

## Proposal of an EPR Based Method for Pollution Level Monitoring in Mangrove Sediments

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O objetivo deste estudo foi o de monitorar o nível de poluição de sedimentos subaquáticos de florestas de mangue utilizando espectroscopias de ressonância paramagnética eletrônica (EPR) e de infravermelho próximo (NIR) e análises químicas. A espectroscopia de EPR foi útil para determinar a presença de radicais livres orgânicos de semiquinonas, bem como, fornecer informações sobre a presença de ferro trivalente em domínios diluídos e concentrados nos espectros das amostras. Concluiu-se que, o uso de EPR e NIR, no intervalo espectral adequado, tornou possível avaliar, de forma não destrutiva, o nível de poluição ambiental em sedimentos subaquáticos costeiros de florestas de mangue. Assim, em um período relativamente curto e imprescindível, as autoridades podem tomar as decisões necessárias a fim de evitar danos ambientais.

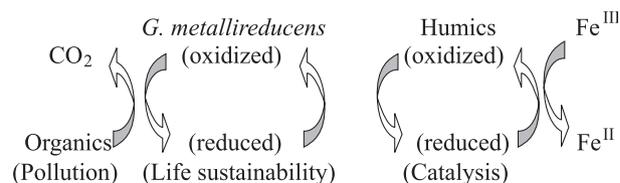
The objective of this study was the monitoring of the pollution level of underwater mangrove sediments using electron paramagnetic resonance (EPR), near-infrared (NIR) spectroscopy and chemical analyses. EPR spectroscopy was useful to determine the presence of organic free radical semiquinone, as well as, to provide information about the presence of trivalent iron in diluted and concentrated or clustered dominion in the sediment samples spectra. It was concluded that by using EPR and NIR spectroscopy measurement, in the appropriate spectral range, it was possible to nondestructively assess the environmental pollution level of coastline underwater mangrove sediments. Though, in a relatively small period of time, the authorities can take the necessary decisions to avoid environmental damages.

**Keywords:** EPR and NIR spectroscopy, mangrove forest, environment monitoring, pollution level, sediments

### Introduction

Specific areas of mangrove ecosystems in Brazil, although very important economically as a space of fish and other marine potential food development and for ecological tourism expansion, are under serious human contamination risk since they are close to urban growing areas. Pollution effects over chemical structures demand the development of physical-chemical analytical methodology to monitor the level of pollution in the mangrove ecosystems. Humic substances act as electron shuttles between microorganisms and Fe<sup>III</sup> oxides or Fe<sup>III</sup>-containing clay minerals.<sup>1</sup> In polluted environment certain microorganisms can reduce humic substances, which then reduce Fe<sup>III</sup>-containing solid phases to access soluble Fe<sup>II</sup>. Scott *et al.*<sup>2</sup> used electron paramagnetic resonance (EPR) spectroscopy to quantify the semiquinone-type free radical concentration in humic substances before and after contact with *Geobacter*

*metallireducens*. Scheme 1 shows the proposed sequence of biochemical and chemical reactions:



**Scheme 1.** Model of humic substances (humics) serving as electron shuttles (catalyst) between humics-reducing microorganisms and Fe<sup>III</sup> oxides. Adapted from Scott *et al.*<sup>2</sup>

Based on an observed increase in free radical concentration after incubation, it is asserted that quinone moieties in humic substances are the mediators of electron transfer reactions between microorganisms and Fe<sup>III</sup> ions. Fe<sup>III</sup> ion is frequently the most abundant potential electron acceptor for the anaerobic oxidation of organic contaminants in polluted subsurface environments.<sup>3</sup> Humics are considered to promote the activity of Fe<sup>III</sup>-

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reducing microorganisms by alleviating the need for direct contact between Fe<sup>III</sup> oxides and Fe<sup>III</sup>-reducing microorganisms.<sup>4</sup>

