International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN 2454-1850

Volume-5, Issue-6, June 2019

Preface

We would like to present, with great pleasure, the inaugural volume-5, Issue-6, June 2019, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development,* and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas

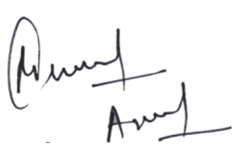
Environmental Research:

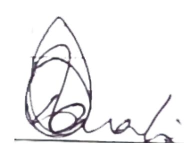
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.





Mukesh Arora
(Editor-in Chief)

Dr. Bhagawan Bharali
(Managing Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Mukesh Arora (Editor-in-Chief)

BE(Electronics & Communication), M.Tech(Digital Communication), currently serving as Assistant Professor in the Department of ECE.

Dr. Bhagawan Bharali (Managing Editor)

Professor & Head, Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat-785013 (Assam).

Dr. Josiah Chidiebere Okonkwo

PhD Animal Science/ Biotech (DELSU), PGD Biotechnology (Hebrew University of Jerusalem Senior Lecturer, Department of Animal Science and Technology, Faculty of Agriculture, Nau, AWKA.

Dr. Sunil Wimalawansa

MD, PhD, MBA, DSc, is a former university professor, Professor of Medicine, Chief of Endocrinology, Metabolism & Nutrition, expert in endocrinology; osteoporosis and metabolic bone disease, vitamin D, and nutrition.

Dr. Rakesh Singh

Professor in Department of Agricultural Economics, Institute of Agricultural Sciences, Banaras Hindu University, Also Vice President of Indian Society of Agricultural Economics, Mumbai

Dr. Ajeet singh Nain

Working as Professor in GBPUA&T, Pantnagar-263145, US Nagar, UK, India.

Prof. Khalil Cherifi

Professor in Department of Biology at Faculty of Sciences, Agadir, Morocco.

Prof. Özhan ŞİMŞEK

Agriculture Faculty, Department of Horticulture, Çukurova University, Adana, 01330 Turkey

Prof. Salil Kumar Tewari

Presently working as Professor in College of Agriculture and Joint Director, Agroforestry Research Centre (AFRC) / Program Coordinator in G.B. Pant University of Agric. & Tech.,Pantnagar - 263 145, Uttarakhand (INDIA).

Goswami Tridib Kumar

Presently working as a Professor in IIT Kharagpur from year 2007, He Received PhD degree from IIT Kharagpur in the year of 1987.

Dr. Mahendra Singh Pal

Presently working as Professor in the dept. of Agronomy in G. B. Pant University o Agriculture & Technology, Pantnagar-263145 (Uttarakhand).

Jiban Shrestha

Scientist (Plant Breeding & Genetics)

Presently working as Scientist (Plant Breeding and Genetics) at National Maize Research Programme (NMRP), Rampur, Chitwan under Nepal Agricultural Research Council (NARC), Singhdarbar Plaza, Kathmandu, Nepal.

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000- March 2002 and DBT overseas Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Muhammad Farooq

Mphil (Food Technology) Gold Medalist

PhD Scholar, Food Science and Biological Engineering, Jiangsu University in China

Production Manager Zeal Food & Beverages Industry Aladand Road, Amandara Batkhela Malakand Agency KP. He developed the skills for Preparation of different fruit pulp preserve Orange, Peach, Guava, Strawberry, and orange squash, orange marmalade.

Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

Table of Contents

Management of *Noorda blitealis* Wlk. on *Moringa oleifera* Lam. using biorationals in the home gardens of Jaffna district, Sri Lanka

K.Sharjana[1], G. Mikunthan[2]

Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Sri Lanka

Abstract— *Moringa oleifera Lam. (Moringaceae) is one of the main crops grown for pods and leaves in Jaffna home gardens in Sri Lanka. After the introduction of Periyakulam 1 (PKM 1) Moringa, a leaf eating caterpillar (Noorda blitealis Wlk.) turned out to be a serious pest causing damage to the leaves. Considering the severity of the damage, this study was carried out to find out the suitable biorationals to manage the pest in an eco-friendly method. Using the leaf disc dipping method biorationals such as 1% neem oil, 3% neem seed kernel extract and 2.5g/L neem leaf extract were used to determine the larval mortality. Consumption of the treated leaf area was measured to determine the larval antifeedant activity for 1% neem oil, 3% neem seed kernel extract, (2.5g/L) neem leaf extract, 15% (g/mL) garlic extract and 75% fermented cow urine and ash solution. Distilled water was used as control in both experiments. The experiments were carried out at a temperature of 28.9 ± 1.13°C and 73% relative humidity in complete randomized design. On the 6th day after treatment larval mortality in 1% neem oil, 3% neem seed kernel extract and 2.5g/L neem leaf extract were 85%, 83.33% and 70% respectively. After 24 hours larvae fed with 1% neem oil, 3% neem seed kernel extract, (2.5g/L) neem leaf extract, 15% (g/mL) garlic extract and 75% fermented cow urine and ash solution showed a larval antifeedant activity of 85.51%, 84.84%, 74.18%, 76.13% and 48.16% respectively.*

Keywords— Moringa oleifera, Noorda blitealis, Biorationals, Home garden.

I. INTRODUCTION

The multipurpose *Moringa oleifera* Lam. belongs to the Family Moringaceae which is a well-known tree grown in the home gardens in Northern Province of Sri Lanka. Leaves, pods, flowers, roots, seeds, bark and wood of the tree have nutritional, medicinal and industrial benefits [1]. Families in Jaffna districts consume the pods and leaves for their daily nutritional requirements. As the demand for the organic *Moringa* leaves has increased in local and foreign markets, many farmers have started to show interest in cultivating *Moringa*. An annual bush type, Periyakulam 1 (PKM 1) *Moringa* which was introduced from India is widely cultivated in resettled home gardens in the Jaffna district.

In recent years, PKM 1 *Moringa* crops were severely attacked by a seasonal pest *Noorda blitealis* Wlk. It is a leaf eating caterpillar which belongs to the Family Pyralidae. The larvae is creamy and sometimes ranges from pale green to cream or pink in colour, feeds on the *Moringa* leaves and in severe conditions they completely defoliate the plant. The affected leaves are skeletonized and appear as translucent sheets and finally dry up. In some conditions especially when the tree is pruned the larvae begin to feed on the stem pith and bark of the tree. In this condition they feed either on the corky content inside the stem heart or on the fleshy bark under the skin [2].

Since there is limited information regarding the pest, there were no effective management practices followed by farmers. Considering the economic status and the health issues of the resettled families and other *Moringa* growers in Jaffna district, this study was carried out to identify suitable techniques to manage the pest with low cost and easily available eco friendly biorationals.

II. MATERIAL AND METHOD

Experiments were conducted in the laboratory at the Faculty of Agriculture, University of Jaffna during February to April 2015. Preliminary tests were done for each experiment to choose the best suitable biorationals for the experiment. The larvae of *N.blitealis* were collected from the leaves, shoots and stems of *Moringa oleifera* in Jaffna district. They were reared in plastic rearing chambers at 28.9 ± 1.13°C and 73% relative humidity. Larvae were fed with fresh PKM 1 *Moringa* leaves for every 6 hours. At the final stage of larvae, they were transferred to another container of 1.5cm thickness of sterilized sandy soil for pupation. When the adults emerged, they were provided with 10% honey on cotton wool and PKM 1 annual *Moringa* leaves as oviposition site and as feed. After the eggs were laid, they were allowed to hatch.

2.1 Efficacy of biorationals on mortality of *N.blitealis*

The experiment was carried out with four treatments including the control. The concentration of each bio-rational used is shown in Table 1.

TABLE 1
BIORATIONALS AND THEIR CONCENTRATIONS USED FOR MORTALITY TEST

Treatment number	Name of the treatment	Concentration
T1	Neem (*Azadirachta indica*) oil	1 % (v/v)
T2	Neem seed kernel extract	3% (w/v)
T3	Neem leaf extract	2.5g/L
T4	Control (Distilled water)	

The concentrations suggested were based on the literature of corresponding botanical results against *N.blitealis* according to the literature and preliminary tests done in the laboratory [2,3]. For each treatment 20 larvae were used with three replicates. Fresh PKM 1 even aged *Moringa* leaf discs were dipped into respective biorationals and were allowed to dry at the room temperature for 30 minutes. Larvae were starved previously for three days and were allowed in rearing chambers. Treated leaf discs were placed in rearing chambers. The number of dead larvae was counted at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} days after treatment.

2.2 Antifeedant activity of *N.blitealis* against biorationals

The antifeedant activity of *N.blitealis* larvae on five biorationals were determined by using leaf dipping method with 24 replicates. The biorationals used for this experiment is shown below in Table 2.

TABLE 2
BIORATIONALS AND THEIR CONCENTRATIONS USED FOR ANTIFEEDANT ACTIVITY TEST

Treatment number	Name of the treatment	Concentration
T1	Neem leaf extract	2.5g/L
T2	Neem oil	1% (v/v)
T3	Neem seed kernel extract	3% (w/v)
T4	Garlic extract	15%(g/mL)
T5	Three days fermented cow urine and ash solution	75%(v/v)
T6	Control (Distilled water)	

Larvae were fed with treated leaf discs in the container. After 24 hours the consumption of the leaf area by the larvae was measured by using grid method. The antifeedant activity was calculated using the following equation (1):

$$\text{Percentage of antifeedant activity} = \frac{C-T}{C+T} \times 100 \qquad (1)$$

Where,

C - Leaf area consumed in the control

T - Leaf area consumed in the treatment

TABLE 3
CLASSIFICATION OF ANTIFEEDANT ACTIVITY

Activity level	Percentage
Strong activity	>80%
Moderate activity	61-80%
Weak activity	40-60%
Little activity	<40%

2.3 Statistical analysis

All experiments were designed according to complete randomization design (CRD) and the data were statistically analyzed using SPSS and SAS packages.

III. RESULTS AND DISCUSSION

3.1 Efficacy of biorationals on mortality of *N.blitealis*

The percentage of mortality of the three biorationals are shown in Table 4. The mortality percentage increased with time period. The percentage of mortality of all biorationals were significantly different from the control in all time periods. The highest mortality of 85% was observed in 1% (v/v) neem oil on the 6th day. Larval mortality percentages in 6th day of 3% (w/v) neem seed kernel extract and 2.5g/L neem leaf extract were 83.33% and 70% respectively. Similar results were reported by studies conducted in India [2], [3].

TABLE 4
EFFECT OF BIORATIONALS ON MORTALITY PERCENTAGE OF *N.blitealis* LARVA IN DIFFERENT INTERVAL

Treatment number	Treatment	Mortality percentage *					
		1st day	2nd day	3rd day	4th day	5th day	6th day
T1	Neem oil 1% (v/v)	3.33^{a}	15.00^{a}	38.33^{a}	51.66^{a}	80.00^{a}	85.00^{a}
T2	Neem seed kernel extract 3% (w/v)	1.66^{a}	15.00^{a}	35.00^{a}	50.00^{a}	76.66^{a}	83.33^{a}
T3	Neem leaf extract 2.5g/L	1.66^{a}	6.66^{ab}	15.00^{b}	40.00^{b}	68.33^{a}	70.00^{a}
T4	Control	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{b}	0.00^{b}

**** All the values with the means of three replicates. Figures having same letters in a column indicate that the values are not significantly different at 0.05 α***

3.2 Antifeedant activity of *N.blitealis* against biorationals

The antifeedant activity of the larvae on five biorationals was significantly different from the control (Table 5). Strong antifeedant activity was observed on 1% (v/v) neem oil and 3% (w/v) neem seed kernel extract with an antifeedant activity of 85.51% and 84.84%. Larvae provided with 15% (v/v) garlic extract and 2.5g/L neem extract showed moderate antifeedant activity of 74.18% and 76.13% respectively. However larvae showed weak antifeedant activity of 48.16% on fermented cow urine and ash treated leaves.

Neem is the source of tetranortriterpenoid azadirachtin and other extractives which have potential value in insect control. It is responsible for both antifeedant and toxic effects in insect [4, 5, 6]. Thus the strong antifeedant activity of *N.blitealis* on 1% (v/v) neem oil and 3% (w/v) neem seed kernel extract is due to the Azadirachtin present in the neem. In this study garlic extract showed a moderate antifeedant activity against *N.blitealis* larvae. According to Amonkar and Banerji, major compounds such as diallyl di-sulphide, diallyl tri-sulphide and diallyl sulfide in garlic are antagonistic to pests [7]. In Ethiopia farmers use fermented cow urine in the indigenous pest management; Banana fly *Drosophila melanogaster* and cowpea aphid *Aphis craccivora* were controlled by fermented cow urine[8].

TABLE 5
ANTIFEEDANT ACTIVITY (AA %) OF *N.blitealis* LARVA

Treatment number	Treatment	Mean	Antifeedant activity %	Antifeedant activity class
T1	Neem leaf extract 2.5g/L	1.37^c	76.13	Moderate activity
T2	Neem oil 1% (v/v)	0.79^c	85.51	Strong activity
T3	Neem seed kernel extract 3% (w/v)	0.83^c	84.84	Strong activity
T4	Garlic extract15% (g/mL)	1.50^c	74.18	Moderate activity
T5	Fermented cow urine and ash solution 75%(v/v)	3.54^b	48.16	Weak activity
T6	Control	10.12^a		

Figures having same letters in a column indicate that the values are not significantly different at 0.05 α

IV. CONCLUSION

Larvae fed with 1% neem oil, 3% neem seed kernel extract showed higher mortality percentages and strong antifeedant activity. In Jaffna home gardens compared to the chemical pesticides, neem based products are easily available and economic to suppress the pest population build-up. The demand of organic *Moringa* leaves in export sector has increased in recent years; therefore there is a need for good quality organic leaves in the market. Neem products such as 1% neem oil, 3% neem seed kernel extract and 2.5g/L neem leaf extract can be sprayed on the leaf surface to prevent leave damage by the larvae.

REFERENCES

[1] Broin, Melanie; de Saint Sauveur, Armelle. 2010. Growing and processing moringa leaves. Moringa Association of Ghana (MAG), Accra, Ghana, pp: 5-57

[2] Satti, A. A., Nasr, O. E. H., Fadelmula, A., and Ali, F. E. 2013. New record and preliminary bio-ecological studies of the leaf caterpillar, *Noorda blitealis* Walker (Lepidoptera: Pyralidae) in Sudan. *International Journal of Science & Nature*, 4(1): 57-62.

[3] Ragumoorthi, K. N. and Arumugam, R. 1992. Control of moringa fruitfly *Gitona* sp., and leaf caterpillar *Noorda blitealis* with insecticides and botanicals. *Indian Journal of Plant Protection*, *20*(1): 61-65.

[4] Mordue (Luntz), A.J., and Nisbet, A.L. 2000. Azadirachtin from neem tree Azadirachta indica: its action against insects. *Anais da Sociedade Entomológica do Brasil*, 29(4): 615-632.

[5] Ascher, K.R.S., 1993. Non conventional insecticidal effects of pesticides available from neem tree, *Azadirachta indica*. *Archives of Insect Biochemechistry and Physiology*, 22: 433-449.

[6] Isman, M.B., 1999. Neem and related natural Products. In F.R.Hall and J.J. Menn (eds.) Biopesticides: Use and Delivery. Humana Press, Totowa, NJ, pp: 139-153.

[7] Amonkar, S.V. and Banerji, A., 1971. Isolation and characterization of the larvicidal principle of garlic. *Science*, 174:1343-1344.

[8] Tesfaye, A. and Gautam, R.D., 2003. Traditional pest management practices and lesser exploited natural products in Ethiopia and India: *Indian Journal of Traditional Knowledge*, 2(2):189-201.

Viability, method and device for horticultural crops with brackish and marine water

Josep García[1], Pedro Monagas[2], Núria Martos[3], Daniel Gálvez[4], Oriol Arnal[5], Montserrat Pujolà[6], Júlia Carol[7]

[1,2,3,4]Centre de Recerca en Seguretat i Control Alimentari de la UPC (CRESCA)

[5]Fundació Privada Aqua Maris

[6,7]Escola Superior d'Agricultura de Barcelona (ESAB)

Abstract— *The method that humanity has adopted to hydrate and thus give life to the plants, imitating the model that was most visible, is the rain. However, the great secret to the contribution of nutrients to the vegetables, the irrigation itself, is on earth, in the groundwater layers and aquifers that hoard and administer the water, keeping every drop of rain and distributing the water through the basins, underground rivers, watering indirectly from the mountain to the sea. The key is in the different circulation velocities of the groundwater because of the nature of the substrates. However, agriculture has taken irrigation from above as we know it and has focused especially on drainage capacity. From this point of view, saline water is not beneficial for irrigated agriculture, but may be the only source of irrigation water in large arid regions, especially in developing countries, where the extreme scarcity of freshwater and the rapidly growing population require more water.*

When considering the possibility of watering with seawater without desalinating, always by means of capillarity systems, it is essential to take into consideration the different strata of soils, the distance to the groundwater, the composition of seawater, the capacity of drainage, chemical reactions of the soil with salts, etc. The modification of any of these parameters can produce effects of salinization, loss of humidity or desertification among others.

This study presents the accumulated experience through the joint collaboration between the Centre for Research in Security and food Control of the Polytechnic University of Catalonia (CRESCA) and the Aqua Maris Foundation in capillary irrigation and it proposes a system and device that allows the controlled development of different vegetal species using brackish and seawater.

Keywords— ***desertification, desalination, reuse, underground stream seawater.***

I. INTRODUCTION

The United Nations, within the framework of its sustainable development programme called Agenda 21, establishes that desertification is the degradation of land in arid, semi-arid and dry sub-humid areas resulting from various factors, including variations climatic and human activities.

Desertification affects about one-sixth of the world's population, 70 percent of all drylands, which represent 3.6 billion hectares, and a quarter of the world's total land surface.

The most obvious impact of desertification, in addition to widespread poverty, is the degradation of 3.3 billion hectares of the total grassland area, constituting 73% of grasslands with a low potential for human and animal load capacity; Decrease in soil fertility and structure in approximately 47 percent of dry areas that are rainfed, marginal farmland; and degradation of irrigated farmland (United Nations 1992).

The main causes of desertification are climate, erosion, ecological factors-the type of soil and ecosystem-and human action. Erosion is the prelude to desertification, because when the air and water drag the surface particles of the soil, it loses the fertile layer, and remains unprotected, being increasingly slow regeneration of the vegetation cover. Difficult-to-drain terrain, torrential rains or drought are other phenomena responsible for desertification.

But the most damaging factor, together with climate change, is human activity. Fires, indiscriminate logging, overexploitation of aquifers, intensive crops, with massive use of chemicals, and some forestry practices (forest or mountain cultivation) are some of the examples of human intervention. In the world there are more than a hundred countries with arid and semi-arid conditions. Africa is the continent most damaged by desertification; He is followed by Asia, Latin America and the Caribbean. Also, southern Europe and Spain (FAO 2000).

In counterpart, about 70% of the entire planet is covered by liquid water, being the most abundant resource in the surface layer of the earth. However, its distribution is very variable: in some regions it is very abundant, while in others it is scarce. However, the total amount of water on the planet does not change (Aparicio 1987) and moreover, about 97,5 % of this 70% of liquid water is saltwater.

