



**UNDER THE AUSPICES OF H. E. THE PRESIDENT OF THE HELLENIC REPUBLIC
MR PROKOPIOS PAVLOPOULOS**

**17TH INTERNATIONAL CONFERENCE
ON CHEMISTRY AND THE ENVIRONMENT**

16 - 20 JUNE 2019 THESSALONIKI, GREECE

Venue:
ARISTOTLE UNIVERSITY RESEARCH DISSEMINATION CENTER
(KEDEA)



CONFERENCE PROGRAMME

	² <i>Institute of Experimental Biology, Faculty of Science, Masaryk University, Czech Republic</i> ³ <i>Academy of Sciences of the Czech Republic, Global Change Research Institute, Czech Republic</i>
19:30-19:45	ID 358. Influence of natural amino acids on CuO nanoparticles antimicrobial activity
	Badetti E.¹, Calgaro L.¹, Falchi L.¹, Bonetto A.¹, Bettiol C.¹, Leonetti B.², Ambrosi E.², Zendri E.¹, Marcomini A.¹ ¹ <i>DAIS - Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari of Venice, Italy</i> ² <i>DMSN - Department of Molecular Sciences and Nanosystems, University Ca' Foscari of Venice, Italy</i>
19:45-20:00	ID 388. Evaluation of the inhibition effectiveness of ampiciline in the corrosion steel in acid solution
	Seiti B.¹, Xhanari K.¹, Bajrami N.¹ ¹ <i>Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania</i>
20:00-20:15	ID 575. The inhibition effect of the amygdali amare biter semen extract in the corrosion of carbon steel in 3 wt.% NaCl solution
	Seiti B.¹, Xhanari K.¹, Lila R.¹, Ylli F.², Alinj A.³ ¹ <i>Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania</i> ² <i>Nuclear Physics Institute, University of Tirana, Albania</i> ³ <i>Department of Chemistry, Faculty of Technical Sciences, 'Ismail Qemali' University, Albania</i>
20:15-20:30	ID 450. Thin gold film electrode for voltammetric determination of Chromium (VI)
	N. Broli, M. Vasjari <i>University of Tirana, Faculty of Natural Science, Albania</i>
19:15-20:15	Monday, Conference Hall C: Session 1.5.C Humic Substances: environmental dynamics and impact on water quality Chairs: Yiannis Deligiannakis, University of Ioannina, Greece Gudrun Abbt-Braun, Chair of water chemistry and water technology, Karlsruhe Institute of Technology, Germany Keynote Speaker: Norbert Hertkorn, Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Research Unit Analytical BioGeoChemistry, Germany
Oral Presentations	
19:15-19:45	ID 522. Keynote Speech Pelagic Sargassum brown algae release significant proportions of phlorotannins into the oceans
	L. Powers^a, N. Hertkorn^b, N. McDonald^{c,d}, P. Schmitt-Kopplin^{b,e}, R. Del Vecchio^f, N. Blough^g, and M. Gonsior^a ^a <i>University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, USA</i> ^b <i>Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Research Unit Analytical BioGeoChemistry, Germany</i> ^c <i>Bermuda Institute of Ocean Sciences, Bermuda</i> ^d <i>GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany</i> ^e <i>Technische Universität München, Chair of Analytical Food Chemistry, Germany</i> ^f <i>University of Maryland, Earth System Science Interdisciplinary Center, USA</i> ^g <i>University of Maryland, Department of Chemistry and Biochemistry, USA</i>
19:45-20:00	ID 371. Antioxidant properties of humic acids extracted from saltmarsh soils (Marano and Grado Lagoon, northern Adriatic Sea)
	Bravo C.^{1,2}, Khakbaz A.¹, Toniolo R.¹, Millo C.³, Contin M.¹, De Nobili M.¹

