



Co-biosorption of copper and glyphosate by *Ulva lactuca*

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ABSTRACT

This study investigated the adsorption of glyphosate (PMG) onto the green algae *Ulva lactuca*. PMG was not adsorbed by *U. lactuca* but PMG was adsorbed when the process was mediated by Cu(II) with molar ratios Cu(II):PMG \geq 1.5:1.

U. lactuca was characterized by water adsorption surface area, FTIR, SEM and EDS. The Langmuir and Freundlich models were applied. Results showed that the biosorption processes for copper and PMG in the presence of copper were described by the Langmuir model ($q_{\max} = 0.85 \pm 0.09 \text{ mmol g}^{-1}$, $K_L = 0.55 \pm 0.14 \text{ l mmol}^{-1}$ and $q_{\max} = 3.65 \pm 0.46 \text{ mmol g}^{-1}$, $K_L = 0.103 \pm 0.03 \text{ l mmol}^{-1}$, respectively). Copper adsorption was greater in the presence of PMG than in the absence of the pesticide and the adsorption can only be represented by the Freundlich model ($K_F = 0.08 \pm 0.01$, $1/n = 1.86 \pm 0.07$).

In all cases studied, the maximum metal uptake (q_{\max}) increased with increasing pH. Surface complexes with a stoichiometry ranging from $\equiv\text{Cu}-\text{PMG}-\text{Cu}$ to $\equiv\text{Cu}-\text{PMG}-\text{Cu}_3$ are suggested as reaction products of the process.

Due to the increasing amounts of PMG applied in Argentina, natural reservoirs present considerable amounts of this herbicide. The value of this work resides in using *U. lactuca*, a marine seaweed commonly found along coastlines all over the world, as a biosorbent for PMG.

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1. Introduction

Herbicide application may be a contamination source for soils and for the aquatic environment since rivers and streams are receptors of toxic wastes generated on land [1]. Due to the increasing amounts of PMG applied in Argentina, natural reservoirs are commonly impacted by this herbicide [2,3].

PMG is an organophosphorous, broad-spectrum, post-emergent, non-selective herbicide, characterized by the presence of a stable carbon to phosphorous covalent bond. It has a moderate persistence in soils with a half-life ranging between 10 and 100 days [4] and is degraded predominantly by co-metabolic microbial processes [5].

The PMG molecule has phosphonate, amine and carboxylate groups that can coordinate with metal ions, especially at near-neutral pH levels where carboxylate and phosphonate are deprotonated [6]. Besides, phosphonate compounds have the ability to coordinate and form strong complexes with free metals, such as copper ions [7,8]. The ability of PMG to coordinate with metal ions places it in a special class of strongly chelating herbicides [9].

PMG has been used for more than 35 years in over 140 countries, from highly industrialized ones (including USA, Germany, France, UK, Russia, Ukraine, Japan, Australia and New Zealand, among others) to those considered to be developing countries. Currently the world uses 2000 million tons of PMG per year and global usage continues to increase. In Argentina application of the herbicide doubled during the last decade, from 93.3 millions of liters in 1993 to 200.8 millions of liters in 2009 [10].

When PMG adsorbs onto soils, it can no longer be biodegraded, so its persistence increases. Moreover, significant amounts of PMG may eventually be transported to other areas before its ultimate degradation [11].

The extensive use of PMG-based herbicides has lead scientists to develop new ways of degrading PMG in order to get rid of excess herbicide remaining in soils and waters.

Adsorption is a potential remediation technique that may be both economic and less hazardous to the environment. Many works describing PMG adsorption onto clay minerals, organic matter, iron and aluminum-oxides have been published [12–14]. PMG adsorption is pH dependent with unfavorable electrostatic conditions existing at high pH levels because organic and inorganic soil components as well as glyphosate are all negatively charged [15].

A new approach to PMG remediation is given by the adsorption of the herbicide onto biological materials mediated by metal adsorption [16,17]. Among the many biosorbents, marine seaweeds are excellent biosorbent materials that have high adsorption

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coefficients because the primary constituent of their cell walls is polysaccharides that interact with free ions in solution. They are much more efficient adsorbents than natural zeolites and active carbon and, depending on pH, polysaccharide groups are either protonated or deprotonated [18,19]. In addition, seaweeds are benign, easy to handle, cost-effective, renewable and have high affinity for metal ions [20]. In recent years the metal biosorption potential of various red, green and brown seaweeds were investigated by many scientists [21–25 and references therein], but there is a lack of information regarding the capacity of this seaweed to be used for PMG adsorption.

Ulva lactuca, a marine seaweed commonly found along the Argentinean coasts has a cell wall made of a polysaccharide fraction described as a highly charged polyelectrolyte, named Ulvan, whose main component is a disaccharide, the β -D-glucurono-syluronic acid (1 \rightarrow 4) L-rhamnose-3-sulfate [26].

The aim of this work is to study the adsorption of PMG onto green algae *U. lactuca* mediated by Cu(II). Copper was chosen because it is very well adsorbed by *Ulva* gender [27,28], it is extensively used for agricultural purposes as a fungicide and also due to its capacity for complex formation.

2. Materials and methods

2.1. Biomass and pretreatment

U. lactuca was collected from Cabo Corrientes, Mar del Plata, Buenos Aires, Argentina. Seaweeds were washed thoroughly with deionized water, then dried overnight at 60 °C and finally stored in desiccators before used. Afterwards, the dried seaweeds were blended in a homogenizer into finer particles. A stainless steel standard sieve was used to obtain seaweeds particles of uniform size (<125 μ m) which were subsequently used for biosorption experiments.

2.2. Solutions preparation

All chemicals utilized were of analytical reagent grade and were used without further purification. All solutions and algal suspensions were prepared using Milli-Q water.

