RESEARCH

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

Parts One

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*"First there was the earth, then the sea,
and then in the sea, life.
From the original life forms were developed
the seaweeds,
Which would provide the sustenance and
shelter for all other life forms to come."
(Anonymous)*

The sea is acknowledged by all as the cradle of life, the primeval soup in which the first cell was born, something indefinable in the passage from the inanimate to animated life and incomprehensible as the beginning of a vital process that in our limitedness we confine between two worlds, the plant world and the animal one. And so, many like to think that a unicellular algae could have had a possible role in this mystery. This could lead to the hypothesis of the oldest fossils found so far in the rocks of the Transvaal, which are 3,200 million years old, through the resemblance of shape between the present-day blue-green algae and the microscopic spheres observed in those rocks by two American researchers, Bartholomew and Lois Anne Nagy (Anzalone and Consonni, 1997).

Algae are certainly amongst the most important vital elements in our ecosystem; for example, of all the other plant species, they are the ones that in the daylight hours provide the greatest supply of oxygen (from 50 to 70%) to the atmosphere that we breathe and the aquatic environment in which they live. In addition, as autotrophic organisms, they represent the first level of the entire food chain as they can photosynthesize from water, carbon dioxide and the energy of the sun the essential elements for their development and survival, but they also represent a source of organic material with a high nutritional and energetic value for all aquatic animals, including the organisms that are symbiotic with themselves (corals, protozoa, sponges and marine worms); in the final analysis, this source is essential, directly or indirectly, for all the other superior organisms that do not have this autonomous capacity of synthesis of nutrients (heterotrophic organisms).

The habitat of algae is a particularly favourable medium for their development, due to the wealth of bioenergy, different nutrients, rare elements and organic-metallic compounds that the algae can store and exploit for all their biological and autotrophic activities. The phenomena that derive from these, including those described, take on surprising importance if, in parallel, account is taken of the fact that the seas and oceans cover three-quarters of the globe's surface, representing an enormous concentrated solution of chemical elements that are constantly taken away from the earth's crust. In addition, the seas and oceans due to their extension also receive the majority of the sun's rays. A significant example of this exceptional vital energy is given by some species of algae (*Macrocystis* spp.) which grow by up to 50 cm. a day, eventually reaching the considerable length of approximately 60 metres.

All in all, this is little known even by the population with an average culture, who continue to consider chlorophyll photosynthesis as the prerogative of green plants only. In addition, in Western countries, where there is no culture regarding the nature and properties of the various types of algae and a tradition of their possible uses, these are generally given little consideration and arouse only some curiosity. The generic terminology of algae itself, (*algae, a*) [1] is used in a holistic way to indicate a world of which very little is known and that is associated mainly with some products of the market of undefined usefulness and in a more negative sense to the macroscopically more visible phenomena of eutrophication, algal blooms and mucilage, i.e. those expressions typical of a considerable deterioration of the environment and of seawater and freshwater environments. The unpleasant sensation of the sight or physical contact with algae and mucilage when bathing in the sea or walking on the beach, along the banks of watercourses or ponds that are polluted to a greater or lesser degree, is fairly common. Those piles of algae in an advanced state of decomposition with an unpleasant odour lead us to consider these organisms as something to be kept away from, not only as far as the negative effects on the environment are concerned, but also regarding human health.

This negative vision has ancient roots in several regions of the Western world and even Virgil and Horace stated that "*Haud est vilior alga*". Recalling then, the ten Biblical calamities at the time of the Pharaohs, at least for six of these (water changed to blood, frogs, mosquitoes, gadflies, the death of animals, ulcers) have been hypothesized as possibly caused, directly or indirectly, by the anomalous phenomena of algal blooms, initially only undesirable and then becoming toxic. These could have caused first the extermination of carnivorous fish in the Nile, followed by an increase in the frog population, the carcasses of which would have fostered a considerable development of larvae and insects. The mounds of algae along the banks, dried and appetizing for the animals due to their particular odour, would however have caused their death due to the presence in them of toxins. Moreover, the animals, drinking the waters of the Nile, would also have swallowed the floating scum that would have been particularly rich in algal toxins. The appearance of ulcers and pustules after the contact of the skin with algae could also be traced back to a photo-sensitising action (Volterra, 1997).

