RESEARCH

USES OF ALGAE IN THE CONTEXT OF PROBLEMS OF THE ENVIRONMENT AND OF PUBLIC HEALTH

3rd part

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ALGAL TOXINS IN SEAWATER

Of the various divisions, that of Pyrrhophyta (Dinoflagellates) contains the highest number of species producing neurotoxins. Saxitoxin is undoubtedly the toxin which is produced in the greatest quantities by almost all the species of Alexandrium (table 5; figure 1). The chemical family it belongs to includes about 12 substances, divided into 4 groups:

- saxitoxin group
- neosaxitoxin group
- tetrodotoxin group
- group of gonyautoxins (from 1 to 4, B1 and B2, from C1 to C4; many of these are in actual fact metabolites of saxitoxin and neosaxitoxin).

The syndrome caused by these toxins, PSP (Paralytic Shellfish Poisoning), and the symptoms of poisoning have already been described in Table 5. It is important to mention that the PSPs are subject to biological magnification according to a mechanism which, starting from the benthonic sediments, continues from the saprophyte zoobenthos to the small predators such as crustaceans, vertebrates, plankton dinoflagellates, to the herbivorous and carnivorous zooplankton and up to vertebrate predators, such as teleosts, amphibians and reptiles. Saxitoxin has in fact been found in seabed sediments contained in resistance cysts, accumulated in both marine molluscs (Saxidomus giganteus in Alaska; Soletellina diphos in Taiwan; Mytilus galloprovincialis in Europe; Mytilus californianus in California; Crassodoma gigantea in North America; Patinopecten yessoensis in Japan) and freshwater molluscs (Corbicula sandai in Lake Biwa in Japan) and in tintinnids (Bruno, 2000). The saxitoxin accumulated in molluscs is metabolised in time and the metabolic intermediates (gonyautoxins) tend to accumulate in tissues and various organs; the toxicity is thus reduced for the mollusc which uses these metabolites for defence from predators. Tetrodotoxin has been found accumulated for defence:

- in Zozimus aeneus and Atergatis floridus crabs;
- in the Californian salamander Taricha torosa, which expels it from the skin for defence;
- in balloon fishes of the Gimnodontides suborder (Ephippion maculatum); these accumulate it in their eggs and ovaries during the reproductive season.

Other genera and species of Dinoflagellates produce DSP, Diarrhetic Shellfish Poisoning, toxins, the syndrome and poisoning symptoms of which have been described in Table 5; these include:

- dinophysistoxins (ocadic acid, DTX-1, -2, -3; figure 2), isolated from molluscs and algae of the Dinophysis and Prorocentrum genera; they are polyethers derived from a fatty acid with 28 carbon atoms; they can reach man after having been accumulated in molluscs and sponges;
- pectenotoxins (PTX 1 – 7; figure 3), isolated from molluscs (Patinopecten yessoensis);
- macrolides (polyether lactones) of a non-diarrhogenic nature;
- yessotoxin (polyether disulfate; figure 4), produced by the dinoflagellate Protoceratium reticulatum (formerly Gonyaulax grindley, not yet reported in Italian seas; the resistance cysts are ubiquitous) and isolated from molluscs (Patinopecten yessoensis) in Japan, Norway, Chile, New Zealand and Italy; it causes toxic effects, different from the diarrhogenic toxins, which act on the cells of the cardiac muscular fibre, with marked intracytoplasmatic oedema; the family includes seven analogues, of which 45-hydroxyyessotoxin is a metabolite elaborated by the contaminated mollusc and homoyessotoxin is produced by a different algal species;
- prorocentrolide (cyclic macrolide with 27 carbon atoms with a hexahydroxy quinoline; figure 5), produced by the benthonic dinoflagellate Prorocentrum lima in which it is found together with ocadic acid (figure 2).

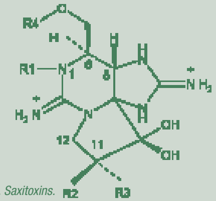


Figure 1. Saxitoxins.

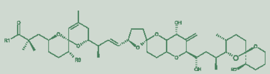


Figure 2. Dinophysistoxins.

