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1 **Aleppo pine seeds (*Pinus halepensis* Mill.) as a promising novel**  
2 **green coagulant for the removal of Congo red dye: optimization via**  
3 **machine learning algorithm**

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33

34 **Abstract**

35 Consideration is now being given to the use of metal coagulants to remove  
36 turbidity from drinking water and wastewater. Concerns about the long-term impact of  
37 non-biodegradable sludge on human health and the potential contamination of aquatic  
38 systems are gaining popularity. Recently, alternative biocoagulants have been  
39 suggested to address these concerns. In this study, using a 1 M sodium chloride (NaCl)  
40 solution, the active coagulating agent was extracted from *Pinus halepensis* Mill. seed,  
41 and used for the first time to remove Congo red dye, the influence of numerous factors  
42 on dye removal was evaluated in order to make comparisons with conventional  
43 coagulants. The application of biocoagulant was shown to be very successful, with  
44 coagulant dosages ranging from 3 to 12 mL L<sup>-1</sup> achieving up to 80% dye removal and  
45 yielding 28 mL L<sup>-1</sup> of sludge. It was also found that biocoagulant is extremely pH  
46 sensitive with an optimum operating pH of 3. Ferric chloride, on the other hand,  
47 achieved similar removal rate with higher sludge production (46 mL L<sup>-1</sup>) under the same  
48 conditions. A Fourier Transform Infrared Spectroscopy and proximate composition  
49 analysis were undertaken to determine qualitatively the potential active coagulant  
50 ingredient in the seeds and suggested the involvement of proteins in the coagulation-  
51 flocculation mechanism. The evaluation criteria of the Support vector machine\_Gray  
52 wolf optimizer model in terms of statistical coefficients and errors reveals quite  
53 interesting results and demonstrates the performance of the model, with statistical  
54 coefficients close to 1 (R= 0.9998, R<sup>2</sup> = 0.9995 and R<sup>2</sup> adj = 0.9995) and minimal  
55 statistical errors (RMSE = 0.5813, MSE = 0.3379, EPM = 0 .9808, ESP = 0.9677 and  
56 MAE = 0.2382).

57 The study findings demonstrate that *Pinus halepensis* Mill. seed extract might  
58 be a novel, environmentally friendly, and easily available coagulant for water and  
59 wastewater treatment.

60

61 **Keywords:** *Pinus halepensis* Mill. seed extract; biocoagulant; Congo red; coagulation-  
62 flocculation mechanism; Support Vector Machine, Gray Wolf Optimizer.

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## 67 **1. Introduction**

68 Synthetic dyes are a significant class of recalcitrant organic compounds that are  
69 frequently found in the environment because of their widespread use in several  
70 industries; they are considered the most polluting of all industrial sectors. Dye  
71 wastewater discharged onto surface waters such as rivers and lakes lowers light  
72 transmission in the water, resulting in the reduction of photosynthesis as well as the  
73 quantity of dissolved oxygen. Several researches stated that certain colors may  
74 degrade and generate carcinogenic aromatic amines that are believed to remain in the  
75 environment for a very long period (Momeni et al., 2018). As a result, there is an  
76 unavoidable requirement for a dye/color removal method that is both successful and  
77 cost-effective under the aforementioned circumstances (Crini et al., 2019; Mouni et al.,  
78 2018).

79 Azo dyes are compounds with one or more azo linkages consisting of a  
80 diazotized amine linked to an amine or phenol. The principal precursors of azo dyes  
81 are aromatic amines. Almost two-thirds of all synthetic dyes are azo compounds.  
82 Indeed, they are the most widely used and structurally diverse class of organic dyes  
83 on the market owing to the vivid colors they exhibit (Ali et al., 2022). Azo dyes are also  
84 stable to light and resistant to microbial degradation or discoloration caused by  
85 washing. Therefore, it is difficult to remove these compounds from wastewater using  
86 conventional wastewater treatment methods. It is estimated that around 10% of dyes  
87 used in textile dyeing procedures do not adhere to fibers and are thus discharged into  
88 the environment (Liu et al., 2022). The recalcitrance of azo dyes, attributable to the  
89 presence of azo bonds and sulphonate groups (Radhika and Aruna, 2022), and their  
90 effects on the environment and human health makes their degradation not only a major  
91 environmental concern but also a challenge (Katheresan et al., 2018).

92 Commercially accessible treatment approaches involve either removal or  
93 destructive techniques depending on the content of the effluent. Coagulation (Hadadi  
94 et al., 2022b; Sun et al., 2021; Tahraoui et al., 2022c), adsorption (Bouchelkia et al.,  
95 2022; Imessaoudene et al., 2022), and membrane separations (Dasgupta et al., 2015)  
96 are frequent removal procedures, whereas biological treatments (Adenan et al., 2022),  
97 advanced oxidation processes (Muniasamy et al., 2020), cavitation (Zampeta et al.,  
98 2021), and incineration are destructive approaches. Regardless of the evolution of

99 technologies, there will always be a requirement for the use of coagulation in water  
100 and wastewater treatment, since it is regarded one of the simplest and cost-effective  
101 methods for enhancing the removal of pollutants from water (Kristianto et al., 2019).

102 Coagulation-flocculation technique refers to a physico-chemical process  
103 involving the use of a coagulant that neutralizes the negative charges in the polluted  
104 water, thereby reducing the electrostatic repulsion of the electric double layer; this is  
105 known as the destabilization process, it begins with an increment in ionic strength,  
106 which helps to promote double-layer compression, and/or with neutralization process  
107 of the particles surface charge by adsorption of anions (Alnawajha et al., 2022). These  
108 destabilized particles aggregate to form more or less large flocs via free precipitation  
109 or air flotation; these flocs are finally separated from the contaminated water (Han et  
110 al., 2022). Coagulation–flocculation has been used efficiently in several industries  
111 because to its simplicity of operation, relatively easy installation, and few energy  
112 requirements. Additionally, owing to the process’s adaptability, coagulation–  
113 flocculation may be employed as a pre-treatment, a post-treatment, as well as the main  
114 treatment of wastewater (Vicente et al., 2022).

115 In reality, the most often employed chemical coagulants are divided into two  
116 groups: those consisting of aluminum, such as aluminum sulfate, sodium aluminate  
117 and aluminum chloride, and those derived from iron salts, such as ferric sulfate, ferric  
118 chloride, ferrous sulfate, or ferric chloride sulfate. Rarely is the usage of these  
119 compounds without consequences. Numerous environmental issues relating to the  
120 long-term toxicity of coagulants/flocculants are currently being explored, particularly for  
121 environmental observers worldwide. In addition to environmental impacts, there are  
122 also health concerns related to their use, metallic-based coagulants/flocculants are  
123 resistant to biodegradation and degradation, and when absorbed, their residuals in  
124 drinking water may have a direct impact on human health and accumulate in body  
125 cells. Indicators of the impact of chemical coagulants/flocculants on human health  
126 include malfunction of the central nervous system, dementia, Alzheimer's disease, and  
127 extreme shaking (Hadadi et al., 2022a). It is also known that they have several  
128 disadvantages, including high cost and high sludge production, which lead to additional  
129 water treatment (Zhang et al., 2022).

130 Biocoagulants and bioflocculants may be a viable substitute for chemical  
131 coagulants and flocculants to minimize environmental pollution and health hazards

132 related with their usage (Šuvalija et al., 2022). Biocoagulants and biofloculants are  
133 derived from living organisms or their components and are completely organic and  
134 biodegradable; hence, they are ecologically beneficial and have a negligible effect on  
135 human health (Adnan et al., 2017). Therefore, to develop these eco-friendly materials,  
136 several steps must be followed before they are used in treatment processing units.  
137 Many biocoagulants and biofloculants produced from various sources have previously  
138 been studied and have shown strong promise as alternatives to chemicals that are  
139 currently widely used, including *Moringa oleifera*, a highly promising alternative  
140 (Hadadi et al., 2022b; Kapse and Samadder, 2021; Nhut et al., 2021), rice starch (Teh  
141 et al., 2014), *Lens culinaris* (Chua et al., 2019), potato starch (Lapointe and Barbeau,  
142 2017), *Dillenia indica* (Manholer et al., 2019), *Jatropha curcas* (Abidin et al., 2011),  
143 *Nephelium lappaceum* (Zurina et al., 2014), etc. Plant-based coagulants are easy to  
144 use, need little processing, and have the potential to provide a sustainable treatment  
145 solution. Despite the fact that a number of biocoagulants have been widely explored  
146 for practical applications, only a handful have been investigated in detail (Chethana et  
147 al., 2016).

148 In this research, we investigate the efficacy of a novel biocoagulant, *Pinus*  
149 *halepensis* Mill. seed extract, in treating synthetic water containing Congo red, a well-  
150 known carcinogenic and poisonous azo dye. As far as we know, the use of this  
151 biomaterial as a coagulant for the removal of dyes has not been previously reported,  
152 and biocoagulation investigations have shown very little interest in it (Hadadi et al.,  
153 2022a). *Pinus halepensis* Mill., commonly referred to as Aleppo pine, is the most  
154 abundant tree in the Mediterranean Basin, particularly in Algeria and Tunisia, and is a  
155 member of the 62 Pinaceae family (Ávila et al., 2022). These trees are characterized  
156 by their rapid growth, high propagule production, and adaptability to their native areas  
157 (Bello-Rodríguez et al., 2020). It has been reported that seeds exhibit a high proteins  
158 content (Al-Ismail et al., 2018), making them potential candidates as biocoagulant. The  
159 use of an active coagulant in the form of an extract has been favored over the direct  
160 use of seed powder; this will decrease the amount of organic matter associated with  
161 active coagulant substances, which increases BOD and COD levels in treated water  
162 (Baptista et al., 2017).

163 In this work, we investigated the influence of parameters such as coagulant  
164 dosage, initial pH, NaCl concentration, slow stirring speed and duration, solution

165 temperature and initial dye concentration on the Congo red removal efficiency.  
 166 Moreover, a Support Vector Machine (SVM) coupled with the Gray Wolf Optimizer  
 167 (GWO) was used to predict the removal rate of Congo red based on the optimized  
 168 parameters of the biocoagulation process. Whereas SVM has lately garnered a  
 169 significant deal of interest as an excellent modeling technique for water treatment  
 170 systems, GWO technique has been utilized to handle a broad range of optimization  
 171 problems, such as enhancing machine learning performance by optimizing model  
 172 hyper-parameters. To our knowledge, such work has never been done before.

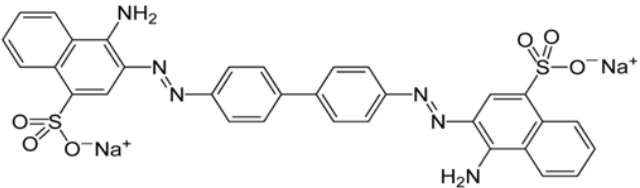
## 173 2. Material and methods

### 174 2.1. Chemicals and materials

175 Hydrochloric acid (HCl), sodium hydroxide (NaOH), Hexane, Bradford reagent,  
 176 Albumin from bovine serum (BSA), Iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ ), of  
 177 high purity ( $\geq 99\%$ ). and NaCl (all from Sigma Aldrich Chemical Company, USA) were  
 178 employed for this investigation; Congo red was purchased from Biochem-  
 179 Chemopharma, France.

180 Congo red dye was chosen as the model substance in this research and was  
 181 dissolved in distilled water at a concentration of  $1 \text{ g L}^{-1}$ ; this stock solution was then  
 182 diluted to generate solutions with the desired concentrations, and the required pH  
 183 value was adjusted by using either 0.1 M HCl or NaOH solutions. Detailed informations  
 184 about the structure and characteristics of the dye are presented in **Table 1**.