Water exists in solid (ICE), liquid and gaseous (water vapor) form that can be observed in oceans, rivers, clouds, rain and other forms of precipitation in frequent changes of state. Thus, surface water evaporates water from clouds precipitate, rain seeps into ground and runs to the sea. The whole of processes involved in the circulation and conservation of water on the planet is called hydrological cycle or, more precisely, geohydrologic cycle (Pidwirny 2006).

Fresh water is a renewable resource, but it is also finite. Around the world there are many signs that human use of water exceeds sustainable levels. The depletion of groundwater, the low flow of rivers and the worsening of pollution levels are among the most obvious indicators of water stress (Postel 2000). Thus, for example, global demand for water has tripled approximately since the mid-twentieth century (McCully 1996).

The World Health Organization (WHO) considers that the adequate amount of water for human consumption (drinking, cooking, personal hygiene, and household cleaning) is 50 L/HAB-day. The necessary contribution to agriculture, industry and, of course, to the conservation of aquatic, fluvial and, in general, freshwater-dependent ecosystems must be added to these quantities. Considering these parameters, it is considered a minimum amount of 100 L/HAB-day. (Howard & Bartram 2003)

The destination applied to fresh water consumed varies greatly from one region to another on the planet, even within the same country. Generally, the high consumption of drinking water is given in rich countries and, within these, urban consumptions double to rural consumptions. At the global level, some 3,600 km3 of freshwater for human consumption are currently being extracted, that is, 1,600 liters/HAB-day, of which, approximately the half is not consumed (it evaporates, infiltrates to the soil or returns to some channel) and, of the other half, it is estimated that 65% is destined to agriculture, 25% to industry and only 10% to domestic consumption.

According to WHO, in 2010, 87% of the world's population, i.e. 5.9 billion people, had sources of potable water supply. On the contrary, almost 39% of the world's population, or more than 2.6 billion people, lacked improved sanitation services. Currently, more than 1.2 billion people consume water without health guarantees, which causes between 20,000 and 30,000 daily deaths and a large number of diseases (WHO & UNICEF 2017).

Water management aims to improve the quantity and quality of the water available. The ways to achieve this are: to regulate the use of surface water and groundwater, to develop alternative water sources, to rationalize their consumption, to control the supply of pollutants and to recover the initial conditions by means of purification processes. The objective of a good water state should be pursued in each watershed, so that the measures relating to surface water and groundwater belonging to the same ecological, hydrological and hydrogeological system are coordinated (Directive 2000/60/EC).

From this perspective, the reuse of purified water is an essential element of the natural water cycle and, in fact, it is envisaged as a measure to solve the problems of water scarcity.

Reuse is very valuable for agriculture, since it guarantees the resource continuously. Its application is a common practice in many areas, especially in the arid and semiarid regions. In this respect, in Spain, in December 2007 the Royal Decree 1620/2007 was promulgated, which established the permitted uses and the criteria of quality, of minimum frequency of sampling, of reference point for the analytical methods and of conformity (BOE December 8^{th} 2007).

On the other hand, seawater is a concentrated solution of inorganic salts that serves as a habitat for countless living beings, with plankton being the most important volume of biomass, consisting mainly of algae (phytoplankton) and microscopic animals (zooplankton). The waters located on the continental shelf are generally greenish due to the presence of chlorophyll and other fito-pigments, and by some substances contributed by the soluble humus of terrestrial origin. It is by far the area of greatest richness and diversity in marine species (Custodio & Llamas 1983).

The composition of the sea water varies according to its origin or marine characteristics, being of higher concentration of salts in warm places with little renovation as in the Mediterranean, and smaller in semi-enclosed places with abundant continental contributions like the Baltic Sea (Grasshoff et al. 1999).

Given the high rates of pollution of rivers, reservoirs and groundwater, an important option is presented: desalination of seawater to obtain consumable water (Lechuga *et al.*, 2007).

As far as the demand for desalinated seawater is concerned, its demand has increased considerably in recent years. This is due, above all, to the serious shortage of water resources that is being suffered in various parts of the world. The three most important processes in the desalination of brackish or marine water are: Reverse Osmosis (RO), Multi-Effect distillation (MED) and instantaneous multi-stage Distillation (MSF) (Veza 2002).

In recent years, the idea that water management should be understood as an instrument in the service of an explicit territorial policy has been reinforced and that it is also supported by the growing demand for integration between water management and Sectoral policies (Del Moral 2009). From this perspective, this study proposes the direct use of seawater, without going through a process of previous desalination, as a fluid to be used in the irrigation of various types of cultivation. To this end, a set of experiences jointly carried out by CRESCA and the AquaMaris Foundation, aimed at demonstrating its viability in both outdoor and greenhouse crops, is collected.

II. MATERIAL AND METHODS

All the experiences that are related in this study have in common that seawater was circulated underground. In this way, by capillarity, seawater was dispersed through the corresponding solid substrate and roots of the selected plant species had access to the moisture and nutrients contained in this fluid as it can be seen in the explanatory diagram of Figure 1.

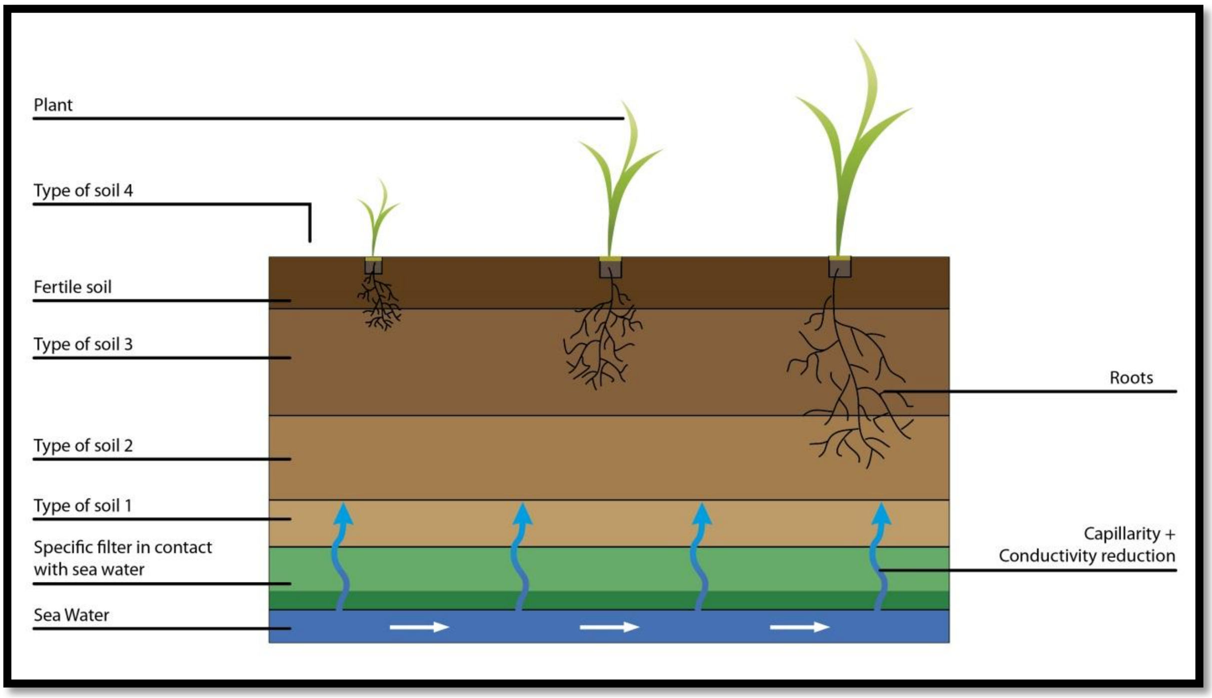


FIGURE 1. Supply of moisture and nutrients to the solid substrate by means of an underground stream of seawater

2.1 Background

In the facilities of the AquaMaris Foundation, a first experience was made to determine the correlation between the distance between the groundwater flow of brackish or marine water and the roots of the selected plant species (radish, lettuce, parsley, cabbage, chard, arugula, basil, endive or tomatoes, as well as an ornamental plant). To this end, a small garden of three levels was built. At the lower level, the different plant species were 40 cm from seawater; In the intermediate level at 80 cm and in the upper to 110 cm. Figure 2 shows schematically the construction work done to develop this experience.

FIGURE2. a) Scheme of the operation of the experimental garden of three levels (40/80/110cm regarding the seawater level b) Actual structure enabled in the AquaMaris Foundation

The type of terrain used in this experiment was a clay composition, with a high capacity for moisture retention and capillarity, but little drainage. In addition, a substrate was incorporated as a filter, consisting of a mixture of materials with very specific physicochemical characteristics, which allowed the passage of water to the upper layers and, simultaneously, was able to significantly reduce the conductivity of the water. This was intended to recover the soil in an easy way, without having to replace it.

Although this experience was subject to the rainfall regime of the Mediterranean basin, the viability of the use of seawater was demonstrated. What's more, a similar experience was designed and developed in the Atacama Desert, with satisfactory results (Gutierrez 2017).

The viability of the use of seawater as an underground supply of moisture and nutrients, in a second phase, was determined to delimit the conditions in which certain crops could be viable. For this purpose, four containers were enabled as shown in Figure 3.

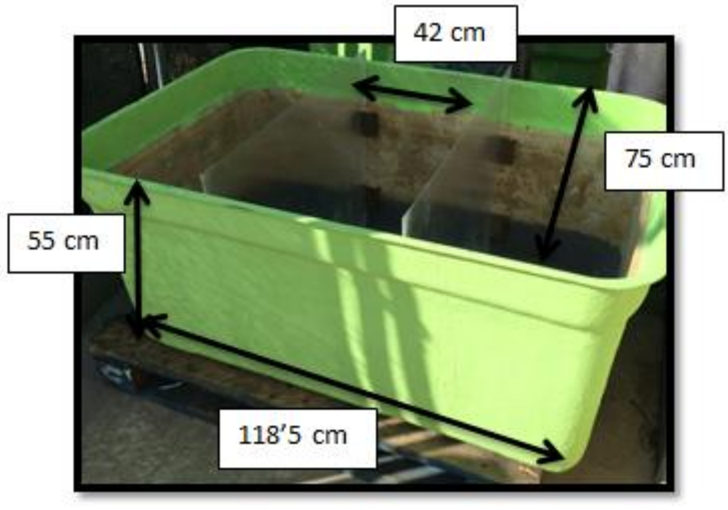


FIGURE 3. Form and measurements of the containers used

At the bottom of these containers a 3 cm thick air chamber was prepared which was subsequently used for the circulation of seawater. These four vessels were separated into two groups. The first of them was left to the mercy of the influence of the rain. On the contrary, the second was kept in guard.

In the two vessels of the first group, two types of substrates were used, one consisting of a mixture of sandstone enriched with compost (A) and the other based on clayey soil of low organic matter content (B).

Each container was subdivided into three zones. In each zone a layer of sandstone was deposited as a filter between the seawater and the corresponding substrate. The different thickness of this sandstone layer was 14 (A1, B1); 27.5 (A2, B2) and 41 (A3, B3) cm. The second layer was a mixture of sandstone and compost at 50%(v/v)and its thickness was adjusted to that of the sandstone layer, so that its thickness was limited leaving a space of 10 cm to the edge of the container. The selected vegetables were: chard, dandelions and tomato cherry and during the first two weeks were irrigated with freshwater (public network) to ensure their roots in the field.

Schematically, the distribution of the selected vegetables in each of these two containers is shown in Figure 4.

FIGURE 4. Distribution of tomatoes, dandelions and chards in containers

On the other hand, the other two containers were protected from the influence of the rain, so that the selected vegetables could only receive water either through the environmental humidity, or from the seawater deposited in the bottom of the vessel renewed periodically, except during the first two weeks, as in the previous case, were irrigated with fresh water, to ensure their roots in the ground.

On this occasion, the selected vegetables were lettuce and tomato cherry and the sandstone layer presented two levels, 25 and 75% of the useful height of the vessel, i.e 15.5 and 46.5 cm, respectively. The second layer, as in the previous experience, was the same mixture of sandstone and plant compost at 50% and its thickness reached up to 10 cm from the rim of the container. The two groups of recipients were allowed to evolve over a three-month period.

On both occasions, a control vessel was used to grow the selected plants with water from the public network. The composition of the land was compost plant 100%.During the growth period of the selected vegetables, the soil moisture and conductivity were monitored.

For the monitoring of the soil moisture, with the help of a spatula, a hole of about 20 cm of depth was made on the surface of the terrain. Once the hole was made, a sample of approximately 10 grams was taken. Later, with the help of a scale, an accurately known amount was weighed in a Petri dish and was introduced in a stove at 110 º C for a period of 24 hours. Then it was introduced into a desiccator to room temperature and weighed again. The percentage of soil moisture was determined from the weight loss.

In containers subject to the influence of rainfall, each time the soil moisture was determined, a sample should be taken in each of the three zones in which each vessel was divided (see Figure 4) and another, corresponding to the control vessel. In the second experience, it was only necessary to take two samples of each container and another one, corresponding to the control vessel.

The soil moisture and the conductivity were determined. To do so, the dry specimen from the previous test was introduced inside a bottle with a screw cap. Immediately, 80 mL of distilled water was added, and mechanical agitation was proceeding, with the help of a magnetic stirrer, for a period of 30 minutes. It was then left to rest for a period of 24 hours and the contents of the vial were filtered. The filtrate was used to determine conductivity by conductivity Jeulin JLC20.

In the containers to which the access to rain was restricted, a control was made of the evolution of the plants through the moisture content in the plant freshly harvested; The content of organic matter, obtained by calcinations and the pH of the Ashes.

The humidity was determined as the loss of weight of the newly harvested vegetable at a temperature of 110 ºC over a period of 24 hours. The organic matter content was determined as the weight loss of the dried vegetable at 110 ºC after being calcined at 470ºC for 4 hours.

The pH was determined in the fraction of soluble ashes, from previous calcination, by means of a Phmetry Thermo Scientic Orion 2 Star.

It was systematically preceded as follows: From each sample, two thirds of the ashes were treated with 100 mL of distilled water in a beaker of 200 mL and kept boiling for a period of 10 minutes. As they were allowed to cool, the sample was kept in agitation with the help of a magnetic stirrer. Once ambient temperature was reached, the contents of the beaker were filtered through a filter paper that had previously been thermally treated at 110 ºC until constant weight. The filtrate was collected and flushed in a 250 mL volumetric flask.

The viability of the growth achieved in this experiment induced to optimize the behaviour of the material layer in contact with the seawater, in order to regulate the migration of salts from the seawater to the substrate.

2.2 Optimization of the material in contact with seawater

The initial objective was to determine the height that, by capillary, would reach seawater in presence of different types of land. For this purpose, specimens made with plastic tubes, of constant diameter (57 ± 5 mm), were prepared and filled with the tested substrates. These tubes were introduced into containers where water height was predetermined, as shown in Figure 5.

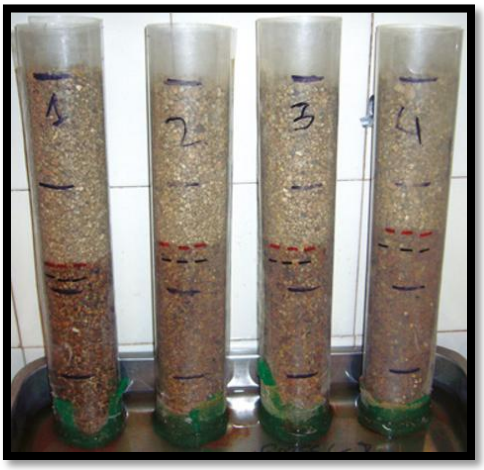


FIGURE 5. Behaviour of specimens with sandstone after four days in contact with seawater

The materials initially selected were: Sandstone of different particle size (coarse, medium and fine), beach sand and compost plant.

Table 1 shows the particle size of sandstone and beach sand, which had previously been dried at 110 ºC, until a constant weight was obtained.

TABLE 1
GRANULOMETRIC ANALYSIS OF SANDSTONE AND BEACH SAND

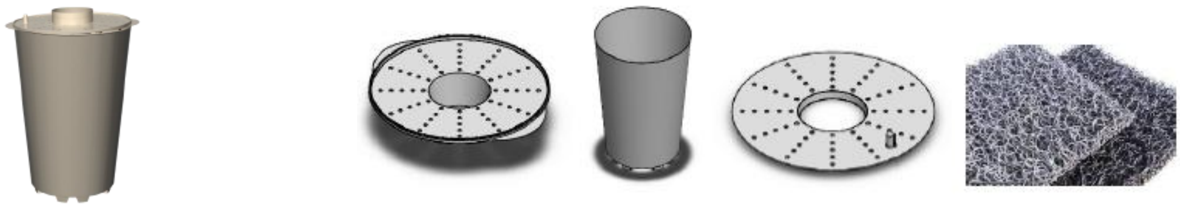
Size	% Thick sandstone	% Medium sandstone	% Thin sandstone	% Beach sand
>2mm	82,5	20,4	2,6	4,3
1-2mm	12,0	31,7	32,3	42,0
<1mm	5,5	34,4	65,6	51,1

In addition to determining the height at which the seawater reached the inside of the specimens, the value of the electrical conductivity was determined at different levels, once it was considered that the water had reached its maximum height. To this end, samples were taken of the substrate contained inside the specimen at different levels and dried at 110 ºC for 24 hours. From the dry material, aliquots of 10 grams were taken and each aliquot was introduced inside a container with a Hermetic seal where 100 mL of distilled water were added. Then, mechanical agitation was proceeded with the help of a magnetic stirrer for a period of 30 minutes. It was then left to rest for a period of 24 hours and the contents of the vial were filtered. The filtrate was used to determine conductivity by a conductivity Jeulin JLC20.

2.3 Tlaloc Device design

From the results obtained in the preceding section, the design of a container suitable for use in a greenhouse was proceeded. Under the name of Tlaloc (Aztec god of Rain and Earth), the device, shaped like a pot, was designed to optimize the function it should develop: to allow the use of seawater as a vehicle for the transport of moisture and nutrients, by capillarity, towards the substrate where the selected plant species are cultivated.

Figure 6 shows the external aspect as well as the various components. The Tlaloc device is composed of three plastic pieces, these are; The flowerpot, the lid and the ventilation system. A PVC curly vinyl grille is also required.



(a) **(b)**

FIGURE 6. a) External appearance of the Tláloc device b) Complementary components

Figure 7 shows an outline of how the Tlaloc device works. Conceived and designed to be used in a greenhouse, the lower supports are bored in order to allow the circulation of seawater that will contact the substrate selected for each type of crop. On the upper part is the cover that incorporates the condensation and ventilation system. In its central part it has a hole where the cylinder is inserted with the plant to be cultivated.

