

Ecological consequence of high aluminium content in acidified estuarine waters: the case of tilapia fishes in lower Casamance (Senegal)

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Abstract

Different examples in the world show that fishes can not tolerate increasing acidity, especially if it is sudden. Aluminium toxicity is now well known. The Casamance estuary, which contains acid sulphate soils associated with strongly acid groundwaters and surface waters, was investigated. Microanalytical methods were used and allowed to detect aluminium in gills of typical fishes sampled in the upstream side of anti-salt dams. They belong to three different genera of the Cichlid family: *Tilapia guineensis*, *Sarotherodon melanotheron heudelotii* and *Hemichromis fasciatus*. Aluminium accumulation was proved on these very resistant fishes and the ecological problem is discussed.

Keywords: Aluminium, surface water, acid sulphate soils, tilapia fishes, microanalysis, acid rain, Casamance, West Africa.

Résumé

Différents exemples dans le monde montrent que les poissons ne supportent pas une augmentation d'acidité de l'eau, en particulier si elle est brusque. Le rôle toxique de l'aluminium présent dans ces eaux a été récemment reconnu. Nous nous sommes intéressés au cas de l'estuaire de la Casamance qui comporte des sols sulfatés acides en relation avec des eaux de nappe et de surface très acides. Des méthodes microanalytiques ont été utilisées et ont permis de détecter de l'aluminium dans les branchies de poissons typiques prélevés en amont de barrages anti-sel. Ils appartiennent à trois genres différents de la famille des Cichlidés : *Tilapia guineensis*, *Sarotherodon melanotheron heudelotii* et *Hemichromis fasciatus*. Ceci permet de mettre en évidence l'accumulation d'aluminium chez ces poissons exceptionnellement résistants et de préciser le problème écologique.

INTRODUCTION

Aluminium in the environment

The average aluminium content in the earth crust is 8.3%. This element is the third abundant element after oxygen (46.6%), and silicon (27.7%), and just before

iron (4.1%) (SALOMON & FÖRSTNER, 1984). In soil, the mean aluminium content is lower (7.2%) (SPOSITO, 1989). Chemical weathering of rocks contributes to the release of Al in soils, especially in the clay fraction.

In the seawater, its mean concentration is 0.01 mg/l, like iron, and Al is the 25th abundant element. In the continental waters, its concentration is dependent on pH values and highly variable. The mean content of this metal is 0.002% in vegetables, 0.00005% in terrestrial mammals (WEDEPOHL, 1970). And, in human blood, its mean is 0.02 mg/l (PERRIN & CERTAIN, 1984).

Aluminium toxicity

Since its origin, life has developed in an Al rich environment. But, this element is usually found as insoluble chemical forms. Under acidic conditions, the aluminium compounds become ionic, in a soluble or exchangeable form in soils (DUCHAUFOR, 1991).

Aluminium is found in very low concentration for the living creatures. Aluminium deficiency has never been recorded, but we know that an excessive level of aluminium can be toxic for vegetal or animal life in several cases. For man it can be a cause of disease in bone, liver, or brain (GALLE, 1986). Its toxicity is also proved for plants (THARWORNWONG & VAN DIEST, 1974; MOORE & PATRICK, in press) and fishes (GALLE *et al.*, 1990).

Examples of high aluminium concentration in water and their ecological consequences

CALLINAN *et al.* (in press) reported seasonally fish mortalities in several northern Australian estuaries. During the first floods, acid sulphate soils are leached and may induce a transient exposure to acid water for aquatic life. The fish mortalities are mainly attributed to the low pH and high aluminium concentration in water. Values of 3.6 and 2.5 mg per liter have been recorded. Severe troubles of ion exchange and respiratory systems are the probable cause of death in fish. Damages to skin and gills of fish were observed. A recent research has shown that an epizootic ulcerative syndrome is caused by an aquatic oomycete fungus belonging to the genus *Aphanomyces*.

In Malaysia, SIMPSON and PEDINI (1987) report a massive destruction of fishes and a lower development of shrimps in acid sulphate soils. Prawn ponds have rapidly expanded in brackish water mangrove areas. The excavation and the drainage of the acid sulphate soils develop strong acidity due to the pyrite oxidation under drying conditions. The leaching of acidity from the dikes of ponds resulted into the decrease of production.

In Philippines, the prawn and fish production is highly variable because of the use of manure and the leaching of acidity (SIMPSON & PEDINI, 1987). A technology scheme, proposed by SINGH *et al.* (1988), allows the reclamation of ponds and the improvement of yields.