185 **Table 1** Congo red properties

Dye	Congo red
Chemical class	Di-azo dye
Chemical formula	$\text{C}_{32}\text{H}_{22}\text{N}_6\text{Na}_2\text{O}_6\text{S}_2$
Molar mass (mol/g)	696.665
Maximum wavelength absorbance ( $\lambda_{\text{max}}$ )	500 nm
Dye content	+ 75 %
Color change at pH	At [pH < 3.0] → Blue color/ At [pH > 5.0] → Red color
Chemical structure	

186

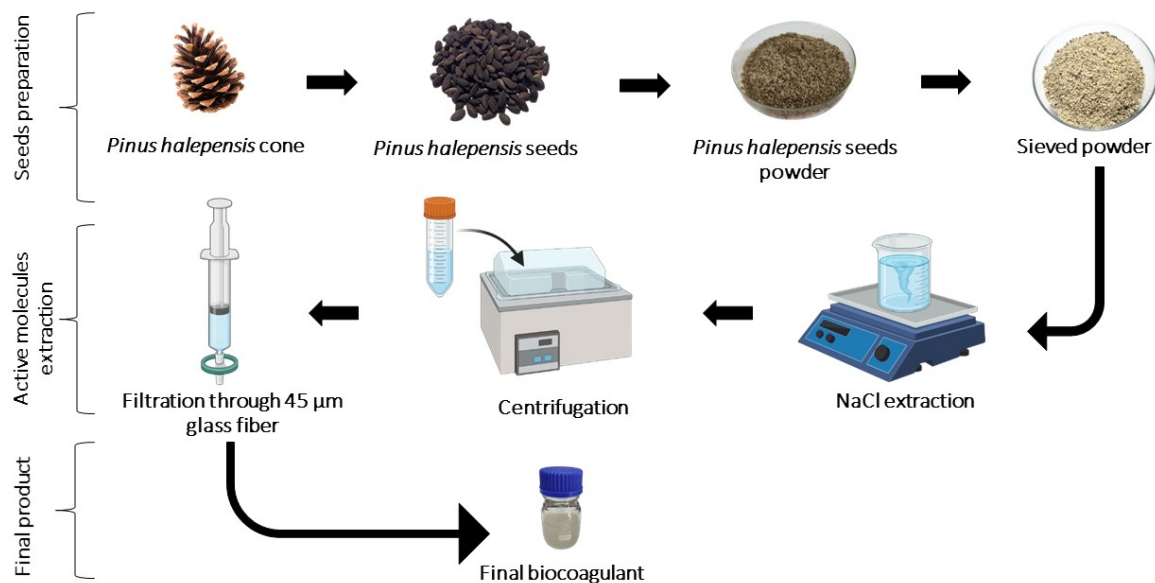
## 187 2.2. *Pinus halepensis* seed preparation and proximate composition analysis

188 *Pinus halepensis* Mill. seeds were purchased from a local herbal market,  
189 thoroughly cleaned using distilled water to remove dust and impurities, oven-dried at  
190 40°C for 24 hours (Memmert GmbH + Co.KG, Germany), crushed and powdered using  
191 a domestic food processor, and sieved through a 500 µm sieve to promote the full  
192 solubilization of active components in the coagulant (Hadadi et al., 2022a). To avoid  
193 any deterioration of the active ingredients, the obtained powder was stored in sealed  
194 amber-glass vial under refrigeration (4°C) until analysis. Moisture and ash contents  
195 were determined using the AOAC method: about 3 g of the sample were dried at 105°C  
196 during 3 hours before being transferred to a desiccator to cool. The moisture content  
197 per 100 g of sample was estimated as the percentage change in weight before and  
198 after oven drying. Ash content was determined by incinerating 5 g of the sample in a  
199 muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 550°C for 6 h. The total  
200 ash per 100 g of sample was represented as the weight of the greyish-white residue  
201 obtained. Crude fat was determined using a Soxhlet apparatus; 20 g of the sample  
202 was extracted in hexane under reflux for 8 h, and the extract was quantified as crude  
203 fat per 100 g of the sample. Proteins content was expressed using the Bradford method  
204 (Kielkopf et al., 2020), total carbohydrates were determined by difference.

## 205 2.3. Coagulant extraction

206 Salt extraction is recognized as a good approach for isolating proteins that may  
207 behave as a polyelectrolyte during the coagulation process. According to previous  
208 studies, NaCl was selected as the most suitable salt, owing to its availability and  
209 economic advantages (Dalvand et al., 2016; Kristianto et al., 2019). The extraction was  
210 performed following the steps shown in **Fig. 1**: 5 g of *Pinus halepensis* Mill. seed  
211 powder were stirred with 100 mL of 0.1 to 2 M NaCl solution for 30 minutes; the  
212 obtained suspension was centrifuged (Hettich Lab Technology, France) at 1006 xg for  
213 10 min and filtered through 45 µm fiberglass. To improve repeatability and avoid aging  
214 effects such as pH, viscosity, and coagulation effectiveness changes caused by  
215 microbial biodegradation during storage process, a fresh solution of the coagulant was  
216 prepared for each sequence of experiments, the resulting extract is referred to as  
217 PhsEXT.





218

219

**Fig. 1. Biocoagulant preparation steps.**

220

#### 221 2.4. Experimental procedure

222 Coagulation experiments were undertaken by using one-factor-at-a-time  
 223 (OFAT) method, the trials were performed in 1 L beakers using a jar-test apparatus  
 224 (VELP Scientifica Srl, Italy), each beaker was filled with 200 mL of the dye solution at  
 225 desired concentrations (50 to 170 mg L<sup>-1</sup>), pH values (3 to 10), dosages of freshly  
 226 prepared PhsEXT (3 to 12 mL L<sup>-1</sup>) and temperatures (10 to 60°C). The biocoagulant-  
 227 dye mixture was rapidly agitated at 200 rpm for 3 min, then the mixing was reduced to  
 228 lower speeds (0 to 110 rpm) for 0 to 30 min; flocs were given time to settle (for 0 to 40  
 229 min). Following that, samples (20 mL) were collected using a volumetric pipette at 3  
 230 cm below the mixture surface (Dalvand et al., 2016) and then centrifuged (1006 xg for  
 231 10 min). The final concentrations of the dye were determined using a  
 232 spectrophotometer (Agilent Cary 60 UV-Vis, USA). The measurement is performed at  
 233 500 nm for the pH range 5–10 and at 562 nm for pH range 3–4. At these wavelengths,  
 234 pH-specific calibration curves are developed and used. All studies were repeated three  
 235 times. The % removal of Congo red dye R (%) was calculated using Eq. (1):

236

237

$$R(\%) = \frac{C - C_f}{C} \times 100 \quad (1)$$

238 where  $C$  ( $\text{mg L}^{-1}$ ) and  $C_f$  ( $\text{mg L}^{-1}$ ) represent the initial and residual dye concentrations,  
239 respectively.

## 240 2.5. Morphology of flocs

241 For morphological observations, an optical microscope (Kern & Sohn, Germany)  
242 equipped with a mobile camera was used. A drop containing flocs of a fresh dye-  
243 PhsEXT mixture was carefully placed on a glass slide and covered with a coverslip. It  
244 was immediately observed at x100 magnification to get the pictures.

## 245 2.6. Support vector machine (SVM)

246 Vapnik developed the support vector machine in the 1990s (Vapnik et al., 1996),  
247 it is founded on the statistical learning theory (SLT) techniques and structural risk  
248 minimisation (SRM) concept (Wang et al., 2022). Its primary applications were  
249 regression analysis and non-linear classification (Bargagli Stoffi et al., 2022). A training  
250 data set of  $N$  points  $(X_i, Y_i)$  is examined in this technique, with  $i = 1, N$ .  $X$  denotes the  
251 inputs of the model and  $Y$  its output. A SVM model appears like:

$$252 \quad \quad \quad 253 \quad \quad \quad y(x) = \omega^T \phi(x) + b \quad (2)$$

254 where  $\phi(\cdot): R^n \rightarrow R^m$  is a non-linear function that maps the finite dimensional space  
255 entry into a higher dimensional space that is implicitly created,  $\omega$  represents a weight  
256 vector, and  $b$  is the bias (Suykens et al., 2002; Vapnik et al., 1996).

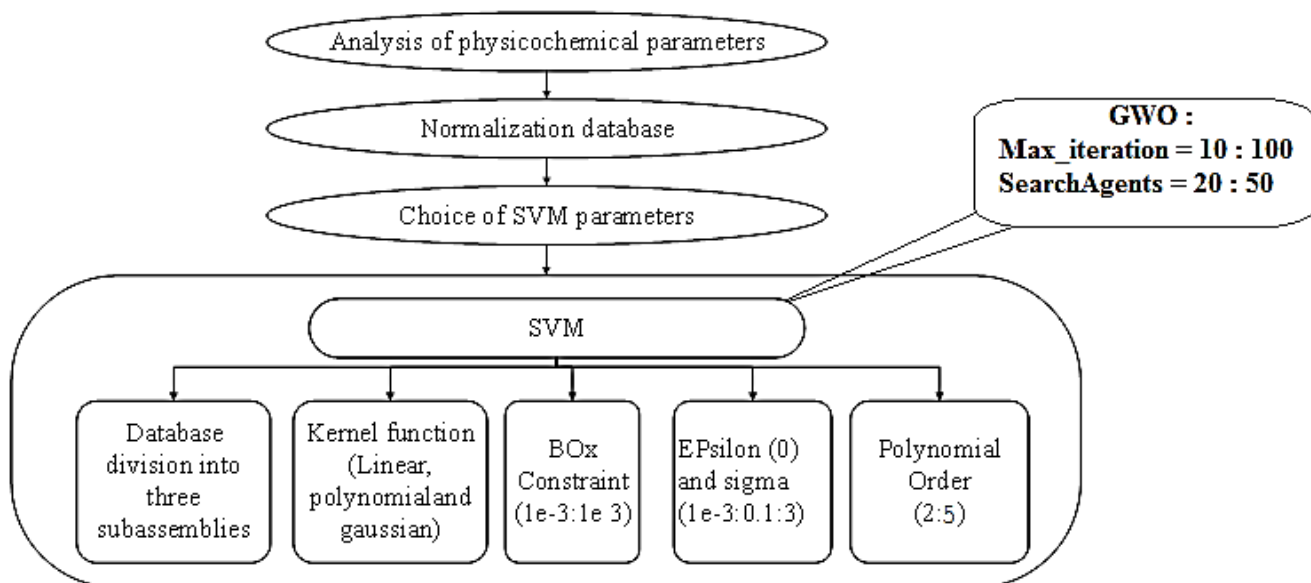
257  
258 In this study, the SVM model was chosen for the prediction of the removal rate of  
259 Congo red by the PhsEXT using the Matlab R2020a software. For this purpose, the  
260 results of the parameter optimization (part "2.4. Experimental procedure") for the  
261 removal of Congo red were compiled into a single database, which has 8 input  
262 parameters and one parameter Release

263 The independent parameters were the slow stirring time (X1), the sedimentation  
264 time (X2), the initial concentration of Congo red (X3), the dose of biocoagulant (X4),  
265 the pH of the solution (X5), the concentration of NaCl (X6), The slow stirring speed  
266 (X7) and temperature (X8). In contrast, the dependent parameter was the removal rate  
267 of Congo red (Y).

268 To obtain the optimal result from SVM, the Gray Wolf Optimizer (GWO) algorithm was  
269 coupled with SVM (SVM\_GWO) in order to optimize the parameters of each kernel

270 function. As a result of its remarkable capacity to enhance model performance via  
 271 hyper-parameter optimization (Colombo, 2017; Mirjalili et al., 2014).

272 To construct the model, the MATLAB software Help guide was used, this design is  
 273 shown in the following illustration:



274

275 **Fig. 2.** Organization chart for SVM mode development and optimization.  
 276

277

Where:

278 • The database was normalized once in the interval [-1, +1] using MATLAB's  
 279 "mapminmax" function. Next, the database was split into two: 80% of the dataset  
 280 for training and 20% of the validation samples.

281 • The kernel functions that have been selected in this study are as follows:  
 282 (Benimam et al., 2020).

283 • Poly (polynomial)

$$284 \quad k(X_i, X_j) = (ax^T + c)^d \quad (3)$$

285

286 • Linear

$$287 \quad k(X_i, X_j) = x^T y + c \quad (4)$$

288 • Gaussian

$$289 \quad k(X_i, X_j) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left\{\frac{-X_i - X_j^2}{2\sigma^2}\right\} \quad (5)$$

290

291 with  $C = \text{BOxConstraint}$ ,  $d = \text{Polynomial Order}$ ,  $\varepsilon = \text{epsilon}$  and  $\sigma^2 = \text{sigma}$  where  $d$ ,  $c$ ,  
 292  $\varepsilon$ , and  $\sigma^2$  are the defined kernel parameters.

293

## 294 2.7. Statistical criteria

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Statistical criteria were used to evaluate the performance and select the best model, including the Correlation Coefficient ( $R$ ), the Coefficient of Determination ( $R^2$ ), the Adjusted Coefficient ( $R^2_{adj}$ ), the Mean Square Error (MSE), the Root Mean Square Error (RMSE), and the Mean Absolute Error (MAE). Error Standard of Prediction (ESP) and Error Prediction of Model (EPM) were also considered. These criteria are calculated considering the following equations (Bousselma et al., 2021 ; Tahraoui et al., 2020, 2021a, 2021b, 2022a, 2022b).

303

$$R^2_{adj} = 1 - \frac{(1-R^2)(N-1)}{N-K-1} \quad (6)$$

304

$$RMSE = \sqrt{\left(\frac{1}{N}\right) \left(\sum_{i=1}^N [(y_{exp} - y_{pred})]^2\right)} \quad (7)$$

305

$$MSE = \left(\frac{1}{N}\right) \left(\sum_{i=1}^N (y_{exp} - y_{pred})^2\right) \quad (8)$$

306

$$MAE = \left(\frac{1}{N}\right) \sum_{i=1}^N |y_{exp} - y_{pred}| \quad (9)$$

307

$$ESP(\%) = \frac{RMSE}{\bar{y}_{exp}} \times 100 \quad (10)$$

308

$$EPM(\%) = \frac{100}{N} \sum_{i=1}^N \left| \frac{(y_{exp} - y_{pred})}{y_{exp}} \right| \quad (11)$$

309

310

311

312

Where  $N$  is the number of data samples;  $K$  is the number of variables (inputs);  $\bar{y}_{exp}$  and  $y_{pred}$  are the experimental and the predicted values respectively;  $\bar{y}_{exp}$  and  $\bar{y}_{pred}$  are the average values of the experimental and the predicted values, respectively (Bousselma et al., 2021; Tahraoui et al., 2021a, 2021b, 2022a, 2022b).

