Table 2. Change in physicochemical properties of different percentages of sludge mixed soil at the beginning of the experiment

	Lime sludge treatment (%)									
Parameters	Control (0)	10	20	30	40	50	60	70	80	100
pH	5.60	7.15	7.22	7.43	8.08	8.62	8.92	9.47	9.94	10.86
Conductivity (mS cm^{-1})	0.162	0.218	0.245	0.266	0.288	0.328	0.347	0.463	0.509	0.765
Water holding capacity (%)	56.46	57.83	59.21	60.86	62.18	63.85	65.19	66.86	68.55	70.9
Organic carbon (%)	0.88	0.84	0.81	0.76	0.68	0.62	0.46	0.38	0.26	0.14
Total nitrogen (%)	0.055	0.055	0.052	0.050	0.048	0.042	0.036	0.028	0.019	0.009
Available phosphorus (mg/kg)	0.644	0.623	0.604	0.536	0.457	0.359	0.324	0.270	0.144	0.078
Exchangeable potassium (meq/kg)	3.46	5.73	9.88	12.57	18.48	21.83	28.65	36.47	41.94	45.38
Sodium (meq/kg)	16.30	34.82	41.39	58.43	77.18	98.56	116.75	146.94	191.36	237.00
Calcium (meq/kg)	40.35	90.62	112.24	146.73	211.16	324.89	411.67	536.30	687.43	752.08
Magnesium (meq/kg)	23.16	27.48	38.25	57.94	68.72	82.59	97.64	118.27	161.91	188.25

lime sludge have been established by increasing the water holding capacity, pH, calcium, potassium and sodium concentrations. In addition, there was no significant change in the quality of soil at lower percentages of sludge but the higher percentages of sludge have an adverse effect on the soil quality, which may lead to reduction of crop production and increased toxicity.

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Rediscovery of naturally occurring seagrape *Caulerpa lentillifera* from the Gulf of Mannar and its mariculture

The cultivation of seaweeds for food, pharmaceutical and industrial uses represents a substantial portion of the world marine harvest. India has yet to fully capitalize on seaweed aquaculture despite its long shoreline with varied climatic conditions[1]. The Food and Agriculture Organization, as early as 1998, included in its recommendation, the expansion of mariculture potential in India through transplantation of *Eucheuma* and *Caulerpa lentillifera*[2]. However, introduction of non-native species for aquaculture in Indian waters caused unforeseen consequences as in the recent case of an introduced species, *Kappaphycus alvarezii*, a red algae used as raw material for the extraction of carrageenan. This commercial effort unfortunately resulted in *K. alvarezii* invasion of the adjacent Gulf of Mannar Marine National Park, resulting in widespread destruction of the endangered coral reef area[3]. For this reason, extreme caution is exercised in non-native introduction into Indian waters despite its large commercial potential.

C. lentillifera, also known as seagrape or green caviar, is a high value seaweed eaten fresh or as a salt-preserved form in Japan, Korea, Philippines and other southeast Asian countries. In Japan, it is cultured in tanks particularly in Okinawa where it is known locally as 'umi-budo' and consumed as fresh salad. Philippine seagrape is called 'lato' and grown in fishponds during the dry season[4]. *C. lentillifera* was first reported to exist in Krusadai island in the Gulf of Mannar in 1955, but was never recorded again despite repeated marine surveys[5]. This species was thought to have become extinct from Indian waters until Mantri in 2004 reported the discovery of this species in Samiani Island situated in the mouth of Gulf of Kutch in the west coast of India[6]. Our marine science discovery programme considered the seagrape project of sufficient national importance to warrant a more extensive survey to locate this species along the Gulf of Mannar where it was originally reported.

A survey using traditional methods is no longer possible because Krusadai Island is part of the protected marine reserve. We were unable to locate distinct beds of seagrapes along the coastal areas of Tuticorin and Mandapam. As a last resort, we attempted an alternative method wherein samples of mixed seaweed species from near shore areas along Kilakarai were collected at low tide, kept moist with seawater and immediately brought to the laboratory. Careful examination of the seaweed biomass revealed the presence of one or more branches of *C. lentillifera* (Figure 1). The biomass comprised predominantly of *C. racemosa* and other minor seaweeds, such as *C. sertularioides, Enteromorpha flexuosa* and *C. peltata*. *C. lentillifera* represented approximately 0.01% of the total collected biomass.