Stock Cu(II) solutions were prepared by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in water. Solutions concentration ranged from 75 μ M to 9 mM and were obtained by diluting the stock Cu(II) solution.

PMG solutions were fresh and prepared daily by dissolving the herbicide in Milli-Q water. All PMG solution concentrations ranged from 50 μ M to 6 mM.

2.3. Experimental procedure

2.3.1. Batch experiments

Copper adsorption by *U. lactuca* was evaluated by batch sorption experiments with 0.1 g of biomass suspended in 100 ml of copper solutions.

Suspensions were kept in constant agitation at constant pH, ionic strength and room temperature. pH was adjusted along the experiment using small aliquots of highly concentrated HCl or NaOH solutions and ionic strength was kept constant (0.1 M) using a KNO_3 solution.

Algal biomass was separated from metal solutions by filtration with cellulose nitrate membrane filters (0.22 μ m). Afterwards, the final metal concentrations in the filtered solutions were

determined and metal uptake (q) was calculated using the following mass balance equation [19]:

$$q = \left[\frac{(C_i - C_{eq})V}{m} \right] \quad (1)$$

where C_i is the initial metal concentration (mM), C_{eq} the equilibrium metal concentration (mM), V the solution volume (l) and m is the dry seaweed weight (g).

The adsorption of PMG onto *U. lactuca* mediated by copper was studied after copper adsorption was evaluated. Batch sorption experiments were performed with 0.1 g of biomass previously suspended in 100 ml of copper solutions and kept in constant agitation. Once copper adsorption equilibrium was reached, small aliquots of PMG solution were added minimizing the dilution factor in order to keep a Cu(II):PMG molar ratio of 1.5:1. pH was kept constant during the whole experiment by adding NaOH drops.

Samples were shaken during 24 h and afterwards algal biomass was separated from PMG/metal solutions by centrifugation at 17,000 rpm using a Sigma 3-18K Centrifuge. Afterwards, the final concentrations of copper and/or PMG in the supernatant solutions were determined and PMG/metal uptake (q) was calculated using Eq. (1).

Control experiments were carried out in the absence of adsorbents in order to determine whether there is any adsorption of copper and/or PMG on the container walls, and if complex formation would influence copper or PMG quantification.

2.3.2. pH effect

The pH dependence of metal and PMG uptake by *U. lactuca* was studied through batch sorption experiments in a pH range from 2 to 5.5. Cu(II) and PMG initial concentrations were 4.5 and 3 mM, respectively, and the algal concentration was 1 g l⁻¹. The pH was adjusted using highly concentrated HCl or NaOH solutions, while ionic strength was 0.1 M. Resulting pH was measured using a Metrohm 644 pH meter with a combined glass microelectrode.

2.4. Metal quantification

Copper concentrations were spectrophotometrically measured using 1 g of sodium diethyldithiocarbamate (DDTC) dissolved in ethanol–water 50% (w/w) and H_2SO_4 (1 mM) as reagent [29]. A yellow complex with Cu(II) is formed at low pH values by DDTC. Absorbance of the corresponding solution was measured at 440 nm with a double beam UV–vis spectrophotometer Shimadzu-Pharmaspec UV-1700.

PMG was evaluated by ion chromatography [30] using a DIONEX DX-100 instrument with a conductivity detector, a sample injection valve, and a 25 μ l sample loop. Two plastic anion columns were coupled in series to serve both as pre-column (DIONEX AG-4) and analytical chromatographic column (DIONEX AS-4). The suppression was made using a micromembrane ASRS-ULTRA II. A mixture of $\text{NaOH}/\text{Na}_2\text{CO}_3$ 4 mM/9 mM was chosen as eluent with a flow rate of 2 ml min⁻¹.

2.5. Reproducibility and data analysis

All experiments were carried out by triplicate. Unless otherwise indicated, all data shown are the mean values from three replicate experiments. Relative standard deviations were below 5% in all cases.

The statistical data analysis was done using the SigmaPlot software package. SigmaPlot uses the Durbin–Watson statistic to test residuals for their independence of each other. The Durbin–Watson statistic is a measure of serial correlation between the residuals. The residuals are often correlated when the independent variable

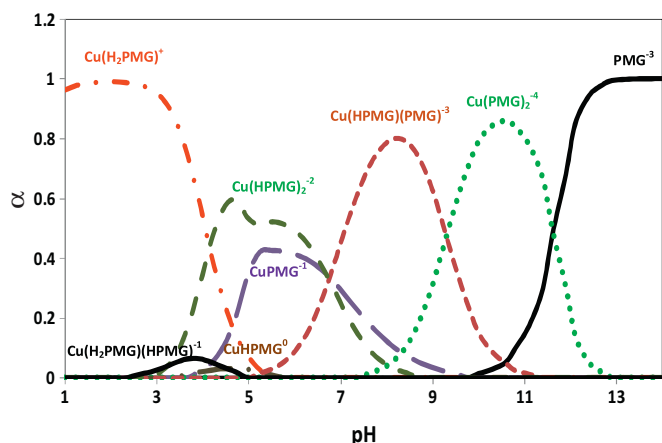


Fig. 1. Speciation diagram for PMG–copper complexes in aqueous solution. (●—), $\text{Cu}(\text{H}_2\text{PMG})^+$; (—), $\text{Cu}(\text{H}_2\text{PMG})(\text{HPMG})^{-1}$; (■—), $\text{Cu}(\text{HPMG})_2^{-2}$; (—), CuPMG^{-1} ; (—), $\text{Cu}(\text{HPMG})(\text{PMG})^{-3}$; (●●●), $\text{Cu}(\text{PMG})_2^{-4}$; (—), PMG^{3-} ; and (—●—), CuHPMG^0 . The speciation calculations were made using Mineql software.

is time and the deviation between the observation and the regression line at a given time is related to the deviation at the previous time.