### 313 3. Results and discussion

#### 314 3.1. Physicochemical characterization

315 The physicochemical characteristics of *Pinus halepensis* Mill. seeds are presented in  
316 **Table 2.**

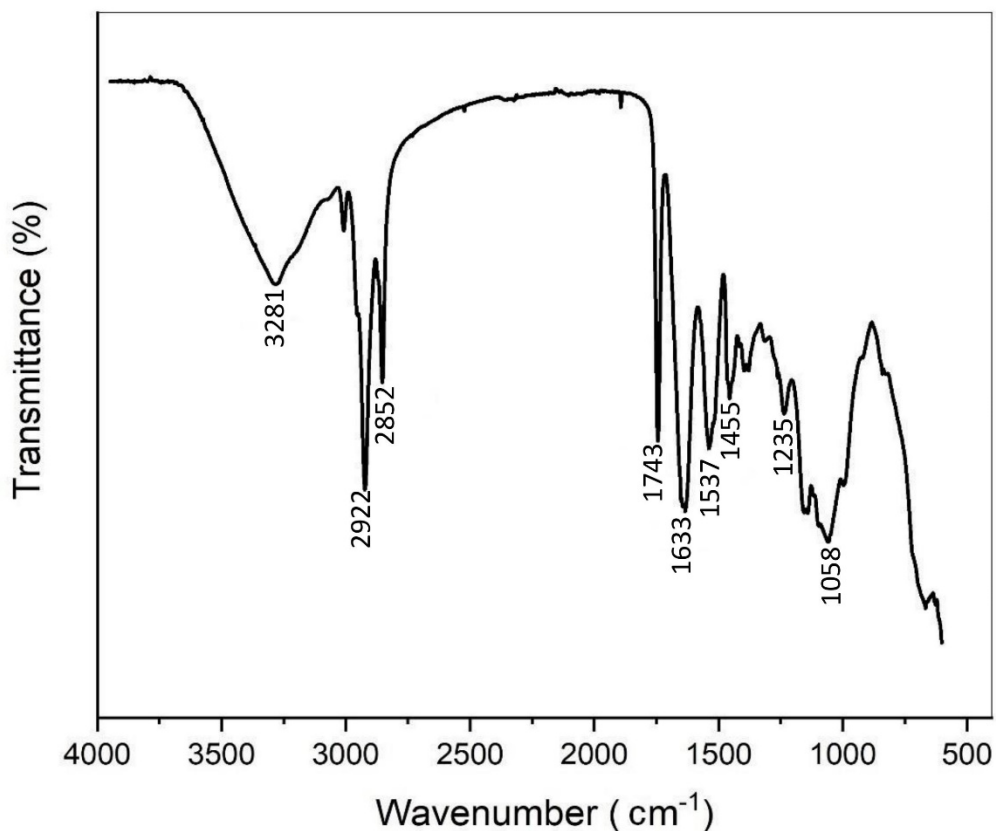
317 The moisture level was 2.3 g/100g, while the ash content was 4.6 g/100g. The  
318 seeds had a considerable amount of fat: 31.10 g/100 g. Although pine seeds are  
319 typically rich in oils, their composition varies based on species and environmental  
320 factors (Dziedziński et al., 2021). Total carbohydrates were 31.4 g/100 g (per  
321 difference). The proximate proteins content reached 30.6 g/100 g. Tukan et al. (2013)  
322 observed that the proteins level of raw or cooked *Pinus halepensis* Mill. seeds could  
323 reach more than 29%; these data suggest a high concentration of proteins capable of  
324 acting as a coagulant agent in our investigation.

325 **Table 2** Chemical composition of Aleppo Pine (*Pinus halepensis* Mill.) seeds. (mean±  
326 SD, n= 3)

Composition	Percentage
Dry matter	97.7±0.6
Ash content	4.6±0.1
Crude proteins	30.6±1.2
Fat	31.1±1.2
Total carbohydrates (by difference)	31.4±1.2

327  
328 **Fig. 3** shows that numerous main peaks with several intensities were detected  
329 at different frequencies in the FTIR spectrum. The broad peak at 3281 cm<sup>-1</sup> can be  
330 ascribed to the stretching vibrations of the –OH groups indicating the presence of the  
331 functional groups alcohol, phenol, carboxylic group and –NH stretching of amide and  
332 amines groups (Ghodke et al., 2021), which appear predominantly in the  
333 carbohydrates, polysaccharides (Saldarriaga-Hernández et al., 2021), proteins and  
334 fatty acid structures present in the *Pinus halepensis* Mill. seeds. The sharp peak in the  
335 vicinity of 2922 cm<sup>-1</sup> was related to the C-H stretching of either alkane (Ghodke et al.,  
336 2021) or carboxylic acid (El-Din et al., 2017). 2852 cm<sup>-1</sup> was related to the –CH– and  
337 CH<sub>2</sub> stretching vibrations found in fatty acids. In the region between 1743 cm<sup>-1</sup> and

338 1633  $\text{cm}^{-1}$ , there are strong peaks linked to C=O bond stretching, carbonyl group is  
339 present in proteins and fatty acids. The peak at 1537  $\text{cm}^{-1}$  is attributed to the bending  
340 of  $\text{NH}_2$  (amine) group (Putra et al., 2020); the presence of this band demonstrates that  
341 *Pinus halepensis* Mill. seeds have a protein structure containing amino groups. Bands  
342 at 1455  $\text{cm}^{-1}$  and 1235  $\text{cm}^{-1}$  correspond to the C-O stretching of carboxylic acids and  
343 phenolic compounds, while that at 1058  $\text{cm}^{-1}$  correspond to the C-O stretching of  
344 polysaccharides (Araújo et al., 2016). Similar FTIR spectra have been observed in  
345 previous studies on natural coagulants (Amran et al., 2021; Hussain and Haydar,  
346 2019).  
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**Fig. 3.** FTIR spectra of *Pinus halepensis* Mill. seeds.

### 353 3.2. Factors affecting the coagulation process

#### 354 3.2.1. Effect of initial pH on Congo red removal

355 pH is a crucial parameter in the coagulation–flocculation process since it  
356 influences the biocoagulant surface charge and the stabilization of the dye wastewater.  
357 **Fig. 4(a)** depicts the influence of pH throughout a pH range of 3 to 10 (Due to Congo  
358 red precipitation, lower pH values were neglected); very acidic conditions promote  
359 effective Congo red removal, with a maximum percentage of 80.46% at pH = 3. The  
360 removal rate decreases dramatically as pH continues to rise. This result is consistent  
361 with previous studies (Chethana et al., 2016; Tie et al., 2015b); Since proteins are  
362 believed to be the active agent in coagulation, their amino groups may get protonated  
363 under acidic conditions, enhancing their interaction with the anionic portion of the dye  
364 (sulfite  $\text{SO}_3^{2-}$  groups). A further rise in pH is expected to cause proteins carboxyl  
365 groups to become negatively charged ( $\text{COO}^-$ ), hence increasing the solubility of Congo  
366 red due to electrostatic repulsion. The proteinaceous profile of *Pinus halepensis* seeds  
367 supported by FTIR and proximate composition analysis results corroborate these  
368 assumptions. (Abidin et al., 2011; Bahrodin et al., 2021) reported that the coagulation  
369 mechanism is charge neutralization when the difference between maximum removal  
370 and minimum removal by using different pH is around 50% or more, which is the case  
371 in our investigation. Also, bridging cannot be considered as a possible mechanism  
372 because unlike charge neutralization, bridging is least affected by pH. however, at  
373 lower pH, the polymeric chains responsible for interparticle bridging will expand and  
374 be able to attach to additional pollutants (Momeni et al., 2018; Zaidi et al., 2022). Using  
375 aluminum sulfate, Vijayaraghavan et al. (2015) observed a 74% Congo red removal  
376 rate under the same acidic conditions and initial dye concentration of  $50 \text{ mg L}^{-1}$ , while  
377 Kristianto et al. (2021) reported that ferric chloride removed 30% of the dye (Congo  
378 red initial concentration =  $50 \text{ mg L}^{-1}$ , pH = 6.5).

#### 379 3.2.2. Effect of coagulant dosage on Congo red removal

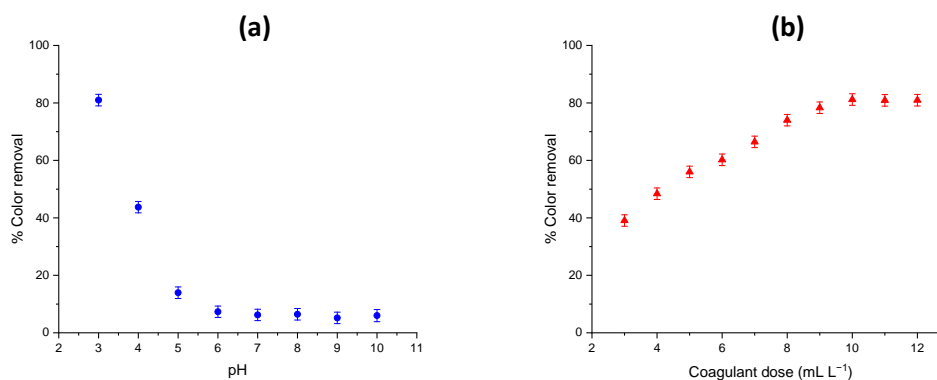
380 Coagulant dosage is a primordial parameter since it determines the cost of the  
381 coagulation–flocculation process (Tie et al., 2015a). The experiment was carried out  
382 to identify the optimal dose of PhsEXT in terms of Congo red elimination.  $50 \text{ mg L}^{-1}$  of  
383 Congo red were treated with an increasing dose of coagulant ( $3$  to  $12 \text{ mL L}^{-1}$ ). We  
384 opted to represent the coagulant dosage in units of  $\text{mL L}^{-1}$  rather than  $\text{mg L}^{-1}$  since the

385 filtering step retains around 70% of the insoluble *Pinus halepensis* Mill. seed powder  
386 during PhsEXT preparation (Nhut et al., 2021).

387 From **Fig. 4(b)**, it is observed that the increase in the biocoagulant dose  
388 increases the percentage removal of Congo red until reaching a plateau, after which  
389 additional increases in dosage did not improve the percentage removal; However,  
390 Congo red removal decreased when the dosage was further increased up to 12 mL/L  
391 (results not shown), this is explained by the fact that the main concept of coagulation  
392 is the destabilization of colloidal matter and suspended particles by the introduction of  
393 positive species. However, when the coagulant dosage increases, there will be an  
394 excess of positive ions, leading the particles to repel one another and the solution to  
395 re-stabilize. A dose of 10 mL L<sup>-1</sup> gives an optimal dye removal rate of 81.17 %. The  
396 interaction between the positive charges of the amino groups and the negative charges  
397 of the anionic dye, as previously stated, is thought to explain the coagulation process  
398 observed in this work, more dye molecules are destabilized by electrostatic attraction  
399 when the coagulant dose is increased, resulting in greater charge neutralization and,  
400 thus, a higher dye elimination % (Chethana et al., 2016; Dalvand et al., 2016; Hadadi  
401 et al., 2022a; Pardede et al., 2018). Shamsnejati et al. (2015) reported the use of  
402 *Ocimum basilicum* as a biocoagulant to eliminate Congo red, achieving a maximum  
403 color removal of 68.5% using 1.6 mg L<sup>-1</sup> (initial Congo red concentration = 50 mg L<sup>-1</sup>).  
404 Under acidic conditions (pH = 3), a solution of 2 % potato starch was found to remove  
405 about 50% of Congo red (Pardede et al., 2018). Mishra et al. (2004) observed that  
406 adding 10 mg L<sup>-1</sup> of *Plantago psyllium* mucilage to an initial dye concentration of 1 mg  
407 L<sup>-1</sup> led to the maximum elimination of golden yellow and reactive black, with 71 % and  
408 35 %, respectively. *Moringa oleifera* and *Phaseolus vulgaris* extracts at 30 mg L<sup>-1</sup>  
409 removed 83 % and 73 % of Congo red, respectively, with an initial dye concentration  
410 of 50 mg L<sup>-1</sup> and pH = 4. (Vijayaraghavan and Shanthakumar, 2015). In comparison to  
411 previous studies, PhsEXT exhibits a high efficiency in the removal of Congo red dye.

412





413

414 **Fig. 4.** Parameters affecting the removal of Congo red dye by the biocoagulant, (a)  
 415 Effect of initial pH of Congo red solutions, (b) Effect of biocoagulant dosage.

416

### 417 3.2.3. Effect of initial Congo red concentration

418 To examine the effect of the initial dye concentration on the coagulation efficiency,  
 419 optimal conditions found earlier (pH = 3, coagulant dose = 10 mL L<sup>-1</sup>) were applied to  
 420 different concentrations of Congo red (50 to 170 mg L<sup>-1</sup>). **Fig. 5(a)** clearly shows that  
 421 increasing the initial Congo red concentration resulted in a decrease in the percentage  
 422 of dye elimination from 80.78 % to 43.61 %.; this trend was noticed in earlier studies  
 423 (Beltrán et al., 2009; Chethana et al., 2016). As the dye concentration increases, the  
 424 coagulant dose becomes insufficient for neutralizing the negative charges in Congo  
 425 red dye, and PhEXT becomes exhausted (Beltrán et al., 2009). In conclusion, treating  
 426 dye wastewater with a higher initial concentration may need a larger dosage of natural  
 427 coagulant by providing more adsorption sites.

