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Freshwater green macroalgae as a biosorbent of Cr(III) ions

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Abstract: The research was conducted to evaluate the possibility of using algae enriched with Cr(III) ions as a feed additive for horses. As a sorbent, macroalga *Cladophora glomerata* was chosen. The results of the kinetic and equilibrium experiments on biosorption of Cr(III) ions are presented. The pseudo-second order model was used for the description of kinetics. Equilibrium of biosorption process was described by Langmuir model. The effect of biosorbent dose: 0.1–1.0 g·L^{-1}, initial metal ions concentration: 100–300 mg·L^{-1} and pH: 3–5 on the biosorption capacity in a batch system was evaluated. These factors played a significant role in affecting the biosorption capacity of biosorbent and the rate constant. Optimal pH for biosorption was 5, biosorbent dose 0.1 g·L^{-1}, initial concentration of Cr(III) ions 300 mg·L^{-1}. The maximum biosorption capacity determined from Langmuir equation was 107.5 mg·g^{-1} (for C_S 1.0 g·L^{-1}, pH 5). The experiments were also performed in a column system and they showed that almost 100% of Cr(III) ions were absorbed after 200 minutes. The FTIR and SEM-EDX technique confirmed binding of Cr(III) ions by the algal biomass. Due to very good biosorption properties, *Cladophora glomerata* can be considered as a carrier of microelement ions in animal feeding.

Keywords: freshwater algae; biosorption; chromium; feed additives; horses.

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Highlights

- Biosorbent dose, initial metal ions concentration, pH and contact time influence biosorption capacity
- Alga *Cladophora glomerata* is a good biosorbent of Cr(III) ions
- Algae could be used as cheap and valuable feed additives for horses

1 Introduction

Chromium is a useful element, for example it is used in leather tanning, wood treatment, as an ingredient of pigments, paints, textile dyes, inks, chemicals, catalysts and metal corrosion inhibitors [1]. Chromium can occur in several oxidation states ranging from -II to VI, but only third and sixth forms are stable in the environment [2,3]. Cr(VI) is known to be highly toxic, mutagenic to living organisms, while Cr(III) is more stable and is approximately 100 times less toxic and 1,000 times less mutagenic than Cr(VI) [4]. Hexavalent chromium is more soluble and better absorbed, than trivalent, when is supplemented directly to the intestine. Trivalent chromium is required for the maintenance of normal glucose and fat metabolism [5] and is necessary for the proper development of humans and animals [1,6]. In the presence of sufficient amounts of chromium, in a biologically active form, much lower amounts of insulin are required [7].

Chromium can be found in many mineral supplements and in a wide range of foods, including whole-grain products, egg yolks, coffee, nuts, high-bran breakfast cereals, fresh fruits, vegetables (e.g., broccoli, beans), brewer's yeast, meat (e.g., poultry, beef, pork), mollusks, unrefined sugars and some brands of wine and beer [1,3]. In the case of oral supplementation, Cr is mostly is reduced to Cr(III) before it gets the small intestine, where it is assimilated. The mechanism of absorption is not fully known, yet. The low bioavailability of inorganic Cr(III) is caused by the creation of non-soluble Cr oxides, interference with other ions (Fe, Zn, V), binding to natural

chelate-forming compounds in pasture, non-optimal niacin quantity and slow conversion to the bioactive form [8]. Chromium is one of the most studied supplements and can improve systemic insulin sensitivity and has positive effect on fasting plasma glucose in mammalian nutrition [9]. Increased tissue sensitivity to insulin may result in hypoglycaemia and increased glucose uptake [10]. In the late 1950s, the Glucose Tolerance Factor (GTF), a dietary agent, was first extracted from Brewer's yeast and it was absent in the diet of rats fed with *Torula* yeast. Animals consuming the diet developed an inability to remove glucose efficiently from the bloodstream, which was reversed after usage of food rich in chromium or by adding synthetic inorganic Cr(III) complexes to the diet [11]. In 1977, GTF was suggested to be a biologically active form of Cr(III) that could substantially lower plasma glucose levels in diabetic mice [12].

Nowadays, the need to assess insulin sensitivity in horses has increased, due to rising number of animals with recognized equine metabolic syndrome (EMS). Decreased tissue sensitivity to insulin in EMS animals leads to persistently high levels of blood glucose and a subsequent pancreatic exhaustion [10].

The most popular form of chromium in dietary supplements is chromium picolinate ($Cr(pic)_3$) [13]. The supplementation with this form can enhance insulin sensitivity especially in aged animals [10]. In the work of Ralston [14] it was shown that chromium L-methionine (0.02 $mg \cdot kg^1$ of body weight) decreased secretion of blood insulin by 30–57% after a standardized meal of grain in normal aged mares. However, in an intravenous glucose tolerance test these effects, in either normal or hyperinsulinaemic aged mares, have not been observed. In comparison, addition of 210 or 420 μg chromium tripicolinate per kg of feed showed increased glucose tolerance after intravenous glucose tolerance testing. In turn, Marycz et al. [15] has recently shown, that *Cladophora glomerata* enriched with Cr(III) improved cytophisiological features and reduced oxidative stress in equine metabolic syndrome derived adipose stem cells. These data show that algae can be used as a potential carrier of chromium in the context of their future clinical application, however, more data is still required. Chromium supplements are easily available to horse owners and are often administered to horses with insulin resistance [16]. Currently, the controversy regarding the effect of chromium supplementation still exists. It is necessary to elucidate the cellular and molecular mechanisms of chromium on carbohydrate metabolism *in vivo*, before specific recommendations in the management of diabetes [3].

In the literature it is suggested that algae are an important food source and can be used directly as valuable feed supplements. This biomass is characterized by low toxicity and cost (when collected from the natural environment) and simultaneously by a high nutritional value and bioavailability to animals [17]. Algae are known to be a rich source of biologically active compounds such as polyunsaturated fatty acids, proteins, vitamins, minerals and pigments [18,19]. In recent years, algae have turned out also to be effective, low cost and promising sorbents [17,20-39]. Dried seaweeds are able to bind and concentrate metal ions from even very diluted aqueous solutions [21]. By using biosorption – a rapid, independent, cellular metabolism process of reversible binding of metal ions from aqueous solutions to the functional groups present on the surface of biomass [20,40], it is possible to obtain the biomass enriched with microelements [17]. This biomass can serve as a feed additive with microelements for animals [17,22,23,40-42]. Previously, this process was mainly used to remove toxic metal ions (e.g., Cu(II), Cd(II), Zn(II), Pb(II), Hg(II), Cr(VI)) from wastewaters [4,20,21,24-27,31,32,36,38,39].

