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The Analysis of Arsenic in the Lipid Phase from Marine and Limnetic Algae

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The arsenic content in the lipid phase extracted from selected marine and limnetic algae has been analysed by use of neutron activation technique. The arsenic content varied from about 0.5 ppm to 5 ppm. The algae were cultivated in the laboratory using enriched cultures. The following types of algae were investigated: *Chlorella ovalis* Butcher, *Chlorella pyrenoidosa* Chick, *Oscillatoria rubescens* (D.C.), *Phaevdactylum tricornutum* Bohlin, *Sceletonema costatum* (Grev.) Cleve.

It has been shown earlier that the arsenic present in different marine organisms as well as in seaweed is in the form of lipid soluble and water soluble arseno organic compounds.[1–4] These compounds can probably be formed in two ways; it is possible that the various marine animals and plants themselves can synthesise compounds using inorganic arsenic which they absorb from their surroundings, or it may be that there is a well defined group or groups of micro organisms which is responsible for the synthesis of these compounds. In the latter case the arseno organic compounds could be transferred *via* the food chain to the more advanced organisms. A combination of the two alternatives may also be a possibility.

As far as we have been able to find out no quantitative data concerning the arsenic content of algae exist. Gautier [5] and others have demonstrated that arsenic is present in marine algae, but in all cases it proved to be difficult to obtain exact data due to small quantities available of algae material for the analysis. Neither is there any information available relating to the form in which the arsenic is present in these algae, although many have considered the hypothesis that the arsenic is present in the form of one or more arseno organic compounds. To clarify this situation it was of interest both to obtain quantitative data relating to the arsenic content in algae, and to obtain more pointers as to the form in which the arsenic is present.

The oil phases in the algae were selected for analysis since the risk of contamination by inorganic arsenic is far smaller for the oil phases than for the water phases. If the oil phase were contaminated with inorganic arsenic

or other inorganic ions, then it is a relatively simple process to remove this contamination. This can for example be achieved by washing the oil with distilled water, or if the amount of oil is small by dissolving the oil in an organic solvent as hexane and then washing this solution in distilled water.

The cultures used in this investigation were *Chlorella ovalis* Butcher, *Chlorella pyrenoidosa* Chick., *Oscillatoria rubescens* (D.C.), *Phaeodactylum tricornutum* Bohlin, *Sceletonema costatum* (Grev.) Cleve, and have been described previously.[6,7] The algae were cultivated in fresh or salt water media enriched with plant nutrients.[8] Arsenic was not added to the media. The only source of arsenic was therefore the arsenic present either in the water samples or as impurities in the components of the enriched solution. The concentration of arsenic in the culture media was of the order of 1 – 3 ppb.

EXPERIMENTAL

The algae were cultivated in a specially built "climate room" designed for the purpose. The details of this room and the conditions observed for the cultivation are described elsewhere.[6] Spherical flasks (2 l) were used to hold the cultures. The flasks stood on a "vibrating" table which was illuminated by fluorescent lamps (Philips TL 40 W/32). These gave an illumination of 6000 lux at the table surface. Every day, once the growth was well under way, one half of the algae solution was filtered off through a glass sinter filter. After the filtration the filter papers with the algae were stored in chloroform. Culture solution was then added to the flasks so that the volume of the solution where the growth occurred remained constant.

It took about 14 days of cultivation to produce enough algae material to allow a sufficiently sensitive analysis of the lipid phase obtained from the algae. The lipids were extracted from the algae specimens by adding methanol and water to the chloroform solution containing the filter with the filtered off algae. The resulting mixture was shaken for about 2 h, and then more water was added so that the chloroform phase became separated from the methanol – water phase. The chloroform phase was then separated, and evaporated. The lipids thus extracted were dissolved in hexane and the solution was then washed twice in distilled water.

The analysis of the oils was carried out using neutron activation. This method is very sensitive and well suited for the analysis of arsenic in oils as long as these do not contain other components which disturb the registration of the induced As-76 activity. The method has been described earlier [9] and only a few of the main points relating to the technique will be mentioned here. The oil specimens were irradiated in quartz ampoules. The oil was transferred to the ampoules by dissolving it in diethyl ether, and then evaporating the ether. The ampoules were then sealed and irradiated together with arsenic standard (As_2O_3 dissolved in 0.01 N HCl) for 2 h in a neutron flux of about 10^{13} n cm^{-2} sec^{-1}. After irradiation, the specimens were transferred to inactive glass vials. Also this transfer was performed by use of a suitable solvent.

The arsenic activity in the irradiated oil specimens was registered using a multichannel γ-spectrometer. The arsenic content in the specimens was estimated by comparing their spectra with the corresponding spectrum taken from the irradiated arsenic standard. For some of the specimens where a small amount of sodium was present it was necessary to wait 3 – 4 days before their γ-spectra could be registered. By this time most of the radioactive sodium isotope Na-24 had disintegrated. It should be mentioned that the bromine content of the oils extracted was small, so that the measurement of the As-76 activity was not disturbed by any activity from the radioactive bromine isotope Br-82 which is created when bromine is irradiated with thermal neutrons.

RESULTS AND COMMENTS

As can be seen from Table 1, the presence of arsenic has been demonstrated in all of the oil specimens extracted from algae. Since the algae were cultivated

Table 1. Yield of and arsenic content in oil extracted from algae cultivated in fresh and salt water.

	Alga	Water	Yield of oil (g)	As ppm
1.	*Sceletonema costatum*	salt water	0.050	1.3
2.	*Chlorella ovalis*	» »	0.032	0.7
3.	*Chlorella pyrenoidosa*	fresh water	0.019	0.5
4.	*Phaeodactylum tricornutum*	salt water	0.062	3.6
5.	*Phaeodactylum tricornutum*	fresh water	0.024	4.8
6.	*Oscillatoria rubescens* (Züricher See)	» »	0.030	0.5
7.	*Oscillatoria rubescens* (Steinsfjorden)	» »	0.016	0.4

under very favourable conditions in the laboratory with, among other things, a sufficient supply of culture solution, the values found for the arsenic contents must be regarded as qualitative and as giving an impression of the algal ability to synthesise arseno organic compounds under such conditions.

Since the arsenic content in the culture solutions probably varied, and was not exactly analysed each time, it is difficult to estimate any enrichment coefficient for arsenic in the algal oils. If the value of 1 – 3 ppb As is accepted (for the culture solutions) then the enrichment coefficient will lie in the region from 200 to 5000.

On the basis of the results obtained, it can be concluded that the arsenic present in lipids extracted from algae is organically bound in the same way as it is in oils extracted from more advanced marine organisms. It is therefore possible that the arsenic which is found in the algae is transferred *via* the food chain to other organisms, and that the algae form an important source for the arsenic which is present in the higher organisms.

If one considers the situation with regard to fish and other aquatic organisms, it seems reasonable to believe that the algae can also synthesise water soluble arseno organic compounds. This possibility is now being more closely investigated in our laboratory, by cultivation experiments where radioactive inorganic arsenic is added to the culture solution.

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