GALLE *et al.* (1990) made a microanalytical study of the intoxication of trouts from acidified stream in the Vosges mountains (France). This acid pollution, associated with aluminium, originated from acid rainfall in low buffered soils.

Casamance example

Since 1970, the main effects of the drought in lower Casamance, especially in the mangrove environment have been:

- (a) decrease of the mangrove vegetation;
- (b) increase of the salinity of seawater, concentrated by evaporation;
- (c) chemical degradation of soils in lowlands;
- (d) decrease of rice production with development of farming systems on the uplands;
- (e) increase of the run-off erosion in the uplands;
- (f) migration of population towards cities (MONTOROI *et al.*, in press).

Natural oxidation of the mangrove pyritic sediments has extended into the mangrove area and the area of acid sulphate soils has increased. The production of sulfuric acid in soils leads to the hydrolysis of the clay fraction of soils and to the release of metal elements, such as aluminium, in the soil solution and in the groundwater (LE BRUSQ *et al.*, 1987). The continental soils (especially red and brownish soils) are also acid but less acid than the lower lands. Exchangeable aluminium is available and erosion can remove this element with the clay and the sand fractions towards the lower part of the watershed. The clay deposition in acid water can originate an increase of the concentration of aluminium (fig. 1).

The Senegalese government has planned increased food production and has promoted the reclamation of tidal lands for rice cultivation (VAN DER KLEI, 1988). Among the applied techniques of reclamation, the construction of anti-salt dams was carried out in several valleys. The main effect of these dams was to stop the intrusion of seawater, but they resulted into the acidification of the upstream lands in the dry season. During the rainy season, the floods from the watershed flushed and leached the soil before the entire flooding of the valley. The improvement of the water management allowed to decrease the salinity and the acidity of the upstream water with periodic releases of water (ALBERGEL *et al.*, 1991). However, water retained a high level of acidity (pH value of 3) and aluminium content (0.2 mmol per liter). Observations showed that fishes can grow in such conditions.

In West Africa, the tilapias are well known for their high ability to endure extreme ecological conditions. They can survive in saline waters with very high levels of salinity during the dry season (*Tilapia guineensis*, *Sarotherodon melanotheron heudelotii* can tolerate salinity up to 90 g/kg). They have a high fecundity rate and almost no predator, due to the high salinity of water (ALBARET, 1987).

This paper presents the results of different aluminium investigations in the tissues of fishes and tries to compare this phenomenon with the ecological consequences of acid rain in temperate regions.

MATERIAL AND METHODS

Water and fish sampling were carried out in different parts of lower Casamance (fig. 2).

Water sampling

The surface waters were sampled in the upstream and downstream sides of the anti-salt dams (Bignona and Djiguinoum valleys). Electrical conductivity and pH were immediately measured after

