CHEMICAL CHARACTERIZATION OF RED CELLS FROM THE BLACK SEA URCHIN *Arbacia lixula* BY X-RAY PHOTOELECTRON SPECTROSCOPY

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1. Comparison between the data acquired on colorless and the red cells spots in triplicate.

To exclude the data being affected by some localized differential charging effects, we have reported for comparison the original C1s, O1s and N1s signals acquired on both samples, on three different spots for colorless and red cells populations, acquired in succession in alternate mode in three consecutive days. Figure S1 refers to colorless cells replicates and Figure S2 to red cells replicates. C1s spectra obtained on different spots of the same sample are quite similar, and some difference in shape between the same peaks can be attributed to a natural variability in composition reported in Table 1 as standard deviation of the At% composition. To validate these results, we have added two tables (Table S2 and S3) reporting the parameters (i.e.: BE, FWHM and Lorentzian/Gaussian (Lw/Gw mix ratio)) used in curves fitting of peaks. No significant broadening can be observed on C1s and N1s colorless and red cells peaks. The FWHM of C1s peak components is quite similar the resolution measured in comparison to the PET standard (1.0 eV). However, a more important widening is observed for the O1s FWHMs in the red cells samples that could be associated to the oxygen chemical differences between the different samples, according to the modified Auger parameters.

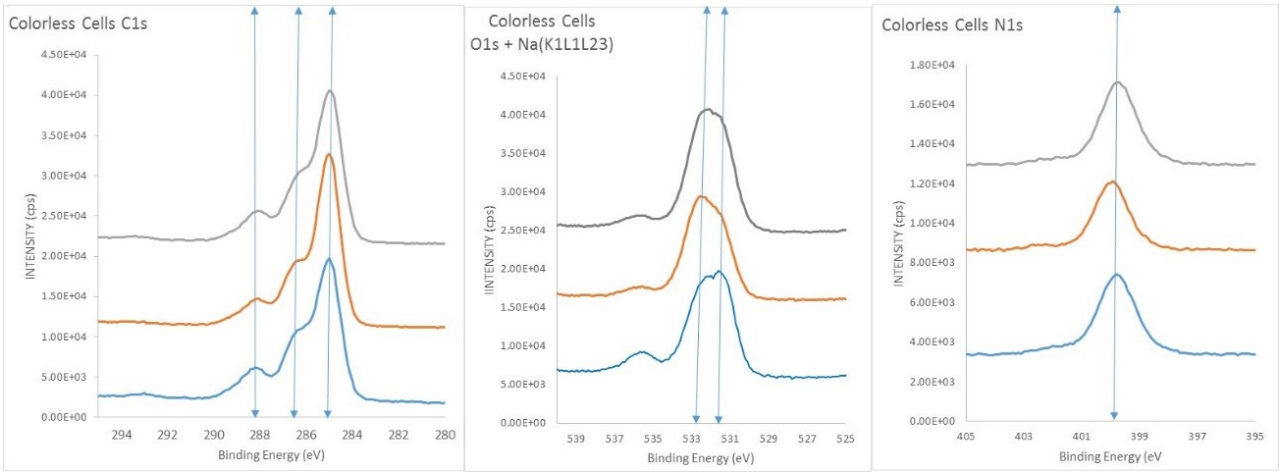

Figure S1. *Comparison of C1s, O1s and N1s peak signals acquired on the three randomly selected spots of colorless cells sample (blue line 1° spot, red line 2° spot and grey line 3° spot).*

