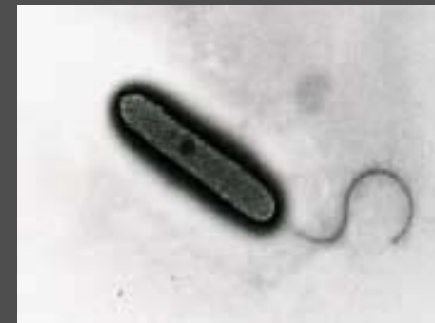


*FLUORESCENT  
BACTERIA SENSING  
IN IRON POLLUTED  
MEDIA*



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## *Abstract*

Since **iron pollution** represents a real threat in the modern industrial era, while, in the mean time, **the environment control using microorganisms** has been developed, the present investigation was dedicated to the **iron sensing** by means of bacterial cultures. *Pseudomonas aeruginosa* was chosen due to its ability of uptake the environmental iron in the form of complex iron compositions named **siderophores**, **characterized by luminescent features**.

## *INTRODUCTION*

Avoidance of iron toxicity through regulation of bacterial iron transport was more and more considered as a viable environmental solution in the frame of a more general issue – the bacterial control in environment. The *Pseudomonas siderophores* are iron chelates in the form of greenish pigments named pyoverdines that are easy to recognize by visual inspection or due to the fluorescence emission under UV irradiation.

Based on *Pseudomonas* an iron biosensor (iron-regulated promoter) was designed by geneticists (Temple et al., 2003) to estimate the relative abundance of iron on some tree blossoms. Our investigation was focused on iron sensing by means of the quantitative relation of *Pseudomonas* pyoverdine fluorescence and the concentration of iron oxides in the culture medium in conditions of iron pollution (simulated by using different concentrations of ferrofluid).

# MATERIALS AND METHODS

*Pseudomonas aeruginosa* samples withdrawn from biological specimens (provided by hospital patients)

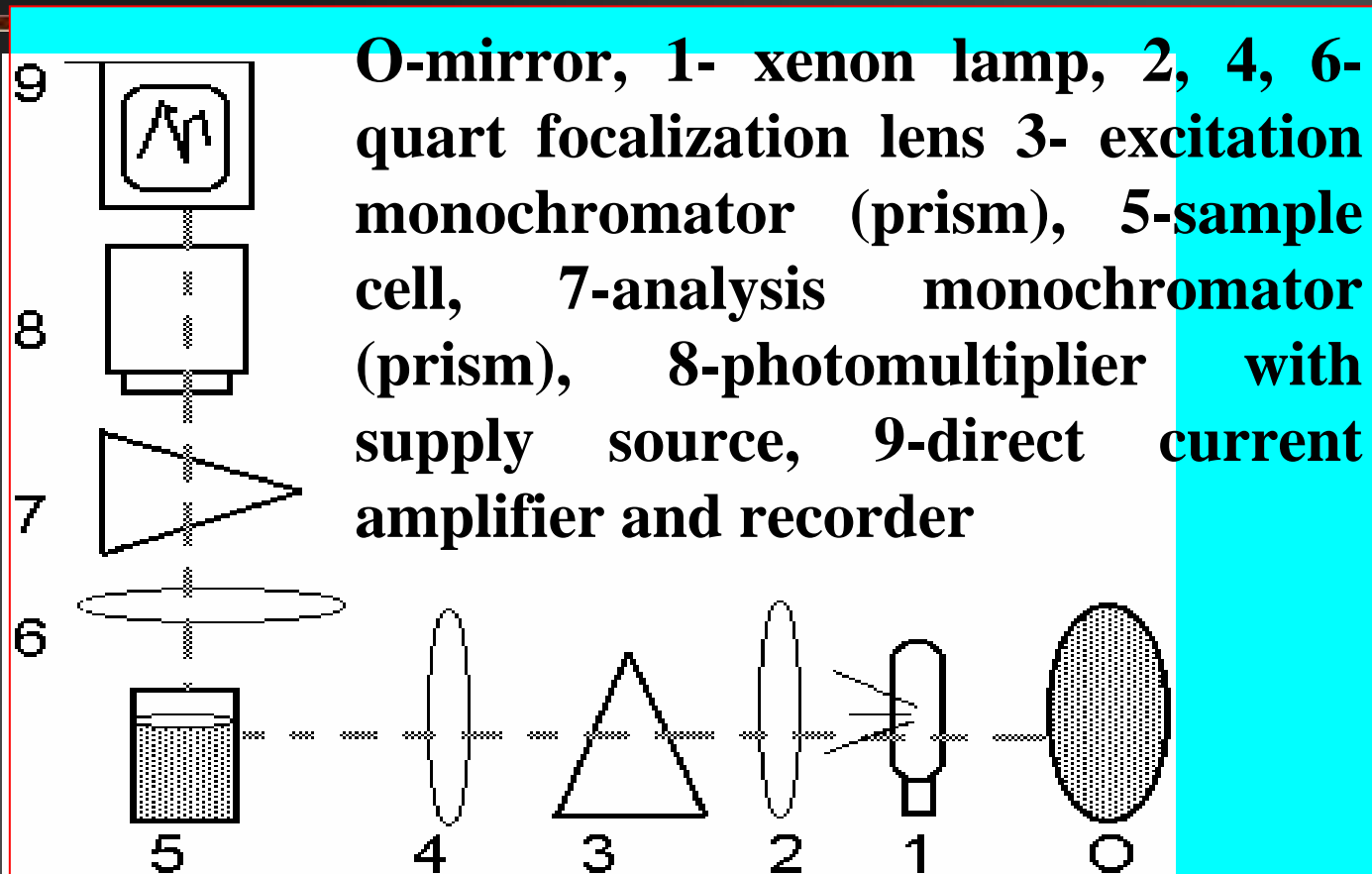
The ferrofluid used for the simulation of iron pollution (Cotae, 1981): magnetite – i.e. iron oxide  $\text{Fe}_3\text{O}_4$ , stabilized with ammonia oleate (ferrophase volume fraction being of 1.5%, the saturation magnetization of 10,34 kA/m and the physical diameter ranging between 4 and 25 nm).