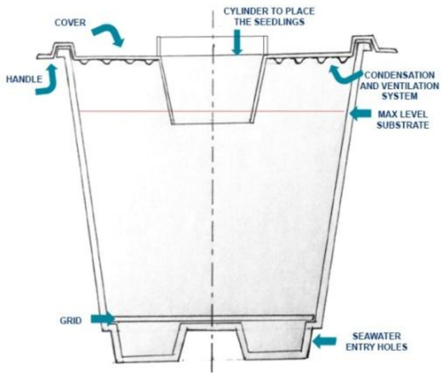


FIGURE 7. Diagram of the Tláloc device

2.4 Experiences with the Tlaloc device

The cultivation of Chard was followed (Beta vulgaris var. Cycle) inside a greenhouse. The plant, of 160 plants, was bought to the company Semilleros Rovira S.C. P located in Cabrera de Mar. Different experimental series were set up in which different experimental conditions were compared, especially the thickness and composition of the different substrates contained inside the 28x18 cm Tlaloc device. Sandstone was used (with a particle size greater than 2 mm), beach sand (with a particle size between 1-2 mm) and Floradur vegetal compost, with an electrical conductivity between 25-50 mS/cm and a pH between 5.5-7. This substrate contains an NPK fertilizer in proportions 18-10-20.

Four different treatments were used plus control. The variation of the treatments was the filter used and the height of this. The filters used were beach sand (A) and sandstone (S) and the filter heights were 6 cm (A1/S1) and 20 cm (A2/S2). The treatment used as a control (C) had only compost.

For each treatment 32 devices were used Tlaloc arranged in 4 trays and in rows of 8 devices per tray.

After 43 days it was collected and the analysis of the product was carried out, preserving a part to make the study of the conservation. To carry out the study of conservation were taken all the chard of the same treatment, were mixed and elaborated 15 trays of porexpan per treatment, of approximately 100g of weight, closed with paper film and were kept in refrigeration for 12 Days at 4 ± 1 ºC. The parameters evaluated were moisture, mineral matter, ascorbic acid, content in Na^+, K^+, Ca^{2+} and Mg^{2+}, colour and various plant measures such as weight, height and leaf amplitude.

The results showed that the conservation of the chard was similar between those irrigated with red water and those irrigated with seawater. In the case of chard irrigated with seawater, it was determined that the height of the filter (6 or 20 cm) was of greater importance than its composition (sand or sandstone) in parameters such as humidity and ascorbic acid.

Figure 8 shows a general view of the interior of the greenhouse after planting the whole chard and after 23 days.

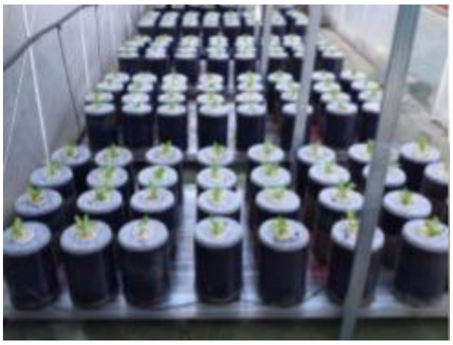


FIGURE 8. General view of the greenhouse a) freshly planted chard b) the same chard after 23 days.

The fresh weight was determined by weighing the whole of the chard collected in each test with an accuracy of ± 0, 1g. The result was expressed in g.

The humidity was determined by the Gravimetric method AOAC 931.04, weighing 50 ± 0,01g of fresh and cut sample, keeping it at 60 ºC, up to constant weight (48 h approximately) in a vacuum stove. The moisture content was calculated by weight difference and expressed as a percentage of water (%). The humidity was determined at the time of collection (day 0) and during the conservation process on days 2, 6, 8 and 12.

The colour of the chard leaves was determined using a KONICA MINOLTA CR-400 colorimeter with aperture size of 8mm and Illuminate D65. The different colour parameters that were determined from the coordinates in the CIELAB space were:

L *: luminosity, Degree of clarity where 0 = black and 100 = white.

A *: chromaticity, Green (-a) and red (+ a).

b *: Chromaticity, Blue (-b) and yellow (+ b).

From these parameters the corresponding colour index (IC) was determined, according to:

$IC = b^* \times 1000 / a^* \times L^*$

The ascorbic acid was determined by the method AOAC 967.21, based on the redox volumetry which has as reagent titrating the DCPI (2.6-diclorofenolindoenol). Before the volumetry, a standardization of the DCPI was performed with a solution of ascorbic acid of known concentration (0.1 g/L). The results were expressed in mg of ascorbic acid/100 g of fresh specimen.

The AOAC 971.33 method was followed for the determination of the mineral matter. Starting from the dry matter, 1.5 ± 0 were weighed, 0001g in an analytical balance Scaltex SBC 33 and calcined at 470 ºC during 4h until obtaining white ashes. The residue obtained was weighed to obtain the% of mineral matter (M.M.) and the% of organic matter (M.O.). Subsequently, the ashes were dissolved in 15 mL of hot 3M nitric acid. Once cooled, the insoluble residue by filtering was separated by a filter without Ashes. The filtrate was carried to a volume of 50 mL. From this acid extract, calcium and magnesium were determined by atomic absorption spectroscopy and sodium and potassium by flame photometry.

III. RESULTS

3.1 Background

The first experimental test, consisting of a structure of 3 levels of height with an artificial groundwater layer of seawater, presented as main objective to have a soil with a drainage capacity related to the depth to the groundwater.

The first plant species used was chard (Beta vulgaris var. Cycle). It was observed that the growth of this species varied considerably from one level to another. But it not only changed the size of the plant (something acceptable because of overexposure to sea water at the lower levels) but also its flavour.

By checking that the chard was growing in all three levels, other species such as arugula, tomato, cabbage, pepper, basil or lettuce were started to be tested.

3.2 Container construction

3.2.1 Evolution of the land

Figure 9 shows the development of chard, dandelion and cherry tomatoes in containers that were exposed to rain. Obviously, the growth was more pronounced in the container with substrate with high content of organic matter.

(a) (b)

FIGURE 9. Influence of the chemical composition of the substrate on the growth of plants after 23 days of planting a) high organic content b) low organic matter content.

The follow-up of the experimental parameters gave the following results:

Soil moisture

The percentage of humidity obtained was higher in the control vessel, which had been irrigated with network water. As far as the humidity determined in the two containers subjected to irrigation with seawater by capillarity is concerned, the container A, with high content in organic matter, presented a higher percentage compared with the container B, with little content in organic matter, especially in the initial rainy period (April), as it can be seen in Figure 10.

As the rain was waning and the ambient temperature was increasing, the difference between the two vessels was lower, with a tendency to equalize to values less than 10%.

In containers that were not subject to the influence of rain, it was found that the humidity of the control vessel was only slightly higher than that of the vessels that received water supply by capillarity. This behaviour, initially not expected, was attributed to different factors, highlighting the composition of the substrate and the fact of not having the influence of the weather.

However, during the development of this experiment, periodically, both the irrigation with fresh water in the control vessel and the seawater in the containers subjected to capillary irrigation with seawater were limited. In this way, the ability to recover moisture depending on the composition of the substrate was revealed.

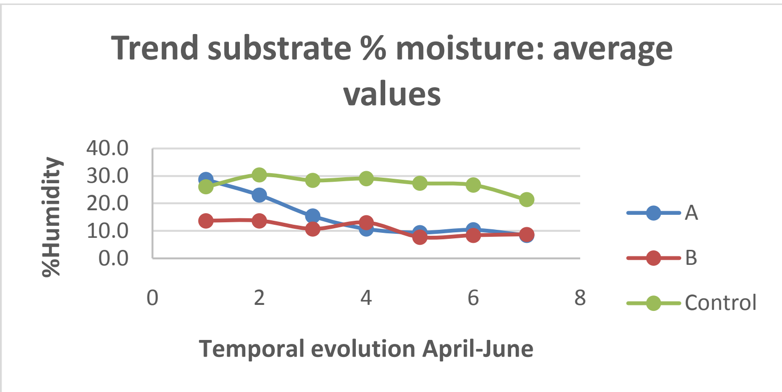


FIGURE 10. Temporal evolution of humidity percentage: A) container with vegetable compost B) container without vegetable compost. Control) control container.

As it can be seen in Figure 11, the value of the moisture percentage experienced a decrease in the three containers. The control vessel and the vessel with a sandstone layer of 25% of the useful height of the vessel presented to each other a much more synchronized behaviour than the vessel with a sandstone layer of 75% of the useful height of the vessel. After an initial period with different alternatives, both substrates ended up converging towards the end of the experience towards values of 30% of humidity. It should be noted that these percentages trebled those obtained in the experiences exposed to the weather.

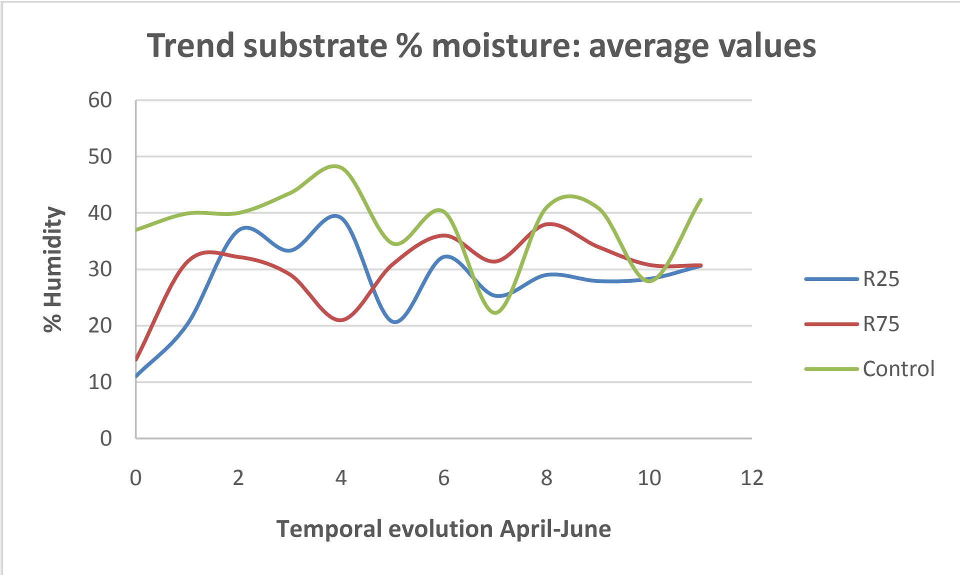


FIGURE 11. Temporal evolution of the humidity percentage: R25) container with a sandstone layer of 25% of the useful height. R75) container with a sandstone layer of 75% of the useful height. Control) control vessel.

3.2.1.1 Conductivity of substrates

In the substrates that had the influence of the weather, the results obtained in the percentage of humidity were complemented by those of the conductivity, since the values obtained in the container B were, systematically, higher than those obtained in container A, as shown in Figure 12.

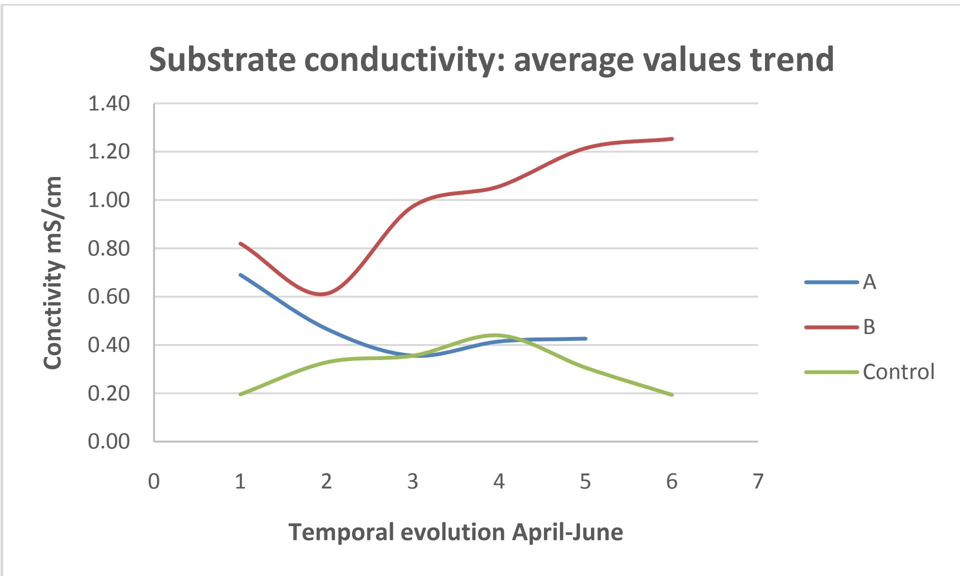


FIGURE 12. Temporal evolution of the substrate conductivity: A) container with vegetable compost. B) Container without vegetable compost. Control) control container.

This behaviour was attributed to the contribution in inorganic compounds of the selected substrate. Only in the case of strong rainfall, greater than 5 L/m2, this parameter provided anomalous results, as a result of the leaching effect of the fallen water on the ground. As far as the control vessel is concerned, freshwater irrigation caused the value of the conductivity to show a tendency lower than that of the selected substrates.

On the other hand, in the substrates those were not subjected to the inclement weather, the values of the conductivity showed a significantly lower dispersion (Figure 13). On the contrary, the experimental values of the control vessel were lower, and, in addition, they experienced a greater dispersion, attributable to manual irrigation with fresh water.

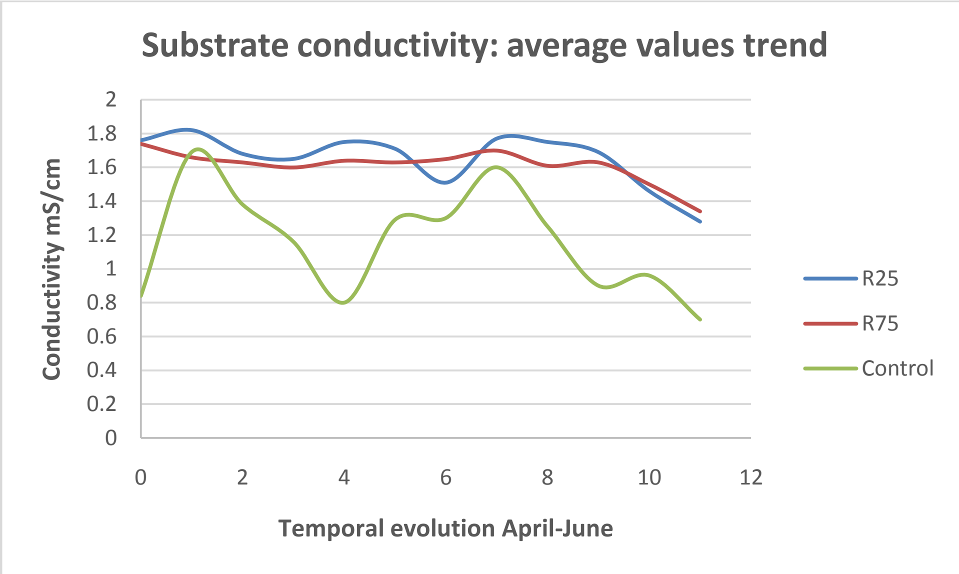


FIGURE 13. Temporal evolution of the conductivity of the: R25) container with a layer of sandstone of 25% of the useful height. R75) container with a layer of sandstone of 75% of the useful height. Control) control vessel.

3.2.2 Evolution of vegetables

First of all, the samples were prepared. In the case of lettuce, samples were taken from the aerial part of the plant and from the roots. Table 2 shows the results of moisture and content in organic matter.

As far as the percentage of humidity is concerned, the lettuce leaf developed in the control vessel showed a slightly higher value than the lettuce leaves developed in vessels with capillary irrigation. This result was consistent with the percentage of humidity determined at 20 cm depth in the different containers.

TABLE 2
MOISTURE PERCENTAGE IN FRESHLY PICKED LETTUCE AND PERCENTAGE OF ORGANIC MATTER AND AVERAGE PH VALUES IN ASHES

Code	% Humidity	% Organic material	pH
Leaf L75	89,96 ± 1,95	72,60 ± 0,50	10,3
Leaf L25	89.93 ± 0,75	64,86 ± 0,65	9,6
Leaf LC	94,23 ± 1,15	67,78 ± 0,45	10,3
Roots L75	67,65 ± 0,88	65,28 ± 1,10	7,9
Roots L25	80.97 ± 1,05	75,29 ± 1,25	9,9
Roots LC	82,97 ± 0,95	84,98 ± 0,95	10,9

L75: lettuce developed in the bowl with 75% useful height of sandstone
L25: lettuce developed in the bowl with 25% useful height of sandstone
LC: lettuce developed in the control container

The percentage of humidity determined in the roots of the plant, the control vessel showed a slightly higher value than the plant developed in the vessel with a layer of sandstone coinciding with 25% of the vessel's useful height. On the contrary, the roots that were developed in the vessel with a layer of sandstone coinciding with the 75% of the vessel's useful height showed a significantly lower value.

On the other hand, the percentage of organic matter determined in lettuce leaves showed a central value corresponding to the plant developed in the control vessel. Paradoxically, the value corresponding to the plant developed in the vessel with a useful height of 75% of sandstone showed the highest percentage. However, when the results were considered for the roots, the control value exceeded by far the other two values.

All the ashes, after being treated with distilled water for 10 minutes, showed alkaline pH values. If the specimen block corresponding to the leaves is considered, these values oscillated between 9.6 and 10.3 pH units. When the block corresponding to the samples for the roots was considered, a greater dispersion was observed, between 7.9 and 10.9 pH units, corresponding to the value of 7.9 to the sample developed in the vessel with a 75% of useful height of sandstone and that of 10.9 to the control vessel.

Table 3 shows the results corresponding to the sherry tomato. Results were obtained from roots, stems, leaves, and fruits. The lower percentage of humidity was determined in roots, being the control vessel where the lowest of the values were determined, whereas in the containers subjected to the capillarity regime with seawater, practically identical values were obtained.

The other three parts considered (stem, leaf and fruit) showed values of the same degree of magnitude, significantly higher than those determined in the roots.

When the values of organic matter were determined, the higher percentages were determined in the roots and, unlike the experience with the values of the humidity, the percentages in organic matter were not of the same order of magnitude in the stems, leaves and fruits.

The lower percentages of organic matter were determined in the stem. While the lesser of them was the one corresponding to the tomato developed in the container with a 25% useful height of sandstone, the other two values were, practically, equivalent.

This situation was repeated with the results determined in the leaves. However, on this occasion the other two results were very different from each other, presenting the highest value the control vessel.

Finally, the values determined in the fruit were very scattered among themselves, presenting the least of them in the control vessel.

As in the experience made with lettuce, all the ashes, after being treated with distilled water to boiling for 10 minutes, presented alkaline pH values. The vast majority of the values determined exceeded the 10 pH units.