C. lentillifera and *C. racemosa* are similar in physical attributes on cursory evaluation, but are quite distinct upon closer inspection. *C. lentillifera* is characterized by thallus consisting of long horizontal stolons with few rhizoidal branches below and many erect, grape-like branches above. The erect branches are populated with many small capitate ramuli crowdedly attached to the main axis. Distinctive of this species is the spherical tips of the short ramular stalk and the base of the globose head (see Figure 2). In contrast, *C. racemosa* has thallus with long horizontal stolons that have fine rhizoidal branches below and erect branches above, with stalked clavate to globose ramuli that are pinnately or irregularly arranged around the main axis.

A comparison of the Mannar Gulf seagrapes with other substrains was made possible by sending salt-preserved seagrapes from India to Philippines for measurements. It was preserved by soaking the freshly harvested seagrapes overnight in 30% salt solution. The dehydration by salt preservation is an established method for long term transport of seagrapes and does not cause any structural changes on rehydration. However, the rehydration process kills the seagrapes, rendering it incapable of regrowth. The preserved seagrapes were rehydrated by washing with freshwater. After an hour, the seagrapes return to its original physical characteristics[4]. Measurements were made by using a magnifier with linear resolution at 0.05 mm (Ted Pella, Inc, Reading, CA, USA). As shown in Figure 2, the Philippine substrain derived from cultured seagrapes from the island of Cebu, is markedly bigger than those collected from the Gulf of Mannar. The *C. lentillifera* from the Gulf of Mannar may represent a distinct substrain, different from that found in other countries. It is therefore unlikely that the species found in this study may have been introduced by accident in the past.

Figure 1. ***a***, Manual sorting of the seaweed biomass upon receipt at the laboratory; ***b***, A single fragment of *C. lentillifera* (indicated by an arrow) within the seaweed biomass; ***c***, Small amount of *C. lentillifera* segregated (indicated by an arrow) from the bulk of collected seaweed biomass; ***d***, Samples of the various species of seaweeds found within the biomass; ***e***, A single *C. lentillifera* plant from the biomass; ***f***, Young growth of *C. lentillifera*.

Measurements (mm)	Gulf of Mannar	Cebu
a. Width of ramuli	1.57 ± 0.15*	2.49 ± 0.10
b. Height of the seagrape from base of the stolon to top of the ramuli	1.76 ± 0.17*	2.57 ± 0.12
c. Distance between each ramuli at the level of the stolon	1.61 ± 0.71*	0.76 ± 0.11
d. Diameter of the stolon	1.18 ± 0.17	1.87 ± 0.10

*values are significant at P<0.004, Student's t-test

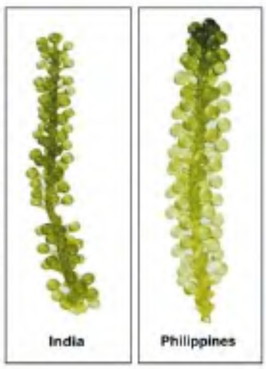

Figure 2. Linear comparison between *C. lentillifera* collected from the Gulf of Mannar (India) and from cultured *C. lentillifera* from the island of Cebu (Philippines). Photographs on the right show side by side comparison of the seagrapes photographed at the same magnification.

