

## REAL ABILITIES AND PROBLEMS OF LASER MONITORING (*IN SITU*) OF OIL POLLUTION IN COASTAL MARINE WATERS

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### ABSTRACT

The paper summarizes results of laboratory and field experiments as well as of computer modelling obtained by the authors in 1998-2000 within the scope of their joint INTAS project, and of the Russian federal program 'Integratsiya'. It has been found that at oil pollution (OP) concentrations in water at concentrations of tens of micrograms per litre the intensity of their fluorescence band is significantly lower than that of aquatic humic substances (AHS), typical of coastal waters. Generally, the OP and AHS fluorescence bands overlap (to a greater or lesser degree at different excitation wavelengths). Thus, it is necessary to solve the problem of extracting small contributions of OP fluorescence. This task is complicated by the effects of a possible interaction between components and changing OP fluorescence band parameters during its presence in the water ('ageing' effect).

In this paper, a possible solution of the indicated problem is investigated by analysing the seawater fluorescence band directly with sensitive instruments, and with the method of fluorimetry with variable gating (a variant of the kinetic fluorimetry). Both methods are used in combination with the application of artificial neural networks (ANN). Spectra obtained for different OP in pure water and in water containing AHS at different concentrations were used as initial data for ANN training. The results of such a treatment of fluorescence spectra of real seawater samples from coastal water areas of the Black Sea, of model samples and computer simulations, have demonstrated the real possibility of estimating OP concentrations in coastal water down to micrograms per litre. It was shown that the application of the ANN technique to the analysis of fluorescence kinetic allows to determine lifetimes of the excited states of fluorophores and their partial contributions, when an information about their fluorescence band shape (with satisfactory accuracy) is not available.

### INTRODUCTION

Express monitoring of oil pollutants (OP) in the seawater remains an actual problem. This problem is extremely important for coastal waters, where consequences of OP are especially dangerous to the environment. The possibilities of developing practically applicable *in situ* methods of oil pollutants fluorescence diagnostics in seawater, i.e. without sampling and preparation, will be discussed in this paper. Only these methods are adequate to the modern problems of ecological monitoring of vitally important seawater areas. However, this problem is complicated because of intensive background signals, which is created by aquatic humic substance (AHS). In coastal waters the concentration of AHS is hundred or thousand times higher than that of oil pollutants.

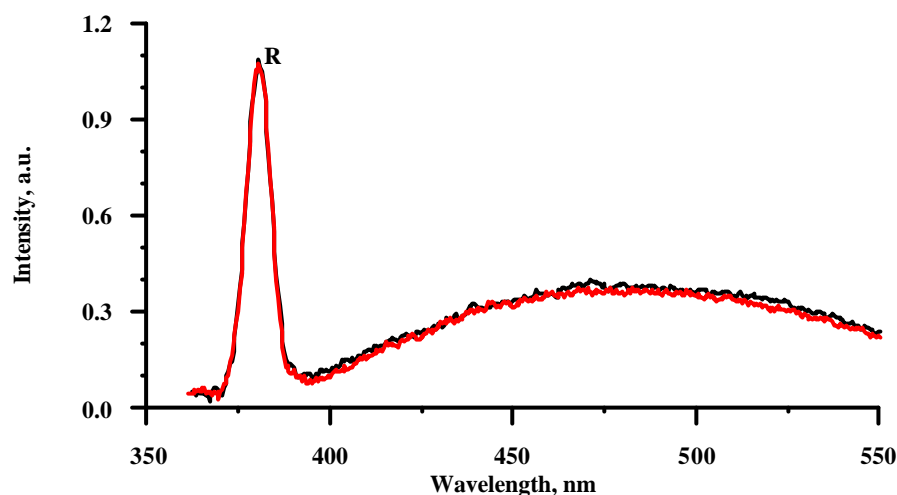
In this paper two approaches are considered, which are capable of solving this problem separately or in combination:

1. The analysis of fluorescence spectra of organic compound mixtures in order to distinguish small contributions of oil pollutants from the generally observed AHS fluorescence band by means of Artificial Neural Networks (ANN).
2. Fluorimetry with variable gating (kinetic fluorimetry) using ANN.

## METHODS AND RESULTS

### Extracting small contributions of oil pollution by analysis of the coastal sea water fluorescence band shapes by means of ANN.

