Romanian Biotechnological Letters *Vol. 15, No. 6, 2010*

ORIGINAL PAPER

Rapid evaluation by UV-Vis and FT-IR spectroscopy of DINOCAP residue in soil: Microbiological implications

Received for publication, September 25, 2010
Accepted, December 16, 2010

ELENA RUSU[1], STEFANA JURCOANE[2], GHEORGHE RUSU[1]
[1] *"Petru Poni" Institute of Macromolecular Chemistry, Aleea Grigore Ghica Voda 41A, Iasi-700487, Romania*
[2] *University of Agronomical Sciences and Veterinary Medicine Bucharest, 59 Marasti, 720229, Romania*
**Corresponding author: Elena RUSU, e-mail: erusu@icmpp.ro, Tel. +40217454*

Abstract

Two spectral methods, namely UV-Vis and FT IR were chosen in order to evaluate the dinitrophenol pesticide DINOCAP. The results obtained by the two spectral methods were similar and they have confirmed the efficiency of bioremediation treatment with bacteria strains of Pseudomonas genus.

Keywords: nitrophenol derivatives, DINOCAP, *Pseudomonas*, FT-IR, UV-Vis

Introduction

The structure of nitrophenol derivatives gives them valuable properties for a large area of applications pertinent to biomedical, pharmaceutical, industrial, agricultural and defence fields, but at the same time it renders also them potential hazardous to the environment. The toxicity due primarily to their ability to uncouple the cellular energy production (with allergic, cytotoxic, mutagenic and cancerogenic effects) and the persistence in environment justified the interest for the determination and detoxification of these compounds [1-10]. The degradation by soil microorganisms is the primary factor in the fate of pesticides in the environment. Living organisms (bacteria belonging to genera *Flavobacterium, Pseudomonas, Moraxella, Arthrobacter, Nocardia, Bacillus* etc) due to their metabolic versatility and evolutionary potential are able to transform many organic pollutants. Different bioremediation techniques have been developed for cleaning up contaminated sites using particular bacteria. The biodegradation of nitroaromatics is affected by several factors. Firstly, the presence itself of NO_2 protects the nitroaromatics from initial attack by oxygenases, but is favourable for reduction. Under anaerobic condition nitroaromatics often either persist or become amine end product in the environment. The chemical structure of nitroaromatics influences its biodegradability; the symmetric location of nitro groups on the aromatic ring limits attack by enzymes involved in the microbial metabolism [11-14].

The diversity and complexity of methods for assessment of toxic compounds increase and become open to greater .scientific debate [14-15]. The difficulties on the quantitative chemical analysis of organic pollutants in environmental samples are linked to both the inherent complexity of these natural matrices and the low analyte levels to be quantified. The fate pathways of pollutants in soil environments are not yet well understood and sometimes, the basic processes have not been evaluated at all. The physical, chemical and biochemical processes with synergistic or antagonistic effects contribute to the variety of products as such or bound irreversibly to soil components and implicit to measurement errors. A survey of analytical methodology developed for estimation of organic pollutants in environmental samples shows that their preliminary separation, such as solvent-extraction is necessary. The

determination of organic pollutants residues in environmental samples is largely performed by relatively expensive and often laborious techniques such as gas (GC) and liquid (LC) chromatography combined with specific detection schemes or liquid scintillation counting (using ^{14}C labelled product in the aromatic ring) [14,16,17]. However, simple, cost-effective and rapid tests for detecting nitroaromatics residues in soil are necessary. Colorimetric and spectroscopic (UV-Vis, fluorescence, FT-IR, Raman) methods together or separate can be used in this sense as well as conventional methods. In spite of their poor selectivity, particularly when similar chemical compounds must be analyzed in complex samples the spectroscopic measurements are useful for small pollutant concentration [17-21]. Molecular absorption spectroscopy has been extensively used for the quantitative determination of compounds in different formulations as well as for the analysis of synthetic mixtures. The use of these techniques have the inherent disadvantage that most active compounds absorb in the UV region and exhibit strongly overlapped spectra that impede their simultaneous determination. The Fourier Transform Infrared (FTIR) spectroscopy allows the analysis of a relevant amount of compositional and structural information concerning environmental samples [22]. The analysis can be performed both on pure compounds and complex multicomponent mixtures, without separation into individual components. IR spectrometry is more sensitive and selective than colorimetric methods. Moreover, FT-IR spectroscopy is an established time-saving method to characterize and analyze microorganisms and monitor biotechnological processes [23].

DINOCAP (Karathane - Rohm and Haas Co., trade name), contact fungicide is a mixture of crotonate of alkyldinitrophenols, accompanying alkyl substituted isomer and aromatic hydrocarbon, as solvent. The 2,6-dinitro-4-octylphenylcrotonate, 2,4-dinitro-6-octylphenyl crotonate (in which "octyl" refers a mixture of 1-methylheptyl, 1-ethylhexyl and 1-propylpentyl groups) are the two important components. It was used frequently in agriculture together other pesticides of dinitrophenol type, such as DNOC and DINOSEB in EU countries. The use of pesticides has been become a must have for a modern agricultural purposes in order both to optimize production and for disease vectors control. The ester group increases the transport rate of the compound to the active site but has no effect on the toxic mechanism [24-26]. The values of the octanol-water partition coefficient, at pH 7 and 20^oC, log P_{ow} is 6.55 and 6.45 for the 2,4-dinitro and 2,6-dinitro isomers, respectively suggested that DINOCAP is strongly hydrophobic and is readily adsorbed onto mineral or organic particles. The degradation study reported that resistance to hydrolysis, biodegradation and photolysis decreases with increasing of pH and environment temperature. At pH 7 the half-life of isomers is about 30 days [27]. Only few works related on the DINOCAP analytical determination are reported. Through the complex make-up of DINOCAP the analysis is challenging among large group of pesticides of nitrophenol type. The study related to effect of DINOCAP on nitrogen transformations in soil revealed that DINOCAP caused stronger inhibition of ammonification [28].

