass. The mathematical optimization of the biostimulation was effectuated by calculating at first, a multiple regression to establish the relationship between the effectiveness of the treatment (reflecting the soil biological quality) as a response variable and the concentration of the fertilizer and the treatment duration as explanatory variables. The obtained linear model was than optimized to get the solutions which were subjected to principal components analysis (PCA) in order to determine the best solution. Finally, all the optimal solutions were represented in a dendrogram to classify them to determine the class containing the best solutions.

Laboratory experiment One of the optimal solutions obtained above is applied in a laboratory test to validate the optimization results. One of the optimal concentrations of $(NH_4)_2SO_4$ and K_2HPO_4 obtained were applied. The contaminated soil (stored at 4°C) was sieved and incubated into bottles containing each 200g of soil to determine the biological activity, the microbial biomass and the percentage of germination of maize seeds after the optimal duration treatment.

Field experiment The optimal solution applied to validate the model in a laboratory experiment is applied in the field in order to estimate the efficiency of the biostimulation in natural conditions. The experiment was carried out in the gas station from which the soil was sampled for the laboratory experiment. The soil analysis for carbon, nitrogen and phosphorus revealed concentrations in approx of those of the soil used in the laboratory experiments. On a parcel situated near the pollution source three soil samples were removed, weighed and the corresponding amounts of fertilizers were calculated. The soil samples were then put in mesh fabric bag, replaced and treated with the fertilizer solution. After the optimal duration given by the model, the soil samples were removed again and were subjected to the same analyzes.

RESULTS

Biological activity The efficiency of biostimulation was evaluated based on the amount of emitted CO_2 expressed by C-CO_2 (mineral carbon). The amount of emitted carbon dioxide by autochthonous microflora in biostimulated samples augmented with time. The best biological activity was observed after 6 and 8 weeks results obtained during this stage were presented in Table 2. The values of C-CO_2 measured were in the soil fertilized with the concentration N3P3 followed by those found in the soil treated with the concentration N4P4. The smaller value of C-CO_2 was obtained in the soil which received the lowest concentration (N1P1) incubated for only 2 weeks. It should be emphasized that the increase in fertilizers concentration is more effective when duration of the treatment is low, but overall, for a treatment of 4 weeks or more, the increase in the dose of fertilizers did not necessarily lead to a more intense biological activity.

Microbial biomass Soil microbial biomass increased with increasing fertilizer dose until the third concentration and then decreased slightly in the soil treated with N4P4. Higher doses stimulated the growth of microorganisms because they have got more nutrients. In all cases, the increasing in duration of treatment induced increasing in microbial activity (Table 2). This value doubled almost every time the duration increased by two weeks especially for the higher fertilizers concentrations (N3P3 and N4P4).

Seed germination When hydrocarbons are degraded sufficiently, the soil becomes less toxic for leaving organisms and for seeds. So, enhancement of the seeds germination indicates that the toxicity is

Table 2. Effect of fertilizers concentration and time duration on microbial activity and biomass and the germination rate of maize seeds.

Treatment	Microbial activity (mgC-CO_2 /100g soil)	Microbial biomass (mg/100g soil)	Germination rate (%)
$T_1N_1P_1$	18.33±1.35	0.28±0.01	62±2.14
$T_1N_2P_2$	26.4±1.55	0.35±0.03	68.33±0.88
$T_1N_3P_3$	43.75±0.83	1.21±0.05	83.5±1.05
$T_1N_4P_4$	38.1±1.37	0.905±0.04	80.66±1.28
$T_2N_1P_1$	36.81±1.01	0.425±0.06	74±0.81
$T_2N_2P_2$	41.4±0.65	1.134±0.1	72.66±0.95
$T_2N_3P_3$	61.6±0.53	2.475±0.22	85.33±1.05
$T_2N_4P_4$	52.6±1.47	1.425±0.1	83.33±1.25
$T_3N_1P_1$	33.7±0.74	0.28±0.01	73.33±1.4
$T_3N_2P_2$	52.33±0.45	1.78±0.05	81.5±1.05
$T_3N_3P_3$	66.73±0.71	5.035±0.25	95.66±1.14
$T_3N_4P_4$	57.33±0.4	2.595±0.08	87.83±1.1
$T_4N_1P_1$	43.45±0.48	1.28±0.1	75.33±2.96
$T_4N_2P_2$	57.81±0.61	2.57±0.21	81.83±0.87
$T_4N_3P_3$	63.06±0.8	3.88±0.13	90±1.48
$T_4N_4P_4$	76.55±3.84	5.835±0.35	90.83±1.4

lowered in the soil. The toxicity of hydrocarbons in the soil was determined by inhibition of seed germination (Table 2).

