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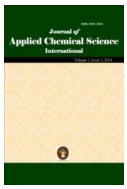

CHEMICAL AND MINERALOGICAL CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY EVALUATION OF SENEGAL'S MUDS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author MBD initiated the research theme and conducted mainly the mudflat analysis. Authors RSG, ANS, MS and GYS designed and conducted the study of the antiinflammatory activity. Authors RSG, MB, CS, MS, AD, DF and AW managed the litterature search, designed and conducted the attapulgite analysis. Author IB collected the samples. Author YN advised on ethical issues, a part of the translation and proofreading. All authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

Clays have been use in therapeutics for a long time and proliferation of thermal mud centers testifies to its effectiveness. In our work, anti-inflammatory activity of Ziguinchor mangrove sludge and Mbodiène attapulgite was evaluated, after a chemical and mineralogical characterization. Results obtained showed for mangrove mud multiple components with mainly varying contents in sodium, aluminum, chlorine, silicon and iron. Attapulgite was predominantly composed of silicon 55.15%, magnesium 13.8%, calcium 11.11%, iron 8.3% and aluminum 7.78%. The cation exchange capacity evaluated with the cobaltihexamine ion was 95 meq / 100 g for Attapulgite versus 6.85 meq / 100 g for the mangrove mud. Mineralogical analysis has shown that mangrove mud consists essentially of kaolinite and palygorskite is the dominant mineral of attapulgite with quartz, crystals of ankerites, dolomites. Anti-inflammatory activity evaluation showed a percentage of inhibition of edema induced in croton oil from 25.23% for attapulgite, to 28.34% for Ziguinchor mud, against 52, 26% for Voltaren Emulgel® as

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reference. These results predict a good way of additional treatment at lower cost for pathologies such as osteoarthritis.

Keywords: Clays; mudflat; attapulgite; antiinflammatory activity.

1. INTRODUCTION

Since a long time, clays constituted important materials in construction of dwellings, utensils or decorative objects, and treatment of certain affections. Mixed with water, clay becomes soft, malleable, shapeable and adherent to the skin [1]. Numerous studies have proven multiple properties of clays [2–8], including pharmaceuticals properties [6,7,9–13]. Indeed, anti-inflammatory, antibacterial, antioxidant and even cytotoxic activities have been proven [14–19].

All of the above activities justifies use of clays as thermal mud in neurological, rheumatological and dermatological conditions.

In this study, the anti-inflammatory activity of muds from a mudflat in Ziguinchor, Casamance (Senegal) has been evaluated. Indeed in these places named "faro", women collect oysters noted their joint pain disappearance, after such activity. In Africa, studies have shown a prevalence of arthritic diseases in women. Obesity is also a contributing factor and is unfortunately a beauty criterion among women in these countries [20–22]. The evaluation of the anti-inflammatory effect of attapulgite from Mbodiene (Senegal) was also carried out. Senegal is a world producer of attapulgite and yet its local valorization remains weak mainly its export for use as animal litter [6].

2. MATERIALS AND METHODOLOGY

2.1 Materials

Muds from Casamance were taken from a "faro" located at the northern entrance of Ziguinchor and the attapulgite from Mbodiene was obtained at Sen-Mines, a private company operating on site.

2.2 Reagents and Apparatus

All reagents used were compliant for analysis. This study required the following devices:

- Hitachi S4700 scanning electron microscope with a field emission gun (FEG), equipped with an EDS microanalysis, a cathodoluminescence detector ranging from ultraviolet to infrared, and a JEOL JSM-IT300 LA EDS variable pressure;
- BRUKER D2 PHASER diffractometer with monochromatic radiation type Kα line of copper.