TABLE 3
HUMIDITY PERCENTAGE IN FRESHLY HARVESTED TOMATO SHERRY AND PERCENTAGE OF ORGANIC MATTER AND AVERAGE PH VALUES IN ASHES

Code	% Humidity	% Organic material	pH
Roots T75	71,16± 1,75	86,03±0,95	9,7
Roots T25	71,99± 1,30	85,00±1,15	10,3
Roots TC	67,65± 1,55	85,74± 1,05	10,8
Stem T75	87,45± 0,85	66,92± 0,60	10,6
Stem T25	89,17± 0,75	54,54± 0,60	9,9
Stem TC	91,96± 0,70	65,83± 0,65	10,1
Leaf T75	85,53± 1,15	77,91± 0,70	8,6
Leaf T 25	86,14± 1,25	68,37± 0,55	9,1
Leaf TC	82,92± 1,20	84,75± 0,60	10,5
Fruit T75	88,83± 1,85	76,04± 0,50	10,0
Fruit T25	87,10± 1,90	68,80± 0,40	10,9
Fruit TC	89,28± 1,85	53,33± 0,55	10,6

T75: Tomato developed in the container with a 75% useful height of sandstone
T25: Tomato developed in the container with a 25% useful height of sandstone
TC: Tomato developed in the control container

3.3 Optimization of the material in contact with seawater

From the selected materials (sandstone, beach sand and compost), the different combinations included in Table 4 were initially tested.

TABLE 4
SELECTED SOIL COMPOSITIONS

	Composition
1	Thick sandstone 100%
2	Medium sandstone 100%
3	Thin sandstone 100%
4	Vegetable compost 100%
5	Sand beach 100%
6	90% Thick sandstone 10% Vegetable compost
7	90% Medium sandstone 10% Vegetable compost
8	90% Thin sandstone 10% Vegetable compost
9	90% Sand beach 10% Vegetable compost
10	50% Thick sandstone 50% Vegetable compost
11	50% Medium sandstone 50% Vegetable compost
12	50% Thin sandstone 50% Vegetable compost
13	50% Sand beach 50% Vegetable compost

The incorporation of compost originated instability in each mixture introduced inside the specimen, so that, with the passage of days the height of all the mixtures decreased significantly. This behaviour was attributed to the high humidity of the compost compared to sandstone and beach sand. Table 5 shows the percentage of humidity determined by weight loss at 110 ºC.

In a second experience it was decided to use the previously desiccated compost at 110 ºC until constant weight, analogous to how it had been done with the sandstone and beach sand. Even so, the experimentation with the compost was definitely left aside because, as much as it reduced its degree of humidity, the difficulties when compacting it in the tubes were very large due to the large amount of air that it contained and did not present the GA Warranties enough to obtain reproducible results.

TABLE 5
WEIGHT LOSS BY DRYING UP TO CONSTANT WEIGHT AT 110ºC

Material	Percentage
Thick sandstone	1,86%
Medium sandstone	2,76%
Thin sandstone	2,58%
Sand beach	Inappreciable
Vegetable compost	62,18%

Finally, coarse sandstone and beach sand were chosen as substrates to be used to systematically determine the height at which sea water rose and the variation of electrical conductivity attributable to this ascent.

Two series of 4 tubes were set, all of them, of the same diameter (57 ± 5 mm) and height (68 cm). In order to establish the influence of water's height contained in the containers, an experience was programmed in which half of the tubes were immersed 2 cm in the water and the other half 3cm,for a period of five days. This experience was made with coarse sandstone and beach sand. Table 6 shows the results obtained with coarse sandstone after the first 5 days, in the 68 cm high specimens.

TABLE 6
HEIGHT OF SEAWATER INSIDE THE SPECIMENS OF 68 CM IN FIVE DAYS

Day	Initial level of water (cm)	Gross height (cm)	Net height (cm)	Ascent speed (cm/day)
3	2	15,9	13,9	4,6
	3	16,6	13,7	4,6
4	2	16,6	14,6	3,7
	3	17,8	14,9	3,7
5	2	17,9	15,9	3,2
	3	18,9	16,0	3,2

After these 5 days, only in the container in which the specimens were submerged 2 cm in seawater, the same initial volume of seawater was added.

Table 7 shows the results obtained with coarse sandstone from the fifth day in the 68 cm high specimens.

TABLE 7
EIGHT OF THE SEAWATER INSIDE SANDSTONE'S SPECIMENS OF 68 CM FROM THE FIFTH DAY

Day	Initial level of water (cm)	Gross height (cm)	Net height (cm)	Ascent speed (cm/day)
5	3,4	18,3	----	-----
	1	18,6	----	-----
7	3,4	20,3	2	1
	1	20,3	1,7	0,8
10	3,4	22,6	4,3	0,9
	1	22,5	3,9	0,8
12	3,4	24,0	5,7	0,8
	1	23,8	5,2	0,7
17	3,4	25,7	7,4	0,6
	1	25,7	7,1	0,6
20	3,4	28,6	10,3	0,7
	1	28,1	9,5	0,6

The results obtained in this experience showed the null influence of the different level of sea water inside the container.

The tested levels were 3 and 4 cm in the experiment made with 28 cm high specimens. The experimental results obtained for five days are shown in table 8.

TABLE 8
HEIGHT REACHED BY THE MARINE WATER INSIDE THE TEST PIECES OF 28 CM

Day	Initial level of water (cm)	Gross height (cm)	Net height (cm)	Ascent speed (cm/day)
3	3	18,1	15,3	5,1
	4	18,6	14,6	4,8
4	3	19,2	16,3	4,1
	4	19,7	15,7	3,9
5	3	20,5	17,6	3,5
	4	20,9	16,8	3,4

On this occasion, the net values of the height reached by the seawater inside the test tube were slightly higher than those of the experience carried out with 68 cm high specimens (see table 6). However, when the average velocity values were considered, a convergence was found in this parameter on the fifth day.

Five days later, in one container the specimens were submerged 3cm in seawater, the same volume with which the experience began, while in the other container no volume of seawater was added.

When the control of seawater height reached inside the specimens was retaken, the reference values were 20.4 and 21.0 cm, respectively. Table 9 shows the results obtained with coarse sandstone from the fifth day in the 68 cm high specimens.

TABLE 9
HEIGHT OF SEAWATER INSIDE THE TEST PIECES OF 28 CM FROM THE FIFTH DAY

Day	Initial level of water (cm)	Gross height (cm)	Net height (cm)	Ascent speed (cm/day)
5	3,4	20,4	----	-----
	1	21,0	----	-----
7	3,4	22,8	2,4	1,2
	1	23,1	2,9	1,4
10	3,4	25,7	5,3	1,1
	1	25,6	5,6	1,1
12	3,4	26,9	6,5	0,9
	1	26,6	5,6	0,8
17	3,4	25,7	7,4	0,6
	1	25,7	7,1	0,6
20	3,4	29,5	9,1	0,6
	1	28,4	7,4	0,5

Both experiences showed a convergence in the values of the average velocity of ascension of seawater by the inside of the specimens, regardless of their height, towards values of the order of 0.6 (cm/day), as it can be seen in Figure 14.

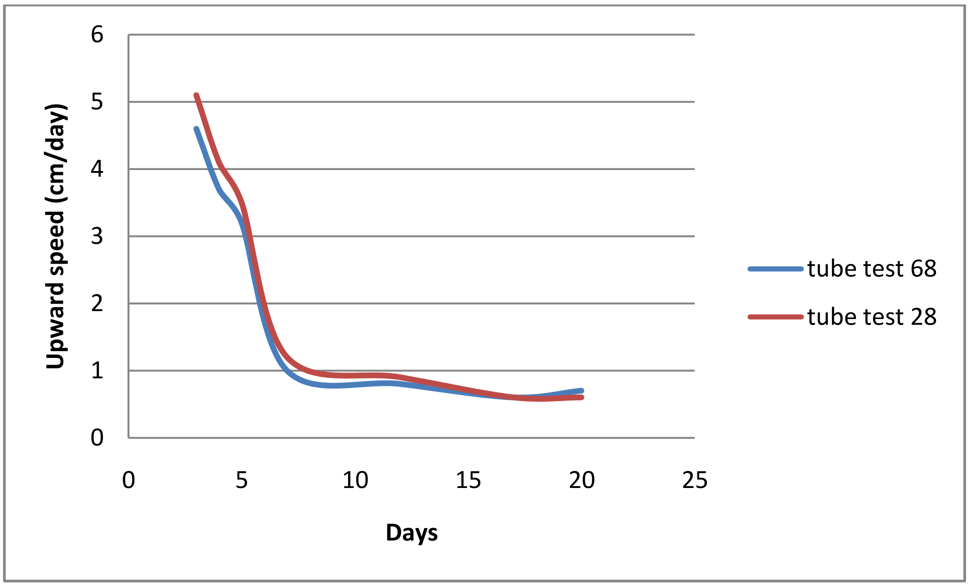


FIGURE 14. Temporal evolution of the ascending velocity of seawater inside the specimens

When the experience with beach sand was repeated, the ascent of the seawater inside the specimens was much smaller, compared to the results obtained with the sand stone. Such is the case that in 35 days the ascent observed in both types of specimens was 9.3 cm which is equivalent to an upward velocity of 0.26 cm/day.

This difference between the values of the ascending velocity was justified by the different particle size of both substrates. Thus, in the coarse sandstone used, the particles of more than 2 mm diameter predominate, while in the sand of the beach the predominant particle diameter was less than 1 mm (see table 1). This difference in the predominant size of the particles caused the degree of compaction to be significantly higher in the beach sand, so that the capillary capacity of this substrate decreased.

On the other hand, samples were taken from inside the specimens at different levels. Experimentally, it was necessary to gradually empty each specimen from the upper part and take the corresponding aliquots at the previously determined heights.

As a reference point, the maximum height delimited by seawater was considered within the container where the specimens were inserted. From that height, the different levels were set. Table 10 shows the experimental values that were considered for the specimens of 68 and 28 cm of height, as well as the corresponding values of the conductivity.

The values of the conductivity were decreased as the aliquots were taken at a higher height than the sea water level in the vessel, with convergent values starting at 17 cm height.

TABLE 10
CONDUCTIVITY VALUES VS HEIGHT REACHED BY SEAWATER INSIDE THE TEST TUBE

Test tube 68 cm height		Test tube 28 cm height	
Height above seawater level (cm)	**Conductivity (mS/cm)**	**Height above seawater level (cm)**	**Conductivity (mS/cm)**
3,6	1,49	0,7	1,99
8,1	0,94	6,7	1,18
12,6	0,76	12,7	0,82
17,1	0,61	18,7	0,63

3.4 Experiences with the Tlaloc device

In order to determine how the different variables that were contemplated in the experiment affected: irrigation water, substrate and filter height, and to obtain a first approximation of the useful life of the product, the study was focused from two slopes.

First, it was determined how it affects the irrigation with seawater on the different quality parameters of the chard as they are: moisture, mineral matter, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} content, ascorbic acid, colour, weight, height and leaf width. Secondly, it was determined how some of these quality parameters evolve over 12 days in the product preserved at 4 ± 1 ºC.

3.4.1 Characteristics of the irrigation water used.

Table 10 shows the characteristics of the two types of water used, the control (water network) and seawater, both obtained in the locality of Badalona.

As expected, seawater presented a very high mineral content, which resulted in that its value of conductivity was of the order of 40 times higher than that of the net water, being the predominant anions chlorides and sulphates while the cations Majoritarian were sodium and magnesium. As far as the pH values are concerned, they were very similar around the neutral pH.

TABLE 10
CHARACTERISTICS OF IRRIGATION WATERS USED IN THE CULTIVATION OF SWISS CHARD

Badalona seawater									
pH: 7,03									
Conductivity: 39,8 mS/cm									
Ions (mg/L)	NH_4^+	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	NO_2^-	NO_3^-
	< 10	260	10060	435	1280	15882	2305	< 10	60
Badalona fresh water									
pH: 7,31									
Conductivity: 985 µS/cm									
Ions (mg/L)	NH_4^+	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	NO_2^-	NO_3^-
	< 1	13	99	87	23	139	88	< 1	7

3.4.2 Effect of seawater on different quality parameters

3.4.2.1 Effect of filter height (6 or 20 cm) on moisture content and mineral matter

With regard to the effects of the height of the filter used, it was observed that those samples with less filter height (6cm), regardless of the material used (sand beach or sandstone), showed a tendency to retain more moisture.

This behaviour was attributed to the largest amount of compost contained in the TLALOC device. In this sense, he highlighted the control device (consisting exclusively of vegetable compost) as the sample of chard with higher moisture content (see table 11). On the contrary, this trend was reversed when the percentage was considered in mineral matter, the sample of control being the one that presented the least of the experimental values. In fact, all samples irrigated with seawater show a mineral content between 4 and 12% higher than the control sample, depending on the treatment.

TABLE 11
PERCENTAGE OF MOISTURE AND MINERAL MATTER ON THE DAY OF HARVEST (D.0) AND EVOLUTION OF HUMIDITY DURING A PERIOD OF 12 DAYS.

Treatments	Initial humidity (%)	Mineral matter (%)	Humidity day 2	Humidity day 6	Humidity day 8	Humidity day 12
A1 (6 cm)	91,05 ± 2,05	35,59 ± 0,55	92,10 ±1,85	92,21 ± 1,60	91,9 ± 1,35	91,67 ± 1,20
S1 (6 cm)	90,82 ± 1,95	39,09 ± 0,65	91,78 ± 1,95	90,42 ± 1,50	90,83 ± 1,55	91,16 ± 1,10
A2(20 cm)	89,22 ± 1,70	43,53 ± 0,45	88,48 ± 1,50	88,04 ± 1,75	88,09 ± 1,50	88,27 ± 1,25
S2(20 cm)	89,99 ± 1,75	39,31 ±0,55	89,05 ± 1,80	89,65 ± 1,65	88,78 ± 1,40	88,39 ± 1,30
Control	94,37 ± 2,15	31,28 ± 0,60	93,13 ± 1,90	93,91 ± 1,45	93,89 ± 1,50	93,83 ± 1,15

A1/S1 Containers with a 6 cm high sand and sand beach filter, respectively
A2/S2 Containers with a 20 cm high sand and sand beach filter, respectively

When the samples of chard kept inside a refrigerator were treated at 4 ± 1 ºC up to a maximum of 12 days, the humidity of the control sample showed higher values than the rest of the samples considered (see Figure 15).

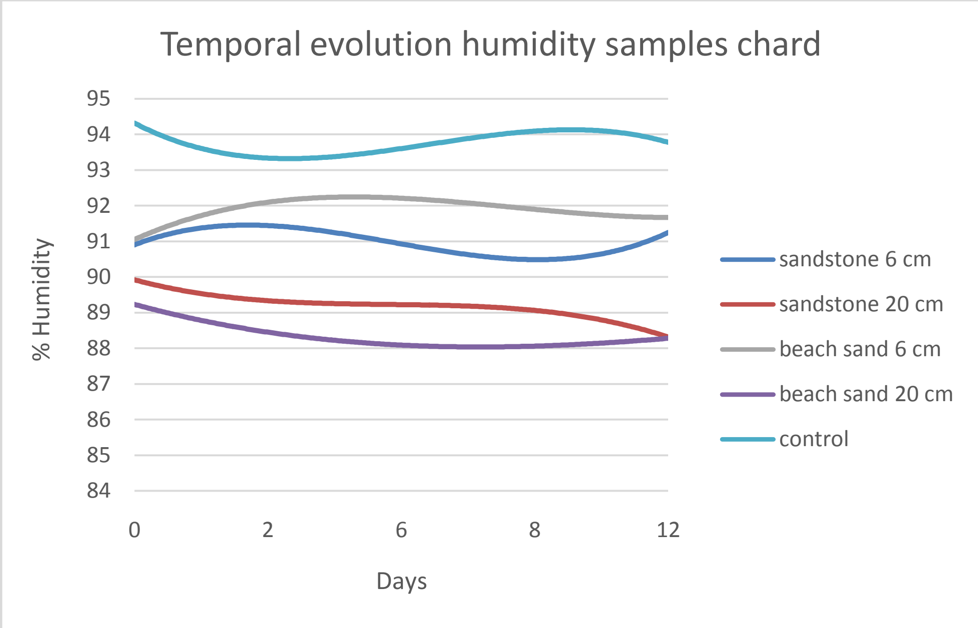


FIGURE 15. Temporal evolution of humidity in chard samples preserved in the fridge

The samples with less filter height (6cm) maintained the tendency to present a higher moisture percentage to the samples with higher filter height (20 cm), regardless of the type of filter considered (sandstone or beach sand). The samples with a 6 cm filter converged, at the end of this time period, towards humidity values of 91% and the samples with a filter of 20 cm did it towards values of 88%, while the control sample presented a value of 94%. This showed the influence of the filter height used: At higher altitude, lower moisture content.

As far as the concentration of alkaline and alkaline metals is concerned, it stands out the low magnesium content in all the treatments. However, when the values obtained with different filter heights (A1/A2) and (S1/S2) are compared, a tendency is observed to obtain higher concentrations with the 20 cm filters.

TABLE 12
CONCENTRATIONS OF ALKALINE AND ALKALINE EARTH METALS IN THE FRESHLY COLLECTED SAMPLES, AFTER BEING DRIED AND CALCINED

Treatment	Na^+ (g/Kg DS)	K^+ (g/Kg DS)	Ca^{2+} (g/Kg DS)	Mg^{2+} (g/Kg DS)
A1 (6 cm)	21,24 ± 0,25	30,41 ± 0,30	13,73 ± 0,15	0,33 ± 0,07
S1 (6 cm)	37,87 ± 0,20	42,03 ± 0,35	26,50 ± 0,25	0,77 ± 0,05
A2(20 cm)	48,90 ± 0,30	41,86 ± 0,30	26,46 ± 0,20	0,76 ± 0,04
S2(20 cm)	55,78 ± 0,25	37,05 ± 0,25	30,91 ± 0,20	0,84 ± 0,06
Control	29,16 ± 0,20	50,82 ± 0,30	21,93 ± 0,25	0,71 ± 0,08

A1/S1 Containers with a 6 cm high sand and sand beach filter, respectively
A2/S2 Containers with a 20 cm high sand and sand beach filter, respectively
DS, Dry sample

When the values obtained in the control vessel were considered, a very high value was observed in the case of potassium, a fact that was attributed to the composition of the compost plant (NPK 18-10-20).

3.4.2.2 Effect of filter height (6 or 20 cm) on ascorbic acid content

The ascorbic acid content in the samples of freshly collected chard showed a different behaviour depending on the height of the filter, regardless of whether it was sandstone or beach sand. Thus, samples from vessels with less filter height (6cm) showed a lower ascorbic acid content than those from a higher-height filter (20 cm). After an irregular evolution during the first 6 days, with the exception of the samples from the containers with a filter of 6 cm of beach sand, the whole of the samples presented convergent values towards 11 mg of ascorbic acid/100 grams Initials of Chard at 12 days. Figure 16 shows this behaviour graphically.

