Environmental Technology
Vol. 33, No. 1, January 2012, 69–75

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Coagulant properties of *Moringa oleifera* protein preparations: application to humic acid removal

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(*Received 16 June 2010; Accepted 20 December 2010*)

This work aimed to characterize the coagulant properties of protein preparations from *Moringa oleifera* seeds in the removal of humic acids from water. Three distinct preparations were assayed, namely *extract* (seeds homogenized with 0.15 M NaCl), *fraction* (extract precipitated with 60% w/v ammonium sulphate) and *cMoL* (protein purified with guar gel column chromatography). The extract showed the highest coagulant activity in a protein concentration between 1 mg/L and 180 mg/L at pH 7.0. The zeta potential of the extract (−10 mV to −15 mV) was less negative than that of the humic acid (−41 mV to −42 mV) in a pH range between 5.0 and 8.0; thus, the mechanism that might be involved in this coagulation activity is adsorption and neutralization of charges. Reduct[illegible]TOC) and dissolved organic carbon (DOC) was observed in water samples containing 9 mg/L c[illegible]eated with 1 mg/L of the extract. A decrease in colour and in the aromatic content of the treate[illegible] These results suggested that the extract from *M. oleifera* seeds in a low concentration (1 mg/L) c[illegible] alternative for removing humic acid from water in developing countries. The extract dose determ[illegible]s not impart odour or colour to the treated water.

Keywords: Moringa oleifera; humic acids; lect[illegible] coagulant

1. Introduction

Humic substances are structurally complex macromolecules that are yellow to black in appearance, acidic and generally heterogeneous; they occur in soils and natural waters as a consequence of the breakdown of plant and animal residues by microbial activity [1], and they account for most of the natural organic matter in surface waters. The removal of these substances has been a main objective of water treatment because of their associated water quality problems: residual colour, taste, odour and trihalomethane formation resulting from chlorination in the water treatment process [2]. Several strategies have been investigated for the removal of humic substances from water, such as activated carbon filtration [3] and photocatalytic degradation on Ti-modified silica [4].

Coagulation is one of the critical operations in water treatment for removing natural organic matter. The formation of insoluble complexes between organic matter and coagulant species, as well as the adsorption on freshly formed hydroxide precipitate, could be the determining mechanism of organic matter removal by coagulation [5]. The coagulants that are frequently used to remove organic contamination are mineral additives including metal salts olyaluminium chloride, aluminium sulphate and synthetic polymers such as polyacrylamide [2,6]. These compounds might have a negative impact on the environment and health. As examples, aluminium ion concentrations above 50 μg/L are potentially toxic to fish and aquatic organisms [7], and polyacrylamide residues (acrylamide) are peripheral nerve toxins that affect man and animals [8]. The environmental side effects of these compounds have increased the interest in the use of natural coagulants because of their abundance, low price, innocuousness and biodegradability. Plant materials such as extracts from *Moringa oleifera* seeds [9–11], *Cactus latifaria* and *Prosopis juliflora* are natural coagulants [12,13] used in the treatment of water for human consumption.

Zeta potential is an analytical method used to evaluate water coagulation [14]. The objective of the zeta potential measurements is to determine the mechanism of coagulation that depends upon the electrostatic forces between charges carried by the colloidal particles [9].

Molinga oleifera, a plant of the Moringaceae family, is commonly known as the horseradish or drumstick tree and is native to the sub-Himalayan region of northwest India, but is also naturalized in Sudan and other parts of Africa. The tree

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ISSN 0959-3330 print/ISSN 1479-487X online

http://dx.doi.org/10.1080/09593330.2010.550323
http://www.tandfonline.com

ranges in height from 5 to 12 m and the fruits (pods) are around 50 cm long. Fully mature, the dry seeds are round or triangular in shape, the kernel surrounded by a light wooded shell with three papery wings [15]. Water-soluble proteins from *M. oleifera* seeds act as effective coagulants for water turbidity removal [16–18] and wastewater treatment [18]. The aqueous extract obtained from *M. oleifera* seeds contains a flocculating protein that works as a clarifying agent of turbid water [19]. Shelled *M. oleifera* seeds have also been used for decontamination of water containing arsenic [20]. Lectins, proteins of nonimmune origin containing two or more binding sites to monosaccharides or oligosaccharides [21], also obtained from *M. oleifera* seeds, have coagulant properties and can remove turbidity from water [22]. The aim of this work was to characterize the coagulant properties of protein preparations from *M. oleifera* seeds (*extract*, *fraction* and *cMoL*) in the removal of humic acids from water.

