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EFFECT OF BIOREMEDIATION ON GENOTOXICITY OF SOIL CONTAMINATED WITH DIESEL OIL

Soil contaminated with diesel oil was made subject to bioremediation conducted in field lysimeters. After 22 weeks the efficiency of the process exceeded 99%. The mutagenic and carcinogenic properties of the soil contaminants were analyzed using the results of the Ames test. The study has produced the following findings. The soil extracts exhibited mutagenic and carcinogenic properties not only immediately after contamination with diesel oil (10 wt.%), but also upon termination of the bioremediation process. The bioremediation process reduced the mutagenicity of the soil. With the exception of particularly sensitive strain of the YG series, the extracts from the bioremediated soil did not increase the number of revertants of the strain of *Salmonella typhimurium* TA 98. Seemingly, upon bioremediation the soil contained either nitro derivatives of organic compounds or any other compounds that are formed during biodegradation of diesel oil. The extracts from bioremediated soil contained compounds requiring and non-requiring metabolic activation. Mutagenic substances were present in the dichloromethane extract from soil alone. Being absent in the water extract, they were not subject to leaching and in consequence posed no ecological hazard.

1. INTRODUCTION

Soil contamination with petroleum products has become an increasingly frequent ecological problem, and the past few years have witnessed significant advances in the technology of removing pollutants of this type [1]. The extent of soil contamination and the efficiency of the remediation process are generally assessed by analyzing (both qualitatively and quantitatively) the composition of the polluting compounds. It is essential to note, however, that the wide variety of the chemicals involved as well as the presence of the metabolites being formed during biodegradation of hydrocarbons seriously limit the reliability of the assessments obtained with the methods mentioned [2], [3]. The properties of the metabolites generated during biodegradation of organic pollutants generally remain unclear or poorly defined. Some of the metabolites may even be

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characterized by a higher toxicity compared with that of the initial substrate; others may exhibit mutagenic or carcinogenic activity [4], [5]. These findings have directed the attention of environmental scientists to the idea of combining instrumental and biological methods for the purpose of soil quality control. Biotests not only provide valuable information on how such pollutants affect living organisms, but they also take into account their synergistic and antagonistic interactions [6], [3]. A biotest is, furthermore, a useful supplement to routine chemical and physical analyses, particularly when the soil is contaminated with multicomponent mixtures. Although many short-term tests are available for genotoxicity assessment, only few of these have been used for the analysis of soil genotoxicity [5], [7]. Among the latter, the test developed by Bruce Ames has found the widest acceptance [8], [9]. The test system proposed by Bruce Ames is commonly used for determining the genotoxicity of single chemical compounds and complex environmental samples [10], [11].

The aim of the study was to assess the influence of the bioremediation process on the genotoxicity of soils contaminated by diesel oil.

2. MATERIALS AND METHODS

2.1. PREPARATION OF SAMPLES

The soil samples were made subject to bioindication tests prior to and after the bioremediation process. Before being subjected to the test, the pollutants present in the effluents from the lysimetric columns were concentrated 3000 times on Amberlite XAD 2 and XAD 8 resins. The soil samples were subjected to extraction with dichloromethane in the Soxhlet apparatus. Upon solvent evaporation, the extracts from contaminated soil were subjected to the test in 0.1 cm^3 of DMSO (dimethylsulfoxide) at concentrations corresponding to 2.5, 2.0, 1.0, 0.5, and 0.25 mg of soil extract per plate.

2.2. BIOREMEDIATION

The tests were conducted in field lysimeters packed with loamy soil monoliths of undisturbed structure and grain size distribution [12]. The soil mass in the column (of a 3000 cm^2 surface area and 80 cm in depth) approached 450 kg. The soil was artificially contaminated with diesel oil and then bioremediated by the in situ method using the following biopreparations: K29 containing foreign strain *Acinetobacter lwoffi* and BIO 1 based on the indigenous microflora of the contaminated soil. The initial content of diesel oil in the soil sample was 10 wt.%. The content of biogenic elements was supplemented with additional nitrogen and phosphorus sources in order to ensure their appropriate proportions to carbon, as required for light soil: C:N:P = 10:1:0.1.