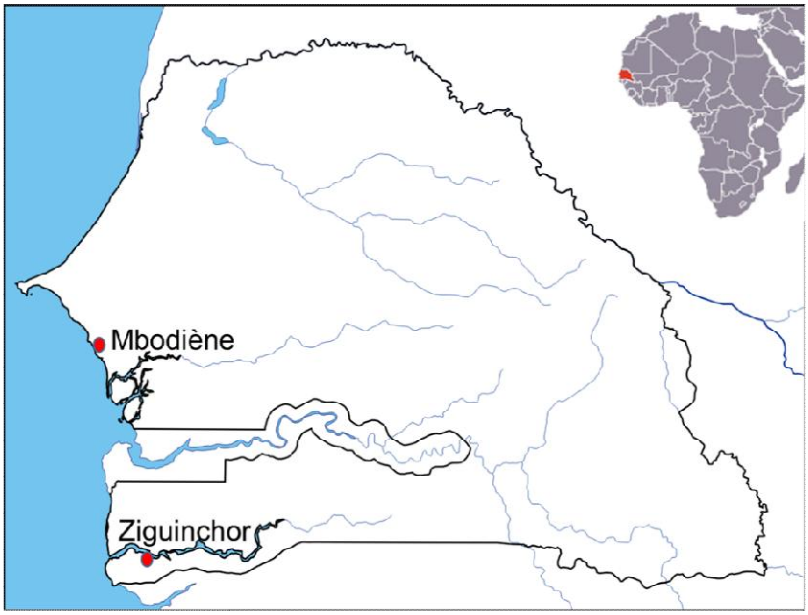

Fig. 1. Sampling of attapulgite and mudflats from Mbodiene and Ziguinchor

2.3 Methodology

- **Physicochemical and mineralogical characterization**

Physicochemical and mineralogical analyze were carried out on clay and attapulgite samples, dried in an oven at 60°C for 48 hours.

- **Anti-inflammatory activity evaluation**

Anti-inflammatory activity evaluation was carried out by using 10 g of each sample mixed by trituration in a porcelain mortar, containing 10 ml of ethanol. The resulting products were then stored in tinted glass vials and were used extemporaneously for anti-inflammatory activity evaluation.

Analysis method derived from Tubaro et al. [23], consists in anti-inflammatory extract of an edema inhibition, caused by local application of an alcoholic solution of croton oil on the ears of mice. For each experimentation, the mice were divided into 5 groups of 5 each, and then weighed, labeled, and deprived of food for 16 hours before the experiment began.

The so-called "simultaneous" (curative) method, where the croton oil and anti-inflammatory extract were applied at the same time, was used.

Mice in the control group were treated locally on the right ear with 10 µl of an alcoholic solution of 1% croton oil.

For mice in the treated groups, 10 µl of the alcoholic solution of 1% croton oil and 10 µl of the test product are applied to the right ear. On the left ear, only 10 µl of the product to be tested is applied.

Finally, regarding the reference group, 10 µl of the alcoholic solution of 1% croton oil and 10 µl of a reference product are applied to the right ear, while on the left ear, 10 µl of the same reference product is applied. A commercial medicinal product based on diclofenac, known as Voltarene Emulgel® ointment, was used as a reference product.

After 6 hours, the mice in the treated groups are anesthetized with ethyl ether. Their ears were then immediately cut along the cartilage, and then weighed with a precision balance. Those mice were subsequently sacrificed.

Evaluation of induced edema was calculated according to the percentage of increase of the right ear's weight (% Incr RE) with the following formula:

$$\% \text{ Incr RE} = \frac{\text{Weight RE} - \text{Weight LE}}{\text{Weight LE}} \text{ X } 100$$

This percentage expresses the intensity of the inflammation induced on the right ear. The average percentage increase was calculated for each group. Anti-inflammatory activity evaluation was obtained by calculating the percentage inhibition of the edema (% Inh edema) relative to the control batch according to the following formula:

$$\% \text{ Inh edema} = \frac{\text{Avg (\%) Incr control} - \text{Avg (\%) Incr treated}}{\text{Avg (\%) Incr control}} \text{ X } 100$$

This percentage expresses the power of inhibition of the edema by the tested substance.

3. RESULTS AND DISCUSSION

3.1 Chemical and Mineralogical Characterization

- **Ziguinchor Mudflat**

Since the mudflat consisted of a mixture of various substances, a physicochemical elementary analysis as representative as possible was carried out. Thus, each SEM-inferred substance is correlated with an elemental analysis (Tables 1 to 11 and Figs. 2 to 12).