### 428 3.2.4. Effect of NaCl concentration

429 Several NaCl solutions with concentrations of 0.1; 0.5; 1; 1.5; and 2 M were selected  
 430 to examine the effect of salt solution concentration on coagulation efficiency. The  
 431 results (**Fig. 5(b)**) shows that the coagulation effectiveness of the biocoagulant  
 432 increases with an increase in salt concentration. Maximum dye removal (81.51%) was  
 433 achieved at 1 M of NaCl. This phenomenon is thought to be caused by the salting-in  
 434 mechanism, which refers to the process of increasing the ionic strength of a solution,  
 435 hence boosting the solubility of the proteins present in *Pinus halepensis* Mill. seeds,  
 436 which are believed to act as a coagulant agent (Dalvand et al., 2016; Madrona et al.,  
 437 2010). A further increase in NaCl concentration is expected to result in the opposite  
 438 phenomenon, salting out; at high salt concentrations, the solubility of proteins reduces

439 dramatically, resulting in poorer extraction and a low Congo red dye removal %  
440 (Megersa et al., 2019; Hadadi et al., 2022b). According to Dalvand et al. (2016) , when  
441 the concentration of NaCl rises, chloride ions may compete with dye molecules for  
442 attraction with the positive charges on the coagulant surface, resulting in decreased  
443 dye removal.

#### 444 *3.2.5. Effect of slow stirring speed on dye removal efficiency*

445 Rapid mixing is often used to promote better chemical dispersion in the  
446 suspension, slow stirring is then applied to allow particles to aggregate in larger  
447 settleable flocs, According to **Fig. 5(c)**, At a slow agitation speed of 30 rpm, the highest  
448 dye removal percentage of 81.80 % was achieved; Increased stirring speed resulted  
449 in a decrease in dye removal; this might be attributable to the breakage of existing flocs  
450 because of disruptive forces (Teh et al., 2014).

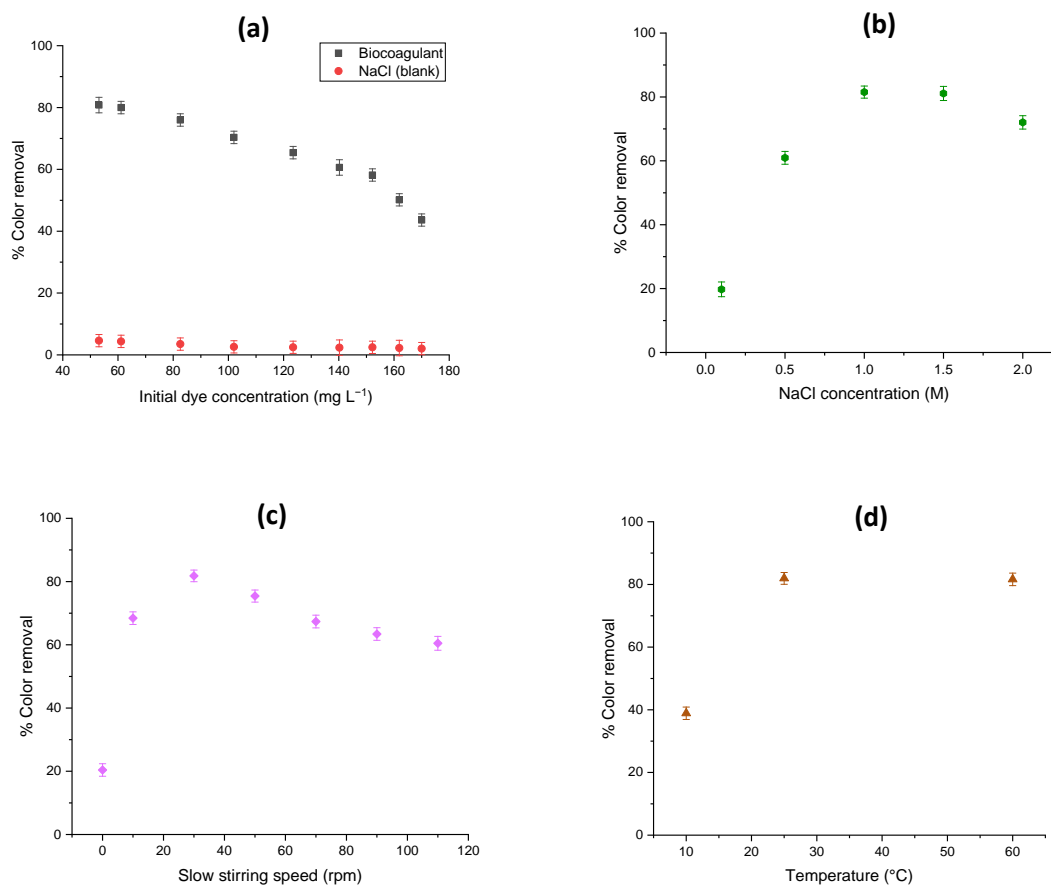
#### 451 *3.2.6. Effect of slow stirring time and floc settling time on dye removal*

452 The settling of generated flocs is the last phase in the coagulation-flocculation  
453 process. A faster floc settling rate is advantageous during the coagulation process and  
454 shows the efficiency of the coagulant (Daverey et al., 2019). The effect of flocculation  
455 mixing time and settling time on Congo red elimination percentages were studied ; time  
456 intervals ranging from 0 to 30 minutes were used (data not shown). flocs rapidly settled  
457 within the first 5 min, with little fluctuation after 10 min. This phenomenon might be  
458 explained by the rapid formation of aggregates of appropriate size that settle rapidly  
459 and easily.

#### 460 *3.2.7. Effect of solution temperature on the dye removal efficiency*

461 Although temperature has not been extensively investigated in earlier studies,  
462 especially when the coagulation-flocculation process is carried out at temperatures  
463 higher than 25°C (Domínguez et al., 2005), given that some textile dyeing effluents are  
464 produced at relatively high temperatures, this parameter must be considered.  
465 Maintaining earlier parameters at their optimum (pH= 3, coagulant dosage= 10 mL/L,  
466 Initial Congo red concentration = 50 mg/L, NaCl concentration = 1 M, Slow stirring  
467 speed = 30 rpm, slow stirring time = 20 min, floc settling time = 30 min), temperature  
468 was varied from 10 to 60°C, **Fig. 5(d)** depicts the results. It is noteworthy that the dye  
469 removal percentage improves from 38.89% to 81.93% when the temperature is

470 increased from 10°C to 60°C; this may be explained by the effect of temperature on  
 471 solution viscosity, which rises as temperature decreases, high viscosity has a negative  
 472 influence on flocs' collision rates, while low temperature is thought to weaken particle  
 473 aggregation in solution, resulting in poor Congo red removal. (Pritchard et al., 2010;  
 474 Rodrigues et al., 2013; Tie et al., 2015a).



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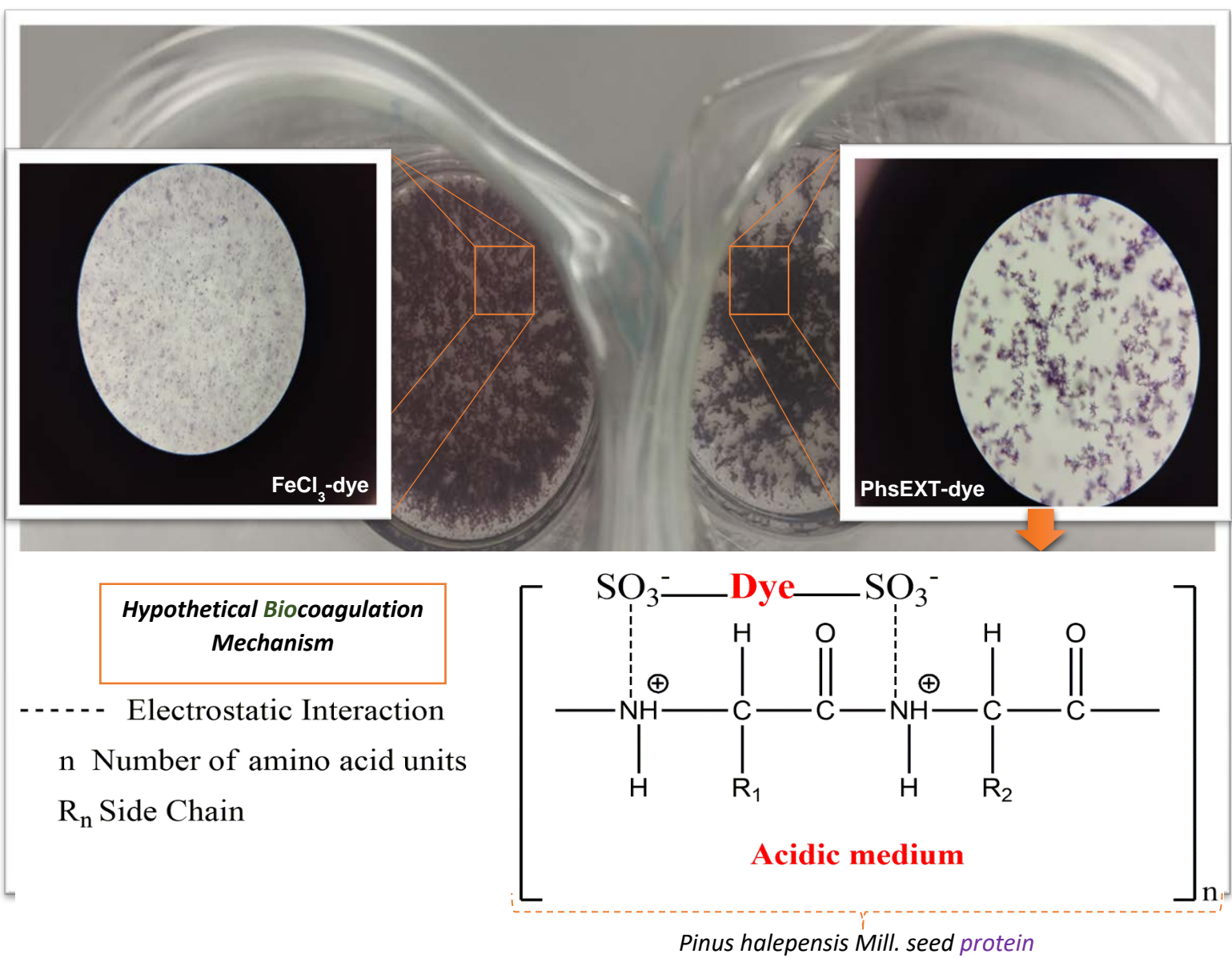
477 **Fig. 5** (a) Effect of Congo red and NaCl (blank) concentrations, (b) Effect of  
 478 extraction solution's concentration (NaCl), (c) Effect of slow stirring speed  
 479 (flocculation step), (d) effect of Congo red solutions temperature. (Optimal conditions  
 480 of each parameter were kept for the next step).

481

### 482 3.3. Morphology of flocs

483 The size and structure of flocs are considered to be critical to the efficient  
 484 functioning of industrial units. The goal of water and wastewater treatment is to remove  
 485 contaminants in the form of solid particles from polluted water. After the solid particles  
 486 are formed, they may be separated from the treated water using sedimentation,  
 487 flotation, filtration, and thickening methods. Consequently, the physical properties of

488 floccs are important in determining their removal efficacy. Using a light microscope, the  
 489 formation of floccs was observed in this study for both FeCl<sub>3</sub>-dye and PhsEXT-dye (**Fig.**  
 490 **6**) (Szygula et al., 2009). Notably, the biocoagulant generated floccs that were thicker  
 491 and denser than FeCl<sub>3</sub>, which produced floccs that were light, dispersed, and more  
 492 fragile. These results suggest that floccs generated with a biocoagulant settle faster  
 493 (Pan et al., 1999); This parameter indirectly measures the efficacy of the coagulation–  
 494 flocculation process (Aziz and Ramli, 2017) and is a good indication of the suitability  
 495 of *Pinus halepensis* Mill. seed-based biocoagulant as an alternative to chemical  
 496 coagulants such as FeCl<sub>3</sub>.



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**Fig. 6.** Microscope observation of floc formation at 100x magnification (pH= 3, dosage 10 mL L<sup>-1</sup>, C = 50 mg L<sup>-1</sup>, sampling at 60 min) with hypothetical biocoagulation mechanism occurring in this study.

### 3.4. Congo red removal Rate modelling using a Support Vector Machine coupled with Gray Wolf Optimizer

In this part, 3 kernel functions were optimized, and each kernel synthesized by the variables must also be optimized: The linear function generated by BOxConstraint, epsilon, and sigma; the Gaussian function synthesized by BOxConstraint, epsilon, and sigma; and the polynomial function generated by BOxConstraint, epsilon, and PolynomialOrder, knowing that PolynomialOrder was optimized from 2 to 5. Using the GWO technique, the variables of each kernel function were optimized.