After 2 weeks, the germination rate was less than 70% in the samples treated with N1P1 and N2P2. It was up to 80% after 2 and 4 weeks in the soils treated with the concentrations N3P3 and N4P4 and for all the tested concentrations after 6 and 8 weeks excepted the first concentration. The germination rate observed after 8 weeks in the soils which received N4P4 and after 6 weeks with the concentration N3P3 exceeded 90%. In these soils the inhibition of seed germination was less than 10%, indicating a very low toxicity due to the hydrocarbons dissipation. The toxicity in the soil decreased during the incubation as measured by seed germination. It must be noted that the highest GR (more than 95%) was obtained in the sample treated with N3P3 after 6 weeks.

Correlation: The biological parameters measured above correlated positively and strongly with each other. The matrix showed good correlation between the germination rate of maize seeds, the C-CO_2 and the microbial biomass (Table 3). This result indicates that when the microbial biomass is enhanced, the microbial respiration is more intensive, indicating an improvement in carbon mineralization. These lead to higher rate of maize seed germination.

Mathematical optimization Since the germination rate of maize is well correlated with the microbial activity and biomass, it was used in the mathematical optimization as indicator of the effectiveness of biostimulation experiment which reflects the restoration of the soil biological quality. The multiple regressions calculated to explain the germination rate of maize seeds by the time duration and the fertilizers concentration are given in Table 4.

Table 3. The correlation matrix of the biological parameters (C-CO_2, Microbial biomass and germination rate)

	C-CO_2	MB	GR
GR	1.0000000	0.8076779	0.8889943
MB	0.8076779	1.0000000	0.9027407
C-CO2	0.8889943	0.9027407	1.0000000

The low probability values indicated that both models are significant. The determination coefficient showed the goodness of fit and suggested that above 83% of the variance is attributed to the variables. The regression models were optimized using genetic algorithms. The fitness function aimed to obtain solutions which gave a germination rate up to 70%, and a population of 80 individuals (solutions) was requested. The individuals of this population are subjected to principal component analysis represented in Fig. 1.

The germination rate was more correlated with the fertilizer concentration than with the time of the treatment. The fertilizer concentration was negatively correlated with the treatment duration. So, when a higher concentration of fertilizers is