In the present paper, we examined the biosorption properties of a freshwater *Cladophora glomerata* towards Cr(III) ions, since this alga is abundant in our climate zone. It is related to the eutrophication process, caused by the intensification of fertilization that increases the concentration of N and P in the water, which efficiently accelerates algae growth [19]. In the literature, the biosorption properties of marine species of *Cladophora* are mainly studied, for example *Cladophora glomerata* collected from the coasts in Turkey was used to bind Pb(II) and Cu(II) ions [24], *Cladophora rupestris* from the Baltic Sea (Poland) to bind Cr(III), Co(II), Cu(II), Zn(II) and Mn(II) ions [17], *Cladophora rivularis* from Caspian Sea (Babolsar, Iran) to remove Pb(II) ions [25], *Cladophora albida* from Qingdao (China) to remove Cr(VI) [26], as well as freshwater *Cladophora* from Tseng-Wen Reservoir (Taiwan) for biosorption of Pb(II) and Cu(II) ions [27].

Taking into account the requirements of horses for highly bioavailable feed supplements with microelements and good biosorption properties of green macroalgae, we propose to use them as a carrier of microelements in animal feed. Therefore, we tested in details the biosorption properties of freshwater macroalga – *Cladophora glomerata*. The research involved experiments on kinetics, equilibrium and selection of the best experimental conditions, as well as biosorption in a column. Binding of Cr(III) ions to the surface of algal biomass was confirmed using FTIR and SEM-EDX technique.

2 Experimental procedure

2.1 Collection of sorbent and its preparation

The biomass of freshwater macroalgae – *Cladophora glomerata* was collected by hand from the surface of the pond in Tomaszówek, Poland (51°27′21″N 20°07′43″E) in October 2016. Then the biomass was air-dried (to assure that cells were not metabolically active). Before the use in biosorption it was milled using grinding mills (Retsch GM300, Haan, Germany). The particle size was 500 μm.

2.2 Biosorption experiments

2.2.1 Kinetics of the biosorption

The kinetic experiments were performed in order to determine the optimal conditions for the biosorption – initial Cr(III) ions concentrations: C_0 100, 200 and 300 mg·L^{-1}, biosorbent content in the aqueous solution: C_S 0.1, 0.5 and 1.0 g·L^{-1} and pH: 3, 4, 5 (based on our previous research [22,23,42]). All processes were conducted in a batch system. pH was measured with pH meter Mettler-Toledo (Seven Multi; Greifensee, Switzerland) equipped with an electrode InLab413 with temperature compensation. The pH of the solutions was adjusted with 0.1 mol·L^{-1} solutions of NaOH/HCl (from POCh S.A., Gliwice, Poland). The solutions of Cr(III) ions were prepared by dissolving appropriate amounts of $Cr(NO_3)_3 \cdot 9H_2O$ (from POCh S.A.) in deionized water.

The biosorption was conducted at 25°C in 250 mL Erlenmeyer flasks containing 100 mL of Cr(III) solutions in a shaker (IKA KS 260 basic, IKA® Works, Inc., Wilmington, USA) at 150 rpm. Samples (7.0 mL) were collected after 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165 and 180 minutes and the biomass was separated from the solution by centrifugation at 4200 rpm for 5 minutes (Thermo Scientific Heraeus Megafuge 40 Centrifuge, UK). The concentration of Cr(III) ions in the solution (4.0 mL) was determined by adding 0.095 g of EDTA (from POCh S.A.), vortexing (VWR International GmbH, Darmstadt, Germany) and boiling in water bath (GFL, Burgwedel, Germany) to complete dissolution. The concentration of metal ions in the solution was determined spectrophotometrically at λ 540 nm (Varian Cary 50 Conc. Instrument, Victoria, Australia) [23] and was read from the previously prepared standard curve for C_0 ranging from 25 to 400 mg·L^{-1}. Biosorption capacity of *C. glomerata* towards Cr(III) ions was calculated from the equation (1):

$$q = (C_0\text{-}C_{eq}) \cdot V/W \quad (1)$$

where: q – is the amount of Cr(III) ions biosorbed by the biomass (mg·g^{-1}) at time t, C_0 – is the initial concentration of Cr(III) ions in the solution (mg·L^{-1}), C_{eq} – is the concentration of Cr(III) ions in the solution at equilibrium (mg·L^{-1}), V – is the volume of the metal solution (L) and W – is the mass of the sorbent (g).

2.2.2 Equilibrium of the biosorption

The equilibrium experiments were performed in Erlenmeyer flasks containing 0.02 g of the biomass and 20 mL of Cr(III) ions solution in a shaker (IKA KS 260 basic) at 150 rpm. The following Cr(III) solutions: 25, 50, 75, 100, 125, 150, 200, 300, 400 mg·L^{-1} were prepared in deionized water. pH 5 of the solutions was adjusted with 0.1 mol·L^{-1} solutions of NaOH/HCl. The contact time was 3 hours determined from kinetic experiments. Samples after biosorption (7.0 mL) were centrifuged at 4200 rpm for 5 minutes and then the concentration of metal ions were determined directly spectrophotometrically by complexation with EDTA [23].

2.2.3 Column biosorption

This experiment was conducted according to the modified procedure described by Djafer et al. (2013) [43]. Biosorption was performed at room temperature (25°C) in a glass column (50 mL). The column was packed with algal powder (5.0 g). The height of the column was 13.5 cm. Then the column was filled with 50 mL of solution with concentration of Cr(III) ions 300 mg·L^{-1} (pH 5). The desired flow rate was established (2.0 mL·min^{-1}). The samples were collected after 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240 minutes. The concentration of metal ions was determined spectrophotometrically with EDTA. After biosorption process, enriched biomass was dried at 40°C, mineralized with nitric acid – 0.5 g of biomass and 5.0 mL of 69% nitric acid (Suprapur, Merck KGaA, Darmstadt, Germany) in a microwave oven StartD (Milestone MLS-1200 MEGA, Bergamo, Italy) and analyzed using ICP-OES technique (Inductively Coupled Plasma – Optical Emission Spectrometer, Varian VISTA-MPX ICP-OES, Victoria, Australia) [17]. The enriched biomass was used for testing with using SEM-EDX and FTIR technique.