2.6. Infrared spectroscopy studies

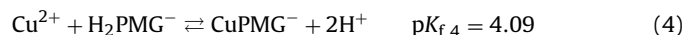
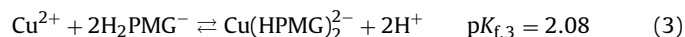
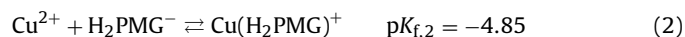
FTIR analysis was performed on samples of solid materials on a Nicolet 8700 FTIR with a DTGS detector working in the spectral range of $4000\text{--}400\text{ cm}^{-1}$, and with 4 cm^{-1} resolution. The samples were examined as KBr disks and data were obtained as % transmittance.

3. Results and discussion

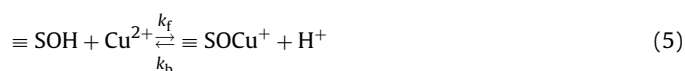
3.1. Cu–PMG adsorption studies

PMG has a zwitterionic structure in aqueous solution and its dissociation constants were previously reported [31–36], (the dissociation reaction pathway Fig. S1 and the acid–base speciation diagram in Fig. S2 are depicted in supporting information). The main species in the working pH range is H_2PMG^- .

The PMG molecule contains amine, carboxylate, and phosphate functional groups that can form strong coordination bonds with metal ions to form aqueous complexes [7,32,34,36,37]. The most stable complexes with copper are depicted in Fig. 1 (the equilibrium constants are detailed in supporting information Table S1). The Eqs. (2)–(4) describe the formation reactions of the most important complexes in the working pH range.



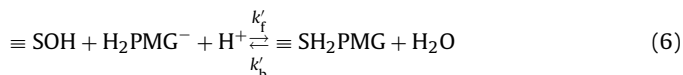
The formation of surface complexes between biomass and metal ions involves its coordination with oxygen donor atoms that constitute algae cell walls, followed by the release of protons from the surface [38], for example:



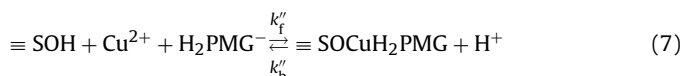
where Cu^{2+} is the free metal ion in solution, $\equiv\text{SOH}$ is the sorbent surface site which is susceptible of coordination, $\equiv\text{SOCu}^+$ is the surface complex formed on the sorbent site at any time, and k_f and k_b

are the kinetic constants for the forward and back reaction steps, respectively.

Also, surface complexes can be formed between some biomass cell walls coordination groups and dissolved ligands, such as PMG, followed in this case by the release of water (or hydroxyls) from the surface [38]:



Certainly, ternary complex formation involving simultaneous or consecutive adsorption of metal ions and ligands is also possible [38]:



Thus, the possible scenarios for the ternary system, copper plus PMG plus alga, are copper adsorption without interaction with PMG, PMG adsorption without copper mediation, and simultaneous adsorption of copper and PMG (Eqs. (5)–(7)). Processes represented by Eqs. (5) and (7) led to an acidification of the media as was experimentally observed.

The isoelectric point (IEP) of most algal cell walls lies between pH 3 and 4; thus, the net overall charge on the cell walls under low pH conditions promotes easier access of anions to positively charged binding sites, as the pH falls below the IEP–pH values [39]. Metals such as Cu(II), are strongly biosorbed [21] at pH values above the IEP, near the apparent dissociation constant of carboxylic acids ($\text{p}K_a$ around 5), where the overall surface charge becomes negative, resulting in weaker electrostatic interactions mostly through ion exchange mechanisms between the surface groups and metal cations but also in covalent bonds, if the pH dependence is slight. Thus, a pH range of 4–7 is widely accepted as being optimal for metal uptake for almost all types of biomass [40]. The opposite effect of pH was observed for anionic species, since the highest biosorption occurs in the pH range of 1.0–2.5 [40]. This could be due to interactions between dissolved anions with protonated nitrogen or sulfur atoms of amine groups or thiol groups in the cell walls. The significant influence of pH on biosorption may be useful since it may allow controlling biomass selectivity for some metal species.

In this sense, PMG, which is negatively charged at the working pH range, can only be adsorbed at low pH values below the IEP of the alga. This fact is clearly shown in Fig. 2, where PMG adsorption in the absence of copper, copper adsorption in the absence of PMG, PMG adsorption at initial copper concentration of 0.40 mM and copper adsorption at initial PMG concentration of 0.59 mM are depicted. From this figure it is possible to conclude that PMG is not adsorbed by the alga in the absence of copper and Eq. (6) should not be considered for the interpretation of the adsorption process, being Eq. (7) the one that represents the adsorption process to be considered.

There was also no adsorption of the herbicide, when simultaneous addition of copper and PMG was made, which would indicate that Cu(II) and PMG complexes (Eqs. (2)–(4)) are not adsorbed on the cell wall in contrast to the results found by Morillo et al. [41] for co-adsorption of glyphosate and copper in soils. The copper surface coverage in the presence of PMG is smaller than the surface coverage in the absence of PMG due to Cu(II)–PMG complex formation that decreases free copper concentration in solution (Fig. 2). Similar results were found by Huguenot et al. [17].

PMG adsorption only occurs if the copper adsorption equilibrium is previously established so the presence of metal is critical to produce the anionic ligand anchor on the surface, which probably occurs by covalent coordination through a phosphonate group as it happens on mineral surfaces [42–44]. Huguenot et al. [17] also found that glyphosate was generally much more sorbed in the

Table 1
Parameters obtained using Langmuir and Freundlich models for the adsorption of Cu (II), copper in the presence of PMG (Cu/PMG) and PMG in the presence of copper (PMG/Cu) by *Ulva lactuca* at pH 5.5. All data shown are the mean values from three replicate experiments. Relative standard deviations were below 5% in all cases.

