

Report on Alkaline Extraction of Aquatic Plants

Introduction

This report is intended to provide the information necessary to carry out this task:

“OMRI is specifically interested in established parameters that set the limits for alkali concentrations needed for actual extraction versus levels beyond which extraction is replaced by nutrient fortification by the alkali and a pH buffer.”

In other words, to answer the question:

What parameter(s) and threshold(s) establish the minimum amount of potassium hydroxide required to extract the active constituents of aquatic plant products?”

In order to provide technically defensible answers to these questions, this review explores the published literature on aquatic plant extracts;¹ sales literature, promotional materials, and websites from suppliers; and information provided by manufacturers to OMRI for review of their products. The review has attached an annotated list of Web sites and publications, and includes abstracts where these were available.

Peer-reviewed scientific literature on the subject is sparse. Articles in scientific journals, reports from scientific meetings, and reviews by reputable scientists (Blunden 1991, Chapman & Chapman 1980), (McHugh 1987, 2003) largely repeat the same information about how commercial aquatic plant extracts are made, much of it fragmentary accounts based on limited information provided by producers. What little is published on the use of aquatic plants offers scant substantive information on agricultural applications. Non-scientific articles and books that refer to the use of aquatic plants as agricultural inputs generally lack critical appraisal.

But before the above question can be answered, it is necessary to pose certain fundamental questions that examine the validity of the premises underlying the use of aquatic plant extracts. I have tried to provide answers to these questions.

What, if any, positive agricultural production responses result from the application of aquatic plant extracts?

Beneficial effects of aquatic plant extracts on crop plants were first reported in 1949, when Maxicrop was introduced as the first commercial product (Yvin 1994). Beneficial effects have usually been attributed to the content of essential trace elements, plant growth regulators (auxins, gibberellins, cytokinins, betaines), and complex

¹ [‘Aquatic plant extracts’ is a generic term chosen for the purpose of the study, taken from the term used in the National Organic Program Rule at 7 CFR 205.601\(j\)\(1\). This review covers mostly species of the families Laminariaceae and Fucaeeae. Other terms used in the literature include ‘algal preparations,’ ‘kelp extracts’ or ‘seaweed extracts.’ Such descriptors may not be taxonomically correct.](#)

polysaccharides in the extracts. Promotional materials also sometimes mention vitamins and enzymes, but these are not regarded as significant by scientific researchers.

How is the agricultural value of aquatic plant extracts assessed?

As is typical of agricultural research, the experimental studies of the effects of aquatic plant extracts vary widely in respect to the kind of tests performed. Researchers have used analytical methods such as mass spectrometry or HPLC to identify and quantify plant growth regulators. Plant growth regulators are also the subject of bioassays that use extract fractions or whole extracts using callus cultures or crop plants. The results from application of known plant growth regulators are compared with results from application of aquatic plant extracts.

Conventional agronomic tests vary in respect to:

- Sources of aquatic plants;
- Extraction or other preparation procedure for the aquatic plants;
- Terrestrial plants on which the extracts are tested;
- Physiologically significant characteristics assessed;
- Economically significant parameters;
- Soil or media conditions.
- Scale of tests, laboratory, greenhouse, field

The sources and extraction procedures are the most important considerations for this study: what aquatic plants were harvested, how they were prepared, geographical locality and on what crop they were used and way they were applied: foliar, root dip, soil drench; dilution and frequency of application. Most of the tests were on arable crops. Significant effects reported include growth rate, yield, number of shoots, root mass, and resistance to insect or fungal damage.

The literature provides an incomplete and fragmentary picture of the function and agricultural value of aquatic plant extracts. A recent review (Edmeades, 2002) of field trials of the efficacy of “natural” liquid fertilizers, including 15 derived from seaweeds, deserves careful consideration: statistical analysis of these published results showed that the distribution of positive and negative responses is consistent with a conclusion that none of these “natural” products have been shown to be significantly effective. It is concluded that the numerous published reports of positive effects are based on much higher doses (10, 100, even 1000-fold higher) than could be considered economical for most field applications, as well as invalid statistical evaluation of results. The argument is made that the aggregate of studies are consistently biased in that researchers are

discouraged from publishing negative results, which are viewed as being of no interest, and so often only positive results are reported.

What factors might affect the effectiveness of aquatic plant extracts?

Possible causes for the unexplained variation and error in estimating the efficacy of aquatic plant extracts include:

- Use of different, perhaps distantly related, or even unspecified species;
- Growth stage, timing of harvest, and post-harvest handling of the aquatic plant;
- Consistency of product processing, storage, and shipment conditions

Younger plants are likely to be richer in plant growth regulators, but lower in phenolics and polysaccharides (Featonby-Smith & Van Staden 1984). Hormone levels may vary by the season, or even by the stage of the lunar cycle (cf. Hofman et al. 1986) during which the aquatic plant was collected.

Because some of the hormones are labile, handling methods may make a significant difference in the levels of biologically active constituents in the extract (Stirk et al. 2004). Significant variables are the age of the harvested aquatic plant, whether or not it is processed promptly while fresh, and storage conditions if it is not processed fresh. If the plants are dried, the temperature and methods used to dry (freeze-dried, slowly sun-dried, or dried quickly in an oven) may result in degradation of hormonal activity. Some processes include freezing before further processing. Quality assurance and quality control procedures can also make a significant difference, and manufacturers may change processing methods or rename products over time. Some distributors may repackage aquatic plants from as many as three different sources under a single label. Because the chain of custody from where the aquatic plant products are harvested to where they are applied is not always maintained, research results often cannot be linked to a specific source or processing method.