2.2.4 Scanning electron microscope (SEM) combined with energy dispersive X-ray (EDX)

The elemental analysis of chromium and its distribution on the surface of investigated *C. glomerata* (mapping) was performed at Wrocław University of Environmental and Life Sciences (Electron Microscope Laboratory, Poland). The samples of *C. glomerata* in a native form, as well as enriched with Cr(III) ions were fixed in 2.5% glutaraldehyde (Merck) and dehydrated by ethanol series (from 50 to 100% concentration). Next, the algal biomass was dried at room temperature, coated with gold (ScanCoat six equipment – Oxford) and observed under the scanning electron microscope (SEM-EVO LS 15, Zeiss, Oberkochen, Germany) using SE detector (15Kv). In the next step, the content and distribution of Cr in the algal biomass were visualized by means of Brucker ScanCoat six equipment – Oxford, according to the previously published protocol [44,45,46].

2.2.5 FTIR analysis of the natural and chromium-loaded *Cladophora glomerata*

For FTIR analyses, KBr discs – 200 mg were ground with 1.5 mg of algal sample (natural and enriched with Cr(III) ions in a column). The spectra were recorded on a Bruker spectrophotometer (Bruker FT-IR IFS 66/s; Billerica, Massachusetts, USA) in the mid IR range (4000–400 cm^{-1}).

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

Biosorption process was used to bind Cr(III) ions from aqueous solution by green macroalga *Cladophora glomerata* and to produce a potential feed additive for horses with equine metabolic syndrome.

3.1 Kinetics of the biosorption

The kinetic experiments are necessary to evaluate the best conditions for biosorption process [22]. Kinetics of biosorption allows the determination of the rate of solute binding on the surface of the biological material and the biosorption capacity at equilibrium. This data provides important information about the possible mechanism of the biosorption process that involves diffusion and chemical reactions. The knowledge of the mechanisms of metal sorption is necessary to scale up the process with packed-bed columns [47]. Among various kinetic models, the models based on the order of chemical reaction – Lagergren (pseudo-I-order, PFO; equation 2) and Ho (pseudo-II-order, PSO; equation 3) are the most often used:

$$\ln\left(q_{eq} - q_t\right) = \ln\left(q_{eq}\right) - k_1 \cdot t \tag{2}$$

$$\frac{dq}{dt} = k_2 \cdot \left(q_{eq} - q_t\right)^2 \tag{3}$$

where: q_{eq} and q_t – are the amounts of adsorbed metal ions on the biosorbent at equilibrium and at time t, respectively ($mg \cdot g^{-1}$) and k_1 and k_2 – are the rate constants of pseudo-first and pseudo-second order model of biosorption (min^{-1} and $g \cdot mg^{-1} \cdot min^{-1}$; respectively).

The correlation coefficients for Ho model are usually higher than for Lagergren. The linearization of both models for the biosorption of Cr(III) ions by *C. glomerata* (pH 5, C_0=300 $mg \cdot L^{-1}$, C_S=1.0 $g \cdot L^{-1}$) is presented in Figure 1. The higher correlation coefficient for the pseudo-second order kinetic (R^2 0.998) and its applicability for fitting the experimental data showed that the biosorption follows this model. This model was used in the present study to describe the biosorption process and the calculated parameters are presented in Table 1. The Pseudo-second order kinetic model is based on the assumption that the rate-limiting step is chemisorption (sharing or exchange of electrons). It can be applied to aqueous solutions in which the biomass is dispersed and the solution is well agitated [42].

Results on the effect of different algal biomass content in the solution (C_S 1.0, 0.5 and 0.1 $g \cdot L^{-1}$) are shown in Figures 2 (a) pH 5, 300 $mg \cdot L^{-1}$, (b) pH 5, 200 $mg \cdot L^{-1}$ and (c) pH 5, 100 $mg \cdot L^{-1}$. It can be noted that the equilibrium state was reached quite quickly. For most cases it was achieved within 90 minutes. After this time, the value of biosorption capacity for all tested concentrations of metal ions remained almost unchanged. Rapid biosorption may be due to physical sorption or ion sorption on the surface of the algal biomass. Slower sorption of metal ions is usually due to other mechanisms involved, such as complexation or micro-precipitation [27].

The initial concentration of metal ions in the solution is an important driving force to overcome all mass transfer resistances of the metal between the aqueous solution and the biomass [48]. In the present study, biosorption capacity at equilibrium (q_{eq}) increased with C_0 – the highest q_{eq} was obtained for 300 $mg \cdot L^{-1}$ (78.1 $mg \cdot g^{-1}$) and the smallest for

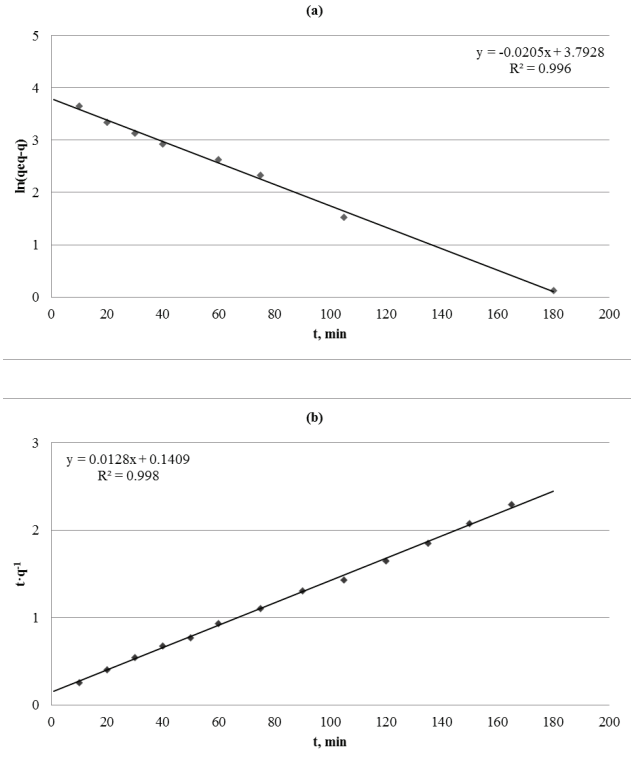

Figure 1: The linearization of pseudo-first and pseudo-second order models for the biosorption of Cr(III) ions by *C. glomerata* (pH 5, C_0=300 mg·L^{-1}, C_S=1.0 g·L^{-1}).

