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Abstract: The Bay of Bourgneuf, France, is a main site of shellfish production. In the marshes along the bay, oyster intensive rearing and fattening need the mass production of microalgae. Salt groundwaters, available in this region, support a large part of this production for aquaculture. Studies carried out by local authorities have brought to the fore the accumulation of lead (Pb) in several samples of algal pastes derived from cultures using salt groundwater. The aim of this study was to compare growth, nutritional value and metal (Cd, Cu, Pb and Zn) uptake of four microalgae grown in two salt groundwaters or in enriched coastal seawater. Cultures of microalgae used in aquaculture (*Haslea ostrearia*, *Phaeodactylum tricornutum*, *Skeletonema costatum* and *Tetraselmis suecica*) were realised at the laboratory under controlled experimental conditions. Results indicated that salt groundwaters provided cultures with, at least, an equal biomass and a nutritional value similar to cultures grown in enriched seawater. There was no difference regarding metal accumulation whatever the culture medium, except when *S. costatum* was grown in one of the salt groundwater in which case its cadmium levels were higher and could be above the French guideline level. These observations questioned on the bioavailability of metals in salt groundwaters. It also underlines the specificity of metal uptake and accumulation by microalgae.

Key words: Saline groundwater; Trace elements; Bioaccumulation; Diatom; Bacillariophycea; Prasinophycea; Growth; Metals; Aquaculture; Marine microalgae cultivation

Résumé : Croissance et accumulation des métaux chez des microalgues produites sur des eaux souterraines salées de la baie de Bourgneuf. La baie de Bourgneuf (France) est un important site de production conchylicole. Dans les marais littoraux, la culture intensive et l'affinage d'huîtres nécessitent la production en masse de microalgues. Les eaux souterraines salées, facilement exploitables dans cette région, sont majoritairement utilisées dans la production des microalgues pour l'aquaculture en baie de Bourgneuf. Des études ont été menées sur les cultures de microalgues sur ces eaux souterraines salées et ont mis en évidence une accumulation de plomb (Pb) supérieure à la norme alimentaire en vigueur dans différents échantillons de pâte d'algue d'*Haslea ostrearia* issus de ces cultures. L'objectif de cette étude est de comparer la croissance, la composition biochimique globale et l'accumulation des métaux (Cd, Cu, Pb, Zn) pour quatre microalgues, utilisées en aquaculture, cultivées sur des eaux souterraines salées provenant de deux forages différents et sur un milieu enrichi à base d'eau de mer. Les cultures sont réalisées au laboratoire dans des conditions expérimentales contrôlées. Les eaux souterraines salées permettent la production de cultures d'une biomasse et d'une qualité biochimique au moins équivalentes à celles réalisées sur le milieu enrichi à base d'eau de mer. Les teneurs en métaux dans les microalgues ne sont pas différentes pour les algues cultivées sur les différents milieux, à l'exception de *Skeletonema costatum* qui, cultivée sur une des eaux souterraines, peut présenter des teneurs supérieures à la norme alimentaire en vigueur. Ces observations soulèvent des questions sur la biodisponibilité des métaux dans les eaux souterraines salées et soulignent la spécificité de l'accumulation des métaux dans les microalgues.

1 Introduction

On the French Atlantic Coast, the Bay of Bourgneuf (46–47◦N, 1–2◦W) is an important site of shellfish production with about 10 000 tons per year of the Pacific oyster *Crassostrea gigas* (Thunberg) and about 2700 tons per year of the blue and Mediterranean mussels *Mytilus edulis* (Linné) and *M. galloprovincialis* (Lamarck) (Barret 2003). Extensive oyster farming takes place on large intertidal flats while oyster intensive rearing and fattening are located in salt ponds in neighbouring marshes. In the latter case, bivalves are fed on outdoor batch cultures of microalgae supplied from salt groundwaters as culture media (Baud and Bacher 1990; Robert 1990; Baud et al. 1991). The salt groundwaters, discovered in this area around 1980, originating from subterranean saline aquifers, are present all along the bay (Robert 1990; Moreau 1998). Their high nutrient content and accessibility allowed the development of low cost production of microalgae (Baud and Bacher 1990; Bacher and Baud 1992; Sauriau et al. 1997; Barillé et al. 2003). *Phaeodactylum tricornutum* (Bohlin), *Skeletonema costatum* (Cleve) and *Tetraselmis suecica* (Butcher) are cultivated in hatcheries to feed oyster larvae and post-larvae (Coutteau and Sorgeloos 1992; Lavens and Sorgeloos 1996). *S. costatum* is the main diatom used in intensive rearing and in broodstock feeding of the oyster *Crassostrea gigas* in the polders of the Bay of Bourgneuf (Baud et Bacher 1990; Muller-Feuga 2000; Barillé et al. 2003). This diatom, well adapted to the quality of the saline groundwater that contains high amounts of ammonia and silica (Baud and Bacher 1990) is an adequate species for bivalve rearing (Baud and Bacher 1990; Muller-Feuga 2000; Barillé et al. 2003). In addition to the oyster fattening, the greening of oyster gills improves the product pre-market value. The diatom *Haslea ostrearia* (Simonsen) is involved in this phenomenon that gives appreciation to oysters. This diatom thrives naturally in ponds on the western coast of France (Robert 1983; Turpin et al. 1999) but in an unpredictable way depending on the variations of environmental factors. Consequently, in order to control the greening of oysters and to improve the final product quality, local producers developed batch cultures of *H. ostrearia*.