Figure 1 displays the typical spectrum of the optical response of water samples from coastal water areas of the Black Sea in the vicinity of Gelendzhik (black line), and the spectrum of the same sample after extracting OP by hexane (red line). These spectra were recorded with a laser spectrometer (on the bases of a  $N_2$  laser). The presence of OP with concentrations of up to at least  $14 \mu\text{g/l}$  does not appear in the fluorescence spectrum. Our task is to learn how to determine small fluorescent contributions of oil pollutants to the general fluorescence band against the background of aquatic humic substance typical of the coastal marine waters.



**Figure 1: Typical fluorescence spectra of the sea-water samples without (red line) and with (black line) OP, concentrations  $14 \text{ mg/l}$ ,  $\lambda_{exc} = 337 \text{ nm}$ . R – Raman scattering band from water molecules.**

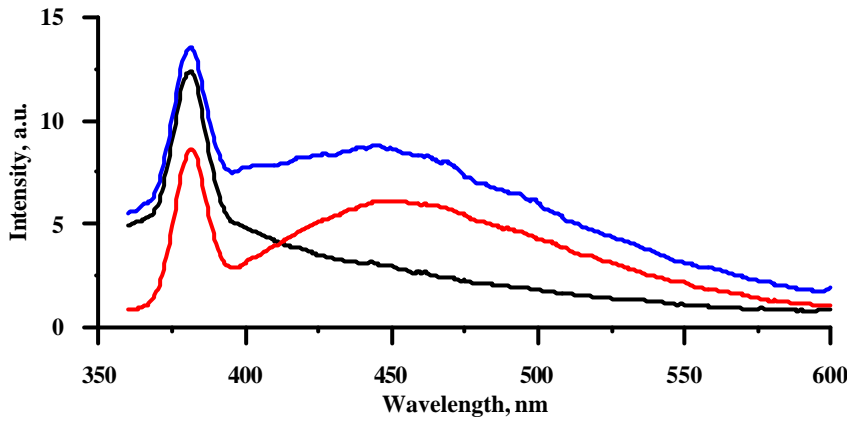
A transition from  $\lambda_{exc} = 337 \text{ nm}$  to  $266 \text{ nm}$  leads to the appearance of fluorescence bands of light oil hydrocarbons in the region of  $310 \dots 360 \text{ nm}$ , i.e. in the “window” between the water Raman scattering band ( $I_{RS}^{max} = 291 \text{ nm}$ ) and the AHS fluorescence band. Simultaneously, however, the fluorescence bands of protein-like compounds show up in the same region (1,2). Their concentrations are high in coastal water, and their fluorescence contributions exceed the fluorescence contribution of OP. The fluorescence bands of heavy oil hydrocarbons overlap with the AHS fluorescence bands as usual. Hence, the problem of extracting the little fluorescence contributions of OP to the background bands (now AHS and protein-like compounds) is important under excitation  $\lambda_{exc} = 266 \text{ nm}$ , too.

For solving this problem, we first carried out a computer experiment with the purpose of estimating the minimal detectable contribution of oil pollutants to the fluorescence band of their mixture with aquatic humic substance.

As the initial data for computer modelling, we used fluorescence spectra of water solutions of humic acids (extracted from soil), oil, Diesel fuel, and their mixtures with different partial concentrations of the components, recorded with a Perkin Elmer model LS50 luminescence spectrometer. Examples of the obtained fluorescence spectra are shown in Figure 2. Values of the fluorescence parameter  $F_0 = N_{fl}^0 / N_{RS}$  ( $N_{fl}^0$  is a number of fluorescence photons,  $N_{RS}$  is a number of Raman scattering photons) of pure substances and their mixtures varied approximately from 0.02 to 20.

In the computer experiment it is assumed that there is no interaction between these components. Spectra of mixture are simulated as a linear superposition of initial spectra of the components, with variable weight coefficients. Partial values of the fluorescence parameter  $\Phi_0$  were used as the weight coefficients.

Using this linear model, fluorescence spectra of mixtures of fulvic acids and oil pollutants have been calculated, and neural networks have been trained on these spectra.