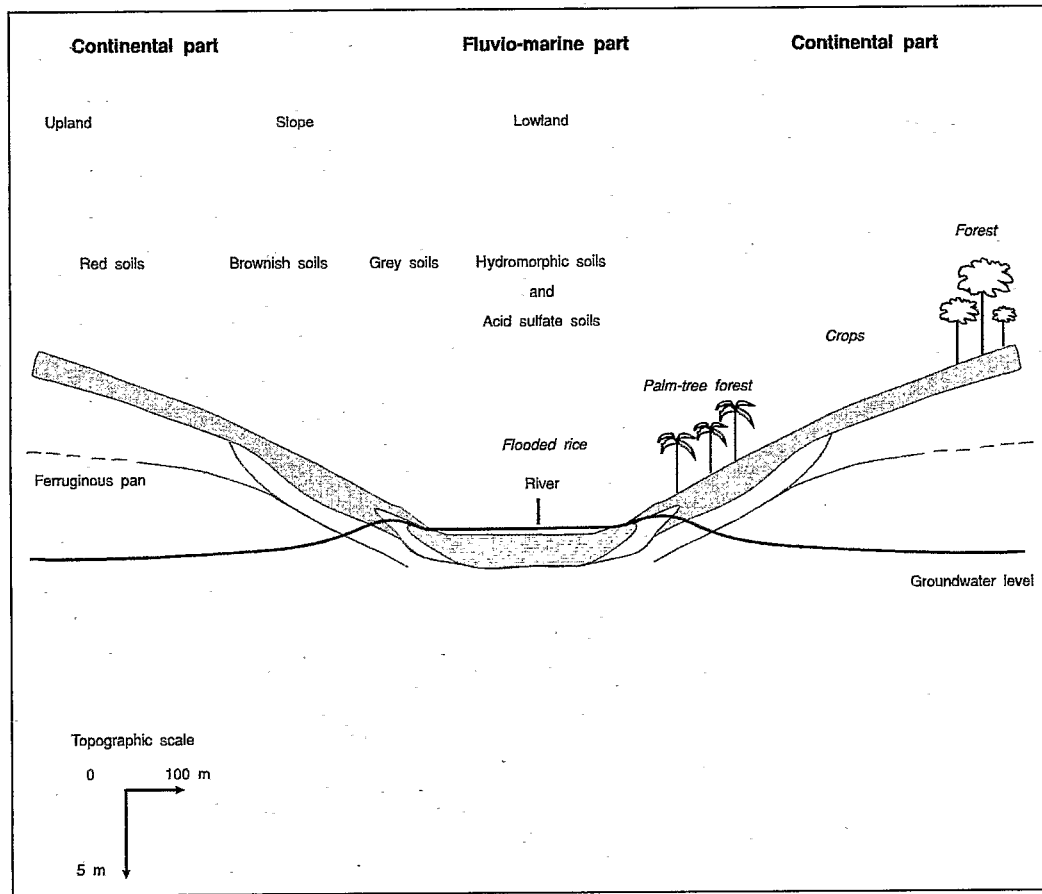


FIG. 1. – Transversal section of Djigounou valley with the soil distribution and the vegetation cover.

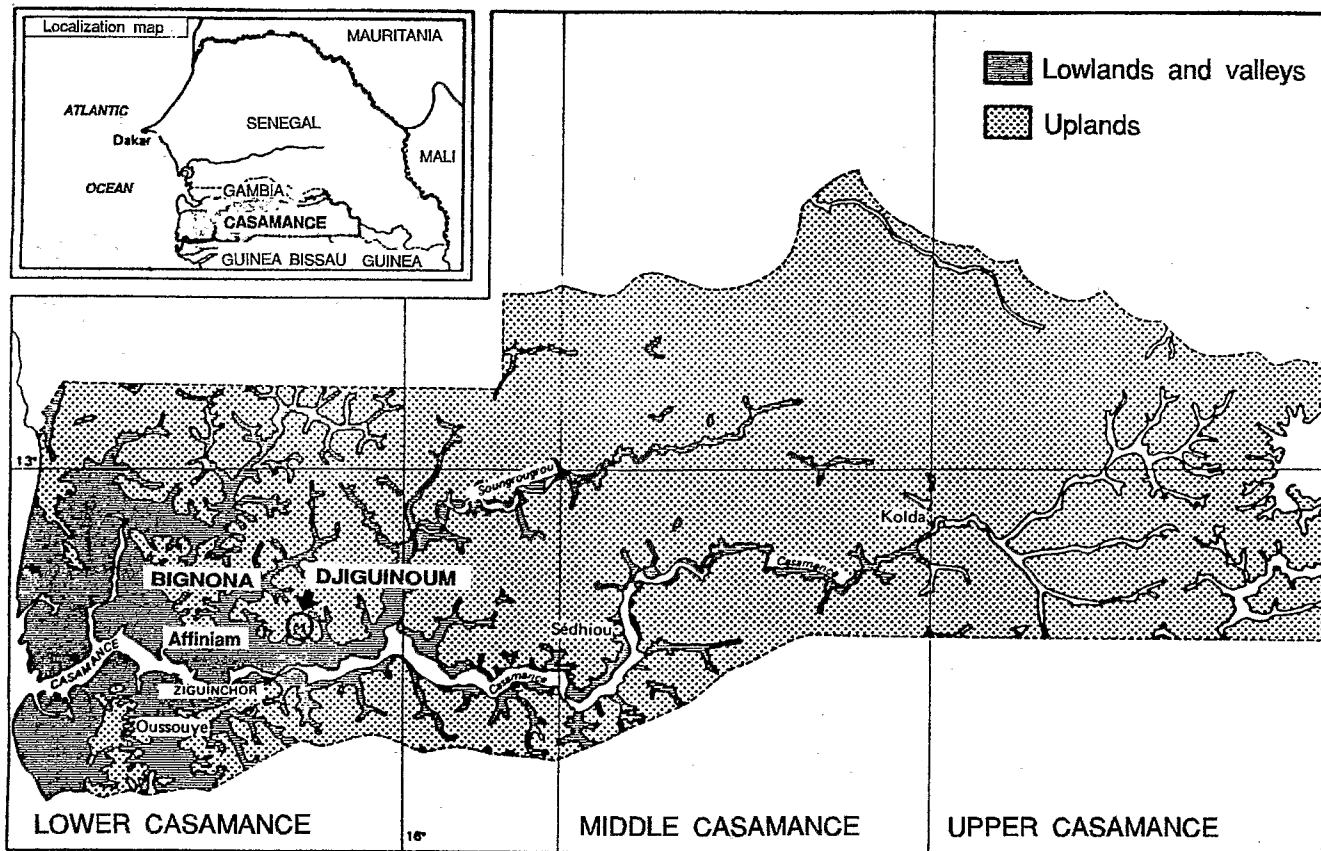


FIG. 2. - Localization of the sampling.