Is alkali extraction necessary to produce an effective aquatic plant extract?

Commercial aquatic plant extracts may be prepared by alkali digestion of aquatic plant, usually followed by neutralization and clarification of the resulting digest, or without alkali, using mechanical disruption or alternatively, freezing, pulverization, and clarification of the thawed slurry (Verkleij 1992). Both alkali and non-alkali extracts have been reported repeatedly to have beneficial effects on crop plants. However, few studies directly compare different extracts, and these generally have shown some differences in effects among different alkali as well as different non-alkali extracts, as well as between alkali and non-alkali products (Stirk & Van Staden 1997). But studies have not demonstrated consistent differences between the effects of alkali and non-alkali extracts, or explained the underlying mechanisms that are responsible for the observed differences.

The incomplete understanding of the mechanisms that appear to cause the beneficial effects of aquatic plant extracts leaves open the possibility that at least some differences exist in the mechanisms underlying the actions of alkali and non-alkali extracts. The diversity in test results of these extracts may result from one mechanism being more effective than another in given situation, but under different conditions the results could be very different. Both kinds of extracts thus might have value, depending on the conditions of use.

Conclusion: Although both alkali and non-alkali extracts may have some value as crop treatments, it is not clear if the latter can provide responses comparable to alkali extracts under all uses and conditions, based on currently available information.

What is the minimum potassium and phosphorus that alkali-extracts of kelp aquatic plant must contain if an effective product is to result?

Most kelp aquatic plant extract manufacturers who use an alkali extraction hydrolysis appear to use potassium hydroxide as the primary alkaline reagent for their digests. Possible alternative alkali reagents are sodium hydroxide, calcium hydroxide, or sodium carbonate. Potassium salts are more expensive than sodium salts, but sodium is objectionable because plants are adversely affected by excess sodium, and calcium salts are prone to form precipitates.

Alkali digestion may be performed at ambient temperatures and pressures, or at elevated temperatures and pressures (autoclave conditions). Some manufacturers maintain that higher-temperature processing reduces the yield of desirable plant growth regulators, so digestion at ambient temperatures requires higher concentrations of alkali to achieve complete digestion. Unfortunately, there have been no published studies focused on comparing extracts produced with different concentrations of alkali, nor digestion at hot versus ambient temperatures. One recent study showed that storage of one commercial aquatic plant extract at 54 °C for 14 days increased the cytokinin content whereas auxin content decreased, indicating that process temperature may indeed have significant, if not always entirely expected, effects (Stirk et al. 2004).

Most manufacturers reduce the high pH of their alkali extracts with acid, most often phosphoric acid, although citric acid may also be used (cf. Acadian Seaplants Liquid Seaweed Concentrate, as stated in OMRI product file). One form of the most widely-used extract product (Maxicrop) is not neutralized, a 25% solution exhibiting a pH of 9-10, although other forms of the product have neutral pH, suggesting that neutralizing the extract is not essential (Stirk & Van Staden 1997). Again, there have been no studies published based on experiments designed to compare high-pH versus neutralized alkali extracts.

Conclusion: Aquatic plant extracts made without alkali treatment, alkali-extracted high-pH extracts, and alkali extracts that have been acid-neutralized, all reportedly have value as crop treatments. However, based on the information currently available, it is not possible to establish the necessity of alkali (potassium hydroxide) in the making of

extracts, nor the necessity of neutralizing with phosphoric acid the resulting high pH. Consequently, it is also not possible to establish a threshold between “extraction” of the active constituents found in aquatic plant products, and “fortification” with the synthetic fertilizer elements (potassium and phosphorus) used in the extraction process.

Suggestions for obtaining definitive answers to the outstanding questions:

The meta-analysis by Edmeades (2002) concluded that even though aquatic plant extracts unquestionably contain plant nutrients and growth regulators, no studies have been done that clearly demonstrate that the extracts have any practical value when they are applied at the levels advocated by the manufacturers, or levels that would be economically justifiable based on the cost of the extracts. A large-scale study (multiple extract products, including pH-neutralized alkali-extracted, non-neutralized alkali-extracted, and non-alkali extracted, applied to various crops) would be required to definitively answer this question. Such a study would have to be carried out by reputable agricultural scientists in order for its results to be widely accepted. It is questionable whether financial support could be found for such a study, in part because university and government agriculture researchers might well be reluctant to advocate the necessity of such a study in light of the doubtful prospect of useful effects being demonstrated, and extract manufacturers and their advocates also might well fear that lack of effects would be clearly demonstrated. The question of the significance of the concentrations of plant growth regulators and nutrients in the extracts need not even be addressed unless efficacy is first demonstrated, but of course the necessary data could be collected by analysis of the products used in the tests.

References

This is a list of the most relevant references that I was able to find. Abstracts are included where they were available, and I have annotated them with boldface and underlining to help the reader find the most relevant information.

Each reference is predated by a number that indicates:

- 1) reference cited in this report
- 2) reference that I read but did not cite in the first draft, but is cited in this report
- 3) reference that I read but did not cite in this report because it was not relevant
- 4) reference that I did not read, usually because the age of the publication suggested obsolescence, or the title or abstract did not indicate relevance or any information not contained in more recent references

General references

2)
Blunden G .
Agricultural uses of seaweeds and seaweed **extracts**
In, Guiry, M. D. and G. Blunden (Ed.). *Seaweed Resources In Europe: Uses And Potential*. xi+432p. John Wiley and Sons, Inc.: Somerset, New Jersey, USA; Chichester, England, UK.