The exact determination of DINOCAP is extremely difficult due to the existence of two main compounds, many isomers and, in addition, remains reactants (with identical ring substituents) from the synthesis which appear in the free phenolic form. Thus significant systematic errors to the evaluation of the areas of the chromatographic peaks appear because when the crotonyl groups on of the DINOCAP esters are methylated yield the same products as from free phenol impurities. The method commonly used for the determination of DINOCAP residues requires careful evaporation of the extracting solvent, steam distillation, extraction of the distillate, evaporation, and finally colour development with a pyridine-water or ethanolic tetraethylammonium hydroxide reagent. It is also observed that DINOCAP forms an intense yellow colour when dissolved in N,N-dimethylformamide without the addition of alkali. The spectral procedures related to phenol derivatives presented in the

literature are very few in numbers. A system gas chromatograph/Fourier transform infrared spectrometer/mass spectrometer was also reported for the determination of DINOCAP [17,25,29,30].

Previous reported results in literature motivated us to study DINOCAP-contaminated soil samples by isopropanol extraction followed by UV-Vis and FT IR spectroscopy. It was shown that monitoring of the conversion of nitro aromatic compounds can be investigated by the two spectral methods. Assessments on the presence of DINOCAP were made on soil samples taken at different stage of experiment and the results are presented herein. The samples were classified as function of treatment step and denoted S1–S5. There was no information about the history and composition of the soils. The results confirmed the potential for metabolism of DINOCAP in soil and efficiency of bioremediation treatment.

Materials and methods

Experimental

Laboratory studies were carried out with arable soil (surface layer 0–10 cm) collected at long-term monitored sites approximately 5 km east from Tecuci, Romania. Intensive and diversified agriculture is observed in this region favoured by its geographic position.

Soils were sampled and manipulated according to ISO 10381-6 (1993). The soil sample, after removal of living material (such as mosses, roots, stones etc.) was crushed to pass a 2 mm mesh sieve and was mixed for preparing "mean sample". The sample materials, before analysis, were kept under normal room conditions with minimal temperature and humidity fluctuations, shielded from incident light. At each sampling, two soil samples were taken per subplot and the analyses were performed as soon as possible. The product used for soil treatment was DINOCAP in its commercial formulations DINOCAP available as an emulsion concentrate containing 350 g aiL^{-1}. In accordance with its ATR FT IR spectrum and literature [25] the band intensity associated with $\nu_{sim}(NO_2)$ at 1345 cm^{-1} and $\nu_{sim}(NO_2)$ at 1545 cm^{-1} being almost equal the product contain predominantly the isomer 2,6-dinitro-6-octylphenyl crotonate (Figure 3). Pesticide treatment schedule has consisted in application of 0.2% DINOCAP aqueous solution (assuming a uniform distribution in the upper 5 cm of the soil and a bulk density of 1.5 g cm^{-3}). The subsequent treatment, consisting in application of a bioremediation solution containing bacteria strains of Pseudomonas genus (known for their ability to grow in media with nitro aromatic compounds as the sole nitrogen source). Tested pesticide concentration for our study was derived from doses that reach soil during application. Soil samples were taken at the start, for untreated (S1) and treated sample with DINOCAP (S2) and at the chosen period –3 day after pesticide application (S3), 6 days after pesticide application (S4) and 3 days after bioremediation treatment (S5).

The isopropyl alcohol was chosen for the extraction procedure, because it is well known its toxicity in bacteria, characteristic important for extraction of samples after bioremediation procedure. From each soil sample 15 g was extracted two times, for 2 h with a volume of isopropyl alcohol equal to two times the weight of the sample for recovery of analyte. The process was performed in an extraction column equipped with frit, cotton layer, tap and stopper. The extracts of each sample were filtered off using a 0.45 micron filter, mixed and centrifuged at 2000 rpm at 20 ^{o}C for 10 min and the supernatants were decanted.

Measurements

The soil samples were characterized by elemental analysis and the results revealed that they contain about 6.22% organic matter and had the nitrogen and moisture content of about 0.04% and 12.38% w/w, respectively.

The UV-Vis spectra of extracts were acquired using SPECORD M42 Carl Zeiss Jena spectrophotometer in a matched pair of 10 mm quartz cells fitted with poly(tetrafluoroethylene) stoppers. Quantitative data were determined by measuring the heights of the absorbance bands [31]. The maximum absorption band at about 258 nm correspond to the band associated with benzene ring and can be interpreted in terms of the strong electron-attracting nature of nitro group that decreases the π-electron density at nucleus.

The stock solutions of DINOCAP (ca. 1.4 g L^{-1}) were prepared from the DINOCAP as received in isopropyl alcohol. With the purpose of having DINOCAP concentrations in conformity to Beer's law, from these solutions, more diluted isopropanol working solutions (ranging between 0.280 and 0.072 g L^{-1}) were obtained before the absorption measurements. Two separate calibration sets of 8 samples were prepared by measuring different volumes of DINOCAP working solutions into 25.0 mL calibrated flasks, and diluting to the mark with blank solutions. The calibration solutions were analyzed within an 8-h time period following preparation. It is important to point out that the absorption spectrum of isopropanol does not interfere with those of DINOCAP in the useful working range. The absorption spectra of soil samples as isopropanol extracts were read in the range 200–500 nm at varying concentrations of DINOCAP. The absorbance of the samples solutions against isopropanol blank at 370 nm were recorded for three repetitive measurements for each sample. The peak absorbance then, were plotted versus the concentration to generate calibration curve. The calibration establish the relation between spectra and reference component concentrations from a set of standard samples, and then the calibration results are employed to estimate the component concentrations in unknown samples. The curves were fit to a straight line according to an equation $A=0.8372C+7.6\times10^{-2}$, where C is the concentration of DINOCAP (in g L^{-1}) in the solution and A is the absorbance at 370 nm measured in a 1 cm quartz cell (correlation coefficient: r = 0.9997). These results provided evidence that this detection method will work for other samples.

The transformation degree of pesticide was obtained by forestalling the reduction in the absorbance for a sample in time.

$$\% \text{ transformation} = (A_o - A_t)/A_o$$

The ATR FT-IR spectral measurements were performed using a Bruker Vertex 70 spectrometer equipped with a single reflection diamond ATR crystal with an incidence angle of 45°. Transmission spectra were obtained using KRS-5 windows. For each spectrum, the average of 100 successive scans, over the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1} was recorded. This range covered the nitro and water bands at 1350 and 1530 cm^{-1}, 1640 and 750 cm^{-1}, respectively and the soil ''fingerprint'' around 800–1200 cm^{-1}. Software OPUS 6.0 (BRUKER) was used for data processing, which was baseline corrected by rubber band method with CO_2 bands excluded. The entire spectra were normalized. In order to follow the possible conversion of nitrocompound by bacteria, it is necessary to record the spectra of the initial media and select the specific/characteristic absorption bands.