In Oriental countries in particular, especially in China and Japan, in central America and in Northern Europe, the coastal populations have a long tradition of making direct use of fresh or dried algae as food. This was already part of the habits and traditions of many ancient peoples; for example, the Aztecs considered algae as a great natural resource, practically ready for use and such as to become fundamental for their sustenance. The nutritional power of algae derives from a chemical composition which is qualitatively and quantitatively very significant, such as proteins and amino acids, fats, carbohydrates and polysaccharides, vitamins, carotenoids, minerals and trace elements. The direct use of algae in the human diet, particularly as an ingredient or seasoning in cooking, is also spreading in Europe, where the collection of wild algae or the production of cultivated algae in controlled basins occurs mainly in France, Ireland and Norway, where it has taken on the characteristics of an industry.

The technologies used, which differ according to the situations and places, include traditional or mechanized systems for the collection of wild or cultured algae, simple manipulations to improve their presentation when they are for direct use in the diet, processing, extraction and more complex transformations for the production of finished products and algal derivatives for widescale distribution in the food, pharmaceutical and cosmetic sectors. These products, considered almost an elitist niche, are presented on the market with a variety of functions, including of a technological type, and nutritional and health properties. In addition, algae represent an important source of chemically defined derivatives (for example hydrocolloids such as alginic acid and alginates, agar, carragenins, or complex raw materials and preparations that have important uses in various sectors. An overview of the various uses,

[1] *In the etymological meaning, the term algae means marine grass. In the texts in English, other terms are found to indicate algae: frog spittle, water mosses, seaweeds (Bold and Wynne, 1985).*

Table 1. Classification of algae according to the habitat (Bold and Wynne, 1985).

Algae	Definitions
Planktonic algae	They live suspended in plankton [2]; at high concentrations of nutrients, the algae present in plankton multiply very rapidly in the form of algal blooms.
Benthonic algae	They live in depth in the benthos [3]; they can be classified as: - epilithic (they live on stones) - epipelic (they live in mud or in the sand) - epiphytic (they adhere to plants) - epizoic (they adhere to animals).
Neustonic algae	They live in the interface between water and the atmosphere.
Subaerial or supralitoral algae	They grow in the area of spray at about sea level; the more properly aerial ones can be: - edaphic (they grow in or on the ground) - epilithic - epiphytic - epizoic - "corticolous" (they grow on the bark of trees).
Intertidal or eulitoral algae	They are exposed to the atmosphere during low tides
Sublitoral algae	They live constantly submerged and are affected by turbidity; they can grow at depths of up to 100 – 200 m; at the latter depth in clear tropical waters.
Algae under the surface of the ground	They can be seen by dampening the soil and illuminating it or by immersing a sample in a sterile so lution of nutrients under illumination. One role of these algae is to stabilize and improve the physical properties of the soil by providing organic material that encourages the aggregation of various particles. Some filamentous algae are formed on ground without protective layers that prevent erosion.
Desert algae	Algae that are found in the desert; they can be classified as: - endedaphic (they live in the ground) - epidaphic (they live on the surface of the ground) - hypolithic (they live on the lower surface of stones on the ground).
Rock algae	They live in the rocks: - chasmolitic (they penetrate the cracks of the rocks) - endolithic (they penetrate the rock).
Thermophile algae	They grow at temperatures between 50 and 73 °C.

both current and potential, in the various countries can be presented as follows:

- **current uses:**
- edible uses of algae consumed as such by man
- as dry forage for animals
- cosmetic products
- pharmaceutical products
- extraction of hydrocolloids and other chemical substances
- fertilizers
- production of paper
- **potential uses**
- sources of fine chemicals by means of pyrolysis
- sources of energetic compounds for digestion
- treatment of waste water
- fine biochemicals

Some of these applications will be discussed in later articles.

The purpose of this first article, however, is to familiarise the less informed reader with more general concepts and knowledge, presenting the suitable context to consider algae in the role they play in the aquatic macrosystem, in the relations with the phenomena of pollution of freshwater and seawater, environmental safety and risks for human health. In this last regard, the fact that the algae for direct use and the other products based on algae that circulate in Italy are mainly imported from countries not belonging to the European Union, may well motivate a deeper analysis of the relative criteria of quality. Another motive of risk is represented by fish products due to the possible presence of algae biotoxins.