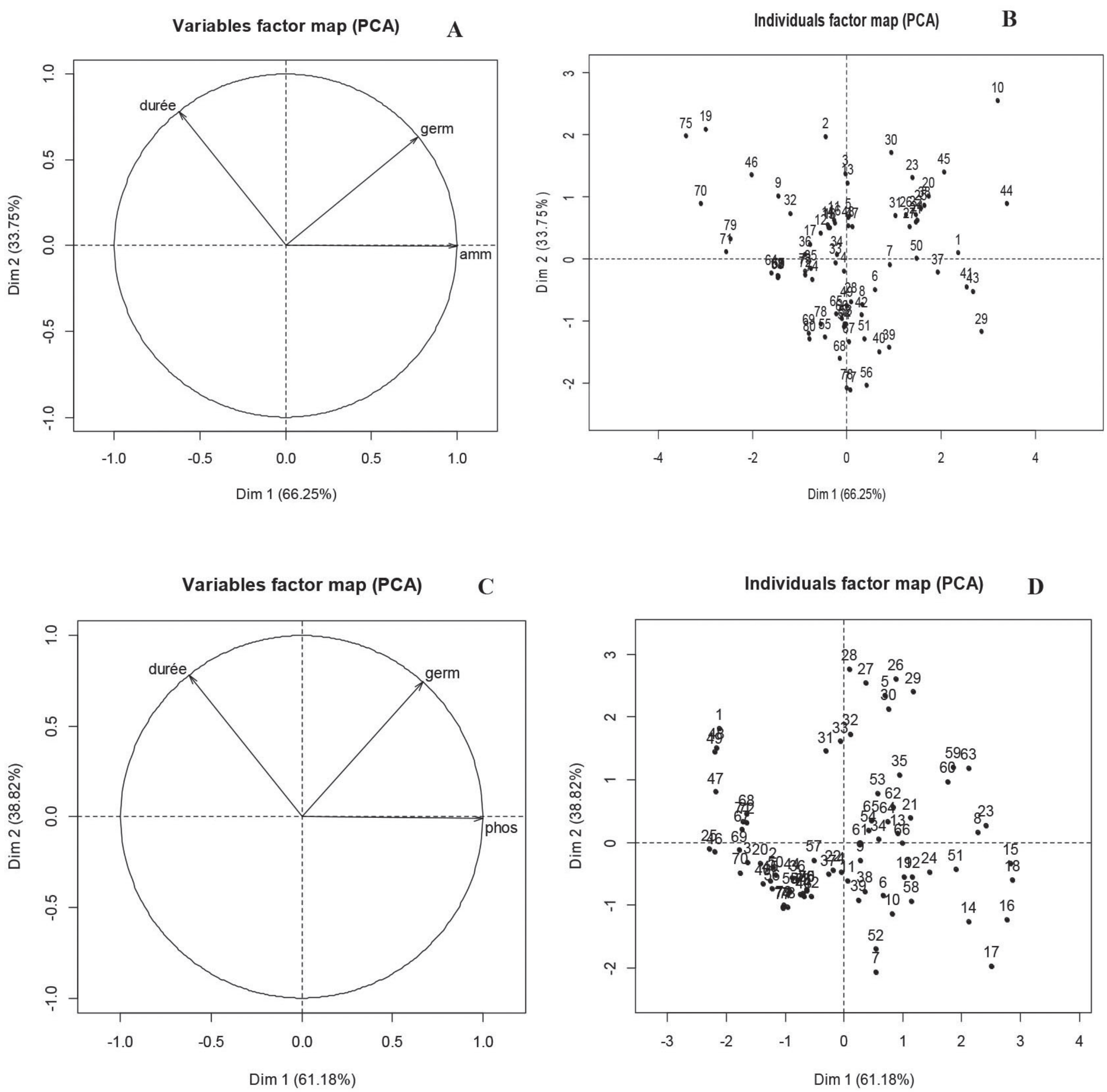

Fig. 1. Principle components analyses. Variables (**A**) and individuals (**B**) factor maps for the first model. Variables (**C**) and individuals (**D**) factor maps for the second model.

Table 4. The calculated regressions between germination rate and the fertilizers concentrations and the treatment duration

Explanatory variables	Regression model	Determination coefficient (R^2)	p-value
Treatment duration and $(NH_4)_2SO_4$ concentration	$GR=2.9933C_N^{***}+1.92D^{***}+55.3^{***}$	0.83	$4.95.10^{-12}$
Treatment duration and K_2HPO_4 concentration	$GR=24.785C_P^{***}+1.92D^{***}+55.3^{***}$	0.83	$4.95.10^{-12}$

GR: germination rate of maize seeds; C_N: $(NH_4)_2SO_4$ concentration; C_P: K_2HPO_4 concentration; D: the treatment duration; the anova showed a very highly significant contribution of the treatment duration, the concentration of each fertilizer and the intercept (***)

applied, less time is needed to improve the germination rate. In the first model, the individual which gave the optimal germination rate was the 10th, characterized by a nitrogenous fertilizer concentration of about 3.975g/100g of soil and a duration time of 6 weeks. In the second model, the best individual was the 26th, characterized by a phosphorous fertilizer concentration of 0.405g/100g of soil and a duration time of about 6.75 weeks. The dendrograms (Fig. 2) showed that the solutions in approx with the optimal solution in the model 1 are contained in the 1st class and those of the second model are contained in the 2nd class. Both the 1st and the 2nd contain 20 individuals.

Laboratory experiment : In the laboratory experiment, one of the optimal solutions was used. An individual was chosen in the class1 of the first model and another one in the class 2 of the second model. Both individuals have treatment duration in approx. So, 7.95g of ammonium sulfate and 0.83g of dipotassium hydrogen phosphate were used in 200g of soil incubated during 6.5 weeks. The obtained germination rate was 86.66%, These results are close to those obtained by the model. The use of