Table 1. Elementary analysis of SEM picture 2

Element	% Mass	% Atomic
O	63.30	79.28
Al	1.13	0.84
Si	1.58	1.12
S	14.53	9.08
Ca	19.06	9.53
Fe	0.40	0.14
Total	100.00	

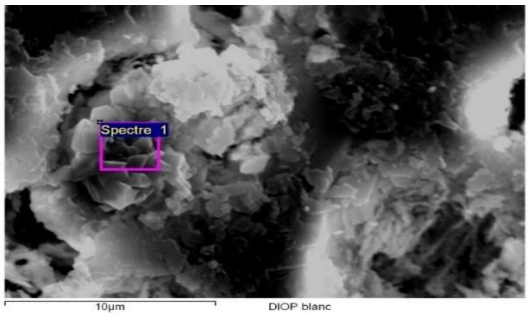

Fig. 2. SEM picture of component of mud

The results showed that the mudflat consists of a mixture of various substances, the most important were calcium, sodium, chlorine, aluminium, silicon, iron, sulfur and magnesium. The Fig. 2 consisted by component with mainly calcium, 19.06% and sulfur 14.53% (Table 1). The Fig. 3 showed component with chlorine (44.24%) and sodium (38.68%) mainly, the calcium was present (8.57%) (Table 2). The component visualized on Fig. 4 contained mainly calcium (24.99%), sodium, magnesium, chlorine and aluminium were in traces (Table 3). The component on Fig. 5 had chlorine (44.08%) and sodium (35.34%) like majoritaries elements, the others, calcium, aluminium, silicium were in weak proportions (Table 4). The component on Fig. 6 contained only calcium (29.73%) and aluminium (0.36%) (Table 5). On Fig. 7, the component had mainly chlorine (49.14%) and sodium (40.64%) in almost similar proportions (Table 6). On Fig. 8, only chlorine (93.50%) and sodium (6.50%) were present (Table 7). The component visualized on Fig. 9 contained mainly chlorine (22.53%), sodium (19.23%), silicium, aluminium, magnesium, sulfur were in weak proportions, calcium and iron like traces were noted (Table 8). On Fig. 10, the component had mainly chlorine (50.89%) and sodium (32.66%) (Table 9). On Fig. 11, sulfur (22.48%), iron (18.51%) and calcium (10.98%) were in significant proportions, silicium and aluminium were traces (Table 10). Endly, on Fig. 12, the component contained sulfur (12.01%), silicium (11.72%), iron (9.70%) like majoritaries elements, calcium and iron were in weak proportions (Table 11).

Table 2. Elementary analysis of SEM picture 3

Element	% Mass	% Atomic
O	7.92	13.53
Na	38.68	45.96
Si	0.59	0.58
Cl	44.24	34.09
Ca	8.57	5.84
Total	100.00	

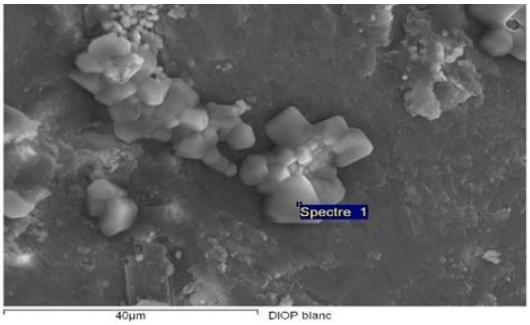

Fig. 3. SEM picture of component of mud

Analyzes of mass variations or T.G.A and differential thermal analysis or D.T.A have revealed that between 0°C and 400°C, successive losses of hydration water or zeolite, of crystallization water, and of constitution water, have been observed. From 400°C, decomposition of the main mineral, kaolinite, was observed. Indeed, the diffraction pattern of the mud specimen confirmed the presence of kaolinite, $Al_2Si_2O_5(OH)_4$.

Table 3. Elementary analysis of SEM picture 4

Element	% Mass	% Atomic
O	73.87	87.36
Na	0.50	0.41
Mg	0.22	0.17
Al	0.20	0.14
Cl	0.21	0.11
Ca	24.99	11.80
Total	100.00	

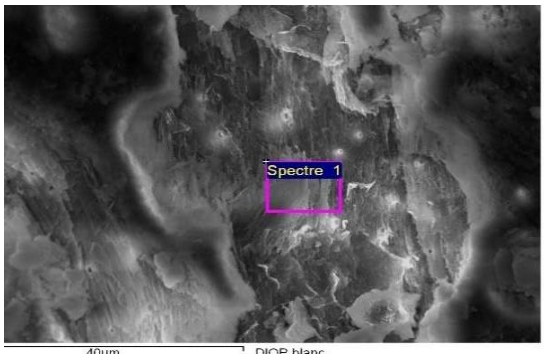

Fig. 4. SEM picture of component of mud

Table 4. Elementary analysis of SEM picture 5

Element	% Mass	% Atomic
O	17.04	26.89
Na	35.34	38.81
Al	1.07	1.00
Si	1.29	1.16
Cl	44.08	31.39
Ca	1.18	0.74
Total	100.00	