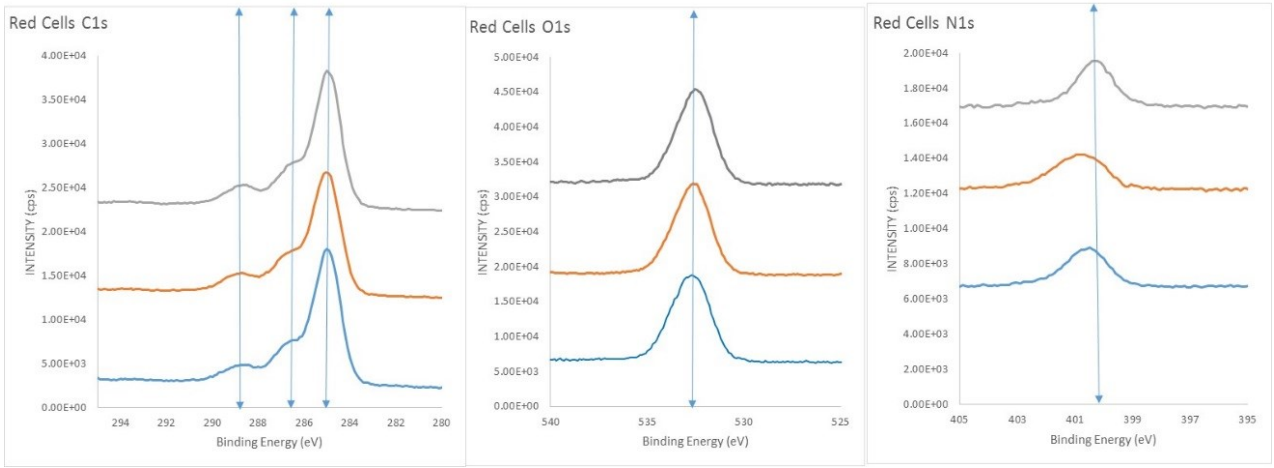

Figure S2. *Comparison of C1s, O1s and N1s peak signals acquired on the three randomly selected spots of the red cells (blue line 1° spot, red line 2° spot and grey line 3° spot).*

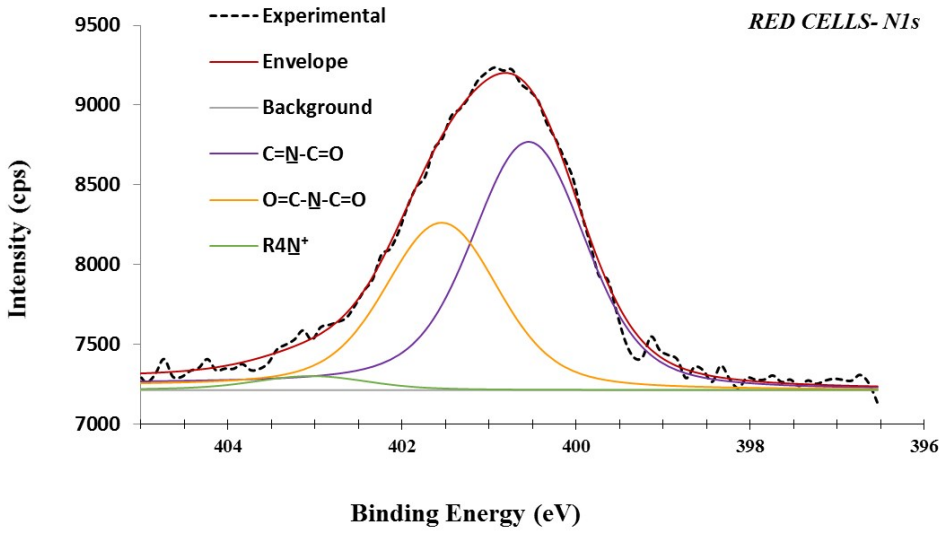

Figure S3. *XPS high-resolution region of red cells (2° spot).*

2. Modified Auger parameter determination

To confirms the chemical shifts attributed to C1s and O1s peak components in the red cells population compared to white cells, we have monitored the modified Auger parameter α' for carbon and oxygen, as an internal parameter not affected from charging effects (Wagner and Josh; 1988)[1], for all the analyzed sample spots. Difference in α' (Δα') refers to the different chemical state of the same element for different samples.

The modified Auger parameter α' (eq. 1) has been calculated as defined from Wagner and Josh (1988)[1]:

$$\alpha' = \text{KE (Auger peak)} + \text{BE (XP peak)} \qquad (1)$$

Where KE is the kinetic energy of the sharpest Auger peak (i.e. C (KLL) and O (KLL)) and BE is the binding energy of the related most intense photo-peak (i.e. C1s and O1s).

A table of the related calculated α'_{oxygen} and α'_{carbon}, averaged on three spots has been added (Table S1). Related peaks have been highlighted with an arrow in Figure S4.

The measured $\Delta\alpha'_{carbon}$ ~ 0.3 eV value related to carbon, referring to C-C/C=C species (the most dominant component for both colorless and red cells), is not significantly different to make a distinction between the chemical carbon species presents on both the cells samples. $\Delta\alpha'_{oxygen}$ ~ 1.2 eV related to the oxygen signals represents an evident real chemical shift that can be attributed to the different oxygenated species on the red cells compared with the colorless ones.

In detail, α'_{oxygen}= 1041.7±0.5 eV value obtained for the colorless cells is within the values range 1041.5 eV-1042.5 eV, attributed to oxygen involved in double bond with carbon in carbonyl and

carboxyl groups and/or in single bond in alcohol /acetal and carboxyl groups (see Wagner plot in NIST database)[2]. Instead, red cells α'_{oxygen}=1042.9±0.3 is very close to the experimental value measured on the dopamine used as standard reference (α'_{oxygen} = 1042.8 eV).