2)
Chapman, V. J. and D.J. Chapman
Seaweeds and their uses.
Chapman and Hall ; New York : Chapman and Hall in association with Methuen, 1980. 3d ed.

4) Fryer, Lee, 1908-
Food power from the sea : the seaweed story / Lee Fryer and Dick Simmons ; ill. by Christine Becker.
New York : Mason/Charter, 1977.

2)
McHugh, Dennis J.
A guide to the seaweed industry / by Dennis J. McHugh.
Rome : Food and Agriculture Organization of the United Nations, 2003.
FAO fisheries technical paper, 0429-9345 ; 441

4)
McHugh Dennis J. (ed.)
Production and utilization of products from commercial seaweeds
Rome : Food and Agriculture Organization of the United Nations, 1987.

Journal and symposium articles

4)

Brain, Keith R.; Lines, D. S.; Booth, M.; Ansell, G.
Enhancement of herbicidal effect by seaweed **extracts**.
NATO Conference Series IV: Marine Sciences (1977), (Mar. Nat. Prod. Chem.), 345-50.

The herbicidal activity of mecoprop [7085-19-0] against *Stellaria media* was enhanced by seaweed ext., by a mechanism probably involving cytokinins. Kinetin [525-79-1] and alginate [9005-32-7] similarly stimulated the effect of mecoprop. The uptake of mecoprop by *S. media* was stimulated by seaweed ext., by methylcellulose [9004-67-5] and by alginate, and was inhibited by kinetin.

4)

Blunden G; Challen S B; Woods D L
Seaweed **extracts** as fertilisers.
Journal of the science of food and agriculture (1968 Jun), 19(6), 289-93.

4)

Blunden, Gerald; Jenkins, Teifryn; Liu, Yan-Wen.
Enhanced leaf chlorophyll levels in plants treated with seaweed **extract**
Journal of Applied Phycology. 8(6). 1997. 535-543.
Application to the soil of an aqueous **alkaline extract** of *Ascophyllum nodosum* resulted in higher concentrations of chlorophyll in the leaves of treated plants in comparison to control plants treated with an equivalent volume of water. Positive results were obtained with all species tested (tomato, dwarf French bean, wheat, barley, maize). When the seaweed **extract** was applied as a foliar spray, similar effects on leaf chlorophyll contents were obtained, except in the case of dwarf French bean plants, for which no significant difference was recorded between test and control plants. When the betaines present in the seaweed **extract** were applied as a mixture in the same concentrations as those in the diluted seaweed **extract** (gamma-aminobutyric acid betaine 0.96 mg L⁻¹, delta-aminovaleric acid betaine 0.43 mg L⁻¹, glycinebetaine 0.34 mg L⁻¹), very similar leaf chlorophyll levels were recorded for the seaweed **extract** and betaine treated plants. This suggests strongly that the enhanced leaf chlorophyll content of plants treated with seaweed **extract** is dependent on the betaines present.

4)

Chapman, V J. ; Whitton, J S. ; Goh, K M.
Kelp **extract** as fertilizer.
N Z J Sci 19, no. 2: 213-214. (1976)

4)

Crouch, IJ; Van Staden, J
Effect of seaweed **concentrate** on the establishment and yield of greenhouse tomato plants.
Journal of Applied Phycology vol. 4, no. 4, pp. 291-296, 1992

Application to the soil of an aqueous **alkaline extract** of *Ascophyllum nodosum* resulted in higher concentrations of chlorophyll in the leaves of treated plants in comparison to control plants treated with an equivalent volume of water. Positive results were obtained with all species tested (tomato, dwarf French bean, wheat, barley, maize). When the seaweed **extract** was applied as a foliar spray, similar effects on leaf chlorophyll contents were obtained, except in the case of dwarf French bean plants, for which no significant difference was recorded between test and control plants. When the betaines present in the seaweed **extract** were applied as a mixture in the same concentrations as those in the diluted seaweed **extract** (gamma-aminobutyric acid betaine 0.96 mg L⁻¹, delta-aminovaleric acid betaine 0.43 mg L⁻¹, glycinebetaine 0.34 mg L⁻¹), very similar leaf chlorophyll levels were recorded for the seaweed **extract** and betaine treated plants. This suggests strongly that the enhanced leaf chlorophyll content of plants treated with seaweed **extract** is dependent on the betaines present.

4)

Durand, Nathalie; Briand, Xavier; Meyer, Christian.

The effect of marine bioactive substances (N PRO) and exogenous cytokinins on nitrate reductase activity in *Arabidopsis thaliana*. *Physiologia Plantarum* (2003), 119(4)

We investigated the effect of exogenous cytokinins and marine bioactive substances contg. seaweed **exts.** (marketed by the ROULLIER Group under the trade name N PRO) on nitrate reductase activity in *Arabidopsis*. Cytokinins, applied either directly in the growth medium or as a foliar spray, did not significantly influence nitrate reductase activity in **exts.** from in vitro grown *Arabidopsis* plants. Conversely, *Arabidopsis* grown in the presence of or sprayed with N PRO had increased nitrate reductase activity. This stimulatory effect of N PRO was even higher when the plants were grown on low nitrate concn., suggesting that these marine bioactive substances may be beneficial for plant growth in adverse nutritional conditions.