sampling. Then, they were filtered (0.45 μm filter) and acidified before analysis, and transferred into polyethylen bottles. The water salinity was too high to allow the use of a chemical separation with the AmberliteIR 120 resin like DRISCOLL method (1984). Aluminium concentrations were measured in filtered and non filtered water samples. Major elements (Na, Mg, K, Ca, Fe, Al, SO_4 , Cl) were determined.

Fish sampling

On the 16/1/1989, seven tilapias (cichlidae family, perciform order), belonging to three different genera: *Tilapia guineensis*, *Sarotherodon melanotheron heudelotii* and *Hemichromis fasciatus* (LEVEQUE *et al.*, 1989; DAGET *et al.*, 1991), were fished in the upstream side of the Djiginoum dam. Their length varied from 9.8 to 13.8 cm and their weight from 20.5 to 69 g. They were immediately stored in 10% formol bottles and sent to France for analysis.

Bulk analysis

The organs (liver, brain) were lyophilized then weighted. Organs (around 50 mg) were dissolved in 2.5 ml nitric acid (suprapur) for 24 hours and then heated at 110°C for two hours. After cooling, 0.5 ml H_2O_2 (suprapur) and 3 ml ammonia were added. Total aluminium content was determined by Graphite Furnace Atomic Absorption (PERKIN-ELMER 5000, ZEEMAN 5000).

Microanalytical methods

Two microanalytical methods and scanning microscopy were used for the study of tilapia gills (GALLE, 1985).

Classical chemical fixation methods were applied (after formol fixation, the organs were hydrated, alcohol dehydrated and embedded into epoxy resin (Epon)). Ultrathin (80 nanometers) and thick (2 micrometers) sections were carried out with a microtome.

Thick sections were deposited on ultra pure gold specimen holder for the investigation with the secondary ion mass microanalysis (SMI 300 CAMECA). This apparatus uses O_2^+ as a primary ion at a tension of 10 kV. Images of the distribution of a given element can be observed on a fluorescent screen or recorded on a photographic emulsion. The detection of such elements is possible at very low concentrations (1 ppm). The observation field is about 250 μm and the spatial resolution 1 μm . (BERRY *et al.*, 1988).

For X-ray microanalysis, two apparati were used:

1) An electron microprobe equipped with a light microscope MS46 (CAMECA) for thick section analysis on terphan plates.

2) A Camebax (CAMECA) microprobe, equipped with a transmission electron microscope and 4 wavelength dispersive spectrometers of high resolving power for ultra thin sections placed on titanium grids.

To prepare the thick or ultra thin section, a carbon film is deposited one side of it to increase its surface conductivity. Then, the following operating conditions were used: electron accelerating voltage: 20-45 kV; probe diameter: 50 nm; probe current: 150 nA. This apparatus gives a X-ray spectrum of a 1 μm diameter area. Spectrum analysis can give qualitative information about the chemical composition of the analysed area. Under these conditions, aluminium concentrations higher than 100 ppm can be detected (GALLE *et al.*, 1990). For X-ray microanalysis, non osmicated materials were used to avoid the possibility of peak interferences with secondary characteristic rays of osmium.

A scanning microprobe JEOL JSM 840 A, was used to obtain gill images on thick sections.

RESULTS AND DISCUSSION

Chemical analysis of waters is reported in tables I and II.

Bulk analysis of organs showed that the mean value (\pm standard deviation) of total aluminium content was:

- in dry brains,

(a) 15 ppm (\pm 7) in 12 non intoxicated trouts from a river from the center of France,

(b) 24 ppm (\pm 3) in 24 trouts from the Vosges,

(c) 1800 (\pm 200) ppm in 4 tilapias from Casamance.

- in dry livers,

(a) 8 (\pm 3) ppm in 7 non intoxicated trouts from a river from the center of France,

(b) 28 ppm (\pm 2) in 8 trouts from the Vosges,

(c) 400 ppm in one tilapia from Casamance.