Taking into account that the bioaccumulation of metals in bivalves results mainly from their trophic uptake (e.g. Géret 2000; Wang 2002), it is relevant to evaluate the transfer of metals from water to algae. Several authors have evaluated the bioaccumulation of metals, in seawater media, by *P. tricornutum* (Zhou and Wangersky 1989; Cid et al. 1995; Kudo et al. 1996; Torres et al. 1998), *S. costatum* and *T. suecica* (Nassiri 1995; Nassiri et al. 1996; Perrein-Ettajani et al. 1999). However, very few studies have been conducted on the bioaccumulation of metals by *H. ostrearia* (Ettajani et al. 1992; Minier et al. 1998). The salt groundwaters used in microalgae cultures in the marshes along the Bay of Bourgneuf contain high concentrations of metals (Moreau 1998). Local authorities carried out metal analyses in algal products and put to the fore the accumulation of non essential metals like Pb and Hg in some cultures of *H. ostrearia*, preventing their use in agri-food or cosmetology (SMIDAP, unpublished study, Table 1).

However, to the best of our knowledge, no study has been specifically been conducted on the bioaccumulation of metals in these species grown in these salt groundwaters. As these groundwaters are used to produce microalgal cultures to feed animals or to be transformed for agri-food or cosmetology, it is important to know the final quality of obtained microalgae. In this study, we aimed to assess the physico-chemical characteristics of salt groundwaters from two different drills in marshes near the Bay of Bourgneuf and their respective influence on growth, total carbohydrate and protein concentrations, and metal (cadmium, copper, lead and zinc) accumulation of four microalgae (*H. ostrearia*, *P. tricornutum*, *S. costatum*, *T. suecica*) was established by comparison with results obtained with the same microalgae grown in coastal seawater based medium.

2 Material and methods

2.1 Sampling

Salt groundwaters were collected from two wells in the polder area of the Bay of Bourgneuf, named “Ifremer station” and “Bouin”. Both salt groundwaters are used to produce microalgae given as food to bivalves. During the pumping, a quick reoxygenation induces the formation of iron oxides to which phosphates associate (Moreau 1998; Partridge 2002). So, the Ifremer salt groundwater cannot be used directly for bivalve rearing due to the risk of gill clogging (Partridge 2002). Hence, prior to its use in bivalve ponds, the Ifremer salt groundwater is oxygenated to transform ammoniacal nitrogen into nitrates and iron oxides are removed (Baud et al. 1991). For this study, both untreated and treated Ifremer groundwater and Bouin groundwater were collected. Seawater was hand-collected on the west coast of Noirmoutier Island (47°N, 2.2°W, France). Chemical analysis and experiments were carried out with untreated Ifremer groundwater, treated Ifremer groundwater, Bouin groundwater and coastal seawater.

Waters for algal cultures were stored in a dark room at 4 °C in 60 L containers, pre-cleaned with a detergent, an acidic solution and rinsed three times with the collected water. Ten samples for each studied water were collected in pre-cleaned polypropylene (6) or glass (4) bottles (100 mL). The method used for cleaning the sample containers was modified from the methods described by Laxen and Harrison (1981). Bottles were soaked with nitric acid (0.5 M) and hydrochloric acid (1 M) during 48 hours, and then rinsed three times with Milli-Q water to remove residual acid.

Two of the sample sets were acidified to pH 2 with 1 mL L^{-1} of concentrated hydrochloric acid (Fischer Scientific, trace analysis quality) for total metal determination. For the analysis of the ammonium concentration, reagents were added *in situ* to the four samples collected in glass bottles. The remaining four samples were brought back to the laboratory and kept frozen until nutrient analyses.