	R1	R2	R3
Ocadic acid	OH	H	OH
Dinophysistoxin-1	OH	CH3	OH
7-Deoxyocadic acid	OH	H	H
Etilocadaato	C2H5OH	H	OH

The NSP toxins responsible for Neurotoxic Shellfish Poisoning (table 5) are brevetoxins (figure 6) produced by the dinoflagellate Karenia brevis (formerly Gymnodinium breve) which act as exciter agents, producing a repetitive activation of the nerve axone. The fish kills by brevetoxins are due to the accumulation in the bivalves during blooms and biological magnification in the case of aquatic mammal kills (Trichechus manatus and Tursiops truncatus).

Other neurotoxins are the ciguatoxins responsible for the ciguatera syndrome characterized by symptoms of paresthesia, neurological and gastrointestinal disorders (table 5). They are produced by the tropical benthonic dinoflagellates Gambierdiscus toxicus and Ostreopsis lenticularis. Ciguatoxins include:

- ciguatoxin (figure 7)
- gambierol (figure 8)
- maitotoxin (figure 9)

which are concerned by biological magnification which reaches the food chain. In fact, the dinoflagellates producing toxins are also epiphytes on the microalgae of the coral barrier where they begin biological magnification on a variety of fish of the Pacific Ocean, consumption of which has led to many cases of poisoning in humans being recorded. The ichthyic families most frequently concerned are: Acanthuridae, Balistidae, Belonidae, Carangidae, Carcharinidae, Holocentridae, Labridae, Lethrinidae, Lutjanidae, Mugilidae, Mullidae, Murenidae, Scaridae, Scombridae, Serranidae and Sphyranidae.

Domoic acid is responsible for Amnesic Shellfish Poisoning, ASP (table 5; figure 10) produced by algae of the Pseudonitzschia genus, which has an excitotoxic effect and induces amnesia in the anterograde memory with a permanent effect in the over sixty-fives. Due to the phenomenon of biological magnification, the consumption of molluscs with a content of domoic acid greater than 20 mg/g of mollusc is prohibited; it takes 18 days or more in uncontaminated seawater to purify the molluscs (Nijjar and Nijjar. 2000).

Allergic contact dermatitis, which is announced first by intense irritation followed, within 3-8 hours, by a conspicuous erythema on the exposed skin and which then evolves into erosive forms, are caused by contact with the tropical marine Oscillatoriaceae producers of aplysiatoxins; these also have a tumorigenous activity due to the activation of protein kinase C (table 5). Twelve aplysiatoxins have been isolated

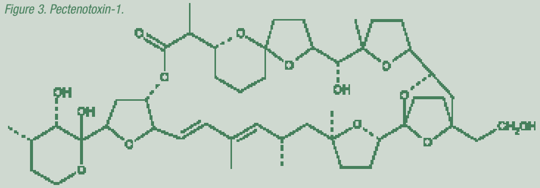


Figure 3. Pectenotoxin-1.

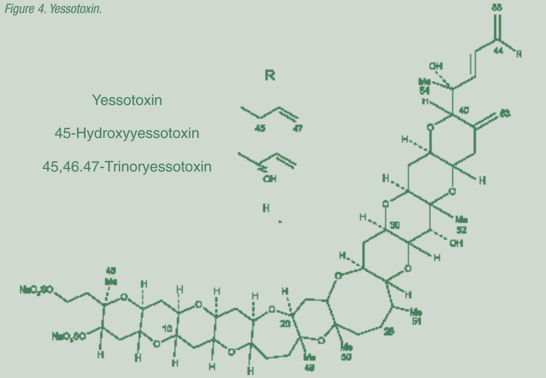


Figure 4. Yessotoxin.

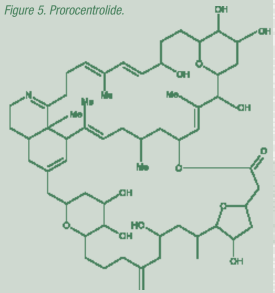


Figure 5. Prorocentrolide.

to date, divided into two classes:

- alkaloids of the indole, such as lyngbyatoxin
- polyacetates, such as aplysiatoxin and debromoaplysiatoxin.

The Oscillatoriaceae grow as epiphytes on macroalgae (Acanthofora spicifora, Laurencia intermedia and Laurencia okamurai) eaten by the coastal populations of Hawaii and Japan for whom aplysiatoxins are a risk factor. Moreover, they also reach the human food chain through the phenomenon of biological magnification by herbivorous fish and molluscs (Siganus fuscescenses, Stylocheilus longicauda and Aplysia kurodai).