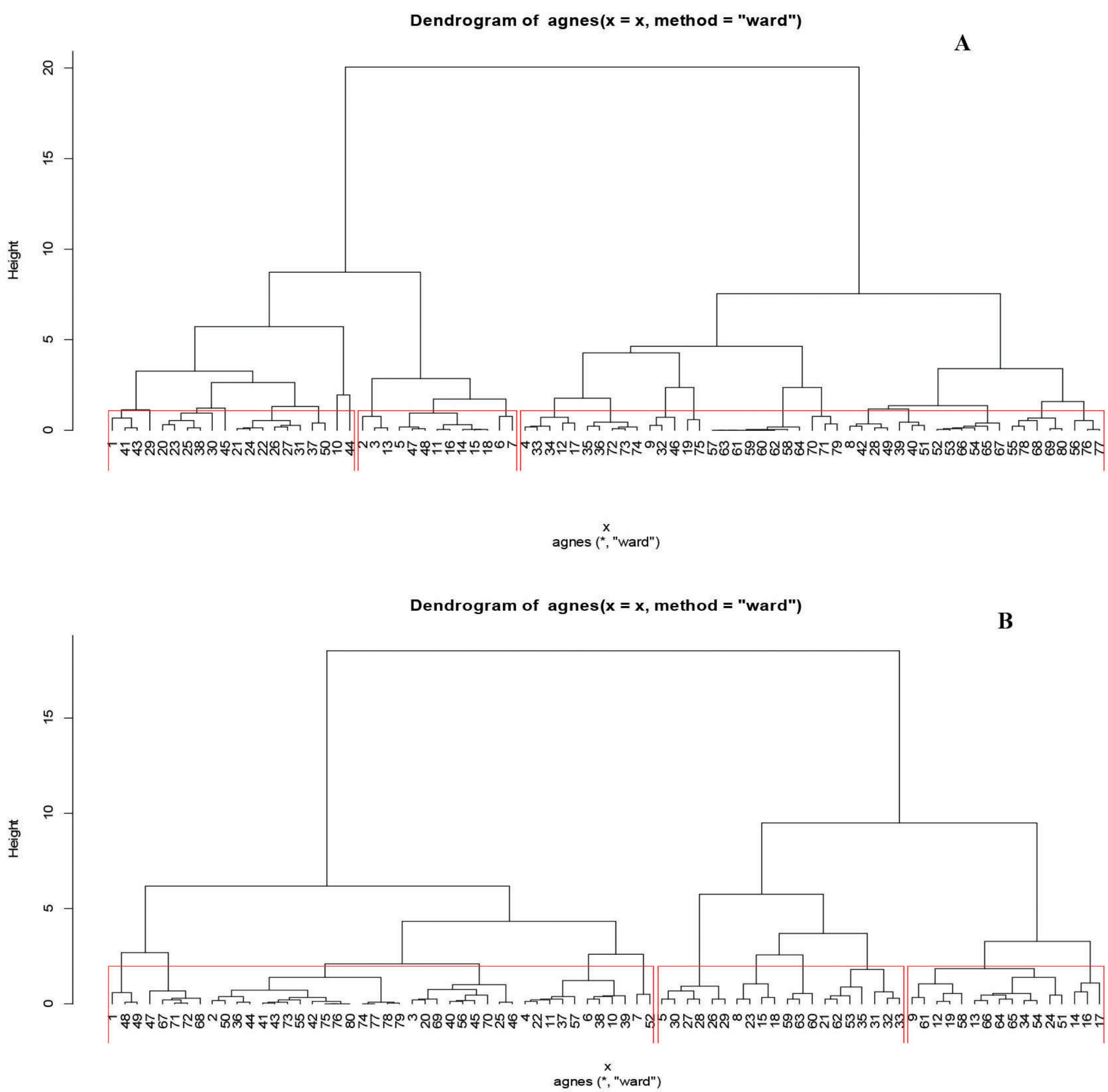

Fig. 2. Dendrograms with classes of the individuals characterized by treatment duration and nitrogen fertilizer concentrations (**A**), or treatment duration phosphorous fertilizer concentrations (**B**).

optimized nutrient and treatment time yielded in results consistent with those generated by the GA. 6 The C-CO_2 and the microbial biomass were about 49mg/100g of soil and 1.92mg/100g of soil respectively (Fig. 3).