1)

Edmeades, D. C.

The effects of liquid fertilisers derived from natural products on crop, pasture, and animal production: A review
Australian Journal of Agricultural Research. 53(8). 2002. 965-976.

The results from field trials measuring the effect of liquid fertilisers derived from organic materials on crop yields are summarised and reviewed. Trials comparing the efficacy of 26 specific products and 2 unnamed generic products were identified. Of these 28 products, 15 were derived from seaweed, 4 from fish waste, 5 were of vegetable origin, and 2 were from animal products. Cereals were the most frequently used test crop (328 recorded treatment effects) followed by root crops (227), legumes (88), pastures (59), and vegetables (52). Fifty-three other treatment effects were recorded on crops such as rape (15), peanuts (8), tobacco (6), and miscellaneous other crops (25). The effects of liquid fertilisers on animal performance were measured in 4 trials. The observed effects of these products on a wide range of crops were normally distributed about zero with an equal number of positive and negative 'responses'. The frequency of statistically significant events, both positive and negative, was consistent with probability theory, assuming that the products are ineffective. The range of observed effects are also

consistent with the normal variability associated with field trial experimentation, taking into account the odd intrusion of other experimental errors. There was no evidence to support the conclusion that at least some product-types or products were effective on some crop-types, or cultivars. Similarly, liquid fertilisers had no effect on animal production when applied as recommended. This conclusion, based on the field evidence, was consistent with, and could be predicted from, independent evidence showing that these products do not contain sufficient concentrations of plant nutrients, organic matter, or plant growth substances (PGSs) to elicit increases in plant growth when applied as recommended.

2)

Featonby-Smith, BC; Van Staden, J

Identification and seasonal variation of endogenous cytokinins in *Ecklonia maxima* (Osbeck) Papenf.

Botanica Marina vol. 27, no. 11, pp. 527-531, 1984

Tentative identification of the cytokinins present in a seaweed concentrate prepared from *E. maxima* revealed the presence of cis and trans ribosylzeatin, trans zeatin, dihydrozeatin and N⁶(Δ^2 -isopentenyl)adenosine. Seasonal changes in the cytokinin levels were determined. Both qualitative and quantitative variations occurred. During summer zeatin, ribosylzeatin and their dihydroderivatives were responsible for most of the detected activity. The cytokinin glucosides increased above the levels of free cytokinins during winter.

4)

Ferreira, M. I.

The efficacy of liquid seaweed **extract** on the yield of canola plants. South African Journal of Plant & Soil. 19(3). 2002. 159-161.

The efficacy of auxin/cytokinin (Kelpak) on its own and in a mixture with the herbicide clopyralid (Lontrel 100), for post-emergence application at various growth stages in canola (*Brassica napus*) was assessed. A **Kelpak** foliar application at 2 l ha⁻¹ applied at the four leaf stage gave a significant increase in yield at Langgewens in 1999 compared to the control. At Tygerhoek, Kelpak at 2 l ha⁻¹ significantly increased canola yields when applied at either the three leaf or five leaf growth stage of canola, but the highest yield, was obtained by Kelpak at 2 l ha⁻¹ at the three leaf average growing stage of canola.

1, 2)

Hofman, PJ; Featonby-Smith, BC; van Staden, J

The development of ELISA and IRA for cytokinin estimation and their application to a study of lunar periodicity in *Ecklonia maxima* (Osbeck) Papenf.

Journal of Plant Physiology vol. 122, no. 5, pp. 455-466, 1986

Changes in the cytokinin-like activity in the seaweed *Ecklonia maxima* during a lunar cycle was determined. Bioassays were performed on lamina, stipe and holdfast material individually. For a more detailed investigation on the cytokinins in the stipe, which is the commercially important component, several immunoassays were developed. An enzyme-linked immunosorbent assay was developed for isopentenyladenosine (IPA), but because of low precision this assay system was not suitable for isopentenyladenine analysis. Sensitive radioimmunoassays were developed for zeatin (Z) and ribosylzeatin (ZR). Bioassay results indicated that cytokinin-like activity in the stipe, lamina and

holdfast material followed similar trends, which suggested a close link to lunar periodicity.

4)

Jeannin I ; Lescure J-C; Morot-Gaudry J-F

The effects of aqueous seaweed sprays on the growth of maize

Botanica Marina. 34(6). 1991. 469-473.

Seaweed **extract** (Goemar GA 14), applied as a foliar spray increased the total fresh matter production of maize [*Zea mays*] seedlings by 15 to 25% over the control. This was reflected in the increase of root and stem mass per plant. However, only minor differences were measured in stomatal resistance just after treatment and in net carbon assimilation rate two weeks after spraying. No other significant differences were observed in gas exchange characteristics. The physiological mechanisms responsible for the beneficial effects of seaweed concentrates on plants and the nature and mode of action of the compounds contained in these concentrates have not yet been elucidated.

2)

Mooney, P. A.; Van Staden, J.

Algae and cytokinins.

Journal of Plant Physiology (1986), 123(1), 1-21.

A review with 148 refs. on use of algal **expts.** as fertilizers in relation to endogenous cytokinins, occurrence and identity of cytokinins in unicellular and multicellular algae, cytokinin formation in relation to phytoeny, and the role of cytokinins in algae growth and development, including cell division and elongation, organogenesis, apical dominance, respiration, and photosynthesis.