After acquiring the outcome of the learning phase, the result was confirmed using the validation database. In order to calculate R, R<sup>2</sup>, R<sup>2</sup>adj, RMSE, MSE, EPM, ESP, and MAE, the generated results (predicted values) were compared with the experimental values in the two phases (the learning phase and the validation phase). The outcomes of these tests are shown in **Table 3**. Note that the findings were denormalized to their actual values in order to be compared to other models.

**Table 3** Performances of the different tested SVM Kernel function.

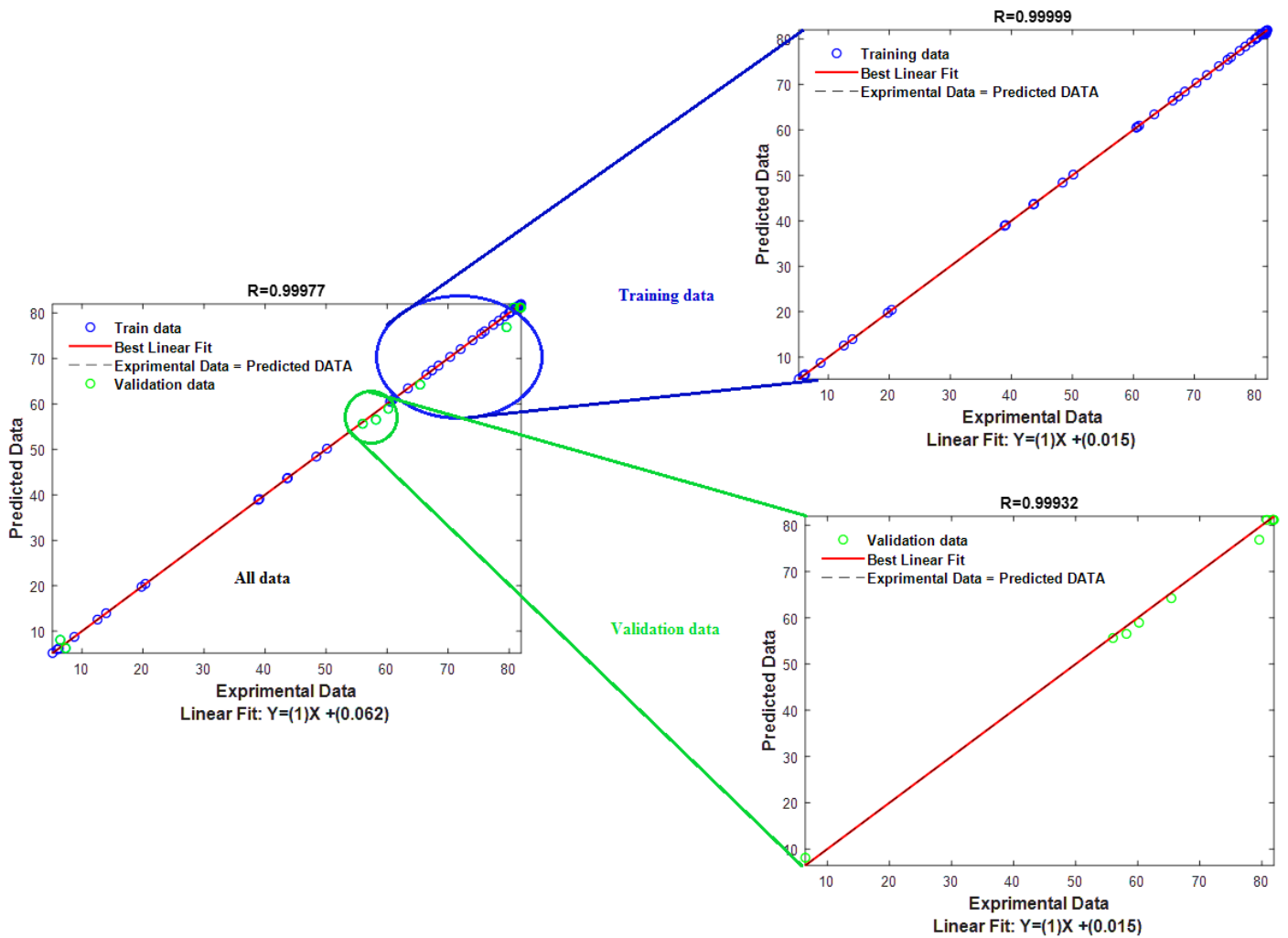
<b>GWO</b>											
<b>Max_iteration=100</b>											
<b>SearchAgents_no=30</b>											
Kernel function	C	$\epsilon$	$\sigma^2$	d		R/R <sup>2</sup> /R <sup>2</sup> <sub>adj</sub>			RMSE/MSE/EPM/ESP/MAE		
						Train	Val	All	Train	Val	All
Linear	540	208.1278	1.2000	/	45	0.7836	0.5543	0.7573	17.6421	14.4266	17.0584
						0.6140	0.3073	0.5735	311.2425	208.1278	290.9878
						0.5283	0.3073	0.5009	67.9624	15.6305	57.6829
									30.4879	20.8748	28.3954
								11.8560	11.1706	11.7213	
<b>Gaussian</b>	<b>1180</b>	<b>1.6758</b>	<b>1.2000</b>	<b>/</b>	<b>45</b>	<b>1.0000</b>	<b>0.9993</b>	<b>0.9998</b>	<b>0.1045</b>	<b>1.2945</b>	<b>0.5813</b>
						<b>1.0000</b>	<b>0.9986</b>	<b>0.9995</b>	<b>0.0109</b>	<b>1.6758</b>	<b>0.3379</b>
						<b>1.0000</b>	<b>0.9932</b>	<b>0.9995</b>	<b>0.0315</b>	<b>4.8643</b>	<b>0.9808</b>
									<b>0.1738</b>	<b>2.1594</b>	<b>0.9677</b>
								<b>0.0252</b>	<b>1.1094</b>	<b>0.2382</b>	
Polynomial	10	23.1264	/	4.8000	45	0.9945	0.8969	0.9917	2.8729	4.8090	3.3429
						0.9890	0.8044	0.9835	8.2534	23.1264	11.1748
						0.9865	0.0218	0.9807	7.4935	3.9188	6.7913
									5.1037	6.3647	5.5646
								1.7791	3.0117	2.0212	

542

543 By comparing the coefficients and the statistical errors, it is obvious that the  
 544 Gaussian function gave the best result; this result is schematized graphically by **Fig.**

545 **7:**

546



548 **Fig. 7.** The relationship between the measured dosage of coagulant and the SVM-  
 549 estimated dose.

550 *3.4.1. Model performance test*

551 Interpolation was used to assess the performance of the generated SVM\_GWO  
 552 model. This was performed by using a cached database comprising 20 experimental  
 553 data points that were not used during model training. **Table 4** summarizes the results  
 554 in terms of coefficients and statistical errors.

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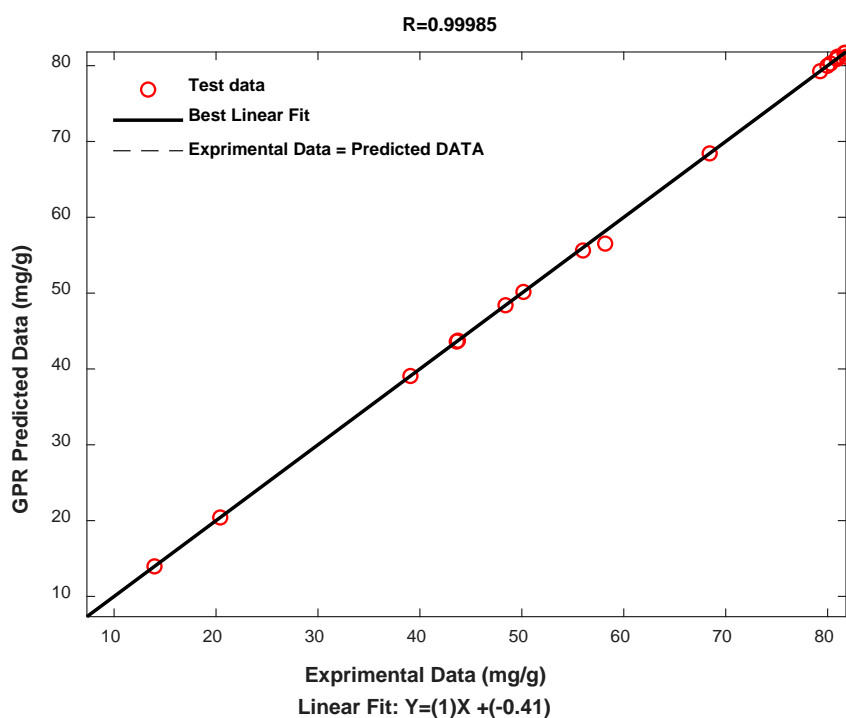
**Table 4** Model test performance.

R/R <sup>2</sup> / R <sup>2</sup> <sub>adj</sub>	RMSE/MSE/ EPM/ESP/MAE
<b>ALL</b>	<b>ALL</b>
0.9998	0.4675
0.9996	0.2186
0.9994	0.9364
	0.7945
	0.1951

560

561 The coefficients shown in **Table 4** demonstrate the effectiveness and  
562 performance of our model.

563 This result was represented graphically (**Fig. 8**) in terms of the experimental  
564 values and the predicted values.



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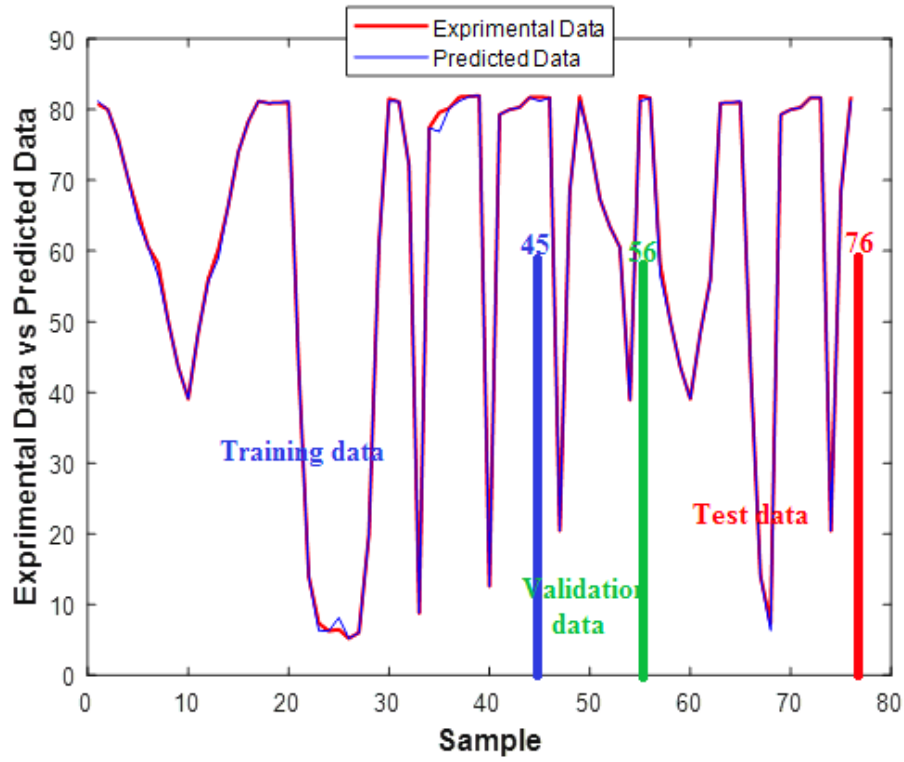
566 **Fig. 8.** Comparison between experimental and predicted values to assess  
567 performance.



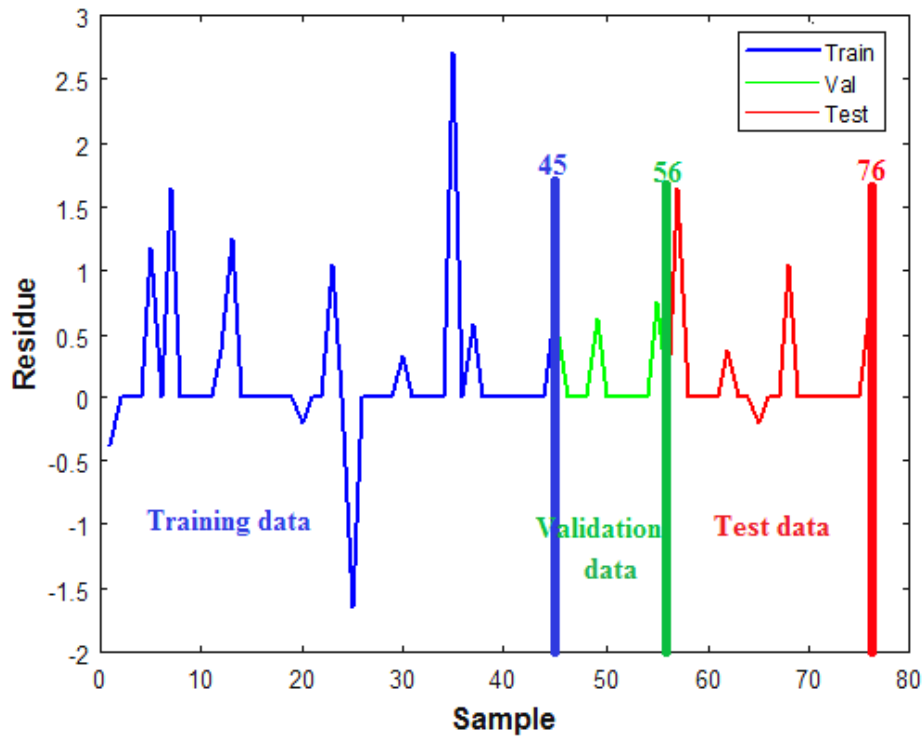
568 3.4.2. *Residues study*

569 The residuals approach was used in this study to investigate the relationship  
570 between the experimental and predicted values on the one hand, and the efficiency  
571 and performance of the chosen model on the other (Tahraoui et al., 2021b, 2022b).  
572 For this purpose, the experimental and predicted values were plotted in parallel as a  
573 function of samples (**Fig. 9(a)**) for all data (including data training, data validation and  
574 data test performance) (Bousselma et al., 2021; Tahraoui et al., 2022a).