Results and Discussion

In the case of contaminated sites the investigation of the behaviour of systems appears to be more realistic, but spatial variation of pesticide concentration is very large. By contrast, the laboratory experiments including microbial degradation of pollutants are typically studied using samples supplied with a single pollutant. The findings can provide a basis for a framework in which field soils containing pesticide can be evaluated. Even in this case the complexity of soil matrix contributes to different behaviour of pollutants. The fate of pollutants in soil environments are not yet well understood because synergistic or antagonistic effects of many and complex physico-chemical or biochemical processes, such as adsorption,

binding to components of soil, reaction with other compounds, biodegradation etc can modify specific pollutants properties.

The analysis of soil sample can be performed on as received sample or on extracts. The ideal method of isolating organic compounds from soil is extraction because organic solvent leaves behind the insoluble inorganic salts and humic and fulvic acids [32]. The spectral analysis of samples are made on the basis of the magnitude and relative intensities of the recorded spectra and in the analogy with the assignments made by other researchers on the similar type of nitro compounds. The accuracy of nitro evaluation increase using the second-derivative spectra.

DINOCAP shows an absorption maximum at 240-250 nm but absorb weakly in the UV-B (near 290 nm) region due to the extended tailing of this absorption and to weak, longer wavelength-induced transitions (Figure 1). In the UV-B region, it is likely that both $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions are occurring. The analysis of the DINOCAP mixture was performed from the absorbance value at about 370 nm.

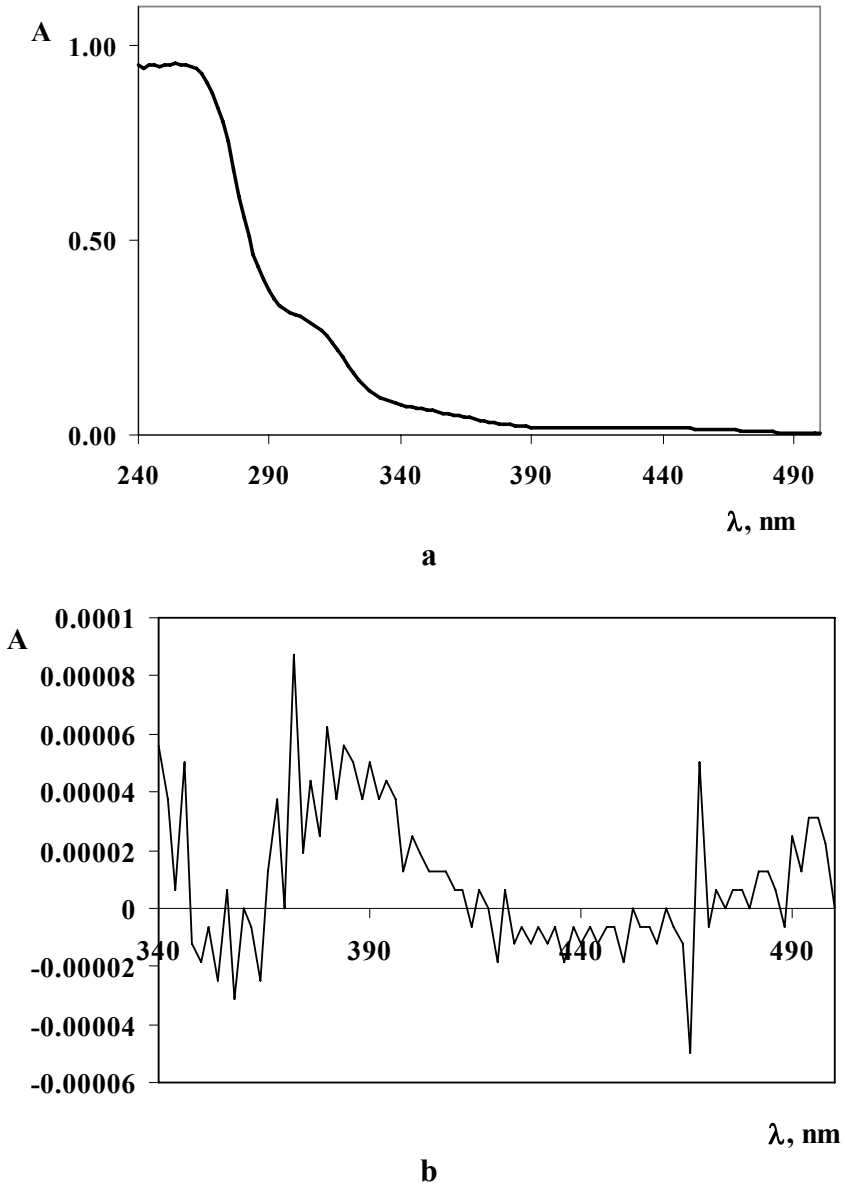


Figure 1. UV-Vis spectrum of Karathane (**a**) raw spectrum; (**b**) second-derivative spectrum

The UV-Vis spectra of soil samples extracted in isopropanol obtained before and after treatment with DINOCAP and after bioremediation are presented in Fig. 2. It was found that the mean decrease in DINOCAP concentration in soil sample was about 82.08% and 69.72% after three days and six days, respectively. After bioremediation, the residual DINOCAP concentration decreased from about 82.08% at day three to 24.56% at day six representing about 57.52% DINOCAP reduction.