The mass variation or thermogravimetric analysis (T.G.A) curve showed endothermic hooks between 0°C and 150°C and between 400°C and 550°C, while differential thermal analysis (D.T.A) showed endothermic peaks between 50°C and 140°C, around 150°C, between 200°C and 270°C and between 400°C and 550°C (Fig. 13).

The mineralogical analysis carried out using the diffractometer showed peaks at 20°, 26° and 31°

which confirmed that the Ziguinchor mudflat consists essentially of kaolinite (Fig. 14).

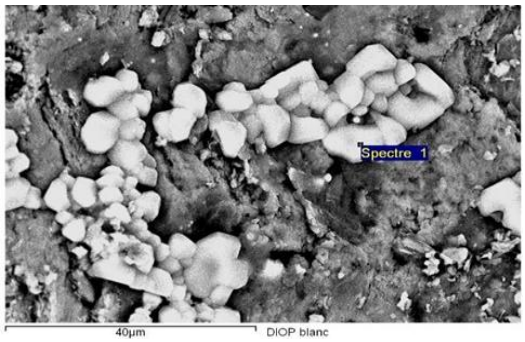

Fig. 5. SEM picture of component of mud

Table 5. Elementary analysis of SEM picture 6

Element	%Mass	%Atomic
O	69.91	85.26
Al	0.36	0.26
Ca	29.73	14.47
Total	100.00	

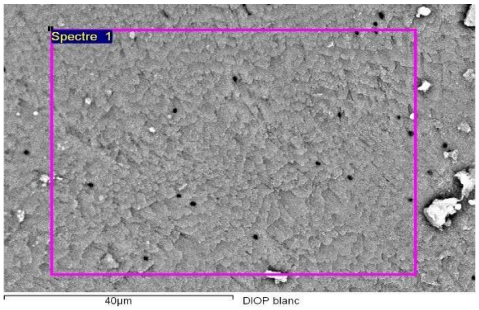

Fig. 6. SEM picture of component of mud

The specific surface area was found to be 87.20 $m^2.g^{-1}$ by the methylene blue method. The cation exchange capacity found using the cobaltihexamine ion method was 6.85 meq/100 g of material. This is consistent with data for kaolinite which has a cation exchange capacity of between 3 and 15 meq/100 g [24,25].

- **Mbodiene Attapulgite**

Regarding the Mbodiene attapulgite, the scanning electron microscope (SEM) images of the plates of the raw attapulgite (Figs. 15, 16) showed a fibrous structure and the presence of ankerite cubes, i.e. calcium carbonates, magnesium, and iron. Other studies have also proven this fibrous structure [14,15,26–28]. The elemental composition determined by X-ray fluorescence spectrometry (Table 12) gave 55.15% of silica, 7.78% of alumina, 8.3% of ferric oxide, 11.11% of calcium oxide, and 13.8% of magnesium oxide. However, the major components are the same and are consistent with the literature [3,5,6]. Therefore, Mbodiene's attapulgite used in this study is a fibrous clay, a hydrated aluminum-magnesium silicate whose formula is $Si_8(Mg_2Al_2)O_{20}(OH)_2(H_2O)_4,4H_2O$.