Me	Langmuir			Freundlich			
	$q = \frac{q_{\max} \times K_L \times C_e}{1 + K_L \times C_e}$	$q_{\max, L} \text{ (mmol g}^{-1}\text{)}$	$K_L \text{ (1 mmol}^{-1}\text{)}$	$q = K_F C_e^{1/n}$	$K_F \text{ (1 mmol}^{-1}\text{)}$	$1/n$	R^2
Cu		0.85 ± 0.09	0.55 ± 0.14		0.22 ± 0.02	0.75 ± 0.09	0.979
Cu/PMG		–	–		0.08 ± 0.01	1.86 ± 0.07	0.998
PMG/Cu		3.65 ± 0.46	0.103 ± 0.03		0.37 ± 0.04	0.73 ± 0.06	0.977

presence of copper, suggesting that the presence of copper promote glyphosate sorption on copper sites by acting as a bridge between glyphosate and the sorbent.

Adsorption studies varying Cu(II):PMG molar ratios were made to find the adequate experimental conditions for the process. Cu(II):PMG molar ratios of 1:3 and 1:1.5 did not show PMG adsorption after 24 h. Results for an equimolar ratio exhibited low herbicide adsorption. Cu(II):PMG molar ratios higher than 1.5:1 showed that PMG adsorption took place during the first minutes of reaction. Under these experimental conditions the retention of the herbicide was large enough to ensure that the experimental error was not significant with respect to the measured value. Thus, further experiments were performed keeping the Cu(II):PMG molar ratio constant and equal to 1.5:1 in such a way that the mass balance and acid base speciation for Cu(II)–PMG complexes and PMG were maintained constant. The results obtained are discussed in the next section.

3.2. Cu–PMG adsorption isotherms

Copper adsorption isotherms, either in the presence or absence of PMG, are comparable at low copper concentrations (Fig. 3), while at copper concentrations up to 1 mM the metal coverage in the presence of PMG has an accumulative profile that does not reach a maximum coverage.

Equilibrium data are commonly analyzed using Langmuir or Freundlich models.

3.2.1. Langmuir model

The Langmuir isotherm is a well known model that indicates a decrease of the available surface sites as the metal ion concentration increases [45]. The Langmuir isotherm assumes

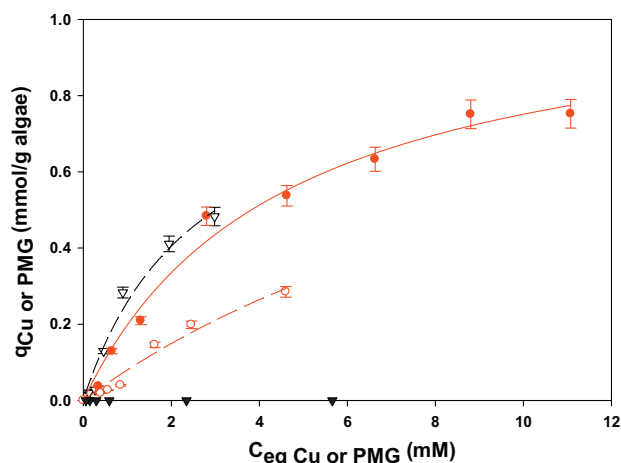


Fig. 2. Adsorption isotherms at pH=3.0, $I=0.1$ M and $T=25$ °C of (▼) PMG in the absence of copper, (●) Cu(II) in the absence of PMG, (▽) PMG in the presence of Cu(II)=0.40 mM, (○) Cu(II) in the presence of PMG=0.59 mM. All data shown are the mean values from three replicate experiments. Relative standard deviations were below 5% in all cases.

monolayer adsorption and is represented by the following equation:

$$q = \frac{q_{\max} + K_L \times C_{eq}}{1 + K_L \times C_{eq}} \quad (8)$$

where q_{\max} is the maximum metal uptake (mmol g^{-1}), meaning the maximum concentration of sorbate on the solid phase, C_{eq} is the metal concentration at the equilibrium on the aqueous media and K_L is the Langmuir equilibrium constant which is related to the free energy of the reaction. Langmuir parameters K_L and q_{\max} and also the determination coefficient (R^2) values for the adsorption of Cu(II), PMG in the presence of copper, and Cu(II) in the presence of PMG by *U. lactuca*, at a Cu:PMG molar ratio of 1.5:1 and at pH 5.5, are given in Table 1. Copper adsorption by *U. lactuca* as well as PMG