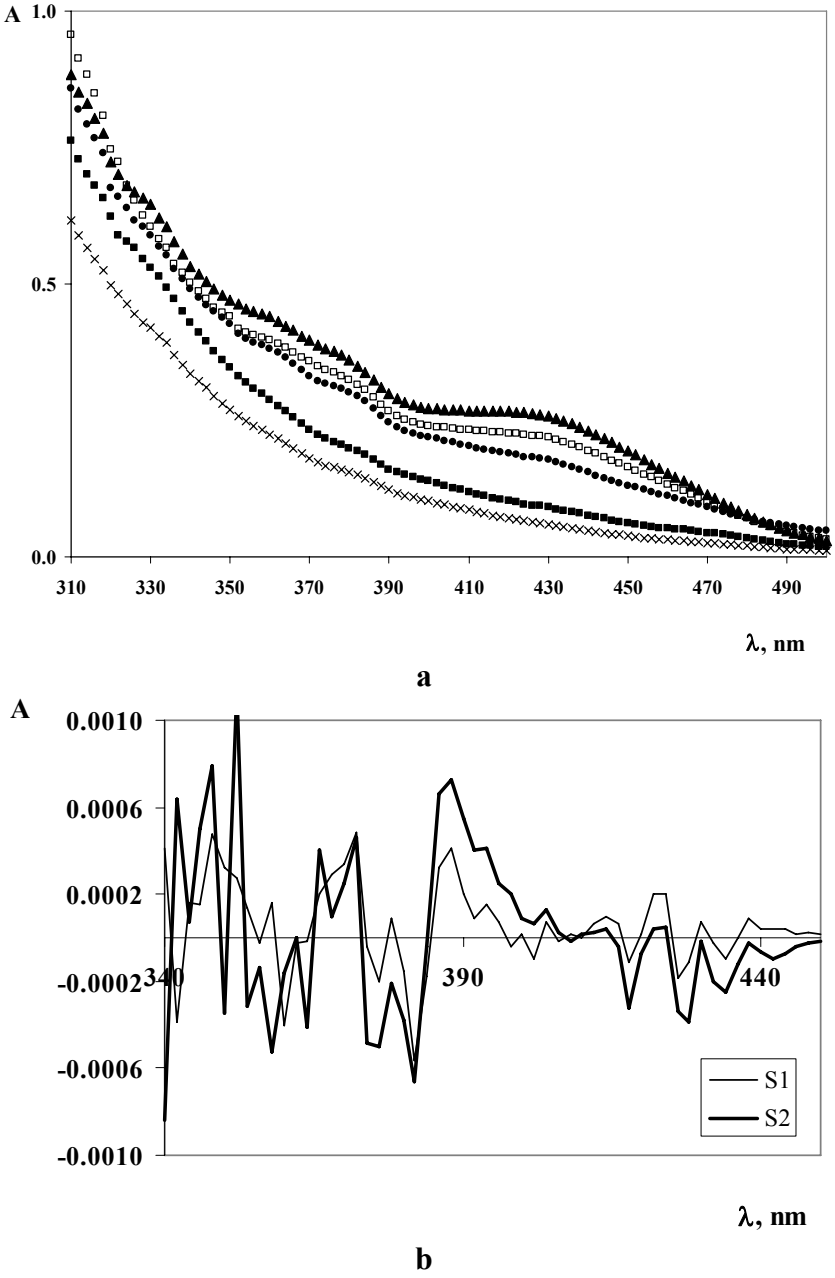


Figure 2. UV-Vis spectra of soil samples extracted in isopropanol in the region of interest – (**a**) raw spectra × - S1, ■ – S2, ● – S3, □ – S4, ▲ – S5; (**b**) second-derivative spectra.

In general, the amount of contaminant that remains in soil after bioremediation depends on contaminant properties, soil characteristics and contaminant history [33]. The results of spectral measurements that evidence the DINOCAP content in the isopropanol extract of the samples revealed that the concentration of DINOCAP decrease from 0.166 gL^{-1} *to 0.088* g L^{-1} at 6 days after pesticide application and to *0.031 g* L^{-1} *at* 3 days after bioremediation treatment.

Qualitative confirmation of the presence of DINOCAP is performed by FTIR spectroscopy. The FT IR spectroscopy can be potential usefulness for determination of nitroderivatives, using the vibration bands of nitro group [34]. DINOCAP, as such shows in FT IR spectrum (see Figure 3) presents many bands due to its complex structure. It was shown that nitro aromatic compounds can be identified in the FT-IR absorption spectra by the characteristic band, NO_2 asymmetric stretching in 1527–1535 cm^{-1} and 1600-1620 cm^{-1} regions [23,35]. The bands observed at 2800-2980 cm^{-1} correspond to CH units from aliphatic segments with variable length. At 1756 and 966 cm^{-1}, respectively DINOCAP exhibits the expected vibrations of C=O group and C=C double bond from ester moiety.