Nitrogen and phosphorus were added in the form of ammonium nitrate, potassium nitrate, ammonium phosphate and potassium phosphate. The tests were conducted for 22 weeks under natural meteorological conditions occurring between July and November, the water level in the ground being made up continually to 60% WHC. In the course of the experiment, the surface soil was oxygenated via agitation. Soil concentration of the petroleum products was determined by gravimetric method and gas chromatography, making use of an N-504 chromatograph with a capillary column (25 cm in length) and a flame ionization detector, under conditions of programmed temperature (30–5 min; 3–1 min; 240–10 min), using helium as carrier gas, and hydrogen and air as detector gas. The temperature of the detector was 300 °C [13].

2.3. AMES TEST

Use was made of the *Salmonella typhimurium* TA 98 and *Salmonella typhimurium* TA 100 strains, obtained from the Ames Laboratory Department of Biochemistry, University of California as well as of the YG 1042 strain, obtained from Professor M. Watanabe, Division of Mutagenesis, National Institute of Hygienic Science, Tokyo [8], [14]. The strains used in the Ames test are nutritional mutants with no ability to synthesize histidine (his^-). The addition of a mutagen to the medium increases the frequency of reversion and accounts for the return to prototrophy, which manifests itself as an increase in the number of revertants in the minimal medium with no histidine [8]. The test was performed in five replications, according to the methods described elsewhere [9], [15]. For the metabolic activation of promutagens use was made of the Aroclor-1254-induced rat liver homogenate; 2,4,7 trinitro-9-fluorenone (TA 98), sodium azide (TA 100 and YG 1042) for direct mutagens, and aminofluorene for indirect mutagens were chosen as positive control. The spontaneous reversion of the test strains of *Salmonella typhimurium* TA 98, TA 100 and YG 1042 was respectively: 37.8±13.2, 92.6±9.8, 118±32.2 (without S9 mix) and 44.3±5.6, 109.5±17.2, 127.5±17.1 (with S9 mix).The results are expressed as mutagenicity ratio (MR), i.e. the ratio of the number of induced revertants (which appeared in the medium with no histidine in response to the contact between the test strains and the soil extracts examined) to the number of spontaneous revertants. According to the procedure, the sample was considered mutagenic when its $MR \geq 2$ and when it demonstrated a linear dose–response relationship.

3. RESULTS AND DISCUSSION

3.1. BIOREMEDIATION PROCESS

The tests have demonstrated that the bioremediation of the soil proceeded at a faster rate when the lysimetric columns had been inoculated with foreign strain of

Acinetobacter lwoffi exhibiting a high degrading activity. The highest extent of diesel oil removal, which was observed during the first three weeks of the experiment, is attributable not only to the activity of the microorganisms, but also to the evaporation of some of the hydrocarbons and to the chemo-oxidation process. The diesel oil concentration in column with *Acinetobacter* strain decreased to 3.0 wt.%, whereas in the lysimeter inoculated with microorganisms of the indigenous microflora of the contaminated soil (BIO 1), the reduction in diesel oil content amounted to 5.8 wt.% (figure 1). After 22 weeks of the experiments, a notable removal of diesel oil was obtained, which was similar in all of the lysimeters. In the K29 column, the quantity of petroleum products decreased to the value of 114.1 mg/kg, and in the BIO 1 column, to the value of 409 mg/kg.