2.2 Water analysis

The following nutrient concentrations in seawater and salt groundwaters were determined with an autoanalyser SKALAR: nitrogen (NO^{-3} and NO^{-2}), inorganic phosphorus and inorganic silicon (Strickland and Parsons 1972). After the collection of the samples, the ammoniacal nitrogen was analysed according to the Koroleff (1969) method modified by Grasshof and Johannsen (1972). The salinity was measured both by refractometry *in situ*, and conductimetry in the laboratory. The alkalinity induced by carbonates was measured according to the method described by Culberson and Hawley (1970) and Strickland and Parsons (1972) based on the reactions of Hansson (1973a,b) and Mehrbach et al. (1973). This method was adapted to salt groundwaters by Moreau (1998).

Total Cd, Cu, Pb and Zn were quantified by differential pulse anodic stripping voltammetry (DP-ASV) on acidified and UV-treated samples according to the method described by Nürnberg (1983). Analyses were performed with a polarograph (EG & G M394) coupled to a voltammetric cell (EG & G model 303A) fitted with a hanging mercury drop electrode, a platinum counter electrode and an Ag/AgCl reference electrode. Samples (10 mL) of water were introduced in the temperature-controlled cell at 20 °C, degassed for 240 s with N_2 to remove the dissolved oxygen, which can interact with the mercury electrode. For the pre-electrolysis, the deposition potential was switched to −1.2 V for 60 s; the bulk solution was stirred from the bubbling step to the end of the pre-electrolysis to ensure the proportionality between the quantity of metals reacting with the mercury drop and the bulk concentration. After the deposition step, an equilibration step of 30 s, without stirring, allowed a

homogeneous distribution of metals on the mercury drop. The stripping step consisted in a potential scanning from −1.2 V to −0.1 V. The currents created by the reduction of the metals from the amalgams were recorded forming peaks at specific potentials for Zn (−1.1 V), Cd (−0.7), Pb (−0.5 V) and Cu (−0.2 V). Metal concentrations were estimated with reference to three additions of a standard mixed metal solution in the bulk analysed according to the same procedure.
The detection limits and the reproducibility of the method were calculated from 10 repetitive analyses of an acidified UV-treated seawater sample, on which the sensitivity was calculated from standard additions. Table 2 shows the obtained values for detection limits.

2.3 Cultures of microalgae

The cultures were performed in erlenmeyers flasks 250 mL, acid-cleaned and sterilized before filling with seawater or salt groundwater. In the experimental conditions used, the strains of selected microalgae could not develop efficiently on coastal seawater. Thus, seawater was enriched with some nutrients (N, P, Si) to obtain similar concentrations as in salt groundwaters and with the f/2 trace metal solution according to Guillard (1982). The enriched seawater medium was sterilized by autoclave (1 bar, 120 °C for 20 min) to prevent the development of microalgae or bacteria naturally present in seawater samples. In the opposite, we did not observe living organisms in these salt groundwaters and Baud and Bacher (1990) did not notice anymore the presence of bacteria or microalgae.
Salt groundwaters were not sterilized by autoclave to avoid a loss of elements by precipitation. As the presence of sedimented particulate matter (iron oxides) has been observed, the sterilization by filtration was excluded to prevent a loss of elements (mainly iron and phosphates) by retention on the filter membrane. The formation of iron oxides did not modify the total metal concentration but only the speciation of metals in salt groundwaters.
All the algal strains were provided by the Nantes Culture Collection (WDCM 856, ISOMer, Université de Nantes, France). *Phaeodactylum tricornutum* (NCC 45, size: 30 μm) and *Skeletonema costatum* (NCC 52, size: 15 μm) were isolated from coastal water of the Bay of Bourgneuf (France). *Tetraselmis suecica* (NCC 62, size: 8 μm) was isolated from a sample of coastal seawater of Le Croisic (Loire-Atlantique, France). *Haslea ostrearia* (NCC 143, size: 70 μm) came from a greening oyster pond in the area of Bourgneuf marshes.
All the culture experiments were performed at 17 °C under 80 μmol photon m^{-2} s^{-1} of irradiance and a 14:10h light:dark cycle. Strains were introduced at 5000 cell mL^{-1} in the flasks filled with 150 mL of enriched seawater or salt groundwater. All experiments were run in four replicates. The cell density (number of cells per mL) was determined daily from the beginning of the exponential phase to the stationary phase using Nageotte (for *H. ostrearia*) or Neubauer (for the other microalgae) hematocymeters.
In an effort to facilitate the presentation of the results, the different media used to grow our microalgae will be represented by the following acronyms: Ifremer salt Groundwater IG (Untreated: UIG; Treated: TIG), Bouin salt Groundwater BG and Enriched coastal SeaWater ESW.