2. Materials and methods

2.1. M. oleifera protein preparations

Moringa oleifera seeds were collected in Recife (northeast Brazil), and a sample is kept as voucher specimen number 63184, IPA, at the herbarium 'Dárdano de Andrade Lima' (Empresa Pernambucana de Pesquisa Agropecuaria, Recife, Brazil). The seeds were ground to a flour, which was then extracted with 0.15 M NaCl for 6 h at room temperature (25 °C) to obtain a saline extract. Extract proteins were precipitated with 60% (w/v) ammonium sulphate for 4 h at room temperature (25 °C). The resulting *fraction* was chromatographed (10 mg of protein) and *cMoL* was purified as described by Santos *et al.* [22]. The extract, fraction and cMoL were used in the experiments described below. The protein was estimated according to Lowry *et al.* [23]. A calibration curve was prepared using bovine serum albumin (BSA) as standard in a range between 0 μg and 400 μg.

2.2. Coagulation activity assays

A coagulation activity assay using small sample volumes was done according to Ghebremichael *et al.* [17], with modifications. Assays were performed as follows: 300 μL of either extract, fraction or cMoL solution with a protein concentration of 1000 mg/L, obtained as previously described, were added to 2700 μL of a humic acid (Sigma Aldrich 53680) solution with a carbon concentration of 10 mg/L and pH 7.0 in a 3 mL cuvette and were instantly homogenized. The coagulant activity of aluminium sulphate at a concentration of 5 mg/L was also tested. A negative control with only humic acid was included. Distilled water was used in the preparation of all solutions. Absorbance was measured at 500 nm every 5 min up to 60 min and then every 10 min up to 150 min using a UV-visible spectrophotometer UNICAM Eiλos γ. Coagulation was expressed as the percentage reduction in the absorbance of the humic acid solution amended with the protein preparation at $t = 150$ min relative to $t = 0$ min.

The effect of pH, in a range from 2.0 to 10.0, and turbidity, simulated with kaolin at concentrations of 0.23 g/L, 0.45 g/L and 0.90 g/L (pH 7.0), on the coagulation activity of the extract was assessed. The optimal extract dosage was also determined. Assays were performed in triplicate at room temperature (25 °C).

Jar tests were performed solely with the extract, at both 1 mg/L (low dosage) and 70 mg/L (high dosage). The test consisted of the instantaneous addition of 40 mL extract solution with a protein concentration of 10 mg/L (or 700 mg/L) to 360 mL of humic acid solution with a carbon concentration of 10 mg/L followed immediately by a rapid mix at 120 rpm for 1 min, slow mix at 30 rpm for 15 min and sedimentation for 30 min. The effects of pH, in a range from 5.0 to 8.0, and turbidity, simulated with kaolin at concentrations of 0.23 g/L, 0.45 g/L and 0.90 g/L (pH 7.0), on the coagulation activity of the extract (1 mg/L and 70 mg/L) and aluminium sulphate (5 mg/L) were also assessed. Total organic carbon (TOC), dissolved organic carbon (DOC) and UV absorbance at 254 nm were assessed at the end of each test. The removal of organic carbon and the reduction in absorbance at 254 nm were expressed as percentages and calculated as the difference between initial and final values divided by the initial values. The initial value refers to the humic acid solution with 10 mg/L of carbon.

The assays were performed in triplicate at room temperature. Samples were taken along time and the absorbance was measured at wavelengths of 254 nm, 280 nm, 400 nm, 465 nm and 665 nm [24]. The absorbencies at 254 and 280 nm are related to the aromatic content of humic substances; absorbance at 400 nm is related to the colour [24] and the ratio of absorbance at 465 nm/665 nm suggests the degree of condensation of the aromatic carbon network [24]. The TOC and DOC were determined at the beginning and end of the Jar tests by sample combustion and infrared carbon dioxide detection (5310 B) according to *Standard Methods* using a Rosemount Analytical, Dohrmann DC-190.