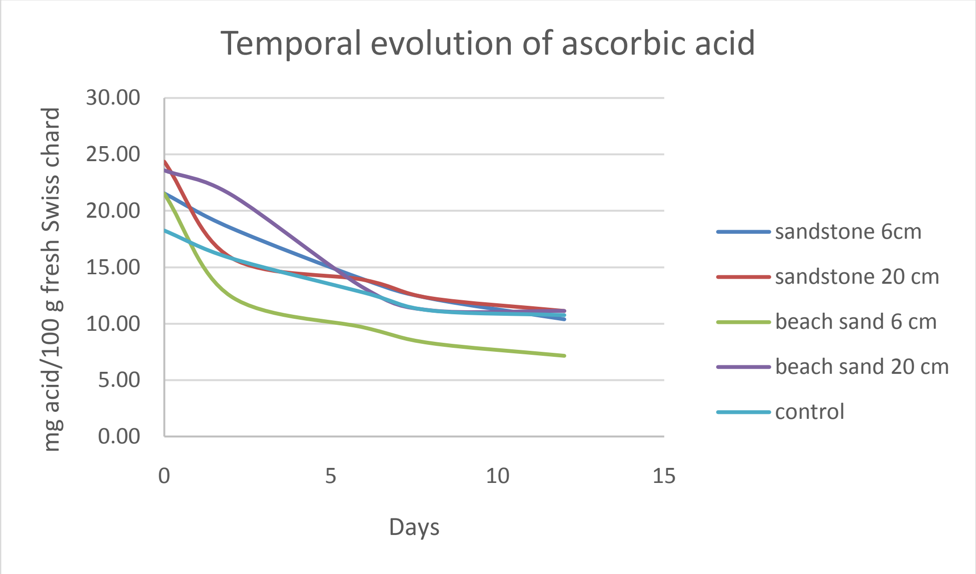


FIGURE 16. Temporal evolution of ascorbic acid during 12 days

3.4.2.3 Effect of filter height (6 or 20 cm) on the weight, height and width of the chard

Samples from vessels with less filter height (6cm) showed a significantly higher weight than the samples coming with a higher filter height (20 cm). Among them, the ones from a sandstone filter presented more weight than those coming from a beach sand filter.

Table 13 shows the relation of weight, height and width of the chard according to the treatment to which they had been subjected.

TABLE 13
AVERAGE VALUES OF WEIGHT, HEIGHT AND WIDTH OF THE FRESHLY HARVESTED SWISS CHARD

Treatment	n	Weight (g)	Height (cm)	Width (cm)
A1 (6 cm)	32	93,08 ± 18,72	16,86 ± 2,11	11,78 ± 1,99
S1 (6 cm)	32	113,03 ± 10,95	17,73 ± 1,71	12,13 ± 2,31
A2(20 cm)	32	56,38 ± 8,86	14,48 ± 1,79	9,58 ± 2,20
S2(20 cm)	32	41,77 ± 12,75	12,30 ± 2,99	8,56 ± 2,26
Control	32	99,47 ± 47,14	17,00 ± 3,10	10,25 ± 1,91

A1/S1 Containers with a 6 cm high sand and sand beach filter, respectively
A2/S2 Containers with a 20 cm high sand and sand beach filter, respectively

3.4.2.4 Effect of filter height (6 or 20 cm) on the colour

From the experimental values of the parameters determined with the colorimeter KONICA MINOLTA CR-400,it was proceeded to calculate the corresponding colour index (I.C.).

The results obtained are shown in table 14. The values of IC obtained allowed cataloguing the chards with a bluish-green colour. Samples from vessels with less filter height (6cm) were closest to the colour of the control sample, especially after being collected and after 6 days.

TABLE 14
EVOLUTION OF THE COLOUR INDEX (I.C.) OF CHARD FOR A PERIOD OF 12 DAYS

Treatment	Initial IC	6^{th} day IC	12^{th} day IC
A1 (6 cm)	-36,93 ± 2,25	-36,08 ± 0,85	-34,29 ± 3,88
S1 (6 cm)	-37,21 ± 3,54	-36,75 ± 2,76	-34,11 ± 3,93
A2(20 cm)	-35,97 ± 4,62	-36,97 ± 2,54	-36,73 ± 3,08
S2(20 cm)	-35,33 ± 4,66	-38,23 ± 3,46	-34,68 ± 2,99
Control	-38,30 ± 1,29	-35,36 ± 1,84	-36,35 ± 1,33

A1/S1 Containers with a 6 cm high sand and sand beach filter, respectively
A2/S2 Containers with a 20 cm high sand and sand beach filter, respectively

IV. DISCUSSION

Current patterns of development and production are unsustainable because they lead to overexploitation of aquifers and rivers, environmental degradation and loss of coastal and indoor wetlands (Marcellesi 2012). In the last fifty years the world irrigated area has doubled and water withdrawals for agriculture have increased (Siebert *et al* 2010).

Life in rural areas is in fact facing very serious problems in practically all countries. The scarcity of good quality water resources is becoming an important issue in arid and semiarid areas. For this reason, the availability of water resources of marginal quality, such as drainage water, saline groundwater and treated wastewater, has become an increasingly considered option (Martínez 1999).

Different studies indicate that brackish water can be used successfully for the production of irrigated crops (Al-Karaki 2006; Niu *et al.* 2010; Jiang *et al.* 2012; Malash *et al.* 2012; Singh & Panda 2012). However, the negative effects on crops may occur from the use of saline water due to the accumulation of salt in the soil (Rengasamy, 2010; Wan *et al*. 2010; Huang *et al*. 2011; Wang *et al*. 2015), especially in regions of limited rainfall (400 mm/year). The extension of the salt accumulation in the different layers of the soil is affected by the specific management of irrigation and local climatic conditions. The dynamics of water in the soil, the structural stability of soil, the solubility of compounds in relation to pH and the movement of nutrients and water play a vital role in the selection and development of salinity tolerant plants.

The incorporation of desalinated seawater into irrigated areas is another alternative that has been booming in recent decades. At the global scale of the main experiences of agricultural irrigation with desalinated water shows that, in many countries with arid or semiarid climate, and that also have a highly technical agriculture, the desalination of brackish waters It represents an additional source of water (ANZECC & ARMCANZ, 2000).

The main advantage of desalination of brackish or marine water is its condition as an inexhaustible water resource and not subject to climatic variations, so strategically it is ideal to systematically increase the availability of water resources for agricultural irrigation in deficit areas. However, only the most technical crops and with higher economic margins can withstand the costs of desalinated water (Martínez & Martín 2014). Starting a desalination plant implies an investment and very high maintenance costs, in addition to a considerable energy consumption that is mainly covered by the use of fossil fuels, with the environmental damage that this involves.

The effect of soil also plays an important role in those soils affected by salinity. Soils also play an important role, especially in those affected by salinity. If a soil is saline, but it is not permeable there will not be enough drainage and therefore there will be a greater accumulation of salts, usually in the area of the roots. For this reason, it is very important to take care of the drainage and aeration of soils affected by salinity (Maas, 1993).

At the time of irrigation, with the traditional irrigation system, water content in soil is maximum, while the concentration of soluble salts is minimal. While water is lost by evaporation and by perspiration, the content of salts around the plant increases. It is for that reason that the more salt content water contains, the more frequent irrigation should be to minimise the impact of water stress on the crop. However, an excess of watering could cause a lack of air in the soil, particularly with those very fine particle soils with little porous space (Maas & Hoffman, 1977).

The tolerance of crops to salinity is expressed as the decrease in the yield of a crop in which a saline concentration has been applied in the root zone, compared to the yield of a crop without the application of a saline solution. (Maas and Hoffman, 1977). This tolerance and the effect that salinity produces on the plant depend on the species and other environmental conditions. (Shannnon & Grieve, 1999).

Temperature, relative humidity, environmental pollution significantly affects the plant's response to salinity. Most crops tolerate soil salinity better when there is a cooler, wetter environment. The combination of environmental factors such as high temperatures (summer temperatures), wind, low humidity (< 50%) Either the drought is more damaging and have a more negative effect on the plant than the salinity itself (Blankendaal *et al.* 1972; Maas, 1993).

The first effects of salinity experienced by the plant are those caused by the osmotic effect. Roots are reduced to length and mass, but not in thickness. The osmotic effect of salinity contributes to reduce the growth rate, changing the colour of the leaves and slowing the normal growth rate. The ionic effects are usually manifested in leaves and meristems. Thus, large amounts of Na^+ and Cl^- accumulate in leaves causing burns.

The symptoms due to lack of nutrients caused by salinity are similar to those suffered by plants irrigated with fresh water, but with lack of nutrients in the substrate. However, not all effects are negative. In spinach, for example, salinity can have a positive effect on disease resistance. On the other hand, the sugar content in the carrot increases with the presence of salts while the starch content decreases in the potato (Maas & Hoffman, 1977; Shannon & Grieve, 1999).

High salinity in soil's pores affect plants in several ways. First, the osmotic potential of the soil experiences a descent; This makes it more difficult for the plant to obtain water from the ground, since the plant should decrease the osmotic potential of its roots below the osmotic potential of the soil in order to obtain water. Second, the ions from the salt that enter the water inside the plant can also cause physiological damage because elevated Na^+ levels can give high toxicity by the plant(Munns *et al.* 2006). This large amount of Na^+ in soil's pores increase competition with the K^+ ions, mineral that is essential for the plant, since the two ions use the same channels to enter inside, giving rise to a lower absorption of K^+ and a deficit of this mine Ral. (De Vos *et al.* 2016).

On the other hand, there are antecedents in which seawater has been used without desalination as irrigation water for crops. Already in 1719, there are experiences carried out in the desert of San José de la Isla, where crops were irrigated with seawater by the Carmelite monks in the area where the convent was located (Esteban-Gómez 1968). Much more recent is the use of sandy and coastal sea-water belts for growing crops in India (Iyengar 1968).

It also highlights the contribution of Dr. Maynard Murray, who, in the mid-twentieth century, after many years devoted to medicine, concluded that the biggest cause of health problems came from a shortage of minerals in food, and this deficit came from the methods used in agriculture. By not having the cultivation of the minerals necessary for its correct growth and development (trace elements), these nutritional deficits passed both to the cattle and to the people, producing a set of alterations and imbalances in the organism (Murray 1976).

In the experiments carried out by CRESCA and the Aqua Maris foundation, it has been proven that, in the case of Swiss chards subjected to the most extreme conditions of the containers isolated from the weather, root growth of the roots has penetrated the growing medium and the filter zone, reaching the interface with the seawater in the last period of growth of the plant just before its collection.

To optimize the growth of other plant species, it is necessary to adapt and improve the composition and height of the filter zone.

V. CONCLUSIONS

The results obtained experimentally allow affirming that the direct use of brackish or marine water as irrigation fluid, as long as it is administered groundwater, is viable. However, this necessary condition is not enough. It is necessary to have a substrate as a filter with certain characteristics (composition and particle size) that allow reducing the saline content and maintaining the humidity at a sufficient height so that the roots of the cultivated vegetables can absorb enough water and nutrients without reaching toxicity limits.

In addition, it is necessary to keep in mind the climatic influence (rain, wind, temperature...) As long as this experience is not carried out inside a greenhouse where the environmental conditions can be regulated.

REFERENCES

[1] ANZECC & ARMCANZ. (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Volume 1. The Guidelines. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand.

[2] Al-Karaki G. (2006). Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Scientia Horticulturae. 109. 1-7. DOI: 10.1016/j.scienta.2006.02.019.

[3] AOAC (2000). Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

[4] Aparicio Mijares F.J. (1987). Fundamentos de Hidrología de Superficie. Ed. Limusa. Noriega Editores, México. 303p.

[5] Blankendaal, M., Hodgson, R. H., Davis, D. G.., Hoerauf, R. A., Shimabukuro, R. H. (1972). Growing plants without soil for experimental use. Agricultural Research Service (DOA), 1251(1), 1-11.

[6] BOE de 8 de diciembre de 2007. Ministerio de la Presidencia. Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas.

[7] Custodio E., Llamas M.R. (1983). Hidrología Subterránea. Editorial Omega, 2ª edición. Barcelona.

[8] De Vos A., Bruning B., Van Straten G., Oosterbaan R., Rozema J., Van Bodegom P. (2016). Crop salt tolerance. Salt Farm Texel. http://www.saltfarmtexel.com/publications.

[9] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

[10] Esteban-Gómez I. (1968). Agricultural cultivation by means of direct irrigation with undesalinated seawater p. 93-106. In: Boyko H. (eds) Saline Irrigation for Agriculture and Forestry. World Academy of Art and Science, Vol 4. Springer, Dordrecht. Doi: 10.1007/978-94-017-6016-4.

[11] FAO. (2000). Organización de las Naciones Unidas para la Agricultura y la Alimentación. 2000. Manual de prácticas integradas de manejo y conservación de suelos.

[12] Grasshoff K., Kremling K., Ehrhardt M. (1999). Methods of Seawater Analysis. Wiley Verlag– VCH, 3th edition.

[13] Gutiérrez, N. (2017) Estudio de factibilidad técnica de riego con agua de mar por capilaridad. Seminario sobre recursos hídricos en agricultura de zonas áridas. Centro de Investigación Tecnológica de Agua en el Desierto (CEITSAZA). Universidad Católica del Norte. Antofagasta. Chile.

[14] Howard G., Bartram J. (2003) Domestic Water Quantity, Service, Level and Health. World Health Organization.

[15] Huang C. H., Xue X., Wang T., De Mascellis R., Mele G., You Q. G., Peng F., Tedeschi A. (2011). Effects of saline water irrigation on soil properties in northwest China. Environmental Earth Sciences, 63, 701–708. DOI: 10.1007/s12665-010-0738-5

[16] Iyengar E.R.R., Kurian T., Tewari A. (1968) Utilization of Sea-water and Coastal Sandy Belts for Growing Crops in India. In: Boyko H. (eds) Saline Irrigation for Agriculture and Forestry. World Academy of Art and Science, vol 4. Springer, Dordrecht. Doi: 10.1007/978-94-017-6016-4

[17] Jiang J., Huo Z., Feng S., Zhang C. 2012. Effect of irrigation amount and water salinity on water consumption and water productivity of spring wheat in Northwest China. Field Crops Research, 137, 78–88. DOI: 10.1016/j.fcr.2012.08.019

[18] Lechuga J., Rodríguez M., Lloveras J. (2007). Análisis de los procesos para desalinización de agua de mar aplicando la inteligencia competitiva y tecnológica, Ingeniería, Revista Académica de la FI-UADY, 11-3, pp. 5-14, ISSN: 1665-529X

[19] Maas, E.V. (1993). Testing crops for salinity tolerance. Salinity Laboratory U.S., USDA-ARS, Riverside, CA.

[20] Maas E.V., Hoffman G. J. (1977). Crop salt tolerance – current assessment. Journal of the Irrigation and Drainage Division, 103, 115-134.

[21] Malash N., Ali F., Fatahalla M., Hatem M., Tawfic S. (2012). Response of tomato to irrigation with saline water applied by different irrigation methods and water management stratigies. International Journal of Plant Production, 2, 101–116. DOI: 10.22069 / IJPP.2012.603

[22] Marcellesi, F. (2012) Cooperación al postdesarrollo. Bases teóricas para la transformación ecológica de la cooperación al desarrollo, Bakeaz, Bilbao 222p.

[23] Martínez V., Martín B. (2014) Antecedentes y problemática de la aplicación de agua marina desalinizada al riego agrícola. Universidad Politécnica de Cartagena.

[24] Martínez, J. (1999) Irrigation with saline water: benefits and environmental impact. Agricultural water management, 40 (2,3) 183-194. https://doi.org/10.1016/S0378-3774(98)00120-6

[25] McCully, P. (1996). Silenced rivers: the ecology and politics of large dams. Zed Books, London, UK

[26] Moral, Leandro del (2009). «New trends in water management, spatial planning and integration of sectorial policies». Revista Electrónica de Geografía y Ciencias Sociales, XIII, 285.

[27] Munns R, James RA, Lauchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany 57, 1025–1043.

[28] https://doi.org/10.1093/jxb/erj100

[29] Murray, M. (1976) Sea energy agricultura. Ed. Valentine & Hendren, Inc.

[30] Niu G, Rodriguez D S, Starman T. 2010. Response of bedding plants to saline water irrigation. HortScience, 45, 628–636. https://doi.org/10.21273/HORTSCI.45.4.628

[31] Pidwirny, M. (2006). The Hydrologic Cycle *Fundamentals of Physical Geography 2nd Edition*.

[32] Postel, S. L. (2000). Entering an era of water scarcity: The challenges ahead. Ecological applications, 10(4), 941-948. DOI: 10.2307/2641009

[33] Rengasamy P. (2010). Soil processes affecting crop production in salt-affected soils. Functional Plant Biology, 37, 613–620. https://doi.org/10.1071/FP09249

[34] Shannon, M.C, Grieve, C.M. (1999) Tolerance of vegetable crops to salinity. Scientia Horticulturae, 78, 5-38.Doi: 10.1016/S0304-4238(98)00189-7

[35] Siebert S., Burke J., Faures J. M., Frenken K., Hoogeveen J., Döll P., Portmann F. T. (2010). Groundwater use for irrigation – a global inventory. Hydrol. Earth Syst. Sci., 14, 1863–1880. Doi:10.5194/hess-14-1863-2010

[36] Singh A., Panda S. N. (2012). Effect of saline irrigation water on mustard (Brassica juncea) crop yield and soil salinity in a semi-arid area of North India. Experimental Agriculture, 48, 99–110.https://doi.org/10.1017/S0014479711000780

[37] United Nations (1992). Managing fragile ecosystems: combating desertification and drought. In: Agenda 21, A/CONF.151/4 (Part II). Geneva: United Nations. pp. 46–66.

[38] Veza J. M. (2002). Introducción a la Desalación de Aguas, 1ªedición Universidad de Las Palmas de Gran Canaria, Gran Canaria, España.

[39] Wan S., Kang Y., Wang D., Liu S. P. (2010). Effect of saline water on cucumber (Cucumis sativus L.) yield and water use under drip irrigation in North China. Agricultural Water Management, 98, 105–113. doi:10.1016/j.agwat.2010.08.003

[40] Wang F., Chen X., Luo G., Han Q. (2015). Mapping of Regional Soil Salinities in Xinjiang and Strategies for Amelioration and Management. Chinese Geographical Science, 25(3): 321-336.

[41] WHO & UNICEF. (2017). World Health Organization and the United Nations Children's Fund. Progress on drinking water, sanitation and hygiene: 2017 update and SDG baseline.

Spotting the sensory preferences of artichokes to improve the consumption of this functional food

María B. Gatti[1], María C. Mondino[2], Stella M. García[3], María C. Ciappini[4*]

[1,4]Universidad del Centro Educativo Latinoamericano. Avda. Pellegrini 1332, (S2000BUN), Rosario, Santa Fe, Argentina.
[2]AER INTA. Arroyo Seco, San Martín 528, (2128) Arroyo Seco, Santa Fe, Argentina.
[2,3]Facultad de Ciencias Agrarias. Universidad Nacional de Rosario. Campo Experimental Villarino. C.C. 14, (S2125ZAA) Zavalla, Santa Fe, Argentina.

Abstract - *Given the greater varietal supply of the globe artichoke and evaluating its use for human consumption, it is important to carry out market positioning work, including the recognition of the newly available cultivars. Sensory analysis comes up as an important instrument to assess the potential of insertion of artichoke into the market. Within this perspective, the aim of the present study was to determine the sensorial quality characteristics of three globe artichoke cultivars produced in the Rosario´s Horticultural Belt and associated with the preference of consumers. The cultivars were Opal, Madrigal and Romanesco. For sensory evaluation, the method called CATA (Check All That Apply) was used. The results of the CATA test were analyzed using multivariate statistics, applying the Correspondence Analysis. The most distinguishing elements between artichokes were the color and aroma. When associating these results with which was the artichoke that consumers liked the most, it was observed that the Romanesco obtained the best rating. Consumers preferred sweet and tender artichokes. This information could guide the production strategies of the horticulturists to offer cultivars that meet these characteristics.*

Key words: artichoques, sensory analysis, CATA.