2.4 Biochemical and chemical analyses

At the stationary growth phase, cultures were filtered, using glass microfibre Whatman GF-F filters, in order to determine the cellular carbohydrate, carotenoid, chlorophyll *a* and protein concentrations by the spectrophotometric methods of Dubois et al. (1956), Richards and Thompson (1952), Lorenzen (1967) and Lowry et al. (1951), respectively. Carbohydrate,

photosynthetic pigment and protein concentrations were used to assess the quality of the algal cultures. To determine the amount of metals accumulated by the algal cells, cultures were filtered using pre-weighted cellulose nitrate membrane Sartorius filters (0.8 μm). Filters were dried at 60 ◦C, weighted to determine algal dry weight and digested with 1 mL concentrated nitric acid (Fisher Scientific, trace analysis). Cu and Zn were determined by flame atomic absorption spectrometry FAAS (Varian SpectrAA 250 Plus) while Cd and Pb were determined by electrothermal atomic absorption spectrometry EAAS (Hitachi Z5000 Polarized Zeeman AAS) in the acidic solutions. Eight blanks were realised in the same analytical conditions to determine detection limits. Table 2 shows the obtained values for the detection limits. Reference material (IAEA 140, sea plant homogenate) was treated and analysed in the same way as the samples; the results of the standard reference material was in good agreement with certified values.

2.5 Statistical analysis

Data on growth, carbohydrate and protein contents and metal bioaccumulation obtained for microalgae grown in groundwaters and enriched seawater were compared using one-way analysis of variance (ANOVA). After checking the normality and the homogeneity of variances, *a posteriori* tests (Tukey tests) were run with ANOVA data. Some series that did not verify normality or homogeneity of variances were tested using ANOVA on ranks and *a posteriori* Dunn's test.

3 Results

3.1 Physico-chemical parameters of waters

Data for pH, alkalinity, nutrients and trace metals are presented in the Table 3. The pH of the salt groundwaters UIG and BG were more acid (pH close to 7) than the pH of SW and TIG (pH close to 8). TIG presented a higher pH than that observed for UIG owing to the transformation of the amoniacal nitrogen in nitrates.

Carbonates and nitrogen were higher in IG than in BG ($p < 0.001$). Both groundwaters (IG and BG) were richer ($p < 0.001$) in major nutrients (N, P, Si) than seawater. A decrease of the concentration of the inorganic phosphorus was observed between UIG and TIG. The concentration of Cd was lower, for all the collected samples, than the quantification limit of the method calculated for our analytical protocol. The Cu concentration of the groundwater BG was higher ($p < 0.05$) than the concentration of the groundwater IG, while, for Zn, the opposite was observed. The level of Cu in seawater was higher than in IG but lower than in BG. Nevertheless, the input of copper from the f/2 enrichment solution should be taken in consideration towards the potential accumulation of Cu in the microalgae.

The highest concentration in Zn was found in seawater. TIG, unexpectedly, contained less Zn than UIG, respectively 1.7 and 5.9 $\mu g L^{-1}$.

TIG had a higher concentration in Pb that could come from the treatment system itself.

3.2 Microalgal growth

The growth curves of the four microalgae cultivated in salt groundwaters and enriched seawater are presented in Figure 1. All four algae grown in groundwaters reached densities (cell mL^{-1}) as high as that observed when grown in ESW. The densities of *T. suecica* and *H. ostrearia* were higher ($p < 0.001$) when cultures were performed in TIG. *T. suecica* had a better development in UIG than in BG or in ESW. *H. ostrearia* could not develop in UIG. The results presented in Figure 1 are those obtained when *H. ostrearia* was grown in the UIG half-

diluted with coastal seawater. The densities of *H. ostrearia* were statistically the same in the cultures realised in dilute UIG, BG or ESW. Densities of *P. tricornutum* grown in BG and ESW were similar and its development was greater in BG than in IG ($p < 0.001$). *S. costatum* reached a higher cell density when cultures grew in IG.

3.3 Biochemical composition of microalgal cells

The partial biochemical composition of microalgal cells is presented in Figure 2. Pigment (carotenoids and chlorophyll *a* + pheopigments), protein and carbohydrate contents are expressed per cell. In general, pigment, carbohydrate and protein concentrations, in cultures grown in one or the other groundwater, were at least equal to or higher than those grown in ESW.