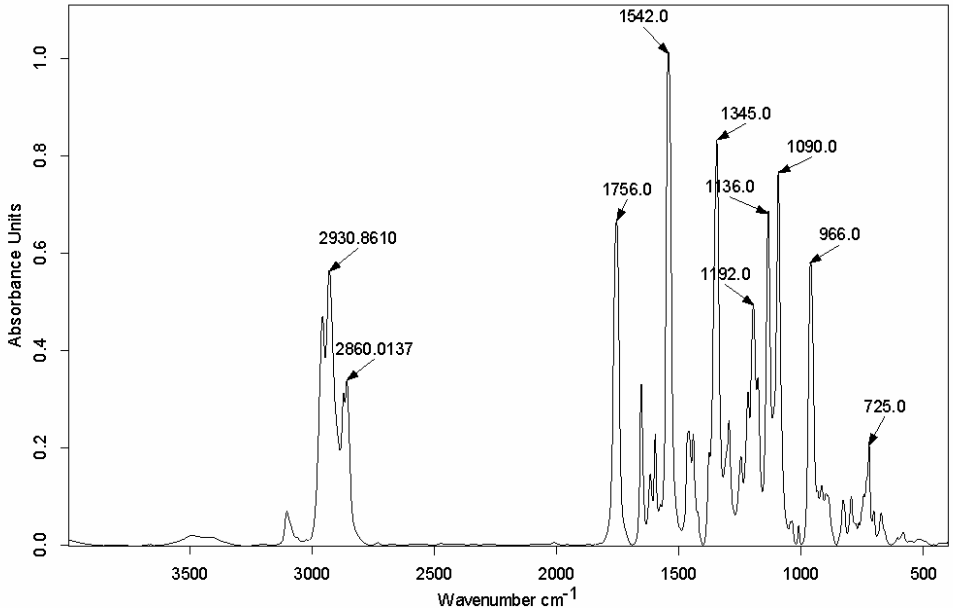


Figure 3. FT IR spectrum of Karathane

In the FT IR spectrum of DINOCAP the most intense bands were the NO_2 peak at about 1542 and 1345 cm^{-1} and those corresponding to CH units from aliphatic segments at 2800-2980 cm^{-1}. Inspection of FT IR spectra reveals that the NO_2 asymmetric and symmetric stretching vibrations are coupled with CC stretching vibrations and in-plane CH bending, and the associated IR intensities are distributed over a large number of vibrations. It was shown that nitro aromatic compounds can be identified in the FT-IR spectra by the characteristic bands that appear in 1550-1475 cm^{-1} ($\nu_{asym(NO)}$) and 1360-1290 cm^{-1}($\nu_{sym(NO)}$) regions. But, the asymmetric $–NO_2$ modes are dominated by the N-O stretch components, while the symmetric $–NO_2$ modes additionally contain major C-N stretching components (34%) [36,37]. The bands at 1620 and 1598 cm^{-1} are assigned to similar complex vibrations consisting of CC stretch, in plane CH bend and NO_2 asymmetric stretch. The CN stretching vibrations is coupled to δ(CH) vibration giving rise to the bands in 1140-1067 cm^{-1} region that are overlapped by the absorption of mineral (δ_{Si-O}) and polysaccharides (ν_{C-O}) constituents. The band at 1548 cm^{-1} has a major contribution from the asymmetric stretch of the two nitro groups and is significantly blue shifted with respect to mono nitroderivative [23,38,39]. The low symmetry of the DINOCAP molecule leads to anomalous splitting of the $\nu_{asym(NO)}$ and $\nu_{sym(NO)}$ bands [40]. The NO_2 out-of plane wag, NO_2 scissors deformation and probably to the N-O stretching appear at around 780, 853 and 872 cm^{-1}, respectively.

We have performed preliminary investigations by ATR FTIR spectroscopy on soil samples in solid state and the corresponding spectra are presented in the Figure 4. We have assigned several of the main bands found in the mid-IR region, but not attempted to identify

the individual compounds in the soil samples. Since the samples surface and content in DINOCAP are different the value of absorbance can vary.

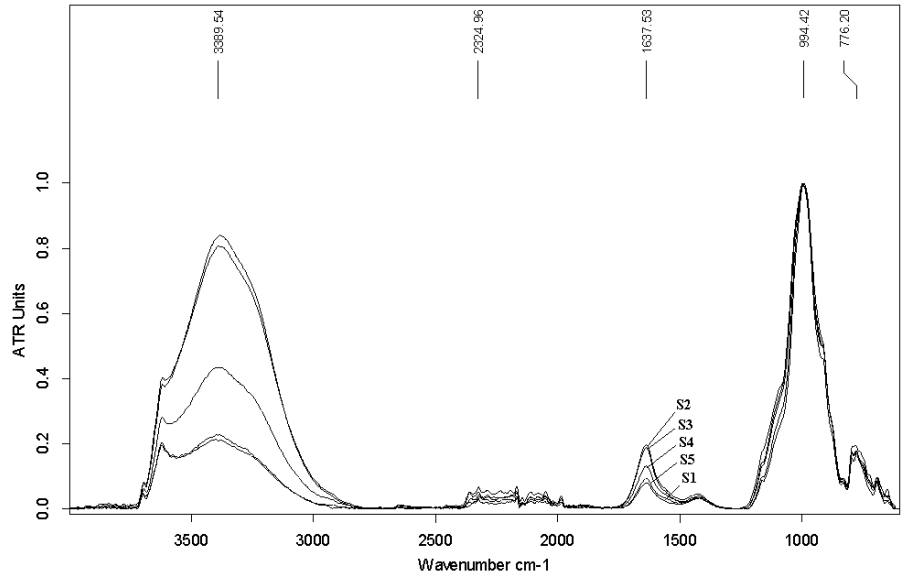


Figure 4. ATR FTIR spectra of soil samples in solid state

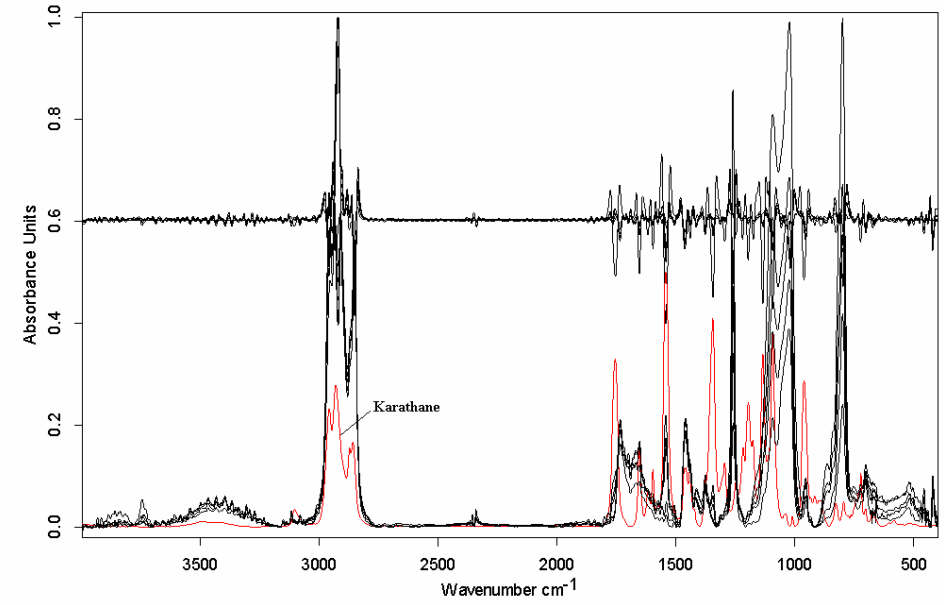
Our initial observation related to ATR FTIR spectra revealed that all spectra of solid sample are dominated by the soil components and exhibit absorption bands at 3400, 2920, 2860 and 1730 cm^{-1}, thus indicating the similarity of the chemical composition, i.e., qualitative identity. These bands are characteristic to soil humic compounds and are also observed in other data given in the literature [32,41]. In the range 2800-3700 cm^{-1} the large band with variable intensity can be associated with stretching vibration of free (3620 cm^{-1}) and bonded OH groups and NH groups (3400 cm^{-1}) from alcohols, phenols and carboxylic acids and amines or amides, respectively. Water, humic substances (2920 and 2860 cm^{-1}, aliphatic CH stretching) and organic component of soil contribute to these vibrations. Its broadness is generally attributed to hydrogen bonding. The water has strong IR absorbance with three prominent bands around 3400 (O-H stretching), 2125 (water association), and 1645 cm^{-1} (H-O-H bending). The C–H bands associated with methyl and methylene groups that usually occur at 2920 cm^{-1} (CH asymmetric stretch) and at 2860 cm^{-1} (CH symmetric stretch) are superimposed as a shoulder of the broad O–H band. Other bands characteristic to humic substances appear at 1630, 1420 and 1100 cm^{-1}. The bands from ATR FTIR spectra, one large (stretching vibration of $\nu_{C=Oas}$ from acids and esters, and $\nu_{C=C}$) at 1540-1650 cm^{-1} with maximum at 1630 cm^{-1} and other for COH (ν_s) in the range 1300-1480 cm^{-1} correspond to unprotoned COO groups. But at 1630 cm^{-1} appear also the vibration for absorbed water, C=C, C=O from amide and benzophenones from humic substances. Each soil sample spectrum appears to have a distinctive ‘‘fingerprint’’ in the 800–1200 cm^{-1} region with maximum at 1050 cm^{-1}, most probably due to its mineral (δ_{Si-O}) and possibly organic (ν_{C-O} of bonds from polysaccharides) constituents [32]. This most intense band has not been completely eliminated in the spectra of calcinated samples. Over a similar period, the infrared spectra obtained from samples free of DINOCAP did not show significant changes.