TABLE I. - Chemical composition (in mmol L⁻¹ of surface waters sampled in the upstream side of anti-salt dams from the Casamance estuary

| Site | Djiguinoum ^o | Djiguinoum* | Bignona* | Affniam* | Affniam ^o |
|--|-------------------------|-------------|----------|----------|----------------------|
| Date | 23.11.90 | 16.1.89 | 16.1.89 | 16.1.89 | January 88 |
| pH | 3.4 | 3.3 | 3.0 | 3.4 | 5.0 |
| Conductivity (dS m ⁻¹) | 6.71 | 11.29 | 6.20 | 10.55 | - |
| Na | 47.3 | 76.4 | 37.4 | 80.6 | 365.7 |
| K | 1.0 | 1.7 | 1.1 | 2.3 | 3.1 |
| Ca | 1.9 | 9.0 | 4.0 | 6.1 | 3.2 |
| Mg | 5.3 | 25.2 | 10.7 | 20.0 | 15.5 |
| Fe | 0.05 | 0.13 | 0.37 | 0.0 | - |
| Al | 0.96 | 1.5 | 1.8 | 0.29 | 0.06 |
| Al _d (filtered/0.45 μ) | 0.81 | - | - | - | 0.01 |
| SO ₄ | 7.3 | 11.0 | 12.5 | 9.3 | - |
| Cl | 58.3 | 102.0 | 39.1 | 93.0 | - |

*: Analysed at the ORSTOM laboratory of Dakar

^o: Analysed at the hydrology laboratory of Clermont-Ferrand

Concentrations of aluminium in brain (liver) are 120 (40) times higher than in non poisoned trouts from Auvergne (France), and 75 (10) times higher than in trouts living in acid streams in the Vosges mountain (France).

According to the bulk analysis results in organs, these total aluminium contents are very high and would be lethal for trouts.

As the tilapia endure extreme ecological conditions, we assume that they can accumulate much more aluminium in their tissues.

TABLE II. - Chemical composition (in mmol L⁻¹ of surface waters sampled in different parts of the Casamance estuary in January 1988.

| Site | pH*** | Na | K | Ca | Mg | Al | Al _d (filtered/0.45 μ) |
|-----------------|-------|-------|------|------|------|-------|--------------------------------------|
| Katakalousse r. | 6 | 671.3 | 13.8 | 9.7 | 58.9 | 0.005 | 0.003 |
| Affiniam +1 r. | 5 | 365.7 | 3.1 | 3.2 | 15.5 | 0.059 | 0.012 |
| Affiniam -1 c. | 6 | 714.8 | 14.4 | 9.9 | 51.1 | 0.013 | 0.004 |
| Affiniam -6 r. | 6 | 652.6 | 13.7 | 9.3 | 53.6 | 0.021 | 0.013 |
| Affiniam -6 r. | 6 | 639.6 | 12.9 | 16.7 | 79.0 | 0.010 | 0.005 |
| Djiromait r. | 6 | 603.0 | 12.0 | 10.9 | 51.8 | 0.050 | 0.012 |
| Guidel +1 r. | 5 | 439.6 | 8.5 | 5.5 | 13.8 | 0.011 | 0.006 |
| Guidel -1 c. | 6 | 578.3 | 12.4 | 9.7 | 45.1 | 0.029 | 0.013 |
| Guidel -1 r. | 5.5 | 680.4 | 15.9 | 16.7 | 50.5 | 0.063 | 0.020 |
| Sea water** | | 467.8 | 9.9 | 10.3 | 53.3 | 0.000 | - |

r.: sampled 1 m from the edge.

c.: sampled in the center of the stream.

+1: water sampled 1 km up the anti-salt dam.

-1: water sampled 1 km down the anti-salt dam.

-6: water sampled 6 km down the anti-salt dam.

Analysed at the hydrology laboratory of Clermont-Ferrand excepted:

** from RODIER (1984)

*** field analysis

Microanalysis

The results are presented on figures 3, 4 and 5.

Ion microscopy reveals an intense emission of aluminium from the basis of the lamellae in gills just where ionocytes are usually found (fig. 4), and from other parts of gill tissue (fig. 5).

And electron probe X-ray on gills of *Hemichromis fasciatus* from Affiniam and Djilakoun allow to determine that aluminium is associated with phosphorus, calcium, and sulfur on the intracellular deposits (fig. 6) and sometimes iron. Further analysis on gill tissues with the microprobe equipped with an electron microscope shows that these aluminium concentrations can be detected in lysosomes, like in Vosgian trouts.

CONCLUSION

In the recent days, in lower Casamance, an ecological change of soil and water condition is evident, especially in the upstream land of anti-salt dams (MONTOROI *et al.*, in press). No catastrophic mortality of fishes was registered.