*Iron supply.* Ferrofluid concentration in the culture medium: 0.003 ml/l to 1.0 ml/l (double serial dilutions) equivalent with the iron oxide pollutant ranging between 0.5 microg/l and 72.0 microg/l.

□ **Pyoverdine** samples preparation: thermal treatment (100 C<sup>0</sup>) of the *Pseudomonas* cell cultures.

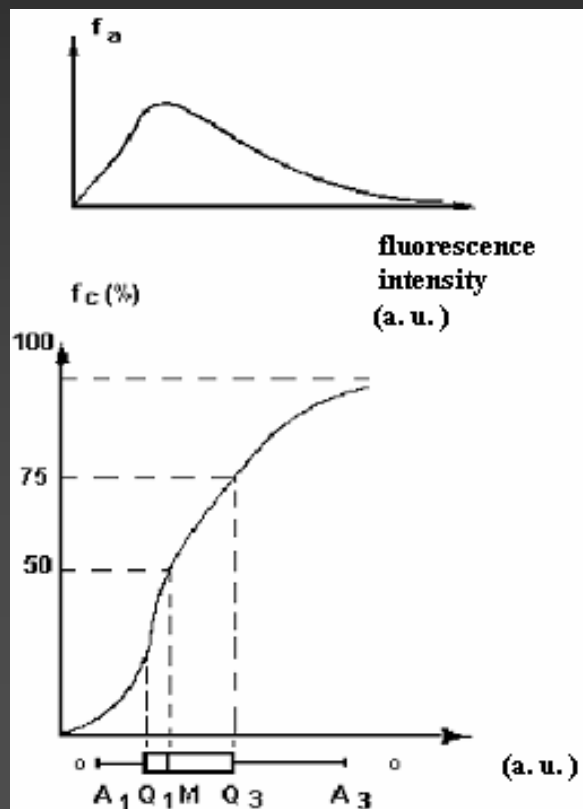
*Spectral devices.* *Pseudomonas* cell density in the test samples was nephelometrically controlled using a C. Zeiss - Jena spectrophotometer. **Fluorescence measurement** was carried out in quartz cells 5 mm width, using a laboratory assembled installation; fluorescence excitation was done using UV light having the wavelength of 300 nm; **fluorescence quenching** was avoided by 1:10 dilution in distilled water.

## *Fluorescence measurement line*



**0**-mirror, **1**- xenon lamp, **2**, **4**, **6**- quartz focalization lens **3**- excitation monochromator (prism), **5**-sample cell, **7**-analysis monochromator (prism), **8**-photomultiplier with supply source, **9**-direct current amplifier and recorder

Statistic comparison was carried out by using the box-plot representation technique: the transformation of a distribution curve into a box with tails and outliers (exceptionally large or small values).