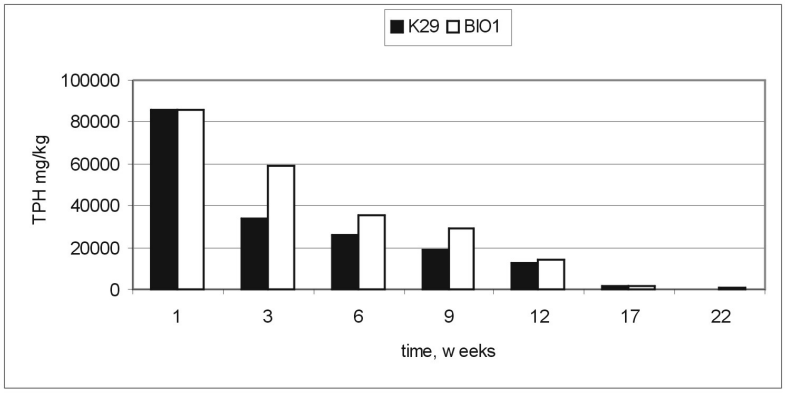

Fig. 1. Average concentration of diesel oil in soil from lysimeters

3.2. GENOTOXICITY ASSESSMENT

No substances of mutagenic or carcinogenic potential were detected in the water extracts from the soil samples analyzed immediately after contamination with diesel oil, or in those analyzed upon the termination of the bioremediation procedure. The calculated values of the mutagenicity ratio were within the range of 1.0–1.9. The substances extracted from the soil with dichloromethane accounted for a rise in the number of revertants of the tester strains. The results obtained have shown that compounds with mutagenic and carcinogenic potential were present in the soil extracts analyzed before (figure 2) and after bioremediation (figures 3 to 4). The extracts from the soils examined were found to include both groups of compounds: those requiring and those not requiring metabolic activation.

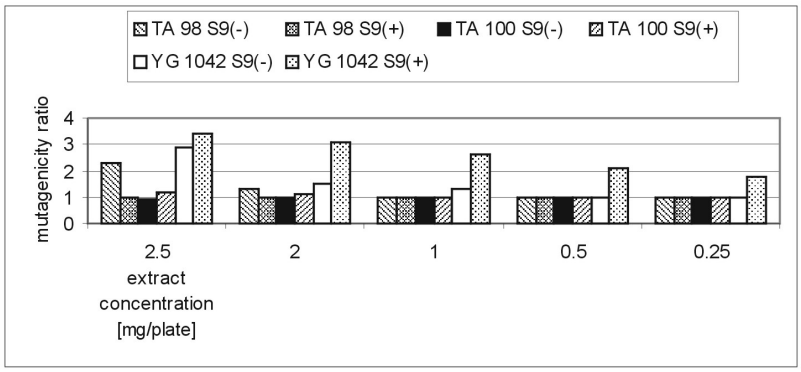

Fig. 2. Mutagenicity ratio of extracts from polluted soil before bioremediation

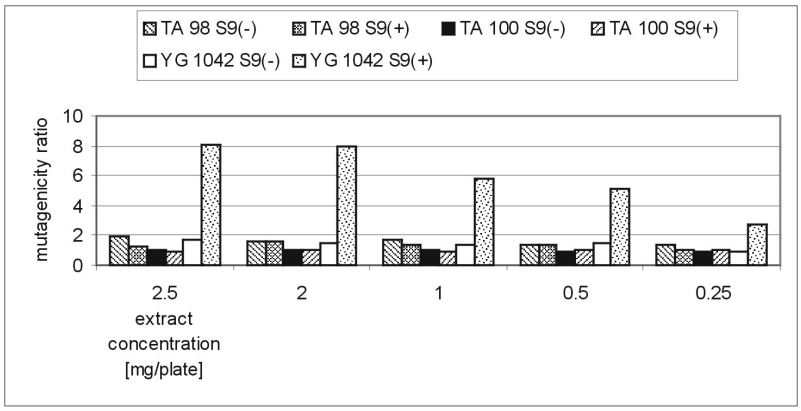

Fig. 3. Mutagenicity ratio of extracts from soil after bioremediation with K29 biopreparation

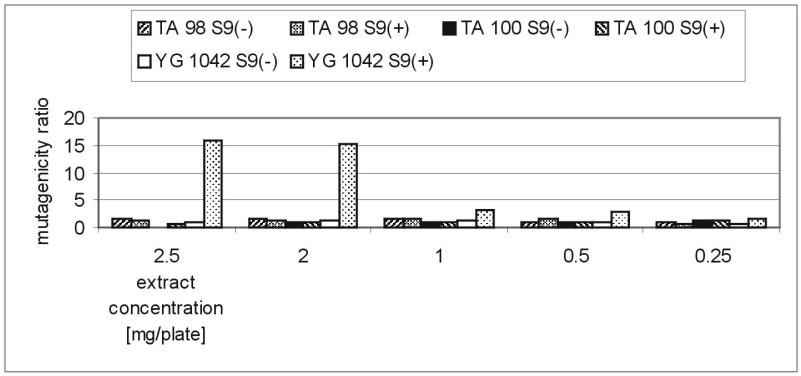

Fig. 4. Mutagenicity ratio of extracts from soil after bioremediation with BIO1 biopreparation