	<p>¹Department of Agricultural Food Environmental and Animal Sciences, University of Udine, Italy</p> <p>²Department of Life Sciences, University of Trieste, Italy</p> <p>³Oceanographic Institute, University of Sao Paulo, Brazil</p>
20:00-20:15	<p>ID 173. A NMR perspective on the effects of drinking water treatment on the structure and composition of dissolved organic matter (DOM)</p>
	<p>Norbert Hertkorn^a, Anna Andersson^b, Elin Lavonen^c, Mourad Harir^{a,d}, Michael Gonsior^e, Philippe Schmitt-Kopplin^{a,d}, Henrik Kylin^b, Susanne Karlsson^b, Kerstin Nilsson^f, Ämma Petterson^g, Helena Stavklint^h and David Bastviken^b</p> <p>^aGerman Research Center for Environmental Health, Helmholtz Zentrum Munich, Germany</p> <p>^bDepartment of Thematic Studies – Environmental Change, Linköping University, Sweden</p> <p>^cNorrvatten, Kvalitet och Utveckling, Sweden</p> <p>^dTechnische Universität München, Chair of Analytical Food Chemistry, Germany</p> <p>^eChesapeake Biological Laboratory, University of Maryland Center for Environmental Science, United States</p> <p>^fVA SYD, Sweden</p> <p>^gNodra, Sweden</p> <p>^hTekniska verken i Linköping, Sweden</p>

Antioxidant properties of humic acids extracted from saltmarsh soils (Marano and Grado Lagoon, northern Adriatic Sea)

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Keywords: humic acids; redox properties; saltmarsh soils; ABTS

1. Introduction

In natural wetlands and submerged soils, humic acids (HA) are involved in numerous redox processes (Keller et al., 2009). HA can be used by facultative anaerobic bacteria as terminal electron acceptors during anaerobic respiration (Lovley et al., 1996) and upon re-aeration, they can donate electrons to oxygen (Klupfel et al., 2014). In addition to acting as redox buffers, HA can stimulate Fe reduction via electron shuttling. HA also mediate electron transfer to organic (e.g. halogenated hydrocarbons) and inorganic (e.g. As, Hg) pollutants, affecting their geochemical cycle.

The antioxidant capacity (AOC) of HA, defined as the capacity to donate electrons by the reduced functional groups, depends on their molecular structure (phenolic groups and quinones act as major reducible moieties in HA) (Nanny and Ratasuk, 2007) and on soil conditions (pH and Eh).

The aim of this work is to quantify the AOC of free and bound HA extracted from saltmarsh soils of the Marano and Grado Lagoon (northern Adriatic Sea) using the ABTS decolorization assay and to link AOC to their geochemical characteristics.

2. Materials and methods

Surface soils were sampled in three saltmarshes of the Marano and Grado Lagoon: Allacciante di Marano (AM) (natural saltmarsh with strong river influences), Allacciante di San Andrea (ASA) (channel-fringing saltmarsh where dredging operations influenced the natural process of soil formation) and Barena di Martignano (BM) (back-barrier saltmarsh where the influence of the open sea is dominant). In each saltmarsh two sampling points were considered.

HA were extracted from soils under a N₂ flux for 1h, first with 0.5 M NaOH (free HA) and then with 0.1 M Na₄P₂O₇ plus 0.1 M NaOH (bound HA) (De Nobili et al., 2008) The extracts were centrifuged (14000 rpm for 20 min) and supernatants were filtered using 0.2 µm cellulose filters. Free and bound HA were precipitated at pH 1 with 6 M HCl, separated by centrifugation, dialyzed against ultrapure water (until Cl⁻ free), frozen and then freeze-dried.

Organic carbon (C_{org}), total nitrogen (N_{tot}) and carbon stable isotope composition (δ¹³C) of bulk soils and HA (free and bound) were determined by a Costech Instruments Elemental Combustion System elemental analyser, coupled with an Isotope Ratio Mass Spectrometer (Thermo Scientific Delta V Advantage). UV-vis spectra were recorded using a Varian Cary 1E spectrophotometer from 220 to 800 nm at a scan rate of 60 nm min⁻¹. ATR-FTIR spectra were recorded with a PerkinElmer Spectrum 100 FTIR spectrometer over an interval from 4000 to 800 cm⁻¹. A linear baseline correction was applied to all spectra. Intensity ratios were calculated for specific pairs of bands (Inbar et al., 1989).