(Koopmans, 1987)

$f_a$  - absolute frequency

a.u. - arbitrary units

$f_c$  - cumulated frequency

A1 - the box left tail

Q1 - the box left edge ( $f_c=25\%$ )

M - the median, ( $f_c=50\%$ )

Q3 - the box right edge ( $f_c=75\%$ )

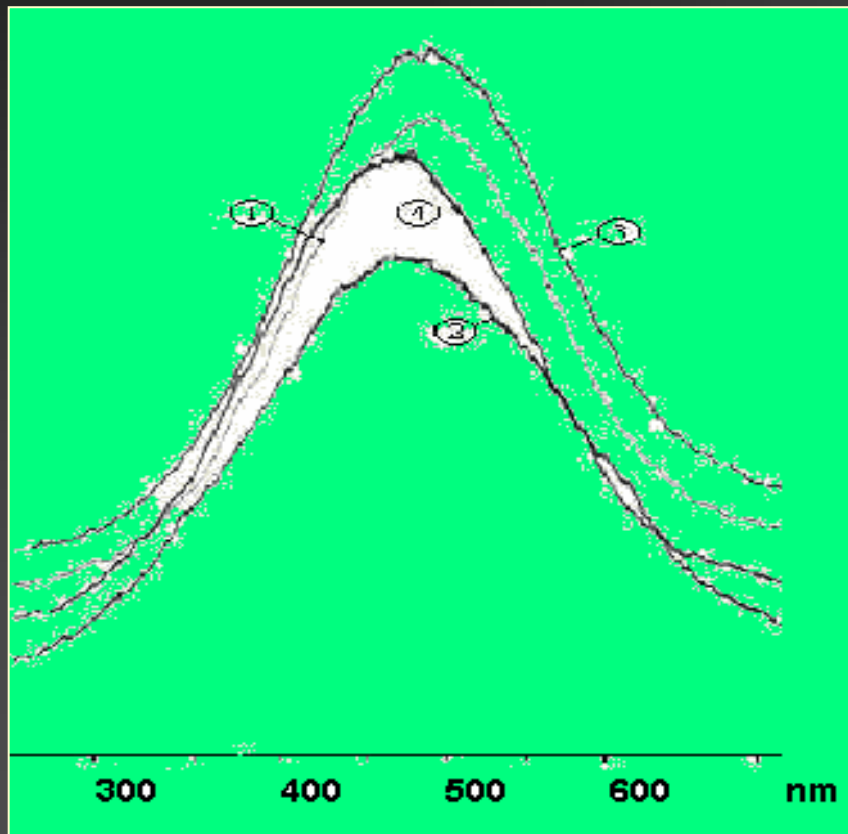
A3 - the box right tail

$A1=Q1-1.5(Q3-Q1)$

$A3=Q3+1.5(Q3-Q1)$

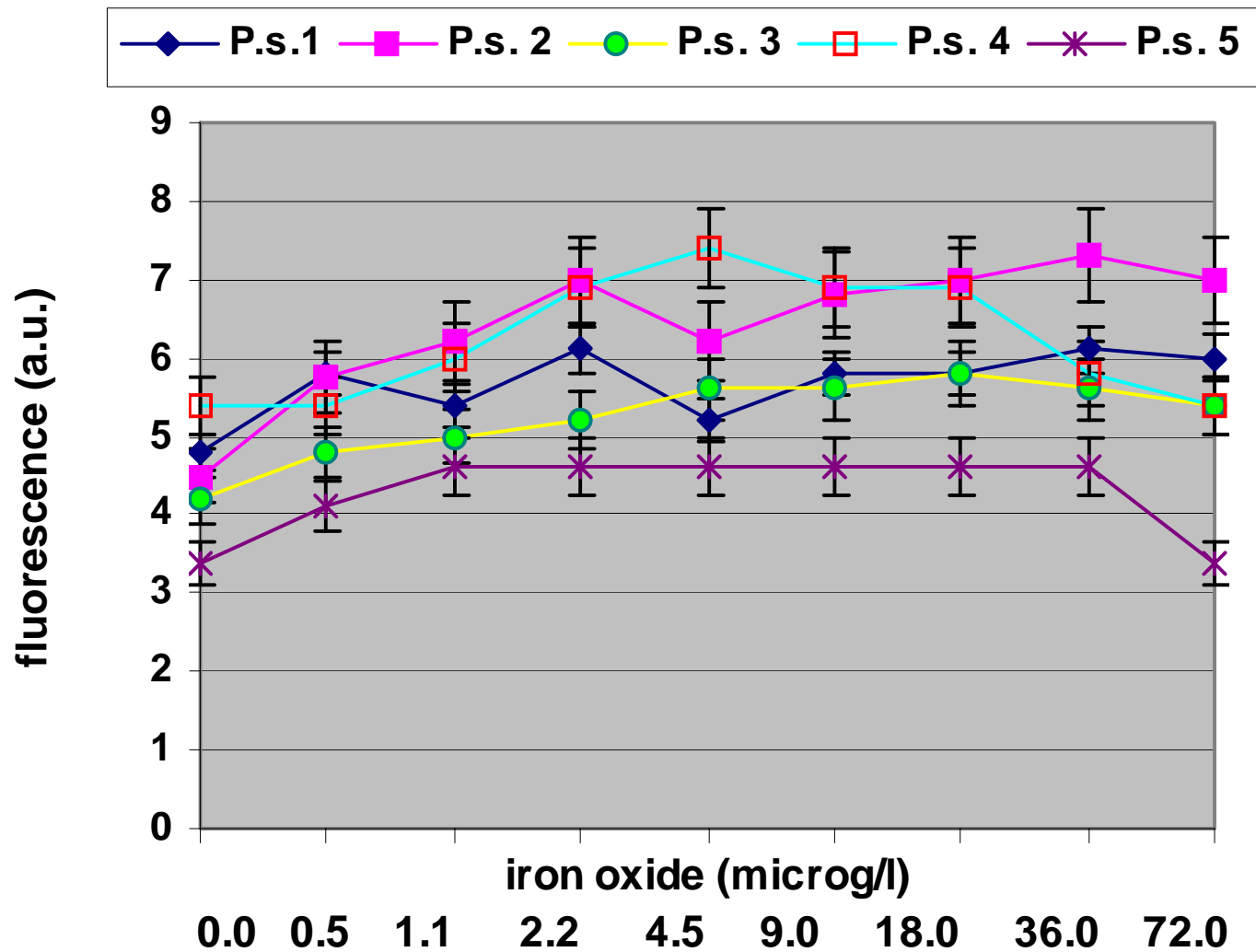
# *RESULTS AND DISCUSSION*

## Fluorescence spectrum of pyoverdine siderophore

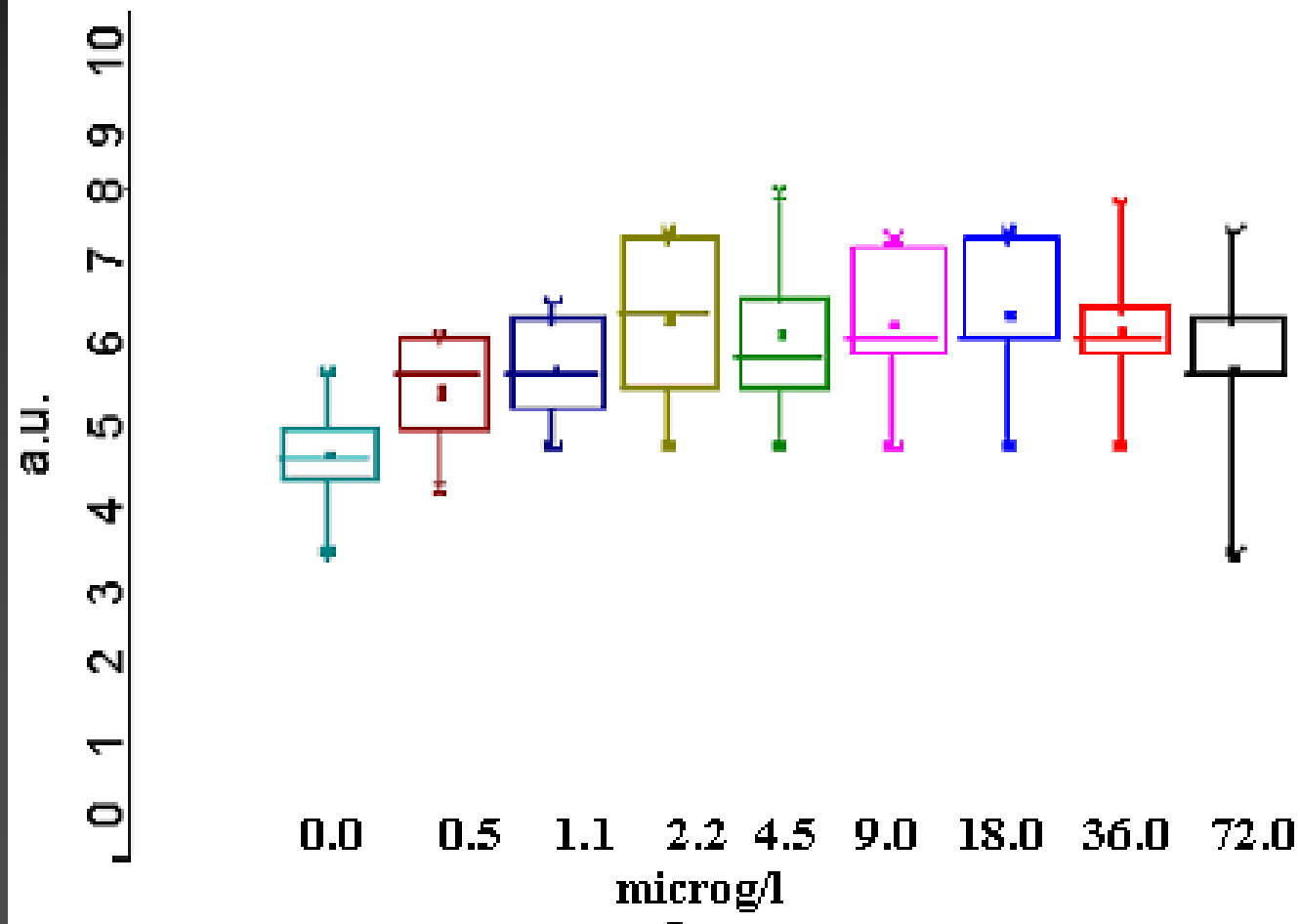


(1)-control sample  
(pyoverdine from  
bacteria grown  
without ferrofluid) (3)-  
ferrofluid 0.0030 ml/l;  
(2)&(4) (origin  
shifted) - ferrofluid  
1ml/l respectively  
0.060 ml/l