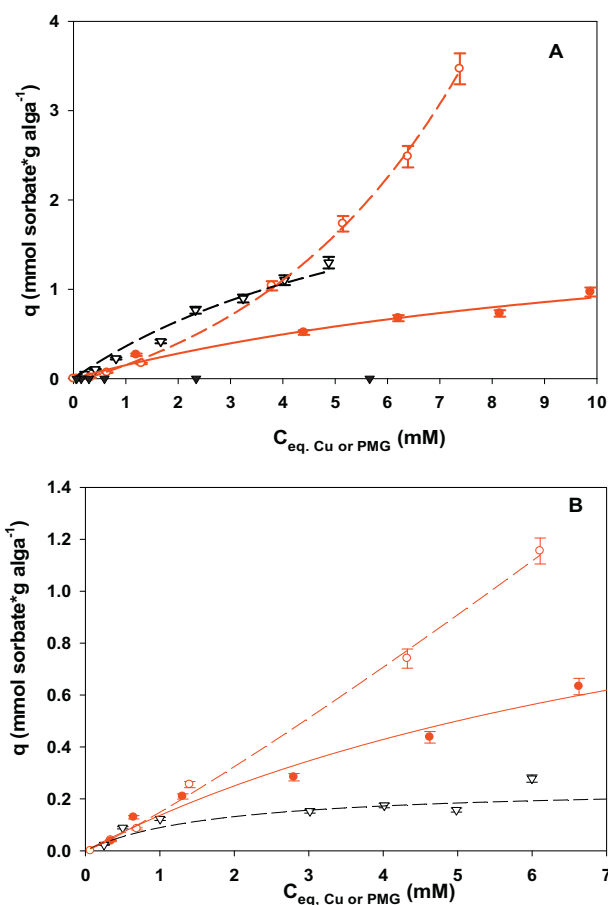


Fig. 3. Adsorption profiles with a Cu:PMG constant molar ratio of 1.5:1 at (A) pH=5.5 and (B) pH=3.0, $I=0.1$ M KNO_3 and $T=25$ °C of (●) Cu(II) in the absence of PMG as a function of Cu(II) equilibrium concentration, (▼) PMG in the absence of copper as a function of PMG equilibrium concentration, (▽) PMG in the presence of Cu(II) as a function of PMG equilibrium concentration and (○) Cu(II) in the presence of PMG as a function of Cu(II) equilibrium concentration. All data shown are the mean values from three replicate experiments. Relative standard deviations were below 5% in all cases.

adsorption mediated by copper can be described by the Langmuir model, while the adsorption of copper when PMG is present cannot be represented by this model (Table 1).

3.2.2. Freundlich model

The Freundlich isotherm assumes a heterogeneous surface with a non uniform distribution of heat of adsorption over the surface. Thus, the Freundlich model describes the adsorption on an energetically heterogeneous surface on which the adsorbed molecules are interactive. This isotherm is represented by Eq. (9).

$$q_e = K_F \times C_{eq}^{1/n} \quad (9)$$

where K_F is the Freundlich constant indicating adsorption capacity and $1/n$ is the adsorption intensity.

The values of K_F and $1/n$ were calculated from the intercept and slope of the plot between $\ln q_e$ vs. $\ln C_{eq}$. The Freundlich isotherm values K_F , $1/n$ and the determination coefficient (R^2) for the adsorption of Cu(II), PMG in the presence of copper, and Cu(II) in the presence of PMG, by *U. lactuca*, are given in Table 1.

Adsorption of copper when PMG is present at a Cu:PMG ratio of 1.5:1, can be represented by the Freundlich model (Table 1).

3.3. Adsorption stoichiometry

The adsorption of copper (or PMG) in the presence and absence of PMG (or copper) and the adsorption of PMG in the presence of copper were represented as a function of the metal equilibrium concentration (Fig. 3).

PMG adsorption onto *U. lactuca* at pH 5.5 (Fig. 3A) and at low copper concentrations have the same profile as the adsorption of the metal and under these conditions the ratio between the adsorbed PMG and adsorbed copper was linear with a slope of 1.36 ± 0.15 and a determination coefficient of $R^2 = 0.9569$ (Fig. S3A). Thus, the stoichiometric ratio was 1.5:1 between both adsorbed species, PMG and copper.

$$[\text{PMG}]_{\text{ads}} = 1.5 \times [\text{Cu}^{+2}]_{\text{ads}} \quad (10)$$

This may indicate that the PMG surface concentration is higher than the copper surface concentration in a factor of 1.5. Therefore, to achieve this surface concentration is necessary that at least 50% of the adsorbed copper atoms are coordinated to one PMG molecule and the other 50% is coordinated to two PMG molecules. Consequently, the metal ions bind to the phosphonate group of the pesticide as described in Eqs. (2)–(4) for the aqueous complex formation.

As the copper concentration in solution increases (Fig. 3A and B), PMG_{ads} to $\text{Cu(II)}_{\text{ads}}$ ratio diminished (Fig. S3A and B), probably because of the formation of new surface $\equiv\text{Cu-PMG}$ complexes and because there might be copper atoms on the alga surface that do not react with PMG, or, because PMG is twisted to bind to an adjacent copper atom forming a bidentate complex ($\equiv\text{Cu}_2$)–PMG. Adsorbed copper concentration in the presence of PMG increases with respect to the adsorption of the metal in the absence of the pesticide leading to the accumulation of an excess of copper on the surface (Fig. 3A and B). At pH 5.5, the increase in copper adsorption is much greater than the increase of PMG adsorption, $q_{\text{PMG,Cu}}:q_{\text{Cu,PMG}} \ll 1$, where both surface coverage have a linear relation with a slope of 0.22 ± 0.005 and a determination coefficient of $R^2 = 0.9955$ (Fig. S3A). This means that on the surface there are four Cu^{2+} by each PMG molecule and also indicates that copper is being accumulated on the surface by forming superficial complexes with stoichiometries varying from $\equiv\text{Cu-PMG-Cu}$ to $\equiv\text{Cu-PMG-Cu}_3$, where now the coordination of the metal to the pesticide also occurs through the second coordination site of phosphonate moiety, or the amino or the carboxyl group of the molecule. Under

these experimental conditions the contribution of the bidentate complexes is minimal.

Metal adsorption at pH 3 and at copper equilibrium concentrations lower than 1.5 mM is comparable, regardless of PMG presence. As well, at higher copper equilibrium concentrations the copper adsorption when PMG is present is higher than in the absence of PMG, whilst pesticide uptake is lower than copper uptake in all concentration ranges studied. At this pH, the ratio between the adsorbed PMG and the adsorbed copper also was linear with a slope of 1.35 ± 0.065 and a determination coefficient of $R^2 = 0.9939$, whilst when the copper equilibrium concentration is higher than 1.5 mM, $[\text{Cu}^{2+}] > 1.5 \text{ mM}$, the slope is 0.093 ± 0.0062 and the determination coefficient is $R^2 = 0.9814$ (Fig. S3B).