Cultures of *P. tricornutum* in Ifremer groundwater (IG) showed higher carbohydrate and protein contents than cultures grown in BG or in ESW. The carotenoid content was higher ($p < 0.001$) in cultures grown in TIG compare to the other three media. Cultures of *S. costatum*, grown in TIG, contained significantly ($p < 0.001$) higher levels of pigments (carotenoids and chlorohyll *a* + pheopigments) compare to the other media, for which pigment cell contents were statistically similar. Protein content in cultures grown in BG was lower ($p < 0.05$) than in the other cultures, whereas carbohydrate concentration was higher in BG or ESW cultures than in IG cultures.
For *T. suecica*, pigment, carbohydrate and protein contents were twice as high ($p < 0.001$) in UIG cultures as those in the other cultures (TIG, BG or ESW). TIG cultures had lower ($p < 0.05$) carbohydrate contents than the other cultures.
Similarly, pigment content was larger in cultures of *H. ostrearia* grown in Ifremer groundwaters. Carbohydrate concentration was higher ($p < 0.05$) in cultures of *H. ostrearia* grown in UIG than in the other three media. The protein concentration for these cultures was also higher when grown in UIG, but only cultures grown in ESW had significant lower protein concentration.

3.4 Microalgal metal uptake

The accumulation of Cd and Cu by microalgae is presented in Figure 3.
No difference of Cd accumulation was observed whatever the medium used to grow *P. tricornutum, T. suecica* or *H. ostrearia*. The Cd concentrations in *T. suecica* and *H. ostrearia* were under the French food regulation standard value (0.5 $\mu g\ g^{-1}$).
The diatom *S. costatum* grown in TIG accumulated more Cd than when it was grown in ESW ($p = 0.036$). *S. costatum* showed Cd concentrations that tended to be higher than the French food regulation standard value when it was grown in TIG or BG, but this tendency was not confirmed statistically.
The accumulation of Cu varied according to the species and the medium. *S. costatum* and *H. ostrearia* accumulated a higher amount of Cu when grown in ESW compared to cultures grown in Ifremer groundwaters. The Cu content in *S. costatum* grown in ESW was also higher than in cultures grown in BG. Among salt groundwaters, no difference was observed for Cu in both microalgae. For *P. tricornutum*, no significant difference in Cu bioaccumulation occurred among the cultures. *T. suecica* showed a lower accumulation of Cu grown in TIG than cultures grown in the other three media.
The quantification of Pb in the microalgae gave results lower than the detection limit of the analytical method, EAAS. The concentration limit of Pb expressed per gram of algal dry

weight was estimated for each alga. A level of Pb higher than the authorised value (5 μg g−1) would have been quantified with our analytical protocol.
The values measured for Zn for the different replicates were too erratic to yield any information about Zn accumulation by microalgae in our experimental conditions.

4 Discussion

In the present study, both salt groundwaters provided cultures with a cell density, carbohydrate and protein cell contents and pigment cell contents similar to that grown in enriched coastal seawater, which suggested similar quality of algal cultures. The advantage using salt groundwater is an overall lower cost since nothing has to be added to the medium, contrarily to seawater that is often enriched with f/2 solutions (Guillard 1982) in mass algal cultures (Lavens et Sorgeloos 1996). However, both groundwaters do not sustain with the same efficiency each species of studied microalgae. Although the drills where the groundwater was collected from are only distant by a few kilometers, the observed differences in their composition and aptitude to be used as growing medium for microalgae were significant. In a previous study, Moreau (1998) noticed this disparity in salt groundwater quality among several drills installed in the marshes along the Bay of Bourgneuf, and put forward gradients of nutrients and salinity following the geographic location. From the coast inland, total ammoniacal nitrogen and Mn concentrations increase while total Fe concentration and salinity decrease. According to the productivity of the three diatoms (*P. tricornutum, S. costatum* and *H. ostrearia*) grown in several groundwaters, this author showed different levels of tolerance/need concerning the total ammoniacal nitrogen (NH_3+NH^+_4) concentration. For these species, the optimal concentration (respectively: 2000, 250−300 and 110−130 μmol L^{-1}) and the toxic concentration (respectively: 8000, 500 and 150−160 μmol L^{-1}) were determined. In the present study, results concerning *S. costatum* and *H. ostrearia* are congruent with the previous observations. *H. ostrearia* did not grow in the groundwater UIG that contains $NH_{3,4}$ concentration about 300 μmol L^{-1}. The dilution of UIG with coastal seawater decreased the $NH_{3,4}$ concentration lower than 180 μmol L^{-1} and allowed the development of the diatom in this medium. Actually, the ammonia concentration (NH_3), that is the toxic form of nitrogen for the microalga, is to be considered rather than the total ammoniacal nitrogen (NH_3+NH^+_4) when discussing nitrogen toxicity. The proportion of NH_3 related to the total ammoniacal nitrogen depends on salinity, pH and temperature (Abeliovich and Azov 1976). In Table 4, we can observe that NH_3 concentrations in all media were lower than the toxic NH_3 concentration level described by Moreau (1998).
Although UIG appeared to be unsuitable to the development of *H. ostrearia*, this groundwater was the most convenient medium for the development of *S. costatum*. This is in agreement with the optimal ammoniacal nitrogen concentration found by Moreau (1998) for this diatom. Baud and Bacher (1990) have already shown that this diatom was well adapted to this kind of medium. On the contrary, regarding the influence of ammoniacal nitrogen concentration in a culture medium, our results for *P. tricornutum* were not consistent with the study of Moreau (1998). UIG would be expected to be the most suitable medium owing to its high concentration in ammoniacal nitrogen. However the highest cell density was observed when our *P. tricornutum* strain was grown in BG.
Higher carbohydrate, pigment and protein levels in cells of *P. tricornutum* grown in TIG were in contradiction with the low cell densities achieved in these cultures. Whereas the cell density in TIG culture was twice lower than that in BG cultures, slightly lower than that in ESW culture and equal to that in UIG culture, the chlorophyll *a* concentration of TIG culture, expressed per mL of culture, was five times higher than that of UIG or ESW cultures and eleven times higher than that of BG cultures, with statistical significance at $\alpha < 0.001$.