I. INTRODUCTION

The cultivation of the globe artichoke (*Cynara cardunculus* var. *scolymus* L.) in Argentina began at the start of 1900s with the arrival of Italian and Spanish immigrants. They introduced the first cultivars and adapted their agricultural practices to the conditions of the local climate and soil. Currently, Argentina is the fourth largest producer of globe artichokes in the world, after Italy, Egypt and Spain [1].

The Horticultural Belt of Rosario (Argentina) has a long tradition in the production of cultivars destined for fresh consumption. The edible portion of the plant consists of the flower buds before the flowers come into bloom. The budding artichoke flower-head is a cluster of many budding small flowers (an inflorescence) together with many bracts, on an edible basis [2]. Artichokes also have nonfoods as their leaves that they are a source of antioxidant compounds, such as luteolin and dicaffeoylquinic acids (cynarin) [3], and the roots contain inulin, an oligosaccharide known to have a positive effect on human intestinal flora, and thus a positive impact on health [4] [5]. It is not only a source of pharmaceutically useful compounds, but also potentially good energy crop [6][2].

At present, different technologies have been used for the production of the globe artichoke, such as the use of seed reproductive materials and drip irrigation, which facilitates their production, expanding the varietal spectrum and prolonging the supply period in the market. The producers have incorporated cultivars with different characteristics in terms of shape, color, texture and taste [2]. However, such differences are not clearly identified by consumers, who are often unaware of the species in question and its mode of consumption.

The quality in fruits and vegetables can have different meanings, according to the different parties involved in the distribution chain. Mainly, it can be divided into product-oriented quality and consumer-oriented quality. When referring to quality from the point of view of the consumer its measurement becomes less tangible and quantifiable. In this case, sensorial analysis becomes a very useful tool, since it allows the identification of important value attributes for consumers, which would otherwise be very difficult to measure [7].

Given the greater varietal supply of the globe artichoke and evaluating its use for human consumption, it is important to carry out market positioning work, including the recognition of the newly available cultivars. The innovative sensorial Check All That Apply (CATA) technique has sprung up in search of a direct link with consumers. The CATA methodology consists in statements used by consumers to mark out as many options as are needed, to express their opinion about the product under analysis. Such methodology is descriptive, not lengthy, and flexible. It can be apply on the consumers without the need for trained appraisers [8].

Techniques involving consumers are apply in knowing the relationship among several factors and help interpret the perception of food by the human being linked to the pleasure experienced in its consumption [9]. Sensory analysis comes up as an important instrument to assess the potential of insertion of new product into the market [8].

Within this perspective, the aim of the present study was to determine which sensory characteristics are preferred by consumers for the globe artichokes. This will allow proposing strategies for their production and commercialization and increasing the consumption of this functional food.

II. MATERIAL AND METHOD

The work was carried out with globe artichoke heads harvested during September and October of 2017 in the area of Rosario (33° 52 18 S, 60° 40 46 W, Argentina). Three cultivars were used: the hybrids Opal and Madrigal were provided by Nunhems Company, the Romanesco was produced using asexual reproduction. It is also known as French variety and being the most traditional farming material in the area.

For the sensory evaluation, the method called CATA (Check All That Apply) was used. It consists on the participants selecting the terms that they consider appropriate for the description of each product from a set of terms provided [9]. For this purpose, the classification shown in Fig. 1 was used [11]. In order to make the descriptors of the shape of artichokes more accessible, terms that were more familiar to consumers were used. The circular and long elliptical forms were jointed under the term "compact", oval and triangular as "ovoid" and the long transverse elliptical form was identified as “flat” (Fig. 1).

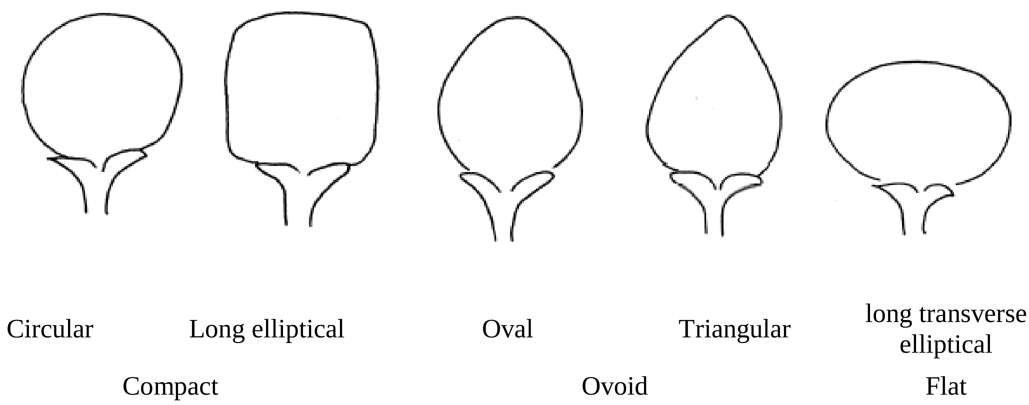


FIGURE 1. Different forms of globe artichoke head.

For the selection of the proper terms for CATA, five previous sessions were conducted with trained sensorial assessors. As a result of this preliminary work, the trained assessors selected 21 terms with the score sheet was drawn (Fig. 2). The color of the bracts was established by differentiating between green and violet. The presence of "hairs or spikes" was explained as very developed flowers that appear when the heads are overripe. Consumers also had to indicate their preference in a hedonic verbal scale of 9 points [12].

Fifty people participated in the CATA test [13], aged between 20 and 60 years old. They were the students and teachers of culinary schools of Rosario, such as the “Asociación de Empresarios Hoteleros Gastronómicos de Rosario” (AEHGAR), “Instituto Superior“ (ISHYR), “Instituto de María de los Ángeles Soso” (MAS) and students of “Licenciatura en Nutrición de la Universidad del Centro Educativo Latinoamericano” (UCEL). Participants were selected considering their knowledge of food, their taste and preference for ingredients, their ability to observe and their vocabulary to describe food.

The heads of the three cultivars were presented raw, in order to evaluate their appearance, and cooked to the point of tenderness to complete the evaluation of the sensorial characteristics.

You will receive three samples of artichokes. For each of them, SELECT ALL the following terms that describe it. Rinse your mouth with water between samples. Finally mark with a cross how much you like this artichoke.
Sample No.: ....

- ☐ Ovoid
- ☐ Flat
- ☐ Compact
- ☐ Green Bracts
- ☐ Violet (purple) bracts

Bracts (Edible portion)

- ☐ Inedible bracts
- ☐ Edible bracts <10%
- ☐ Edible bracts <50%
- ☐ Edible bracts >50%

Bottom(fund)

- ☐ Bitter taste
- ☐ Sweet taste
- ☐ Fresh herb flavor
- ☐ Tomatoe flavor
- ☐ Olive flavor
- ☐ Herbaceous aroma
- ☐ Aroma of olive oil
- ☐ Chlorophyll aroma
- ☐ Tomato aroma
- ☐ Tender texture
- ☐ Fibrous texture
- ☐ With presence of hairs or spikes

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely — Neither like nor dislike — Like extremely

FIGURE 2. Example of CATA score sheet

The results of the CATA test were analyzed using multivariate statistics, applying the Correspondence Analysis (CA) methodology by R-project v.3.5.0 [14]. CA is a multidimensional scaling multivariate technique that uses non-metric data in the crossed design to create percentage maps including all variable categories [15].

III. RESULTS

TABLE 1
DESCRIPTOR SELECTION FREQUENCIES OBTAINED WITH THE CATA QUESTIONNAIRE

	Ovoid	Flat	Compact	Green Bracts	Violet bracts	Inedible bracts	Edible bracts <10%	Edible bracts <50%	Edible bracts >50%	Bitter	Sweet	Fresh herb flavor	Tomato Flavor	Olive flavor	Herbaceous aroma	Aroma of olive oil	Chlorophyll aroma	Tomato aroma	Tender texture	Fibrous Texture	Presence of hair or spikes
Romanesco	35	4	25	9	47	0	9	25	14	21	15	14	1	9	28	9	3	0	36	14	34
Madrigal	42	3	25	47	11	0	15	24	11	32	8	15	0	8	29	3	8	0	32	23	9
Opal	33	8	12	16	34	2	16	17	12	35	7	16	1	5	25	8	5	1	15	24	35

Table 1 shows the number of times that consumers marked each statement. It was observed that the descriptors inedible bracts, tomato flavor and aroma were practically not chosen. The most selected terms were "Ovoid", "Green or Violet bracts", "Bitter taste", "Herbaceous aroma", "Tender texture" and "Presence of hair or spikes". In order to better appreciate the relationship between the CATA terms and the hedonic points, a Correspondence Analysis (CA) was conducted. The inedible bracts, tomato flavor and aroma were excluded from the statistical analysis so as not to generate distortions.

Fig. 3 shows the Correspondence Analysis factorial map. Its first dimension (Dim 1) explained 73.86% of the differences found between the globe artichokes. The most distinguishing elements were the color, Aroma of olive oil and Chlorophyll aroma. Madrigal was associated with Green bracts and Chlorophyll aroma, while the Romanesco and Opal artichokes were related with the Aroma of olive oil and the violet bracts. The "presence of hairs" was a descriptor close to the Opal artichokes.

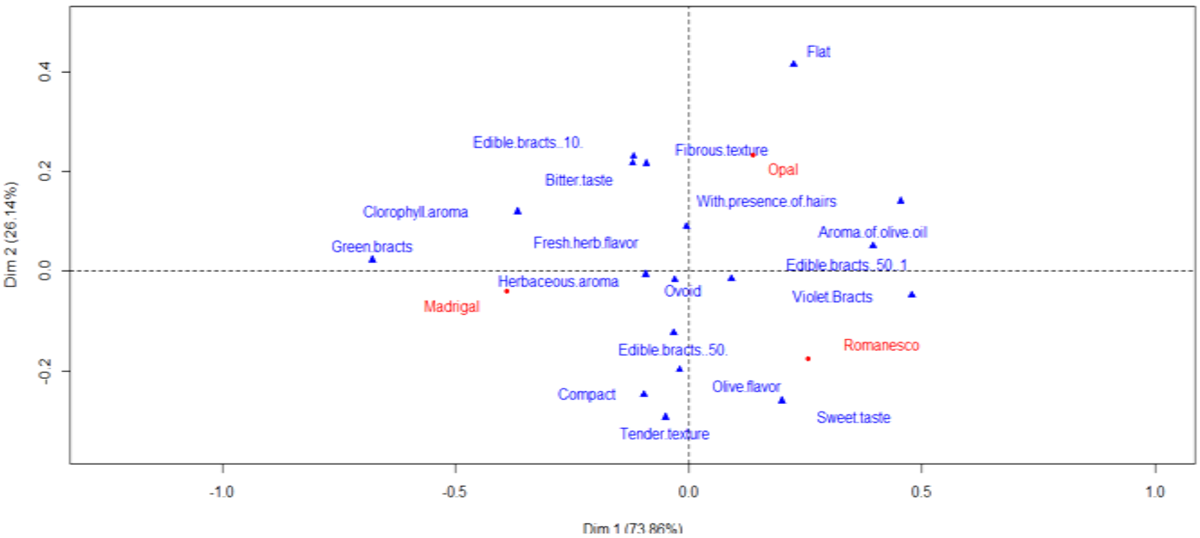


FIGURE 3. Factorial Map obtained by Correspondence Analysis on the CATA evaluations on Romanesco, Madrigal and Opal artichokes.

The second dimension (Dim 2) explained the 26.14% of variations. The descriptors of form “flat” and “compact”, the “bitter” and “sweet” taste, and the “fibrous” and “tender” terms of texture differentiated the samples. Romanesco was described as the sweetest. The Opal was described as bitter, fibrous and flat.

Fig, 4 shows the Correspondence Analisys map of the artichoke consumers preferences. While Opal was closest of phrases that express dislike, Madrigal was near to the neutral phrase “Neither like or dislike”, but the phrases related to liking too. Romanesco is between the phrases “Like extremely” and “Dislike very much”.

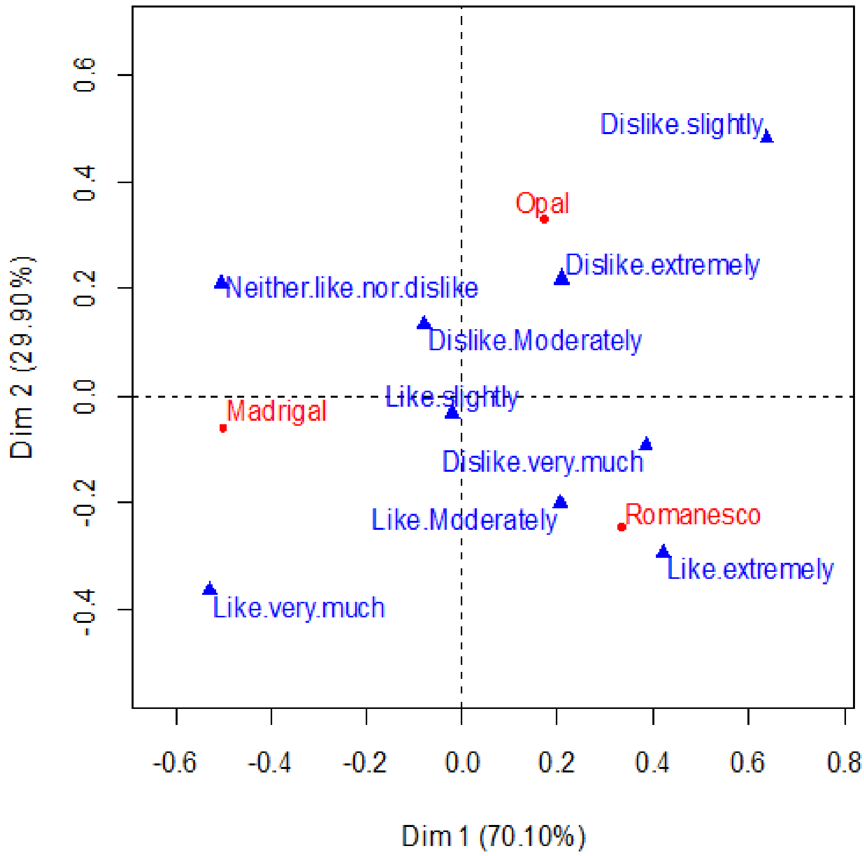


FIGURE 4. Correspondence Analysis on the preference of the consumers for the different artichokes cultivars: Romanesco, Madrigal y Opal.

In short, the Romanesco had consumers who appreciated it but it also had detractors, Madrigal was more homogenous in preference, and Opal had the more negative comments. Participants preferred tender and sweet artichokes, characteristics mainly offered by the Romanesco. Madrigal was accepted because it is not bitter or fibrous either.

Food preferences are related to culture and culinary habits. While in Spain green and small heads were preferred (Blanca de Tudela), in Italy they opted for the violet and median variety (Romanesco). In Bretagne (France), they preferred green and large heads (Camus de Bretagne) and in Provence (France), violet and medium heads (Violeta de Provenza) [16]. The color of the head was the variable that had the most influence on the habits of consumption of each zone [17].

Consumers from Texas (USA) preferred fresh, large and green artichokes compared to small and violet canned cultivars, since the taste, the freshness and the aspects related to the nutrition were the three main factors that influence in the decisions of purchase of the artichoke consumers [18].

IV. CONCLUSION

The CATA technique made it possible to obtain information about the sensorial characteristics of the three globe artichoke cultivars that were evaluated. It proved to be an important tool for researching consumer market.

The preference of consumers was associated with the differentiator's sensory attributes. They like sweet and tender artichokes as Romanesco. This information could guide the production strategies to offer cultivars that meet these characteristics.

It is necessary to continue this line of work, to deepen the knowledge of the different cultivars in relation to consumers and the use of artichokes.

REFERENCES

[1] FAO Organization. Major Food And Agricultural Commodities And Producers – Countries By Commodity. Archived from the original on 2013-01-14. Retrieved Feb 20, 2017.

[2] Sonnante G, Pignone D, Hammer K. The Domestication of Artichoke and Cardoon: From Roman Times to the Genomic Age. Annals of Botany 100, 1095–1100, 2007.

[3] Lattanzio V, Kroon P, Linsalata V, Cardinali A. Globe artichoke: A functional food and source of nutraceutical ingredients. Journal of Functional Foods, 1(2), 131-144, 2009.

[4] Pandino G, Lombardo S. Globe artichoke leaves and floral stems as a source of bioactive compounds, Industrial Crops and Products, 44, 44-49, 2013.

[5] Falco B, Incerti G, Amato M, Lanzotti V. Artichoke: botanical, agronomical, phytochemical, and pharmacological overview. Phytochemistry Reviews, 14(6), 993–1018, 2015.

[6] Lima SH, Ryu JM. Ethanol fermentation from artichoke powder using Saccharomyces cerevisiae KCCM50549 without pretreatment for inulin hydrolysis. Bioresource Technology, 102(2), 2109–2111, 2011.

[7] Driesener C, Romaniuk J. Comparing methods of brand image measurement. International Journal of Market Research, 48, 681 – 689, 2006.

[8] Belusso AC, Nogueira BA, Breda LS, & Mitterer-Daltoé ML. Check all that apply (CATA) as an instrument for the development of fish products. Food Science and Technology, 36(2), 275-281. Epub May 20, 2016.https://dx.doi.org/10.1590/1678-457X.0026

[9] Naes V, Brockhoff P, & Tomic O. Statistics for sensory and consumer science (pp. 282). 2010. United Kingdom: John Wiley and Sons.

[10] Valentin D, Chollet A, Lelievre M, Abdi H. Quick and dirty but still pretty good: a review of new descriptive methods in food science. Food Science and Technology, 47(8), 1563-1578, 2012.

[11] Dellacecca V, Magnifico V, Marzi V, Porceddu E, Scarascia Mugnozza GT. Contributo alla conoscenza delle varietà di carciofo coltivate nel mondo. Atti 2º Congresso Internazionale di Studi sul Carciofo. 199-316. 1974.

[12] ISO 11136. Sensory analysis -- Methodology -- General guidance for conducting hedonic tests with consumers in a controlled area. 2014. International Organization for Standardization, Geneva, Switzerland.

[13] Ares G, Jaeger SR. Check-all-that-apply questions: Influence of attribute order on sensory product characterization. Food Quality and Preference, 28(1), 141-153, 2013.

[14] Abdi H, Williams IJ, Valentin D, Bennani Dosse M. Statis and DISTATIS: optimum multi table principal component analysis and three way metric multidimensional scaling. Wiley Interdisciplinary Reviewers: Computational Statistics, 4, 124-167, 2012.

[15] Hair J., Black W., Babin B., Anderson R., & Tatham R. Análise multivariada de dados (6th ed.). 2009. Porto Alegre: Bookman.