# The pyoverdine fluorescence in five *Pseudomonas* strains (average values and standard deviations) in iron polluted medium



# Comparison of the fluorescence intensity distribution curves by using the box-plot representation



The analysis of the distribution curves obtained for the 9 series of 5 bacterial strains, by means of box-plot representation:

- no exceptional large or low values exist within the 9 data series taken into account;
- the most symmetrical distribution corresponds to the control data series (the average value is overlapped on the median line in the box center and the tails are equal);
- for the highest iron oxide concentration the dispersion (the box length) is the largest the Q1 box edge median being shifted up to the median

- the asymmetry of the distributions that correspond to **iron polluted media** is given by the **higher weight of higher values** which determines the shift of the Q3 edge toward the A1 tail (the upper tail is zeroed); this occurred for 5 of the 8 data series;
- the **median** corresponding to the **highest iron oxide level** has the **lowest position** among all the 9 distribution curves, (**concordant with the lack of the statistical significance** of the fluorescence modification by **the highest iron oxide level**).

The **statistical significance** of the differences between the average values of fluorescence intensity in iron polluted media in comparison to the control (**t-test**)

Iron oxide concentration	0.5 microg/l	1.1 microg/l	2.2 microg/l	4.5 microg/l
Statistic significance	0.05	0.01	0.01	0.01
Iron oxide concentration	9.0 microg/l	18.0 microg/l	36.0 microg/l	72.0 microg/l
Statistic significance	0.01	0.01	0.01	>0.05*