Table 1: The parameters of pseudo-second order model of biosorption kinetics.

C_0, mg·L^{-1}	pH	C_s, g·L^{-1}	q_{eq}, mg·g^{-1}	k_2, g·mg^{-1}·min^{-1}	R^2
Effect of C_S					
300	5	1	78.1	0.00116	0.998
300	5	0.5	80.7	0.00185	0.997
300	5	0.1	128.2	0.00177	0.992
200	5	1	74.6	0.00208	0.997
200	5	0.5	122.0	0.00081	0.998
200	5	0.1	49.5	0.00029	0.996
100	5	1	63.3	0.00195	0.994
100	5	0.5	52.9	0.00199	0.997
100	5	0.1	52.1	0.00160	0.999
Effect of pH					
300	5	1	78.1	0.00116	0.998
300	4	1	46.1	0.00399	0.998
300	3	1	12.7	0.01760	0.999
Effect of C_0					
100	5	1	63.3	0.00195	0.994
200	5	1	74.6	0.00208	0.997
300	5	1	78.1	0.00116	0.998

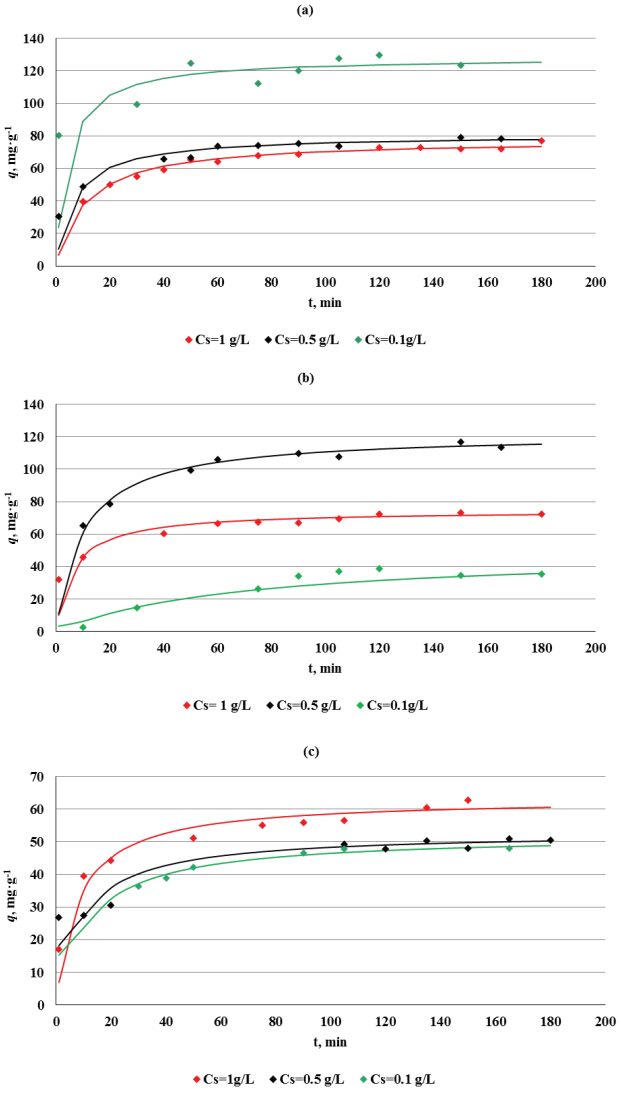

Figure 2: The kinetics of biosorption of Cr(III) ions at different contents of biosorbent (a) pH 5, 300 mg·L^{-1}, (b) pH 5, 200 mg·L^{-1}; (c) pH 5, 100 mg·L^{-1}.

100 mg·L^{-1} (63.3 mg·g^{-1}) for the biomass content 1.0 g·L^{-1} (Table1). It should be noted that the difference in biosorption capacity between 200 and 300 mg·L^{-1} was slight (4.7%). This means that for higher Cr(III) ion concentrations (C_0 > 200 mg·L^{-1}), the available sites for biosorption became fewer and the saturation of the sorption sites was observed for lower concentration. The same trend was observed in our previous study – biosorption of Cr(III) ions by marine *Enteromorpha prolifera*, but there was a slight difference between 300 and 400 mg·L^{-1} (difference of about 7%) [23]. Lower initial metal ion concentrations necessary to reach the maximum biosorption capacity are beneficial from the economical point of view – lower amount of inorganic salts are needed to prepare the initial solutions.

Usually, biosorption capacity increases rapidly with an increase in the initial metal ions concentration. Then the process subsequently proceeds more slowly until the equilibrium is reached [49]. It was found that generally with the increase of C_0, k_2 decreased – increasing concentration of Cr(III) ions in the solution could reduce their diffusion in the boundary layer of the algal biomass [50]. The same trend was observed in many works, for example in the work of Calero et al. for biosorption of Cr(III), Cd(II) and Pb(II) by olive stone [49] or for biosorption of Cr(III) ions by marine macroalga *Enteromorpha prolifera* [23]. According to the literature data, k_2 usually decreases with increasing initial metal ions concentration, which is a fact related to the interpretation of k_2 as a time-scaling factor – the higher is C_0, the longer time is required to reach an equilibrium [51].

As seen from the results presented in Table 1, the biosorption process is the most efficient for the following process parameters – biomass content: 0.1 g·L^{-1}, initial concentration of chromium ions: 300 mg·L^{-1} and pH 5 – q_{eq} is equal to 128.2 mg·g^{-1}. It was found that the lowest content of the biosorbent in the solution (0.1 g·L^{-1} among 0.5 and 1.0 g·L^{-1}) with the highest concentrations of metal ions (300 mg·L^{-1} among 100 and 200 mg·L^{-1}) resulted in the highest sorption capacity what is in agreement with literature data [27]. Although, for low biomass content in the biosorption process a high capacity is obtained, a small amount of biomass is enriched. Taking into account production of feed additives from the enriched biomass, these experimental conditions, especially low C_S would be unbeneficial. Therefore, for future experiments we recommended the content of biomass in the solution 1.0 g·L^{-1}.