Table 6. Elementary analysis of SEM picture 7

Element	%Mass	%Atomic
O	10.22	16.84
Na	40.64	46.61
Cl	49.14	36.55
Total	100.00	

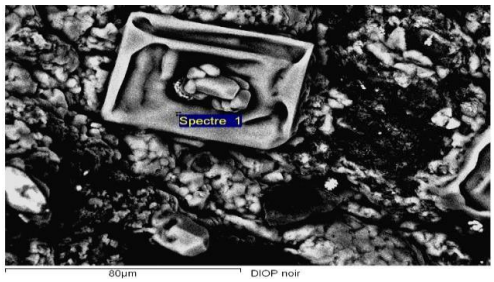

Fig. 7. SEM picture of component of mud

Table 7. Elementary analysis of SEM picture 8

Element	%Mass	%Atomic
Na	6.50	9.68
Cl	93.50	90.32
Total	100.00	

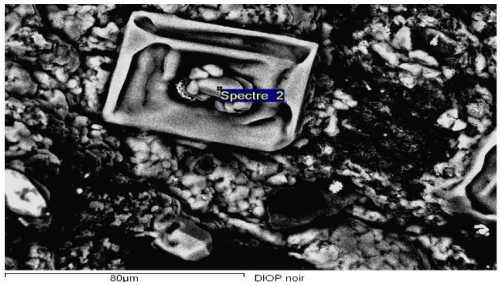

Fig. 8. SEM picture of component of mud

The T.G.A plot (Fig. 17) showed only endothermic peaks. The first endothermic plateau at about 150°C corresponded initially to the hydration water or zeolite. At about 300°C, the loss of the crystallization water took place, and then towards 500°C, the disappearance of the water of constitution, that is to say dehydroxylation, took place, and finally at around 750°C, we have the decomposition of the mineral.

Table 8. Elementary analysis of SEM picture 9

Element	%Mass	%Atomic
O	46.37	60.65
Na	19.23	17.50
Mg	1.65	1.42
Al	2.87	2.23
Si	4.43	3.30
S	1.50	0.98
Cl	22.53	13.30
Ca	0.62	0.32
Fe	0.80	0.30
Total	100.00	

Fig. 9. SEM picture of component of mud

Table 9. Elementary analysis of SEM picture 10

Element	%Mass	%Atomic
O	16.45	26.48
Na	32.66	36.57
Cl	50.89	36.96
Total	100.00	

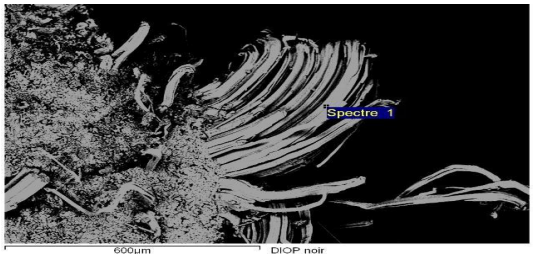

Fig. 10. SEM picture of component of mud

The D.T.A plot (Fig. 17) showed endothermic peaks between 50°C and 100°C, between 250°C and 300°C, and around 450°C, which corresponded respectively to the departures of the zeolite water, the crystallization water, and the constitution water. Decarbonation would happen around 650°C, quartz decomposition around 750°C, and finally palygorskite decomposition around 850°C.

Table 10. Elementary analysis of SEM picture 11

Element	%Mass	%Atomic
O	43.54	64.94
Al	1.77	1.57
Si	2.72	2.31
S	22.48	16.73
Ca	10.98	6.54
Fe	18.51	7.91
Total	100.00	

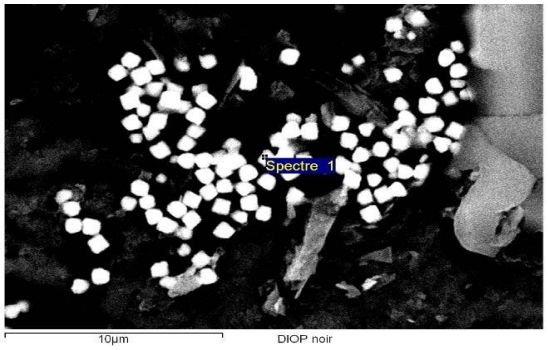

Fig. 11. SEM picture of component of mud

Table 11. Elementary analysis of SEM picture 12

Element	%Mass	%Atomic
O	58.92	75.39
Al	3.75	2.84
Si	11.72	8.54
S	12.01	7.67
Ca	3.91	2.00
Fe	9.70	3.55
Total	100.00	

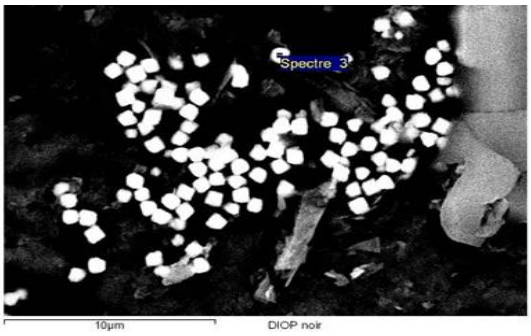

Fig. 12. SEM picture of component of mud

The diffractograms of the raw attapulgite (Fig. 18) showed a majority presence of palygorskite (82%), quartz and ankerite. The study of the clay fraction of

the Mbodiene attapulgite revealed 15% of smectite and 3% interstratified [29].