Table S1. *Modified Auger parameters for colorless cells and red cells. The error represent the standard error obtained from the variability observed in the three different sample spots randomly selected on both colorless and red cells. The pairs of peaks C1s (O1s) and related C(KLL) (O(KLL)), used to calculate α' were acquired in the same survey scan. Three pairs were used for both colorless and red cells.*

	Colorless Cells α' (eV)	Red Cells α' (eV)	Δ α' (eV)	Dopamine α' (eV)
C1s, CKLL	546.3±0.1	546.57±0.2	0.3	
O1s, OKLL	1041.7±0.5	1042.9±0.3	1.2	1042.8

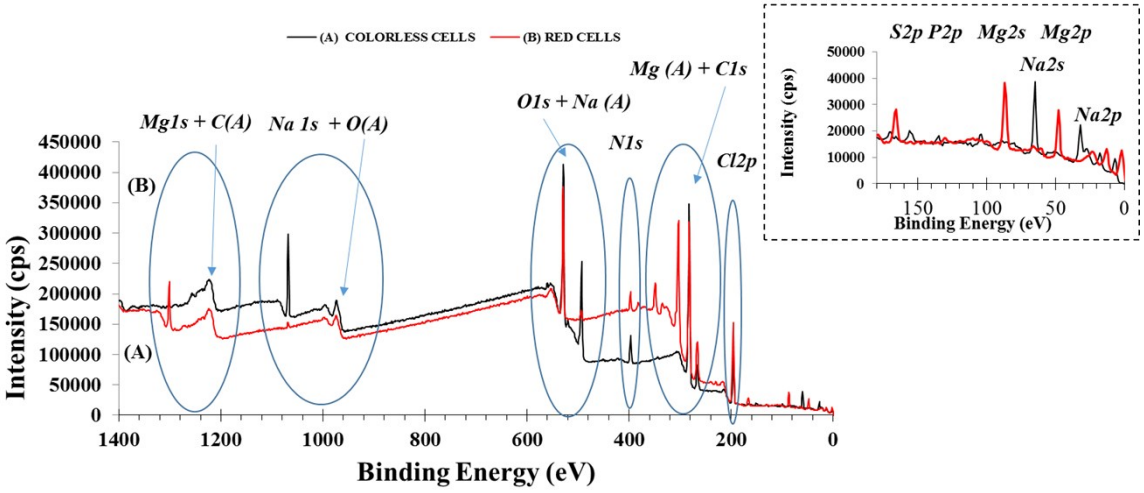

Figure S4. *Survey XPS spectra for colorless (A) and red (B) cells samples, as obtained original data.*

3. Comparison between C1s acquired on red cells before and after X-ray irradiation.

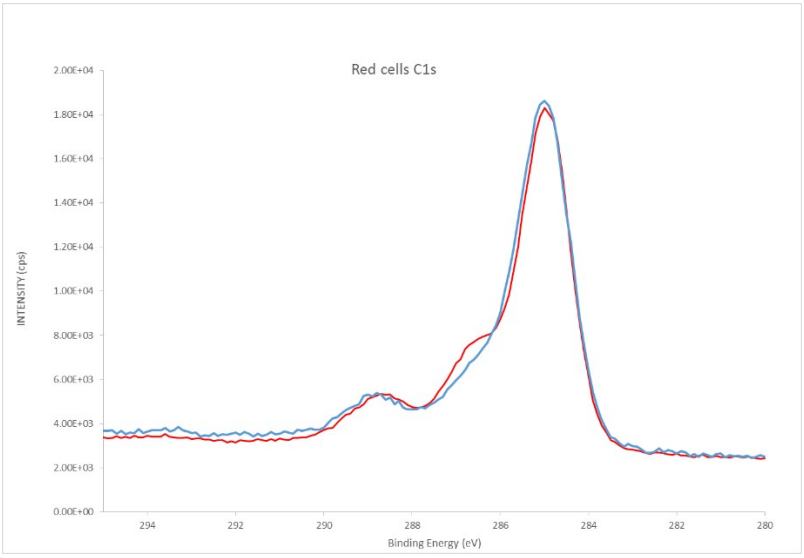

Figure S5. *C1s XPS spectra for red cells sample at the beginning (red line, spot 3) and at the end of the complete analysis (blue line, spot 3).*

4. C1s peaks for Na_2CO_3 and $MgCO_3$

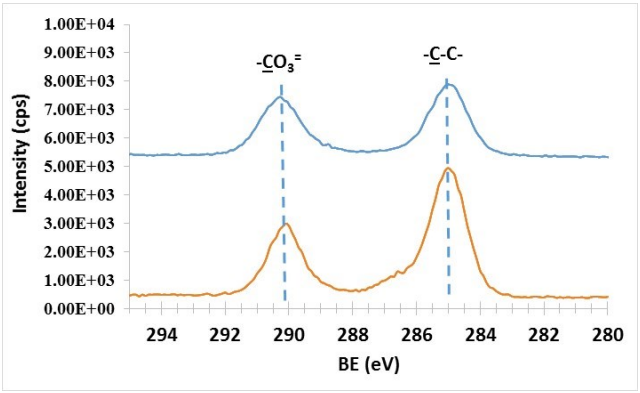

Figure S6. *XPS high-resolution region of C1s peaks for $MgCO_3$ (blu line) and Na_2CO_3 (yellow line), respectively*