[16] Macua González JI. Evaluación agronómica de variedades españolas y otras europeas de alcachofa (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) en las condiciones del Valle del Ebro. Tesis Doctoral. 2009. Universidad Pública de Navarra. España. 139 p.

[17] Aubert S, Foury C. Couleur et pigmentation anthocyanique de l´artichaut. In: Atti. 3er Cong. Int. Studi Sul carciofo. Industria Gráfica Laterza. Bari. Italia. 57-76, 1981.

[18] Segovia MS, Palma MA, Leskovar DI. Factors affecting consumer preferences and willingness to pay for artichoke products. Acta Hort. (ISHS) 1147, 271-280, 2016.

Hepatitis A virus and environmental quality indicators in aquatic ecosystems for oyster farming in the Northeast of the State of Pará, Brazil

José Raul Rocha de Araújo Júnior[1],
Denise Suéllen Amorim de Sousa Santos[2], Mônica Cristina de Moraes Silva[3], Dielle Monteiro Teixeira[4], Elivam Rodrigues Vale[5], Walber Victor de Moraes Pinto[6], Bruno Santana Carneiro[7], Vanessa Salete de Paula[8], Yvone Gabbay Mendes[9], Heloísa Marceliano Nunes[10], Lena Líllian Canto de Sá Morais[11]
[1,2,3,4,5,6,7,9,10,11]Instituto Evandro Chagas/SVS/MS, Belém-PA (BRAZIL) 67030-000
[8]Laboratório de Virologia Molecular - Fundação Oswaldo Cruz, Rio de Janeiro-RJ (BRAZIL) 21040-900

Abstract - *Research into the occurrence of microbiological contaminants, including hepatitis A virus (HAV), in river waters intended for oyster farming is of extreme importance for public health. This study aimed to detect the occurrence of HAV in the aquatic environment for ostreiculture in northeastern Pará, Brazil, and correlate with microbiological, physico-chemical and climatological variables. The HAV research was based on the method of water concentration by filtration membrane adsorption-elution and in the organic flocculation method with skim milk, followed by Nested-PCR. Quantification of coliforms, Enterococci and heterotrophic bacteria was performed. The physico-chemical variables were measured with multiparametric probe and spectrophotometry. Positive samples were purified and submitted to sequencing. From March 2017 to December 2018, 203 samples of river water were collected and analyzed in the municipalities of Augusto Corrêa, Curuçá, Salinópolis and São Caetano de Odivelas. In 10.8% of the analyzed samples the HAV RNA was detected, in all localities the HAV was classified in genotype IB. There was no significant difference between the concentration methods of the water samples. The only physicochemical variable that most influenced HAV detection was dissolved oxygen. Regarding the bacterial indicators, the highest statistical significance occurred with thermotolerant coliforms and Escherichia coli. The detection of HAV in the four municipalities studied shows that the virus is circulating in the aquatic environment and, therefore, in the community. In this context, more effective hygienic-sanitary measures are necessary in these communities dedicated to oyster farming.*

Keywords— Hepatitis A Virus, Quality Indicators, Water and Ostreiculture.

I. INTRODUCTION

The hepatitis A virus (HAV) belongs to the order of *Picornavirales*, family *Picornaviridae*, genus *Hepatovirus*, species *Hepatovirus A* [1]. It is an icosahedral symmetry virus, not enveloped, with a diameter of 27 to 32 nanometers. Classified in six genotypes, according to the phylogenetic analysis of the complete VP1 protein sequence. Genotypes I, II, and III are found in humans, being subdivided into subgenotypes IA and IB; IIA and IIB; IIIA and IIIB [2].

HAV is transmitted enterally by the ingestion of contaminated food and water, causing Hepatitis A, a self-limiting, infectious, symptomatic or asymptomatic disease of benign evolution, with the occurrence of fulminant cases. HAV infection is prevalent throughout the world, but with different epidemiology according to age of exposure and immunization [3].

With increasing pollution of the aquatic system, microorganisms that cause water-borne diseases can occur in oysters due to contamination by human waste or chemical pollution [4]. The quality of seawater where oysters are grown, as well as the animal itself, is of extreme importance for public health, since food and / or water contaminated by pathogens are the main causes of the occurrence of gastrointestinal diseases in Brazil [5]. Due to the various contaminants and forms of contamination of the aquatic environment, it is necessary to use quality micro-organisms that ensure the absence of other pathogens. The most commonly used indicators are coliform bacteria, total coliforms, thermotolerant bacteria, and Escherichia coli. However, depending on the environment studied, heterotrophic bacteria and Enterococci can be used to evaluate water quality [6].

One of the most widely used methods in environmental virology is based on the adsorption of virus particles to the filter media by loading interaction and subsequent elution of the virus by a pH adjusted solution [7]. Another method of viral concentration is organic flocculation, where an alkaline proteinaceous solution containing glycine with meat extracts or skimmed milk is used, promoting the recovery of virus particles adsorbed on the flakes [8].

After the concentration processes, molecular tests are carried out by PCR techniques, which has allowed advances in the detection of enteric viruses. However, the assay is susceptible to inhibitors found in aquatic environments [9]. The higher the level of water pollution, the lower the detection efficiency of the viral genome, the presence of particulate matter or suspended solids in water [10].

The investigation of the contamination of water in relation to enteric viruses using molecular techniques may be impaired due to the large volume of water in the environment in relation to the low concentration of viruses in these environments, and also by the possible presence of inhibitors of enzymatic reactions that can be found in these samples that compromise the detection of viral genomes [9].

This study aimed to detect the occurrence of HAV in the aquatic environment for ostreiculture in northeastern Pará, Brazil, and correlate with microbiological, physico-chemical and climatological variables.

II. MATERIAL AND METHOD

2.1. Description of the study

This is a cross-sectional field research that was carried out in a descriptive way, with a quantitative analysis approach developed from March 2017 to December 2018.

2.2. Study area

The samples were collected in four different rivers for ostreiculture, in the municipalities of Salinópolis, Augusto Corrêa, Curuçá and São Caetano de Odivelas. The municipalities belong to the Meso-region of the Northeast of the State of Pará, Brazil.

2.3. Sampling

The water samples were collected in three distinct points (mouth, oyster and spring) of a river in each municipality studied. In São Caetano de Odivelas, an extra point was added due to the occurrence of a point of launch of a rainwater gallery near the ostrich. A total of 203 water samples were collected: 48 samples in Salinópolis, 45 in Augusto Corrêa, 48 in Curuçá and 62 in São Caetano de Odivelas.

2.4. Collection and storage of samples

At each collection point the samples were stored in previously sterilized plastic polypropylene bottles and kept at ± 4 ° C until the time of analysis, which did not exceed 24 hours in any opportunity. Two liters of water were collected for each method of concentration of the virus particles, one liter for the physico-chemical analyzes and 500 mL for quantification of the microbiological indicators of water contamination. All methodology complied with the recommendations of ISO 5667-14, Standard Methods for the Examination of Water and Wastewater [11] and CETESB [12].

2.5. Chemical physical analysis

The pH, Temperature, Electrical Conductivity (CE), Total Dissolved Solids (STD), Salinity and Dissolved Oxygen (OD) parameters were analyzed by potentiometry in a previously calibrated Professional Plus YSI® multiparameter probe. Turbidity was determined by spectrophotometry on the HACH® DR3900 equipment. The analytical methods employed for the determination of the physicochemical parameters obeyed the procedures and recommendations described in the Standard Methods for Examination of Water and Wastewater [11] and Procedures Manual HACH-Spectrophotometer DR-2800.

2.6. Quantification of microbiological indicators

The most probable number (MPN/100mL) of total coliforms, thermotolerant, Escherichia coli, Enterococcus and heterotrophic bacteria was determined using the COLLILERT 18 / QUANTI-TRAY®, ENTEROLERT / QUANTI-TRAY® and SimPlate TM for chromogenic substrate method HPC Unit Dose from IDEXX Laboratories, Inc. © from IDEXX Laboratories, Inc., following the manufacturer's recommendations and the Standard Methods for the Examination of Water and Wastewater [11].

2.7. Concentration of viral particles

2.7.1 Method of organic flocculation with skimmed milk

One liter of the sample was acidified with hydrochloric acid (1N HCl) for pH 3.5 adjustment. 100 mL of 0.01% w / v pre-flocculated skim milk solution was added to the acidified sample. The samples were kept under stirring for eight hours, followed by resting for an additional eight hours for sedimentation of the flocculated material. The supernatant was carefully removed and the final volume containing the pellet centrifuged at 7000 x G for 30 minutes at 12ºC. After removal of the centrifuged solution supernatant, the pellet was resuspended in 8 mL of 0.2 M phosphate buffer pH 7.5 (1: 2 0.2 M Na2 HPO4 and 0.2 M NaH2 PO4). Once dissolved, phosphate buffer was added to a final volume of 10 mL. The concentrate was stored at -20 ° C [13].

2.7.2 Adsorption-elution method on filter membranes

Two liters of the samples were acidified with hydrochloric acid (6N HCl) to adjust pH 5, when necessary. Subsequently, after concentrating on 0.45 µm and 142 mm diameter pore HA membranes (MILLIPORE), after the sample was passed through the membrane, 350 mL of sulfuric acid (5 mM H2SO4 pH 3.0) was filtered for elimination of cations. Elution was performed with 15 mL of 1 mM NaOH solution pH 10.5 for 10 minutes over orbital shaking. The eluate was neutralized with 50 ul H2SO4 (50mM) and 50µL TE 100x (pH 8). The concentrate was stored at -20 ° C [14].

2.8. Ultrafiltration

Ultrafiltration was performed by means of a membrane using a vacuum system. The samples concentrated by the adsorption-elution method were reconcentrated in Amicon Ultra-15 (MILLIPORE) device at 5000 RPM for 15 minutes at 4 ° C, obtaining a final volume of 2 mL, stored at -20 ° C until extraction of the nucleic acid.

2.9. Molecular analysis

2.9.1 Viral RNA Extraction

RNA extraction was performed with commercial QIAamp Viral RNA kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's recommendations.

2.9.2 Preparation of Complementary DNA (cDNA)

After extraction of the nucleic acid, the cDNA was prepared from 9.5 µL of the extracted RNA, with a random primer Pd (N) 6 (Invitrogen) and reverse transcription using Super Script III (Invitrogen), with a cycling of 25 ° C for 5 minutes, 50 ° C for 60 minutes and 70 ° C for minutes.

2.9.3 Nested RT-PCR

For detection of HAV by the Nested RT-PCR technique, after reverse transcription, the VP1/2A junction region of the HAV genome was amplified (409 bp). The sequences of the external primers used were F6-CTATTCAGATTGCAAATTAYAAT and F7-AAYTTCATYATTTCATGCTCCT (Y = C or T), and internal primers F8-TATTTGTCTGTYACAGAACAATCAG and F9-AGGRGGTGGAAGYACTTCATTTGA (R = A or G, Y = C or T) [15].

2.9.4 Sequencing

Sequencing of the positive samples was performed from the 2nd round product of Nested-PCR, purified with the commercial BigDye XTerminator Purification Kit (Applied Biosystems) according to the manufacturer's instructions. For the reaction, the BigDye Terminator Cycle Sequencing kit was used. 3.1 (Applied Biosystems) and the 3130xl Genetic Analyzer (Applied Biosystems) following the manufacturer's recommendations. Briefly, two µL of the purified genetic material was used in the preparation of the sequencing reactions, which is composed of 250ng of each of the primers (F8 and F9), in separate reactions, together with the reaction buffer and BigDye, totalizing a volume end of 20 µL. The reactions were subjected to 25 cycles where the steps of denaturation occurred at 96 ° C, hybridization at 50 ° C and extension at 60 ° C. After the cycling of the sequencing reaction, the DNA was precipitated (125 mM EDTA, ethanol) to remove excess reagents that might interfere with obtaining the data during electrophoresis.

2.9.5 Sequence analysis and genotyping

The sequences were edited and aligned in the Geneious version 8.1.3 program and compared to prototype sequences of the HAV genotypes, available from the National Center for Biotechnology Information (NCBI) database. The comparative analysis was performed with BLAST (BlastN) to compare the nucleotide sequence to be analyzed against available sequences in the NCBI database. To evaluate the existence of recombination between the sequences, the phi-test of the program Splits tree version 4.13.1 was applied.

2.9.6 Phylogenetic analysis

The alignment was manually inspected in the Geneious program version 8.1.3 and corrected insertion and deletion regions shared by more than one strain. In order to evaluate the genetic distance and the choice of the nucleotide substitution model, MrModeltest was used to select the best evolutionary model for the phylogenetic tree generation process, where the Neighbor-Joining method was selected.

The evolutionary distances were calculated using the Tamura-Nei method. The rate variation between the sites was modeled with a gamma distribution (shape parameter = 1). All ambiguous positions were removed for each pair of sequences. There were a total of 225 positions in the final dataset. The visualization of the phylogenetic tree was performed in the Mega 6.06 program.

2.10. Statistical analysis

The G test was applied to analyze the correlation of independence of HAV detection with biotic and abiotic variables. For the analysis of variance between the biological indicators with biotic and abiotic variables, the Kruskal-Willis test was used. The Pearson test analyzed the correlation between biological indicators and abiotic variables. Fisher's exact test was used to analyze the relationship between the viral concentration methods used in the study.

III. RESULTS AND DISCUSSION

Oyster farming in the Northeast of the State of Pará is an income alternative carried out alongside or parallel to traditional fishing, during periods of detention or low fish production. Oyster farming makes it possible for the fishermen to take part in family life, serving as a complementary source of income. In the study period, there were no reports of hepatitis A outbreaks in the communities, however the occurrence of HAV in the study areas demonstrates a possible source of contamination of the cultivated molluscs, which may have a negative impact on public health, production and commercialization of these foods.

3.1 Occurrence of HAV

HAV-RNA was detected in 10.8% (22/203) of the analyzed water samples. All positive samples were confirmed by sequencing. HAV was detected in 11.1% (5/45) of Augusto Corrêa samples, 12.5% (6/48) of Curuçá, 10.4% of (5/48) Salinópolis and 9.7% (6 / 62) of São Caetano de Odivelas, this difference was not significant ($p = 0.688$ Test G).

As for the distribution of HAV positive results among the sampling points, a positivity of 30.4% (8/22) was observed among the samples collected at the source of the rivers, 31.8% (7/22) at the point of oyster culture, 31.8% (7/22) at the mouth and 4.5% (1/22) at the extra point, there being no statistical significance ($p = 0.9835$ Test G).

As for the concentration of water samples used in this study, HAV was detected in 4.4% (9/203) of the samples concentrated by the membrane adsorption-elution method and in 7.4% (15/203) of the samples. samples concentrated by organic flocculation with skim milk, this difference in the data was not statistically significant ($p > 0.05$ Fischer exact test). In only two instances were the samples positive by both methods. This fact calls for the use of at least two methods of water concentration for this type of study in order to obtain better results. In the case of salt water, Moresco (2011) emphasized the method of concentration by organic flocculation in relation to membrane filtration (adsorption-elution). Rigotto [16] detected HAV in 8.3% of the samples, using the adsorption-elution method, but using a pre-filter to reduce the presence of debris in the samples, demonstrating the need for adaptations according to the type of virus or matrix analyzed .

Of the 22 HAV positive samples, 30.4% (7/23) were concentrated by the membrane adsorption-elution method, 56.5% (13/23) only by the organic flocculation method with skim milk and 8.7 % (2/23) by both methods. The detection of HAV has also been reported by concentrating seawater samples by the polyethylene glycol (PEG) precipitation method [17]. The use of PEG may increase the recovery of some viral species, such as Poliovirus, but it may also be a potent inhibitor of PCR reactions [18].

In fact, the use of molecular biology tools to detect microorganisms in environmental samples may be limited due to the presence of polymerase chain reaction inhibitors, for example, humic acid may adsorb proteins or enzymes interfering in the sites of activity of the polymerase or chelate the cofactors of the enzymes, the cations of $Ca2 +$ and $Mg2 +$ [19]. It is also possible that marine bacterial enzymes can degrade the viral capsid, being a thermolabile biological factor active against enteric viruses, including HAV [19]. To decrease the action of possible inhibitors of the PCR reaction, the genetic material can be diluted 1:10, mainly in viral quantification reactions [18]. Rigotto [16] observed that the detection of HAV in six samples was only possible after dilution of the same. In the present study there was no analysis of diluted samples.

In two separate studies on seawater in Santa Catarina, Rigotto [16] detected HAV in 16% of the samples using Nested RT-PCR and Moresco [18] detected the HAV in 51.5% of seawater samples using the quantitative RT q-PCR method. This improved detection efficiency corroborates with a study by [15], in which HAV was detected in 23% of water samples by nested-RT-PCR and 92% by RT-qPCR, demonstrating a lower influence of enzymatic inhibitors on the samples in quantitative tests and the need for more a method of viral detection. As only the Nested RT-PCR was performed for the analysis of the samples from this study, it was not possible to compare the qualitative and quantitative results.

From the 22 sequences of hepatitis A virus genome fragments obtained from the positive samples, it was possible to perform the phylogenetic analysis in 19 sequences, observing a nucleotide similarity of 98.4 to 100.0%. Samples were grouped with strains M14707 (wild-type), AY323047 (Rio de Janeiro) and AF268396 (Brazil), all genotype IB, with nucleotide identity varying from 98.2 to 100.0%. Phylogenetic analysis included complete genomic sequences which are available from GENBANK (Fig. 1).

Mendes [20] analyzed 144 samples from the Rodrigo de Freitas Lagoon, Rio de Janeiro, detected the HAV by RT q-PCR in 21.53% of the samples and only 3.47% by Nested RT-PCR, all of which were genotyped as genotype IB. The genotyped samples showed a small genetic difference between them, demo nstrating the circulation of different strains. In the State of Pará there are few studies that report the occurrence of HAV genotype in aquatic matrices, mainly in brackish or salt water. Santos [21] analyzed freshwater samples in Mosqueiro Island, Pará, and detected three samples of genotype IA and 11 of genotype IB, similar to IB isolates from Brazil.

In bivalve molluscs, IB and IB subgenotypes are the most commonly found in Brazil, France, China and Japan. Hepatitis A outbreaks involving IB genotype were associated with oyster ingestion in the Middle East, Egypt and Morocco [22]. In a study in Tunisia, the occurrence of genotype IA was observed both in clinical samples and in samples of oysters and culture water [23].