To complete the picture of the possible aspects connected with algal blooms in marine environments, discussed so far with reference to algal toxins, the syndromes and symptoms of poisoning that they can cause in man directly or through the phenomenon of biological magnification, two other phenomena are to be reported: "red tides" and the transfer of toxic algae, in the state of resting cysts, in geographically different habitats through water in the holds of cargo ships.

The "red tides" occur when the warmer surface water rises by heating or due to the supply of freshwater on top of deeper waters more rich in nutrients. In this case, the fast-growing algae are reproduced, exhausting the nutrients in the superficial layers and saving the nutrients that are below the pyknocline. Only mobile algae, such as the dinoflagellates (with a migratory capacity of up to 10 m/day), can have access to this deep layer, reproducing until producing coloured blooms (with 20 millions of cells/litre or more) which rise in the day to reach conditions of greater heat and light in the surface waters, although poor in nutrients (Bruno and Melchiorre, 2000).

The toxic algal species can spread in coastal territories other than those of origin through the transfer of resting cysts in the hold waters of cargo ships (Bruno and Marchiori, 2002). Indeed, the unloading of goods entails the rebalancing of the ship by means of pumping seawater into the empty holds; this water is then discharged by the cargo into the loading port of new goods. Often the algae transferred this way readapt to their new environment. A recent example of this phenomenon is that of the colonization of the coasts of Liguria by the tropical algae Ostreopsis ovata which has subsequently continued to expand along the costs of Tuscany, Lazio, Puglia, Marche and Emila-Romagna.

The documented evidence on the transfer into the food chain of the phycotoxins produced during algal blooming due to the phenomenon of biological magnification concern, for example, the accumulation of microcystines in the hepatopancreas of edible bivalve molluscs, in the liver of salmon, in the larvae of crabs. Microcystines have also been found in freshwater organisms: mussels, molluscs (Anadonta woodiana), fish (including Cyprinus carpio, Carassius carassius, Hypomenesius transpacificus) and freshwater shrimp (crayfish, Pacifastacus leniusculus) (Chu, 2000).

The provisions taken in this respect aim to prohibit or limit the sale of polluted fishery products or with parameters of reference that are higher than the established requisites of health and hygiene, or implement principles of health protection aimed to guarantee the quality of waters which require protection and improvement to be suitable for fish life. In particular, the measures on the quality of waters. not only seawater and freshwater, but also water for aquaculture for fish and molluscs, are to be found in the Italian Legislative Decree no, 152 of 11th May 1999.

All the laws and regulations regarding fishery products and those for the protection of the aquatic environment are container in the book by Rizzatti and Rizzatti (2001) to which reference should be made for further discussion of the question. However, with specific reference to algae, the following ordinances are recalled.

- Legislative Decree no. 530 of 30th December 1992, which lays down rules for the production and commercialisation of live bivalve molluscs (Lamellibranchia filtering molluscs, i.e. Echinodermata, tunicates and marine gastropods) for direct human consumption or transformation before consumption. This ordinance pays attention to algal biotoxins that have been accumulated by bivalve molluscs through absorption of plankton containing toxins. Attachment A of the decree, which shows the health and hygiene requisites of live bivalve molluscs for direct human consumption, specifies under letters f) and h) below, that they:

"f) must contain algal biotoxins of the PSP type (Paralytic Shellfish Poison) in quantities not exceeding 80 micrograms per 100g of flesh.

h) they must not give a positive response for toxins (Diarrhetic Shellfish Poison) to the analysis methods as per art. 15, section 1, letter e)."

Compliance with these requisites is binding (art. 3):

- for domestic production that takes place in classified zones as follows according to specific requisites established by special regulations: zone A (live bivalve molluscs produced there may be for direct human consumption); zone B (the molluscs can be for direct human consumption only after treatment in a purification or fish farming centre in an area having the microbiological, biological, chemical and physical requisites prescribed for zone A); zone C (the period of fish-farming of bivalve molluscs produced in this area must not be less than two months);
- for production from third countries which must be subjected to health and hygiene inspections when they are imported (art. 11).

The purification systems, where the live bivalve molluscs which fail to meet the requisites in the first analysis under attachment A must be sent, must use exclusively potable water under Presidential Decree no. 236 of

Figure 6. Brevetoxins.

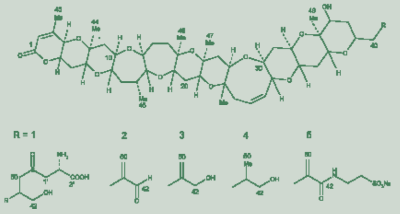


Figure 7. Ciguatoxin.