The study has revealed a decrease in the mutagenicity of the soil upon bioremediation. This is to be attributed primarily to the removal of contaminants and, also, to no response of the TA 98 strain, which failed to develop an increase in reversion typical of the response to the extract from the freshly contaminated soil. The new test strain of the YG series was found to be particularly sensitive to the compounds persisting in the soil upon bioremediation; an increase of mutagenicity ratio and linear dose–response relationship were observed for soil before and after bioremediation (figure 5). In some instances, the number of revertants exceeded the relevant number for the extract from the soil before bioremediation. Hence, it can be assumed that upon bioremediation the soils contained nitro derivatives of organic compounds or some other compounds which are formed during biodegradation of diesel oil. It is essential to emphasize the fact that the quantity of the petroleum products detected in the soil by analytical methods was not very high, and thus was not indicative of any threat to living organisms, including humans. The extract content in the soil differed from one sample to another. In freshly contaminated samples, the extract content was high, amounting to 95,000 mg/kg, whereas that in the bioremediated soil was low and averaged 409 mg/kg and 114 mg/kg for BIO 1 and K29, respectively. As can be seen, the health hazard posed by freshly contaminated soil was substantially higher than that posed by the bioremediated soil. Regretfully, our attempt to precisely define the groups of the compounds responsible for the increase in the mutagenic activity of the soil has failed. Presumably, they are products of incomplete biodegradation (or of the biotransformation) of hydrocarbons. According to BROWN et al. [16], direct mutagens, e.g. nitrosamines, may originate in the soil from natural products or from the chemicals being the components of mineral fertilizers or pesticides. The mutagens detected in the soil upon bioremediation belonged to the group that required metabolic activation as well as to the group that exerted a direct impact on the genetic material of the tester bacteria.

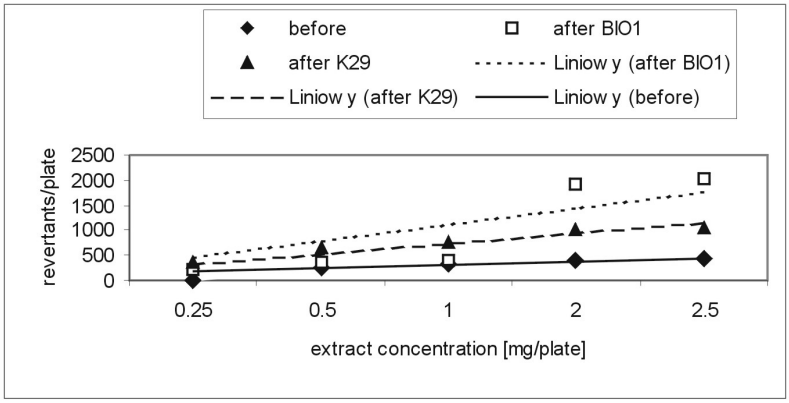

Fig. 5. Effect of soil extracts before and after bioremediation on number of revertants of *Salmonella typhimurium* YG 1042 strain (with S9 mix)

The threat associated with the presence of mutagenic substances in the bioremediated soil was comparatively low, as they were strongly bonded to the soil, which prevented their leaching from soil to water. No mutagenic substances were detected in the soil extracts. It seems advisable, however, to take into account the possibility that the physicochemical properties of the soil will undergo changes favouring desorption of mutagenic substances from the soil and their release into the soil solution.

And this implies that bioindication tests should be conducted not only on contaminated soil, but also on reclaimed grounds, particularly if they are to be used for agricultural or recreational purposes. Agricultural and recreational uses are not recommendable for the bioremediated soils being examined in our present study because of the possibility that their components with mutagenic and carcinogenic potential will then be released into the environment.