The hydroquinone moieties produced as the result of electron transfer to HS can abiotically reduce Fe<sup>III</sup> oxides, or Fe<sup>III</sup>-containing clay minerals, producing Fe<sup>II</sup> and regenerating oxidized HS that can again serve as an electron acceptor in the metabolism of Fe<sup>III</sup>-reducing microorganisms.<sup>5</sup>

Semiquinone radical, the first reduction product of quinone, is EPR active and Fe<sup>II</sup>, the first reduction product of Fe<sup>III</sup>, is not EPR active. EPR measurements of the semiquinone radical in solid or dissolved HS require greater semiquinone concentration than normally exists in this case, as semiquinone radical acts at the catalytic level.<sup>6</sup> By the other hand, no correlation was found between the reduction power of HA and the semiquinone content as measured by EPR spectroscopy. In consequence, a hypothesis for abiotic electron transfer reactions of HA, involving complexed Fe<sup>III</sup> as mediator, was proposed.<sup>7</sup>

Although, in general a large amount of data is required for calibration and validation of environmental analysis model,<sup>8</sup> in this work we utilize a simple method using EPR spectroscopy in order to predict environmental quality by analyzing only a few number of samples. Instead of measuring quantitatively the semiquinone concentration, we analyze the Fe<sup>III</sup> ions EPR signal of the whole sediment. EPR spectroscopy is a suitable tool for the studies of the environmental organic and inorganic matter complexes with paramagnetic ions. That technique may provide useful information about oxidation states, modes of coordination and geometry of the ligand sites.<sup>9-11</sup> If the sample place is polluted, the action of microorganisms in oxidizing the contaminants is very intense. The level of semiquinone resulting from the reduction of quinone moieties is high and that of Fe<sup>III</sup> species will be lower, as can be seen by means of the scheme presented above.

Chemical bonds between light atoms such as C-H, O-H, and N-H generally have high vibrational frequencies and strong vibrational overtones detectable in the near infrared (NIR) spectroscopy region. These spectroscopic features make this region suitable for registration aspects of the chemical composition of organic sediment samples. We used in this work the chemometric method principal components analysis (PCA), to analyze the NIR spectroscopy data set to classify the samples according with similar characteristics. With the objective of known more about chemical structures of under mangrove forest sediments, and the need of the development of a prompt and effective analytical method to correlate those chemical structures with the local pollution level, encouraged us to make this study.