In the spectra of sample under study can be evidenced bands that can be correlated with vibrations of DINOCAP molecule. Due to the position of the band with maximum at 1050 cm^{-1} from spectra of solid soil samples it can’t be evidenced vibration corresponding to C=C double bond from trans crotonate residue at about 966 cm^{-1}. Also, nitro group has a weak band at about 1040 cm^{-1}, but due to other soil constituents this band is not visible in the

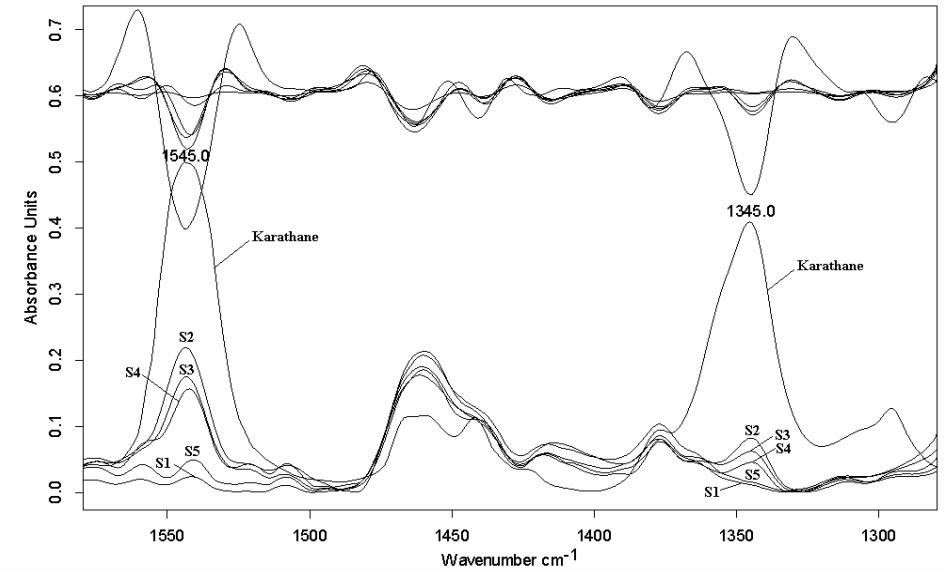
soil spectra. As matter of fact, different series of experiments on soil samples present in the literature revealed that the band of nitro aromatic compounds can be affected by the presence of other compounds (i.e. carbonates, proteins, water). Taking into account all these for samples under study was of interest the band with maximum at about 1620 cm^{-1} attributed to asymmetric vibration of NO_2 groups coupled with other vibrations consisting of CC stretch and in plane CH bend.

As shown in Figure 4, an increase of the band in the region corresponding to the nitro group was observed after treatment with DINOCAP. As is expected due to natural environment and especially after bacteria application the decreasing of the DINOCAOP content in soil occurs. This behaviour is reflected in FT IR spectra where the intensity of the nitro band has been diminished..

The bands characteristic to nitro group was better evidenced in the case of isopropanol extracts of samples as is presented in their spectra (see Figure 5).



a



b

Figure 5. FTIR spectra of soil samples extracted in isopropanol and second derivatives: **a** – full spectra, **b** – 1300 – 1550 cm^{-1} region

As can be seen in Figure 5, the spectrum of samples obtained after extraction with isopropanol and that of the DINOCAP is somewhat similar thus indicating that practically DINOCAP was extracted from the samples when isopropanol was used. The intensity of nitro band, as is shown in Figure 4 and 5 seems to follow the increasing/decreasing of nitroaromatic content in sample. As expected the high intensity of the absorption band correlated with asymmetric vibration of nitro groups was observed in the case of sample designed S2 taken immediately after treatment with DINOCAP. It is also observed that the intensity of this band decrease after treatment with DINOCAP. The bioremediation favoured decreasing of DINOCAP from soil and the intensity of NO_2 band is close to that ascribed to untreated soil sample. The $\nu_{asym(NO)}$ mode decreases in frequency with a concomitant increase in position of the $\nu_{sym(NO)}$ in going from mixture of solvent from DINOCAP formulation solution to isopropanol solution. The relative intensities of the $\nu_{(NO)}$ bands are sensitive to differing modes of surface interactions. This behaviour may be due to nitrobenzene interactions with the solvent or other compounds from extract [42]. Evaluation of the spectra showed that the intensity of the asymmetric vibration of nitro group decrease in the time and this behaviour can be correlated with decreasing of DINOCAP content in samples. On can estimated that DINOCAP (S3) declined and 81.60% remain after 3 days in soil, but after 6 days the percent of DINOCAP in soil sample (S4) become 74.70 %. The bacteria seem to contribute to the decrease of the DINOCAP content with 36.00%, thus after bioremediation (S5) this reaches the value of 29.40%. The results are in agreement with those obtained by UV-Vis spectroscopy. It isn't observing the presence of amine group in the FT IR spectra.