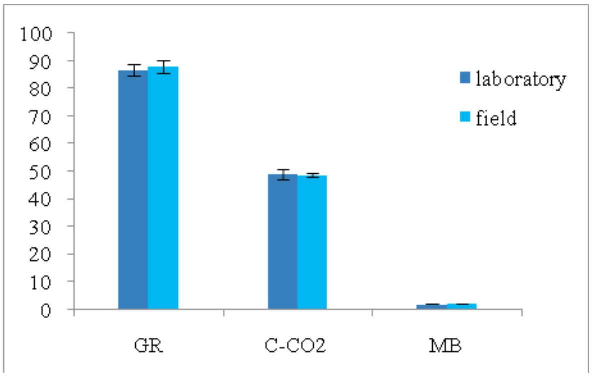

Fig. 3. Results of C-CO_2, MB and GR in the laboratory and the field experiments

Field experiment : After application of the optimal solutions of fertilizers concentrations, the soil was analyzed in laboratory after 6.5 weeks of treatment. The observed germination rate was 88% for maize seeds and the values obtained for the C-CO_2 and microbial biomass were respectively about 48.7mg/100g of soil and 2.15mg/100g of soil (Fig. 3). The results of the field experiment were slightly better than those of the laboratory experiment. The optimized concentrations of fertilizers and treatment duration were efficient even on the field.

DISCUSSION

The application of biostimulation with inorganic nutrients had favorable effect on the microbial respiration, and thus, on carbon mineralization (Braddock *et al.*, 1997; Margesin and Schinner, 2001; Ghaly *et al.*, 2013; Wu *et al.*, 2017; Roy *et al.*, 2018). The microbial population was greater (Margesin and Schinner, 2001; Abid *et al.*, 2014; Wu *et al.*, 2017; Bahmani *et al.*, 2018; Koshlaf, 2019). Several studies correlated the loss in hydrocarbons with the microbial biomass and activity. Siles and Margesin (2018) showed that high TPH removal rate was obtained after addition to the contaminated soil of inorganic fertilization. Safdari *et al.* (2018) found a percentage of hydrocarbon degradation of about 82%. Abid *et al.* (2014) noted relationship between the soil bacterial number and the removal rate of TPH. Yaman et bin Faisal (2020) also found that the biostimulation of a hydrocarbon-polluted soil resulted in the reduction of 74% of TPH with a number of degrading bacteria two order of magnitude higher in comparison to control.

The degradation of gasoline and diesel oil in the soil by the stimulated indigenous bacteria enhanced the germination of maize seeds, since the soil was less toxic. The optimal duration of biostimulation was around 6 weeks. These results are in concordance with those of Wu *et al.* (2016), who observed a loss of 60% of TPH 6 weeks after the application of the biostimulation, and a plateau of degradation in the seventh week. A plateau has been also observed by Rostami *et al.* (2013) around the fifth week of incubation at 25°C of nutrient amended soil. Margesin and Schinner (2001) also noted that the removal of the hydrocarbons deceased with time because of a probable formation of polar compounds and the loss of labile compounds which led to a decline in microbial activity. The GA optimization for the fertilizers gave optimal concentrations of 3.975g and 0.405g/100g soil of ammonium sulfate and dipotassium hydrogen phosphate salts respectively. These concentrations are not the highest tested. Similar results were obtained by Braddock *et al.* (1997). Indeed, the authors have observed the greatest microbial activity at the lowest level of nutrient addition. Ramasdass *et al*. (2018) noted that the addition of the fertilizers at recommended bioremediation levels resulted in significant inhibition of hydrocarbon degradation. The optimal concentrations obtained by the GA optimization permit to economize 0.627kg of $(NH_4)_2SO_4$ and 0.361kg of K_2HPO_4 per m^2 of soil over depth of 20cm (with a density of $1.9g/cm^3$). In the laboratory and field experiments, the results confirmed the efficiency of the optimal solutions calculated by the GA. The high germination rate showed that the soil in both experiments was less toxic to maize seeds. Pelaez *et al*. (2013) found that more than 71% of total petroleum hydrocarbons have been degraded in biostimulation field experiment in less than 7 weeks. These authors have also observed a degradation rate of 72.6% in a laboratory biostimulation treatment after 8 weeks. Suja *et al.* (2014) have noted that the dissipation rate of TPH was higher in the field. This can be explained by the fact that the conditions in the field, which is an open system, are different and the loss in hydrocarbons is not du only to degradation but also to volatilization and leaching. According to Lors *et al.* (2012) the most important diminution in hydrocarbons concentration in the soil was observed between 4 and 8 weeks with a slightly faster degradation in the field of 4- and 5-ring PAHs.

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