2.3. Zeta potential

The zeta potentials of the humic acid (10 mg/L of carbon), kaolin (0.5 g/L) and extract (700 mg/L protein) were determined using a Malvern Zetasizer instrument equipped with the zeta potential cell DTS1060 at 20 °C. At the end of the Jar test, a sample was taken to assess the zeta potential of the mixture extract (70 mg/L) plus humic acid (9 mg/L of carbon). Zeta potential values were derived from the electrophoretic mobility using the Smoluchowski approximation [25].

3. Results and discussion

3.1. Coagulation assays

Coagulation assays of a humic acid solution (9 mg/L carbon) with protein preparations from *M. oleifera* seeds

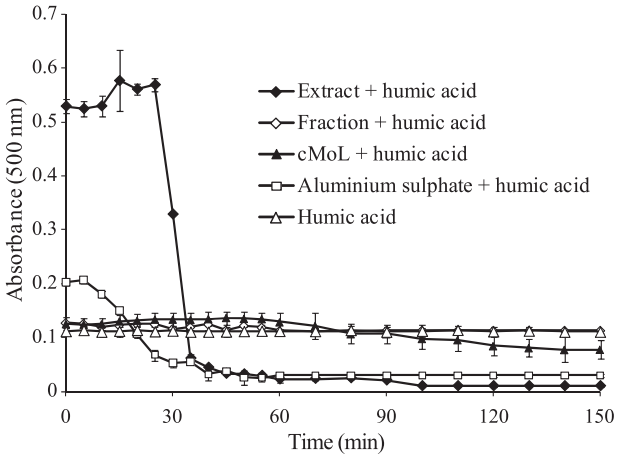

Figure 1. Absorbance measured (500 nm) along time during a coagulation assay of a humic acid solution (9 mg/L carbon) with *M. oleifera* extract, fraction and cMoL (100 mg/L protein) and aluminium sulphate (5 mg/L). A negative control with only the humic acid was included. Values represent the mean of three assays (± standard deviation).

(extract, fraction and cMoL) were performed first with a small volume of extract (3 mL) and then with higher volumes (40 mL) using the well-known Jar test method. The humic acid concentration used in the experiments was in the range of those found in surface waters (0.1 mg/L to 20 mg/L) [26]. UV-visible spectroscopy and zeta potential measurements were used to monitor coagulation activity.

The results from the coagulation assays done with a small volume of extract at pH 7.0 showed that the addition of the extract (100 mg/L protein) to a humic acid solution (9 mg/L carbon) induced a higher reduction in the absorbance at 500 nm than the use of similar concentrations of fraction and cMoL (Figure 1). The coagulation activity of the extract was higher than that of the aluminium sulphate (5 mg/L), 98% and 85%, respectively. The effect of pH, kaolin concentration and coagulant concentration on coagulation activity was studied only for the extract because it presented the highest coagulation activity and is the least expensive of the three protein preparations.

The pH corresponding to the maximum coagulation activity with an extract concentration of 180 mg/L protein was 7.0 (Figure 2a); a sharper decrease in activity was observed for higher and lower pH values. A natural macromolecular coagulant obtained from *C. latiforia* presented the lowest coagulant activity at pH 6.0 and the highest at pH 10.0 [12]. In this respect, the use of the extract from *M. oleifera* is more advantageous because its optimum pH is compatible with the one observed in natural systems. Kaolin has been used in research to simulate turbidity in water. The kaolin concentration used in the current experiments simulated a low turbidity water [27]. The present study suggested that kaolin at a concentration of 0.90 g/L had a coagulant activity comparable to that of the extract (180 mg/L) and aluminium sulphate (5 mg/L) (Figure 2b).

The coagulation activity at pH 7.0 as a function of extract concentration was high (>90%) for low concentrations of the extract (1.90 mg/L protein); increasing the extract concentration did not increase coagulation activity (Figure 2c). On the contrary, for a very high concentration of the extract (280 mg/L protein), the coagulation activity was considerably reduced in comparison to the one obtained at the lowest concentration of the extract (1 mg/L protein). This result is explained by the formation of a stable suspension that did not settle, conferring a high turbidity to the water. For very low concentrations of the extract (0.1 mg/L and 0.5 mg/L protein) coagulant activity was not observed.