The methylene blue method made it possible to find a specific surface area of the order of 94.18 $m^2.g^{-1}$. The determination of the cation exchange capacity of the raw palygorskite with a solution of 0.05N cobaltihexamine was 95 meq/100 g of material.

3.2 Antiinflammatory Activity Evaluation

Evaluation of the anti-inflammatory activity (Table 13) of the Ziguinchor mudflat and Mbodiène attapulgite by the croton oil-induced edema protocol gave respective inhibition percentages of 28.34 and 25.23. This indicates a similar activity of mudflat compared to attapulgite, although less than that of the reference product, Voltarene Emulgel®. This anti-inflammatory activity of the mudflat was already observed by women collecting oysters in these mudflats.

However, the mode of action of kaolinite is still unclear due to its low cation exchange capacity and surface area, its 1:1 monolayer structure, which makes it a preferred excipient for delay administration or for targeted action [25]. It is especially curious that kaolin-carrageenan mixture is often used to induce pain and inflammation in joints, in the protocols for studying these phenomena [30–32].

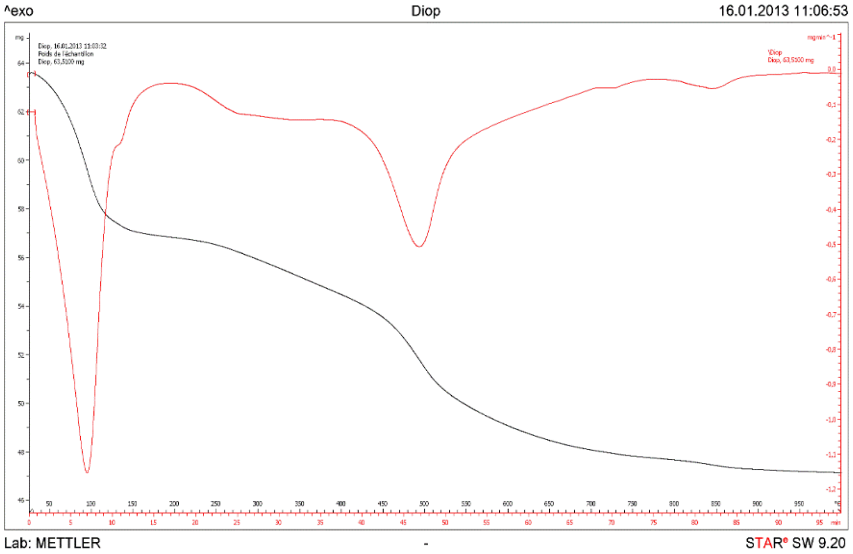