4)

Patier, Pascale ; Yvin, Jean-Claude ; Kloareg, Bernard ; Lienart, Yvette ; Rochas, Cyrille .

Seaweed liquid fertilizer from *Ascophyllum nodosum* contains elicitors of plant D-glycanases

Journal of Applied Phycology. 5(3). 1993. 343-349.

The structure of the major component contained in a liquid seaweed **extract** prepared from *Ascophyllum nodosum* (Phaeophyta, Fucales) was investigated. The **extract** was fractionated by gel permeation chromatography, and the various fractions were analysed by GLC, HPLC, ¹³C NMR spectroscopy and mass spectrometry. All fractions were derivatives of the branched beta-D-(1 fvdarw 3) glucan known as laminaran. They were capable of eliciting D-glycanase activities (laminaranase, alpha-amylase) in *Rubus fruticosus* suspended-cell cultures.

4)

Steveni C M ,Norrington-Davies J , Hankins S D .

Effect of seaweed concentrate on hydroponically grown spring barley

Journal of Applied Phycology. 4(2). 1992. 173-180.

Spring barley (*Hordeum vulgare* cv. Triumph) was grown hydroponically over a 6-week period. Two treatments were incorporated either into the hydroponic solutions or sprayed onto the plants at rates of 1 ml per 3 litres. The treatments applied were: (i) a seaweed **concentrate** prepared from *Ascophyllum nodosum* (L.) Le Jolis (marketed as Maxicrop Triple), (ii) a 'Trace element' treatment incorporating the micro and macro nutrients added to the seaweed **extract** base to produce the formulated product Maxicrop Triple and (iii) a control treatment. Irrespective of

the mode of application, plants treated with Maxicrop Triple grew faster than plants under either of the two other treatments. Elevated growth rates were also found for the 'Trace element' treated plants when incorporated into the hydroponic solution. At the final harvest, plants with Maxicrop incorporated into the hydroponic solution showed increases from 56-63% over the control treatment for the growth characteristics measured. 'Trace element'-treated plants produced increases of between 25-45%. When the treatments were sprayed the effect was less pronounced. Maxicrop Triple increased growth characters by 35-38% and the 'trace element' treatment gave increases in the range of 2-13%.

2)

Stirk, W. A ; Van Staden, J.

Comparison of cytokinin- and auxin-like activity in some commercially used seaweed **extracts**

Journal of Applied Phycology. 8(6). 1997. 503-508.

Six commercially used seaweed **extracts** were tested for cytokinin- and auxin-like activity using the soybean callus bioassay and the mung bean rooting bioassay respectively. All the seaweed **extracts** tested showed cytokinin-like activity. Seamac caused the most callus growth with activity being concentrated at R-f 0.9 which co-chromatographed with iso-pentenyladenine and its riboside. The other five seaweed **extracts** yielded activity at R-f 0.7-0.8. This co-chromatographed with zeatin and zeatin riboside. All the seaweed **extracts** tested improved rooting of mung beans. Kelpak made from *Ecklonia maxima* (Osbeck) Papenf. gave the best rooting response.

4)

Stirk, W. A.; Van Staden, J.

Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L.

Journal of Applied Phycology (1997), 9(4), 327-330.

Marine Adenine Cytokinins (MAC) is a new com. seaweed product made from *Fucus serratus* L. Cytokinins were isolated from this ext. by cation exchange, paper and high performance liq. chromatog. Cytokinins were detected and isolated by bioassay guided fractionation using the soybean callus assay. Trans-zeatin, dihydrozeatin and iso-pentenyladenosine were identified by GC-MS as being the dominant cytokinins present in MAC.

1, 2)

Stirk WA, Arthur GD, Lourens AF, Novak O, Strnad M, van Staden J

Changes in cytokinin and auxin concentrations in seaweed concentrates when stored at an elevated temperature

Journal Of Applied Phycology 16 (1): 31-39 2004

Two seaweed **concentrates** were made from the kelps *Ecklonia maxima* and *Macrocystis pyrifera* using a cell burst method. Cytokinin- and auxin-like activities were measured using the soybean callus and mungbean bioassays, respectively. Cytokinin- like activity was detected in both seaweed concentrates, being equivalent to approximately 50 mug L-1 kinetin. Auxin-like activity was also detected in both concentrates with the *E. maxima* derived **concentrate** having higher biological activity, equivalent to 10(-5)-10(-4) M indole-butyric acid. Two replicates of each **concentrate** were stored at 54degreesC for 14 days to accelerate the effects of storage. Both fresh and stored samples of the

two seaweed concentrates were analysed for their endogenous cytokinin and auxin content. The samples were purified using a combined DEAE-Sephadex octadecylsilica column and immunoaffinity chromatography based on wide-range cytokinin and IAA specific monoclonal antibodies. These **extracts** were analysed by HPLC linked to a Micromass single quadrupole mass spectrophotometer. Eighteen and nineteen different cytokinins were detected, respectively, in the two concentrates, with trans-zeatin-O-glucoside being the main cytokinin present. Accelerated storage of the concentrates caused an increase in the total cytokinin concentration with a large increase in the aromatic meta-topolin. Indole-3-acetic acid was the main auxin in both seaweed concentrates. Indole conjugates, including amino acid conjugates, were also quantified. The total auxin concentration decreased with accelerated storage for both concentrates.