Although the aluminium poisoning of trouts is well studied (GALLE, 1992) and shows biological consequences (lower growth and reproduction), it is the first time that we reveal, for the tilapias, an aluminium accumulation in gill cells. According to the bulk analysis results in organs, the total aluminium content is very high and would be lethal for trouts.

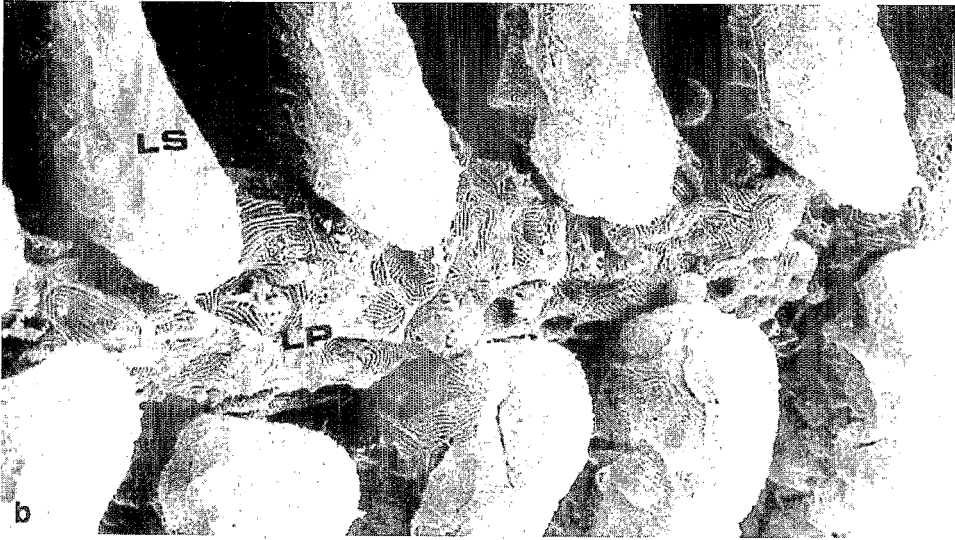
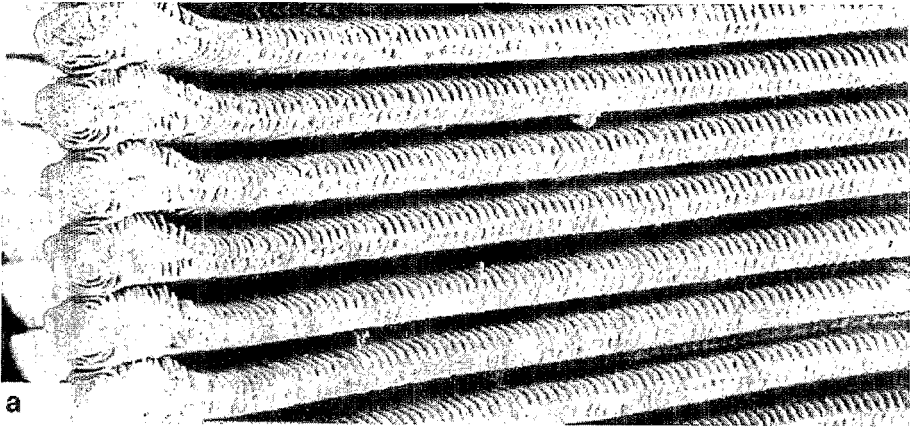


FIG. 3. — *Sarotherodon melanotheron heudelotii* from Affiniam. Gills. Scanning microscope pictures showing the important exchanging surface of lamellae. We can see primary lamellae (LP) and secondary lamellae (LS).

a: (X50)

b: (X1000)

Comparison Vosges/Casamance

Each acid rainfall leaches the soils and causes a temporary and strong increase of acidity and aluminium content in the upstream freshwater.