*The limitations of the Pseudomonas iron sensing.*

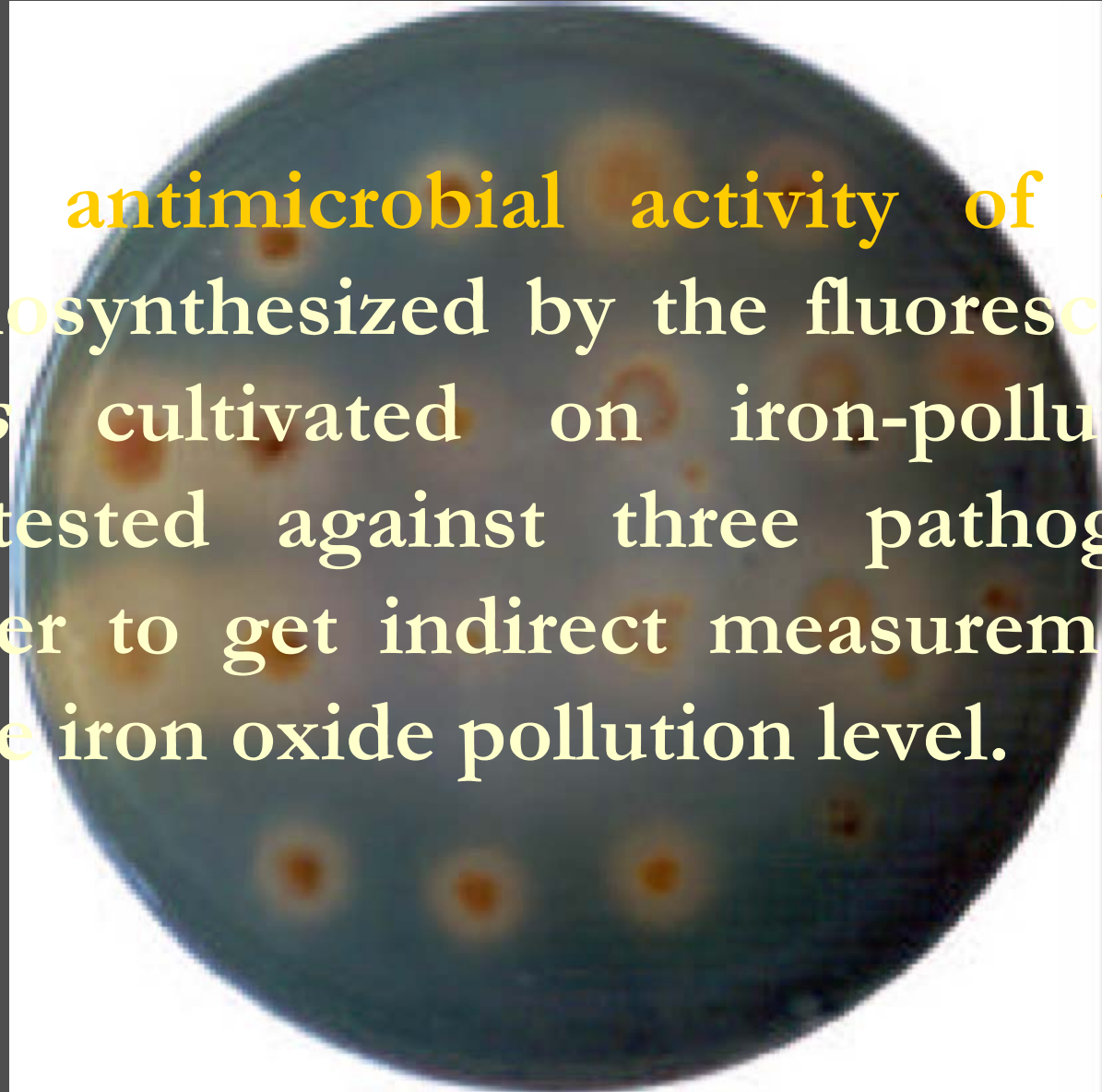
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The ability of *Pseudomonas* strains of scavenging the iron is limited, in the case of this experiment to the ferrofluid level of 0.5 ml/l (iron oxide level being 36 microg/l), suggesting that for higher iron concentration the growth of the iron consuming bacteria is affected.

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**Complementary investigation** was carried out applying the agar diffusion **microbiological test**.

The **putative antimicrobial activity** of the **pyoverdine** biosynthesized by the fluorescent *Pseudomonas* cultivated on iron-polluted media was tested against three pathogen germs in order to get indirect measurement method for the iron oxide pollution level.



Though for relatively high ferrophase concentrations two of the three germs (*Sarcina lutea* and *Staphylococcus aureus*) have responded coherently, for relatively low concentrations the experimental data are rather spread. More, in the range of high ferrophase concentrations, a rapid saturation tendency of the pyoverdine antimicrobial activity versus iron oxide concentration was revealed – which is not convenient for a sensitive measurement method.

The third germ (*Bacillus cereus*) showed no sensitivity to the pyoverdine action.

## ***CONCLUSION***

Considering the statistical significance of the fluorescence enhancing following the enhancing of iron oxide level, one can consider that the accuracy of this measurement method is acceptable as a biosensing tool able to control environment iron pollution.

The method can be useful when a calibration curve for fluorescence intensity is established, for instance in the case of aqueous natural media polluted with iron oxide and hosted by fluorescent *Pseudomonas*. Further improvements are designed in order to validate bacterial biocontrol possibilities.

## References - selection

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