During the first stages of the reaction, copper adsorption in the presence and absence of PMG is similar (Fig. 3 and supporting information Fig. S3), which may indicate that the presence of PMG does not affect previous copper adsorption and that PMG is accumulated on the surface.

The ratio, $q_{\text{PMG,Cu}}:q_{\text{Cu,PMG}} \ll 1$ suggests that there are copper surface sites that are not coordinated to PMG on the algae surface, and/or that the PMG is attached to the surface through the phosphonate group by binding to two adjacent coppers and forming a bridging bidentate complex as occurs with mineral surfaces [13,42]. The metal uptake growth markedly with respect to PMG uptake when copper equilibrium concentrations are up to 2 mM (when the ratio $q_{\text{Cu,PMG}}:q_{\text{PMG,Cu}}$ is between 1 and 2 (Fig. S4).

This is also an indicative that $\equiv\text{Cu-PMG-Cu}$ to $\equiv\text{Cu-PMG-Cu}_2$ monodentated complexes are formed where the chain moiety of the PMG molecule is twisted and coordinate another copper cation by the amino group. In this case the formation of bidentate or bridging bidentate complexes is negligible because the copper uptake $q_{\text{Cu-PMG}}$ is much higher than that obtained at equal copper concentrations when copper adsorption takes place in the absence of PMG.

3.4. pH influence

The pH influence on *U. lactuca* biosorption capacity was studied in series of batch biosorption experiments at pH values of 2, 3, 4 and 5.5 (Fig. S5). The initial concentrations for copper and PMG were 4.5 and 3.0 mM, respectively.

PMG adsorption in the presence of copper has a similar profile to the adsorption of copper in the absence of PMG whilst copper biosorption in the presence of PMG exhibit a higher dependence on pH.

At low pH, the functional groups of the cell walls are protonated, which means that the majority of the binding sites are occupied by protons decreasing the seaweed metal biosorbent capacity. As pH increases the binding sites are deprotonated and available for metal coordination, then biosorption increases.

Adsorption of ligands such as PMG presents an opposite behavior, the adsorption decreases with pH increase [38,41–44]. However, PMG biosorption follows the same pattern as the copper biosorption (Fig. S5), which means that both processes are cooperative and not independent.

The increasing shape of the adsorption with the pH could suggest that the Cu–PMG complex positively charged is the species that has a higher tendency to be adsorbed, but at pH up to 4 the concentration of this species in aqueous solution go down drastically (Fig. 1) resulting in the decrease of the $q_{\text{PMG,Cu}}$ as opposed to what is observed experimentally

The adsorption of copper in the absence of PMG leads to an increase of the positive charge on the surface (Eq. (5)). The change on the surface charge (Δq) will be equal to the copper surface coverage: $\Delta q = q_{\text{Cu}}$.

The subsequent adsorption of the molecule of PMG, negatively charged, on the surface leads to the formation of a bridge complex resulting in a decrease of the positive surface charge followed by the stabilization of the system

Because of the acid–base equilibrium of PMG depicted in Fig. S1 the charge of the ternary surface complex will be dependent of pH as is detailed in Table S2. Consequently, the total change of the surface charge could be calculated using the equations described in Table S3 and assuming that

- (1) each copper cation replace a proton from the surface leaving a positive charge excess on the surface
- (2) the PMG is attached to the surface as the predominant species determined by acid–base equilibrium outlined in Fig. S1

Although the system alga–Cu–PMG adsorbs much more copper than the system alga–Cu the excess of surface charge is more positive in the absence of PMG as can be seen in Fig. S6. Thus, the copper accumulation mediated by the PMG molecule presence does not lead to a significant charge augmentation because could take place surface reactions compensating the charge increasing.

3.5. Algal characterization

The specific surface area of *U. lactuca* was determined by water adsorption (S_w) [46] at room temperature and relative humidity of 56% and the obtained value was $292 \pm 6 \text{ m}^2 \text{ g}^{-1}$.

The surface morphology of *U. lactuca* was analyzed by scanning electron microscopy (see supporting information Fig. S7) and results revealed the surface structure of the alga where heavy metals and/or PMG are adsorbed.

The EDS spectra of the alga before and after being used as a biosorbent of copper (see supporting information Fig. S8) and PMG, mediated by Cu(II), with a PMG:Cu(II) molar ratio of 1:1.5, and pH values of 2, 3, and 5.5 are shown in Fig. 4. PMG cannot be detected by this technique since its molecule consists of the same chemical elements as the alga cell wall (N, C, P and O). Therefore, it is only possible to distinguish the presence of copper on the alga surface.

The value of adsorbed copper increases with increasing pH, with surface copper values of 0.96, 4.91, 5.42 and 6.39% (w/w) for pH values of 2, 3, 4 and 5.5, respectively. It is noteworthy that the values of copper adsorption in the system in the absence of PMG are 0.42, 1.48, 1.61 and 2.52% (w/w) for pH values of 2, 3, 4 and 5.5, respectively [47]. These differences between % (w/w) values in the presence and absence of PMG show that the accumulation of copper in the surface is enhanced due to the presence of the herbicide.

3.6. FTIR

A PMG infrared spectrum is depicted in Fig. 5A. The IR bands are assigned as follows: OH stretching at 3454 cm^{-1} ; hydrogen bonded P–OH at $2900\text{--}2500 \text{ cm}^{-1}$; ν_{asym} and ν_{sym} of carboxylate group at 1730 cm^{-1} and 1423 cm^{-1} , respectively; ν (PO_3H) 1268 cm^{-1} ; ν_{sym} and ν_{asym} of P–O[−] and $\text{PO}_2(\text{OH})^-$ groups are at 1092 and 1158 cm^{-1} ; ν (P–OH) at 916 cm^{-1} ; δ (PO_3H) at 195 and 776, respectively [32,48]; δ (NH_2) at 1560 cm^{-1} ; δ (CH_2) at 1332 cm^{-1} ; and ν (CCNC) at 1078 cm^{-1} [49].