AOC was determined using the 2,2'-azino-bis-(3-ethylbenzothiazolinesulfonic acid) radical cation (ABTS^{•+}) decolorization assay. The ABTS^{•+} was generated according to Re et al. (1999) at two different pH: 4.79 (0.1 M citrate buffer) and 7.00 (0.1 M phosphate buffer). For measurements, the ABTS^{•+} solution was diluted to an absorbance of 0.70 at 734 nm. After adding spikes (20, 50, 100, 200, 300, 400 µL) of HA solutions (0.5 g L⁻¹), the absorbance decrease at 734 nm was measured continuously for 18 min. The AOC, expressed as mmol e⁻ g_{HA}⁻¹, was calculated considering the decrease in absorbance measured 30 s after addition of HA.

3. Results and discussion

C_{org}, N_{tot} and δ¹³C values of bulk soils and free and bound HA are reported in Table 1. These results highlight the presence of a geographical gradient among the three saltmarshes. The amount of C_{org} present in soils decreases from the innermost saltmarsh (AM) toward the most external one (BM). At the same time, δ¹³C values become less negative, reflecting the decreasing contribution of terrestrial inputs. This is confirmed by C/N ratios. Free and bound HA reflect the same trend of bulk C_{org}. On the other hand, bound HA well differentiate from free HA on the basis of their C/N ratio.

Table 1: C_{org}, N_{tot}, δ¹³C and C/N values of bulk soils, free and bound HA.

Station	Soil				Free HA				Bound HA			
	C _{org} %	N _{tot} %	δ ¹³ C ‰ V-PDB	C/N	C _{org} %	N _{tot} %	δ ¹³ C ‰ V-PDB	C/N	C _{org} %	N _{tot} %	δ ¹³ C ‰ V-PDB	C/N
AM1	4.5	0.4	-24.9	11.1	52.0	6.5	-25.9	8.0	51.6	4.3	-25.6	11.9
AM3	4.9	0.4	-25.1	12.2	49.5	5.6	-25.5	8.8	53.3	3.9	-25.7	13.7
ASA1	1.6	0.2	-19.0	9.3	n.d.	n.d.	-19.4	n.d.	48.5	5.1	-19.2	9.6
ASA3	4.8	0.4	-21.4	10.9	48.7	6.4	-21.6	7.6	52.4	5.2	-21.4	10.1
BM1	0.4	0.1	n.d.	9.1	53.4	8.8	-22.5	6.1	56.2	6.0	-22.9	9.4
BM3	0.5	0.1	-19.4	9.1	50.9	8.8	-19.1	5.8	51.0	5.3	-18.5	9.7