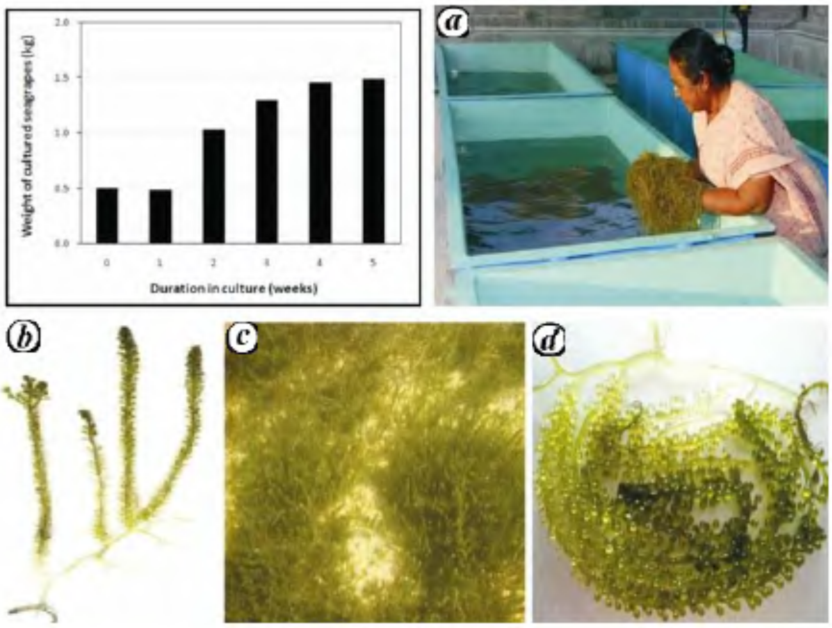

Figure 3. Growth profile of *C. lentillifera* in artificial tank culture after inoculation with 0.5 kg of seagrapes. ***a***, Tank monoculture of *C. lentillifera* after 5 weeks of growth showing the large biomass; ***b***, Typical artificially grown seagrape showing long branches; ***c***, *C. lentillifera* growing on the bottom substratum in the tank; ***d***, Fully grown *C. lentillifera* after 5 weeks in culture, with longer rhizomes and extended stolon.

A total of 16 grams of live seagrapes were collected manually from the biomass harvested from the wild on 27 July 2008. These were transferred to a 1 m^3 fibre glass tank filled with 0.4 m^3 of seawater. The seagrape fragments were planted directly on the 8 cm deep muddy substratum at the bottom of the tank. The culture was protected from direct sunlight and dilution by rainwater using a translucent polyethylene cover. Half the seawater in the tank was replaced with fresh seawater every 4 days. Fertilizers were not added since there was adequate nutrition in normal seawater to sustain growth of the seagrapes at low biomass level. From the initial 16 g of live seagrapes, the cultured biomass grew to 12.4 kg by 24 January 2009, i.e., after 6 months of tank culture. The seagrapes did not show any growth for the first week after planting and grew vigorously thereafter. There was a slowdown in the growth of seagrapes during the 4th and 5th week of tank culture. The seagrapes grew well in artificial culture, with an estimated doubling time of 15 days (Figure 3).

The rediscovery of *C. lentillifera* in the Gulf of Mannar is serendipitous since exploration of near shore areas failed to identify existing natural beds. *C. racemosa*, the predominant species in the Gulf of Mannar, may have overwhelmed the pre-existing *C. lentillifera* over the last 50 years. Nevertheless, finding fragments of *C. lentillifera* successfully co-existing with *C. racemosa* suggest, that search for species thought to be extinct should include careful evaluation of the entire seaweed harvest besides searching for distinct established natural beds. It also suggests that distinct natural beds may be present in still undiscovered areas and that *C. lentillifera* fragments may have broken away from established beds and carried by currents or other organisms.

Besides being a high value food item, recent studies point to other industrial applications, such as in waste remediation[7,8] and in medicine[9–12]. The commercial applications of *C. lentillifera* in India are possible only when mariculture technologies are developed. Any sizeable industry cannot rely on extractive, uncontrolled harvesting from natural beds. Thus, the discovery of native population of *C. lentillifera* in the Gulf of Mannar in concert with the successful demonstration of cultivation of this species opens unique commercial opportunities in the future. To prevent crop loss and to have better control over the quality of seagrapes, land-based mariculture of *C. lentillifera* in artificial tanks is a preferred culture method. The acceptability of cultured Indian seagrapes (referred to as kadalthiratchai in Tamil), in the world market will require substantial efforts in research and development to improve crop quality to match market requirements. Besides being a high value food item, recent studies point to other industrial applications, such as in waste remediation[7,8] and in medicine[9–12]. Improved technologies in processing, packaging and transport of fresh and preserved seagrapes need to be achieved to enable successful entry into local as well as overseas markets.

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