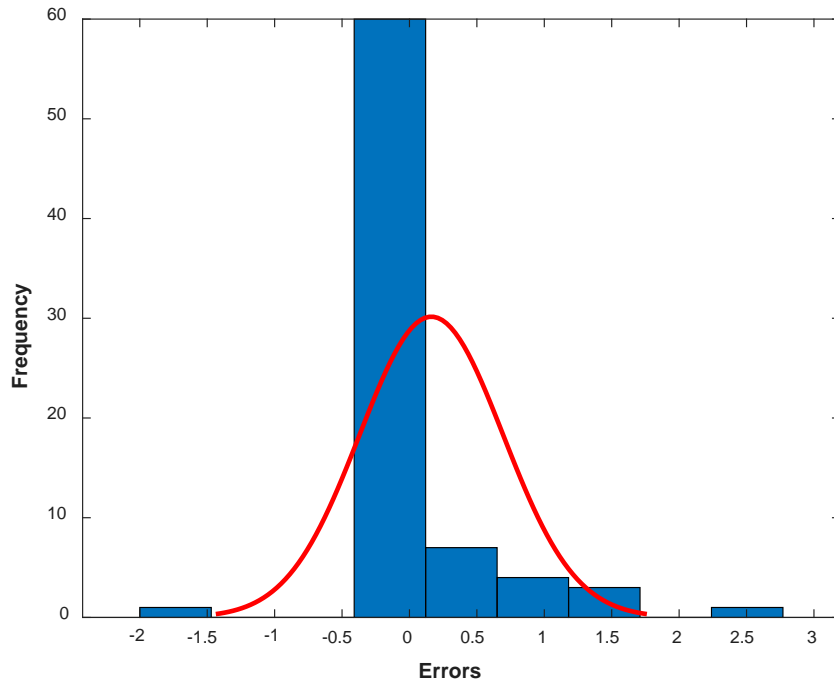
575 In contrast, for all data, the error was determined as the difference between the  
576 experimental and predicted values (including data training, data validation and data  
577 test performance). This error was plotted graphically (**Fig. 9(b), (c) and (d)**) by three  
578 methods (residue, frequency and instances) (Tahraoui et al., 2021a, 2021b, 2022b).



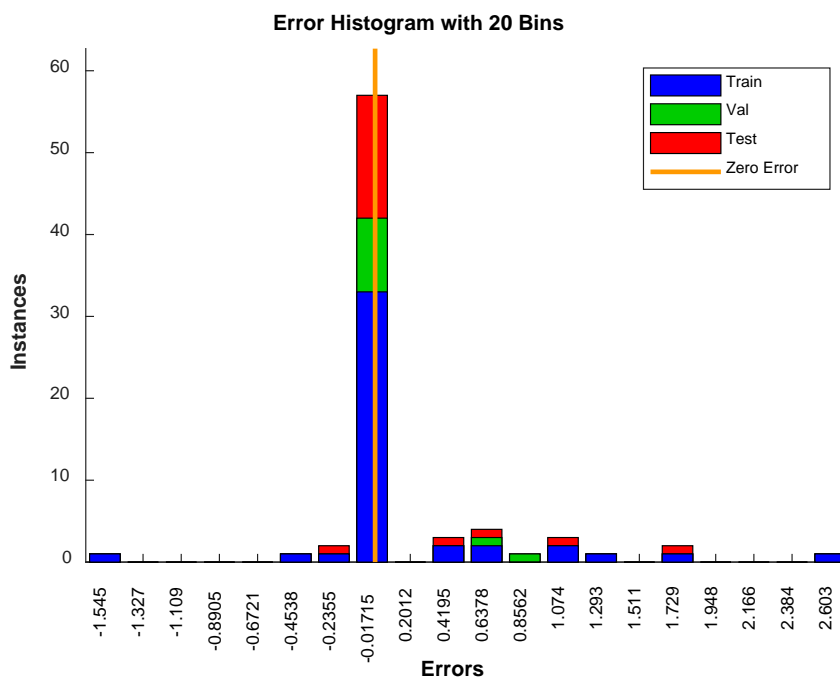
(a)



(b)



(c)



(d)

**Fig. 9.** Residuals relating to the models established by the different techniques according to the estimated values: (a) Relationship between experimental and anticipated sample data, (b) Residues relating to the models established, (c) Frequency distribution of errors, and (d) Instances distribution of errors.

**Fig. 9(a)** shows the strong convergence between the experimental values and the predicted values, confirming once again the efficiency of the obtained model.

579 Moreover, **Fig. 9(b)** shows the small errors obtained in the three phases. Indeed,  
580 in the learning phase the error did not exceed 3%, the error values were 1% in the  
581 validation phase, and 2% for the test phase. It can therefore be concluded that the  
582 errors obtained from our model are very small. This was confirmed by examining **Fig.**  
583 **9(c) and (d)**, where **Fig. 9(c)** shows that the high frequency was obtained at error 0.  
584 Similarly for **Fig. 9(d)**, where most errors were around zero for all three phases  
585 (training, validation, and testing data). All these demonstrations and interpretations  
586 strongly demonstrate the efficiency and performance of our model.

587

### 588 3.5. Optimization of the optimal analysis and Validation

589 An optimization method was carried out using GWO to find the optimal conditions  
590 for the elimination of Congo red by the biocoagulant.

591 The optimization results revealed ideal conditions for small concentrations, namely  
592 up to 150 mg/L ( $X_1 = 20$  min,  $X_2 = 30$  min,  $X_3 = 50$  and  $150$  mg L<sup>-1</sup>,  $X_4 = 10$  mL L<sup>-1</sup>,  
593  $X_5 = 3$ ,  $X_6 = 1$ ,  $X_7 = 30$  rpm, and  $X_8 = 25^\circ\text{C}$ ). For concentrations greater than 150 mg  
594 L<sup>-1</sup>, the same preliminary conditions as those obtained for the concentration less than  
595 150 mg L<sup>-1</sup> apply, the only difference is that the temperature must be increased to  $60^\circ\text{C}$   
596 ( $X_8 = 60^\circ\text{C}$ ) for an elimination rate of 53.23 % to be achieved. These findings were  
597 validated in the laboratory by applying the ideal conditions at various doses (50, 150,  
598 and 170 mg L<sup>-1</sup>). The optimization results are shown in **Table 5** with an error rate of  
599 less than 2%, demonstrating our model's effectiveness once again.

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604 **Table 5** Results of the removal rate of Congo red under the optimal conditions.

GWO: X1 = 20 min, X2 = 30 min, X3 = 50 mg L <sup>-1</sup> , X4 = 10 ml L <sup>-1</sup> , X5 = 3, X6 = 1 M, X7 = 30 rpm and X8 = 25°C	
The predicted removal rate of Congo red (%)	81.17
The experimental removal rate of Congo red	81.93
Error (%)	0.79
GWO: X1 = 20 min, X2 = 30 min, X3 = 150 mg L <sup>-1</sup> , X4 = 10 mL L <sup>-1</sup> , X5 = 3, X6 = 1 M, X7= 30 rpm and X8= 25°C	
The predicted removal rate of Congo red (%)	56.52
The experimental removal rate of Congo red	58.17
Error (%)	1.65
GWO: X1 = 20 min, X2 = 30 min, X3 = 170 mg L <sup>-1</sup> , X4 = 10 mL L <sup>-1</sup> , X5 = 3, X6 = 1 M, X7= 30 rpm and X8 = 60°C	
The predicted removal rate of Congo red (%)	53.23
The experimental removal rate of Congo red (%)	51.83
Error (%)	1.4

605

606 3.6. *Comparison of Pinus halepensis Mill. seed extract coagulating performances*  
 607 *with ferric chloride (FeCl<sub>3</sub>)*

608 For comparative purposes, 50 mg L<sup>-1</sup> of Congo red dye were treated with different  
 609 amounts of ferric chloride (results not shown), to do so, optimal conditions obtained  
 610 earlier were taken into consideration. Using the Imhoff cones method, the quantity of  
 611 wet sludge generated by the two coagulants (FeCl<sub>3</sub> and PhsEXT) was also studied.  
 612 According to **Table 6**, PhsEXT and FeCl<sub>3</sub> demonstrated nearly identical removal rates  
 613 (81.22 % and 83.78 %, respectively); FeCl<sub>3</sub> generated higher sludge quantity (about  
 614 18 mL more) comparing to PhsEXT. Similar results have been reported by (Choudhary  
 615 et al., 2019; Kristianto et al., 2019). Organic polymers typically generate less sludge  
 616 than chemical coagulants since they don't contribute in adding weight to the water or  
 617 react chemically with some other ions to form precipitates. Thus, the sludge formed by  
 618 PhsEXT was volume-reduced and compressed. This can also be attributed to the

619 difference between flocs formed when  $\text{FeCl}_3$  and the biocoagulant were used (Dalvand  
620 et al., 2016). This shows that substituting chemical coagulants with natural ones may  
621 reduce sludge handling costs. The use of conventional coagulants generates a  
622 massive volume of non-biodegradable sludge. This sludge is often disposed of in  
623 conventional landfills, as there is currently no rule tightly regulating the management  
624 of aluminum and iron in sludge. It has been demonstrated that the use of  
625 biocoagulants/biofloculants significantly reduces the amount of sludge generated  
626 during treatment operations by up to 30% (Kurniawan et al., 2020). Additionally, since  
627 all *Pinus halepensis* byproducts are biodegradable organics, the sludge may be utilized  
628 as a fertilizer as long as no heavy metals are present in the treated water. The present  
629 findings demonstrate the great efficiency of PhsEXT as a natural coagulant substitute  
630 for traditional ones.

631 **Table 6** Comparison of *Pinus halepensis* Mill. seeds based biocoagulant with ferric  
632 chloride ( $\text{FeCl}_3$ ) in coagulating Congo red. (Mean  $\pm$  SD, n = 3)

Coagulant	Coagulant dosage (mL L <sup>-1</sup> )	% Dye removal	Sludge volume (mL L <sup>-1</sup> )
<b>PhsEXT</b>	10	81.2 $\pm$ 3.7	28 $\pm$ 1
<b>FeCl<sub>3</sub></b>	10	83.8 $\pm$ 3.6	46 $\pm$ 1

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634

#### 635 **4. Conclusion**

636 The findings of the current investigation show that PhsEXT has significant potential  
637 as a natural coagulant and may thus be considered an interesting contribution in the  
638 field of natural resources development. At pH = 3, optimum performance was achieved  
639 with a biocoagulant dosage of 10 mL L<sup>-1</sup> and an initial Congo red concentration of 50  
640 mg L<sup>-1</sup>, yielding 81% of dye removal. Under acidic conditions, PhsEXT performed  
641 similarly to ferric chloride, yet produced less sludge and larger flocs. Seed's proximate  
642 composition and FTIR analysis corroborate the theory that charge neutralization  
643 (proteins— $\text{SO}_3^{2-}$  groups of the dye) is the main mechanism occurring in this study. Due  
644 to the very high statistical coefficients (R = 0.9998, R<sup>2</sup> = 0.9995, and R<sup>2</sup> adj = 0.9995)

645 and significantly lower statistical errors (RMSE = 0.5813, MSE = 0.3379, EPM =  
646 0.9808, ESP = 0.9677, and MAE = 0.2382), the SVM\_GWO model exhibited  
647 remarkable accuracy. In addition, the efficacy of the model was demonstrated using a  
648 variety of methodologies, including test interpolation and residual analysis. This  
649 highlights the advantages of combining SVM with GWO.

650 The outcomes of this work indicate that *Pinus halepensis* seeds as a biocoagulant  
651 has a promising future in the wastewater treatment field and may be a good alternative  
652 to chemical coagulants/flocculants in order to reduce environmental pollution and  
653 health hazards associated with their usage.

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### **Acknowledgements**

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659

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661 The study is not dependent on any particular funding source in any form.

662

### **Compliance with Ethical Standards**

664 The submitted work is an original article. It does not contain any human/animal  
665 experiments. The paper is original, has not been submitted to another journal for  
666 simultaneous review, and has not been published in any form or language elsewhere.

### **Data availability statement**

668 The authors certify that the data backing the study's conclusions are included in the  
669 publication [and/or] its supplemental materials.