**Figure 2: Fluorescence spectra of German oil in distilled water (black line), fulvic acid in distilled water (red line) and their mixture in distilled water (blue line),  $\lambda_{exc}=337$  nm.**

The application of neural networks to the model (simulated) spectra shows that by means of this technique, one can determine the contribution of oil pollutants down to a value of the fluorescence parameter  $F_0^{OP} = 0.02$  against the background of humic acid fluorescence up to values of  $F_0^{HA} = 20.0$ . In this case, the error in determining the  $F_0^{OP}$  parameter did not exceed 10%. When the value of  $F_0^{OP}$  is increased the error decreases. Thus, the average relative error over the whole range of  $F_0^{OP}$  and  $F_0^{HA}$  is about 2% (see Table 1, columns 1 and 2). However, these estimations are derived for an ideal case, where the fluorescence bands of the components are known. Besides that, the interaction between components, the influence of aquatic humic substance on the water Raman scattering band, and the effects of oil ‘ageing’ are neglected.

It is obvious that these factors hamper the procedure of distinguishing the contribution of oil pollutants to the fluorescence emission of unpolluted seawater. Therefore, an experiment capable of taking the described effects into account was done. The neural network was trained on real fluorescence spectra of mixtures of humic acids with oil pollutants and with Diesel fuel. Therefore, all the mentioned factors were taken into account automatically. Table 1 compares the results of applying the two described types of neural networks to simulated and experimental spectra.

**Table 1: Average relative errors ( $\epsilon_{FA}, \epsilon_{GO}$ ) of the definition of the humic acids and German oil fluorescence parameters ( $F_0^{FA}, F_0^{GO}$ ) by means of ANN analysis of the fluorescence bands.  $\lambda_{exc} = 337$  nm.**

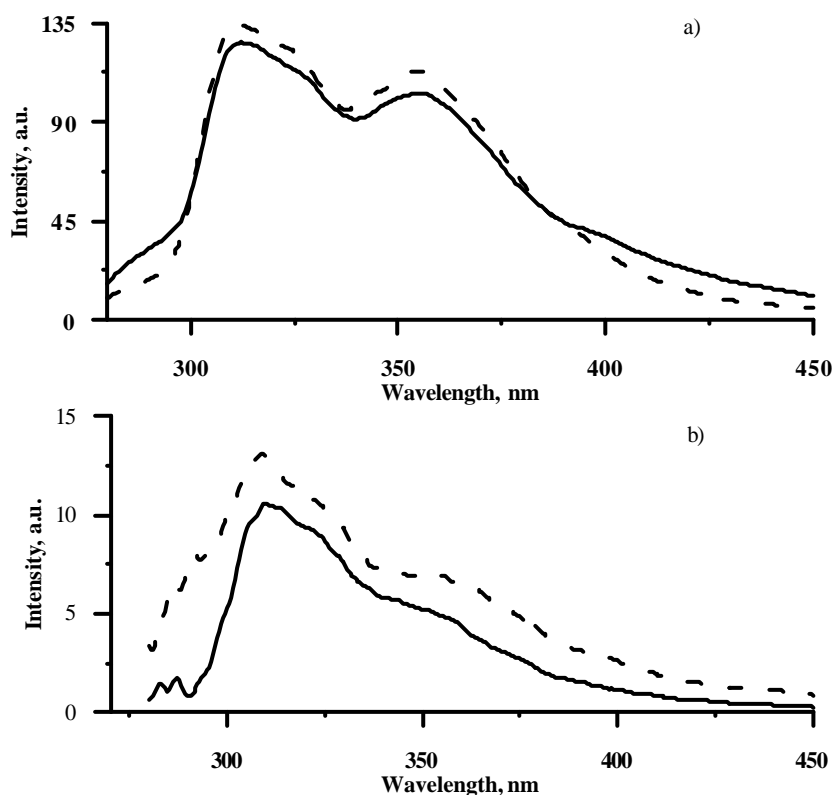
ANN trained on a 'linear model', for the model set spectra		ANN trained on a 'linear model', for the experimental set		ANN trained on experimental spectra, for the experimental set	
1	2	3	4	5	6
$\epsilon_{FA}, \%$	$\epsilon_{GO}, \%$	$\epsilon_{FA}, \%$	$\epsilon_{GO}, \%$	$\epsilon_{FA}, \%$	$\epsilon_{GO}, \%$
2.1	1.6	12.0	27.1	9.6	12.8