Many algal blooms, reported both in freshwater and in seawater, are of a toxic species and therefore represent an alarming cause of possible health risks. The extermination of aquatic organisms, phenomena of allergies for humans following contact when bathing, dangerous contamination of fish products and algae for direct human use, of water supplies for drinking water and, in the final analysis, of the whole food chain which uses polluted water sources are described. Neurotoxic, hepatotoxic and gastro-intestinal effects of a certain gravity and which can even be fatal at times in the exposed populations are also described.

Concerned in particular by the aforementioned algae phenomena are those rivers, lakes, seas or water supplies with a limited water turnover or with a low hydrodynamism and rich in nutrients such as compounds of phosphorus and nitrogen that are the residue of various human activities. The greenhouse effect also contributes to this environmental degradation causing a decrease in the levels of the water supply and therefore an increase in the concentration of nutrients. The problem concerns the whole planet as man is mainly responsible for this situation. The often unmonitored disposal of domestic, urban and industrial waste waters constantly flowing into the various watercourses, finally reaching lakes and the sea and thus causing their pollution[4].

At this stage, an attempt can be made to add a new meaning to the negative aspects of the algae mentioned at the beginning of this introduction, and recognise in these organisms, through their algal blooms even if toxic, a role as sentry to alert us in a macroscopic way that we are exaggerating in continuing to contaminate the aquatic environment.

The consequences are very serious and the solutions that appear, already in part started by the European Union, if they seem obvious in the first analysis, on the operative level are complex, slow and particularly expensive. It may be of use to reflect a little on these points and mention Legislative Decree no. 152 of 11th May 1999, which defines the general discipline for the protection of the surface waters, seawater and subterranean water. This sets 31st December 2016 as the date to reach the objective of environmental quality corresponding to a "*good*" state, but "*by 31st December 2008, each classified body of superficial water or part of it must achieve at least the requisites of the "sufficient" state as per attachment 1*" (art. 5, section 3). The objectives pursued by Legislative Decree 1999/152 (art. 1) are:

a) to prevent and reduce pollution and implement the reclamation of polluted waterbodies;
b) to improve the state of the waters and adequately protect waters for special uses;
c) to pursue sustainable and durable uses of water resources, with priority for drinking water;
d) to maintain the natural capacity of self-purification of waterbodies, as well as the capacity to sustain extensive and highly diversified animal and plant communities.

If these objectives and the rules laid down to implement them appear praiseworthy and encouraging, however the temporal limits foreseen to benefit from a future improvement of the aquatic environment are surprising, because they are the expression of a worrying current state of

[2] *Plankton is the group of plant and animal organisms (including protozoa, celenterates, crustaceans, larval states of vertebrates and other invertebrates) which live constantly suspended in sea and fresh water environments, from the surface to great depths, without coming into relation with the bed for their entire vital cycle or part of it. The organisms of plankton have a poor capacity of autonomous movement and their movements are generally due to the movement of the currents and winds. Surface plankton consists mainly of unicellular algae.*

[3] *The benthos is the whole of the aquatic organisms that remain constantly in more or less close relation with the sea bed; the sessile, crawling, ambulant or natant benthos are distinguished, depending on whether the organisms live on the bed or crawl, walk or swim on it.*

[4] *The term is thus defined by Legislative Decree no. 152 of 11th May 1999 (Charter I, art. 1, section 2, letter z): "Pollution: the discharge made directly or indirectly by man into the water environment of substances or energy the consequences of which are such as to endanger human health, harm living resources and the water ecological system, compromise the attraction or obstacle other legitimate uses of the waters".*

Table 2a. Classification of algae and their significant characteristics: common name, pigments and plastids, reserve products (Bold and Wynne, 1985).