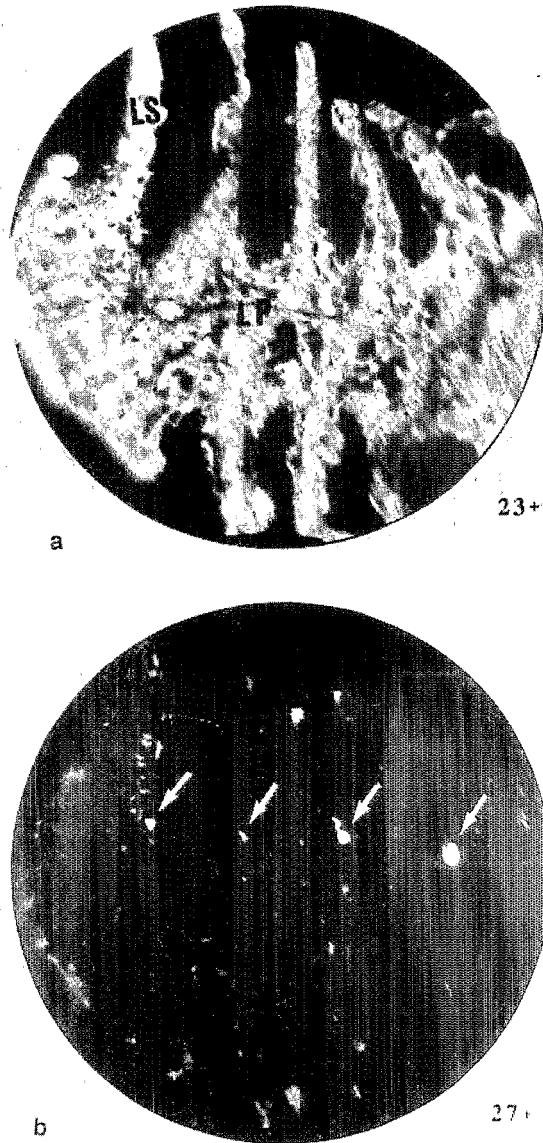


FIG. 4. — *Hemichromis fasciatus*. Gills. Ion images from the same area.

a) Ion image on mass 23 corresponding to $^{23}\text{Na}^+$ distribution. This image is useful to see the analysed tissue histology. We can see secondary lamellae (LS) and primary lamellae (LP). (X380).

b) Ion image on mass 27 corresponding to $^{27}\text{Al}^+$ distribution. This image shows local and recurrent emissions of aluminium precisely from the basis of secondary lamellae (arrows) where chloride cells are usually found, but only from one side of the primary lamellae. (X380)

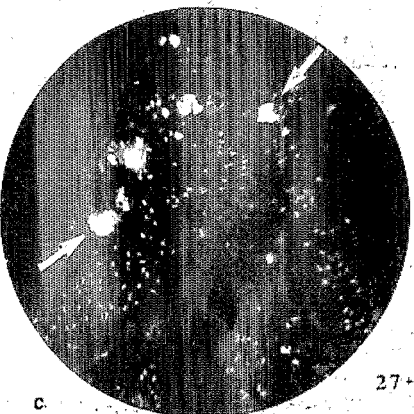
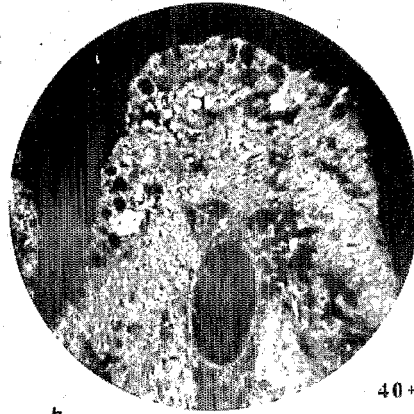
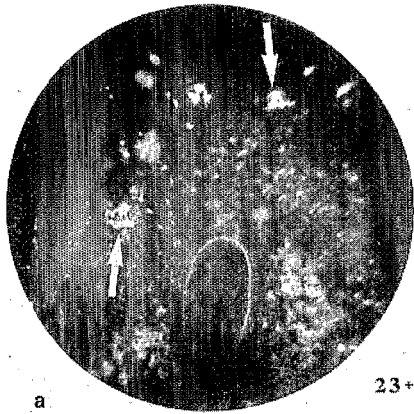


FIG. 5. — *Tilapia guineensis*. Gills. Ion images from the same area near a blood vessel.

a) Ion image on mass 23 corresponding to $^{23}\text{Na}+$ distribution. This image is useful to see the analysed tissue histology. We can see an area of gill tissue with a blood vessel, and some spots with a higher concentration (arrows). (X380).

b) Ion image on mass 40 corresponding to $^{40}\text{Ca}+$ distribution. (X380).

c) Ion image on mass 27 corresponding to $^{27}\text{Al}+$ distribution. This image shows several important emissions of aluminium in gill tissue (arrows). (X380).

Each rainfall on acid sulphate soils has the same effect on the brackish water or the seawater. In this case, the organisms are used to high mineralization. Therefore, they should be less sensitive.