Solution pH is an important parameter that controls sorption process. pH influences site dissociation, the solution chemistry of the metals that involves hydrolysis, complexation by organic and/or inorganic ligands, redox reactions and precipitation [24,26]. Therefore, we did not perform experiments for pH higher than 5, since higher pH (> 5.5) caused precipitation of Cr(III) ions as $Cr(OH)_3$ [28]. The relationship between chromium uptake and solution pH (3, 4 and 5), under constant process conditions (300 mg·L^{-1}, 1.0 g·L^{-1}, 25°C) is illustrated in Figure 3 and in Table 1. The highest q_{eq} was recorded for pH 5 (78.1 mg·g^{-1}). The decrease in pH value resulted in decrease in the biosorption capacity – for pH 3 it was 6 times lower than for pH 5. This may be attributed to the fact that at low pH, the solution is protonated due to specific pK_a values of the sorbates and the functional groups and competition between protons and metal ions in the solution lead to reduced biosorption [29]. A similar trend was observed in other studies regarding biosorption at different pH [23, 30, 31]. In the case of second-order adsorption rate constant, the effect of pH was inverse – the higher pH, the smaller k_2. The same trend was observed in our previous study – biosorption of Cr(III) ions by marine *Enteromorpha prolifera* [23]. This can result from the size and geometry of complexes of chromium cations – that actual radii of complexes with charge < 3 ($CrOH^{2+}$ dominates at higher pH, at pH 4 ~ 74%) are significantly larger and better sorbed than that for a simple chromium(III) ion, which dominate in pH 3 [52].

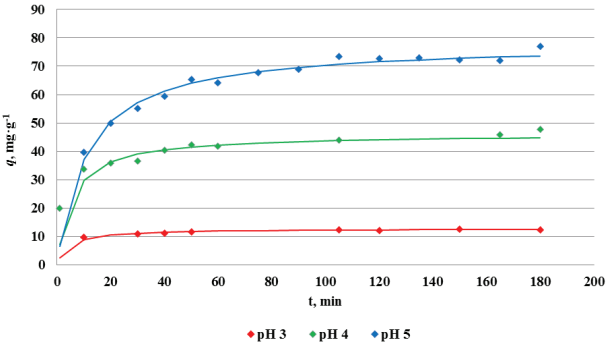

Figure 3: The kinetics of biosorption of Cr(III) ions at different pH of solution (C_S 1.0 g·L^{-1}, 300 mg·L^{-1})

3.2 Equilibrium of the biosorption

There are several mathematical models that are used to describe the equilibrium of the biosorption process. Among them we can distinguish: Langmuir, Freundlich, Sips, Redlich–Peterson, Temkin and Dubinin-Radushkevich. According to the literature data, the equilibrium between the solid and liquid phases in biosorption is mostly described by the Langmuir, Freundlich and Dubinin-Radushkevich equations [40]. The parameters of these model determined after their linearization are presented in Table 2.

The highest correlation coefficient (0.997) of the Langmuir model and its applicability for fitting the experimental data show that the biosorption of Cr(III) ions by alga follows this model. For the experimental conditions determined in the kinetic studies (pH 5, 1.0 g·L^{-1}), the maximum biosorption capacity of *C. glomerata* towards Cr(III) ions was 107.5 mg·g^{-1} and the parameter b was equal to 0.05 L·mg^{-1} (from Langmuir equation). In Figure 4, Langmuir biosorption isotherm for Cr(III) ions by alga is presented. Mostly, q_{max} obtained for *Cladophora* was higher than the value of q_{max} reported in the literature for other algae (Table 3). However, it was 2 times lower than

Table 2: The parameters obtained from Langmuir, Freundlich and Dubinin-Radushkevich models.

Model	Equilibrium model			Linearization		
Langmuir	$q_{eq} = \frac{q_{max} b C_{eq}}{1 + b C_{eq}}$		(4)	$\frac{1}{q_{eq}} = \frac{1}{b \cdot q_{max}} \cdot \frac{1}{C_{eq}} + \frac{1}{q_{max}}$		(5)
Parameters	q_{max} (mg·g^{-1})	b (L·mg^{-1})		R^2		
Metal ion – Cr(III)	107.5	0.05		0.997		
Freundlich	$q_{eq} = K C_{eq}^{1/n}$		(6)	$\ln q_{eq} = \ln K + \frac{1}{n} \ln C_{eq}$		(7)
Parameters	n	K		R^2		
Metal ion – Cr(III)	5.02	28.4		0.446		
Dubinin–Radushkevich	ln q_{eq} = ln q_{max} – $\beta' \varepsilon^2$ (8); $\varepsilon = R'T \ln (1 + 1/C_{eq})$ (9)					
Parameters	q_{max} (mg·g^{-1})	β (mol^2·kJ^{-2})		E (kJ·mol^{-1})	R^2	
Metal ion – Cr(III)	129.5	0.0024		14.4	0.505	

where: q_{max} – is the maximum possible quantity of metal ions adsorbed per gram of adsorbent (mg·g^{-1}), b – is the Lanmuir constant related to the affinity of binding sites for the metal ions (L·mg^{-1}), K – is a Freundlich coefficient indicating the adsorption capacity, n – is a Freundlich coefficient indicating the intensity of biosorption, β – is a constant related to the mean free energy of biosorption per mole of the biosorbate (mol^2·kJ^{-2}), ε – is a Polanyi potential, which is equal to $R \cdot T \cdot \ln(1+1 \cdot C_{eq}^{-1})$, where R (kJ·mol^{-1}·K^{-1}) – is the gas constant and T (K) – is the absolute temperature [23,40]

Table 3: The maximum biosorption capacity towards Cr(III) ions obtained for different algae.

Algae	Classification	Process parameters				Reference
		C_s, g·L^{-1}	pH	T °C	q_{max}, mg·g^{-1}*	
Cladophora rupestris	*Chlorophyta*	1.0	5	25	112.0	[17]
Pithophora varia	*Chlorophyta*	1.0	5	20	60.6	[22]
Pachymeniopsis sp.	*Rhodophyta*	0.1	4.5	25	225	[32]
Sargassum sp.	*Phaeophyta*	1.0	4	30	68.9	[34]
Spirulina sp.	*Cyanobacteria*	0.1	5	25	90.9	[35]
Chlorella vulgaris	*Chlorophyta*	1.0	5	25	30.2	[36]
Polysiphonia nigrescens	*Rhodophyta*	10	4	25	16.1	[37]
Sargassum sp.	*Phaeophyta*	1.0	4	23	53.5	[38]
Spirogyra	*Chlorophyta*	2.0	5.8	-	38.2	[39]
Cladophora glomerata	*Chlorophyta*	1.0	5	25	107.5	Present work

* the maximum biosorption capacity determined from Langmuir isotherm

q_{max} for red marine alga *Pachymeniopsis* sp. [32]. It can also be noted that the maximum biosorption capacity varies considerably depending on the species of used algae and experimental conditions. In the work of Witkowska et al., (2013) different biomasses, that are commonly used as feed ingredients, were enriched with Cr(III) ions. The highest q_{max} was reached for barley (45.9 mg·g^{-1}), soy beans (43.1 mg·g^{-1}) and corn beans (42.1 mg·g^{-1}) [42]. Comparing these results it can be stated that alga *Cladophora* is a promising biosorbent for Cr(III) ions.