Divisions	Common name	Pigments and plastids in photosynthetic species	Reserve products
Cyanophyta (Cyanobacteria)	Green and blue-green algae	chlorophyll a; c-phycocyanine; allophycocyanine; c-phycoerythrin; β-carotene; various xantophylls	Granules of cyanophycine (arginine and aspartic acid); polyglucose (similglucose)
Prochlorophyta		chlorophyll a, b; various carotenoids, of which β-carotene and zeaxanthin are prevalent	starch-similar
Chlorophyta	Green algae	chlorophyll a,b; α-, β- and γ-carotene and various xantophylls; 2-5 thylakoids/starch	starch (amylose and amylopectin) (oil in some)
Charophyta	Stoneworts	chlorophyll a, b; α-, β- and γ-carotene and various xantophylls; variously associated thilakoids	starch (amylose and amylopectin)
Euglenophyta	Euglenoids	chlorophyll a, b; α-, β- and γ-carotene and various xantophylls; 2-6 thilakoids/starch, sometimes many	paramylon (β-1,3-glucopyranoside), oil
Pheophyta	Brown algae	chlorophyll a, c; β-carotene and fucoxanthin and various other xantophylls; 2-6 thilakoids/starch	laminaran (β-1,3-glucopyranoside preponderant); mannitol
Chrysophyta	Yellow and golden algae (including diatoms)	chlorophyll a, c (c absent in some); α-, β- and ε-carotene and various xantophylls, including fucoxanthin in Chrysophyceae, Bacillariophyceae and Prymnesiophyceae; 3 thilakoids/starch	chrysolaminaran (β-1,3-glucopyranoside preponderant); oil
Pyrrhophyta	Dinoflagellates	chlorophyll a, c; β-carotene and various xantophylls; 3 thilakoids/starch	starch, α-1,4-glucan (oil in some)
Chryptophyta	Crypto-monadines	chlorophyll a, c; α-, β- e ε-carotene; specific xantophylls (alloxanthin, crocoxanthin, monadoxanthin); phycobilin; 2 thilakoids/starch	starch, α-1,4-glucan
Rhodophyta	Red algae	Chlorophyll a (d in some Florideophyceae); R- e C-phycocyanine, allophycocyanine; R- and B-phycoerythrin; a- and b-carotene and various xantophylls; non-associated single thilakoids	Floridean starch (similar to amylopectin)

deterioration and pollution of waters, with consequences for our algae as well.

NOTES ON THE CLASSIFICATION OF ALGAE

According to systematic botany, algae are placed between the tallophytes (together with schizomycetes or bacteria, mixomycetes, fungi and lichens), the external morphology of which is very highly differentiated from group to group, whilst the internal structure, unicellular or multicellular, is very simple.

Algae do not have a vascular system, flowers or roots and their vegetative body cannot be separated into stem and leaves. The special characteristics that differentiate them in particular from other green plants are linked to their method of reproduction (Bold and Wynne, 1985):

- in unicellular algae the organisms themselves can act as gametes;
- in some pluricellular algae the gametes can be produced in a special unicellular or "gametangium" structure;
- in others the "gametangium" is multicellular and each cell, as it is fertile, represents a gamete.

The rough classification and the relative common name take into account the colour given by special pigments present in the algae: blue-green, green, brown, yellow and red algae. Scientific classification comprises ten divisions for algae (Bold and Wynne, 1985):

- Cyanophyta or Cyanobacteria[5] (Blue-green algae)
- Prochlorophyta
- Chlorophyta (Green algae)
- Charophyta
- Euglenophyta
- Pheophyta (Brown algae)
- Chrysophyta (Yellow algae)
- Pyrrhophyta
- Cryptophyta
- Rhodophyta (Red algae).

This classification, in addition to the pigmentation, mainly takes into account the morphological differences and partially other phytochemical and biological criteria that include the reproductive characteristics and the features of the vital cycle. This is then followed by a long and complex sub-division into classes, orders, families, genii and species, which cannot be shown here. We will only list the orders and families (in brackets) of the blue-green, brown, green and red algae as these are the commonest, including for the purposes of this series of articles:

- Blue-green algae or Cyanophyta or Cyanobacteria: 1. Chroococcales; 2. Chamaesiphonales (Pleurocapsaceae; Dermocarpaceae; Chamaesiphonaceae); 3. Oscillatoriales (Oscillatoriaceae; Nostocaceae; Scytonemataceae; Stigonemataceae; Rivulariaceae).
- Green algae or Chlorophyta: 1. Volvocales (Polyblepharidaceae; Chlamydomonadaceae; Phacotaceae; Volvocaceae; Astrephomenaceae; Spondylomoraceae); 2. Tetrasporales (Palmellaceae; Tetrasporaceae); 3. Chlorococcales (Chlorococcaceae; Characiaceae; Protosiphonaceae; Characiosiphonaceae; Hydrodictyaceae; Oocystaceae; Scenedesmaceae); 4. Chlorosarcinales (Borodinellaceae); 5. Ulotrichales (Ulotrichaceae; Microsporaceae; Cylindrocapsaceae); 6. Sphaeropleales (Shaeropleaceae); 7. Chaetophorales (Chaetophoraceae; Aphanochaetaceae; Coleochaetaceae); 8. Trentepohliales (Trentepohliaceae); 9. Oedogoniales (Oedogoniaceae); 9. Ulvales (Percursariaceae; Monostromataceae; Ulvaceae; Schizomeridaceae; Prasiolaceae); 11. Cladophorales (Cladophoraceae; Anadyomenaceae); 12. Acrosiphoniales (Acrosiphoniaceae); 13. Caulerpales (Codiaceae; Udoteaceae; Caulerpaceae; Derbesiaceae; Phyllosiphonaceae; Dichtomoswiphonaceae); 14. Siphonocladales (Siphonocladaceae; Valoniaceae); 15. Dasycladales; 16. Zygnematales (Zygnemataceae; Mesotaeniaceae; Desmidiaceae).
- Brown algae or Pheophyta: 1. Ectocarpales (Ectocarpaceae; Ralfsiaceae); 2. Chordariales (Myrionemataceae; Elachistaceae; Leathesiaceae; Chordariaceae); 3. Sporochnales; 4. Desmariestiales; 5. Cutleriales; 6. Sphacelariales; 7. Tilopteridales; 8. Dictyotales; 9. Dictyosiphonales; (Striariaceae; Punctariaceae; Dictyosiphonaceae); 10. Scytosiphonales (Scytosiphonaceae); Laminariales (Chordaceae; Laminariaceae; Lessoniaceae; Araliaceae); 12. Fucales (Fucaceae; Sargassaceae; Cystoseriaceae; Hormosiraceae); 13. Durvillaeales.
- Red algae or Rhodophyta: a) Bangiophycidae sub-class: 1. Porphyridiales (Porphyridiaceae; Goniotrichaceae); 2. Compsopogonales (Compsopogonaceae; Erythropeltidaceae; Boldiaceae); 3. Balgiales (Bangiaceae). b) Florideophycieae sub-class: 1. Batrachospermales (Batrachospermaceae); 2. Palmariales (Palmariaceae); 3. Nemaliales (Achrochaetiaceae; Nemaliaceae; Helminthocladiaceae; Chaetangiaceae); 4. Gelidiales; 5. Bonnemaisoniales (Bonnemaisoniaceae); 6. Cryptonemiales (Dumotiaceae; Gloiosiphoniaceae; Cryptonemiaceae; Kallymeniaceae; Choreocolaceae); 7. Corallinales (Corallinaceae); 8. Gicartinales (Solieriaceae; Gigartinaceae; Phyllophoraceae; Graciliariaceae); 9. Rhodymeniales (Rhodymeniaceae; Champiaceae); 10. Ceramiales (Ceramiaceae; Dasyaceae; Delesseriaceae; Rhodomelaceae).

The forms vary from very simple, in the case of algae made up of a single cell, to a surprising complexity as in the case of the "giant kelps" and "rock weeds"; as a consequence, the dimensions range from a few mm (for example, 2 – 8 mm in chlorella) to approximately 60 metres in *Macrocystis*. Typical expressions associated with the form of the algae are, for example (Bold and Wynne, 1985):

- unicellular
- colonial
- filamentous
- membranous or leaf-shaped
- tubular
- lamina-shaped
- root-shaped
- stem-shaped
- leaf-shaped
- phloema- or book-shaped

Numerous characteristics and curiosities on algae have been described in a vast range of literature; an attempt at a synthesis is made below (Bold and Whynne, 1985):