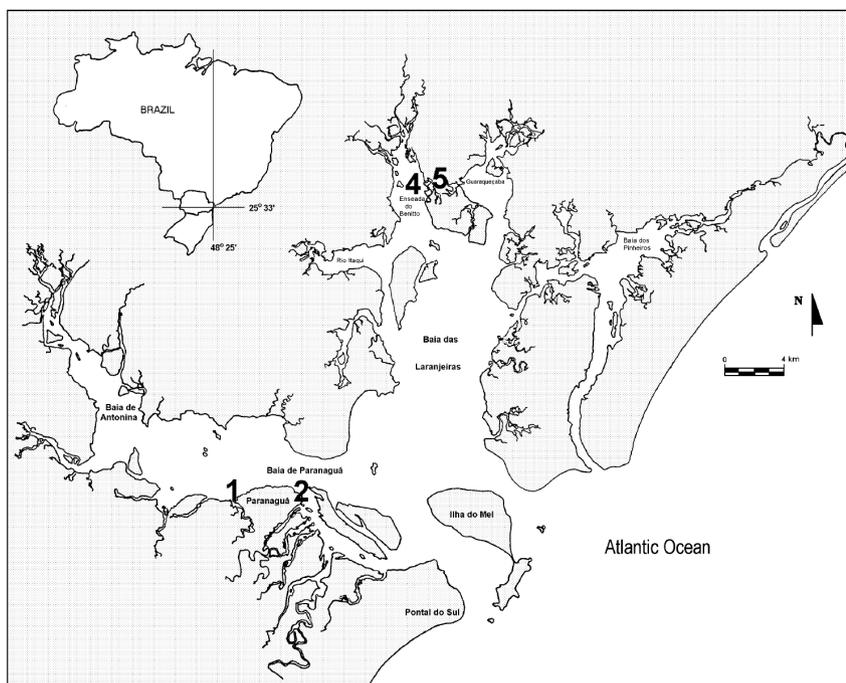
## Experimental

### *Sampling stations*

The Paranaguá Bay, where the organic sediment samples were collected for this study (Figure 1), extends to the West and to the North of the Paraná State, Brazil, coastline. Its estuarine complex (480, 25' W, 250, 33' S) is the largest estuary of the Paraná State coast and it stretches into the continent from its access bars sided by an island called Ilha do Mel (Honey Island). Its restricted mouth, in consequence of the barrier caused by this island, creates excellent conditions for considerable mangrove development. The Paranaguá City has about 250,000 inhabitants and is the main city inside the Bay. It influences adjacent areas with domestic effluents and depends economically from the important Paranaguá Port, with a great movement of petrol, agricultural products and fertilizers transportation ships, mainly during the crop period and exportation of the soggy. There is a commercial agreement to construction of a big ethanol exportation structure inside the Paranaguá Port, there is also a potential for industrial development, and some already installed factories discharge waste into the inland waters. These activities produce enough chemical contaminants to create a localized debilitation of the mangrove forest around the Port. In this area, considered polluted (P), two representative samples of the region were collected (Figure 1, sampling stations 1 and 2). On the other hand, far from the Paranaguá City, but also inside the Bay, in a clean location, there is a place called Benito, full of beautiful mangrove forest. In this area, considered non-polluted (NP), two sediment representative samples of the region were collected (Figure 1, sampling stations 4 and 5). Sediment samples were taken from the surface (sample 1 and 4) or from the seabed position with deep of 1 m from the water surface (sample 2 and 5). After collected from the sites, the sediment samples were stored and transported in plastic bags until reach to the laboratory. There, the samples were dried over laboratory rows, reduced to powder and sifted in fine nylon sieve.

### *Analytical methods*

Total Al, Mn, Fe, Co and Cu analyses were carried out on a Varian model 250 PLUS atomic absorption spectrophotometer. For that purpose, solutions obtained by the sediment samples treatment with concentrated HNO<sub>3</sub>:HCl, 3:1, were conveniently introduced into air-acetylene, or nitrous oxide-acetylene, flames. Coefficients of variations between replicate metal analyses averaged 1%.



**Figure 1.** Paranaíba Bay map and the position of samples collected of polluted (P) (1 and 2) and non-polluted (NP) (4 and 5) sampling stations.

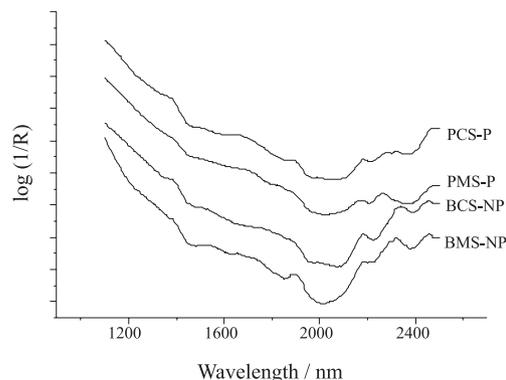
A Bruker ESP 300E spectrometer operating at a frequency of 9.7 GHz (X-band), with 100 kHz modulation frequency and 2.024 G of modulation amplitude was used to obtain the EPR spectra. All spectra were normalized. The “weak pitch”, which is a standard free radical sample, supplied by Bruker into a quartz tube ( $g = 2.0028$ ) was used to estimate accurate  $g$ -values.

NIR spectra of the powdered sediment samples were registered at room temperature using a NIR Cary, 5G –UV-Vis-NIR spectrophotometer. The instrument measures diffuse reflectance that is transformed to absorbance ( $A$ ) similar values according to the relationship,  $A = \log(1/\text{reflectance})$ . Data were collected at 4 nm intervals between 1100 and 2500 nm. One NIR spectrum was collected from each sediment sample. All spectra were normalized. Principal components analysis (PCA), implanted in the MATLAB package, was used to analyze the NIR data set.