Fig. 13. T.G.A and D.T.A plots of the Ziguinchor mudflat

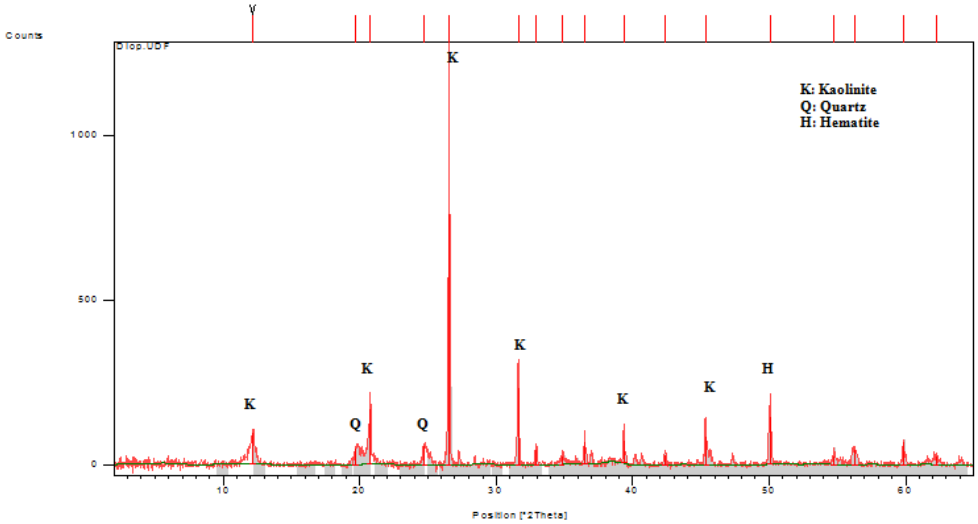

Fig. 14. Diffractogram of the Ziguinchor mudflat

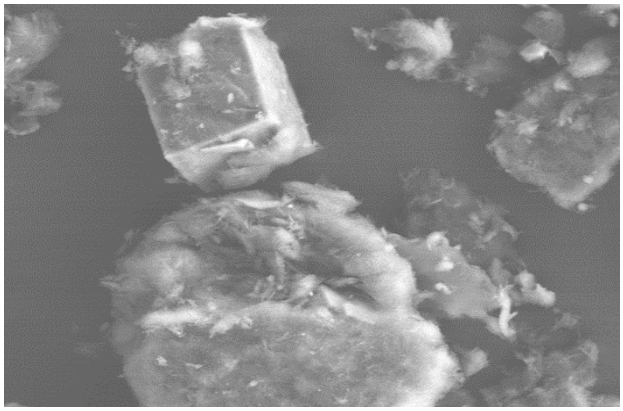

Fig. 15. SEM picture Mbodiène attapulgite with ankerite cubes

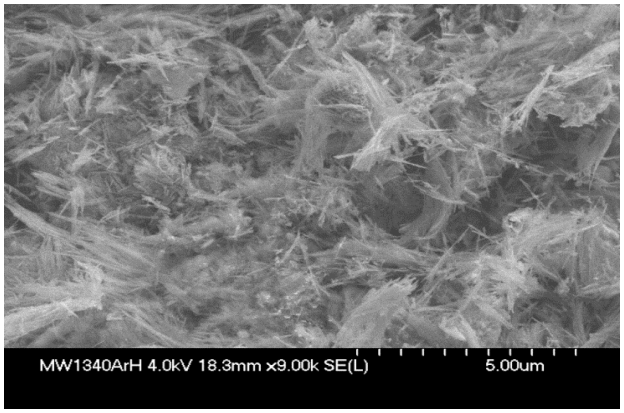

Fig. 16. SEM picture of raw Mbodiène attapulgite

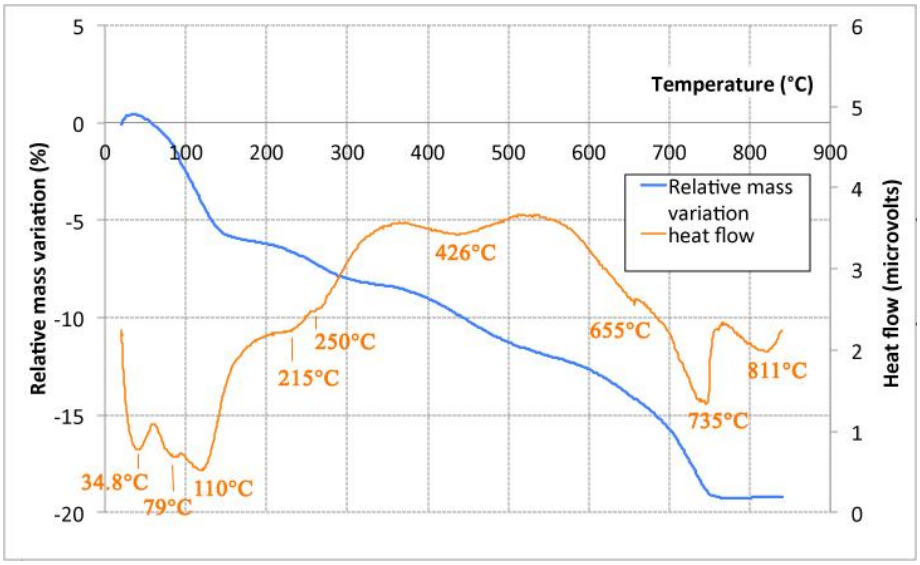

Fig. 17. T.G.A and D.T.A plots of the Mbodiene attapulgite sample

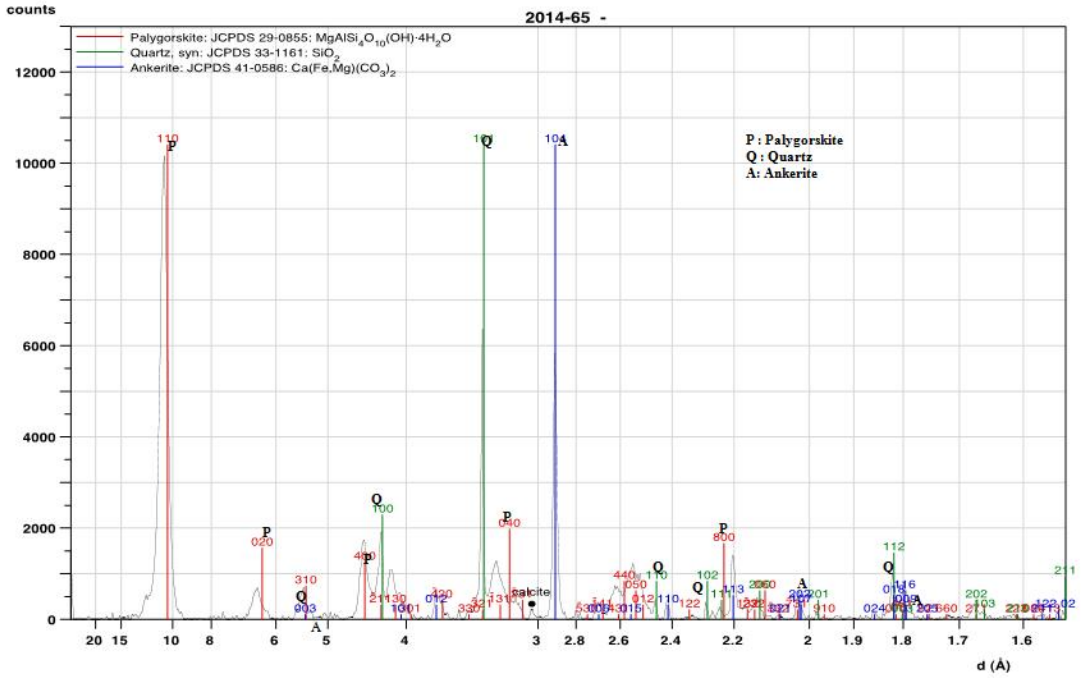

Fig. 18. Diffractogramm of the raw attapulgite sample from Mbodiène

Table 12. Chemical composition of Mbodiène attapulgite sample by X-Ray fluorescence spectrometry

Constituent	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	TiO_2	P_2O_5	K_2O	SO_3	Cl
Content (%)	55,15	7,78	8,3	11,11	13,80	0,99	0,78	0,97	0,35	0,33

The presence of hydroxyl groups in kaolinite and palygorskite could explain the anti-inflammatory activity of the mudflat and attapulgite.

Table 13. Results of the anti-inflammatory activity evaluation

Group	% Incr RE	SEM	% Inh edema
Control	52,55	3,75	-
Reference	25,08	8,15	52,26
Treated mud	37,65	3,35	28,34
Treated attapulgite	39,29	7,17	25,23

Percentage of increase of the right ear's weight (% Incr RE) inhibition of the edema (% Inh edema) SEM: Standard Error Mean

4. CONCLUSION

Clays have been of interest for a very long time and with the elucidation of their structures, chemical compositions, and properties, their uses have multiplied.

The chemical characterization the muds of Ziguinchor and the attapulgite of Mbodiène showed many important elements for life like calcium, iron, sodium, chlorine, magnesium. The mineralogic study found respectively for the samples kaolinite and palygorskite as the dominant minerals.

The results of the evaluation of the antiinflammatory activity by the croton oil-induced edema protocol showed an anti-inflammatory power of the mudflat and attapulgite with similar edema inhibitory percentages between the two.

According to this results, the use of clays such as mudflat and attapulgite could contribute to better management of rheumatic, neurological and dermatological conditions. Indeed, there is an increased interest for the use of natural therapies of lesser cost and side effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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