For the biosorption of Cr(III) ions by *Cladophora glomerata* we also calculated the dimensionless constant separation factor (R_L) which is expressed as:

$$R_L = \frac{1}{1 + b \cdot C_0} \quad (10)$$

For R_L values between 0 and 1 – the biosorption is favorable, for R_L >1 – unfavorable, for R_L = 1 – linear, while for R_L = 0 irreversible [53]. In our case, the value of R_L was 0.0560 what indicates favourable biosorption. Jafari and

Senobari (2012) also showed that biosorption of Pb(II) ions by *Cladophora rivularis* was favourable since R_L was in the range $0 < R_L < 1$ [25].

From the Freundlich equation, the n parameter was determined which is higher than unity, that also indicates favourable adsorption [23]. This is in agreement with the obtained R_L value for biosorption of Cr(III) ions by *Cladophora glomerata*. The Dubinin–Radushkevich model allowed the determination of the parameter E – is the mean free energy (kJ·mol^{-1}) which can be calculated from the formula:

$$E = \frac{1}{(2\beta)^{0.5}} \tag{11}$$

This parameter gives information about the type of biosorption mechanism – chemical ion exchange or physical biosorption. The value of E in the range between 8 and 16 kJ·mol^{-1} indicates a chemical ion-exchange process [51]. It can be supposed that biosorption of Cr(III) ions by *Cladophora glomerata* occurs through ion exchange, since E value is equal to 14.4 kJ·mol^{-1}.

3.3 Column biosorption

The biosorption process can be performed under continuous mode in a packed bed column. In the case of practical application it is the most effective method because it efficiently utilizes the sorbent capacity and results in a better quality of the effluent [33,54]. The process of chromium ion biosorption in a column is influenced by the sorption equilibrium and the mass transfer [55].

The bioremoval efficiency (R) calculated from the following formulae (12):

$$R(\%) = \frac{C_0 - C_f}{C_0} \cdot 100\% \tag{12}$$

was 30.9% after 180 minutes for the biosorption of Cr(III) ions in the batch system (pH 5, C_0 300 g·L^{-1}, 1.0 g·L^{-1}) and 97.7% after the same time for the biosorption in a column system (pH 5, C_0 300 g·L^{-1}, 5.0 g of the biomass), where C_f – is a final concentration of Cr(III) ions at time t.

The changes in Cr(III) ion concentration in the solution collected after a given time are presented in Figure 5. It can be noted that after 20 minutes only 50% of Cr(III) ions were adsorbed, but after 200 minutes almost 100%. Table 4 shows the content of certain elements in the algal biomass before and after biosorption in column. It can be seen that the content of chromium in the

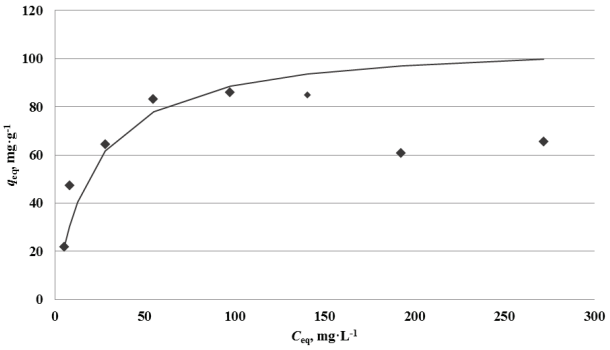

Figure 4: Langmuir isotherm for biosorption of Cr(III) ions by *Cladophora glomerata* (C_S 1.0 g·L^{-1}, pH 5).

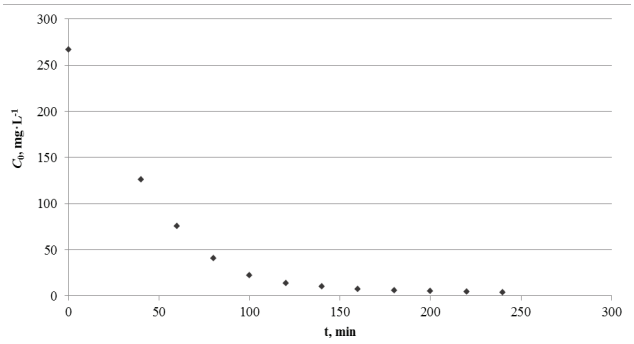

Figure 5: The changes in Cr(III) ions concentration in the solution during the biosorption process

enriched biomass increased 3000 times when compared with the natural algal biomass. Biosorption is thus an efficient method increasing the content of elements in the biomass. Additionally, it was found that the dominating mechanism of the biosorption process was ion exchange between light metal ions naturally bound with functional groups and Cr(III) ions in the solution, in accordance with literature data [20,28]. The main light metal ions involved in the exchange were K and Mg. Their content in the natural biomass decreased from 25 122 and 2951 mg·kg^{-1} to 199 and 1048 mg·kg^{-1} in enriched biomass, respectively.

Due to the possibility of using enriched biomass as a feed component, the elements that have a key impact on the treatment of animals and prevention of the development of metabolic syndrome were selected. In addition, the table lists the recommended requirements from Kentucky Equine Research– expressed in mg·kg^{-1} of dry matter of feed for the individual elements for working horses. The National Research Council does not have a specific recommendation for chromium. According to the guidelines of Pagan et al. the recommend dose of chromium is 5.0 mg/day [56]. In ICP-OES the content of all forms of chromium is determined. Assuming that the given values

Table 4: Characterization of the biomass before and after biosorption and recommendation for working horses.