Table S2. *Binding energy and parameters used for fitting C1s, O1s, N1s and Cl2p signals on colorless cells samples adsorbed on coverslip substrate, reported as the average obtained on three sample spots. Peak assignments refer to literature data, the NIST standard reference database available on line and experimentally acquired standards when disposable (i.e. $MgCO_3$ and dopamine).*

	Peak fit component	BE (eV)	σ_{BE} (eV)*	FWHM (eV)	σ_{FWHM} (eV)**	Lw/Gw %	Assignment
C_{1s}	(1)	285.00	0.00	1.19	0.04	25	C-C/C=C
	(2)	286.40	0.03	1.19	0.04	25	C-N/C-N-C=O/C-O-H(C)
	(3)	288.02	0.03	1.19	0.04	25	C-N-C=O/C=O/O-C-O
	(4)	288.96	0.02	1.19	0.04	25	-COOH
O_{1s}	(1)	531.4	0.1	1.55	0.01	25	C=O/N-C=O/COO(H)
	(2)	532.7	0.1	1.55	0.01	25	C-O-H(C)/O-C-O
N_{1s}	(1)	399.8	0.1	1.47	0.07	30	C-N/C-N-C=O
	(2)	402.1	0.3	1.47	0.07	30	$C\text{-}NH_3^+$
	(3)	402.8	0.1	1.51	0.001	30	R_4N^+
$Cl2_{p3/2}$	(1)	198.9	0.1	$1.24(2p_{3/2}\text{-}2p_{1/2})$	0.03	25	Cl^-

∗ σ_{BE} (eV): represents the standard errors of binding energy (BE, in eV) calculated from the fitting results obtained analyzing 3 independent sample spots of the colorless cells.
∗∗σ_{FWHE} (eV): represents the standard errors of the full with half maximum (FWHM in eV) calculated from the fitting results obtained analyzing 3 independent sample spots of the colorless cells.

Table S3. *Binding energy and parameters used for fitting C1s, O1s, N1s and Cl2p signals on red cells samples adsorbed on coverslip substrate, reported as the average obtained on three sample spots. Peak assignments refer to literature data, the NIST standard reference database available on line and experimentally acquired standards when disposable (i.e. $MgCO_3$ and dopamine).*

	Peak fit component	BE (eV)	σ_{BE} (eV)*	FWHM (eV)	σ_{FWHM} (eV)**	Lw/Gw %	Assignment
C_{1s}	(1)	285.00	0.00	1.23	0.02	22	C-C/C=C
	(2)	285.83	0.05	1.23	0.02	22	$C_{\alpha}\text{-}C_{qu}$=O
	(3)	286.9	0.1	1.23	0.02	22	C-O-C=O/N-C=O-N
	(4)	288.3	0.1	1.23	0.02	22	C-N-C=O/C=O/O-C-O
	(5)	289.2	0.1	1.23	0.02	22	C(π - π* satellite)
O_{1s}	(1)	532.4	0.1	1.81	0.01	25	$C\text{-}O\text{-}H(C)/O\text{-}C\text{-}O/C_{qu}\text{=}O$
	(2)	533.6	0.1	1.81	0.01	25	C_{Ar}-OH
N_{1s}	(1)	400.6	0.1	1.51	0.04	30	C=N-C=O
	(2)	401.6	0.1	1.51	0.04	30	O=C-N-C=O
	(3)	403.04	0.04	1.51	0.04	30	R_4N^+
$Cl2_{p3/2}$	(1)	199.0	0.1	$1.58(2p_{3/2}/2p_{1/2})$	0.08	25	Cl^-

∗ σ_{BE} (eV): represents the standard errors of binding energy (BE, in eV) calculated from the fitting results obtained analyzing 3 independent sample spots of the colorless cells.
∗∗σ_{FWHE} (eV): represents the standard errors of the full with half maximum (FWHM in eV) calculated from the fitting results obtained analyzing 3 independent sample spots of the colorless cells.

References

[1] C.D. Wagner and A. Josh, J. Electron Spectrosc. Relat. Phenom., 1988, 47, 283-313.

[2] NIST, X-Ray Photoelectron Spectroscopy Database, https://srdata.nist.gov/xps/main_search_menu.aspx