The carbohydrate and protein concentration increase observed when *P. tricornutum* was cultured in TIG were not as important as the chlorophyll *a* concentration increase. The increase of chorophyll *a* in *P. tricornutum* cells grown in TIG compared to the other media could be associated to a higher need in energy in order to reduce nitrates (the nitrogen form found in TIG) into ammonium (the nitrogen form used in the cell metabolism). A similar relation between nitrate reduction and photosynthesis was established for cyanobacteria (Serrano et al. 1981; Flores et al. 1983, 2005). In this study, such a relation has not been put forward for the other three microalgae.

Regarding the metal enrichment in groundwaters, cultures did not present systematic accumulation of metals (Cd, Cu and Pb) suggesting a low bioavailability of these metals for the selected microalgae, except for *Skeletonema costatum* that presented high cadmium concentrations. These observations are not congruent with the earlier studies in which high levels of Pb have been found in *H. ostrearia* cultures (SMIDAP, pers. comm.). These studies had been performed using relatively different experimental conditions. Actually, previous experiments were realised in outdoor batch cultures in 600 L tanks whereas cultures in the present study were performed in small volume close flasks with 150 mL of medium in controlled conditions (temperature, irradiance). Firstly, outdoor batch cultures are exposed to atmospheric Pb which is the major source of this metal (Nriagu and Pacyna 1988). Moreover, using smaller volumes of culture (Erlenmeyer *versus* tank) implies a higher surface/volume ratio promotingmetal adsorption on the flask, and a shorter culture period reducing the contact period between algae and metals. That can explain the difference of metal accumulation between the previous and the present studies. Furthermore, metals, adsorbed onto the precipitates formed during the oxygenation of the water, could be released with a slow kinetic following the consumption of dissolved metal forms by algae in the medium. In batch cultures performed in the Bay of Bourgneuf (Taraud, pers. comm.), they use semi-continuous culture techniques which consists in the renewing of 70% of the total volume with salt groundwater while the 30% of the remained culture is used as algal *inoculums* (Lavens and Sorgeloos 1996). Then, accumulation of inorganic matter could occur at the bottom of the tank and could constitute a potential reservoir of metals. Another difference between the various studies was the analysed algal product. In the previous studies, cultures were centrifuged to obtain algal pastes that were used for metal analyses. In the present study, cultures were filtered in order to retain cells, which clearly reduced metal contamination due to experimental techniques.

Among the four tested algal strains, *P. tricornutum* and *S. costatum* present higher concentrations of Cd and Cu than the other microalgae. Yet, in earlier studies, Cd accumulation in *T. suecica* has been shown higher than in *S. costatum* (Perrein-Ettajani et al. 1999; Ettajani et al. 2001) and in *P. tricornutum* (Ng et al. 2005). The equilibrium of metal forms found in salt groundwaters would be different from seawater owing to the pH and nutrients contents and would affect specific metal uptake rates of the different strains. Regarding *H. ostrearia*, this diatom is at least five fold longer than the other microalgae and thus, the cells present a smaller surface/volume ratio that can partly explain a lesser metal uptake. One of the microalgae tended to accumulate Cd reaching levels above the safety guidance limits. That was however not established statically. This statement needs to be confirmed by further experimentations. Contrarily to the previous observations, the *H. ostrearia* strain used in this studied did not present higher accumulation of metals than the other microalgae.