The increase in soil mutagenicity upon bioremediation has also been observed by other researchers [5]. They have demonstrated that this increase was not correlated with the content of PAHs, and that it should be attributed to the formation of new mutagens during PAH biodegradation. BROWN, DONNELLY and THOMAS [17] conducted bioindication tests on agricultural soils. In their tests, the mutagenicity of 1 gram of soil was comparable with the mutagenicity of the extract from 1 cigarette. According to these investigators, the mutagenicity of the soil is related to past agricultural practices (such as the application of biocides and fertilizers) or to the contamination from the exhaust gases emitted by the vehicles used for soil cultivation [17], [18]. The increase in the quantity of the mutagens migrating in the soil together with precipitation water has been reported by BROWN and DONNELLY [16]. They have found that the leachate from the soil analyzed immediately after contamination with petrochemical wastes posed a lower hazard than did the leachate from the same soil analyzed after 100 days. Seemingly, the biological processes occurring there accounted for the release of hazardous metabolites. It can also be assumed that the impact exerted by the hydrocarbon mixture differed considerably from the expected one. As a consequence of the antagonistic interactions between the components of the mixture, the biological properties of some compounds may have been masked. It was possible to define them only after the majority of the readily biodegradable waste components had been removed. Our studies have confirmed the observations made by BISTO [19], who has demonstrated that the assessment of soil mutagenicity requires that the pollutants should be extracted not only with water but also with organic solvents.

In the past few years, an increasing significance has been attached to the fact that the reduction of petroleum product concentration in the soil fails to guarantee the desired quality of the soil. Despite all the efforts made by technologists to upgrade the soil decontamination, complete cleanup is still infeasible. Some part of the non-readily biodegradable components as well as those strongly bonded to the soil matrix persist in the soil. It can furthermore be expected that the metabolites originating as a result of biodegradation also persist in the soil. Qualitative identification of the hydrocarbons (as well as

their metabolites) persisting in the soil will not be fully reliable when performed with instrumental methods only, all the more they occur in a wide diversity and at trace concentrations. It is also difficult to assess the biological properties of the polluting compounds accumulated in the soil. In this context, wider significance is now being attached to the ecotoxicological assessment of the soils that are to be bioremediated.

4. CONCLUSIONS

1. The bioremediation process was efficient and accounted for an almost complete removal of diesel oil from the soil.

2. Soil extracts contained genotoxic compounds regardless of whether they were analyzed immediately upon contamination or after the bioremediation process.

3. The environmental impact associated with the presence of mutagenic substances in the soil examined was comparatively low, which is attributable to the following facts:

(a) the substances in question have not been leached from the soil by precipitation water,

(b) the content of substances extractable with dichloromethane was low in the bioremediated soil, averaging 409 mg/kg and 114 mg/kg for BIO 1 and K29, respectively.

4. There is an unquestionable need to perform bioindication tests not only of contaminated but also reclaimed soils, particularly if they are to be used for agricultural or recreational purposes.

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WPŁYW BIOREMEDIACJI NA GENOTOKSYCZNOŚĆ GLEBY SKAŻONEJ OLEJEM NAPĘDOWYM

Bioremediację gleby skażonej olejem napędowym prowadzono w lizymetrach polowych. Skuteczność procesu oczyszczania po 22 tygodniach wyniosła ponad 99%. Właściwości mutagenne i rakotwórcze zanieczyszczeń zawartych w glebie analizowano na podstawie wyników testu Amesa. Wykazano, że zarówno ekstrakt z gleby świeżo skażonej olejem napędowym (10% mas.), jak i z gleb po bioremediacji miał właściwości mutagenne i rakotwórcze. Stwierdzono obniżenie mutagenności gleby dzięki bioremediacji. Ekstrakty z oczyszczonych gleb nie zwiększały liczby rewertantów szczepu *Salmonella typhimurium* TA 98, a jedynie szczególnie wrażliwego szczepu testowego z serii YG. Można przypuszczać, że w glebach tych po oczyszczeniu były obecne związki stanowiące nitrowe pochodne związków organicznych lub inne związki powstające w procesie biodegradacji oleju napędowego. W ekstraktach z oczyszczonych gleb stwierdzono obecność zarówno związków wymagających, jak i niewymagających aktywacji metabolicznej. Substancje mutagenne były obecne jedynie w dichlorometanowych ekstraktach z gleby, ale nie w wyciągu wodnym. Nie podlegały zatem wymywaniu wodą i dlatego nie stwarzały potencjalnego zagrożenia ekologicznego.