On the other hand Pseudomonas, bacteria used for bioremediation presents bands in the ATR FTIR spectra in the following waverange: 3300 cm^{-1} for nucleic acids structures, 3000–2800 cm^{-1} for cell membrane fatty acids, 1800–1500 cm^{-1} for cell proteins, 1500–1400 cm^{-1} for fatty acids, 1500–1200 cm^{-1} for proteins and phospholipids, 1200–900 cm^{-1} for glycopeptides and phosphate groups of nucleic acids constituents, 900–550 cm^{-1} for less defined cell constituents [43]. After bioremediation of samples it is observed in ATR FTIR spectra the appearance of peaks in the range 2850-2920 cm^{-1} that could be associated with concurrent biomass formation.

To confirm its identification, an aliquot of Dinocap extract was treated with N,N-dimethylformamide and an intense yellow colour was developed.

Conclusions

Because of the inherent complexity of soils, with properties that vary both spatially and temporally, the sampling and testing procedures designed to investigate soil characteristics of interest must be carefully chosen. The non-uniformity within the sampling area is considered one important source of error of results. Both FT-IR and UV-Vis procedures are costly and time-consuming. The inconclusive results can be also motivated by use of different sample corresponding to different stage of soil treatment.

Taking into account all these and the complex structure of DINOCAP and its ability to interact with organic matter and clay it could be performed its assessment better through the combined use of different techniques.

The variation of DINOCAP content in the soil sample can be monitored by UV-Vis spectroscopy taking into consideration the absorption band at 370 nm. Previously, the extraction of DINOCAP into isopropanol must be performed.

The DINOCAP in soil samples can be identified by the peaks at about 1530 cm^{-1} and 1350 cm^{-1} in the FT-IR absorption spectra. The variation of intensity of these peaks can be used for monitoring of the degradation of nitro aromatic compounds. The solid sample and

their isopropanol extracts were used in this sense. The results of spectral analysis demonstrated the response of DINOCAP to bacteria presence.

Acknowledgments

The financial support of this work by MedCT-ANCS, CNMP 2746/2007 (Grant 31-017/2007) is gratefully acknowledged.

References

1. J. D. RODGERS, N. J. BUNCE, Treatment methods for the remediation of nitroaromatic explosives, Wat. Res., 35, 2101–2111 (2001).
2. R. PODLIPNÁ, A. NEPOVÍM, P. SOUDEK, M. VÁGNER, T. VANĚK, Biol. Plantarum, 51, 367-371 (2007).
3. V. L. GEMINI, A. GALLEGO, V. TRIPODI, D. CORACH, E. I. PLANES, S. E. KOROL, Microbial degradation and detoxification of 2,4-dinitrophenol in aerobic and anoxic processes, Int. Biodeterior. Biodegrad., 60, 226–230 (2007).
4. O. MUTER, A. VERSILOVSKIS, R. SCHERBAKA, M. GRUBE, D. ZARINA, Effect of plant extract on the degradation of nitroaromatic compounds by soil microorganisms, J. Ind. Microbiol. Biotechnol., 35, 1539–1543 (2008).
5. K.-S. JU, R. E. PARALES, Nitroaromatic compounds, from synthesis to biodegradation, Microbiol. Mol. Biol. Rev., 74, 250-272 (2010).
6. L. SCHMALZ, A. WEBER, S. TRÄNCKNER, Determination of polar nitroaromatic compounds in soils and the impact of the soil properties on the extraction results, Anal. Chim. Acta, (2010), in press.
7. M. ARAVENA, C., R. FIGUEROA, C. OLEA-AZAR, V. J. ARÁNJ, ESR, Electrochemical and ORAC studies of nitro compounds with potential antiprotozoal activity, Chil. Chem. Soc., 55, 244-249 (2010).
8. H. WACKERBARTH, L. GUNDRUM, C. SALB, K. CHRISTOU, W. VIÖL, Challenge of false alarms in nitroaromatic explosive detection-a detection device based on surface-enhanced Raman spectroscopy, Appl. Optics, 49, 4367-4371 (2010).
9. D. J. L. PRAK, A. N. WADDLE, Solubilization of nitroaromatic compounds from multi-component mixtures into nonionic surfactant micellar solutions, Separ. Sci. Technol., 45, 732 – 739 (2010).
10. G. DROCHIOIU, Laser-induced ATP formation: Mechanism and consequences. Photomed. Laser Surg., 28, 573-574 (2010).
11. S. K. WALIA, S. ALI-SADAT, G. R. CHAUDHRY, Influence of nitro group on biotransformation of nitrotoluenes in Pseudomonas putida strain OU83, Pestic. Biochem. Phys., 76, 73–81 (2003).
12. C. ZHANG, G. N. BENNETT, Biodegradation of xenobiotics by anaerobic bacteria, Appl. Microbiol. Biotechnol., 67, 600–618 (2005).
13. D. PAUL, R. SINGH, R. K. JAIN, Chemotaxis of Ralstonia sp. SJ98 towards p-nitrophenol in soil, Environ. Microbiol., 8, 1797–1804 (2006); M. KULKARNI, A. CHAUDHARI, Microbial remediation of nitro-aromatic compounds: An overview, J. Environ. Manage., 85, 496–512 (2007).
14. N. EISENHAUER, M. KLIER, S. PARTSCH, A. C. W. SABAIS, C. SCHERBER, W. W. WEISSER, S. SCHEU, No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration, Appl. Soil Ecol., 42, 31–36 (2009).
15. K. KARIM, S. K. GUPTA, Continuous biotransformation and removal of nitrophenols under denitrifyingconditions, Water Res., 37, 2953–2959 (2003).
16. A. ÜZER, E. ERÇAĞ, R. APAK, Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction, Anal. Chim. Acta, 534, 307–317 (2005).
17. Y. PICÓ, C. KOZMUTZA, Evaluation of pesticide residue in grape juices and the effect of natural antioxidants on their degradation rate, Anal. Bioanal. Chem., 389, 1805–1814 (2007).
18. W. W. KILGORE, K. W. CHENG, Fungicide-miticide mresidues, extraction and determination of DINOCAP residues in fruits, J. Agric. Food Chem., 11, 477-479 (1963).
19. M. NATANGELO, S. MANGIAPAN, R. BAGNATI, E. BENFENATI, R. FANELLI, Increased concentrations of nitrophenols in leaves from a damaged forestall site, Chemosphere, 38, 1495-1503 (1999).
20. P. AYSAL, A. AD AMBRUS, S. J. LEHOTAY and ANDREW CANNAVAN, Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction, J. Environ. Sci. Heal. Part B, 42, 481–490 (2007).
21. Z. C. LAGOBO, O. B.YAPO, V.MAMBO, V. P. HOUENOU, G. DROCHIOIU, Rapid spectrophotometric assay of dinitrophenol pesticides. Rev. Chim. (Bucharest), 2010, in press.