670

### **Competing interests**

672 The authors state that they do not have any conflicting interests.

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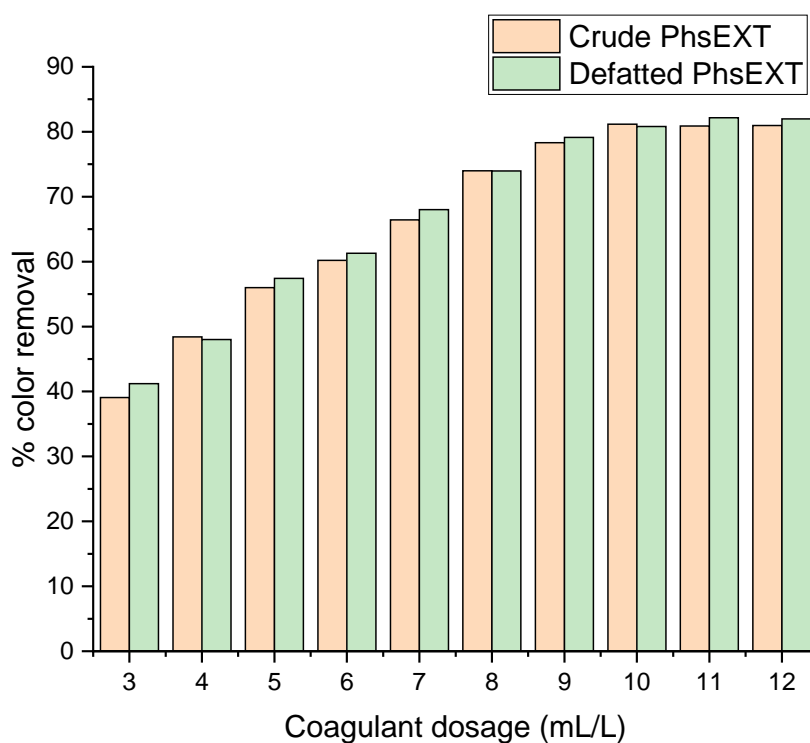
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## 960 **Supplementary Materials**

### 961 *1. Influence of defatting Pinus halepensis Mill. seed on coagulation efficiency*

962 the presence of oil might inhibit the extractability of protein and, ultimately, the efficacy  
963 of the coagulation process; this phenomenon has been described in a number of prior  
964 works (Camacho et al., 2017; Marobhe and Sabai, 2021; Yimer and Dame, 2021). In  
965 this regard, a preliminary investigation was conducted on the influence of delipidation  
966 on the Aleppo pine seeds coagulation efficiency for the removal of Congo red dye. A  
967 hexane extraction was performed according to the methodology given by (Chales et  
968 al., 2022). The oil extraction was conducted using a Soxhlet extractor equipment with  
969 hexane as the solvent and a solid/solvent ratio of 0.05 g/mL over a period of 3 h (20  
970 cycles) to achieve a high oil-removal yield in comparison to other removal approaches  
971 (batch) or other solvents (ethanol, ethyl acetate, and acetone). The resulting defatted  
972 powder was subjected to the same extraction procedure depicted in the manuscript, 5  
973 g were stirred vigorously with 100 mL of 1 M NaCl solution, the coagulation  
974 performance of crude and defatted seeds was then evaluated, and the results are  
975 presented in the following figure:



976

977 **Fig. 1.** Effect of coagulant dosage on the color removal efficiency of crude and  
978 defatted PhsEXT (pH = 3; [NaCl] = 1 M; T = 25°C)

979

980 As shown in **Fig. 1**, there is a little variation in the removal efficiency of Congo red with  
981 seeds that have been defatted, suggesting that this step does not greatly enhance the  
982 process and might be skipped, especially because one of our primary objectives was  
983 to undertake the dye removal by the novel green coagulant with as few steps as  
984 possible to preserve the eco-friendly nature of the investigation. Such results have  
985 been reported by (Chales et al., 2022), indeed, the defatting step of *Moringa oleifera*  
986 seeds (MOS) did not improve the turbidity removal capacity of seeds' saline extract,  
987 but rather reduced the cytotoxicity of the treated water and generated a significant by-  
988 product (edible MOS oil).

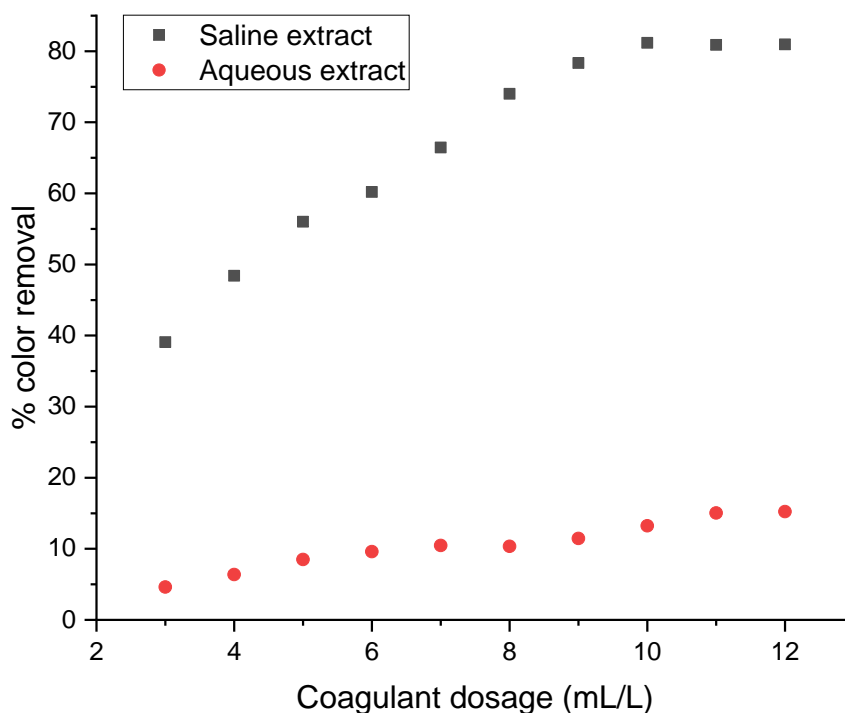
## 989 2. *Pinus halepensis* Mill. seed extract (PhsEXT) coagulating agent

990 Concerning the possible influence of carbohydrates on the coagulation  
991 performance, a preliminary investigation was carried out in order to confirm or refute  
992 this possibility. Indeed, as is well known in the domain of biocoagulation-  
993 bioflocculation, natural coagulants are typically made up of carbohydrates and protein,  
994 with polysaccharide and amino acids functioning as the building blocks, either through  
995 charge neutralization, generally attributable to amino acids, or bridging phenomenon,  
996 usually linked to polysaccharide. The above mentioned phenomena are the primary  
997 mechanisms that govern biocoagulation-bioflocculation activity (Panwar et al., 2022).

998 A number of studies have reported the involvement of carbohydrates in the  
999 coagulation process (Mardarveran and Mohd Mokhtar, 2020) through the bridging  
1000 mechanism. Our preliminary investigation involved examining the efficacy of an  
1001 aqueous extract of Aleppo pine seeds according to the methodology reported in those  
1002 previous studies. The dried *Pinus halepensis* seeds were sieved to a particle size of  
1003 0.5 mm. Then, 5 g of the dried raw materials was soaked in 100 ml of distilled water  
1004 and stirred vigorously for 30 minutes. The obtained extract was centrifuged and filtered  
1005 according to the same methodology depicted in the manuscript and tested on a 50  
1006 mg/L Congo red solution, the results are presented in **Fig. 2** (results for saline extract  
1007 were incorporated for comparative purposes).

1008





1009

1010 **Fig. 2.** Effect of coagulant dosage of saline and aqueous PHsEXT on the removal of  
 1011 Congo red (pH = 3; Congo red concentration = 50 mg/L; [NaCl] = 1 M; T = 25°C)

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1013 As can be observed in **Fig. 2**, there is a noticeable difference in the efficacy of  
 1014 the two seed extracts. The saline extract significantly outperforms the aqueous one,  
 1015 with an elimination rate of 80%, whereas the aqueous extract exhibits negligible Congo  
 1016 red removal. As stated in the manuscript, results and discussion section, (3.2.4. *Effect*  
 1017 *of NaCl concentration*), the increase in salt concentration promotes a better protein  
 1018 extraction through the salting-in mechanism, which refers to the process of increasing  
 1019 the ionic strength of a solution to boost solubility of protein leading, ultimately, to a  
 1020 dramatical increase in Congo red removal (from 0.1 M to 1 M NaCl).

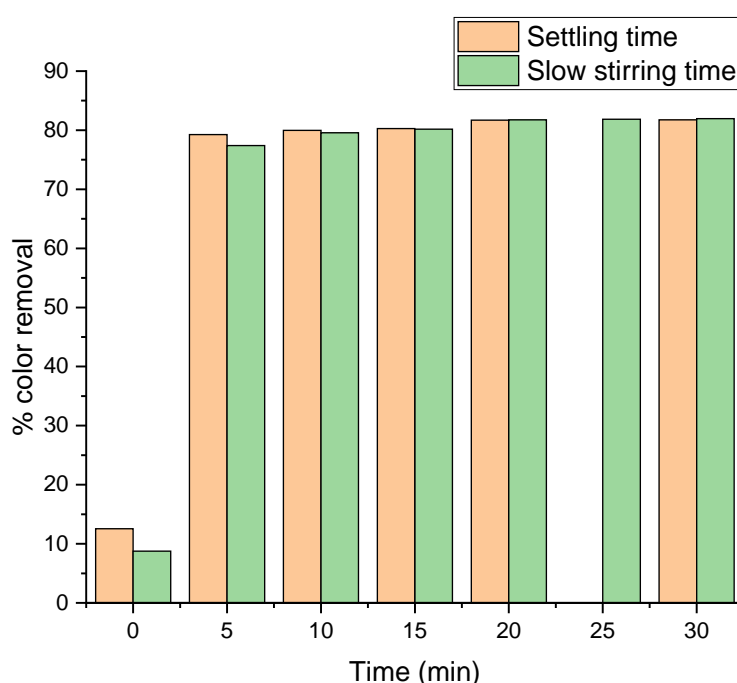
1021 Based on the data presented in **Fig. 2** and the section on the effect of NaCl  
 1022 concentration, it is obvious that proteins are the main protagonists in the coagulation  
 1023 process in this research.

1024 This theory could also be confirmed by the effect of pH on coagulation efficiency  
 1025 (3.2.1. *Effect of initial pH on Congo red removal*), indeed, (Abidin et al., 2011; Bahrodin

1026 et al., 2021) reported that the coagulation mechanism is charge neutralization when  
1027 the difference between maximum removal and minimum removal by using different pH  
1028 is around 50% or more, which is the case in our investigation. Also, bridging cannot be  
1029 considered as a possible mechanism because unlike charge neutralization, bridging is  
1030 least affected by pH. however, at lower pH, the polymeric chains responsible for  
1031 interparticle bridging will expand and be able to attach to additional pollutants (Momeni  
1032 et al., 2018; Zaidi et al., 2022).

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### 1034 3. Effect of slow stirring time and floc settling time on dye removal



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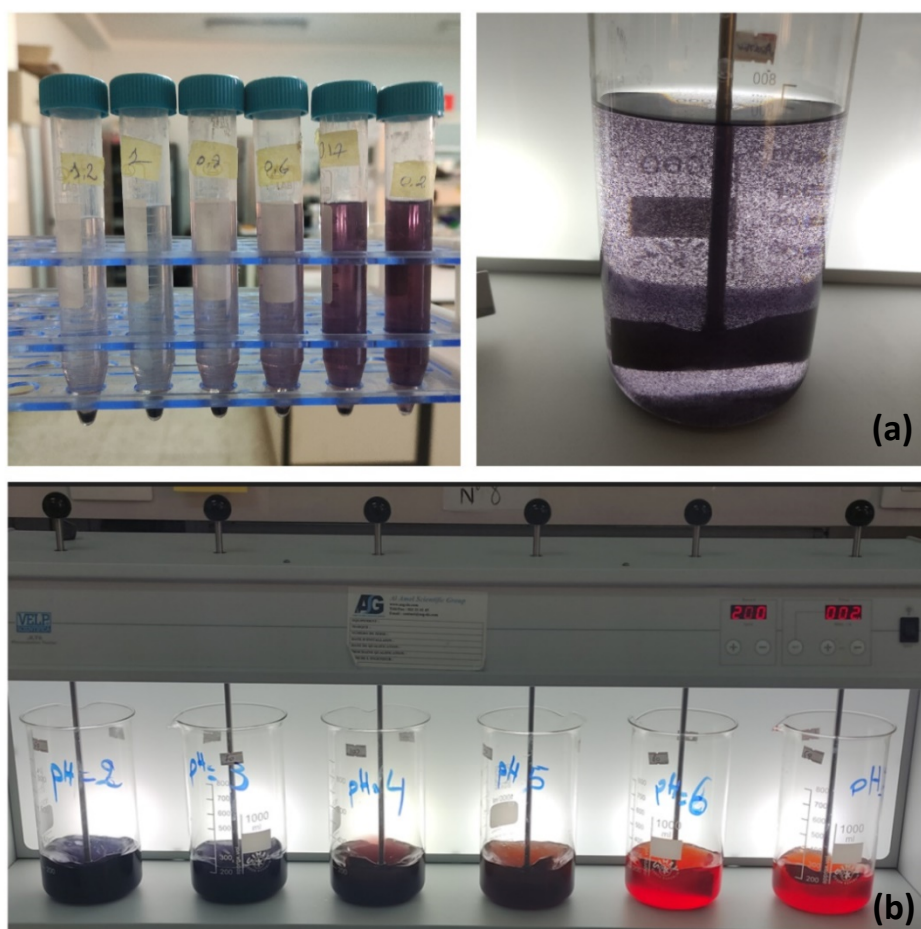
1036 **Fig. 3.** Effect of stirring time (flocculation stage) and settling time on dye removal  
1037 efficiency.