Average errors, which are shown in columns 3 and 4, are the errors of the measured fluorescence parameters of the experimental spectra of the German oil and fulvic acid mixtures by means of ANN trained on the emulated spectra. A comparison of columns 1 and 2 with columns 3 and 4 reveals that the errors rapidly increases when compared with the earlier described ideal cases. This is apparently due to interaction and aging processes described above, and the high noise of the input data. To improve this, ANN trained on experimental spectra were used where the interaction and ‘ageing’ are considered automatically (Table 1, columns 5, 6). Indeed, the error decreased in comparison with columns 3 and 4, but remained above that of the ideal case (columns 1, 2). The reason

is the decrease of the number of spectra used for the ANN training, in contrast to the case where the ANN is trained on simulated spectra (i.e. on a model).

Thus, in the ideal case, processing of spectra by means of neural networks may yield very good results. However, under natural conditions, one should account for the above-mentioned factors. This has been shown with two possible approaches for field measurements. In the first approach ANN training is based on the spectra of natural water samples measured without any sample processing, for which independent data (for example by means of extraction) of the oil pollution concentration are available. It is obvious that for monitoring purposes the data base volume should be quite large, but it is difficult to provide this data base in field experiments. In the second approach (which could be named ‘model’), the training process is based on simulated spectra, which are combined from seawater spectra and spectra of the oil pollutants that typically occur in that area. In a first approximation such spectra could be summed up following a linear model and neglecting the influence between the components and the ‘ageing’ effect. In a second approximation such influences could be included as a result of their specific analysis. Choosing the reference spectra for the ANN training is one of the critical problems of this method.

However, the choice of standard spectra, which are the best fits of a real water pollution, is a common problem of all known oil pollution analysis methods, including the UNESCO method (3). This will be the goal of future investigations to solve this problem. In this paper the following approach was chosen: The spectra (266 nm excitation) of samples taken in the Blue, Gelendzhik, and Novorossiisk bays were analysed. The same samples were used to extract oil pollutants with hexane by the standard UNESCO method (3). A qualitative comparison of fluorescence spectra of such extracts with those of Diesel fuel and machine oil solutions in hexane revealed that the shapes of the fluorescence bands of the Diesel fuel and machine oil solutions differ noticeably, and the fluorescence bands of the extracts were close by shape to one or the other of these bands (Figure 3).



**Figure 3: Fluorescence bands of hexane extracts (solid line) and hexane solutions (dashed line),  $\lambda_{exc} = 266$  nm. Extract of water samples a) from station 26, Novorossiisk bay (depth 0 m) and solution motor oil in hexane; b) from station 4, Gelendzhik bay (depth 0m) and solution of Diesel fuel in hexane.**

It is therefore assumed that in the sampling area the water was polluted by these oil products. This result was not evident *a priori*, since the process of OP formation in the water column is highly complicated and depends on many factors. In particular, fluorescence properties of OP depend on a correlation of truly soluble fractions and oil emulsion. The fluorescence band of a soluble fraction

greatly differs from a fluorescence band of the pure oil and is stable in time. In the beginning a fluorescence band of an emulsion is close to that of the pure oil, but changes noticeably in time (4).

First, seawater samples were presented to the ANN trained on fulvic acids and Diesel fuel mixtures. In this case the ANN did not identify the spectra of these samples. This is due to the AHS in the investigated region which differ from the humic acids. It is also possible that the spectra of the real OP differ from those of the Diesel fuel.

Secondly, the neural networks were trained on the spectra of simulated 'mixtures' of the solution (emulsion) of Diesel fuel with real seawater samples, free from oil pollution. The seawater samples were taken in the area of Yuzhnaya Ozereevka, 200 meters off the shore, from a 1 metre depth.

The ANN derived the appropriate oil pollution concentration only for the sample with a measured concentration of 13  $\mu\text{g/l}$  (this sample was obtained above the sank vessel 'Admiral Nakhimov'). This concentration should correspond to an  $F_0^{DF}$  value of 0.7 in the original sample, but the ANN gives  $F_0 = 0.4$ . Thus, the sensitivity of these networks reveals  $F_0 \geq 0.4$  for real seawater spectra at a Diesel fuel concentration of 13  $\mu\text{g/l}$ , the error in this case being about 70%. In cases with  $F_0 < 0.4$  the ANN did not reveal reliable results. As shown above, there are several reasons the sensitivity limit being ten times higher than in the ideal case: the errors (noise), the neglected interaction, and mainly the rough approximation of the real spectra of the components (oil pollution and AHS) in natural water as reference spectra used for ANN training. In future investigations we will pay special attention to this problem<sup>1</sup>.