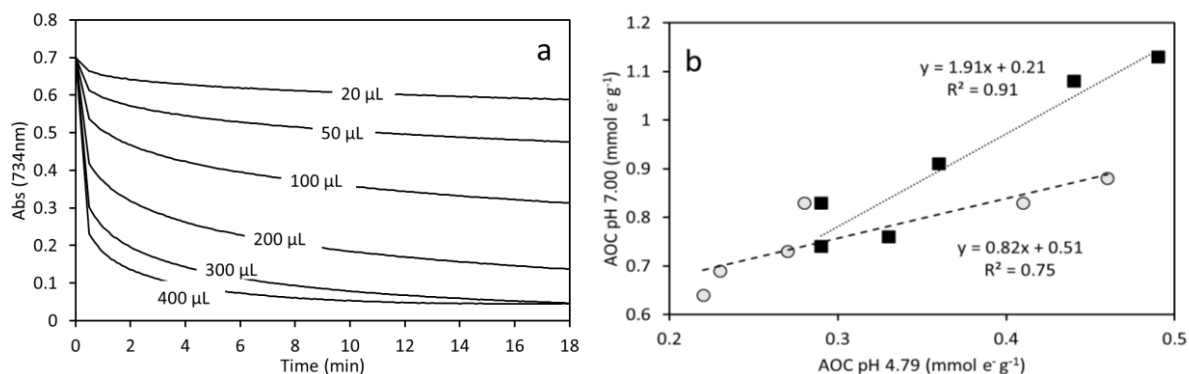


Figure 1: a. Time course of absorbance at 734 nm for the reactions of ABTS⁺ with increasing quantities of HA (BM3 free HA, as example). b. Correlation between the AOC measured at pH 4.79 and 7.00 for free (grey circles) and bound (black squares) HA.

A stable end-point of the reaction between ABTS⁺ and HA was not reached during the time allowed for the experiment (Figure 1a). This suggests that at least two mechanisms may be involved: a faster one, that occurs at the beginning of the reaction and a slower one that lasts over time. We decided to calculate the AOC after 30 s of reaction because long periods of time are not environmentally significant considering that, to support electron transfer for biological reactions, fast processes are involved.

We determined the AOC both in acid and neutral conditions. For all samples, the AOC is higher at pH 7.00 compared to pH 4.79 (Figure 1b), probably due to a greater dissociation of phenolic groups. Moreover, bound HA present a higher AOC (about +20%) compared to that of free HA: this can be attributed to increased molecular complexity and degree of aromaticity in the bound HA (as highlighted by FTIR and UV-vis spectra).

Both free and bound HA show a decrease of AOC values moving from the innermost saltmarsh (AM) to the most external one (BM). The negative correlation between $\delta^{13}\text{C}$ and AOC values (Figure 2a) and the positive correlation between C/N ratio and AOC values (Figure 2b) are consistent with each other and confirm that HA with more terrestrial influence have a higher AOC. This could be due to a major phenolic character of terrestrial-derived HA. FTIR band ratios and SUVA₂₅₄ support this hypothesis.

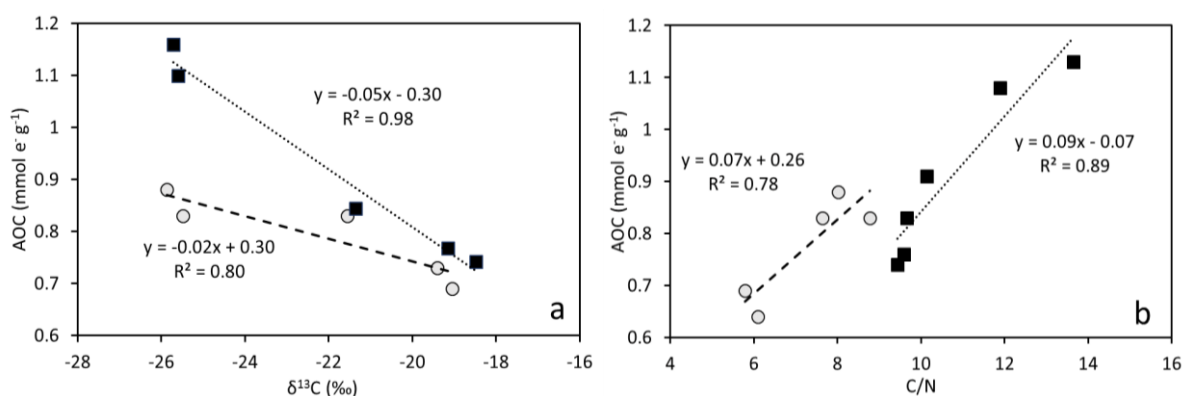


Figure 2: Antioxidant capacity as a function of $\delta^{13}\text{C}$ (a) and C/N ratio (b) of free (grey circles) and bound (black squares) HA.

4. Conclusions

This study demonstrates that the AOC of HA extracted from saltmarsh soils is strongly related to their geochemical characteristics. Future studies will focus on the kinetics and the mechanisms involved during electron transfer from HA to the ABTS⁺, to highlight the processes involved in the fast and slow steps of the ABTS⁺ reduction.

References

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