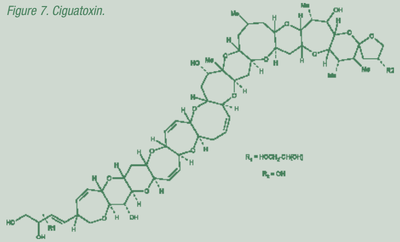


Figure 9. Maitotoxin.

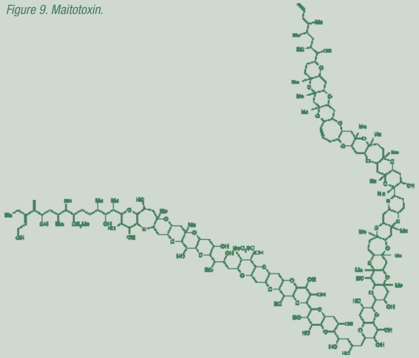


Figure 10.Domoic acid.

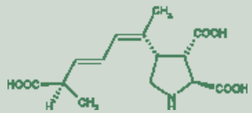


Figure 8. Gambierol.

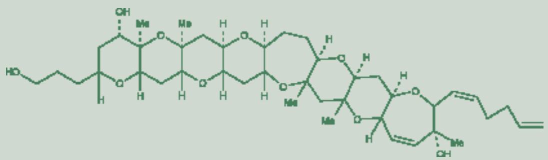
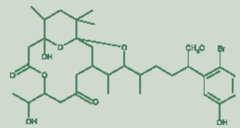


Figure 11. Bromo-aplyasiatoxin
e debromoaplysiatoxin
(formula without Br).



24th May 1988, or systems for the supply of clean seawater (attachment B, point 4).

- Ministerial Decree of 1st September 1990 (Official Journal no. 218 of 18th September 1990), which contains the methods of analysis to determine the algal biotoxins in bivalve molluscs as well as to determine qualitatively and quantitatively the phytoplanktonic populations in seawater for mollusc breeding.
- Legislative Decree no. 531 of 30th December 1992, on the health regulations applicable to the production and commercialization of fishery products. This ordinance prohibits the commercialization (art. 5) of fishery products (all seawater or freshwater animals or parts of these, including their eggs or roe, excluding aquatic mammals, frogs and protected aquatic animals) "containing biotoxins such as ciguatoxin or toxins that paralyse muscles."

Amongst products from the sea, special attention must also be given to algae for direct use as food, such as spirulina and chlorella. Contamination by microcystines or other algal biotoxins released by blooms of Cyanobacteria occur (including Anabaena, Microcystis, Aphanizomenon, Nodularia and Cylindrospermopsis), taking place in the vicinity of the areas of collection of these edible algae. Reports in this respect have been made for blue-green algae (Cyanobacteria) marketed in the form of tablets or capsules and taken as dietary supplements, to which some adverse reactions in consumers have been associated, with complaints of nausea, diarrhoea, asthenia, numbness and formication of the hands and feet (Draisci et al., 2001).

As this represents a potential ranger for the health of consumers, research on the detection of the possible presence of biotoxins in algal products for direct human consumption are particularly required. In this respect, reference is made to the study of some samples of spirulina in tablets or capsules marketed in Rome which were analysed for the possible presence of neurotoxins correlated with anatoxin-a using an SRM (selected reaction monitoring) LC-MS-MS micromethod specially developed (James et al., 1999). The neurotoxins forming the object of the study were: anatoxin-a and homoanatoxin-a and their degradation products, dihydroanatoxin-a, epoxyanatoxin-a, dihydrohomoanatoxin-a and epoxyhomoanatoxin-a. Of these, only dihydroxyhomoanatoxin-a (9-10 mg g-1) and a new isomer of epoxyanatoxin-a (18 and 19 mg g-1) were identified respectively in 3/5 and 2/5 of the samples (Draisci et al., 2001). These studies suggest the importance of checking imported algae for direct human consumption.

A similar problem has affected the commercialization of lyophilised algae from the American Lake Klamath, which, offered as dietary supplements, have proven to be contaminated by phycotoxins from various species. It seems that the same main species, Aphanizomenon flos-aquae, under particular conditions, itself produces saxitoxin toxins (Ecker et al., 1981; Elder et al., 1993; Gentile, 1971; Phinney e Peek, 1961).

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