It is expected that the detection threshold of  $F_0^{OP}$  can be highly improved using fluorescence saturation. It has been found (5) that the dependence of the saturation factor  $G$  on  $F$  may be approximated with a linear function  $G \approx 1 + b \cdot F$  at not too high values of the photon flux  $F$  of the pumping radiation. Estimations showed that the ratio ( $b_{AHS}/b_{OP}$ ) differs from 1.5 to 3 for different oil pollutants at 337 nm excitation (5). This means that under laser excitation, the fluorescent contribution  $F_0^{OP}/(F_0^{AHS} + F_0^{OP})$  increases with the intensity of excitation  $F$ , improving the possibility of detecting this contribution. A full-scale solution of the inverse problem of fluorescence saturation for a mixture of organic compounds is complex enough. However, algorithms based on neural networks developed to solve the saturation fluorimetry problem for single-component solutions (emulsions) (6,7) provide a basis for solving such a problem for two-component solutions as well.

#### **Fluorimetry with variable gating (kinetic fluorimetry).**

A second approach is the method of fluorimetry with variable gating. This is another way to increase the fluorescent contribution  $F_0^{OP}/(F_0^{AHS} + F_0^{OP})$ : by optimising the gate duration and the delay time, it is possible to utilise the difference between the fluorescence decay times of the components (8). In the general case of fluorimetry with variable gating, the set of fluorescence intensity values for a mixture of organic compounds is measured at different wavelengths and at different delay times. It is possible to use three algorithms for processing this data:

- to obtain a dependence of the time constant of the exponent approximating the kinetic curve, on fluorescence wavelength, and using it to identify the pollutant (9 and bibliography in this book);
- to determine the component concentrations for a mixture of fluorophores on the basis of the algorithm treating the full spectral-temporal matrix and of the calibration procedure (10);
- to identify the dependence of the fluorescence photon number  $N_{fl}$  (over the entire spectrum) in the receiver gate-on delay time (kinetic curves), and use as a basis for solving the inverse problem of determination of the fluorescence decay time for all components and of their partial values  $F_{0i}$ .

<sup>1</sup> The realization of this program during an expedition on the Black Sea in September 2000 led to a great improvement of the results: the error of determination of the parameter  $F_0^{OP}$  was decreased to 17% in the real samples of seawater (this footnote was added upon correction of the article in October 2000).

The last approach has been studied since it does not require a knowledge of the OP fluorescence band shape, and, therefore, obviates the main difficulty involved in other methods. The preliminary results show that the precision of the determination of oil pollutant concentrations is lower since the information about the contour of the fluorescence band and hence an initial information is not used. Moreover, the precision of determining the fluorescence lifetime  $\tau$  and of partial contributions is lowered if the condition of a laser pulse length and detector gate duration smaller than the fluorescence lifetime is not met. Therefore, it was estimated, to what extent the neural network is capable to solve the problem under these conditions.

In this experiment, solutions (emulsions) of Aldrich humic acid, German oil, Diesel fuel, and tryptophan (trp) in distilled water were used. The 4<sup>th</sup> harmonic of a YAG:Nd laser ( $I_{exc} = 266$  nm,  $t_{las} = 7$  ns) was used, and signals were recorded with a UV-enhanced optical multichannel analyser (DeltaTek, Russia) with  $t_{gate} = 10$  ns and with a delay time between 0 and 50 ns in 2 ns steps. The fluorescence decay curves  $N_{fl}(t_{delay})$  of the analysed substances are shown in Figure 4.

The neural network was trained on model kinetic curves. Two fluorescence models were selected:

- 1) A single-component model, where the solutions of the kinetic equations, calculated for  $t$  in the range from 1 to 40 ns, were the inputs and the sought lifetime  $t$  was the output.
- 2) A two-component model for substances containing two types of fluorophores. This corresponds to a three-parameter inverse problem with the parameters  $t_1$  and  $t_2$  of the fluorophores, and the ratio of their partial fluorescent contributions. The kinetic curves calculated in the parameter ranges shown in Figure 4 were the inputs to the neural networks.