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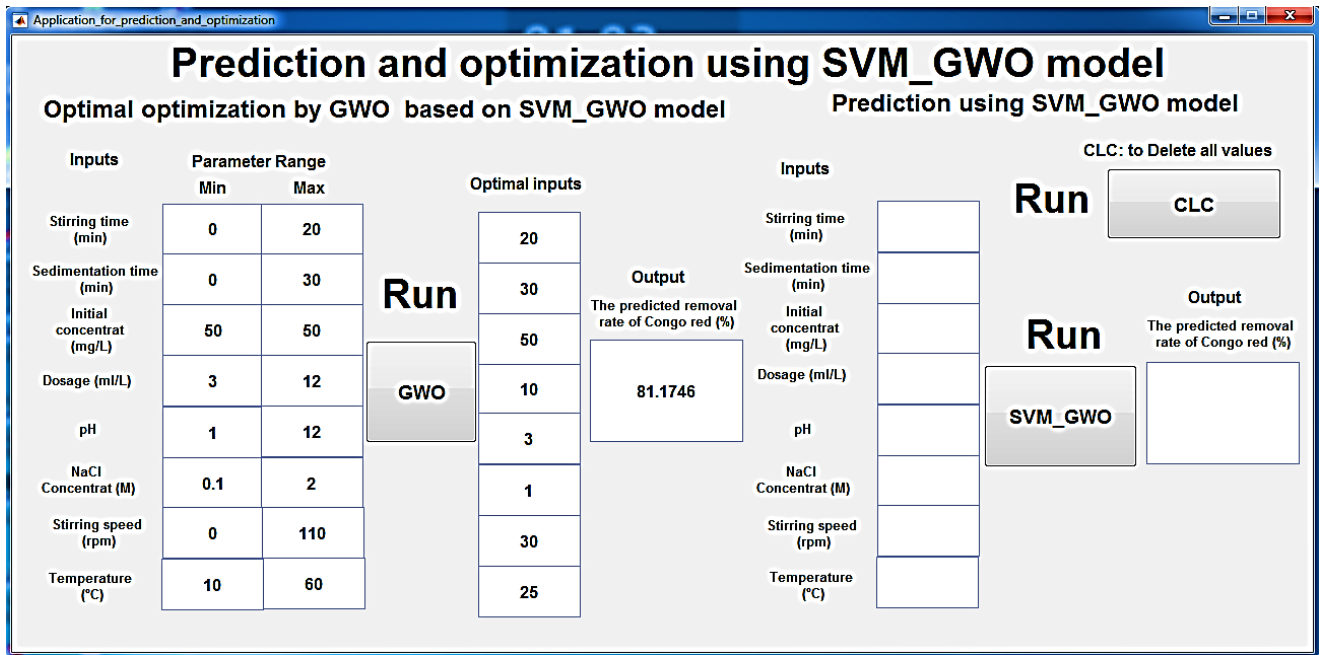


**Fig. 4. (a)** Representation of the discoloration process after treatment with *PhsEXT*,  
**(b)** Jar-test apparatus used in this study.

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#### 4. Interface for optimization and prediction

1060 To provide a simple way to implement the optimization and predict the removal rate  
1061 of Congo red by biocoagulant, an interface was designed using the MATLAB guide for  
1062 optimization and prediction (**Fig. 5**). This interface tool has been converted into an  
1063 executable application under Windows. This powerful direct-to-use application predicts  
1064 the output by selecting values input by SVM\_GWO. In addition, the application also  
1065 helps to find an optimal solution by GWO.  
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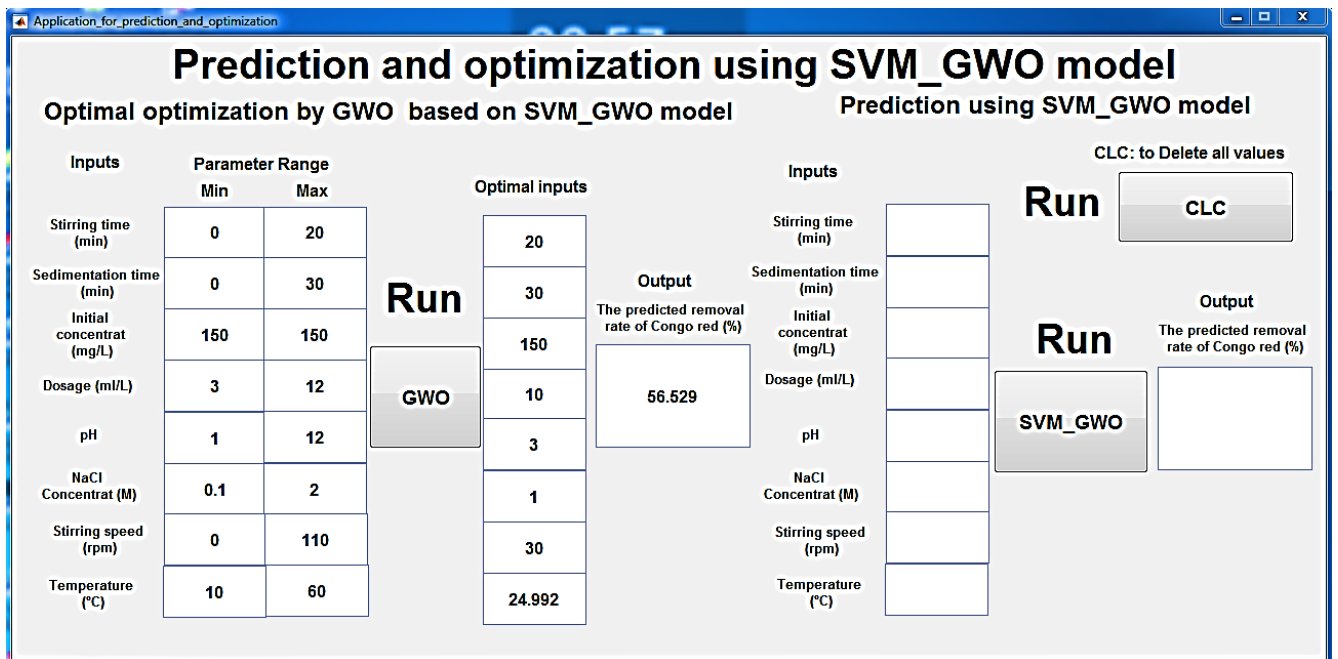


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(a)

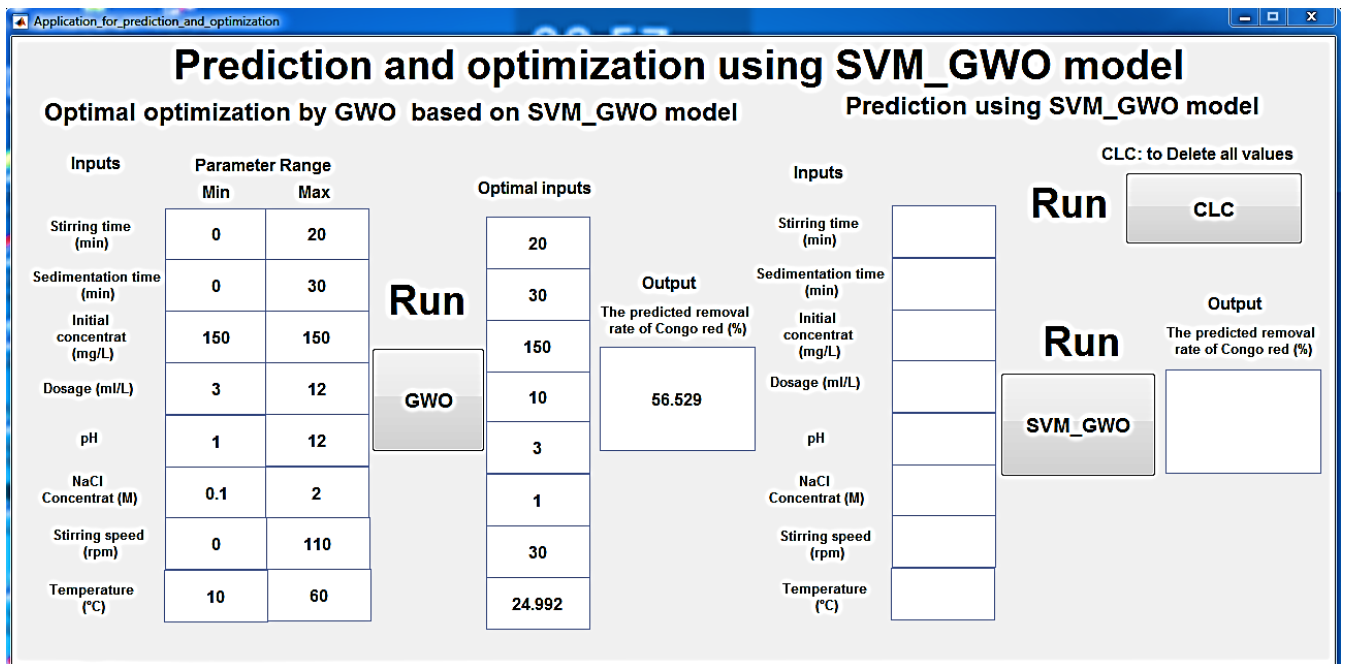


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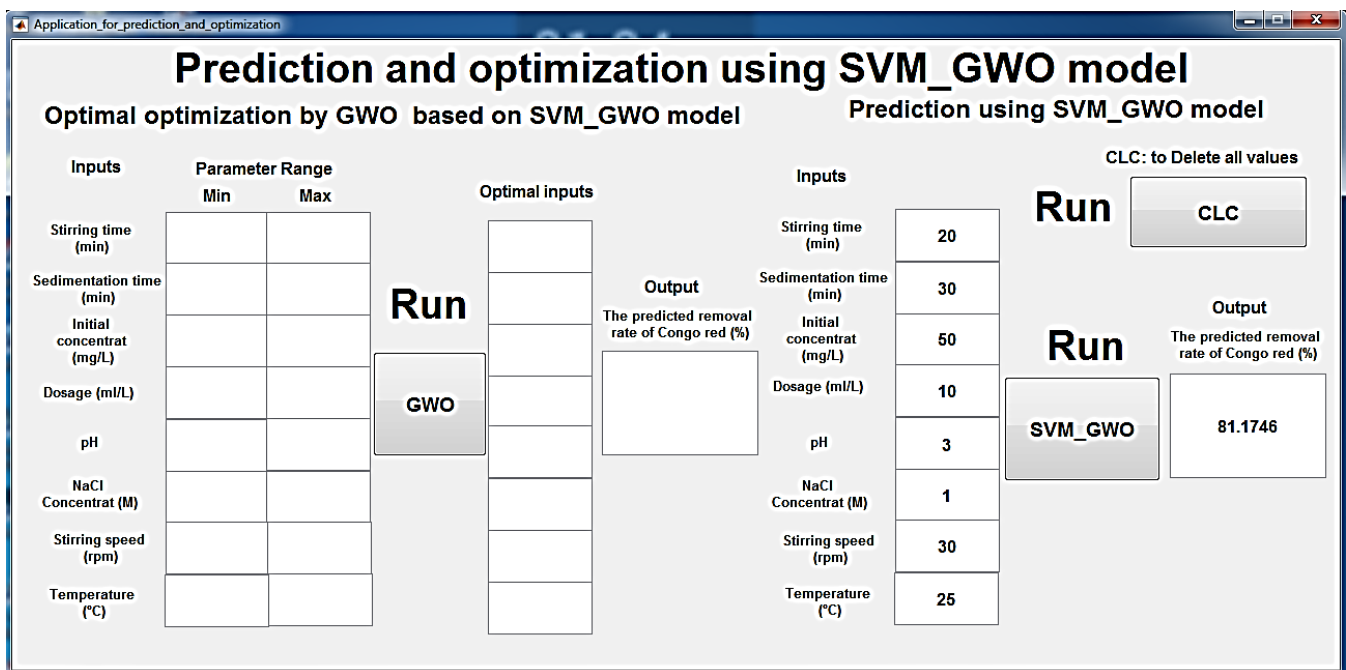
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(b)



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(c)



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(d)

1078 **Fig. 5.** MATLAB Interface: Optimization of optimal using GWO for (a) concentration  
1079 50 mg/L, (b) concentration 150 mg/L, (c) concentration 170 mg/L, and (d) prediction  
1080 the removal rate of Congo red with SVM\_GWO model.

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1084 **Supplementary Materials references**

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