Element	Before biosorption (mg·kg^{-1} dry mass)	After biosorption (mg·kg^{-1} dry mass)	Recommendation (mg·kg^{-1} of dry feed)
Cr	6.16±0.92	17 951±3 590	0.4-0.5
Cu	4.90±0.73	1.84±0.28	10-15
Fe	1 650±330	2 068±414	40-50
Mn	589±88	521±78	40-60
Zn	16.0±2.6	29.5±4.4	40-60

refer to Cr(III) ions it can be determined that only 0.3 g of enriched biomass is sufficient to supply recommended daily amount of chromium ions. The addition of 10 g of enriched alga constitutes 0.2% of recommended copper demand, 46% for iron, 10% for manganese and 0.6% for zinc. These experiments confirmed that algae *Cladophora glomerata* can be successfully used as feed additives for horses suffering from EMS.

On the basis of the obtained results, biosorption in a pilot plant will be performed next in order to produce appropriate amounts of enriched algal biomass that will be used in feeding experiments on horses.

3.4 Scanning electron microscope (SEM) combined with energy dispersive X-ray (EDX)

The scanning electron microscope (SEM) combined with energy dispersive X-ray (EDX) was used to investigate chromium ion distribution on the surface of investigated *C. glomerata* before and after biosorption process. This technique allows the precise visualization of the places where chromium ions were bound to the biomass. The performed SEM-EDX investigation (mapping technique), showed that *C. glomerata* has been enriched evenly in chromium ions in the biosorption process (Figure 6 – E, F, K, L). The "red spots" were distributed densely on the surface of *C. glomerata*, which indicates on the presence of Cr ions on the tested biomass. No morphological differences were observed between these two biomasses, which indicates, that the biosorption process *per se* does not affect morphology of investigated algae (Figure 6 – G, J). Moreover, the EDX analysis showed the higher total content of chromium ions in enriched *C. glomerata* (0.05±0.01 wt%) when compared to the native, non-enriched biomass (4.78±1.17 wt%) (difference statistically significant for $p<0.05$, test *t*). Interestingly, it was observed, that in the course of biosorption process, chromium ions agglomerate on the surface of *C. glomerata* (Figure 6 – E, F, K asterisk). It might be due to the accumulation of zoospores and gametes on the surface of biomass, which induces the agglomeration of chromium ions.

3.5 FTIR analysis of natural and chromium-loaded *Cladophora glomerata*

In the present work, we used FTIR analysis to determine the chemical functional groups in the algal biomass that are responsible for the binding of chromium ions from the aqueous solutions. The cell wall matrix of green algae contains several macromolecules, including complex heteropolysaccharides, proteins that can provide amino, carboxyl, and sulphate groups [25]. FTIR spectra of natural *Cladophora glomerata* biomass and that loaded with Cr(III) ions are shown in Figure 7. The FTIR spectroscopy is an important and useful analytical technique, which allows the detection of the vibration characteristics of functional groups present on the surface of the biosorbent [21,25,28]. In the present study it was found that the broad band at 3400 cm^{-1} is associated with stretching vibrations of O-H bonds from hydroxyl groups of polysaccharides and the water adsorbed in the sample. A number of bands about 2900 cm^{-1} are assigned to stretching vibrations of C-H bonds of aliphatic groups. The 1450 and 1355 cm^{-1} bands are connected with the bending vibrations of the hydroxyl groups. The bending and scissoring vibrations of C-H bonds are observed in the form of bands at approximately 1430, 1315 cm^{-1} (CH_2) and 1370 and 1280 cm^{-1} (CH). A wide band with a maximum of about 1245 cm^{-1} is associated with the overlap of a series of deformation and breathing vibrations of pyranose ring and bonds O-H and C-H. The 1205 cm^{-1} band comes from deformation vibrations of O-H bonds. A series of sharp bands at wave numbers 1160, 1108, 1076, 1060, 1033, 1010 and 990 cm^{-1} are associated with stretching vibrations of C-O, C-C and rocking vibrations of groups CH_2. A band at approximately 895 cm^{-1} is associated with deforming (folding) vibrations of the pyranose ring, as well as a weak band at approximately 750 cm^{-1} (breathing vibrations). Signal at 660 cm^{-1} is associated with C-OH out-of-plane bending vibrations. The protein components of algal material are visible in the form of bands of approximately 1655 cm^{-1} (I amide band) and 1520 cm^{-1} (II amide band). The remaining band characteristics for proteins overlap with signals of the polysaccharide component of the analysed material. The series of bands at 2511, 1790, 1428, 872 and 711 cm^{-1} are derived from bicarbonate ions which