5 Conclusion

The results obtained in the present study are congruent with previous studies regarding the interest of using salt groundwaters to grow microalgal cultures for bivalve rearing and fattening. Salt groundwaters provide cultures with a high nutritional value for a low cost. The previous observations of metal accumulation in *H. ostrearia* were not verified in this experiment; this diatom presents lower metal levels than the other ones (*P. tricornutum* and *S. costatum*). The experimental conditions previously used for the studies realised at the request of the local authorities presented several differences compared to those adopted in our study explaining the lower accumulation observed in our cultures. It would be interesting to realise long term experiments in different culture volumes to determine the effects on the metal/microalgae interaction of the culture time and of the ratio between the surface of the flask/tank walls and the volume of culture. The study of metal repartition in groundwaters by determining the metal speciation and the complexation capacity of such culture media would allow a better understanding of the metal bioavailability. Furthermore, it seems important to measure the pH and the algal exudates production all along the culture in order to assess the impact of the algal development itself on the metal accumulation. Indeed, Sunda et al. (2005) reported that, when the cell concentration in a batch culture becomes sufficiently high, the complexation of metals is higher owing to an increase in the pH of the medium and the release of metabolites by the algae.

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Table 1. Accumulation of cadmium and lead by *Haslea ostrearia*, results obtained with five samples of algal paste during the experimentations designed by the local authorities (SMIDAP, unpublished study, May 2000).

	Haslea ostrearia (algal paste)					French food regulation standard
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	
Cd ($\mu g\ g^{-1}$ DW)	0.18	0.42	0.14	0.31	0.56	0.50
Pb ($\mu g\ g^{-1}$ DW)	11.00	8.70	10.63	6.50	6.30	5.00

Table 2. Detection limits of the analysed metals. Differential pulse anodic stripping voltammetry (DP-ASV) on acidified and UV-treated samples; atomic absorption spectrometry (AAS).

Detection limits	Cd	Cu	Pb	Zn
DP-ASV ($\mu g\ L^{-1}$)	0.08	0.22	0.16	0.65
AAS ($\mu g\ g^{-1}$)	0.01	0.41	0.29	0.69

Table 3. Physico-chemical characteristics of salt groundwaters (Untreated Ifremer Groundwater UIG, Treated Ifremer Groundwater TIG and Bouin Groundwater BG), coastal seawater (SW) and enriched coastal seawater (ESW): pH, salinity, alkalinity, nutrient and trace metal concentrations. Mean • ± confidence interval.

	UIG Nov. 2003	TIG Nov. 2003	BG Nov. 2003	SW Dec. 2003	ESW
Salinity	31.4	32.4	29.5	34.3	34.3
pH	7.31	8.16	7.12	8.03	8.11
Alkalinity induced by carbonates (μmol L^{-1}):					
- CO_2, HCO_3^-, CO_3^{2-}	9.32	9.30	5.99	2.74	9.84
Inorganic nitrogen (μmol L^{-1})					
- Ammoniacal nitrogen (NH_4^++NH_3)	324 ± 0.2	14 ± 7	85 ± 9	22 ± 6	100
- Nitrites (NO_2^-) + Nitrates (NO_3^-)	0.4 ± 0.5	285 ± 18	1.6 ± 0.4	0.2 ± 0.8	0.9 ± 0.3
Inorganic phosphorus (μmol L^{-1})	13.92 ± 0.8	11.51 ± 0.91	14.18 ± 0.54	0.28 ± 0.06	10
Inorganic silicon (μmol L^{-1})	234 ± 14	260 ± 27	215 ± 9	6 ± 3	200
Cd (μg L^{-1})	<0.08	<0.08	<0.08	<0.08	<0.08
Cu (μg L^{-1})	0.9 ± 0.3	0.9 ± 0.3	5.0 ± 3.2	2.0 ± 0.1	17.4 ± 0.3
Pb (μg L^{-1})	1.0 ± 0.2	2.7 ± 0.2	1.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
Zn (μg L^{-1})	5.9 ± 1.4	1.7 ± 0.3	2.1 ± 0.3	11.3 ± 0.6	14.4 ± 2.0

Table 4. Concentration in total ammoniacal nitrogen $NH_{3,4}$ and in ammonia NH_3 (caculated with Johnasson and Wedborg's equations, 1980) in the groundwaters and the enriched seawater.