Also, FTIR infrared bands of free PMG–Cu complex in aqueous solution at 1144, 1091, 1033 and 994 cm^{-1} were assigned to P–O[−] and $\text{PO}_2(\text{OCu})$ frequency groups and the frequency at 945 cm^{-1} was ascribed to P–OCu group [48].

U. lactuca infrared spectra showed bands at wave numbers of 1650, 1550, 1420, 1325, 1260, 1115, 1050, 837 and 790 cm^{-1} (Fig. 5B), and these results are in agreement with the assignments made by others authors [50]. The asymmetrical stretching band at 1650 cm^{-1} and the weaker symmetric stretching band at

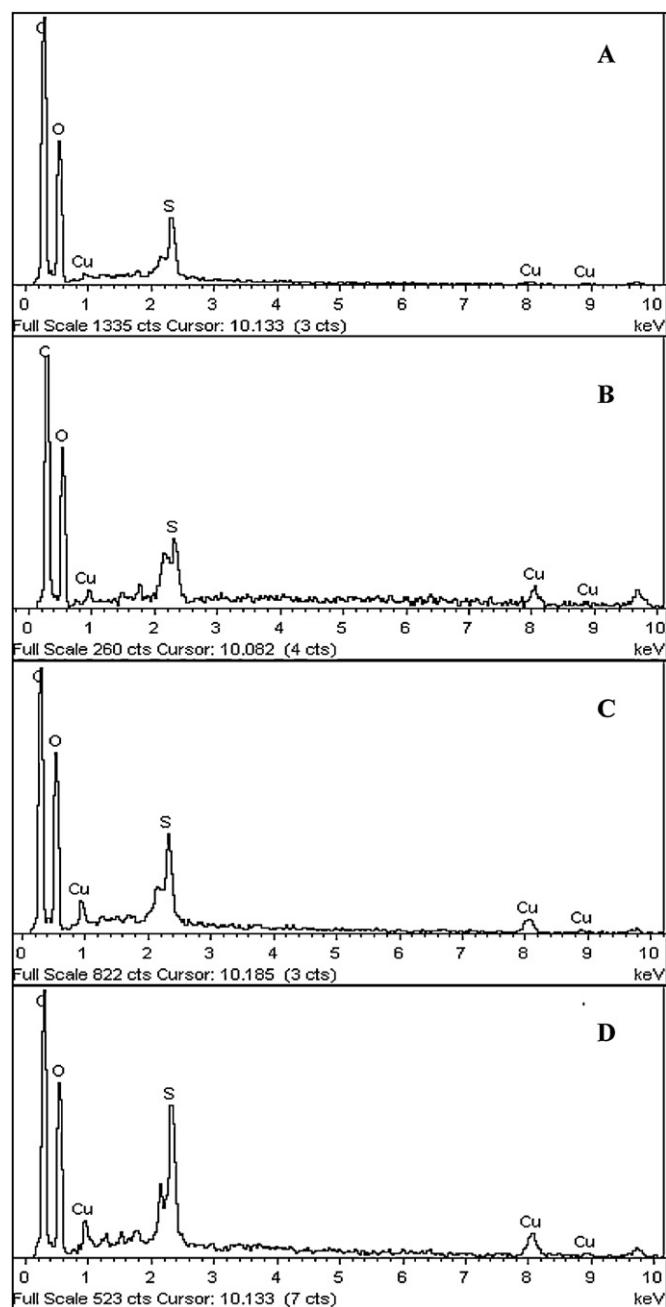


Fig. 4. EDS images of *U. lactuca* at constant ionic strength and at: (A) pH 2; (B) pH 3; (C) pH 4 and (D) pH 5.5 after the adsorption of Cu(II) and PMG at a Cu:PMG constant molar ratio of 1.5:1.

1420 cm^{-1} [50 and references cited therein] are assigned to the carboxylate group, while the strong band at 1260 cm^{-1} is assigned to sulfate esters [50,51]. The $1200\text{--}1000 \text{ cm}^{-1}$ region is dominated by sugar ring vibrations overlapping with stretching vibrations of (C–OH) side groups and the (C–O–C) glycosidic bonds vibration [50 and references cited therein]. Also, the band at 790 cm^{-1} is related to sugar cycles. Ulvan spectra have a maximum absorption band at around 1050 cm^{-1} probably due to C–O stretching from the two main sugars, rhamnose and glucuronic acid [50].

The *Ulva*–Cu–PMG samples (prepared at pH 5.5) FTIR spectra show that the alga bands at 1100 and 1320 cm^{-1} decrease while PMG concentration increases (Fig. 5C and D) stating that PMG is bonded to the surface and there might be blocking of the COO[−] and C–OH groups [52]. After coordination, it should be expected that