- algae are ubiquitous; they are even found in desert areas and prosper in the areas of perennial snow and Antarctic rocks; they live in hot springs and grow at temperatures between 50 and 73.0 °C (thermophile algae); others can live endozoically in various protozoa, celenterates, molluscs and worms and as endophytes or endosymbionts in other plants;
- algae are redistributed in the environment (aquatic, aerial or terrestrial) by being conveyed in various ways by the tides, currents and movement caused by the winds, movements of animals (for example, coleopters and aquatic birds), ships and planes; the air currents transport edaphic algae and those present in dry ditches and on the sides of lakes, at times representing a cause of allergy for humans;
- it is possible to find algae that are still vital even in the dust of domestic environments;
- algae can be classified according to the habitat in which they live (table 1), the botanical and pharmacognostic characters (table 2a and table 2b) and their shape;
- aquatic algae grow in water with a low saline content (< 10 ppm; fresh water) or in seawater with a higher content of solutes (generally 33 - 40 ppt, parts per thousand); extreme values of salinity are found in Laguna Madre in Texas, where salinity reaches 100% in the dry seasons, or in the Mountain Lake in Virginia where the total solutes are only 3,6 ppt [6];
- factors limiting the growth of algae are the concentration of dissolved oxygen, carbon dioxide and many other substances, temperature and turbidity; the last characteristic prevents the penetration of light and therefore the photosynthesis and growth of the algae.

[5] *Blue-green algae belong to this division and together with the viruses, bacteria and Prochlorophytae, have the prerogative of being procariots, i.e. their cells do not have a nucleus that is separate from the rest of the cytoplasm; this characteristic makes these organisms different from all the other living organisms, the eucariots, with a nucleus separate from the cytoplasm by a well defined cellular membrane. Blue-green algae are amongst the most diversified and common organisms which, due to their taxonomic and morphological characteristics, lend themselves to a dual classification, deemed appropriate, however, by the scientific community: a) Cyanophytae, as they have a photosynthesizing system of chlorophyll a, which is present in the form of free lamellae (thylakoids) in the cytoplasm, more or less towards the periphery, i.e. not included in the chloroplasts, small bodies typical of all the other algae and eucariotic plants; in addition to this, the function, the structure and the morphology of an algal type allow the application of the taxonomic rules of the International Code of Botanical Nomenclature (I.A.P.T., 1972); b) Cyanobacteria, as they have characteristics of bacteria meeting the principles of the International Code of Bacteria Nomenclature (A.S.M., 1972).*

[6] *According to the saline content, lakes are classified as oligotrophic (< 100 ppm of solutes), eutrophic (greater saline contents), alkaline (pH > 7; hard-water lakes) and acid (pH < 7; soft-water lakes) and characterized by a different flora.*

Table 2b. Classification of algae and their significant characteristics: cell wall, number of flagella and insertions, habitat (Bold and Wynne, 1985).

Divisions	Cell wall	Number of flagella and insertions	Habitat *
Cyanophyta (Cyanobacteria)	α- and ε- diaminopimelic acid, glucosamine, alanine, etc.	absent	fw, bw, sw, t
Prochlorophyta	peptidoglycan	absent	sw
Chlorophyta	cellulose in many (β-1,4-glucopyranoside), hydroxy-proline glucosides; xylanes and mannans; or wall absent; calcified in some	1,2-8 many, equal, apical	fw, bw, sw, t
Charophyta	cellulose (β-1,4-glucopyranoside); some calcified	2, equal, subapical	fw, bw
Euglenophyta	absent	1,3 (-7) apical, subapical	fw, bw, sw, t
Pheophyta	cellulose, alginic acid and sulphated mucopolysaccharides (fucoidane)	2, unequal, lateral	fw (very rare), bw, sw
Chrysophyta	cellulose, silica, calcium carbonate, mucilaginous substances and sometimes chitin; or wall absent	1-2, unequal or equal and apical	fw, bw, sw, t
Pyrrhophyta	cellulose or absent; mucilaginous substances	2, one trailing, one equatoria	fw, bw, sw
Chryptophyta	absent	2, unequal subapical	fw, bw, sw
Rhodophyta	cellulose, xylanes, various sulp hated polysaccharides (galactanes) calcifications in some; alginates in Corallinaceae	absent	Fw (some), bw, sw (the majority)

* fw = freshwater; bw = brackish water; sw = sea water; t = terrestrial: soil, rocks, etc..