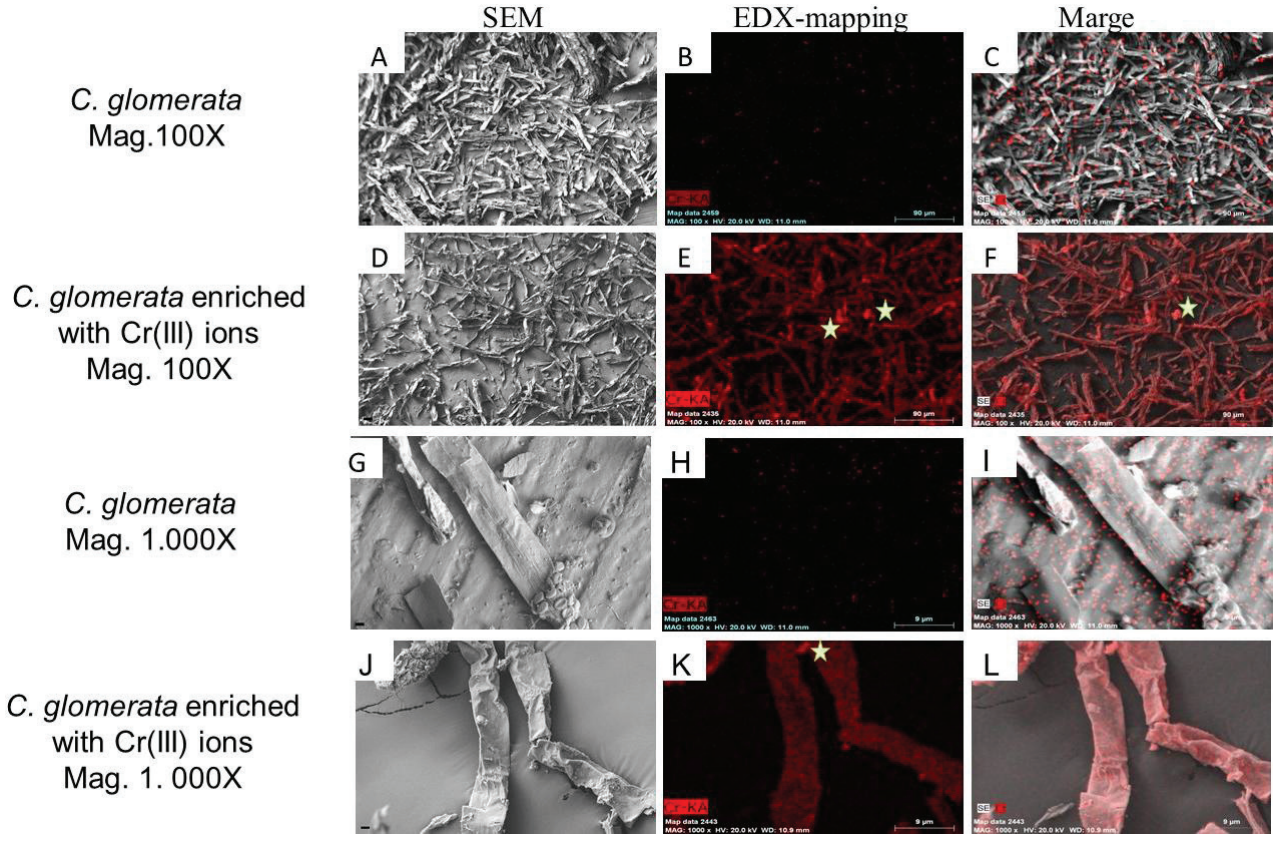

Figure 6: Scanning electron microscope images of native *C. glomerata* (A, G) and enriched with Cr(III) ions (D, J) performed under the 100X and 1000X magnification, respectively. EDX microphotographs and Cr distribution in native *C. glomerata* (B, H) and enriched with Cr(III) ions (E, K). Marge microphotographs of *C. glomerata* in native form (A, G) and enriched with Cr(III) ions (D, J).

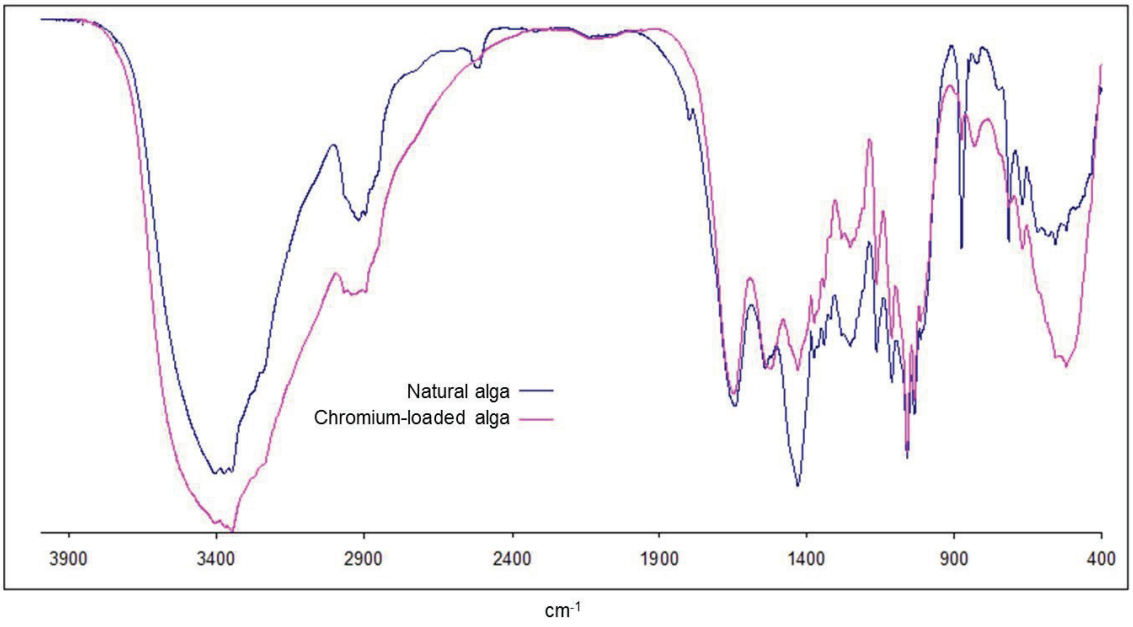

Figure 7: FTIR spectra for the natural and chromium-loaded *Cladophora glomerata*.

are strongly reduced in the sample treated with chromium solution due to the decomposition of HCO_3^- ions. The main differences between the tested biomasses are observed for the wave number range from approximately 1000 to 1500 cm^{-1}. The FTIR spectra of the natural and enriched with Cr(III) ions biomass revealed that the metal ions from aqueous solution can be bound to the carboxyl, hydroxyl and amino groups present on the surface of algal biomass. Also in other studies it was found that for the biomass enriched in optimal experimental conditions, carboxyl groups played a predominant role in the sorption of metal ions by algae [27,28].

4 Conclusions

The evaluation of the biosorption properties of *Cladophora glomerata* was based on the equilibrium modeling and kinetic studies. Physicochemical factors, such as pH, contact time, initial metal ion concentrations, biosorbent dose have been found to play a significant role in the affecting the capacity of biosorbent. Optimal pH for biosorption was 5, biosorbent dose 0.1 $g \cdot L^{-1}$, initial concentration of chromium ions in the solution 300 $mg \cdot L^{-1}$, and time to attain equilibrium state was 90 minutes. In column experiment, after 200 minutes almost 100% of Cr(III) ions were absorbed. The study shows that *Cladophora glomerata* is a good biosorbent of Cr(III) ions and could be successfully used as cost-effective and valuable feed additive for horses with metabolic syndrome. SEM-EDX technique confirmed binding of Cr(III) ions by the algal biomass. FTIR analysis showed that mainly carboxyl, hydroxyl and amino groups presented on the surface of algal biomass participated in the biosorption of Cr(III) ions by *Cladophora glomerata*. Only 0.3 g of enriched biomass would be sufficient to supply the recommended daily amount of chromium to horses. Breeders are beginning to understand the impact of dietary intervention for the treatment of horses with metabolic syndrome. The optimal amounts of biologically active chromium in a horse diet will ensure much lower amounts of insulin. *In vivo* studies on the supplementation of horses with algal biomass enriched with Cr(III) ions need to be performed.

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