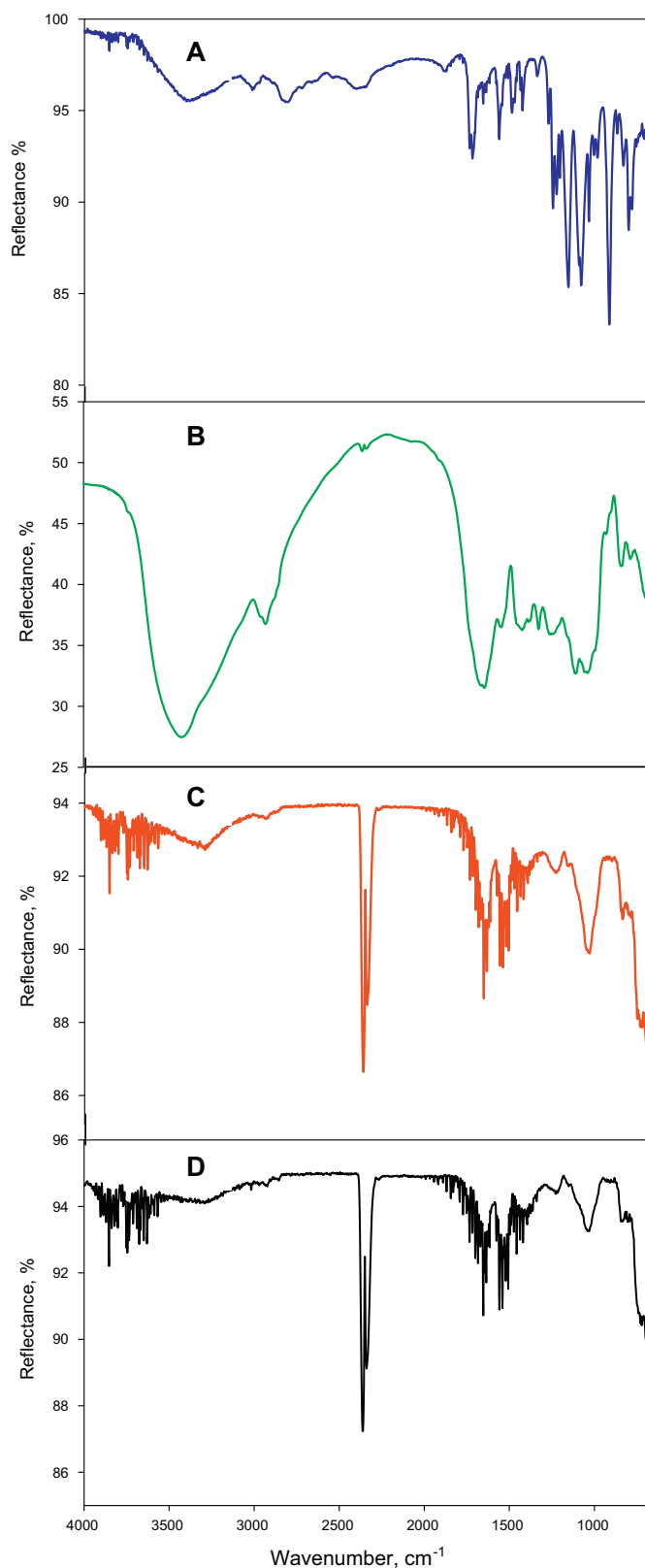


Fig. 5. FTIR spectra of (A) PMG (■); (B) *U. lactuca* (■); (C) *U. lactuca*-Cu-PMG 3 mM (■); and (D) *U. lactuca*-Cu-PMG 6 mM (■).

the band of carboxylate group that is assigned to ν_{sym} at 1423 cm^{-1} shift to lower frequencies due to the generation of Cu–O bond and an increase of the ν_{asym} at 1730 cm^{-1} because the C=O double bond is partially reconstructed. Nevertheless this frequency is displaced to lower wave numbers probably due to the fact that the C=O group is not coordinated and participates in hydrogen bonding with a water molecule or is involved in weak secondary bonds to the metal center of a neighboring complex [53]. Both interactions produce a weakening of this partially reconstructed C=O bond as is also discussed for Zn–glycine complexes formation [53]. Moreover, the stretching bands at 916 cm^{-1} and 795 cm^{-1} assigned to P–OH and $\text{PO}_2(\text{OH})^-$ are moved to frequencies that were assigned to P–O⁻ and $\text{PO}_2(\text{OCu})$ groups indicating that the phosphonate group is not protonated and is coordinated to copper atoms.

FTIR assignments are in agreement with the adsorption stoichiometry changes discussed in Section 3.3 where the surface complexes stoichiometry is varying from $\equiv\text{Cu-PMG-Cu}$ to $\equiv\text{Cu-PM-Cu}_3$.

4. Conclusions

Results demonstrated that *U. lactuca* biosorbs PMG in the presence of copper.

PMG adsorption only occurs if the copper adsorption equilibrium is previously established so the presence of metal is critical to produce the anionic ligand anchor on the surface, which probably occurs by covalent coordination through phosphonate groups as it happens on mineral surfaces.

PMG adsorption mediated by copper onto *U. lactuca* and copper adsorption onto *U. lactuca* can both be described by the Langmuir model but copper adsorption in the presence of PMG cannot be described by this model. Freundlich model can be used to describe all the adsorption isotherms.

PMG and copper at pH 5.5 are accumulated on the surface forming complexes with stoichiometry from $\equiv\text{Cu-PMG-Cu}$ to $\equiv\text{Cu-PMG-Cu}_3$, where the coordination of the metal to the pesticide also occurs through the amino or carboxyl group of the molecule.

The herbicide uptake at pH 3 is lower than the copper uptake in all concentration ranges studied. The $q_{\text{PMG,Cu}}$ is half of the $q_{\text{Cu,PMG}}$, which may indicate that the pesticide is coordinated to one of every two coppers adsorbed on the surface of the alga.

The presence of copper and PMG on the alga surface was confirmed by SEM/EDS and FTIR.

A new approach on PMG remediation is given by the adsorption of this herbicide mediated by metal adsorption onto biological materials, such as marine seaweeds, since the PMG molecule has chemical groups that can coordinate with metal ions. Thus, the obtained results suggest that *U. lactuca* could be a new material with a high potential to be used as a biosorbent for copper and PMG.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.colsurfb.2012.12.047>.

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