22. I. KÖGEL-KNABER, Analytical approaches for characterizing soil organic matter, Org. Geochem., 31, 609-625 (2000).
23. M. GRUBE, O. MUTER, S. STRIKAUSKA, M. GAVARE, B. LIMANE, Application of FT-IR spectroscopy for control of the medium composition during the biodegradation of nitro aromatic compounds, J. Ind. Microbiol. Biotechnol., 35, 1545–1549 (2008).
24. V. ZITKO, D. W. MCLEESE, W. G. CARSON, H. E. WELCH, Toxicity of alkyldinitrophenols to some aquatic organisms Bulletin of Environ. Contam. Tox., 16, 508-515 (1976).
25. H. MAYER, R. KUCKUK, F. HEIMLICH, A. N. DAVIES, J. NOLTE, Multidimensional spectroscopic identification of the pesticide Dinocap, J. Mol. Struct., 349, 361-364 (1995).
26. M. ÇELIK, F. ÜNAL, D. YÜZBAŞIOĞLU, M. A. ERGÜN, O. ARSLAN, R. KASAP, In vitro effect of Karathane LC (DINOCAP) on human lymphocytes, Mutagenesis, 20, 101—104 (2004).
27. a. *http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/98eva/dinocap.pdf; b. http://sitem.herts.ac.uk/aeru/footprint/en/index.htm*
28. J. ČERNOHLÁVKOVÁ, J. JARKOVSKÝ, J. HOFMAN, Effects of fungicides mancozeband dinocapon carbon and nitrogen mineralization in soils, Ecotox. Environ. Safe., 72, 80–85 (2009).
29. A. ÜZER, E. ERÇAĞ, H. PARLAR, R. APAK, H. FILIK, Spectrophotometric determination of 4,6-dinitro-o-cresol (DNOC) in soil and lemon juice, Anal. Chim. Acta, 580, 83–90 (2006).
30. J. A. ARANCIBIA, G. M. DELFA, C. E. BOSCHETTI, G. M. ESCANDAR, A. C. OLIVIERI, Application of partial least-squares spectrophotometric-multivariate calibration to the determination of 2-sec-butyl-4,6-dinitrophenol (dinoseb) and 2,6-dinitro-p-cresol in industrial and water samples containing hydrocarbons, Anal. Chim. Acta, 553, 141–147 (2005).
31. D. STAHL, D. TILOTTA, Screening method for nitroaromatic compounds in water based on solid-phase microextraction and infrared spectroscopy, Environ. Sci. Technol., 35, 3507-3512 (2001).
32. R. J. COX, H. L. PETERSON, J. YOUNG, C. CUSIK, E. O. ESPINOZA, The forensic analysis of soil organic by FTIR Forensic Sci. Int., 108, 107-116 (2000).
33. L. BONTEN, Improving bioremediation of PAH contaminanted soils by thermal pretreatment, Thesis Wageningen University, Wageningen, The Netherlands, (2001).
34. R. LINKER, I. SHMULEVICH, A. KENNY, A. SHAVIV, Soil identification and chemometrics for direct determination of nitrate in soils using FTIR-ATR mid-infrared spectroscopy, Chemosphere, 61, 652–658 (2005).
35. S. ARMENTA, G. QUINTÄ S, A. MORALES, S. GARRIGUES, M. DE LA GUARDIA, FTIR approaches for Diuron determination in commercial pesticide formulations, J. Agric. Food Chem., 53, 5842-5847 (2005).
36. G. N. ANDREEV, B. SCHRADER, H. TAKAHASHI, D. BOUGEARD, I. N. JUCHNOVSKi, Normal coordinate analysis of m-dinitrobenzene-d_0 and -d_4 and -(^{15}N, ^{15}N), Can. J. Spectrosc., 29, 145-147 (1984).
37. J. H. S. GREEN, H. A. LAUWERS, Vibrational Spectra of Benzene derivatives-XIII The dinitrobenzenes, Spectrochim. Acta, 27A, 817-824 (1971).
38. I. AHMAD, T. J. DINES, C. H. ROCHESTER, J. A. ANDERSON, IR study of nitrotoluene adsorption on oxide surfaces, J. Chem. Soc., Faraday Trans., 92, 3225-3232 (1996).
39. R. A. NYQUIST, S. E. SETTINERI, Infrared Study of Substituted Nitrobenzenes in Carbon Tetrachloride and Chloroform Solutions, Appl. Spectrosc., 44, 1552-1557 (1990).
40. S. BOYD, G. SHENG, B. TEPPEN, A.T. JOHNSTON ,Mechanisms for the adsorption of substituted nitrobenzenes by smectite clays, Environ. Sci. Technol., 35, 4227-4234 (2001).
41. J. C. ROCHA, A. H. ROSA, M. FURLAN, An Alternative Methodology for the Extraction of Humic Substances from Organic Soils, J. Braz. Chem. Soc., 9, 51-56 (1998).
42. C. JOHNSTON, M. F. DE OLIVEIRA, B. J. TEPPEN, G. SHENG, S. A. BOYD, Spectroscopic study of nitroaromatic-smectite sorption mechanisms, Environ. Sci. Technol., 35, 4767-4772 (2001).
43. Z. FILIP, S. HERMANN, An attempt to differentiate Pseudomonas spp. and other soil bacteria by FT-IR spectroscopy, Eur. J. Soil Biol., 37, 137-143 (2001).