Jar-test assays done with the extract (1 mg/L) and aluminium sulphate (5 mg/L) showed that both reduced the TOC, DOC and UV absorbance at 254 nm of a humic acid solution (9 mg/L carbon) in the pH range assayed, though the reduction was significantly higher with aluminium sulphate (Table 1). The use of a higher concentration of the extract, 70 mg/L, entailed the addition of organic carbon (increase in TOC and DOC) which was detrimental to water quality. In the pH range tested, significant differences were not observed in the removal of TOC, DOC and UV absorbance at 254 nm. Earlier studies have shown that the use of *M. oleifera* does not significantly alter the water pH after treatment [9]. In the presence of both kaolin and extract (1 mg/L) a higher reduction of TOC, DOC, and UV absorbance at 254 nm was observed (Table 1). The dose of kaolin that presented the best results was 0.9 g/L. Thus, kaolin is not a suitable compound to simulate turbidity in water because it also acts as a coagulant agent for humic acids.

The Jar-test results for coagulation of humic acid solution (9 mg/L carbon) with extract (70 mg/L) as a function of pH and kaolin concentrations were characterized by UV-visible spectroscopy at several wavelengths and the results are presented in Table 2. Humic acid solutions treated with the extract showed the lowest aromatic content and colour at pH 7.0 and in the presence of 0.9 g/L kaolin. The highest 465 nm/665 nm ratio was obtained for the extract at pH 7.0 and with 0.23 g/L kaolin. A low 465 nm/665 nm ratio is thought to reflect a high degree of condensation of aromatic constituents, whereas a high 465 nm/665 nm ratio infers a low degree of aromatic condensation and the presence of relatively large proportions of aliphatic structures [24].

3.2. Zeta potential

Zeta potential was used to study the interaction between humic acid (10 m/L carbon), extract (700 mg/L) and kaolin (0.5 g/L) in the pH range 5.0 to 8.0, as depicted in Figure 3. The zeta potential of the humic acid (10 mg/L carbon) was considerably more negative (−40 mV to −42 mV) than those of the extract (−10 mV to −15 mV) and the kaolin suspension (−2 mV to −7 mV). The humic acid–extract mixture presented a zeta potential slightly more positive