## Results and Discussion

### NIR spectroscopy

We will first use NIR spectra (Figure 2) to investigate the possibility of discrimination among the sediment samples. In Figure 3, a PCA of the four sediment samples NIR spectra displays normal segregate regions (PC1) with the non-polluted, BMS-NP and BCS-NP samples in one region, and the polluted, PMS-P and PCS-P, samples



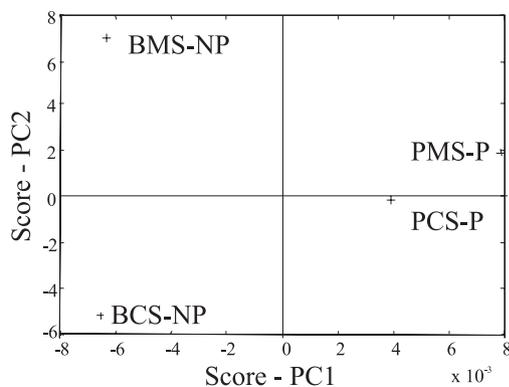
**Figure 2.** Whole sediment samples diffuse reflectance near infrared (NIR) spectra from polluted, PCS-P and PMS-P, and non-polluted, BCS-NP and BMS-SP, sampling stations.

on the other region. NIR analysis has been applied more effectively for sediments rich in organic matter.

We can identify three regions corresponding to the four sediment samples where PC1 differentiates between the polluted and non-polluted samples, while PC2 differentiates between the mangrove and channel samples. The polluted PCS-P and PMS-P samples are more similar than the non-polluted BMS-NP and BCS-NP samples.

### Metals contents

Al, Mn, Fe, Co and Cu concentrations determined by atomic absorption spectroscopy in the sediment samples



**Figure 3.** Principal Components Analysis (PCA) of whole sediment samples near infrared (NIR) spectra from polluted, PCS-P and PMS-P, and non-polluted, BCS-NP and BMS-SP, sampling stations.

were higher in non-polluted than that in polluted samples (Table 1). Both the polluted and non-polluted sediment samples presented Al and Fe detected in higher amounts than Mn, Co and Cu. Sediment sample concentrations for Fe are good parameters for the pollution level estimation. Normally the non-polluted samples present higher iron contents.<sup>2</sup> In the present case the Al, Mn, Fe, Co and Cu concentrations decrease with the enhance of the pollution degree of the studied sediment samples indicating that the prediction for iron is also applicable for the other studied metals.

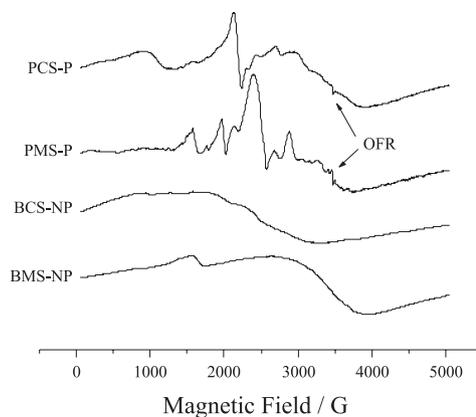
**Table 1.** Total quantity ( $\text{mg kg}^{-1}$ ) of aluminum (Al), manganese (Mn), iron (Fe), cobalt (Co) and copper (Cu) in the sediment, Paranaguá Chanel (PCS), Paranaguá Mangrove (PMS), Benito Chanel (BCS) and Benito Mangrove (BMS). P stands for polluted and NP for non-polluted

Sample	Al	Mn	Fe ( $\text{mg kg}^{-1}$ )	Co	Cu
PCS-P	5840	72.66	4600	2.97	4.95
PMS-P	1940	10.05	2090	ND	2.83
BCS-NP	11870	123.37	15220	9.88	5.88
BMS-NP	37440	143.38	34970	5.60	18.33

### EPR spectroscopy

EPR spectra of the whole sediment samples, at a sweep range of 5000 G, are shown in Figure 4.