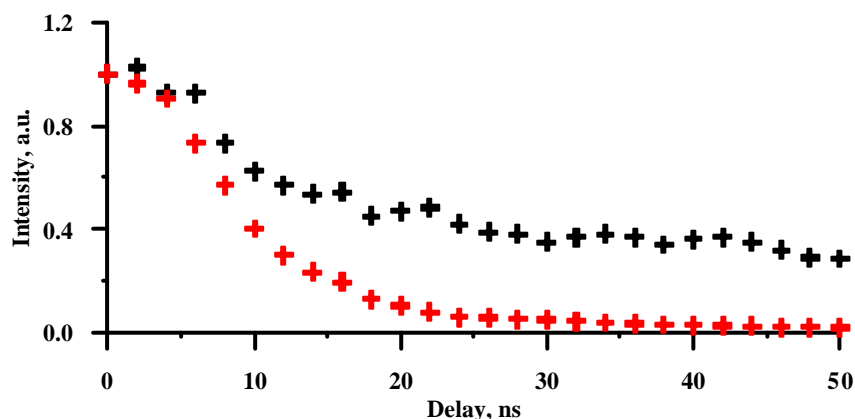


Figure 4: Experimental kinetic curves of dissolved-emulsified German oil (black line) and a solution of humic acid (red line) in distilled water.  $I_{exc}=266$  nm.

Table 2: Parameters  $t_1$ ,  $t_2$ , and  $F_{01}/F_{02}$  for Aldrich humic acid (AHA), German oil (GO), Diesel fuel (DF) and tryptophan (trp), determined by means of the artificial neural networks method in single- and two-component models.

Object	One-component model	Two-component model		
	$t \pm s$ /ns	$t_1 \pm s$ /ns	$t_2 \pm s$ /ns	$F_{01}/F_{02} \pm s$
trp	$4.4 \pm 0.1$	-	-	-
AHA	$3.3 \pm 0.1$	$3.6 \pm 0.8$	$14.6 \pm 1.1$	$3.6 \pm 0.6$
GO	-	$0.7 \pm 0.2$	$20.6 \pm 6.2$	$7.4 \pm 1.2$
DF	-	$3.1 \pm 0.9$	$21.8 \pm 2.1$	$7.4 \pm 1.2$

Since there is no concurrence in the literature regarding the number of fluorophores in the substances under investigation, it was interesting to present the recorded kinetic curves for them to both neural networks and to compare the results (Table 2). The obtained lifetimes values are in good qualitative agreement with known literature data. This holds mainly for trp and humic acid (11).

## CONCLUSIONS

The high diagnostic ability of ANN makes it possible to determine oil pollution in coastal waters, where the oil fluorescence is less than the fluorescence of humic substances and protein-like compounds which form the background bands. The direct analysis of the fluorescence band shape using ANN allows to determine values of  $\rho^{OP} = 0.02$  of the fluorescence parameter in the presence of a background of  $\rho^{AHS} = 20$  from humic substances in the ideal case. In realistic conditions this still holds with a value of  $\rho^{OP} = 0.1$ . A further progress will depend on a successful study of the nature of humic substance and oil fluorescence bands, and the regularities of their characteristics under different conditions in seawater.

The ANN technique allows a diagnostic variant of kinetic fluorimetry to be realised with a limited size of initial information, first of all with an exact knowledge of the fluorescence band shapes. The fluorescence parameter  $\rho^{OP}$  and the fluorescence lifetimes determined by this method leads to a rough identification of oil pollution. It can be carried out more exactly using the method of non-linear fluorimetry. To develop non-linear fluorimetry for a multi-component mixture of organic compounds is a subject of future investigations.

The analysis showed that the full-scale development of a strictly scientifically established method of *in situ* fluorescence diagnostics of oil pollutants in coastal waters is very complicated. However, the results obtained in this study show that it is possible to solve this problem on the basis of the approaches developed here. For this purpose the most interesting task will be to create a complex method including all approaches described in this paper.

## ACKNOWLEDGEMENTS

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