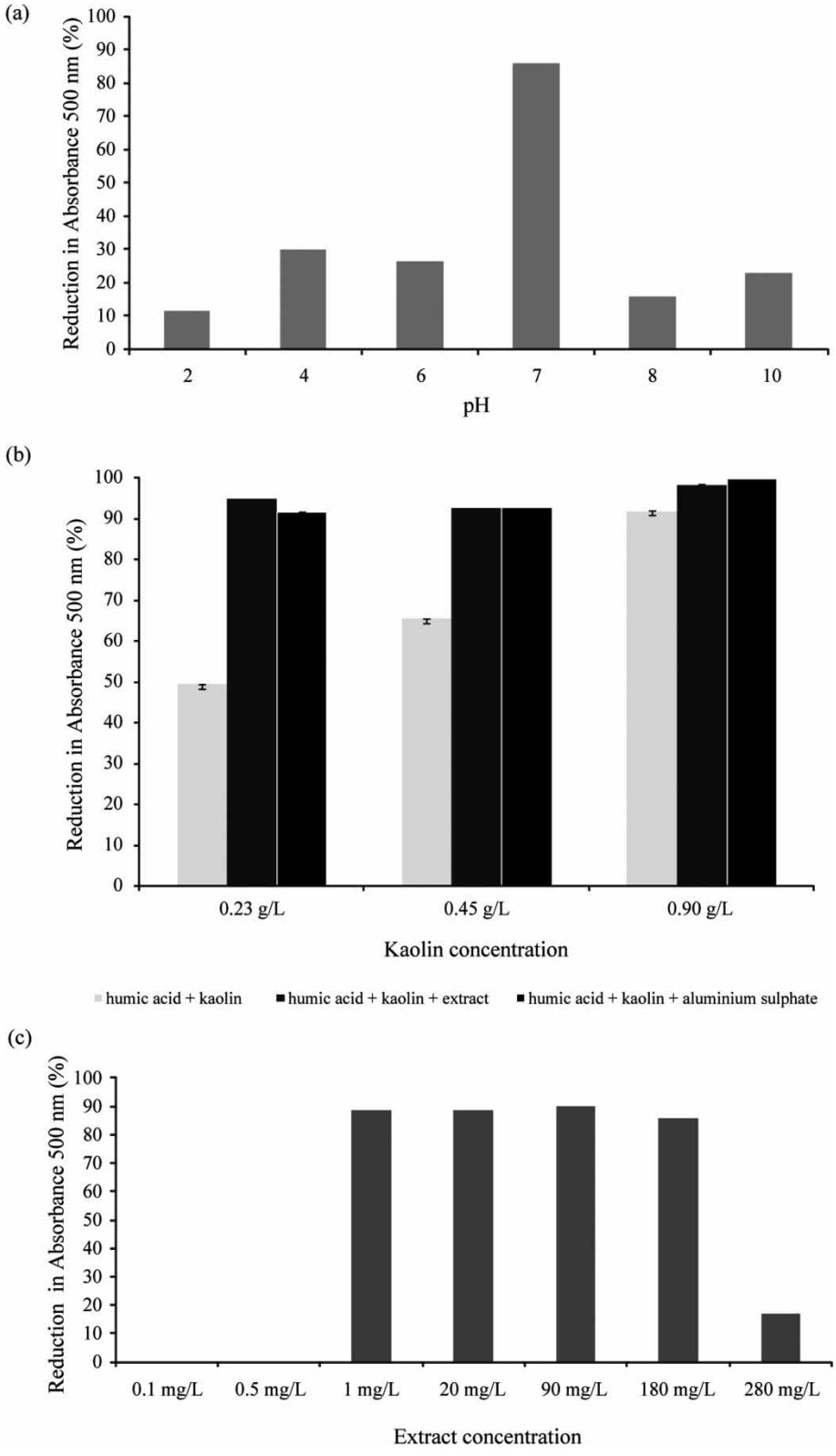

Figure 2. Percentage reduction in absorbance (500 nm) of the humic acid (9 mg/L carbon) as a function of (a) pH for an extract concentration of 180 mg/L, (b) kaolin concentrations for an extract concentration of 180 mg/L and pH 7.0, (c) different extract concentrations at pH 7.0. Values represent the mean of three assays (± standard deviation).

Table 1. Jar-test results for coagulation of a humic acid (9 mg/L) at different pH values and kaolin concentrations with extract from *M. oleifera* seeds.

	Treatment	TOC removal (%)	DOC removal (%)	Removal of UV absorbance at 254 nm (%)
pH 5	Extract 1 mg/L	31	30	8
	Extract 70 mg/L	0	0	1
	Aluminium sulphate 5 mg/L	61	73	85
pH 6	Extract 1 mg/L	25	18	14
	Extract 70 mg/L	0	0	5
	Aluminium sulphate 5 mg/L	52	73	74
pH 7	Extract 1 mg/L	25	18	14
	Extract 70 mg/L	0	0	14
	Aluminium sulphate 5 mg/L	63	72	76
pH 8	Extract 1 mg/L	31	38	12
	Extract 70 mg/L	0	0	31
	Aluminium sulphate 5 mg/L	64	80	87
Kaolin 0.23 g/L	Extract 1 mg/L	39	36	25
	Extract 70 mg/L	0	0	44
	Aluminium sulphate 5 mg/L	92	67	83
Kaolin 0.45 g/L	Extract 1 mg/L	43	57	60
	Extract 70 mg/L	24	0	75
	Aluminium sulphate 5 mg/L	82	98	75
Kaolin 0.90 g/L	Extract 1 mg/L	80	70	85
	Extract 70 mg/L	15	0	85
	Aluminium sulphate 5 mg/L	100	100	85

Table 2. UV-visible absorbance intensities of a humic acid solution (9 mg/L) and after the Jar test for coagulation of humic acid with extract (70 mg/L) at different pH values and kaolin concentrations.