4)

Stirk WA, Novak O, Strnad M, van Staden J

Cytokinins in macroalgae

Plant Growth Regulation 41 (1): 13-24 2003

Thirty-one seaweeds were collected from the warmer KwaZulu-Natal coast and the cooler Cape waters (South Africa). Plant material was extracted with 70% ethanol supplemented with deuterium labelled standards of all known isoprenoid cytokinins. The samples were then centrifuged and purified by combined DEAE-Sephadex3 octadecylsilica column and immunoaffinity chromatography and finally analysed for cytokinins by HPLC-linked mass spectrometry and a photodiode array detector. The cytokinin profiles were similar in all the macroalgae regardless of their taxonomy and growing locality. The main type of isoprenoid cytokinins present were zeatins with cis forms being more common than trans forms and isopentenyladenine (iP) derivatives. Only a few dihydrozeatin-type cytokinins were detected at very low levels in only nine species. Aromatic cytokinins were also present but at lower levels and were represented by benzyladenine (BA) and ortho- and meta-topolin derivatives. The topolins were present in greater diversity and concentrations than BA. For all the cytokinin types, the free bases, O-glucosides and nucleotides were the most common with no N-glucosides being detected and ribosides present at very low levels. The results suggest that different pathways for regulating cytokinin concentrations operate in macroalgae than in higher plants.

4)

Tay, S. A. B.; Palni, L. M. S.; MacLeod, J. K

Identification of cytokinin glucosides in a seaweed **extract**.

Journal of Plant Growth Regulation (1987), 5(3), 133-8.

Zeatin-O-glucoside, its dihydro deriv., and dihydrozeatin riboside-O-glucoside have been identified as the main cytokinin-O-glucosides in Seacol, a com. preparation from Tasmanian giant bull kelp marketed as a liquid org. fertilizer. The anal., which also indicated the presence of zeatin riboside-O-glucoside, was carried out by gas chromatog.-mass spectrometry of the aglucons using the stable isotope diln. method.

4)

Tay, Stephen A. B.; MacLeod, John K.; Palni, Lok Man S.; Letham, David S.

Detection of cytokinins in a seaweed **extract**.

Phytochemistry (1985), 24(11), 2611-14.

trans-Zeatin, trans-zeatin riboside, their dihydro derivs., isopentenyladenine and isopentenyladenosine were identified and quantified in Seasol a com. ext. of Tasmanian giant bull kelp, *Durvillea potatorum*, that is marketed as a fertilizer and soil conditioner.

4)

Temple, W. D.; Bomke, A. A.; Radley, R. A.; Holl, F. B.
Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop growth and nitrogen nutrition under varying soil moisture regimes.

Plant and Soil (1989), 117(1), 75-83.

A greenhouse expt. was designed to test the effects of kelp (*M. integrifolia* and *E. maxima*) **concs.**, when prepd. as foliar sprays, on bean (*Phaseolus vulgaris*) growth and N nutrition under three soil moisture regimes. Plant growth and developmental responses in this greenhouse expt. have demonstrated the effectiveness of the two kelp foliar sprays as plant growth regulating substances. Bean growth and developmental responses to the kelp foliar spray treatments were dependent on the soil moisture regime to which they have been subjected. Although the two kelp foliar sprays had varying and sometimes contrasting effects on bean growth and N nutrition, which were dependent on the soil moisture treatment, their developmental effects upon the no. of nodes, shoot/root ratio, leaf area ratio and specific leaf area were quite similar. A soybean callus bioassay demonstrated the presence of cytokinin-like substances and a callus growth antagonist in the kelp **conc.** Increasing diln. of the kelp **conc.** disproportionately reduced the callus growth antagonist relative to the growth promoting or cytokinin-like activity.

2)

Verkleij F N

Seaweed **extracts** in agriculture and horticulture a review
Biological Agriculture & Horticulture. 8(4). 1992. 309-324.

In biological agriculture and horticulture diluted **extracts** of seaweed are applied to promote growth, prevent pests and disease and improve the quality of the products. The efficacy of the **extracts** is probably based upon plant hormones (mainly cytokinins) and trace nutrients present in the **extracts**. A survey of the recent literature is given concerning the properties of seaweed **extracts**, methods of application, their effects and putative working mechanisms. The significance of seaweed **extracts** in biological agriculture and horticulture is evaluated, regarding environmental aspects of production and use.

4)

Warman, P. R. ; Munro-Warman, T. R. .

Do seaweed **extracts** improve vegetable production?

Fragoso, M. A. C. [Editor], van Beusichem, M. L. [Editor]. Developments in Plant and Soil Sciences; Optimization of plant nutrition. 1993. 403-407. Developments in Plant and Soil Sciences. Vol. 53.

1)

Whapham, CA; Blunden, G; Jenkins, T; Hankins, SD; McLachlan, JL (ed)
Significance of betaines in the increased chlorophyll content of plants treated with seaweed **extract**.

Seaweed **extract**, prepared by alkaline extraction of *Ascophyllum nodosum*, applied either to the soil or to the foliage of tomato plants, produced leaves with higher chlorophyll levels than those of control plants. The effects on leaf chlorophyll content were investigated using a cucumber bioassay procedure devised for cytokinins. The seaweed **extract** was shown to increase the chlorophyll levels of the cucumber cotyledons, but "peaks" of activity were obtained when widely different concentrations were used. The possibility that these effects were the result of predicted from, independent evidence showing that these products do not contain sufficient concentrations of plant nutrients, organic matter, or plant growth substances (PGSs) to elicit increases in plant growth when applied as recommended.