The spectra of the polluted samples PCS-P and PMS-P show sharp lines (arrows), almost immersed into broad lines, with  $g = 2.0037$ , typical of the organic free radical (OFR) *ortho*- or *para*-semiquinone absorption.<sup>12</sup> This fact is suggestive of a more anoxic (more polluted) condition on the environment of the sampling stations 1 and 2 (Figure 1). Spectra for the polluted samples PCS-P and PMS-P showed also features of several narrowed EPR line absorptions below 3000 G. Concerning the iron ions, EPR



**Figure 4.** Electron paramagnetic resonance (EPR) spectra (300 K) measured non-destructively on whole sediment samples from polluted, PCS-P and PMS-P, and non-polluted, BCS-NP and BMS-NP, sampling stations.

spectroscopy is active for  $\text{Fe}^{\text{III}}$  in low or high spin complexes at low or high concentration. For  $\text{Fe}^{\text{II}}$  ions EPR is effective only for high spin complexes at very low temperature (*ca.* 4 K). There are two main regions typical for  $\text{Fe}^{\text{III}}$  ions in high spin complexes in the EPR spectra. The first one is at magnetic fields below 3000 G, with  $g$ -values around 9.0 (*ca.* 750 G), 5.0 (*ca.* 1400 G), 4.3 (*ca.* 1500 G), and 3.5 (*ca.* 1750 G) and 2.8 (*ca.* 2500G). This region of the EPR  $\text{Fe}(\text{III})$  spectrum is called of diluted dominion.<sup>10</sup> All sediment samples showed a wide line of absorption (Figure 5) that in the case of the non-polluted samples are extended practically over all the magnetic field range used. These wide lines occur due to the presence of  $\text{Fe}^{\text{III}}$  oxides,  $\text{Fe}^{\text{III}}$ -containing clay minerals or other  $\text{Fe}^{\text{III}}$  species in relatively great concentration on the samples.<sup>9-11,13,14</sup>  $\text{Fe}^{\text{III}}$  occurring in this domain is referred to as concentrated or “clustered”  $\text{Fe}^{\text{III}}$  ions domain. EPR spectra for sediment samples BCS-NP and BMS-NP suggest that the origin of these wide lines is due to a large concentration of  $\text{Fe}^{\text{III}}$  ions forming complexes with constituents of the inorganic-organic matter, besides iron oxides. This fact is in agreement with the higher concentration of iron in the two non-polluted samples mainly in the BMS-NP that presented the higher Fe concentration (Table 1). The wide EPR lines of those two sediment samples suggest also an oxic condition for the sample stations environment 4 and 5.

### Conclusions

The chemometric method principal component analysis (PCA) of sediment samples NIR spectra displays four points corresponding to the four sediment samples where PC1 differentiates between the polluted and non-polluted samples, while PC2 discriminates between the mangrove and channel samples. The polluted samples, PCS-P and

PMS-P, are more similar in chemical structures, according the NIR spectra, than the non-polluted BMS-NP and BCS-NP. Al, Mn, Fe, Co and Cu concentrations determined by atomic absorption spectroscopy were higher in the non-polluted than that in the polluted sediment samples. EPR spectroscopy was useful to determine the presence of organic free radical semiquinone in the polluted sediment samples and provided information also about the presence of Fe<sup>III</sup> ions in diluted dominion in the polluted sediment samples, PCS-P and PMS-P, and in concentrated or clustered dominion in the non-polluted sediment samples. Both those EPR are as foreseeing from the model for humic substances serving as electron shuttles (catalyst) between humics-reducing microorganisms and Fe<sup>III</sup> oxides (Figure 1). EPR and NIR spectroscopy methods presented in this paper were constituted in useful tool to relatively rapid analysis for the underwater sediment pollution level determination.

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