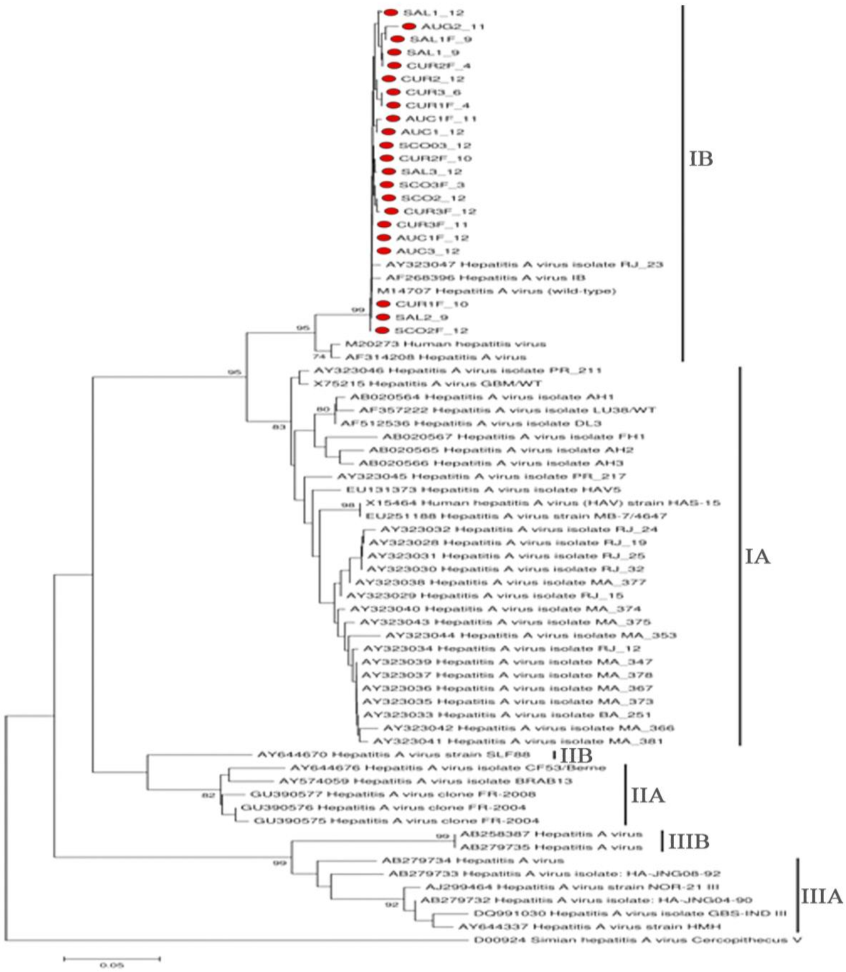


FIGURE. 1 - Neighbor-joining algorithm phyogenetic tree built on sequences of hav genetics studied, compared with sequences available in the data bank of the national center for biotechnology information (NCBI).

3.2 Climate variables

According to the website of the National Institute of Meteorology (INMET), the highest rainfall rates occurred in the months of March to July 2017 and from January to July 2018, indicating the rainy season in the study area. Regarding the seasonality of HAV occurrence, 4.0% (4/100) of the positive samples in the dry period and 17.5% (18/103) in the rainy season were observed, a highly significant factor ($p = 0.0034$ Test G). The higher the rainfall the greater the transport of solids to water, pathogenic organisms can remain attached to particles, using them as a substrate and form of protection and resistance [8].

Of the analyzed samples, 18.2% (37/203) were acidic (pH range between 5.28 and 6.99) and 81.2% (166/203) were basic (pH variation between 7.01 and 12 , 06). Catuxo et al. [24] observed values different from those obtained in the present study, relating them to the possible influence by leaching of organic acids to water. In quiescent and organic-rich waters, a great variation of organic and inorganic acids occurs and, therefore, they are often more acidic [25]. As for the occurrence of HAV in acidic or basic waters, 18.8% (4/22) occurred in acid medium and 81.2% (18/22) in basic medium, and although the highest number of HAV positive samples was found in basic water, this fact did not present statistical significance ($p> 0.05$ Fisher's exact test), as found by Hernandez-Morga et al. [26] who found statistical significance between pH and HAV RNA detection. HAV is more stable at acidic pH, even at pH close to 1, which would reproduce the pH of the human stomach [27]. The resistance of HAV to a more acidic medium is due to its extremely cohesive capsid [28].

3.3 Physico-chemical variables

Regarding the electrical conductivity, the parameter varied between 1.32 μS / cm and 111.7 μS / cm in the studied municipalities. A statistically significant difference was observed when EC values were compared between the municipalities and seasonality, especially in the rainy season ($p <0.05$ Kruskal-Wallis test). Generally, values above 100 μS / cm may indicate impacted environments [12], therefore, all the samples of this study would be within the acceptable limit. The highest values found for electrical conductivity occurred in the rainy season, which corroborates with the values found by Kiyatake [29], in a study carried out in São Caetano de Odivelas, possibly because of the smaller influence of the ocean along the estuary in this period.

The turbidity ranged from 1.0 to 247.5 nephelometric units (UNT), with an overall mean of 22.0 NTU. The relation of the turbidity with the municipality and with the period of highest rainfall index was statistically significant ($p <0.05$ Kruskal-Wallis test). It was also observed that, in the four studied rivers, the turbidity values were higher in the rainy season, a fact that can be justified by the loading of inorganic and organic material from the river bed by rainfall.

The analysis of the total dissolved solids data showed statistically significant relation with the municipalities studied and with respect to the occurrence of rain during the collection period ($p <0.05$ Kruskal-Wallis test). The STDs are directly related to rainfall, ie, the higher the rainfall, the higher the solids dilution in the water [30]. In the municipalities of Curuçá and São Caetano de Odivelas, the highest averages of STD were observed in the rainy season, different from the municipalities of Augusto Corrêa and Salinópolis. The fact that the rivers studied in Curuçá and São Caetano de Odivelas have less distance between their margins and lower depth corroborates a higher concentration of STD.

Salinity is used as a parameter for water classification. Salt water has a high concentration of salt, the salinity is equal to or greater than 30 parts per million (ppm). Brackish water has salinity between 0.5 ppm and 30.5 ppm. Fresh water has a salinity of less than or equal to 0.5 ppm [31]. The salinity of the samples ranged from 0.09 to 42.08 ppm in the studied municipalities, 2.9% (6/203) of freshwater samples, 77.3% (157/203) of salt water and 19.8 % (40/203) of salt water. Statistical analysis showed that, in relation to the sampling point, the rivers of the four municipalities presented the highest concentration of salinity at the mouth and lower concentration at the source. ($p <0.05$ Kruskal-Wallis test). The concentration of salinity was inversely proportional to the values of rainfall, data also found by Kiyatake [29] in a study in waters in the same rivers, possibly due to the ions being more concentrated in the water. In a study carried out in a river in Santa Maria da Vitória, Bahia, Loss [32] verified that the farthest point of the mouth had a lower mean salinity due to the dilution of the salt water with the fresh water of the river, corroborating with the findings of this river study. The concentrations of salinity found in this study corroborate a study by Mok et al. [33] in water intended for oyster farming in Hansan Geojeman, Korea, which observed lower levels of salinity in the rainy season, even with the temperature occurring between 8.8 ºC and 25.5 ºC.

Dissolved oxygen (OD) ranged from 0.0 to 14.0 mg / L, with an average of 5.0 mg / L. In this study, 18.2% (37/203) of the samples were below 5 mg / L O_2, limit established for DO by Resolution CONAMA 357/2005 [31], therefore unsuitable for oyster cultivation. Both the comparison between the municipalities and the collection points are not statistically significant ($p <0.05$ Kruskal-Wallis test).

The statistical analysis of this study demonstrated that the only physicochemical variable that presented statistically significant results when compared to the occurrence of HAV was OD ($p < 0.05$ test G), different from that found by Hernandez-Morga et al. [26], who observed that the variable that most possessed statistical significance with HAV detection was salinity.

3.4 Microbiological indicators of water quality

In the analysis of the occurrence of HAV in the study environment in relation to the bacterial indicators studied, there was statistical significance between the occurrence of HAV with thermotolerant coliforms and E. coli ($p < 0.05$ test G). The occurrence of HAV in samples with acceptable levels of microbiological indicators of water quality demonstrates the need to include virus research in the tests already performed in water quality analysis in order to improve epidemiological and sanitary surveillance.

In the analysis of variance of the mean concentrations of thermotolerant coliforms (CTT) and E. coli, obtained in the studied rivers, it was observed that there was no statistically significant difference between them, as well as when the collection points of the same river ($p > 0.05$ Kruskal-Wallis test). However, the analysis of concentrations of Total Coliforms (CT) and Enterococci was significantly positive in the source of the studied rivers ($p < 0.05$ Kruskal-Wallis test), the highest values were detected in the rivers. In a study carried out in Rio de Santa Maria da Vitória, Bahia, Loss (2012) [32] verified that the four collection points, including the one closest to residences, presented contamination by fecal residue, containing CT and Enterococci, probably by the release of debris from the sewage in the river water. In addition to sewage or faeces, organic plant materials, insects and reptiles may also be sources of Enterococci [34].

IV. CONCLUSION

The detection of HAV, genotype IB, in the four municipalities studied shows that the virus is circulating in the aquatic environment and, therefore, in the community. We suggest the use of more than one concentration method with a view to reducing false-negative results, since even though no significant difference was observed between the concentration methods of the water samples for HAV detection, in only two the results were concordant.

The results obtained in this research are important to evaluate the occurrence and concentration of microbiological and physicochemical contaminants in oyster farming areas in the Northeast of Para. In this context, it is necessary to make more hygienic-sanitary measures in the communities dedicated to the cultivation of this food so that it meets the minimum standards established in current legislation, aiming at the expansion of oyster farming in the region and greater sanitary security for consumers.

REFERENCES

[1] INTERNATIONAL COMMITTEE ON TAXONOMY OF VIRUS (ICTV). Master Species List 2015. Available in: <https://talk.ictvonline.org/taxonomy>. Access in: January 22, 2018

[2] SANTOS, N. S.; ROMANEGS, M. T. V.; WIGG, M. D. "Hepatites virais" In: Virologia Humana, 3. ed., N. S. Santos. Rio de Janeiro: Guanabara Koogan, 2015. p. 863-929, 2015.

[3] MACKINNEY-NOVELO, I.; BARAHONA-GARRIDO, J.; CASTILLO-ALBARRAN, F.; SANTIAGO-HERNNDEZ, J. J.; MENDEZ-SANCHEZ, N.; URIBE, M.; CHAVES-TAPIA, N. Clinical course and management of acute hepatitis A infection in adults. Annals of Hepatology, v. 11, n. 5, p. 652-657, 2012.

[4] FLEMING, L. E.; BROAD, K.; CLEMENT, A.; DEWAILLY, E.; ELMIR, S.; KNAP, A.; POMPONI, S. A.; SMITH, S.; SOLO, G.; WALSH, P. Oceans and human health: Emerging public health risks in the marine environment. Marine Pollution Bulletin, v. 53, n. 10, p. 545-560, 2006.

[5] VELAZQUEZ, T. H.; OLIVEIRA, A. J. F. C.; BARBIERI, E.; ANDRADE, V. C.; PEREIRA, C. R. Análises microbiológicas de ostras e de águas coletadas em bancos naturais e em ostreiculturas. In: CONGRESSO DE INICIAÇÃO CIENTÍFICA DA UNESP, 22., 2010, São Paulo. Anais... São Paulo: Universidade Estadual Paulista, 2010.

[6] BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Vigilância e controle da qualidade da água para consumo humano. Brasília: Ministério da Saúde, 2006.

[7] CASHDOLLAR, J. L.; WYMER, L. Methods for primary concentration of viruses from water samples: a review and meta-analysis of recent studies. Journal of Applied Microbiology, v. 115, p. 1-11, 2013.

[8] SILVA, J. P. Avaliação da qualidade da água superficial utilizada no sistema de abastecimento público do município de Belém (PA). 2010. 122 f. Dissertação (Mestrado em Recursos Hídricos e Saneamento Ambiental) – Universidade Federal do Pará, Belém, 2010.

[9] BORGES, C. R. Aplicação de métodos moleculares e de cultivo celular no monitoramento de vírus entéricos no ambiente aquático. 2009. 125 f. Tese (Doutorado em Biotecnologia) – Universidade Federal de Santa Catarina, Florianópolis, 2009.

[10] PRADO, T.; MIAGOSTOVICH, M. P. Virologia ambiental e saneamento no Brasil: uma revisão narrativa. Cadernos de Saúde Pública, v. 30, n. 7, p. 1367-1378, 2014.

[11] APHA/AWWA/WEF. Standard Methods for the Examination of Water and Wastewater, 21 ed., Washington, D.C., 2005.

[12] CETESB - COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL. Guia nacional de coleta e preservação de amostras: água, sedimento, comunidades aquáticas e efluentes líquidos. São Paulo, 2011.

[13] CALGUA, B.; MENGEWEIN, A.; GRUNERT, A.; BOFILL-MAS, S.; CLEMENTE-CASARES, P.; HUNDESA, A.; WYN-JONES, A. P.; LOPEZ-PILA, J.C.; GIRONES, R. Development and application of a one-step low cost procedure to concentrate viruses from seawater samples. Journal of Virological Methods, v. 153, n. 2, p. 79-83, 2008.

[14] KATAYAMA, H.; SHIMASAKI, A.; OHGAKI, S. Development of a virus concentration method and its application to detection of enterovirus and norwalk virus from coastal seawater. Applied and Environmental Microbiology, v. 68, p. 1033-1039, 2002.

[15] DE PAULA, V. S.; BAPTISTA, M. L.; LAMPE, E.; NIEL, C.; GASPAR, A. M. C. Characterization of Hepatitis A Virus Isolates From Subgenotypes IA and IB in Rio de Janeiro, Brasil. Jornal of Medical Virology, v. 66, p. 22-27, 2002.

[16] RIGOTTO, C.; VICTORIA, M.; MORESCO, V.; KOLESNIKOVAS, C. K.; CORREA, A. A.; SOUZA, D. S. M.; MIAGOSTOVICH, M. P.; SIMOES, C. M. O.; BARARDI, C. R. M. Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. Journal of Applied Microbiology, v. 109, n. 6, p. 1979-1987, 2010.

[17] MASACHESSI, G.; PISANO, M. B.; PREZ, V. E.; MARTINEZ, L. C.; MICHELENA, J. F.; MARTINEZ-WASSAF, M.; GIORDANO, M. O.; ISA, M. B.; PAVAN, J. V.; WELTER, A.; ELTER, A.; NATES, S. V.; RÉ, V. Enteric viruses in surface waters from Argentina: molecular and viable virus detection. Applied and Environmental Microbiology, v. 84, n. 5, p. e0327-37, 2018.

[18] MORESCO, V. Detecção e quantificação de patógenos entéricos virais em amostras de água do mar. 2011. 129 f. Dissertação (Mestrado em Biotecnologia) – Universidade Federal de Santa Catarina, Florianópolis, 2011.

[19] PATTI, A. M.; SANTI, A. L.; GABRIELI, R.; FIAMMA, S.; CAULETTI, M.; PANA, A. Hepatitis A virus and Poliovirus 1 inactivation in estuarine water. Water Research, v. 21, n. 11, p. 1335-1338, 1987.

[20] MENDES, A. C. O. Monitoramento do vírus da hepatite A na Lagoa Rodrigo de Freitas do Município do Rio de Janeiro, RJ. 2009. 80 f. Dissertação (Mestrado em Biologia Parasitária) – Instituto Oswaldo Cruz, Rio de Janeiro, 2009.

[21] SANTOS, D. S. A. S. Detecção molecular e genotipagem do vírus da hepatite A em praias da ilha de Mosqueiro, Estado do Pará, Brasil. 2015. 109 f. Dissertação (Mestrado em Virologia) – Instituto Evandro Chagas, Ananindeua, 2015.

[22] PARK, S.; JEONG, H.; JUNG, J.; KIM, K.; KIM, M.; KIM, Y.; OH, Y.; JUNG, K. Monitoring the Hepatitis A Virus in Oyster from Korea. Advances in Microbiology, v. 5, n. 13, p. 825-831, 2015.

[23] GHATBI-KHELIFI, H.; SDIRIL, K.; FERRE, V.; HARRATH, R.; BERTHOME, M; BILLAUDEL, S.; AOUNI, M. A 1-year study of the epidemiology of hepatitis A virus in Tunisia. Clinical Microbiology and Infection, v. 13, n. 1, p. 25-32, 2007.

[24] CATUXO, V. T. S.; SOUSA, P. H. C.; SILVA, C. N. C.; MELO, O. A.; GOMES, A. C. S. Avaliação de aspectos físico-químicos da água de cinco corpos hídricos situados na microrregião da cidade de Castanhal-PA. In: CONGRESSO TÉCNICO CIENTÍFICO DA ENGENHARIA E DA AGRONOMIA, 2017, Belém. Anais... Pará: Conselho Federal de Engenharia e Agronomia, 2017.

[25] PEDROZO, C.; KAPUSTA, S. Indicadores ambientais em ecossistemas aquáticos. Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, v. 1, p. 72, 2010.

[26] J Appl Microbiol. 2009 May;106(5):1579-90. doi: 10.1111/j.1365-2672.2008.04125.x. Epub 2009 Feb 16. Detection and characterization of hepatitis A virus and Norovirus in estuarine water samples using ultrafiltration--RT-PCR integrated methods. Hernandez-Morga J1, Leon-Felix J, Peraza-Garay F, Gil-Salas BG, Chaidez C.

[27] SCHOLZ, E.; HEINRICY, U.; FLEHMING, B. Acid stability of Hepatitis A virus. Journal of General Virology, v. 70, p. 2481-2485, 1989.

[28] D'ANDREA, L.; PÉREZ-RODRÍGUEZ, F. J.; COSTAFREDA, M.I. Bases moleculares do comportamento da hepatite um vírus exposto a alta pressão hidrostática. Applied and Environmental Microbiology. v. 8, n. 20, p. 6499-505, 2014.

[29] KIYATAKE, D. M. Avaliação sanitária de água de cultivo e de ostras da Zona do Salgado, Nordeste do Estado do Pará, Brasil. 2011. 97 f. Dissertação (Mestrado em Ecologia Aquática e Pesca) – Universidade Federal do Pará, Belém, 2011.

[30] PIRATOBA, A. R. A.; RIBEIRO, H. M. C.; MORALES, G. P.; GONÇALVES, W. G. Caracterização de parâmetros de qualidade da água na área portuária de Barcarena, PA, Brasil. Revista Ambiente & Água, v. 12, n. 3, p. 352-359, 2017.

[31] BRASIL. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente (CONAMA). Resolução nº 357, de 17 de março de 2005. Brasília, 2005.

[32] LOSS, S. M. Vírus entéricos e indicadores bacteriológicos de poluição fecal em amostras de água na Região da Ilha das Caieiras, na Baía de Vitória, ES. 2012. 76 f. Dissertação (Mestrado em Engenharia Ambiental) – Universidade Federal do Espírito Santo, Vitória, 2012.

[33] MOK, J. S.; LEE, K. J.; KIM, P. H.; LEE, T. S.; LEE, H. J.; JUNG, Y. J.; KIM, J. H. Bacteriological quality evaluation of seawater and oysters from the Jaranman-Saryangdo area, a designated shellfish growing area in Korea: Impact of inland pollution sources. Marine Pollution Bulletin, v. 108, n. 1-2, p. 147-154, 2016.

[34] GRANT, S. B.; SANDERS, B. F.; BOEHM, A. B.; REDMAN, J. A.; KIM, J. H. Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surface zone water quality. Environmental Science Technology, v. 35, p. 2407-2416, 2001.

AD Publications

Sector-3, MP Nagar, Rajasthan, India

www.adpublications.org, www.ijoear.com, info@ijoear.com