4)

Wu YT, Lin CH

Analysis of cytokinin activity in commercial aqueous seaweed **extract**
Gartenbauwissenschaft 65 (4): 170-173 (2000)

TTC viability staining method as a bioassay for cytokinin activity was refined using the soybean (*Glycine max* L.) suspension cultures by detecting cell viability and plotting a standard curve ($R-2 = 0.98$) and using different concentrations of kinetin in soybean medium as control. When kinetin concentrations are between 0.02 and 2 mg/l, it can improve cell viability. Soybean cell cultures are sensitive to the presence of kinetin. When cytex dilutions are between 5x and 5000x, it can improve cell viability as well. Thus it is concluded that cytex contains the biological activity similar to cytokinin. Establishment of a standard curve ($R-2 = 0.91$) using enzyme-linked immunosorbent assay (ELISA) for a natural cytokinin (DHZR), we found that cytex contains about 6.5 pmole/0.1ml DHZR which shows cytokinin like biological activity in soybean cell culture. The results are consistent with TTC method. We conclude that TTC can be one of the bioassays for cytokinin in suspension cell culture.

1, 2)

Yvin, Jean-Claude.

New approach to the mode of action of seaweed **extracts** in agriculture.
Comptes Rendus de l'Academie d'Agriculture de France (1994), 80(2),
103-12. **written in French**

A review with 20 refs. Seaweed **exts.** used in agriculture are reviewed. After an approach of biol. effects induced on treated plants by the seaweed cream GA 14, the modes of action of such seaweed **exts.** used by means of foliar sprays are discussed. Among the active substances, phytohormones, betaines and, as a new approach, oligosaccharides are described and their resp. biol. functions on plants reviewed.

4)

Zhang, Xunzhong; Schmidt, R. E.; Ervin, E. H.; Doak, S

Creeping bentgrass physiological responses to natural plant growth regulators and iron under two regimes

Hortscience. 37(6). October, 2002. 898-902.

Creeping bentgrass (*Agrostis palustris* Huds.) is an extensively used cool-season grass for fine turf areas such as golf course putting greens, but suffers from poor summer stress tolerance. These studies were conducted to investigate the influences of natural plant growth

regulators (NPGR) and Fe on creeping bentgrass photochemical activity (PA), antioxidant superoxide dismutase (SOD) activity, root growth and leaf color under two fertilization regimes. The bentgrass was maintained in well-watered field conditions or water-stressed glasshouse conditions. A mature bentgrass was treated monthly during the field season with seaweed (*Ascophyllum nodosum* Jol.) extract (SWE) at 50 mgcndotm⁻² or humic acid (HA) at 150 mgcndotm⁻² or in combination with or without FeSO₄ at 520 mgcndotm⁻² and grown under a low or a high fertilization regime. Foliar application of SWE+Fe increased PA (14% to 15%), while applications of SWE+HA or SWE+HA+Fe increased SOD activity (49% to 114%) of creeping bentgrass in Summer 1997 and Summer 1998. There was no significant fertilizationXNPGR interaction for PA and SOD activity. Bentgrass PA was increased by 13% to 46% when treated with NPGR with or without Fe compared to the control measured in May. The addition of Fe with each NPGR application improved fall and winter leaf color. All NPGR and Fe treatments increased root mass (17% to 29%) in Aug. 1997 and 1998, except HA alone in 1998. Under sustained low soil moisture (-0.5 MPa) conditions, application of NPGR with or without Fe increased PA and SOD activity. The data indicate that SWE and HA enhance the physiological function of 'Southshore' creeping bentgrass, resulting in improved root growth regardless of low or high fertilization regime. However, addition of Fe to these NPGR served primarily to improve late season leaf color. The results suggest that, in addition to maintaining adequate plant-nutrients, applications of natural PGRs, such as SWE and HA, prior to and during summer abiotic stresses would be beneficial.

**Product information for aquatic plant extract products on the OMRI lists, http://www.omri.org/crops_generic.pdf and <
http://www.omri.org/crops_alpha.pdf>**

Several of these products seem to have vanished from the market; they are not listed on their manufacturer's Web sites and/or are not found by a Google search. Some manufacturers cannot be found under the names on the OMRI list.

Several products make vague claims as to benefits conferred by their use without making any claims as to specific ingredients/content of the product. Very few Web sites present product label information or a MSDS (seemingly the only source of information about the pH of these products). No manufacturer's Web site offers information about alkali extraction or neutralization with acid, and for many no information at all is presented about how the extract is made.

Although OMRI has product information files about these products, they do not appear on the OMRI lists:

- Acadian Seaplants Liquid Seaweed Concentrate
- Thorvin kelp for Plants

Product	Manufacturer	Species	Extraction method	Alkali / acid	Nutrient / growth regulator claims	Label use claims	Comments
Acadian Organic Powder 0.5-0.3-16, =New Era Organic Powder = TAP Organic Powder	Acadian AgriTech =Acadian Seaplants	Ascophyllum nodosum	Solar dried, ground to powder	No info	K 3% (OMRI analysis 18%)	No info	No info on this product on Web site
Agri-Gro Ultra	Agri-Gro Marketing, Inc.	"kelp"	No info	No info	No info	16-32 oz/acre	
Alg-A-Mic	BioBizz	"seaweed"	"cold pressed"	none	"trace elements and hormones of vegetable origin. Natural amino acids and vitamins are also added"	20 to 50 ml per 10 l water	
ASL Organic Technical Powder 1-0.1-20	Acadian Seaplants Limited = Acadian AgriTech	Ascophyllum nodosum	Solar dried, ground to powder	none	No info	No info	No liquid products or claims on Web site
BioFlora SW - 3	Global Organics, LLC	No info	No info	No info	No info	No info	No info on bioflora.com Web site
Bio-Genesis High Tide™ SeaWeed	Green Air Products, Inc.	"seaweed"	No info	No info	Potassium (0-0-4.5), micronutrients.	1-2 tsp/gal foliar	http://www.greenair.com/bio-genesis.htm#Bio-Genesis%20High%20Tide%20Sea weed

Product	Manufacturer	Species	Extraction method	Alkali / acid	Nutrient / growth regulator claims	Label use claims	Comments
Crop Micro Organic	Ag Connection Sales Inc / Assure Crop	No info	No info	No info	No info	No info	Web site "under construction"
Cyto-Gem Organo Organic	Natural Resources Group	Ascophyllum nodosum	"alkaline hydrolysis"	No info	"auxins, hormones, indoles and cytokinins"	3 to 4-1/2 gal/acre/crop	http://www.callnrg.com/filecabinet/Cyto_Gem_Organo.pdf
Dr. Earth Seaweed Concentrate 0-0-4.5	Dr. Earth, Inc.	Ascophyllum nodosum	No info	No info	Potassium, "essential growth hormones, including cytokinins, auxins and gibberellins"	No info on Web site	Kelp meal only kelp product on Web site
Fog Island Kelp Organic Fertilizer	Coast of Maine	Ascophyllum nodosum	"low temperature vacuum evaporation"	none	"0.0-0.0-2.0"	1 tbs/gal foliar spray	No other nutrient claims
Goemar MZ Organic	Laboratoires GOËMAR	A. nodosum (?)	"Patented freeze-burst process", no alkali	Ph2.2-2.7	Manganese (Mn) 78 g/l, Zinc (Zn) 39 g/l (salts added)	5 l/ha per application	
Kelp Sea Life Liquid Concentrate	Natural Farmer [mfr. Listed as J&G Agrow-Tek on Acorn Organic Web site]	Ascophyllum nodosum	No info	No info	"complete N-P-K and micronutrients"	1-4 qt/a foliar (3x), 1-3 gal/a irrig.	
Kelpro	Tecniprosesos Biologicos, S.A. de C.V.	No info	No info	No info	No info	No info	No Web presence
Kelprosoil	Productos del Pacifico, S.A. de C.V.	Macrocystis pyrifera	No info	No info	"auxin, Cytokinin and Gibberelic Acid"	5-80 L/hectare (various crops)	
Maxicrop Soluble Seaweed Powder 1-0-4	Maxicrop, Inc.	Ascophyllum nodosum	Alkaline, not neutralized	KOH pH 9-10 in 25% solution	"minor elements" "biogrowth stimulants" 20.9% K by OMRI analysis	3 gal/acre for corn [NCR soil additives list]	No product-specific info on Web site
Organic BioLink Seaweed-29	Westbridge Agricultural Products	"seaweed"	No info	No info pH 8.3 (MSDS)	4.5% K, "natural plant hormones"	1 qt/acre/ 3weeks	Label: http://www.westbridge.com/eng/organic/Labels/Biolink/OBL_Seaweed29.pdf http://www.westbridge.com/eng/or

Product	Manufacturer	Species	Extraction method	Alkali / acid	Nutrient / growth regulator claims	Label use claims	Comments
							ganic/MSDS/OBL_Seaweed_29.pdf
Organic TRIGGRR	Westbridge Agricultural Products	1.7% Seaweed (Ascophyllum nodosum)	No info	No info pH 3.8 (MSDS)	No info, "designed to improve nutrient and water uptake"	"dilute in sufficient water to assure adequate and even coverage... Water/Acre: Aerial All 3 to 20 gal, Field Crops 5 to 50 gal, Ground Row Crops 10 to 100 gal, Tree Crops 50 to 400 gal	Label: http://www.westbridge.com/eng/organic/Labels/PGR/Organic_TRIGGRR.pdf http://www.westbridge.com/eng/organic/MSDS/Organic_TRIGGRR.pdf
Symspray (Organic)	Agro-K Corp	Ascophyllum nodosum	No info	No info	Calcium Carbonate, Ca 0.5%	1.2-5 l/acre	http://www.agro-k.com/labels/agro-pdf/1Gal_SymsprayOrg.pdf
Synergy™	Green Air Products, Inc.	"fermented plant and seaweed extracts" "1.7% Seaweed"	No info	No info	"natural hormones, amino acids, betaines" "not a fertilizer"	Foliar spray 0.5-2 tsp/gal	http://www.greenair.com/biogenesis.htm
TAP Organic Powder 1-0-17 See Acadian Organic Powder	Acadian Seaplants Limited						Product not listed on Web site
Zone Biologicals Foliar Supplement	Zone Products, Inc.	No info	No info	No info	No info	No info	This product not listed on Web site, see next entry
Zone Kelp-Gro	Zone Products, Inc.	"Ascophyllum Nodosum, Sargassum Natans and Fluitans "	No info	No info	No info	No info	http://www.bioag.ab.ca/products/biohumates/kelpgrow.html