		UV-visible absorbance			
		254 nm	280 nm	400 nm	465 nm/665 nm
pH 5	Humic acid	0.511	0.407	0.119	6.600
	Extract + humic acid	0.507	0.239	0.031	2.125
pH 6	Humic acid	0.470	0.386	0.108	6.100
	Extract + humic acid	0.447	0.086	0.015	–
pH 7	Humic acid	0.447	0.380	0.106	6.666
	Extract + humic acid	0.382	0.072	0.013	2.500
pH 8	Humic acid	0.556	0.463	0.139	4.050
	Extract + humic acid	0.385	0.296	0.040	1.647
Kaolin 0.23 g/L	Humic acid	0.189	0.285	0.095	4.727
	Extract + humic acid	0.248	0.145	0.039	4.400
Kaolin 0.45 g/L	Humic acid	0.161	0.129	0.036	2.000
	Extract + humic acid	0.112	0.080	0.015	1.250
Kaolin 0.90 g/L	Humic acid	0.084	0.067	0.012	1.000
	Extract + humic acid	0.066	0.040	0.003	0.500

than the one of the extract alone in the pH range studied. The zeta potential of both extract and extract–humic acid suspensions ranged between +20 and −20 mV, and were characteristic of unstable suspensions. In contrast, the humic acid solution presented a zeta potential more negative than −20 mV in the pH range studied and can be considered stable [28]. Based on the significantly different zeta potential values of the humic acid and the extract, the mechanism that might be involved in this coagulation activity is adsorption and neutralization of charges [9].

The results obtained in the present work suggest that in the presence of the humic acid the extract formed flocs that settled well, as was assessed in the coagulation activity essay and inferred from the zeta potential values. However, the removal of the humic acid by adsorption on the surface

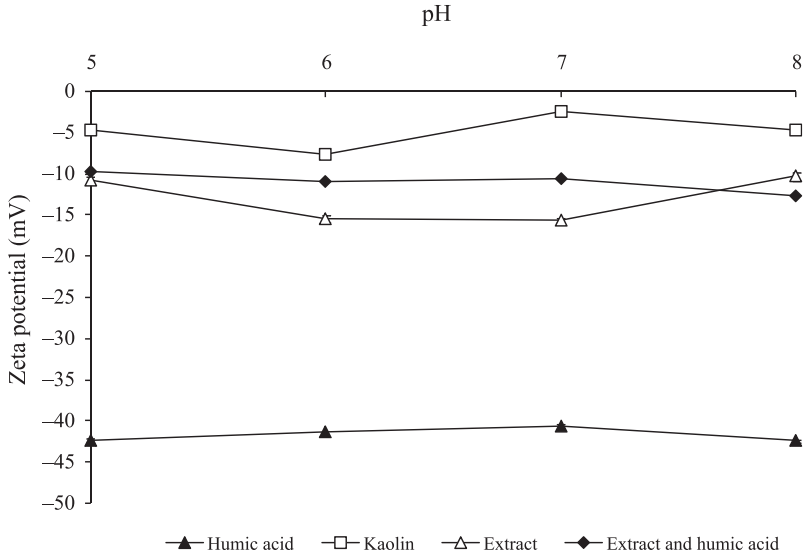

Figure 3. Zeta potential versus pH values for humic acid (10 mg/L carbon), kaolin (0.5 g/L), extract (700 mg/L protein) and humic acid.extract solution collected at the end of the Jar-test assays. Values represent the mean of three assays (± standard deviation).

of the flocs was not very high, between 13% and 38% of the DOC, as demonstrated in the Jar test.

4. Conclusions

The extract, a protein preparation obtained from *M. oleifera* seeds, at a concentration of 1 mg/L was able to remove total and dissolved organic carbon and to reduce the aromatic content and colour of water with a humic acid content of 9 mg/L carbon in a pH range between 5.0 and 8.0.

The use of the extract from *M. oleifera* seeds to coagulate humic acids from water in developing countries can be an interesting natural alternative to traditional methods, for example the application of aluminium sulphate. The dose of extract recommended in the present study, 1 mg/L, does not impart colour or odour to the treated water.

Acknowledgements

The authors acknowledge the financial support from the Portuguese Fundacao para Ciencia e a Tecnologia (FCT), through the post-doctoral grant SFRH/BPD/37349/2007, and the Brazilian programmes: Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), VALNATURA ALFA and the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES).

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