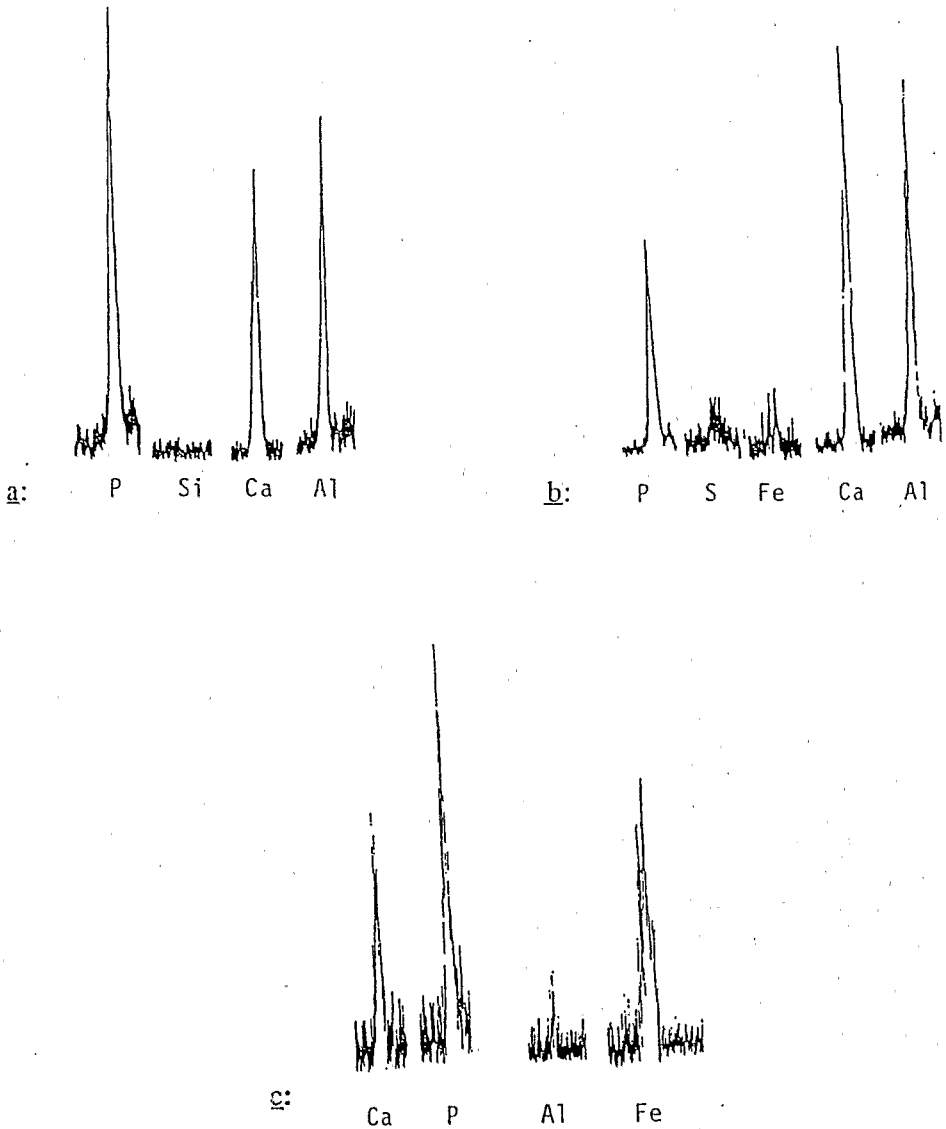


FIG. 6. — *Hemichromis fasciatus* from Affiniam (a,b) and Djilakoun (c). X-Ray spectra of several metals in intracellular aluminium deposits. We can see that aluminium is associated to phosphorus, calcium, sulfur, and sometimes iron.

Consequences for the environment

Man belongs to the trophic chain and the consumption of fishes issued from acid water may induce aluminium accumulation. But, we know that on the one hand, an intestinal barrier exists and reduces the levels of ingested aluminium and on the other hand there is a renal mechanism of active elimination of this element in blood (except for dialyzed humans, GALLE, 1986).

The leaching of soil acidity with the release of water at the anti-salt dam can lead to rapid disease on other organisms in the Casamance stream. The high sensitivity of prawns to variations of acidity, was shown in other countries. If the reclamation policy of acid sulphate soils is developed in the future, the impact on the fish resource must be evaluated to prevent an economic crisis in the region.

It can be seen that a sudden change in water chemical parameters (especially pH and aluminium concentration) is much more poisonous than a gradual change in acid sulphate soils (SIMPSON & PEDINI, 1987; CALLINAN *et al.*, in press) as is observed in other cases (GALLE, 1992). So we can expect an important mortality of tilapias to happen in lower Casamance, as well as in Queensland (CALLINAN *et al.*, in press); especially in the upper land of anti-salt dams in the case of flush effect due to an important rainfall.

Therefore further studies are necessary to understand the biological mechanism and also to evaluate an ecological hazard.

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