	$NH_{3,4}$ (μmol L^{-1})	NH_3 (μmol L^{-1})
Untreated Ifremer Groundwater (UIG)	324	1.98
Dilute UIG	173	1.66
Treated Ifremer Groundwater (TIG)	14	0.58
Bouin Groundwater (BG)	85	0.34
Enriched SeaWater (ESW)	100	3.70

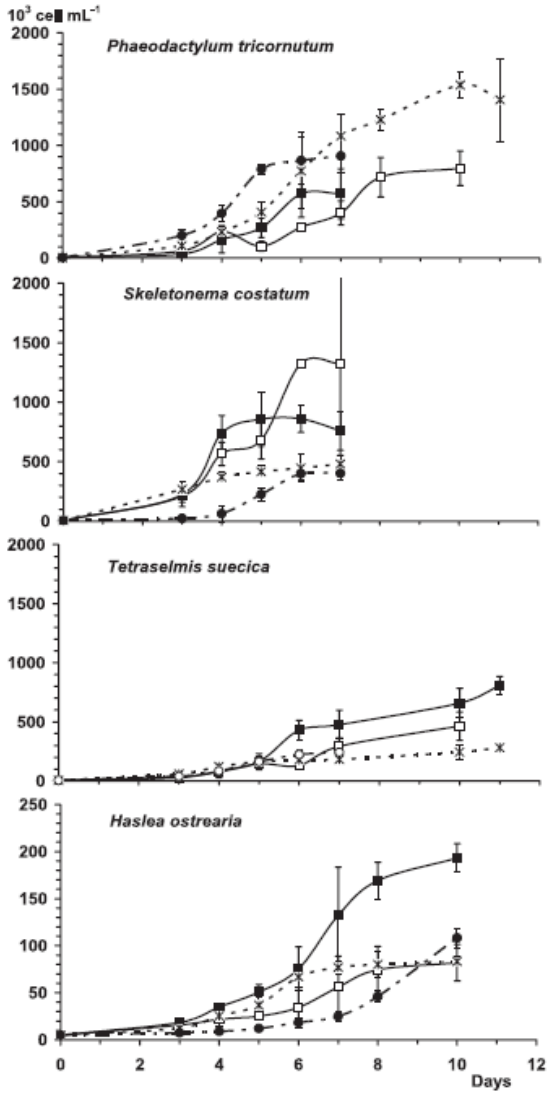

Figure 1. Growth (10^3 cell mL^{-1}) of microalgae (*Phaeodactylum tricornutum*, *Skeletonema costatum*, *Tetraselmis suecica*, *Haslea ostrearia*) in (−□−) Untreated Ifremer Groundwater (UIG), (–■–) Treated Ifremer Groundwater (TIG), (–×–) Bouin Groundwater (BG) and (–●–) Enriched SeaWater (ESW). Mean • ± confidence interval.

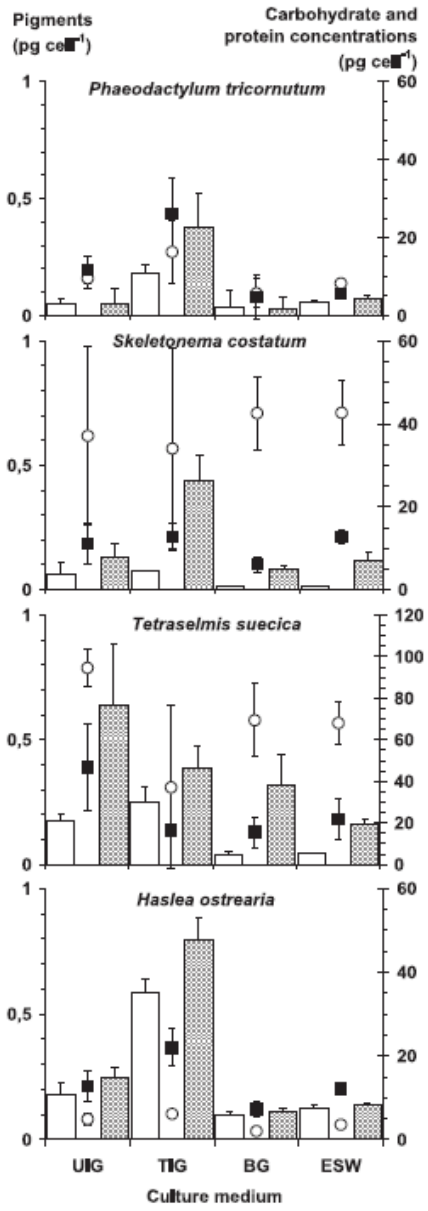

Fig. 2. Carotenoid (□), chlorophyll *a* and pheopigment (▨), carbohydrate (○) and protein (■) contents of cultures (pg cell^{-1}) grown in Untreated Ifremer Groundwater (UIG), Treated Ifremer Groundwater (TIG), Bouin Groundwater (BG) and Enriched SeaWater (ESW). Mean ± confidence interval.